UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

ASSOCIAÇÃO ENTRE POLIMORFISMOS DOS GENES *UGT1A1*, *IL1A*, *NFKB1*, *PAR1*, *TP53* E *UCP2* E A SUSCEPTIBILIDADE AO CÂNCER DE PULMÃO

Esdras Edgar Batista Pereira

BELÉM
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Autor: Esdras Edgar Batista Pereira
Orientador: Prof. Dr. Ney Pereira Carneiro dos Santos
Orientador: Prof. Dr. João Farias Guerreiro

Tese submetida ao programa de Pós-Graduação em Genética e Biologia Molecular, do Instituto de Ciências Biológicas, da Universidade Federal do Pará, como requisito para obtenção do título de Doutor em Genética e Biologia Molecular.

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Banca Examinadora

Prof. Dr. Ney Pereira Carneiro dos Santos
Instituição: Universidade Federal do Pará Assinatura:___________________________

Prof. Dr. João Farias Guerreiro
Instituição: Universidade Federal do Pará Assinatura:___________________________

Profa. Dra. Marianne Rodrigues Fernandes
Instituição: Universidade Federal do Pará Assinatura:___________________________

Prof. Dr. Paulo Pimentel Assumpção
Instituição: Universidade Federal do Pará Assinatura:___________________________

Profa. Dra. Verônica Marques Zembrzuski
Instituição: Fundação Osvaldo Cruz Assinatura:___________________________

Prof. Dr. Sidney Emanuel Batista dos Santos
Instituição: Universidade Federal do Pará Assinatura:___________________________

Prof. Dr. Rommel Mario Rodrigues Burbano (Suplente)
Instituição: Universidade Federal do Pará Assinatura:___________________________

Prof. Dr. Vinicius de Albuquerque Sortica (Suplente)
Instituição: Universidade Federal de Alagoas Assinatura:___________________________

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(Isaías 40:31)

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RESUMO

O câncer de pulmão é uma das neoplasias mais frequentes no mundo. O câncer de pulmão de não pequenas células (CPNPC) representa a grande maioria das neoplasias pulmonares. Por ser uma doença complexa, sua formação ocorre em vários estágios, decorrentes de interações entre fatores de risco ambientais, como tabagismo, e susceptibilidade genética individual. Nosso objetivo foi investigar associações entre polimorfismos genéticos e o risco para o câncer de pulmão. Trata-se de um estudo, caso-controle, que incluiu 276 indivíduos com e sem câncer. As amostras foram analisadas para os polimorfismos do gene *UGT1A1* (rs8175347), *NFKB1* (rs28362491), *PAR1* (rs11267092), *TP53* (rs17878362), *IL1A* (rs3783553), *UCP2* (INDEL 45-pb) e genotipados em PCR, seguido de análise de fragmentos em que aplicamos um conjunto previamente desenvolvido de marcadores ancestrais informativos. Usamos regressão logística para identificar diferenças nas frequências alélicas e genotípicas entre os indivíduos. Indivíduos com genótipo Del/Del do polimorfismo *NFKB1* (rs28362491) (p=0.018; OR=0.332) demonstraram efeito protetor para o desenvolvimento do CPNPC, similar ao observado nas variantes do *PAR1* (rs11267092) (p=0.023; OR=0,471) e do *TP53* (rs17878362) (p=0,041; OR=0,510). Além disso, indivíduos com o genótipo Ins/Ins do polimorfismo *IL1A* (rs3783553) demonstram maior risco para o CPNPC (p=0,033; OR=2,002), assim como os voluntários com Del/Del do *UCP2* (INDEL 45-pb) (p=0,031; OR=2,031). Para o *UGT1A1* (rs8175347), indivíduos com o alelo TA7 têm maior chance de desenvolver adenocarcinoma de pulmão (p=0,035; OR:2,57), assim como aqueles com genótipos relacionados de atividade enzimática reduzida ou baixa: TA6/7, TA5/7 e TA7/7 (p=0,048; OR:8,41). Indivíduos com TA7/7 homozigotos têm maior chance de desenvolver o carcinoma de células escamosas de pulmão (p=0,015; OR=4,08). Os seis polimorfismos investigados podem contribuir para a susceptibilidade especificamente ao CPNPC, adenocarcinomas e carcinomas de células escamosas.

Palavras-chave: Susceptibilidade; câncer pulmão não pequenas células; adenocarcinoma; carcinoma de células escamosas; *NFKB1; PAR1; TP53; IL1A; UCP2; UGT1A1.*

ABSTRACT

Lung cancer is one of the most frequent neoplasms in the world. Non-small cell lung cancer (NSCLC) represents the vast majority of lung neoplasms. As it is a complex disease, its formation occurs in several stages, resulting from interactions between environmental risk factors, such as smoking, and individual genetic susceptibility. Our objective was to investigate associations between genetic polymorphisms and lung cancer risk. This is a case-control study that included 276 individuals with and without cancer. The samples were analyzed for polymorphisms of the *UGT1A1* (rs8175347), *NFKB1* (rs28362491), *PAR1* (rs11267092), *TP53* (rs17878362), *IL1A* (rs3783553), *UCP2* (INDEL 45-pb) genes and genotyped by PCR, followed by fragment analysis in which we apply a previously developed set of informative ancestral markers. We used logistic regression to identify differences in allele and genotypic frequencies between individuals. Individuals with the Del/Del genotype of the *NFKB1* polymorphism (rs28362491) (p=0.018; OR=0.332) demonstrated a protective effect for the development of NSCLC, similar to that observed in the variants of *PAR1* (rs11267092) (p=0.023; OR=0.471) and *TP53* (rs17878362) (p=0.041; OR=0.510). In addition, individuals with the *IL1A* polymorphism Ins/Ins genotype (rs3783553) demonstrate a higher risk for NSCLC (p=0.033; OR=2.002), as do volunteers with *UCP2* Del/Del (INDEL 45-pb) (p=0.031; OR=2.031). For *UGT1A1* (rs8175347), individuals with the TA7 allele are more likely to develop lung adenocarcinoma (p=0.035;OR:2.57), as are those with related genotypes of reduced or low enzyme activity: TA6/7, TA5 /7 and TA7/7 (p=0.048; OR:8.41). Individuals with homozygous TA7/7 are more likely to develop squamous cell carcinoma of the lung (p=0.015; OR=4.08). The six polymorphisms investigated may contribute to susceptibility specifically to NSCLC, adenocarcinomas and squamous cell carcinomas.

Keywords: Susceptibility; non-small cell lung cancer; adenocarcinoma; squamous cell carcinoma; *NFKB1; PAR1; TP53; IL1A; UCP2; UGT1A1*.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

AAVD - Escala de Avaliação das Atividades Avançadas de Vida Diária

ABVD - Atividades Básicas de Vida Diária

ACCP - *American College of Chest Physicians*

AIMS - marcadores informativos de ancestralidade autossômica

AIVD - Atividades Instrumentais de Vida Diária

CEP - Comité de Ética em Pesquisa

CNS - Comissão Nacional de Saúde

CPNPC - Câncer de Pulmão de Não Pequenas Células

CPPC - Câncer de Pulmão de Pequenas Células

DNA - Ácido Desoxirribonucleico

DPOC - Doença Pulmonar Obstrutiva Crônica

EBUS - Ultrassonografia Endobrônquica

FPI - Fibrose Pulmonar Idiopática

HUJBB - Hospital Universitário João de Barros Barreto

HWE - Equilíbrio de Hardy-Weinberg

IARC - *International Agency for Research on Cancer*

INCA - Instituto Nacional do Câncer

NPO - Núcleo de Pesquisa em Oncologia

PEP-CT – Tomografia Computadorizada por emissão de pósitrons

PS-ECOG - *Performance Status* do *Eastern Cooperative Oncology Group*

ROS - Espécies Reativas de Oxigênio

SIM - Sistema de Informação sobre Mortalidade

SUS - Sistema Único de Saúde

TC - Tomografia Computadorizada

TCLE - Termo de Consentimento Livre e Esclarecido

UFPA - Universidade Federal do Pará

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CAPÍTULO I. INTRODUÇÃO

1. CONSIDERAÇÕES GERAIS

O câncer é uma doença multifatorial, com fatores de risco modificáveis e não modificáveis. Os não modificáveis referem-se a fatores intrínsecos, como mutações espontâneas que podem surgir como resultado de erros aleatórios na replicação do DNA (WU *et al*., 2018). Esse erro favorece a expressão descontrolada de genes, alterando a conservada capacidade de estabilidade genômica (CHAMMAS, 2012; MAVROGONATOU, PRATSINIS, KLETSAS, 2020). Esse desequilíbrio entre a replicação e os mecanismos de morte celular, ocasionado por distúrbios entre oncogenes e genes supressores de tumor, favorece um conjunto de capacidades funcionais que levam as células da normalidade até a formação dos tumores malignos (HANAHAN, 2022).

O câncer de pulmão está entre os tipos de câncer mais prevalentes na população mundial, representando 11,4% de todos os cânceres registrados, e são responsáveis por 18% das mortes por câncer (SUNG *et al*., 2021). Essa frequência varia entre os países, dependendo de suas características demográficas, taxa de tabagismo e nível de desenvolvimento econômico (BADE, CRUZ, 2020). É dividido em duas categorias, câncer de pulmão de pequenas células (CPPC), responsável por 15-20% dos casos; e câncer de pulmão de não pequenas células (CPNPC), que representa 80-85% dos casos (DUMA, DAVILA, MOLINA, 2019; SALTOS, SHAFIQUE, CHIAPPORI, 2020).

O principal fator de risco para o desenvolvimento do câncer de pulmão é o tabagismo, pois as substâncias contidas no tabaco favorecem a inflamação e a carcinogênese. No entanto, têm-se demonstrado que a formação dessa neoplasia pode ocorrer em vários estágios, com interações sinérgicas e complexas entre fatores de risco ambientais e a susceptibilidade genética individual (WANG *et al*., 2015; TSAO *et al*., 2016).

Estudos sugerem uma possível associação entre as variações genéticas germinativas com o risco do desenvolvimento do câncer de pulmão (ZHONG *et al*., 2013; KIM *et al*., 2014; WANG *et al*., 2015). Essa associação tem como chave os polimorfismos de genes relacionados ao metabolismo e controle do ciclo celular, que pode ser o gatilho inicial ou fazer parte de múltiplas alterações que ocorram no processo de carcinogênese (GRUDNY *et al*., 2013; GISBERGEN *et al*., 2015). No entanto, ainda é necessária uma maior compreensão da relevância da variabilidade da sequência de genes do DNA e a susceptibilidades ao câncer de pulmão (LATORRE-PELLICER *et al*., 2016).

As neoplasias pulmonares desenvolvem-se de forma assintomática ou com sintomas inespecíficos, isso associado à ausência de um protocolo de detecção precoce contribui para o diagnóstico tardio, influenciando diretamente sobre o prognóstico, a letalidade e os gastos com a doença (CHUDGAR *et al.*, 2015; LEE *et al.*, 2016).

Segundo Lee *et al.* (2016) quando o CPNPC é diagnosticado precocemente, a taxa de sobrevida em cinco anos aumenta para 80%, mas a mesma reduz para 15% no diagnóstico tardio. Estima-se que o custo médio total por paciente com câncer de pulmão, submetido à internação hospitalar seja de cerca 30 mil reais, que poderiam ser reduzidos com políticas de prevenção e diagnóstico precoce (GOOZNER, 2012; INCA, 2015; KNUST, PORTELA, PEREIRA, 2015).

Estudos investigam a viabilidade do diagnóstico precoce em indivíduos com maior risco para o desenvolvimento do câncer de pulmão, como tabagistas de longa data com idade avançada. Os instrumentos pesquisados para essa finalidade são a radiografia torácica, a citologia de escarro e a tomografia computadorizada (TC). A TC de baixa dose tem demonstrado uma redução de 20% das mortes pela doença. No entanto, sua aplicação ainda possui limitações (PATZ *et al.*, 2014; CHUDGAR *et al.*, 2015; YOUSAF-KHAN *et al.*, 2015).

A utilização de biomarcadores de susceptibilidade, como polimorfismos genéticos, baseia-se na perspectiva de utiliza-los como um teste de rastreio populacional, associando-os a fatores de risco clínicos já estabelecidos, o que facilitaria um diagnóstico precoce. A identificação das associações de polimorfismos com a neoplasia pulmonar poderá permitir, futuramente, o rastreio de indivíduos com maior susceptibilidade de desenvolver a doença, antes dos primeiros sintomas, permitindo instituir o monitoramento e o tratamento precoce, diminuindo o risco da morbidade e mortalidade da doença.

O presente estudo buscou entender a associação entre os polimorfismos de biomarcadores genômicos e a carcinogênese pulmonar, identificados no sangue periférico, o que favorece o esclarecimento e a compreensão da susceptibilidade individual ao desenvolvimento deste tipo tumoral em adultos.

O ensaio utilizado para investigação desses elementos moleculares possui características ideais para um *screening* populacional: metodologia minimamente invasiva, com aplicabilidade relativamente fácil e rápida, de baixo custo, exigindo pouca quantidade de DNA do paciente. Sua aplicação na rotina clínica poderá auxiliar na predição do estado de saúde de forma interdisciplinar, embasar estratégias de prevenção, facilitar a gestão e a elaboração de futuros planos de cuidados em oncologia.

1.1 EPIDEMIOLOGIA DO CÂNCER DE PULMÃO

O câncer de pulmão, carcinoma broncogênico ou carcinoma pulmonar, é a neoplasia maligna de origem no epitélio respiratório inferior (SIDDIQUI, VAQAR, SIDDIQUI, 2021). Essas células epiteliais possuem a capacidade de se dividir e são susceptíveis a sofrer alterações proliferativas que podem resultar em lesões pré-neoplásicas. É importante esclarecer que cerca de 95% dos tumores pulmonares são carcinomas e 5% são miscelânias de outras neoplasias, como linfomas, sarcomas, mesoteliomas e carcinoides, que não se enquadram nesse termo (KUMAR; ABBAS, ASTER, 2013; BERNARDI *et al*., 2016).

No início do século passado o câncer de pulmão era considerado uma doença rara, mas atualmente é visto como um problema de saúde pública (GROOT *et al*., 2018). A doença tem sido o tipo de câncer mais frequente no mundo durante muitos anos. Representa aproximadamente 11,4% (2,2 milhões) do total de cânceres registrados no mundo e é responsável por 18% (1,8 milhões) das mortes por câncer (Figuras 1 e 2), sendo que dois terços desses óbitos estão ligados ao tabagismo (INCA, 2019; SUNG *et al*., 2021). Estima-se um aumento de 2% por ano na sua incidência mundial, ocorrendo principalmente em regiões menos desenvolvidas, onde se concentram 58% dos casos (INCA, 2017; ARAUJO *et al*., 2018).

A epidemiologia do câncer de pulmão nos principais países desenvolvidos tem sido relatada de forma consistente, mas nas regiões menos desenvolvidas essa prevalência é menos descrita. Isso mostra que devido o câncer de pulmão possuir uma significativa prevalência mundial e uma relação direta com variabilidade internacional nas tendências de crescimento populacional, índice do desenvolvimento humano, envelhecimento e hábito de fumar, a sua epidemiologia global requer monitoramento contínuo (CHENG *et al*., 2016).

Estudos demonstram que essa neoplasia tem sido mais frequente em homens (67% dos casos), podendo ter pequenas diferenças entre as regiões geográficas do mundo. Nos Estados Unidos os homens representam aproximadamente 52% dos casos, mas em outras partes do mundo, como no Leste da Ásia, Norte da Europa, Austrália e Nova Zelândia, é mais frequente entre as mulheres (DIDKOWSKA *et al*., 2016). No entanto, no mundo, observa-se um declínio na tendência das taxas de incidência dessa neoplasia entre os homens, ao contrário do que vem sendo observado com relação às taxas de incidência nas mulheres, possivelmente reflexo dos padrões de adesão e cessação do consumo do tabaco (INCA, 2020).

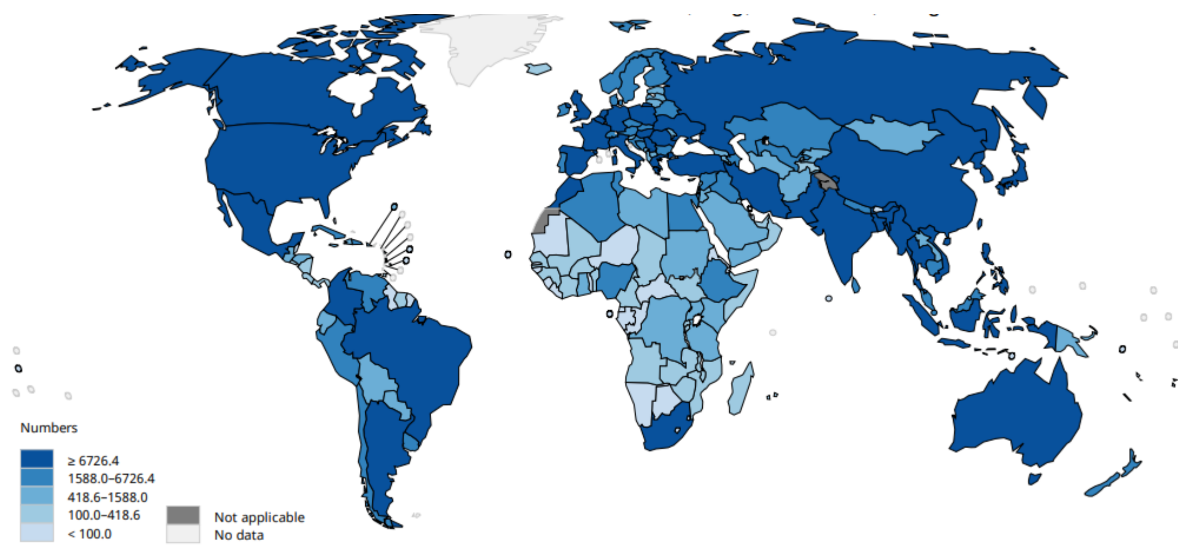

Figura 1. Prevalência do Câncer de Pulmão no Mundo.
Fonte: WHO/ IARC, 2020.

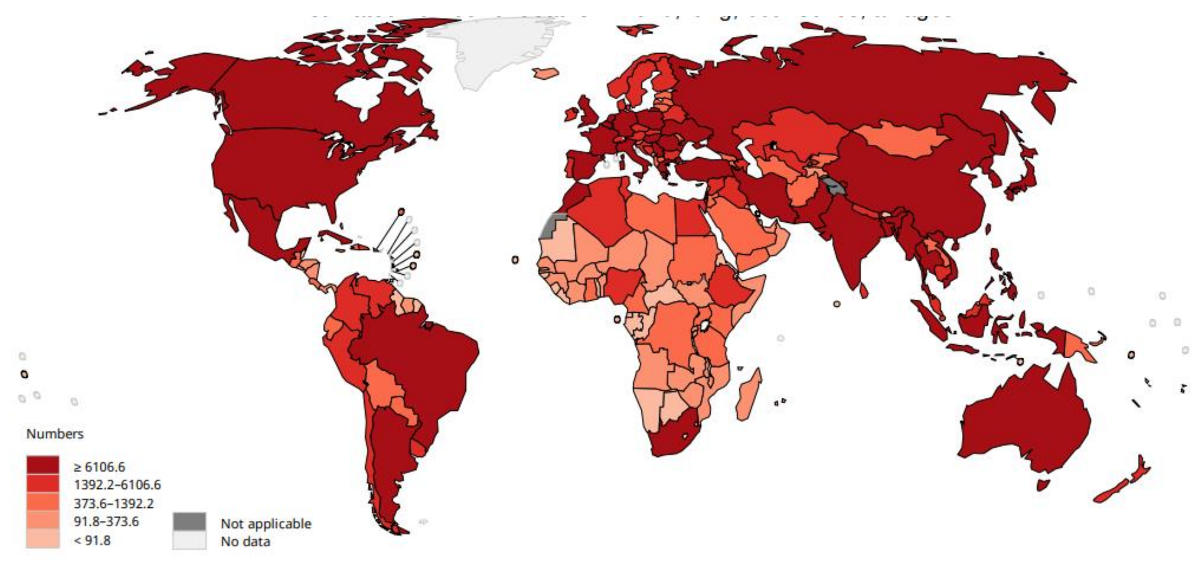

Figura 2. Mortalidade por Câncer de Pulmão no Mundo.
Fonte: WHO/IARC, 2020.

Nessa perspectiva global, nota-se um crescimento do consumo de tabaco, associada tendências ascendentes da mortalidade por câncer de pulmão, especialmente nos países em desenvolvimento. Em países mais desenvolvidos a tendência de incidência de câncer de pulmão reverteu ou se estabilizou, mas, apesar desse declínio o número absoluto de mortes continuará a crescer. Isso reflete no número de mortes por câncer de pulmão no mundo, que deve crescer até 3 milhões até 2035. Esse aumento será mais acelerado em regiões com baixo índice de desenvolvimento humano (DIDKOWSKA *et al*., 2016).

No que se refere à idade do diagnóstico, observa-se que cerca 31% dos casos no mundo são diagnosticados entre 65 e 74 anos e a população idosa compreende 68% do total de casos investigados, como observado na Figura 3 (CRUZ, TANOUE, MATTHAY, 2011; WHO/IARC, 2013; HOWLADER *et al.*, 2015). Nas próximas décadas, o envelhecimento populacional aumentará exponencialmente, principalmente entre os octogenários, o que certamente contribuirá para o aumento dos casos de câncer de pulmão após os 70 anos, hoje estimado em 30% (PRESLEY *et al.*, 2018).

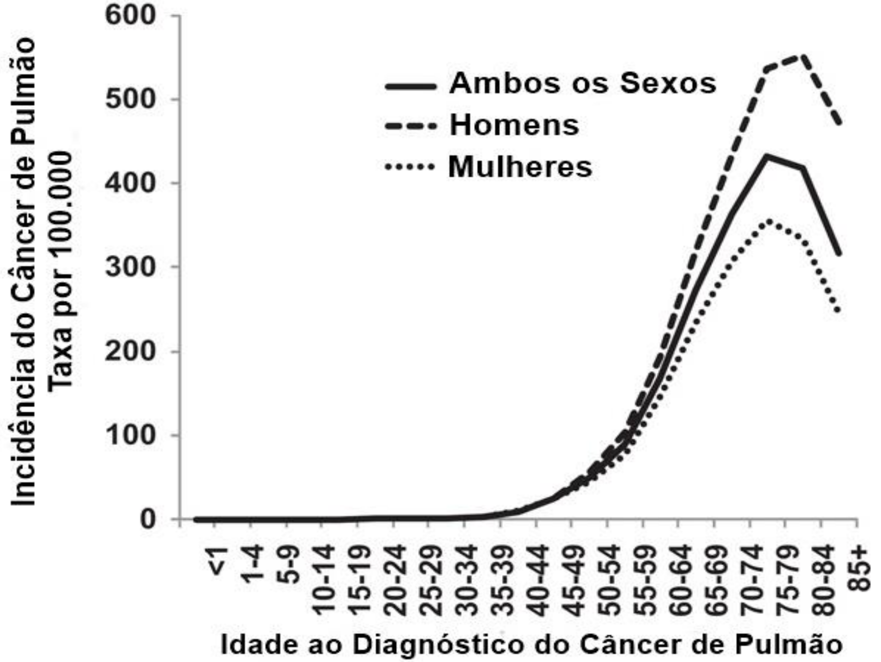

Figura 3. Esquema da Incidência de Câncer Pulmão, específica por idade, no mundo.
Fonte: Adaptado de Cruz *et al.* (2011).

No Brasil, estima-se que em 2020 a incidência de câncer de pulmão seria de 30.200 novos casos, sendo 17.760 entre homens e de 12.440 entre as mulheres, o terceiro mais frequente em homens e quarto mais frequente em mulheres (Figura 4). Regionalmente, a frequência dos novos casos foi diferenciada, onde, para os homens, mostrou ser o segundo mais frequente nas regiões Sul e Nordeste e o terceiro no Norte, Centro-Oeste, Sudeste, e para as mulheres, o terceiro mais frequente nas regiões Sul e o quarto nas demais regiões (INCA, 2020).

No estado do Pará estimou-se aproximadamente 560 novos casos de câncer de pulmão para 2020, 32% na cidade de Belém, sendo mais frequente em homens do que em mulheres (INCA, 2020). Entre os anos de 2015 e 2020 houve aumento de 57,7% dos casos de internação por câncer de pulmão no estado do Pará (SIH/SUS, 2021). Nesse período, se observou um

aumento de óbitos, sendo mais frequente a partir de 60 anos, correspondendo a aproximadamente 77,8% dos casos (SIM/SUS, 2021).

Localização primária	Casos	%	Homens	Mulheres	Localização primária	Casos	%
Próstata	65.840	29,2%			Mama feminina	66.280	29,7%
Cólon e Reto	20.540	9,1%			Cólon e Reto	20.470	9,2%
Traqueia, Brônquio e Pulmão	17.760	7,9%			Colo do útero	16.710	7,5%
Estômago	13.360	5,9%			Traqueia, Brônquio e Pulmão	12.440	5,6%
Cavidade Oral	11.200	5,0%			Glândula Tireoide	11.950	5,4%
Esôfago	8.690	3,9%			Estômago	7.870	3,5%
Bexiga	7.590	3,4%			Ovário	6.650	3,0%
Linfoma não Hodgkin	6.580	2,9%			Corpo do útero	6.540	2,9%
Laringe	6.470	2,9%			Linfoma não Hodgkin	5.450	2,4%
Leucemias	5.920	2,6%			Sistema Nervoso Central	5.230	2,3%

* Números arredondados para múltiplos de 10

Figura 4. Distribuição proporcional dos dez tipos de câncer mais incidentes estimados para 2020 por sexo, exceto pele não melanoma.
Fonte: INCA (2020).

Estudos revelam que a taxa de sobrevida global em 5 anos do câncer de pulmão é sombria, com aproximadamente 15% nos países desenvolvidos e 5% nos países em desenvolvimento (MAILK, RAINA, 2015). É geralmente detectado em estágios avançados, 80-92% dos pacientes são diagnosticados nos estádios III e IV, influenciando diretamente sobre o prognóstico, a letalidade e os gastos com a doença. Segundo Lee *et al.* (2016) quando o câncer de pulmão é diagnosticado precocemente, a taxa de sobrevida de cinco anos aumenta para 80%, mas a mesma reduz para 15% quando o diagnóstico é tardio e o tratamento é ineficaz.

1.2 ETIOLOGIA E PATOGENIA

A etiologia do câncer de pulmão pode ser entendida como o reflexo das consequências da associação entre fatores ambientais e alterações genômicas múltiplas, ou seja, exposição a agentes etiológicos e susceptibilidade individual a esses agentes (CRUZ, TANQUE, MATHAY, 2011; BERNARDI *et al.*, 2016). Essa interação sinérgica entre fatores de risco múltiplos pode ter consequências diretas no risco para o desenvolvimento do câncer de pulmão. Entre os exemplos já bem conhecidos encontra-se o tabagismo, que associado a outras substâncias como asbesto, radônio e radicais livres é responsável por cerca de 90% dos casos

de câncer do pulmão em homens e 70% em mulheres (ALBERG *et al*., 2013; SIDDIQUI, VAQAR, SIDDIQUI, 2021).

1.2.1 Tabagismo e Carcinogênese Pulmonar

O principal fator de risco para o desenvolvimento do câncer de pulmão é o tabagismo, pois as substâncias contidas no tabaco favorecem a inflamação e a carcinogênese. Diversos estudos revelam que o tabagismo é o causador primário de 80-90% dos casos de câncer pulmonar. Esse risco depende de diferentes aspectos, como consumo médio, tempo de tabagismo, tempo de cessação tabágica, idade de início, tipo de produto do tabaco e padrão de inalação (PARKIN *et al*., 2004; TSAO *et al*., 2016).

O tabagismo não cria apenas riscos pessoais, mas a inalação passiva da fumaça do tabaco no ambiente, realizada pelos fumantes passivos, também pode causar câncer de pulmão em indivíduos não fumantes, aumentado de 20 a 30% o risco de câncer de pulmão (KUMAR; ABBAS, ASTER, 2013). Estima-se que o tabagismo passivo cause 21.400 mortes por ano em todo mundo (ALBERG, 2013).

Com o fumo o indivíduo entra em contato com mais de 5.000 substâncias químicas, muitas delas carcinogênicas, sobretudo os hidrocarbonetos policíclicos aromáticos, nitrosaminas, aminas aromáticas, aldeídos, compostos orgânicos (benzeno, cloreto de vinil) e inorgânicos (arsênico, cromo, radônio, chumbo e polônio). Os dois primeiros são os carcinógenos mais potentes presentes na fumaça do cigarro (BERNARDI *et al*., 2016). As variedades de ações biológica desses agentes é caracterizada pela presença de agentes cancerígenos, tóxicos, irritantes, promotores de tumores, coagentes cancerígenos e agentes inflamatórios (HECHT, 2012).

Os agentes químicos e físicos presentes no tabaco podem agir em múltiplos estágios do processo de carcinogênese (Figura 5). O contato direto dos carcinógenos com o tecido normal, leva a formação de aditivos e mutação em genes como *RAS, MYC, TP53, P16, CDKN2A, FHIT* e outros. As vias AKT (proteína quinase B) e PKA (proteína quinase A) são ativadas pela nicotina, o que favorece à redução de apoptose e aumento da angiogênese tecidual. O tabagismo promove diretamente a inflamação e interfere nas barreiras naturais de proteção do corpo (MACHADO, LEAL, 2013).

Essas modificações do DNA, causadas por esses agentes químicos, são críticas no processo carcinogênico. Quando essas alterações não são reparadas causam erros de codificação durante a replicação do DNA, resultando em uma mutação permanente. Se essa mutação ocorrer em uma região crítica de um oncogene como o *KRAS* ou um gene supressor de tumor como o *TP53*, o resultado é inegavelmente a perda dos mecanismos normais de controle do crescimento celular e, consequentemente, o desenvolvimento de câncer (HECHT, 2012).

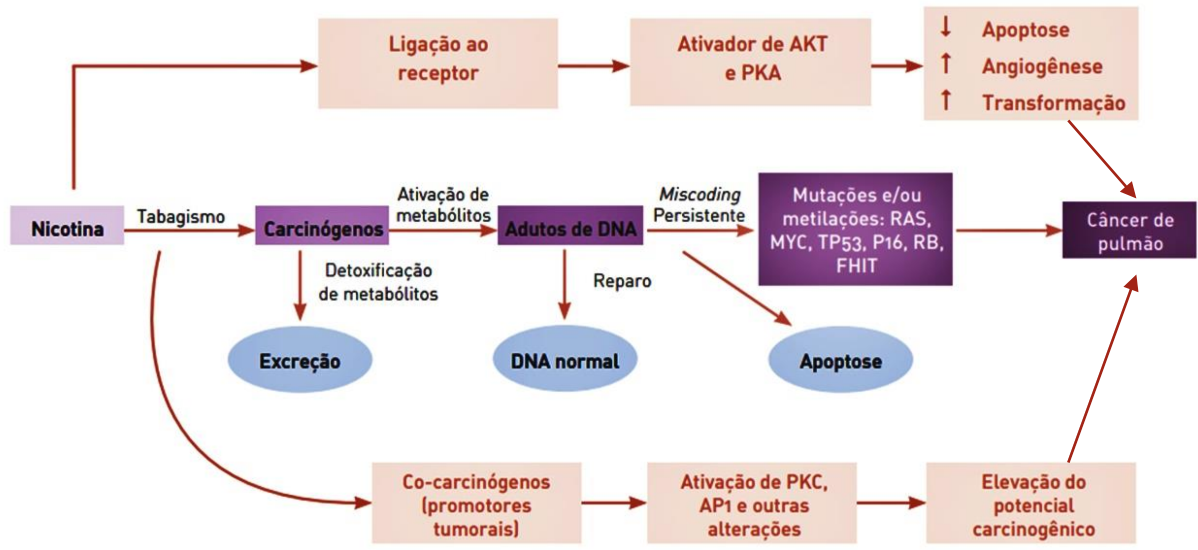

Figura 5. Processo de Carcinogênese desencadeado pelo Tabaco.
Fonte: Machado e Leal (2013).

1.2.2 Envelhecimento e Carcinogênese Pulmonar

A maioria dos cânceres surge em indivíduos com mais de 60 anos. À medida que a população mundial vive mais e atinge idades mais avançadas, o câncer está se tornando um importante problema de saúde pública (FANE, WEERARATNA, 2020). O envelhecimento é um fenômeno universal, individual e inevitável, que se inicia no nascimento e continua até a morte, capaz de conduzir o organismo a múltiplas disfunções celulares e reações moleculares, afetando a homeostase, comprometendo a capacidade funcional e favorecendo o aparecimento de doenças (TEIXEIRA, GUARIENTO, 2010; GIGLIO, KARNAKS, TODARO, 2012; PALLIS *et al*., 2014; BERBEN *et al*., 2021).

Na busca do entendimento do processo de envelhecimento, postularam-se diversas teorias biológicas, classificadas em evolutiva, celular-molecular e sistêmica. Os mecanismos biológicos envolvidos em cada grupo de teorias explicam o fenótipo da senescência humana,

revelando a complementaridade entre as teorias e a complexidade do entendimento da etiologia do envelhecer (TEIXEIRA, GUARIENTO, 2010; JIN, 2010).

Com a evolução do conhecimento das bases moleculares e celulares da vida e das doenças, o envelhecimento vem sendo submetido à intensa investigação. Isso inclui o entendimento de mecanismos intrínsecos, eventos acidentais, sinais genéticos programados, mutações ou danos no DNA nuclear ou mitocondrial, proteínas danificadas e anormais, ligação cruzada, glicação, formação de radicais livres, e componentes celulares específicos, como gene, cromossomo, mitocôndrias ou telômeros (CEFALU, 2011).

Os chamados *hallmarks* do envelhecimento já foram postulados e constituem características que determinam o fenótipo do envelhecimento: instabilidade genômica, redução do telômero, alterações epigenéticas, perda de proteases, desequilíbrio de nutrientes, disfunção mitocondrial, senescência celular, esgotamento de células tronco e comunicação intercelular alterada (Figura 6). Essas marcas se manifestam durante o envelhecimento normal, o seu agravamento experimental acelera o envelhecimento e a sua melhoria experimental retarda o processo de envelhecimento normal (LOPEZ-OTIN *et al*., 2013).

Os fenômenos bioquímicos envolvidos na senescência favorecem o aparecimento de doenças crônicas, entre elas o câncer (GIGLIO, KARNAKS, TODARO, 2012; AUNAN, CHO, SØREIDE, 2017). O envelhecimento contribui para a carcinogênese de duas maneiras: primeira, o passar do tempo leva ao acúmulo de células com diferentes aberrações moleculares; segunda, o envelhecimento está associado a alterações substanciais na homeostase interna. Isso aumenta a susceptibilidade a agentes cancerígenos, a carcinogênese e a redução dos mecanismos de proteção (FALANDRY *et al*., 2014; PALLIS *et al*., 2014).

O câncer se desenvolve da expressão descontrolada de genes, ocasionada por condições que ultrapassam ou mesmo subvertem a conservada capacidade de estabilidade genômica, culminando no crescimento desordenado das células. É um desequilíbrio entre a replicação e o mecanismo de morte celular, que ocorre fundamentalmente, ocasionado por distúrbios entre oncogenes e genes supressores de tumor (INCA, 2011; CHAMMAS, 2012).

Figura 6. *Hallmarks* do Envelhecimento.
Fonte: adaptado de Lopez-Otin *et al*. (2013).

Esse desequilíbrio envolve características comuns, capazes de promover a doença neoplásica, com capacidades intrínsecas e extrínsecas à célula tumoral, os chamados *hallmarks* do câncer: manutenção de sinalização proliferativa, fuga da supressão de crescimento, fuga da destruição imune, imortalidade, inflamação, invasão e metástase, angiogênese, instabilidade e mutação genômica, resistência à morte celular e desequilíbrio energético (Figura 7) (HANAHAN, WEINBERG, 2011). E mais recentemente foram incorporadas mais quatro características: desbloqueio de plasticidade fenotípica, reprogramação epigenética não mutacional, microbiomas polimórficos e células senescentes (HANAHAN, 2022).

O câncer e o envelhecimento aparentemente são processos antagônicos, visto que o câncer é a consequência de um ganho anormal de aptidão celular, e o envelhecimento uma perda de aptidão. Com a senescência, a célula é impedida de proliferar, o que seria uma proteção ao surgimento das neoplasias (GIGLIO, KARNAKS, TODARO, 2012 AUNAN, CHO, SØREIDE, 2017).

No entanto, ambos compartilham origens comuns, por exemplo, o acúmulo de danos celulares com o passar do tempo é aceito como causa geral do envelhecimento, que pode eventualmente proporcionar vantagens aberrantes a certas células, produzindo o câncer (LOPEZ-OTIN *et al*., 2013). Assim, o acúmulo de mutações pode ser visto como um guia

comum tanto da senescência quanto da carcinogênese (FALANDRY *et al*., 2014; ZINGER, CHO, BEN-YEHUDA, 2017).

Figura 7. *Hallmarks* do Câncer.
Fonte: adaptado de Hanahan & Weinberg (2011).

O dano no DNA é uma alteração estrutural, com instabilidade genômica e mutações, que ocorre por causas externas (radiação ionizante, radiação UV, agentes químicos, metais pesados, etc.) e por causas internas (metabolismo celular, reações de hidrólise e produção de radicais livres) (BERBEN *et al*., 2021). Quando o DNA não é reparado, ou quando o reparo é ineficaz ou impreciso, há possibilidade de dois caminhos: a manutenção do erro genômico e consequentemente o câncer; ou a interferência no processo de transcrição e tradução celular, ativando os mecanismos do envelhecimento (SERRANO, BLASCO, 2007; BERNSTEIN *et al*., 2013; AUNAN, CHO, SØREIDE, 2017).

Os mecanismos de reparo do dano celular oferecem resistência ao envelhecimento e ao câncer. No entanto, o câncer e a longevidade necessitam de proliferação celular. Assim, os sistemas que limitam a proliferação celular executam ação contra o câncer, mas favorece o envelhecimento. O equilíbrio global entre estes mecanismos garante aptidão e uma vida livre do câncer (SERRANO, BLASCO, 2007; BERBEN *et al*., 2021).

O envelhecimento também é um dos principais fatores de risco para as doenças pulmonares, incluindo a doença pulmonar obstrutiva crônica (DPOC), a maioria dos tipos de câncer de pulmão e fibrose pulmonar idiopática. A compreensão da senescência pulmonar inclui o entendimento dos *hallmarks* do envelhecimento anteriormente citados (LOPEZ-OTIN *et al*., 2013; LOWERY *et al*., 2013).

Para o parênquima pulmonar existem 10 desses marcos do envelhecimento, agrupados em fatores intrínsecos e extrínsecos da célula. Os fatores intrínsecos são senescência celular, instabilidade genômica, atrito de telômeros, alterações epigenéticas, perda de proteases, detecção de nutrientes desregulada e disfunção mitocondrial. Os extrínsecos são exaustão de células-tronco, desregulação da matriz extracelular e comunicação intercelular alterada. A influência desses fatores pode variar dependendo da patologia pulmonar (MEINERS, EICKELBERG, KÖNIGSHOFF, 2015). No câncer de pulmão todos os 10 *hallmarks* estão envolvidos na patogênese da doença (Figura 8), e todos são comuns aos *hallmarks* do câncer (HANAHAN, WEINBERG, 2011; LOPEZ-OTIN *et al*., 2013).

Figura 8. *Hallmarks* do Câncer de Pulmão e Envelhecimento Pulmonar.
Fonte: Meiners, Eickelberg, Königshoff (2015).

A interação entre esses fatores intrínsecos e extrínsecos, muitas vezes determinados por uma susceptibilidade genética ou epigenética individual, associada a fatores a fatores ambientais, como o tabagismo, favorece o desenvolvimento de doenças crônicas, como o câncer. O próprio surgimento de outras doenças crônicas pulmonares como a DPOC e a fibrose

pulmonar idiopática possuem vias patogênicas que se sobrepõem às envolvidas no surgimento do câncer de pulmão (Figura 9) (MEINERS, EICKELBERG, KÖNIGSHOFF, 2015).

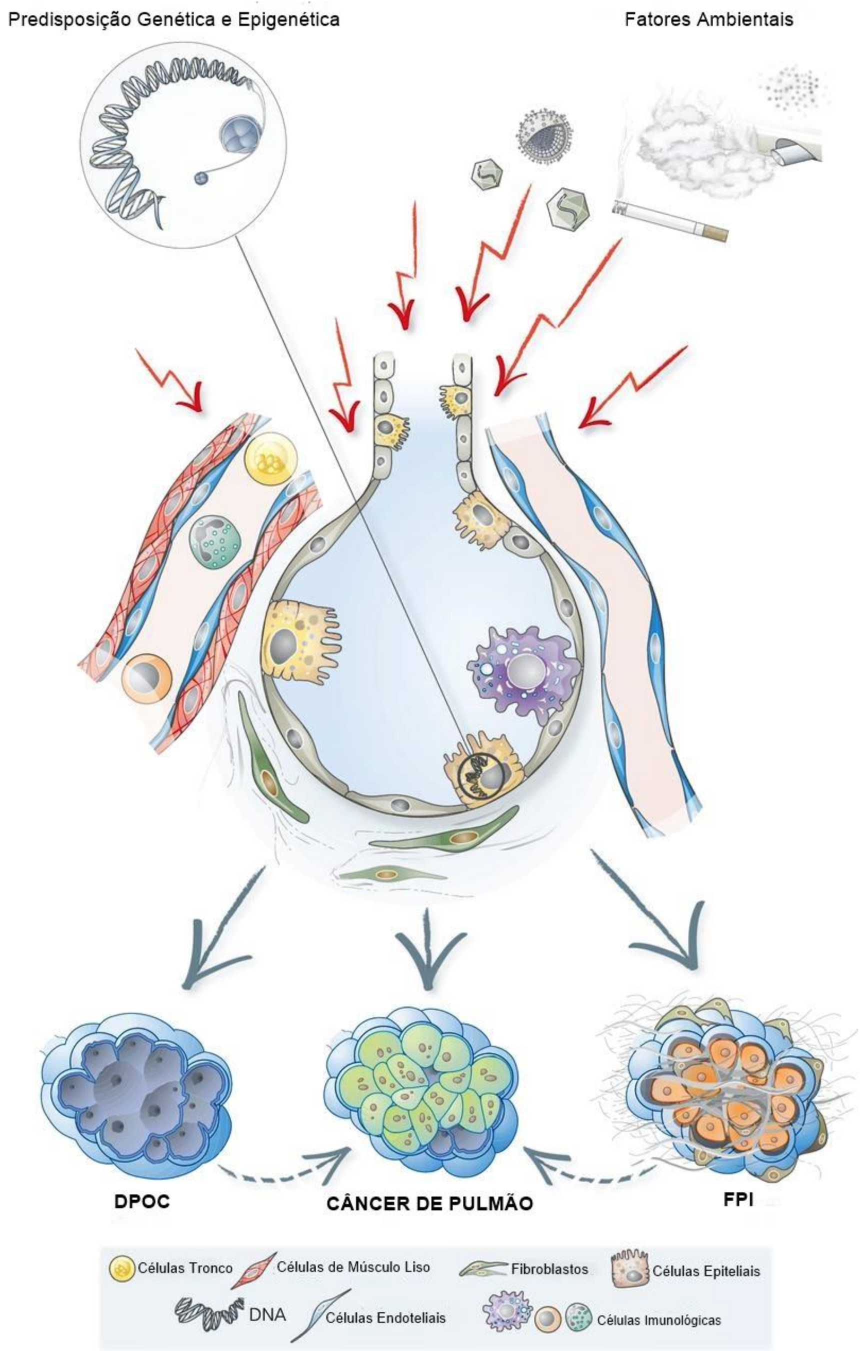

Figura 9. Interação entre fatores genéticos e epigenéticos individuais com fatores ambientais (Ex. tabagismo) e o surgimento de doenças crônicas pulmonares**.**
Fonte: Meiners, Eickelberg, Königshoff (2015).

O surgimento do câncer de pulmão se inicia como uma pequena lesão, de aspecto firme e cinza-esbranquiçado. Inicialmente são como massas intraluminais, que invadem a mucosa

brônquica ou formar grandes massas volumosas, empurrando o parênquima pulmonar nas proximidades. Por vezes, essas grandes massas podem formar cavitações secundárias e necrose central ou desenvolvem áreas focais de hemorragia. Esses tumores podem estender-se à pleura, invadir a cavidade e a parede torácica, e se espalhar para estruturas intratorácicas próximas. As metástases podem ocorrer por via linfática ou hematogênica (KUMAR; ABBAS, ASTER, 2013).

A metástase do câncer de pulmão mostra-se como um processo multifacetado, que se desenvolve desde o estabelecimento do tumor, seguido da migração celular, com invasão vascular, linfática e hematológica, extravasam até a criação do foco metastático à distância. Os focos preferenciais para metástases do carcinoma de pulmão são o cérebro, ossos e glândulas suprarrenais, outros órgãos estão envolvidos geralmente na fase tardia da doença. Mas, dependendo do tipo histológico, há também um local metastático preferencial, como a metástase hepática para carcinoma pulmonar de células pequenas (CPPC) e metástase cerebral para o adenocarcinoma (POPPER, 2016).

1.3 TIPOS HISTOLÓGICOS

O câncer de pulmão se desenvolve a partir das células do epitélio respiratório e pode ser dividido em duas categorias, o câncer de pulmão de pequenas células (CPPC), responsável por 15-20% dos casos; e o câncer de pulmão de não pequenas células (CPNPC), que representa 80-85% dos casos, esse é subdividido em três principais tipos patológicos: adenocarcinoma, carcinoma de células escamosas e carcinoma de grandes células (CRUZ, TANOUE, MATTHAY, 2011; LEE *et al.,*2016). Em alguns casos pode haver combinação de diferentes tipos histológicos, como, por exemplo, a associação do carcinoma de pequenas células e adenocarcinoma (KUMAR; ABBAS, ASTER, 2013).

1.3.1 Adenocarcinoma

O adenocarcinoma de pulmão é responsável entre 50% e 60% dos casos de CPNPC, cerca de 40% dos casos de carcinoma pulmonar (LATIMER, MOTT, 2015; ZHENG, 2016). O adenocarcinoma pode manifestar-se lentamente, com o crescimento preferencialmente

periférico, de tamanho menor que os demais subtipos tumorais, mas tendem a desenvolver metástases ainda nos estágios iniciais do desenvolvimento da doença (KUMAR; ABBAS, ASTER, 2013).

É uma neoplasia que frequentemente atinge a pleura visceral com lesões destrutivas e cicatrizes no parênquima pulmonar ou a hiperplasia de pneumócitos, que são os locais de origem de focos adenomatosos. A maioria dos adenocarcinomas origina-se nas vias respiratórias periféricas, a partir de células claviformes (*club-cell*) ou de pneumócitos do tipo II. O perfil imunoistoquímico característico é dado pela positividade de CK7 e TTF-1 e negatividade de CK20, embora variações possam ocorrer dentro dos subtipos de adenocarcinoma. São o mais comum tipo de câncer de pulmão em não fumantes e em mulheres (GEIB, ROITHMANN, 2013; BERNADI *et al.*, 2016).

1.3.2 Carcinoma de Células Escamosas

O carcinoma de células escamosas, também conhecido como epidermóide ou espinocelular, representa cerca de 30% dos CPNPC e de 20% a 25% dos cânceres pulmonares (LATIMER, MOTT, 2015; ZHENG, 2016). Manifesta-se preferencialmente com lesão na porção central do pulmão, próximo aos brônquios principais e eventualmente se espalham para linfonodos hilares (KUMAR; ABBAS, ASTER, 2013). Seu crescimento é lento, saindo de pequenas tumorações, mas podendo atingir grandes dimensões, associadas à necrose, cavitações e hemorragias (ZHENG, 2016).

O tumor é formado por células epiteliais contendo pontes intercelulares e ceratinização individual ou sob a forma de pérolas córneas. As células tumorais não possuem estrutura glandular ou produção de mucina. São divididos em subtipos queratinizados, não queratinizados e basalóides (KUMAR; ABBAS, ASTER, 2013; ZHENG, 2016).

A mucosa com a lesão pode exibir áreas de metaplasia escamosa, displasia ou carcinoma *in situ*, sugerindo a natureza broncogênica da neoplasia. Esse tumor pode ser: bem diferenciado, quando mostra características cito e histológicas das células escamosas; moderadamente diferenciado, se apresenta características intermediárias entre o bem e o pouco diferenciado; e pouco diferenciado, aquele em que a produção de ceratina e/ou a presença de pontes intercelulares são discerníveis com dificuldade ou as células são indiferenciadas (BERNARDI *et al.*, 2016; ZHENG, 2016).

Quanto ao perfil imunohistoquímico, os carcinomas de células escamosas tipicamente não expressam CK7 e CK20 e poucos expressam CK718. A grande maioria dos casos não expressa o TTF-1, podendo o mesmo ocorrer em 7% dos casos. No entanto, podem expressar, citoqueratinas de baixo peso molecular, como AE1/AE3 e CK5/6 (GEIB, ROITHMANN, 2013).

É o tipo histológico mais associado ao tabagismo, o menos heterogêneo do ponto de vista morfológico e mais comum em homens. Além disso é o que apresenta disseminação para fora do tórax mais tardiamente do que os outros tipos histológicos (KUMAR; ABBAS, ASTER, 2013; BERNARDI *et al*., 2016).

1.3.3 Carcinoma de Grandes Células

O carcinoma de grandes células representa 10% dos carcinomas de pulmão e cerca de 12,5% dos cânceres CPNPC (LATIMER, MOTT, 2015). Essa neoplasia maligna epitelial é indiferenciada, com ausência de características citológicas de carcinoma de células pequenas e sem diferenciação glandular ou escamosa e possui comportamento altamente agressivo, evoluindo rapidamente para óbito (KUMAR; ABBAS, ASTER, 2013; BERNARDI *et al*., 2016).

A tumoração apresenta-se com uma lesão predominantemente periférica, subpleural, não se associa a segmento brônquico e caracteriza-se por formar grandes massas com áreas de necrose e hemorragia (ZHENG, 2016). Essa neoplasia maligna é composta de grandes células, apresentando núcleos grandes e centrais, oval ou poligonal, com nucléolo evidente, citoplasma abundante e membrana celular bem definida; a relação núcleo/citoplasma é a mais alta entre as neoplasias pulmonares (BERNARDI *et al*., 2016; ZHENG, 2016).

1.3.4 Carcinoma de Pequenas Células

O carcinoma de pequenas células representa cerca de 10% dos casos de câncer de pulmão (ZHENG, 2016). É uma neoplasia altamente maligna, detendo o pior prognóstico entre os tumores pulmonares. É comum, no diagnóstico da doença já existir metástases linfonodais e

na medula óssea (BERNARDI *et al*., 2016). O histórico de tabagismo está presente em praticamente todos os casos de CPPC (BERNARDI *et al*., 2016).

A lesão dessa neoplasia é geralmente localizada centralmente nas principais vias aéreas, com células tumorais pequenas em comparação com outros tipos de câncer de pulmão, geralmente menor que o diâmetro de 3 linfócitos maduros (MATHIAS, UGALDE, ROSA, 2013). Apresenta cromatina fina e granular, sem nucléolos proeminentes, com citoplasma escasso e as bordas celulares discretas. Há também uma alta taxa apoptótica e presença frequente de extensa necrose tumoral. A classificação da OMS divide o CPPC em 2 subtipos: CPPC puro e CPPC combinado contendo um componente do CPNPC (ZHENG, 2016).

O diagnóstico é baseado em microscopia de luz usando lâminas de rotina coloridas com hematoxilina-eosina (ZHENG, 2016). Um ou mais marcadores de diferenciação neuroendócrina, tais como cromogranina, enolase específica do neurônio, Leu-7 e sinaptofisina são detectados em aproximadamente 75% dos casos (MATHIAS, UGALDE, ROSA, 2013). E o TTF-1, que é expresso em quase 90% dos casos (ZHENG, 2016).

O tumor geralmente tem uma apresentação clínica com linfadenopatia maciça e central, com síndromes paraneoplásicas, pois o tumor pode produzir e secretar ACTH, serotonina, hormônio antidiurético, calcitonina, estrógenos e hormônio de crescimento (LATIMER, MOTT, 2015; BERNARDI *et al*., 2016).

1.4 APRESENTAÇÃO CLÍNICA

A maioria dos cânceres de pulmão não apresenta sintomas nos estados iniciais da doença e quase sempre são sintomáticos no diagnóstico, pois, nesses casos, a maioria dos pacientes já possui doença avançada no momento do diagnóstico. Não existem sinais e sintomas específicos para o câncer de pulmão, como expõem o Quadro 1 (GEIB, ROITHMANN, 2013; SIDDIQUI, SIDDIQUI, 2019).

Estima-se que cerca de 15% podem ser assintomáticos, principalmente nos estágios iniciais em que o diagnóstico é observado em exame de imagem. Para os demais, os sintomas podem ser causados pelo tumor primário: tosse, dispneia, hemoptise, chiado localizado; disseminação intratorácica: por exemplo, dor torácica, síndrome de Horner, obstrução da veia cava superior; metástases distantes: dor óssea; e causados por síndromes paraneoplásicas: como

a síndrome do hormônio antidiurético inadequado, principalmente para os casos de CPPC (GEIB, ROITHMANN, 2013; LATIMER, MOTT, 2015).

Quadro 1. Sintomas e sinais mais frequentes na apresentação de pacientes com câncer de pulmão.

Sinais e Sintomas	Variação de Frequência (%)
Tosse	8 -75
Emagrecimento	0 - 68
Dispneia	3 - 60
Dor Torácica	20 – 49
Hemoptise	6 – 35
Dor Óssea	6 – 25
Baqueteamento Digital	0 – 20
Febre	0 – 20
Fraqueza	0 – 10
Síndrome de Compressão da Veia Cava Superior	0 – 4
Disfagia	0 – 2
Vigilância ou Estridor	0 – 2

Fonte: Geib e Roithmann (2013).

1.5 DIAGNÓSTICO E ESTADIAMENTO

O *screening* para câncer de pulmão em assintomáticos, considerados de alto risco, já é recomendado em diversos países, indicado quando: idade entre 55 e 80 anos; carga tabágica ≥ 30 maços/ano; e tabagismo atual ou interrupção nos últimos 15 anos, sendo a Tomografia Computadorizada (TC) de baixa dose o método preconizado (LATIMER, MOTT, 2015).

Todos os pacientes com suspeita clínica de câncer de pulmão devem ser submetidos à anamnese e exame físico detalhado, objetivando identificar sinais e sintomas sugestivos de disseminação local ou à distância, determinar a presença de outras morbidades clínicas, avaliar o *status* funcional, da função pulmonar e as condições de saúde (GEIB, ROITHMANN, 2013).

A avaliação para o diagnóstico do câncer de pulmão inclui três etapas simultâneas: diagnóstico tecidual, estadiamento e avaliação funcional (LATIMER, MOTT, 2015). De acordo com as diretrizes do *American College of Chest Physicians* (ACCP), a avaliação inicial do paciente com suspeita de câncer de pulmão deve ser concluída dentro de 6 semanas em pacientes com sintomas toleráveis e sem complicações (SIDDIQUI, SIDDIQUI, 2019)

Nesse processo de diagnóstico a tomografia computadorizada de tórax e abdômen superior, com uso de contraste, deve ser realizada em todos os casos suspeitos. Isso porque,

esse exame de imagem é capaz de caracterizar o tumor primário e a sua relação com as estruturas torácicas, avaliando o possível envolvimento de linfonodos mediastinais e de a presença de metástases à distância. Sua indicação também auxilia os médicos a decidir o local ideal da biópsia (GEIB, ROITHMANN, 2013; SIDDIQUI, SIDDIQUI, 2019).

A realização da biópsia estabelece o diagnóstico tecidual do câncer de pulmão, que auxiliará o planejamento adequado do tratamento. Pode ser realizada através da abordagem do tumor primário, linfonodos regionais ou sítios metastáticos. O melhor local para realização da biópsia deve ser aquele mais facilmente acessível e/ou que forneça o estadiamento mais avançado da doença (GEIB, ROITHMANN, 2013).

As abordagens mais comuns para a biópsia do tumor primário são a fibrobroncoscopia com biópsia endobrônquica ou transbrônquica, a biópsia percutânea guiada por método de imagem (ecografia ou tomografia computadorizada) e a via cirúrgica, seja por toracotomia ou toracoscopia. A broncoscopia convencional funciona melhor para lesões centrais, enquanto a aspiração transtorácica por agulha guiada por tomografia é tipicamente o método de primeira linha para lesões periféricas (LATIMER, MOTT, 2019).

Após a obtenção do diagnóstico histopatológico, o estadiamento clínico desempenha um papel crucial no estabelecimento do tratamento de pacientes com câncer de pulmão, bem como sua influência na morbidade e na previsão da sobrevida da doença (SIDDIQUI, SIDDIQUI, 2019). Esse estadiamento TNM é baseado em diretrizes que são revisadas constantemente à medida que mais dados são disponibilizados para fornecer os marcadores prognósticos mais precisos, sendo a 8ª edição utilizada a partir de 2018 (LIM *et al.*, 2018).

O estadiamento tumoral (T) é determinado pelo tamanho do tumor primário no eixo longo medido na reconstrução multiplanar e seu envolvimento com estruturas adjacentes. O estadiamento nodal (N) avalia a carga tumoral nos linfonodos regionais hilares e mediastinais. Os estágios nodais são capazes de separar consistentemente os pacientes em diferentes grupos prognósticos. O estadiamento de metástases (M) é definido pela presença de metástases além dos linfonodos regionais (Quadro 2) (LIM *et al.*, 2018; SIDDIQUI, SIDDIQUI, 2019). Por isso, acredita-se que o melhor acesso à TC por emissão de pósitrons (PEP-CT) e ultrassonografia endobrônquica (EBUS) para amostragem de linfonodos mediastinais aumentam a precisão do estadiamento do câncer de pulmão (JONES, BALDWIN, 2018).

Esse estadiamento pode ser agrupado em estágios, que auxiliará na determinação do tratamento, morbidade e sobrevida (Quadro 3). É extremamente importante que o estadiamento clínico seja feito com precisão e vigilância, principalmente para o CPNPC. Para o CPPC

também pode ser realizado da mesma maneira, mas uma abordagem muito mais direta é usada para doenças limitadas e extensas (LIM *et al*., 2018; SIDDIQUI, SIDDIQUI, 2019).

Quadro 2. Estadiamento TNM para o câncer de pulmão.

Tumor Primário (T)	
Tx	Não pode ser avaliado, não visualizado na imagem
T0	Nenhuma evidência de tumor primário
Tis	Carcinoma in situ
T1	Tumor ≤ 3 cm, envolto por parênquima ou pleura visceral e longe de brônquio principal (a partir de brônquio lobar):
T1a	≤1 cm
T1b	> 1 - ≤ 2 cm
T1c	> 2 - ≤ 3 cm
T2	Tumor > 3 cm e ≤ 5 cm, ou tumor que apresenta: Atelectasia/pneumonia pós-obstrutiva; Invasão do brônquio; Invasão da pleura visceral; Invasão local do Diafragma
T2a	> 3 - 4 cm
T2b	> 4 - ≤5 cm
T3	Tumor > 5 - ≤7 cm ou uma das seguintes características: Invasão local da parede torácica, pericárdio parietal, nervo frênico ou presença de nódulo de satélite (mesmo lobo)
T4	Tumor > 7 cm ou uma das seguintes características: Invasão no mediastino, traqueia, coração/grandes vasos, esôfago, vértebra, carina, nervo laríngeo recorrente; Nódulo satélite (lobo diferente, mesmo pulmão)
Linfonodo Regionais (N)	
Nx	Linfonodos regionais não podem ser avaliados
N0	Sem metástase de linfonodos regionais
N1	Metástase em linfonodo ipsilateral peribrônquico e/ou hilar e intrapulmonar, incluindo envolvimento por extensão direta
N2	Metástase em linfonodos mediastinais e/ou subcarinais ipsilaterais
N3	Metástase no mediastino contralateral, hilar contralateral, escaleno ipsilateral ou contralateral ou linfonodo(s) supraclavicular
Metástases à Distância (M)	
M0	Sem metástase distante
M1a	Nódulo(s) tumoral(s) separado(s) em um lobo contralateral; nódulos pleurais ou derrame pleural ou pericárdico maligno
M1b	Metástase extratorácica única ou envolvimento de um único nó distante (não regional)
M1c	Múltiplas metástases extratorácicas em um ou vários órgãos

Fonte: 8ª Classificação TNM para o Câncer de Pulmão - Lim *et al.* (2018).

Quadro 3. Estágio geral pelo agrupamento do Estadiamento TNM.

<table>
<tr><th>T / M</th><th>N0</th><th>N1</th><th>N2</th><th>N3</th></tr>
<tr><td>T1a</td><td>IA1</td><td rowspan="5">IIB</td><td rowspan="5">IIIA</td><td rowspan="5">IIIB</td></tr>
<tr><td>T1b</td><td>IA2</td></tr>
<tr><td>T1c</td><td>IA3</td></tr>
<tr><td>T2a</td><td>IB</td></tr>
<tr><td>T2b</td><td>IIA</td></tr>
<tr><td>T3</td><td>IIB</td><td rowspan="2">IIIA</td><td rowspan="2">IIIB</td><td rowspan="2">IIIC</td></tr>
<tr><td>T4</td><td>IIIA</td></tr>
<tr><td>M1a</td><td colspan="4" rowspan="2">IVA</td></tr>
<tr><td>M1b</td></tr>
<tr><td>M1c</td><td colspan="4">IVB</td></tr>
</table>

Fonte: 8ª Classificação TNM para o Câncer de Pulmão - Lim *et al.* (2018).

O estadiamento da capacidade funcional também é necessário ser estabelecido, tendo em vista que muitos pacientes com câncer de pulmão podem apresentar idade avançada, outras doenças associadas e estado nutricional debilitado, fatores esses que podem influenciar na resposta ao tratamento para doença, seja através da ressecção cirúrgica, radioterapia e quimioterapia. Assim, instrumentos como o *Performance Status* do *Eastern Cooperative Oncology Group* (PS-ECOG) (Quadro 4) podem ser aplicados com intuito de prever a tolerância dos pacientes ao tratamento (CORREA *et al*., 2012; LATIMER, MOTT, 2015).

Quadro 4. Estadiamento Funcional através da escala *Performance Status* do *Eastern Cooperative Oncology Group.*

Pontuação	Descrição
0	Totalmente ativo, capaz de exercer atividade de antes da doença sem restrição
1	Restrito à atividade fisicamente extenuante, mas ambulatorial e capaz de realizar trabalhos leves ou sedentários (por exemplo, tarefas domésticas leves, trabalho de escritório)
2	Ambulatorial e capaz de todo o autocuidado, mas incapaz de realizar quaisquer atividades de trabalho, acima e acima de 50% das horas de vigília
3	Capaz de apenas autocuidado limitado, confinado a uma cama ou cadeira por mais de 50% das horas de vigília
4	Completamente incapacitado, incapaz de qualquer autocuidado, totalmente confinado a uma cama ou cadeira
5	Morto

Fonte: Latimer & Mott (2015).

A avaliação inicial da função pulmonar é direcionada para o risco associado ao tratamento planejado e pela qualidade de vida que o paciente alcançaria. Para avaliação do risco aumentado de complicações perioperatórias após ressecção pulmonar, o teste

ergoespirométrico com exercício padronizado é recomendado. Especificamente para função pulmonar, casos críticos e em uma situação clínica do paciente é complexa, recomenda-se que os pacientes sejam avaliados por uma equipe interdisciplinar composta por pneumologistas, cirurgiões torácicos, oncologistas e radiologistas (GOECKENJAN *et al*., 2011).

Existem recomendações detalhadas para a avaliação da operabilidade funcional em pacientes com câncer de pulmão. Paciente com volume expiratório forçado (VEF 1) superior a 80% do normal (VEF 1> 80% do normal) podem ser encaminhados para cirurgia torácica. Se estiver abaixo de 80% da normal, é necessária uma avaliação mais aprofundada com espiroergometria. Se isso mostrar um consumo máximo de oxigênio acima de 75% da norma ou se o valor for maior que 20 mL/min/kg, o paciente é um candidato para cirurgia. No entanto, se o consumo máximo de oxigênio for inferior a 40% do normal ou inferior a 10 mL/min/kg, deve-se assumir a inoperabilidade funcional (HAMMERSCHMIDT, WIRTZ, 2009).

1.6 TRATAMENTO

O tratamento do câncer de pulmão é complexo, encontra-se em constante evolução e tem cido bem detalhado em diretrizes de práticas baseadas em evidências. Os resultados do mesmo sobre a morbimortalidade podem ser melhorados para pacientes avaliados e tratados por uma equipe multidisciplinar, incluindo oncologistas, cirurgião torácico e uma equipe multiprofissional em um centro de tratamento de câncer de pulmão (LATIMER, MOTT, 2015).

A abordagem terapêutica para o câncer de pulmão é frequentemente multimodal, onde a radioterapia e a quimioterapia podem ser administradas simultaneamente como radioquimioterapia. Quimioterapia, radioterapia e radioquimioterapia podem preceder a cirurgia (terapia neoadjuvante) ou podem segui-la (terapia adjuvante) (HAMMERSCHMIDT, WIRTZ, 2009). A terapia instituída varia dependendo do tipo histológico e do grau de avanço da doença. Os CPPCs comportam-se de forma agressiva e são tratados de maneira não cirúrgica na maioria dos casos, enquanto os CPNPC são gerenciados por uma combinação de cirurgia e terapia adjuvante (ZHENG, 2016).

Os CPNPC em 25% a 30% dos casos são diagnosticados nos estágios iniciais. Para o CPNPC no Estágio I, o tratamento é primariamente cirúrgico, e oferece a melhor chance de cura. O procedimento cirúrgico inclui, entre outras coisas, lobectomia, bilobectomia (remoção de dois lobos pulmonares adjacentes) e pneumonectomia com linfadenectomia mediastinal

sistemática, preferencialmente com uma abordagem minimamente invasiva, como a cirurgia torácica vídeo assistida (HAMMERSCHMIDT, WIRTZ, 2009). A quimioterapia e radioterapia adjuvantes não mostraram benefícios nesse Estágio. Para o CPNPC no Estágio II, o tratamento preferido é a cirurgia seguida de quimioterapia adjuvante (SIDDIQUI, SIDDIQUI, 2019).

Os CPNPC em Estágio III, é o grupo mais heterogêneo, consistindo em uma ampla variação de invasão tumoral, bem como envolvimento linfonodal, sendo necessária a combinação de diferentes modalidades terapêuticas. O primeiro passo deve ser estabelecido separando os pacientes que possuem lesões ressecáveis ou irressecáveis (GEIB, ROITHMANN, 2013). Nos ressecáveis a abordagem é similar ao do Estágio II. Para os irressecáveis o domínio é da radioquimioterapia (SIDDIQUI, SIDDIQUI, 2019).

Os CPNPC em Estágio IV, 40% a 50% dos casos, são considerados incuráveis, e a terapia visa melhorar a sobrevida e aliviar os sintomas, por isso a necessidade da indicação precoce dos cuidados paliativos (HAMMERSCHMIDT, WIRTZ, 2009; LATIMER, MOTT, 2015). Seja pela apresentação da doença avançada no diagnóstico ou pelo desenvolvimento de recidiva de um CPNPC previamente tratado, o tratamento sistêmico é a principal modalidade terapêutica (GEIB, ROITHMANN, 2013). Para um paciente com um adequado *performance status* indica-se a quimioterapia baseada em platina associada a uma segunda droga (taxano, gencitabina, vinorelbina), favorecendo um tempo de sobrevida de cerca de 10 meses (SIDDIQUI, SIDDIQUI, 2019),

Os casos CPPC são muito sensíveis à quimioterapia, no entanto há uma taxa de recorrência muito alta, sendo necessário estabelecer um tratamento de acordo com o estágio da doença. Para os CPPC, nos Estágios de I a III (30% a 40%), é indicada uma combinação de quimioterapia à base de platina e radioterapia. Em alguns casos, CPPC limitado em Estágio I, é indicada cirurgia seguido de quimioterapia adjuvante (HAMMERSCHMIDT, WIRTZ, 2009; MATHIAS, UGALDE, ROSA, 2013). Já para os CPPC nos Estágios IV (60% a 70%), CPPC extenso, que inclui metástase distante, é indicado o tratamento com quimioterapia à base de platina (SIDDIQUI, SIDDIQUI, 2019). A taxa de sobrevida em cinco anos é praticamente zero para CPPC em estágio extenso (LATIMER, MOTT, 2015).

1.7 BIOMARCADORES DE RISCO PARA O CÂNCER DE PULMÃO

Com a evolução da compreensão da carcinogênese pulmonar, associada à aplicação do conhecimento desenvolvido por pesquisas translacionais, houve o surgimento de métodos que que facilitam a identificação dos riscos intrínsecos para a doença. Essa capacidade de identificar com precisão, a nível molecular, a susceptibilidade individual para o risco de câncer de pulmão oferece uma ferramenta que traz significativos benefícios para a saúde pública (ALBERG *et al.*, 2013).

O conhecimento da biologia molecular pode explicar a susceptibilidade de neoplasias pulmonares, através de polimorfismos germinativos de vários genes envolvidos no metabolismo, no reparo do DNA (*XPD, XRCC1 e MGMT*) e no controle do ciclo celular (*p53*). Por exemplo, os polimorfismos de genes de metabolismo, como das famílias CYP e GST, estariam diretamente associados ao controle de agentes carcinogênicos. Associações como essas podem direcionar intervenções terapêuticas e preventivas para o câncer de pulmão no futuro (YOUNES, ABRÃO, 2015).

Existem ensaios moleculares baseados em tecidos tumorais, que investigam diretamente o genoma da neoplasia, identificando aberrações somáticas e alterações epigenéticas em genes chaves da carcinogênese pulmonar, bem como o reflexo de alterações a nível transcricional, preteômica e metabolômica. Mas há também, ensaios não invasivos, que não abordam diretamente o tumor, utilizando os mais variados tipos de abordagem (sangue periférico, fluidos, vias aéreas, etc), baseados em estudos caso-controle, com populações assintomáticas (ALBERG *et al.*, 2013).

Especificamente no sangue periférico, investigando alterações genéticas e epigenéticas, estudos epidemiológicos baseados em variantes da linha germinativa em relação ao risco de câncer de pulmão começaram por estudar SNPs em genes candidatos que codificam enzimas em vias bioquímicas específicas, com ênfase no metabolismo de carcinógenos, reparo de DNA e inflamação (ALBERG *et al.*, 2013; YOUNES, ABRÃO, 2015).

Entre as alterações genéticas, já estudadas e conhecidas para o desenvolvimento do câncer de pulmão, existem mutações ativadoras ou amplificações de proto-oncogenes, tais como nos genes *BRAF, EGFR, ERBB2, KRAS, NRAS, PIK3CA* e família MYC, e mutações inativadoras, deleções ou hipermetilação de regiões promotoras dos genes supressores tumorais como *LKB1, BRG1, MYC, PTEN, P16, RB e TP53* (ZUKIN, DIENSTMANN, ARAÚJO, 2013; BERNICKER, ALLEN, CAGLE, 2019).

As variantes candidatas incluídas no presente estudo compõem um painel de marcadores, que podem estar ligados à carcinogênese: controle do ciclo celular (*TP53*), homeostase (*PAR1*), resposta imunológica (*IL1A e NFKB1*) e vias metabólicas (*UCP2 e UGT1A1*). Todas essas ligadas à susceptibilidade ao câncer.

1.7.1 Gene *TP53*

O gene *TP53* possui sua localização citogenética no cromossomo 17 (região 17p13.1). Esse gene é responsável pela codificação da proteína p53, uma proteína com função de fator de transcrição, que responde a modificações celulares, desencadeando a regulação de genes que induzem a parada do ciclo celular, apoptose, senescência, reparação de DNA e as alterações no metabolismo. Em células normais, a atividade da p53 é baixa, mas quando ocorre, por exemplo, danos no DNA os níveis dessa proteína aumentam, impedindo que células anormais se proliferem (KIM, ZHANG, LOZANO, 2016; KAMP, WANG, HWANG, 2016).

Devido sua capacidade de regulação do ciclo celular, a p53 também passou a ser observada como uma proteína que regula o envelhecimento. Sua ação pode conduzir à apoptose excessiva, que leva à atrofia tecidual, degeneração e perda da capacidade regenerativa. No entanto, a mesma pode ter efeitos que favorecem a longevidade, pois eliminam células danificadas ou disfuncionais, impedindo sua proliferação (KEIZER, LABERGE, CAMPISI, 2010).

Estudos apontam que existem mais de 45 mil mutações somáticas e germinativas e pelo menos 100 polimorfismos no gene *TP53*. O entendimento da heterogeneidade dessas mutações e polimorfismos, que causam alterações estruturais e funcionais, é necessário para a compreensão dos papéis desse gene em células normais e em processos patológicos (PETITJEAN *et al*., 2007; SAGNE *et al*., 2013; LEROY, ANDERSON, SOUSSI, 2014).

1.7.1.1 Polimorfismo no Gene *TP53* (rs17878362)

O gene *TP53* apresenta polimorfismos que podem induzir alterações da expressão do gene e, consequentemente, a perda do controle homeostático da célula. Na Figura 10, é observado o polimorfismo desse gene, a inserção de 16pb no íntron 3 (rs17878362). Esse é um polimorfismo bastante comum, que pode provocar a redução do nível de transcrição da proteína

p53, sugerindo que este polimorfismo provoca uma alteração no processamento do RNAm da proteína, justificando sua associação com o desenvolvimento de doenças como o câncer (SAGNE e*t al*., 2013; WU *et al*., 2013; SAGNE *et al*., 2014; VYMETALKOVA *et al*., 2015).

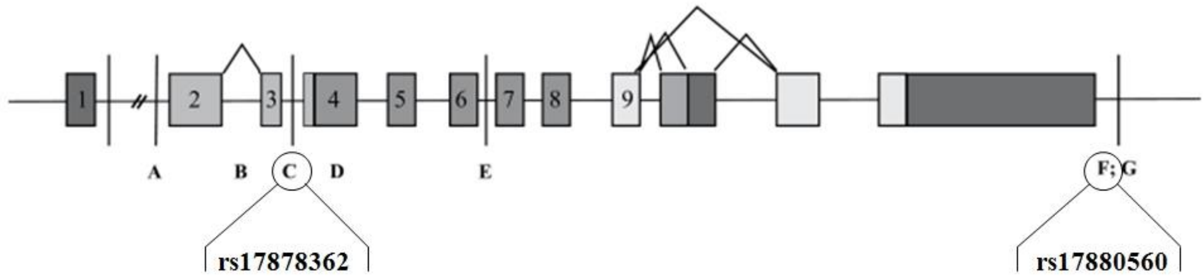

Figura 10. Polimorfismos do Gene *TP53*.
Fonte: adaptado de Sagne *et al*. (2014).

1.7.2 Gene *PAR1*

O gene *PAR1* localiza-se no cromossomo 5 (região 5q13.3), composto por 2 exons que abrangem cerca de 27kb (KAHN *et al.,* 1998; BAHUO, 2007). Expressa a isoforma de uma proteína que atua como receptor ativado por protease (PAR-1), membro da superfamília de receptores acoplados à proteína G, altamente expresso em plaquetas, fibroblastos, células endoteliais, células renais, células T, células do sistema nervoso e musculoesquelético (MARTINO *et al*., 2013; YUN, SANG, 2015).

O PAR1 contribui para muitos processos fisiopatológicos, como crescimento, desenvolvimento, proliferação, mitogênese, ativação plaquetária, inflamação e angiogênese (MARTINO *et al*., 2013). Os polimorfismos no gene *PAR1* podem alterar a expressão de PAR-1 e, assim, influenciar no desenvolvimento de doenças crônicas, entre elas o câncer (YUN, SANG, 2015).

1.7.2.1 Polimorfismo do Gene *PAR1* (rs11267092)

Esse polimorfismo ocorre na posição -506, com a inserção/deleção de 13 pb, na região promotora do gene, conforme mostra a Figura 11 (MARTINO *et al*., 2013). Já foi associado à angiogênese, regulando a liberação de fatores pró-angiogênicos e antiangiogênicos, como o

fator de crescimento endotelial vascular (VEGF), endostatina e metaloproteinases de matriz, associados à susceptibilidade e prognóstico de alguns tipos de cânceres (MARTINO *et al.*, 2013; OLIVEIRA, 2018). Estudos apontam que o alelo de deleção está relacionado a um melhor prognóstico nos cânceres de mama, estômago e esôfago (LURJE *et al.*, 2010; EROĞLU, KARABIYIK, AKAR, 2012).

Figura 11. Polimorfismo do Gene *PAR-1*
Fonte: adaptado de Zhang *et al.* (2015).

1.7.3 Gene *IL1A*

O gene *IL1A* localiza-se no cromossomo 2 (região 2q14.1), composto por 7 éxons que abrangem aproximadamente 10kb. Expressa uma das isoformas da IL-1, que é sintetizada em vários tipos de células como macrófagos, queratinócitos, linfócitos B e fibroblastos. Essa é multifuncional e está relacionada à comunicação celular (autocrina e paracrina), envolvida no processo inflamatório, na resposta imunológica e na hematopoiese. A *IL1A* é produzida como uma proteína longa, retida na célula, que sofre clivagem pela proteína calpaína, tornado-a mais curta e madura. Essa então é secretada pelas células do sistema imunitário para influenciar as funções de outras células (WERMAN *et al.*, 2004; HUANG *et al.*, 2015).

Estudos apontam que a IL1A desempenha um papel importante na regulação da resposta imunitária na promoção ou bloqueio dos processos de tumorigênese, proliferação celular, angiogênese, invasão e metástase. Esses achados conflitantes justificam-se pela variabilidade genética, que pode afetar a resposta imune e inflamatória (WANG *et al.*, 2016).

1.7.3.1 Polimorfismo do Gene *IL1A* (rs3783553)

Esse polimorfismo caracteriza-se por ser uma inserção/deleção de 4pb na região 3'UTR. Essa região é o local de ligação de microRNAs (miR-122 e miR-378) responsáveis pela regulação da expressão da proteína *IL1A*. Na existência de um polimorfismo nessa região, a força de ligação do microRNA com o RNAm é alterada, comprometendo a regulação da expressão protéica (HUANG *et al*., 2015; YAN *et al*., 2015).

Essa falha de regulação tem sido associada a diferentes níveis de expressão da *IL1A*, relacionada ao desenvolvimento de diversos tipos de câncer e com a osteoartrite (YANG *et al*., 2015; MA, ZHOU, 2016). Estudos sugerem que essa variação dentro da região 3'UTR pode ter duas consequências: a variação pode afetar o local de regulação, alterando a estabilidade do microRNA no RNAm; ou pode criar um local alvo de um outro microRNA, conforme exposto na Figura 12 (SLABY *et al*., 2012).

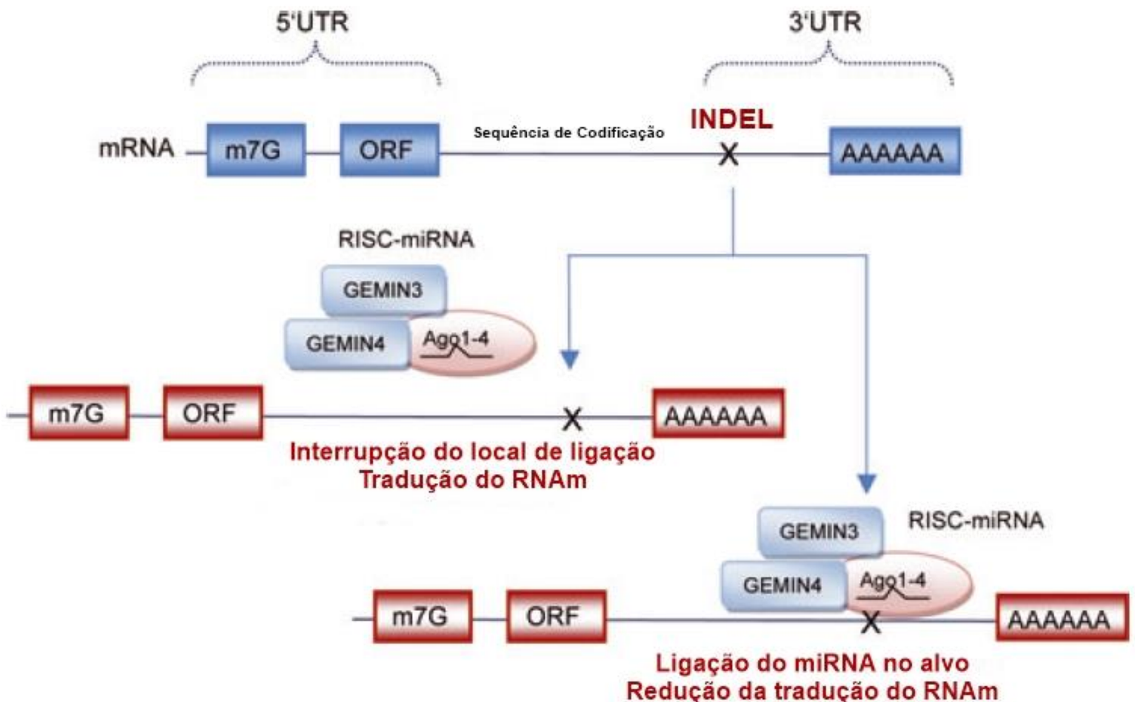

Figura 12. Esquema de interação do Polimorfismo INDEL do gene *IL1A* e miRNA.
Fonte: adaptado de Slaby *et al*. (2012).

1.7.4 Gene *NFKB1*

O gene *NFKB1* está localizado no cromossomo 4 (região 4q24), composto por 25 éxons que abrangem um tamanho de aproximadamente 156kb. Esse gene é responsável pela expressão das proteínas p105 e p50. A proteína p50 é originada da remoção da sequência C-terminal, mediada pelo proteassoma 26S, da proteína p105 (Figura 13). A p50 é um fator de transcrição importante em vários processos fisiológicos e patológicos, incluindo a proliferação e diferenciação celular, resposta inflamatória e imunitária, apoptose, sobrevivência celular, reações ao estresse celular e ao desenvolvimento de câncer (YU, MAN, HUANG, 2009; YANG *et al*., 2014; LI *et al*., 2015).

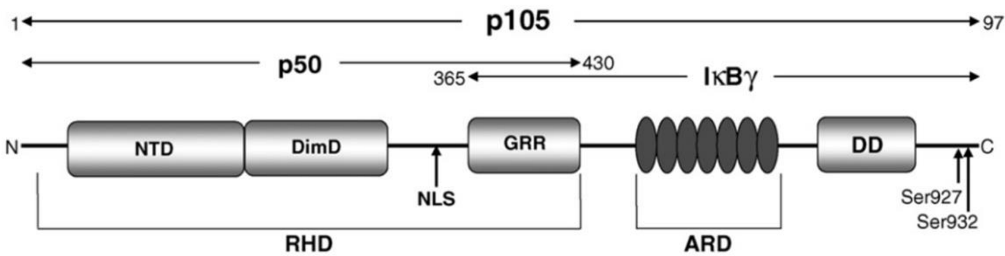

Figura 13. Estrutura da Proteína p105.
Fonte: adaptado de Pereira e Oakley (2008).

Em geral o complexo da *NFKB1* encontra-se inativado no citoplasma, ligado a proteínas inibidoras de NFkB, chamadas de IkBs. Existem várias vias que conduzem à ativação da *NFKB1*, por estímulos intra e extracelulares, resultantes da exposição celular a citocinas inflamatórias, exposição à luz ultravioleta, hipóxia, aumento das espécies reativas de oxigênio e produtos bacterianos e virais. Esses estímulos ativam o complexo cinase IkB (IKK), que promove a remoção da sequencia C-terminal, ativando a subunidade p50. Essa por sua vez desloca-se para o núcleo e regula a expressão de genes das mais variadas funções biológicas (YAMINI, 2015; CARTWRIGHT, PERKINS, WILSON, 2016).

Entre as proteínas reguladas pela NFKB1 estão as mediadoras da resposta inflamatória, que convergem com a proliferação celular, apoptose e angiogênese. O papel da p50 na resposta aos danos no DNA revela que esta subunidade é necessária para a manutenção do genoma e que sua perda resulta no comprometimento da longevidade e o desenvolvimento de doença crônica e do câncer (KOLOVOU *et al*., 2014; YAMINI, 2015; ZHANG *et al*., 2015; CARTWRIGHT, PERKINS, WILSON, 2016).

1.7.4.1 Polimorfismo do Gene *NFKB1* (rs28362491)

O gene *NFKB1* possui um polimorfismo funcional entre duas regiões promotoras, caracterizado pela inserção/deleção de 4pb (-94ins/del ATTG, rs28362491), como observado na Figura 14. Com a presença da deleção de 4pb se observou uma perda da ligação de proteínas nucleares, levando à redução significativa da atividade do promotor e, consequentemente, a níveis mais baixos de proteína p50. Assim, este polimorfismo parece estar associado a várias doenças, tais como câncer e doenças inflamatórias (YANG *et al*., 2014; CHEN, CAI, LIANG, 2015; LAI *et al*., 2015; WANG *et al*., 2015).

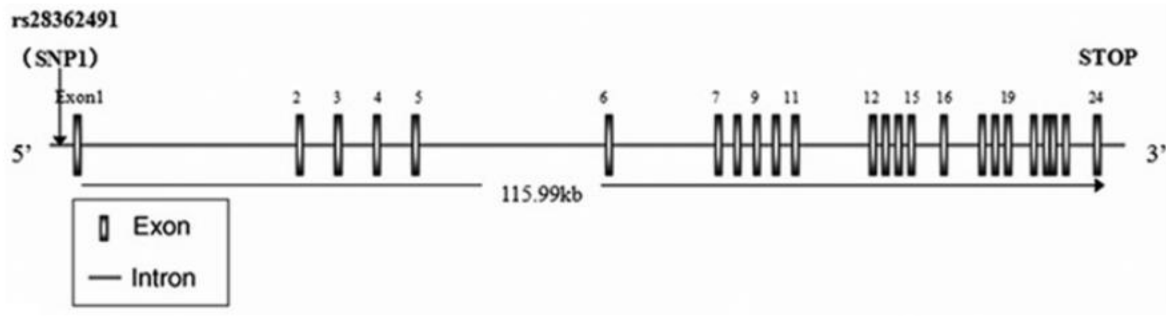

Figura 14. Polimorfismo do Gene *NFKB1* (rs28362491).
Fonte: Yang *et al*., 2014.

1.7.5 Gene *UCP2*

O gene *UCP2* está localizado no cromossomo 11 (região q13.4), composto por 8 éxons, abrangendo cerca de 8kb. É responsável pela expressão da proteína UCP2, uma proteína transportadora mitocondrial, que desacopla a fosforilação oxidativa da produção de ATP, pela dissipação do gradiente de prótons gerados na membrana interna mitocondrial, reduzindo a produção de ATP pela cadeia respiratória mitocondrial. Além de regular a produção de ATP mitocondrial, a UCP2 regula também a geração de espécies reativas de oxigênio (ROS) (HASHEMI *et al*., 2014; TODA, DIANO, 2014).

Essa regulação da geração de ROS, garantida quando ocorre a sobre-expressão da UCP2, previne o dano oxidativo nos tecidos (HASHEMI *et al*., 2014). A redução desse estresse oxidativo é importante na regulação da sobrevivência celular e do envelhecimento (KIM *et al*., 2016). No entanto, a desregulação da sua expressão pode contribuir para o desenvolvimento de

doenças metabólicas, neurodegenerativas, aterosclerose e o câncer. A hiperexpressão da UCP2 tem sido observada como um mecanismo de regulação positiva para a carcinogênese, o que promove a redução do estresse oxidativo e o crescimento celular (ROBBINS, ZHAO, 2011).

1.7.5.1 Polimorfismo do Gene *UCP2 (INDEL 45-bp)*

O gene *UCP2* possui um polimorfismo caracterizado pela inserção/deleção de 45bp na região 3'UTR do éxon 8 (Figura 15). Estudos afirmam que uma das variações desse polimorfismo pode influenciar na quantidade da proteína UCP2, devido às alterações na estabilidade do RNAm ou nas taxas de tradução (YANOVSKI *et al*., 2000; MERCER *et al*., 2007).

Essas alterações permitem estabelecer a associação desse polimorfismo com o comprometimento metabólico, aumento do peso e a obesidade. Esses fatores podem promover o aumento do estresse oxidativo, aumentando o risco do desenvolvimento de doenças crônicas e degenerativas, entre elas o câncer (HEIDEMA *et al*., 2010; HASHEMI *et al*., 2014; MUTOMBO, YAMASAKI, SHIWAKU, 2013).

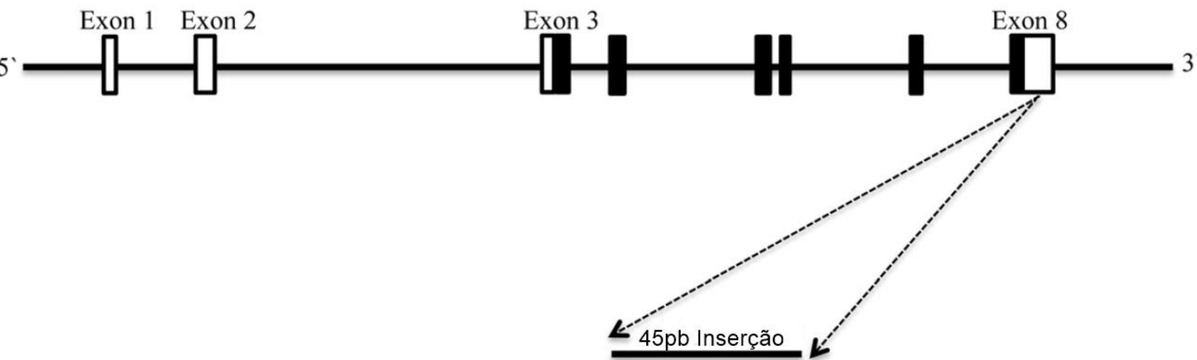

Figura 15. Polimorfismo do Gene *UCP2*.
Fonte: adaptado Hashemi *et al*. (2014).

1.7.6 Gene *UGT1A1*

O gene *UGT1A1* é originário da enzima UDP-glucuronosiltransferase, que é ativa na via metabólica da glicuronidação, uma das principais vias de biotransformação dos xenobióticos (STEVENTON, 2020; SISSUNG *et al*., 2020). As enzimas UGT1A1 foram observadas na

glicuronidação do estradiol e de um precursor do potente carcinógeno orgânico benzo(α)pireno-7,8-diidrodiol-9,10-epóxido (BPDE), encontrado na fumaça do cigarro (BARBARINO *et al.,* 2014). Este gene tem sido associado ao desenvolvimento de vários tipos de câncer: cólon, mama e próstata. A enzima UGT1A1 desempenha um papel importante na desintoxicação e metabolização de vários carcinógenos (BAJRO *et al*., 2012; CLENDENEN *et al*., 2013).

1.7.6.1 Polimorfismo do Gene *UGT1A1* (rs8175347)

O polimorfismo do Gene *UGT1A1* (rs8175347) ocorre na região promotora do gene. A variante alélica TA7 é caracterizada por sete repetições de timina-adenina (TA) dentro da região promotora, ao contrário do alelo TA6 de tipo selvagem que tem seis repetições TA (Figura 16). Esta repetição extra prejudica a transcrição gênica adequada (FANG, LAZARUS, 2004; TAKANO, SUGIYAMA, 2017; PATEL *et al*., 2020). Isso resulta em uma redução de 25 a 70% na atividade da enzima, dependendo da presença de um ou dois alelos variantes de TA7, respectivamente, que reduzem a glicuronidação (STRASSBURG, 2008; BELKHIR *et al*., 2018; NELSON *et al*., 2021). Os genótipos encontrados estão associados ao grau de atividade enzimática. Na literatura, esses genótipos podem ser agrupados como: atividade enzimática normal (TA6/6), reduzida (TA6/7 e TA5/7) e baixa (TA7/7) (NELSON *et al*., 2021).

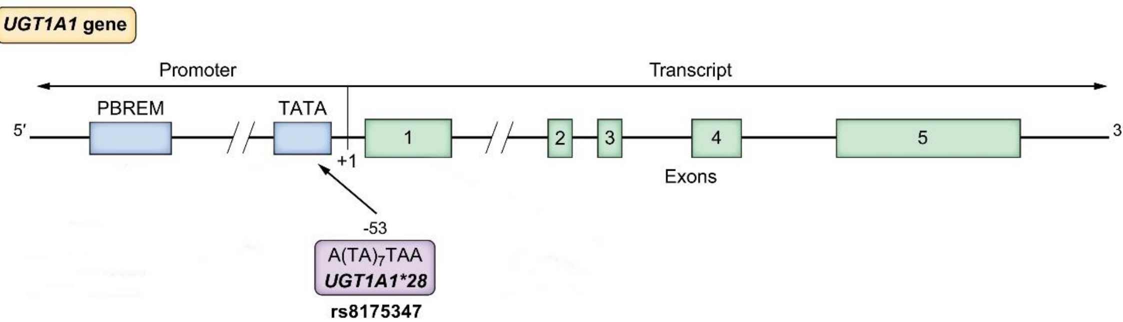

Figura 16. Polimorfismo do Gene *UGT1A1* (rs8175347).
Fonte: adaptado de Vitek & Tiribelli (2021).

CAPÍTULO II. OBJETIVOS

2.1 OBJETIVO GERAL

- O objetivo do presente estudo foi investigar as possíveis associações entre a variabilidade genética individual e a susceptibilidade ao desenvolvimento do câncer de pulmão.

2.2 OBJETIVOS ESPECÍFICOS

- Demonstrar o perfil clínico de pacientes com câncer de pulmão.
- Descrever o perfil de ancestralidade de pacientes com câncer de pulmão.
- Investigar os polimorfismos nos genes *TP53, PAR1, IL1A, NFKB1, UCP2 e UGT1A1* em pacientes com câncer de pulmão e no grupo controle.
- Comparar a variabilidade de polimorfismos nos genes *TP53, PAR1, IL1A, NFKB1, UCP2 e UGT1A1* em pacientes com câncer de pulmão e no grupo controle.
- Associar a variabilidade de polimorfismos nos genes *TP53, PAR1, IL1A, NFKB1, UCP2 e UGT1A1* com a susceptibilidade ao câncer de pulmão.

CAPÍTULO III. APLICABILIDADE

As neoplasias pulmonares desenvolvem-se de forma assintomática ou com sintomas inespecíficos, isso associado à ausência de um protocolo de detecção precoce estabelecido que contribui para o diagnóstico tardio, influenciando diretamente sobre o prognóstico, a letalidade e os gastos com a doença (CHUDGAR *et al*., 2015; LEE *et al*., 2016).

Segundo Lee *et al*. (2016) quando o CPNPC é diagnosticado precocemente, a taxa de sobrevida de cinco anos aumenta para 80%, mas a mesma reduz para 15% no diagnóstico tardio. Estima-se que o custo médio total por paciente com câncer de pulmão, submetido à internação hospitalar seja de aproximadamente 28 mil reais, podendo variar entre 12 e 49 mil reais, que poderiam ser reduzidos com políticas de prevenção e diagnóstico precoce (GOOZNER, 2012; INCA, 2016; KNUST, PORTELA, PEREIRA, 2015).

Estudos investigam a viabilidade do diagnóstico precoce em indivíduos com maior risco para o desenvolvimento do câncer de pulmão, como tabagistas de longa data com idade entre 55 e 80 anos. Os instrumentos pesquisados para essa finalidade são a radiografia torácica, a citologia de escarro e a tomografia computadorizada (TC). A TC tem demonstrado uma redução de 20% das mortes pela doença. No entanto, sua aplicação ainda possui limitações (PATZ *et al*., 2014; CHUDGAR *et al*., 2015; YOUSAF-KHAN *et al*., 2015).

A análise de biomarcadores baseia-se na perspectiva de utiliza-los como um teste de rastreio populacional, associando-o a fatores de risco clínicos já estabelecidos, o que facilitará um diagnóstico precoce. A identificação de associações desses biomarcadores com a neoplasia pulmonar poderá permitir, futuramente, o rastreio de indivíduos com maior susceptibilidade de desenvolver a doença, antes dos primeiros sintomas, permitindo instituir o monitoramento e o tratamento precoce, diminuindo o risco da morbimortalidade da doença.

A proposta do presente estudo demonstra-se aplicável, pois busca entender a associação entre os polimorfismos de biomarcadores do DNA e a carcinogênese pulmonar, identificados no sangue periférico. Esse conhecimento esclarece a compreensão da susceptibilidade individual ao desenvolvimento dessa neoplasia em adultos jovens e idosos.

O ensaio utilizado para investigação desses elementos moleculares possui características ideais para um *screening* populacional: metodologia minimamente invasiva, com aplicabilidade relativamente fácil e rápida, de baixo custo e que exige pouca quantidade de DNA do paciente. Sua aplicação na rotina clínica poderá auxiliar na predição do estado de saúde de forma interdisciplinar, embasar estratégias de prevenção, facilitar a gestão e a elaboração de futuros planos de cuidados.

CAPÍTULO IV. MATERIAIS E MÉTODOS

4.1 ASPECTOS ÉTICOS

O estudo foi realizado da aprovação do Comitê de Ética em Pesquisa (CEP) do Núcleo de Pesquisa em Oncologia (Anexo A), do aceite do Hospital Universitário João de Barros Barreto (Anexo B), da autorização dos voluntários a partir da assinatura do Termo de Consentimento Livre e Esclarecido - TCLE (Apêndice A), após os mesmos terem sido informados dos riscos e benefícios da avaliação ao qual foram submetidos, de acordo com a Resolução do Conselho Nacional de Saúde (CNS) 466/2012, que consta de todas as informações acerca da pesquisa.

4.2 TIPO DE ESTUDO

Trata-se de um estudo transversal analítico comparativo, desenvolvido a partir da avaliação clínico-funcional e análise de polimorfismos genéticos.

4.3 AMOSTRA

A pesquisa contou com 276 participantes recrutados das instituições coparticipantes, divididos em dois grupos: 196 pacientes sem câncer de pulmão (Grupo Controle) e 80 pacientes com câncer de pulmão (Grupo Caso).

4.3.1 Critérios de Inclusão

Foram aceitos no estudo, indivíduos de ambos os gêneros, com idade igual ou superior a 30 anos, oriundos das duas instituições listadas acima. O Grupo Controle foi composto por indivíduos sem câncer, com e sem diagnóstico de outras doenças crônicas. O Grupo Caso incluiu pacientes com diagnóstico fechado de câncer de pulmão (confirmado em exame histopatológico). Os pacientes foram inclusos se demonstrassem interesse em participar da pesquisa através da assinatura do TCLE. Em casos de analfabetismo ou incapacidade motora para escrever, os indivíduos que contribuíram com o estudo necessitaram estar acompanhados por seu responsável e convidados a assinar o TCLE ou deixar sua impressão digital, após a explicação detalhada pelos pesquisadores do presente estudo.

4.3.2 Critérios de Exclusão

Foram excluídos do estudo, indivíduos que apresentaram dificuldade para responder os questionários e/ou impossibilidade de realizar os testes propostos na avaliação, os que no momento da aplicação da avaliação apresentaram sinais de instabilidade hemodinâmica (alteração da frequência cardíaca, da pressão arterial, da frequência respiratória, da saturação periférica de oxigênio e da temperatura) ou impossibilidade de realizar a coleta de sangue periférico. No grupo controle, foram excluídos pacientes sob internação hospitalar, com diagnóstico de doença infecciosa aguda e sob investigação clínica de câncer de qualquer origem.

4.4 PROCEDIMENTOS

Os procedimentos para o desenvolvimento da pesquisa foram realizados em seis etapas: aplicação da avaliação clínica e funcional, coleta de sangue total, extração e quantificação do DNA, genotipagem para determinação da ancestralidade individual, genotipagem para investigação dos biomarcadores, os três últimos de leucócitos do sangue periférico.

4.4.1 Avaliação Clínica e Funcional

A avaliação clínica e funcional caracteriza-se por ser um instrumento interdisciplinar, baseada em escalas e testes quantitativos, permitindo uma visão global da saúde dos pacientes (Apêndice B). Foi subdividida em: Avaliação Sociodemográfica, Clínica e Epidemiológica e da Funcionalidade.

4.4.1.1 Avaliação Sociodemográfica

A avaliação sociodemográfica coletou dados que facilitaram a caracterização da amostra, incluindo: identificação, sexo, idade (anos), agrupamento etário, estado civil, escolaridade, profissão e ocupação.

4.4.1.2 Avaliação Clínica e Epidemiológica

Esse item destina-se a investigar as possíveis doenças diagnosticadas, as possíveis queixas de saúde, comorbidades presentes, histórico de câncer na família e hábitos de vida

(tabagismo, etilismo e sedentarismo). Especificamente para o grupo de pacientes oncológicos foram coletadas as variáveis: tipo de câncer, tipo de biópsia realizada, resultado citopatológico, resultado histopatológico, realização de imunohistoquimica, estadiamento e tempo de diagnóstico.

A presença de comorbidades foi avaliada através da Escala de Comorbidades de Charlson, instrumento que considera o número e a severidade das comorbidades. Essa escala inclui 19 condições, com pesos de valores 0 a 6 (0 para aquelas condições fora as 19 presentes na escala), atribuídos mediante o risco de mortalidade, sendo posteriormente somados, com uma pontuação que varia de 0 a 43 pontos, ajustado pela idade (HALL *et al*., 2004; PERKINSA *et al*., 2004; MARCHENA-GOMEZ *et al*., 2009).

4.4.1.3 Avaliação da Funcionalidade

A avaliação da capacidade funcional inclui a aplicação da Escala de Katz (Atividades Básicas de Vida Diária - ABVD), a Escala de Lawton (Atividades Instrumentais de Vida Diária - AIVD), Escala de Avaliação das Atividades Avançadas de Vida Diária (AAVD), Escala *Performance Status* do *Eastern Cooperative Oncology Group* (PS-ECOG), a avaliação dos sistemas funcionais e avaliação funcional nutricional.

A Escala de Katz avalia as ABVD, referentes às tarefas necessárias para o cuidado com corpo ou autopreservação, composta por 6 domínios, com um escore total que varia de 6 a 18 pontos, permitindo classificar o paciente como independente (6 pontos), semidependente (7 a 16 pontos) e dependente (acima de 16 pontos) (QUARESMA, 2008; AFONSO *et al*., 2013; JUNG *et al*., 2013).

A Escala de Lawton avalia as AIVD, referentes às tarefas necessárias para o cuidado com o domicílio ou atividades domésticas, composta por 9 domínios, com um escore total que varia de 9 a 27 pontos, permitindo classificar o paciente como dependente (9 pontos), semidependente (10 a 18 pontos) e independente (19 a 27 pontos) (NOGUEIRA, GARCIA, 2008; MELO, SAINTRAIN, 2009).

A Escala para Avaliação das AAVD, referente às atividades produtivas, recreativas e sociais, é composta de 12 perguntas, com um escore total que varia de 12 a 36 pontos, e quanto maior a pontuação melhor a funcionalidade do paciente (RODRIGUES, NERI, 2012; FIGUEIREDO *et al*., 2013; OLIVEIRA, NERI, D’ELBOUX, 2013).

A Escala PS-ECOG, avalia como a doença afeta as habilidades de vida diária do paciente, com um escore que varia de 0 a 5 pontos, permitindo classificar o paciente com o

índice 0 (atividade normal), 1 (sintomas da doença, mas deambula e leva seu dia a dia normal), 2 (fora do leito por mais de 50% do tempo), 3 (no leito mais de 50% do tempo, carente de cuidados mais intensivos), 4 (preso no leito) e 5 (morto) (ECOG, 2006; CORRÊA *et al.*, 2012).

A avaliação dos sistemas funcionais inclui os testes apropriados para a análise da cognição (Escala Mini-Exame do Estado Mental – MEEM), humor (Escala de Depressão Geriátrica de Yesavage – GDS 15) e mobilidade (Teste *Timed Up and Go* – TUG; Escala de Mobilidade e Equilíbrio Tinetti – TINETTI), indicada pela sua simplicidade, rapidez, portabilidade, fidedignidade e pela sua utilidade como indicadora da presença de incapacidades (MORAES, 2012).

A avaliação funcional nutricional foi realizada através da Miniavaliação Nutricional (MAN), um instrumento rápido é fácil de ser aplicado, com o poder de detectar o risco elevado de desnutrição. Composta por 18 itens divididos em Triagem e Avaliação Global, com um escore que varia de 0 a 30 pontos, permitindo classificar o paciente com estado nutricional normal (27 a 30 pontos), sob risco de desnutrição (17 a 23,5 pontos) e desnutrido (<17 pontos) (CALVO *et al.*, 2012; URREA, MESEGUER, 2013).

4.4.2 Coleta do Sangue Total

A investigação dos polimorfismos foi realizada utilizando uma amostra do sangue total (4ml) do paciente, coletado em tubo EDTA utilizando métodos bem estabelecidos e por profissionais devidamente capacitados, evitando possíveis complicações provenientes dessa etapa da pesquisa.

Esse material foi devidamente armazenado, congelado a -20°C, no Núcleo de Pesquisa em Oncologia, para posterior análise relacionada exclusivamente ao presente estudo. Após a análise, o material biológico foi devidamente descartado, conforme normas vigentes de órgãos técnicos competentes, respeitando-se a confidencialidade e a autonomia do sujeito da pesquisa.

4.4.3 Extração, Quantificação e Diluição do DNA

Para análise dos polimorfismos foi necessária a extração do DNA genômico de leucócitos do sangue periférico, usando o Kit Mini Spin Plus (P.250, Biopur, Biometrix, Brasil). O kit abrange as seguintes etapas: lise da amostra, ligação do DNA genômico à membrana do tubo, lavagem da membrana e eliminação do etanol e eluição do DNA genômico (Figura 17), seguindo o protocolo para extração do sangue total de humanos (Anexo C).

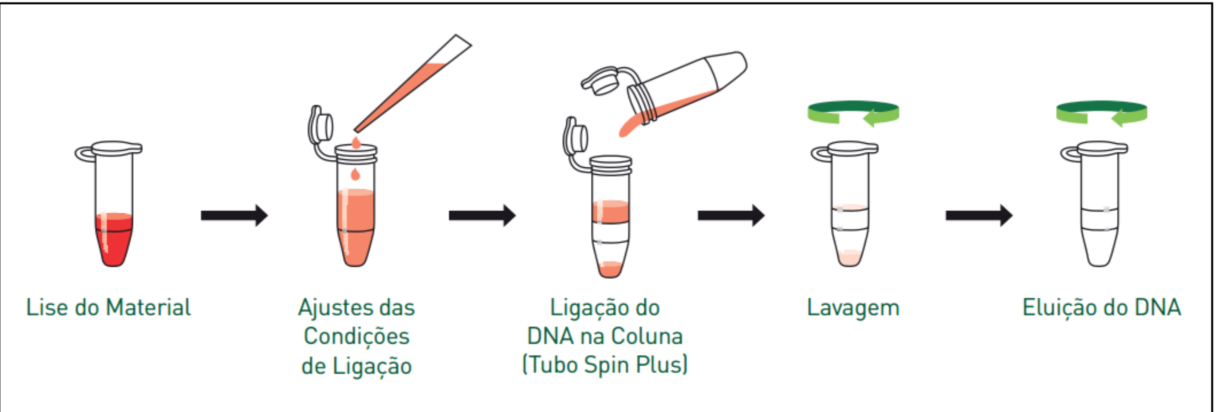

Figura 17. Extração do DNA genômico.
Fonte: Biopur, 2014.

A quantificação do DNA foi realizada no Espectrofotômetro NanoDrop 1000 (Thermo Scientific NanoDrop 1000; NanoDrop Technologies, Wilmington, DE). Depois de quantificada a mostra foi diluída para uma concentração de 10ng/ul.

4.4.4 Controle Genômico da Ancestralidade

A ancestralidade genômica foi investigada realizada de acordo com Santos *et al*. (2010), utilizando 61 marcadores informativos de ancestralidade autossômica (AIMS). Foram realizadas três reações de PCR multiplex, cada uma com 16 marcadores. Os amplicons de PCR foram analisados por eletroforese usando o sequenciador ABI *Prism 3130* e o *software GeneMapper* ID v.3.2. As proporções individuais de ancestralidade genética Europeia, Africana e Ameríndia foram estimadas usando o *software Structure* V.2.3.3, assumindo três populações parentais (europeia, africana e ameríndia) (CARVALHO *et al*., 2015).

4.4.5 Seleção dos Marcadores

Os seis polimorfismos do estudo foram genotipados (Quadro 5) por uma reação de PCR multiplex seguida de uma eletroforese capilar. Para as amplificações foram utilizados 0,5 µL do kit QIAGEN Multiplex PCR (QIAGEN, Alemanha), 1,0 µL de Q-solution, 1,0 µL de Primer Mix, 2,0 µL de água e 20 ng de DNA. A PCR foi realizada seguindo o protocolo a seguir: uma desnaturação inicial a 95ºC por 15 minutos, 35 ciclos a 94 ºC por 45 segundos, 60ºC por 90 segundos e 72ºC por 60 segundos, seguidos de uma extensão final de 70ºC por 30 minutos. A análise dos amplicons de PCR foi realizada por eletroforese usando o sequenciador ABI *Prism 3130* e o *software GeneMapper* ID v.3.2.

Quadro 5. Caracterização Técnica dos Polimorfismos Investigados.

Gene	Identificação	Tipo	Comp.	Iniciadores	Amplificação
IL1A	rs3783553	INDEL	4 pb	F-5'TGGTCCAAGTTGTGCTTATCC3' R-5'ACAGTGGTCTCATGGTTGTCA3'	230-234 pb
NFKB1	rs28362491	INDEL	4 pb	F-5'TATGGACCGCATGACTCTATCA3' R-5'GGCTCTGGCATCCTAGCAG3'	366-370 pb
PAR1	rs11267092	INDEL	13 pb	F-5'AAAACTGAACTTTGCCGGTGT3' R-5'GGGCCTAGAAGTCCAAATGAG3'	265-277 pb
TP53	rs17878362	INDEL	16 pb	F-5'GGGACTGACTTTCTGCTCTTGT3' R-5'GGGACTGTAGATGGGTGAAAAG3'	148-164 pb
UCP2	INDEL 45bp	INDEL	45 pb	F-5'CCCACACTGTCAAATGTCAACT3' R-5'CCATGCTTTCCTTTTCTTCCT3'	119-164 pb
UGT1A1	rs8175347	VNTR	2 pb	F-5'CTCTGAAAGTGAACTCCCTGCT3' R-5'AGAGGTTCGCCCTCTCCTAT3'	133/135/137/139 pb

INDEL. Inserção/Deleção. VNTR. Número Variável de Repetições em Tandem,

4.5 ANÁLISE ESTATÍSTICA

Foi realizada uma análise descritiva dos dados referentes à caracterização da amostra, utilizando a frequência absoluta, porcentagens, média e desvio padrão. As variáveis quantitativas foram primeiramente submetidas ao teste Kolmogorov-Smirnov para análise da distribuição de normalidade.

As frequências alélicas e genotípicas dos polimorfismos foram determinadas por contagem direta dos alelos, e em seguida foi calculado o equilíbrio de Hardy-Weinberg (HWE)

utilizando o software Arlequin 3.5.1.2. As proporções individuais das ancestralidades genéticas europeia, africana e ameríndia foram estimadas através do software Structure 2.3.3.

Para avaliar as diferenças entre os grupos estudados, foram utilizados o teste Qui-Quadrado e o Teste Exato de Fisher para fazer a comparação da distribuição da frequência dos alelos e dos genótipos dos polimorfismos. Para a análise de risco dos polimorfismos sobre a susceptibilidade ao câncer de pulmão, foi realizada uma Regressão Logística controlada por covariáveis: gênero, idade e tabagismo. Todas as análises estatísticas foram realizadas usando o pacote estatístico do software SPSS 20.0, respeitando o nível de significância de 5% ($p<0,05$).

CAPÍTULO V. ASSOCIATION BETWEEN POLYMORPHISM OF GENES *IL1A, NFKB1, PAR1, TP53* AND *UCP2* AND SUSCEPTIBILITY TO NON-SMALL CELL LUNG CANCER

Article

Association Between Polymorphism of Genes IL-1A, NFKB1, PAR1, TP53 and UCP2 and Susceptibility to Non-Small Cell Lung Cancer in the Amazon

Esdras E. B. Pereira [1,2,3], Antônio A. C. Modesto [1,2], Bruno M. Fernandes [2], Rommel M. R. Burbano [1,2], Paulo P. Assumpção [2], Marianne R. Fernandes [2*], João F. Guerreiro [1], Sidney E. B. dos Santos [1,2] and Ney P. C. dos Santos [1,2]

[1] Laboratory of Human and Medical Genetics, Institute of Biological Science, Federal University of Pará, Belem 66077-830, Brazil; esdrasedgarbp@gmail.com (E.E.B.P.); antonioacm@ufpa.br (A.A.C.M.); rommelburbano@gmail.com (R.M.R.B.); joaofg@ufpa.br (J.F.G.); sidneysantos@ufpa.br (S.E.B.d.S.); npcsantos.ufpa@gmail.com (N.P.C.d.S.)
[2] Oncology Research Center, Federal University of Pará, Belem 66073-005, Brazil; brunomelllo1@gmail.com (B.M.F.); assumpcaopp@gmail.com (P.P.A.); fernandesmr@yahoo.com.br (M.R.F.)
[2] Instituto Tocantinense Presidente Antônio Carlos (ITPAC), Abaetetuba 68440-000, PA, Brazil
* Correspondence: fernandesmr@yahoo.com.br

Abstract: Non-small cell lung cancer (NSCLC) represents the great majority of lung neoplasms. It is formed in multiple stages, with interactions between environmental risk factors and individual genetic susceptibility, with genes involved in immune and inflammatory response paths, cell or genome stability, metabolism, among others. Our goal was to investigate the association between five genetic polymorphisms (*IL-1A, NFKB1, PAR1, TP53* and *UCP2*) and the development of NSCLC in the Amazon. The study included 263 individuals with and without cancer. The samples were analyzed for polymorphisms of *NFKB1* (rs28362491), *PAR1* (rs11267092), *TP53* (rs17878362), *IL-1A* (rs3783553) and *UCP2* (INDEL 45-pb), genotyped in PCR, followed by an analysis of fragments in which we applied a previously developed set of informative ancestral markers. We used a logistic regression model to identify differences in the allele and genotypic frequencies among individuals. Individuals with a Del/Del genotype of polymorphism *NFKB1* (rs28362491) (p=0.018; OR=0.332) demonstrated a protective effect for NSCLC development, similar to that observed in the variants of *PAR1* (rs11267092) (p=0.023; OR=0.471) and *TP53* (rs17878362) (p=0.041; OR=0.510). Also, individuals with the Ins/Ins genotype of polymorphism *IL-1A* (rs3783553) demonstrated greater risk for NSCLC (p=0.033; OR=2.002), as well as volunteers with Del/Del of *UCP2* (INDEL 45-pb) (p=0.031; OR=2.031). The five polymorphisms investigated can contribute towards NSCLC susceptibility in the population of the Amazon region.

Keywords: genetic polymorphism; biomarker; non-small cell lung cancer; susceptibility

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1. Introduction

Lung neoplasm is among the prevailing cancer types in the world, representing a little over 11% of all cancers and responsible for 18% of the deaths by the disease.[1] Non-small cell lung cancer (NSCLC) represents up to 85% of that neoplasm.[2,3] In Brazil, that prevalence may reach 90% and most cases are diagnosed in the advanced stages of the disease.[4,5]

The formation of lung neoplasms may occur in several stages, with synergistic and complex interactions between the environmental risk factors, such as smoking and individual genetic susceptibility.[6] That susceptibility is associated to genetic polymorphisms, which include genes, involved in the metabolism, activation of carcinogens in tobacco

smoke, DNA repair, regulation of the cell cycle, homeostasis, immune response, among other paths.[7,8]

The alterations present in those genes may generate imbalances in those paths and trigger the development of neoplasms. In this study, five genetic variants relevant for those paths were researched: *IL-1A* (rs3783553), *NFKB1* (rs28362491), *TP53* (rs17878362), *PAR1* (rs11267092) and *UCP2* (INDEL 45-pb). Those genes and its polymorphisms have been associated to other cancers, including breast, colorectal, gastric, prostate, head and neck cancers and leukemias, in different populations.[7,9-18]

The choice for those markers was based on the fact that they are potential influencers in the development of neoplasms. Thus, identifying the associations of those polymorphisms with NSCLC may enable tracking individuals with greater disease susceptibility, before the first symptoms, enabling monitoring and early treatment, reducing the risk of the disease's morbidity and mortality.

The goal of this work was investigating the association between five polymorphisms in genes related to immune and inflammatory response paths (*IL-1A, NFKB1* and *PAR1*), cell or genome stability (*TP53*), metabolism (*UCP2*) and the development of NSCLC in the Amazon.

2. Materials and Methods

2.1. Ethical Compliance

It is an observational, case-control study, approved by the research Ethics Committees of the Oncology Research Center, under the CAAE protocol number: 37386214.3.0000.5634, and by the João de Barros Barreto University Hospital, under CAAE protocol number: 37386214.3.3001.0017, both in the city of Belém-Pará, within the Amazon region of Brazil. All volunteers signed a free and clarified term of consent.

2.2. Case and Control

The participants were recruited in public health centers, from both genders, not belonging to the same family group and from the same socio-economic level. Data and samples from 263 individuals were collected, whereas 67 patients had NSCLC (case group), defined and classified in the histopathological exam, and 196 patients had no type of cancer (control group). Both groups had demographic and clinical data survey, which included age, gender and smoking.

2.3. DNA Extraction and Quantification

The extraction of leukocytes' genomic DNA from the peripheral blood was carried out using a Mini Spin Plus Kit (P. 250, Biopur, Biometrix) pursuant to the manufacturer's recommendations. The DNA's concentration and purity were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific NanoDrop 1000; NanoDrop Technologies, Wilmington, DE, EUA).

2.4. Genotyping

The five genetic polymorphisms were genotyped by a multiplex PCR reaction followed by a capillary electrophoresis. The primers detailed in Chart 1 were used for the amplifications. The analysis of the PCR amplicons was made based on an electrophoresis using the ABI Prism 3130 sequencer and the GeneMapper ID v.3.2 software.[19]

Chart 1. Technical characteristics of the markers studied.

Gene	ID	Type	Length	Primers	Amplicon
IL-1A	rs3783553	INDEL	4 pb	F-5'TGGTCCAAGTTGTGCTTATCC3'	230-234 pb
				R-5'ACAGTGGTCTCATGGTTGTCA3'	

NFKB1	rs28362491	INDEL	4 pb	F-5'TATGGACCGCATGACTCTATCA3'	366-370 pb
				R-5'GGCTCTGGCATCCTAGCAG3'	
PAR1	rs11267092	INDEL	13 pb	F-5'AAAACTGAACTTTGCCGGTGT3'	265-277 pb
				R-5'GGGCCTAGAAGTCCAAATGAG3'	
TP53	rs17878362	INDEL	16 pb	F-5'GGGACTGACTTTCTGCTCTTGT3'	148-164 pb
				R-5'GGGACTGTAGATGGGTGAAAAG3'	
UCP2	-	INDEL	45 pb	F-5'CCCACACTGTCAAATGTCAACT3'	119-164 pb
				R-5'CCATGCTTTCCTTTTCTTCCT3'	

F: Forward; R: Reverse; INDEL: Insertion / Deletion.

2.5. Analysis of the Hardy-Weinberg Equilibrium (HWE)

The allele and genotype frequency of the polymorphism was determined by a direct count of the alleles, followed by the calculation of the Hardy-Weinberg Equilibrium (HWE) using the standard parameters of the Arlequin 3.5.1.2 software (Swiss Institute of Bioinformatics, Bern, Suíça). All polymorphisms were shown to be present in the HWE.

2.6. Genetic Ancestry Analysis

The genotyping was performed to analyze the ancestry of the samples, performed according to Ramos *et al.*,[20] using 61 informative markers of autosomal ancestry in three PCR multiplex reactions. The amplicons were analyzed by electrophoresis using the ABI Prism 3130 sequencer and the GeneMapper ID v. 3.2 software (Applied Biosystems, Life Technologies, Carlsbad, CA, EUA). The individual proportions of European, African and Amerindian genetic ascendancy were estimated using the Structure v. 2.3.3 software (Stanford University, Stanford, CA, EUA), assuming three parental populations.[21]

2.7. Statistic Analysis

All statistical analysis were made using the SPSS 20.0 statistics package (IBM, Armonk, NY, EUA). For the comparative analysis between the study groups as to demographic and clinical variables, Pearson's chi-square and the Mann-Whitney test were both applied. To analyse the association of polymorphisms with a lung cancer risk, a logistics regression was made, estimating the odds ratio (OR) and their reliability intervals of 95% (IC). Variables gender, age and smoking were controlled in that multivariate analysis to prevent confusion association. A significance level of $p < 0.05$ was considered for all statistical analyses.

3. Results

In the results related to demographic and clinical aspects, we may observe that the groups differed as to gender, age and smoking. Most NSCLC patients were male, with a 60-year average, and had a smoking history (Table 1). The ancestry analysis performed revealed that the case and control groups had a similar ancestry genome profile, with a larger European contribution for both populations (Table 1).

Table 1. Demographic and clinical characteristics of the investigated groups.

Characteristics	Case (n = 67)	Control (n = 196)	p-value
Gender			
Male	47 (70.1%)	62 (31.6%)	<0.001[a*]
Feminine	20 (29.9%)	134 (68.4%)	
Ages (years)			
Mean (±sd)	60.4 (±12.3)	70.5 (±8.2)	<0.001[b*]

Smoking			
Never smoked	13 (19.4%)	93 (47.4%)	<0.001[a]*
Smoker	54 (80.6%)	97 (49.5%)	
Ancestry			
European	47.1 (±16.9)	45.2 (±17.0)	0.521[b]
Amerindian	30.3 (±13.6)	30.6 (±14.8)	0.912[b]
African	22.6 (±12.1)	24.2 (±13.8)	0.498[b]

a. Chi-square Test. b. Mann-Whitney Test. *. p-value < 0.05.

The analysis of the genotype and allele distribution revealed that polymorphisms NFKB1 (rs28362491), PAR1 (rs11267092) and TP53 (rs17878362) presented variations associated to the protection for the development of NSCLC. Whereas the variations of the polymorphism of gene IL-1A (rs3783553) and UCP2 (INDEL 45-pb) were associated to the risk of developing the disease (Table 2).

For *NFKB1* (rs28362491), where genotype Del/Del also demonstrated a protection for the development of the NSCLC in comparison with different genotypes (p=0.018; OR=0.332; IC95%= 0.133 - 0.825). That is similar to what was observed for polymorphisms PAR1 (rs11267092) and *TP53* (rs17878362), where their Del/Del genotypes also demonstrated association of protection for the development of NSCLC (Table 2).

Differently, the polymorphism of the IL-1A (rs3783553) gene presented its Ins/Ins genotype associated to a higher risk for developing NSCLC (p=0.033; OR= 2.002; IC95%: 1.059 - 3.546), similar to Allele Ins. Similar results were identified for the polymorphism of gene UCP2 (INDEL 45-pb), where individuals with the Del/Del genotype had about twofold the risk of developing the disease compared to different genotypes (p=0.031; OR=2.031; IC95%= 1.067 - 3.868) (Table 2).

Table 2. Genotypic and allelic distributions of investigated polymorphisms for non-small cell lung cancer patients compared to the control group.

Polymorphisms	Case (n = 67)	Control (n = 196)	p-value[a]	OR (IC95%)
IL-1A (rs3783553)				
Del/Del	5 (7.5%)	32 (16.3%)		Ins/Ins *vs* Others
Ins/Del	33 (49.3%)	107 (54.6%)	0.033*	
Ins/Ins	29 (43.2%)	57 (29.1%)		2.002 (1.059 - 3.546)
Alelo Del	43 (32.1%)	171 (43.6%)	0.033*	0.499 (0.264 - 0.944)
Alelo Ins	91 (67.9%)	221 (56.4%)		2.002 (1.059 - 3.546)
NFKB1 (rs28362491)				
Del/Del	13 (19.4%)	46 (23.5%)		Del/Del *vs* Others
Ins/Del	35 (52.2%)	97 (49.5%)	0.018*	
Ins/Ins	19 (28.4%)	53 (27.0%)		0.332 (0.133 - 0.825)
Alelo Del	61 (45.5%)	189 (48.2%)	0.981	1.009 (0.500 - 2.033)
Alelo Ins	73 (54.5%)	203 (51.8%)		0.992 (0.492 - 1.999)
PAR1 (rs11267092)				
Del/Del	23 (34.3%)	108 (55.1%)		Del/Del *vs* Others
Ins/Del	32 (47.8%)	75 (38.3%)	0.023*	0.471 (0.247-0.971)
Ins/Ins	12 (17.9%)	13 (6.6%)		
Alelo Del	78 (58.2%)	291 (74.2%)	0.130	0.477 (0.183-1.244)
Alelo Ins	56 (41.8%)	101 (25.8%)		
TP53 (rs17878362)				

Del/Del	34 (50.7%)	129 (65.8%)		Del/Del *vs* Others
Ins/Del	29 (43.3%)	61 (31.1%)	0.041*	0.510 (0.267-0.974)
Ins/Ins	4 (6.0%)	6 (3.1%)		
Alelo Del	97 (72.4%)	319 (81.4%)	0.568	0.655 (0.153-2.789)
Alelo Ins	37 (27.6%)	73 (18.6%)		
UCP2				
Del/Del	39 (58.2%)	88 (44.9%)		Del/Del *vs* Others
Ins/Del	22 (32.8%)	90 (45.9%)	0.031*	
Ins/Ins	6 (9.0%)	18 (9.2%)		2.031 (1.067 - 3.868)
Alelo Del	100 (74.6%)	266 (67.9%)	0.617	1.367 (0.401 - 4.663)
Alelo Ins	34 (25.4%)	126 (32.1%)		0.731 (0.214 - 2.495)

Ins. Insertion. Del. Deletion. OR *odds ratio;* IC confidence interval. a. Logistic regression adjusted for gender, age and smoking. *p-value < 0.05.

4. Discussion

Lung cancer is the second type of cancer with the highest volume of diagnosis in both genders globally, prevailing more among male individuals aged over 65 and frequently associated to smoking.[1,22,23] Specifically in the Brazilian population, that neoplasm is the third most common among males and fourth among females, whereas more than 8% of the cases are related to smoking.[4,24] That is also maintained for NSCLC, where a larger prevalence was observed among men over 60.[5,25,26] And that is similar to what was observed in this study, where individuals with NSCLC were shown to be mostly men above 60 with a history of smoking.

The frequency of lung neoplasm among males is typically associated to tobacco consumption, since the ratio of men smokers is larger than women smokers. Men are also exposed to carcinogens in some labor activities, which contributes to a higher prevalence in that group.[27-29] However, those differences vary among countries, according to their level of socio-economic and cultural development, associated to tobacco consumption and exposure to both intrinsic and extrinsic risk factors.[1,30]

In terms of age, we observe that at least one third of the lung neoplasm cases are diagnosed between 65 and 74 years of age, whereas the elderly encompass nearly two thirds of all cases.[1] The aging process is associated to genomic modifications, favoring the accumulation of cells with different molecular alterations that modify internal homeostasis, increasing individual susceptibility to carcinogens and, in result, lung carcinogenesis.[31,32]

In this study, upon assessing individual susceptibility to the development of NSCLC, we may observe relevant results in respect to the polymorphisms of *NFKB1* (rs28362491), *PAR1* (rs11267092) and *TP53* (rs17878362), all three associated to the reduction of the risk for developing NSCLC. Whereas the polymorphisms of *IL1A* (rs3783553) and *UCP2* (INDEL 45-pb) are associated to a higher risk for developing that neoplasm.

The *NFKB1* gene expresses the NFkB1 (p50/p105) transcription factor, activated by several intracellular and extracellular stimuli, favoring the repression of gene transcription.[18,33] In polymorphism *NFKB1* (rs28362491), it occurs from the *NFKB1* gene-promoting region and is related to a susceptibility to multiple diseases associated to inflammation, immunity and tumorigenesis.[34,35] In our study, individuals with the Del/Del genotype of that polymorphism presented a protective effect against NSCLC development. Other studies also identified associations of that polymorphism with lung cancer.[36,37] That is because the genotype is associated to lower transcriptional activity and reduction of p50/p105 expression, acting as a counterpart to the tumorigenesis events.[18,38]

The same was observed with polymorphism *PAR1* (rs11267092), where individuals with genotype Del/Del had less chances of developing NSCLC. Gene *PAR1* expresses the

PAR1 receptor, which regulates physiological processes of the cardiovascular, respiratory, neurological systems, inflammation, embryogenesis and carcinogenesis.[20,39] The *PAR1* (rs11267092) polymorphism occurs in the gene promoting region and modulates PAR1 production and activity, influencing many physiological events.[40] The reduction of mRNA expression by PAR1 correlates to the reduction of invasive properties of some types of cancers.[41,42] In addition, it has been observed that the Del allele is associated to a better prognosis in some kinds of solid cancers,[38,43] which corroborates the results of this study.

A similar outcome was observed in polymorphism *TP53* (rs17878362), where the individuals with genotype Del/Del presented lower risk for NSCLC. Gene *TP53* encodes the p53 protein, which is regulated by multiple mechanisms in response to a broad range of antiproliferative responses.[9] Polymorphism *TP53* (rs17878362) is associated to many types of cancers, owing to the alterations in gene expression and protein function.[9,16] Corroborating our results, studies show that the Del/Del genotype of that polymorphism is associated to higher levels of TP53 mRNA and a greater DNA repair capability than for alleles Ins/Del and Ins/Ins, granting more protection against carcinogenesis events.[9,44,45]

In turn, polymorphism *IL-1A* (rs3783553) presented association with the risk of NSCLC development. Individuals with genotype Ins/Ins presented twofold the susceptibility to neoplasm compared to other genotypes. Gene *IL-1A* expresses interleukin IL-1A, a pro-inflammatory cytokine produced by monocytes and macrophages, released in response to cell injury, which may influence proliferation, angiogenesis and tumor invasion, among other carcinogenic events.[46] Polymorphism rs3783553 is associated to the regulation of IL-1A expression levels, for interrupting a linking site to miRNA-122 and miRNA-378, and is thus associated to several kinds of malignant neoplasms.[47,48] Studies indicate that the Ins/Ins genotype and the Ins allele of that polymorphism were also associated to a greater risk of developing cancer in general,[48] corroborating our findings.

In this study, the polymorphism in *UCP2* (INDEL 45-bp) also revealed an association with NSCLC susceptibility. Individuals with genotype Del/Del presented twofold the risks for developing the disease. Gene *UCP2* expresses uncoupling protein 2 (UCP2), which acts as proton transporters in mitochondria, involved in energy homeostasis, thermogenesis, among other metabolic phenomena.[49] That gene and its variants have been associated to chronic diseases and some types of cancer.[50,51] Polymorphism *UCP2* (INDEL 45-bp) may alter the mRNA stability of UCP2.[52] According to Esterbauer et al.[53] the half-life of mRNA from individuals with the deletion allele is greater than those that have the insertion allele, which may indicate greater expression. That greater expression may influence carcinogenesis, since the latter mitigates the production of reactive species of oxygen (ROS), protecting the neoplastic cell of the apoptotic path,[54,55] which clarifies the results obtained in this study.

Our findings reinforce the results of other studies with other populations, which had a more expressive sample number, but also observed a relationship between these genes and carcinogenesis. It is one of the few that investigated the association of these five variants with NSCLC, being extremely important for the Amazon, because it is an extremely mixed population, unique, little studied and with a lack of epidemiological and genetic information about the disease in the region. In the future, the use of this information as a screening tool will be able to identify individuals with greater susceptibility to NSCLC, facilitating the establishment of preventive measures and early diagnosis, reducing the cost to health services and the morbidity and mortality rates from this neoplasm.

5. Conclusions

The five polymorphisms investigated show a significant association with NSCLC in the population of the Amazon region, whereas the genotypes of the polymorphisms of *NFKB1* (rs28362491), *PAR1* (rs11267092) and *TP53* (rs17878362) were associated to a lower risk for disease development and the genotypes of polymorphisms *IL-1A* (rs3783553) and of *UCP2* (INDEL 45-pb) were associated to a growth in the susceptibility to that malignant neoplasm.

Author Contributions: E.E.B.P. designed the study, processed the data, and wrote the article. B.M.F. contributed to the writing of the article. A.A.C.M. contributed to the genotyping and data analysis. R.M.R.B., P.P.A., S.E.B.d.S., M.R.F., J.F.G. and N.P.C.d.S. were the project coordinators. All authors have read and agreed to the published version of the manuscript.

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References

1. Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* **2021**, 71 (3), 209-249. DOI: 10.3322/caac.21660.
2. Duma, N.; Santana-Davila, R.; Molina, J. R. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clin Proc* **2019**, 94 (8), 1623-1640. DOI: 10.1016/j.mayocp.2019.01.013.
3. Saltos, A.; Shafique, M.; Chiappori, A. Update on the Biology, Management, and Treatment of Small Cell Lung Cancer (SCLC). *Front Oncol* **2020**, 10, 1074. DOI: 10.3389/fonc.2020.01074.
4. Araujo, L. H.; Baldotto, C.; Castro, G.; Katz, A.; Ferreira, C. G.; Mathias, C.; Mascarenhas, E.; Lopes, G. L.; Carvalho, H.; Tabacof, J.; et al. Lung cancer in Brazil. *J Bras Pneumol* **2018**, 44 (1), 55-64. DOI: 10.1590/S1806-37562017000000135.
5. Ferreira, C. G.; Abadi, M. D.; de Mendonça Batista, P.; Serra, F. B.; Peixoto, R. B.; Okumura, L. M.; Cerqueira, E. R. Demographic and Clinical Outcomes of Brazilian Patients With Stage III or IV Non-Small-Cell Lung Cancer: Real-World Evidence Study on the Basis of Deterministic Linkage Approach. *JCO Glob Oncol* **2021**, 7, 1454-1461. DOI: 10.1200/GO.21.00228.
6. Tsao, A. S.; Scagliotti, G. V.; Bunn, P. A.; Carbone, D. P.; Warren, G. W.; Bai, C.; de Koning, H. J.; Yousaf-Khan, A. U.; McWilliams, A.; Tsao, M. S.; et al. Scientific Advances in Lung Cancer 2015. *J Thorac Oncol* **2016**, 11 (5), 613-638. DOI: 10.1016/j.jtho.2016.03.012.
7. Cavalcante, G. C.; Amador, M. A.; Ribeiro Dos Santos, A. M.; Carvalho, D. C.; Andrade, R. B.; Pereira, E. E.; Fernandes, M. R.; Costa, D. F.; Santos, N. P.; Assumpção, P. P.; et al. Analysis of 12 variants in the development of gastric and colorectal cancers. *World J Gastroenterol* **2017**, 23 (48), 8533-8543. DOI: 10.3748/wjg.v23.i48.8533.
8. Eaton, K. D.; Romine, P. E.; Goodman, G. E.; Thornquist, M. D.; Barnett, M. J.; Petersdorf, E. W. Inflammatory Gene Polymorphisms in Lung Cancer Susceptibility. *J Thorac Oncol* **2018**, 13 (5), 649-659. DOI: 10.1016/j.jtho.2018.01.022.
9. Sagne, C.; Marcel, V.; Amadou, A.; Hainaut, P.; Olivier, M.; Hall, J. A meta-analysis of cancer risk associated with the TP53 intron 3 duplication polymorphism (rs17878362): geographic and tumor-specific effects. *Cell Death Dis* **2013**, 4, e492. DOI: 10.1038/cddis.2013.24.
10. Xia, H.; Chen, Y.; Meng, J.; Liang, C. Effect of polymorphism on polymorphism on IL1A to cancer susceptibility: Evidence based on 34,016 subjects. *Artif Cells Nanomed Biotechnol* **2019**, 47 (1), 3138-3152. DOI: 10.1080/21691401.2019.1646750.
11. Kulmann-Leal, B.; Ellwanger, J. H.; Chies, J. A. B. CCR5Δ32 in Brazil: Impacts of a European Genetic Variant on a Highly Admixed Population. *Front Immunol* **2021**, 12, 758358. DOI: 10.3389/fimmu.2021.758358.
12. Karami, S.; Sahel, S.; Pouria, P.; Farhad, T.; Maomé, H.; Gholamreza, B. Lack of association between 4-base pair insertion/deletion (rs3783553) polymorphism within the 3'UTR of IL1A and breast cancer: A preliminary report. *Gene Reports* **2021**; 23, 101067. DOIi:10.1016/j.genrep.2021.101067.
13. Tajbakhsh, A.; Farjami, Z.; Nesaei-Bajestani, A.; Afzaljavan, F.; Rivandi, M.; Moezzi, A.; Abedini, S.; Asghari, M.; Kooshyar, M. M.; Homaei Shandiz, F.; et al. Evaluating the Association between. *Iran J Public Health* **2021**, 50 (3), 583-591. DOI: 10.18502/ijph.v50i3.5604.

14. Aguilar, E.; Esteves, P.; Sancerni, T.; Lenoir, V.; Aparicio, T.; Bouillaud, F.; Dentin, R.; Prip-Buus, C.; Ricquier, D.; Pecqueur, C.; et al. UCP2 Deficiency Increases Colon Tumorigenesis by Promoting Lipid Synthesis and Depleting NADPH for Antioxidant Defenses. *Cell Rep* **2019**, 28 (9), 2306-2316.e2305. DOI: 10.1016/j.celrep.2019.07.097.
15. Zhao, L.; Yin, X. X.; Qin, J.; Wang, W.; He, X. F. Association Between the TP53 Polymorphisms and Breast Cancer Risk: An Updated Meta-Analysis. *Front Genet* **2022**, 13, 807466. DOI: 10.3389/fgene.2022.807466.
16. Hashemi, M.; Bahari, G.; Sarhadi, S.; Eskandari, E.; Narouie, B.; Taheri, M.; Ghavami, S. 4-bp insertion/deletion (rs3783553) polymorphism within the 3'UTR of IL1A contributes to the risk of prostate cancer in a sample of Iranian population. *J Cell Biochem* **2018**, 119 (3), 2627-2635. DOI: 10.1002/jcb.26427.
17. Li, L.; Zhang, Z. T. Genetic Association between NFKBIA and NFKB1 Gene Polymorphisms and the Susceptibility to Head and Neck Cancer: A Meta-Analysis. *Dis Markers* **2019**, 2019, 6523837. DOI: 10.1155/2019/6523837.
18. Yazdani, Z.; Mousavi, Z.; Ghasemimehr, N.; Kalantary Khandany, B.; Nikbakht, R.; Jafari, E.; Fatemi, A.; Hassanshahi, G. Differential regulatory effects of chemotherapeutic protocol on CCL3_CCL4_CCL5/CCR5 axes in acute myeloid leukemia patients with monocytic lineage. *Life Sci* **2020,** 240, 117071. DOI: 10.1016/j.lfs.2019.117071.
19. Pinto, P.; Salgado, C.; Santos, N. P.; Santos, S.; Ribeiro-dos-Santos, Â. Influence of Genetic Ancestry on INDEL Markers of NFKβ1, CASP8, PAR1, IL4 and CYP19A1 Genes in Leprosy Patients. *PLoS Negl Trop Dis* **2015**, 9 (9), e0004050. DOI: 10.1371/journal.pntd.0004050.
20. Ramos, B. R.; D'Elia, M. P.; Amador, M. A.; Santos, N. P.; Santos, S. E.; da Cruz Castelli, E.; Witkin, S. S.; Miot, H. A.; Miot, L. D.; da Silva, M. G. Neither self-reported ethnicity nor declared family origin are reliable indicators of genomic ancestry. *Genetica* **2016**, 144 (3), 259-265. DOI: 10.1007/s10709-016-9894-1.
21. Carvalho, D. C.; Wanderley, A. V.; Amador, M. A.; Fernandes, M. R.; Cavalcante, G. C.; Pantoja, K. B.; Mello, F. A.; de Assumpção, P. P.; Khayat, A. S.; Ribeiro-Dos-Santos, Â.; et al. Amerindian genetic ancestry and INDEL polymorphisms associated with susceptibility of childhood B-cell Leukemia in an admixed population from the Brazilian Amazon. *Leuk Res* **2015**. DOI: 10.1016/j.leukres.2015.08.008.
22. Thandra, K. C.; Barsouk, A.; Saginala, K.; Aluru, J. S. Epidemiology of lung cancer. *Contemp Oncol (Pozn)* **2021**, 25 (1), 45-52. DOI: 10.5114/wo.2021.103829.
23. Rodak, O.; Peris-Díaz, M. D.; Olbromski, M.; Podhorska-Okołów, M.; Dzięgiel, P. Current Landscape of Non-Small Cell Lung Cancer: Epidemiology, Histological Classification, Targeted Therapies, and Immunotherapy. *Cancers (Basel)* **2021**, 13 (18). DOI: 10.3390/cancers13184705.
24. Brasil. Ministério da Saúde. Instituto Nacional de Câncer José Alencar Gomes da Silva. *Estimativa 2020: Incidência de Câncer no Brasil*. Rio de Janeiro: INCA; 2019. Available online: https://www.inca.gov.br/sites/ufu.sti.inca.local/files//media/document//estimativa-2020-incidencia-de-cancer-no-brasil.pdf (accessed on 15 November 2021).
25. de Sá, V. K.; Coelho, J. C.; Capelozzi, V. L.; de Azevedo, S. J. Lung cancer in Brazil: epidemiology and treatment challenges. *Lung Cancer (Auckl)* **2016**, 7, 141-148. DOI: 10.2147/LCTT.S93604.
26. Nicolau, J. S.; Lopez, R. V. M.; de Moraes Luizaga, C. T.; Ribeiro, K. B.; Roela, R. A.; Maistro, S.; Katayama, M. L. H.; Natalino, R. J. M.; de Castro, G.; Neto, J. E.; et al. Survival analysis of young adults from a Brazilian cohort of non-small cell lung cancer patients. *Ecancermedicalscience* **2021**, 15, 1279. DOI: 10.3332/ecancer.2021.1279.
27. Delva, F.; Margery, J.; Laurent, F.; Petitprez, K.; Pairon, J. C.; Group, R. W. Medical follow-up of workers exposed to lung carcinogens: French evidence-based and pragmatic recommendations. *BMC Public Health* **2017**, 17 (1), 191. DOI: 10.1186/s12889-017-4114-1.
28. Stapelfeld, C.; Dammann, C.; Maser, E. Sex-specificity in lung cancer risk. *Int J Cancer* **2020**, 146 (9), 2376-2382. DOI: 10.1002/ijc.32716..
29. Ragavan, M.; Patel, M. I. The evolving landscape of sex-based differences in lung cancer: a distinct disease in women. *Eur Respir Rev* **2022**, 31 (163). DOI: 10.1183/16000617.0100-2021.
30. Barta, J. A.; Powell, C. A.; Wisnivesky, J. P. Global Epidemiology of Lung Cancer. *Ann Glob Health* **2019**, 85 (1). DOI: 10.5334/aogh.2419.
31. Meiners, S.; Eickelberg, O.; Königshoff, M. Hallmarks of the ageing lung. *Eur Respir J 2015*, 45 (3), 807-827. DOI: 10.1183/09031936.00186914.
32. Schneider, J. L.; Rowe, J. H.; Garcia-de-Alba, C.; Kim, C. F.; Sharpe, A. H.; Haigis, M. C. The aging lung: Physiology, disease, and immunity. *Cell* **2021**, 184 (8), 1990-2019. DOI: 10.1016/j.cell.2021.03.005.
33. Concetti, J.; Wilson, C. L. NFKB1 and Cancer: Friend or Foe? *Cells* **2018**, 7 (9). DOI: 10.3390/cells7090133.
34. Luo, J. Y.; Liu, F.; Zhang, T.; Tian, T.; Luo, F.; Li, X. M.; Yang, Y. N. Association of NFKB1 gene rs28362491 mutation with the occurrence of major adverse cardiovascular events. *BMC Cardiovasc Disord* **2022**, 22 (1), 313. DOI: 10.1186/s12872-022-02755-x.
35. Dimitrakopoulos, F. D.; Kottorou, A. E.; Kalofonou, M.; Kalofonos, H. P. The Fire Within: NF-κB Involvement in Non-Small Cell Lung Cancer. *Cancer Res* **2020**, 80 (19), 4025-4036. DOI: 10.1158/0008-5472.CAN-19-3578.
36. Oltulu, Y. M.; Coskunpinar, E.; Ozkan, G.; Aynaci, E.; Yildiz, P.; Isbir, T.; Yaylim, I. Investigation of NF-κB1 and NF-κBIA gene polymorphism in non-small cell lung cancer. *Biomed Res Int* **2014**, 2014, 530381. DOI: 10.1155/2014/530381.
37. Yin, J.; Yin, M.; Vogel, U.; Wu, Y.; Yao, T.; Cheng, Y.; Sun, Z.; Hou, W.; Wang, C. NFKB1 common variants and PPP1R13L and CD3EAP in relation to lung cancer risk in a Chinese population. *Gene* **2015**, 567 (1), 31-35. DOI: 10.1016/j.gene.2015.04.059.
38. Wang, Y.; Chen, L.; Pan, L.; Xue, J.; Yu, H. The association between NFKB1-94ins/del ATTG polymorphism and non-small cell lung cancer risk in a Chinese Han population. *Int J Clin Exp Med.* **2015**, 8 (5), 8153-7.

39. Amador, M. A.; Cavalcante, G. C.; Santos, N. P.; Gusmão, L.; Guerreiro, J. F.; Ribeiro-dos-Santos, Â.; Santos, S. Distribution of allelic and genotypic frequencies of IL1A, IL4, NFKB1 and PAR1 variants in Native American, African, European and Brazilian populations. *BMC Res Notes* **2016**, 9, 101. DOI: 10.1186/s13104-016-1906-9.
40. Castaño-Rodríguez, N.; Kaakoush, N. O.; Goh, K. L.; Fock, K. M.; Chionh, Y. T.; Sutton, P.; Mitchell, H. M. PAR-1 polymorphisms and risk of Helicobacter pylori-related gastric cancer in a Chinese population. *Anticancer Res*. **2012**, 32(9):3715-21.
41. Darmoul, D.; Gratio, V.; Devaud, H.; Lehy, T. Laburthe M. Aberrant expression and activation of the thrombin receptor protease-activated receptor-1 induces cell proliferation and motility in human colon cancer cells. *Am J Pathol*. **2003**, 162(5):1503-13. DOI: 10.1016/S0002-9440(10)64283-6.
42. Lurje, G.; Husain, H.; Power, D. G.; Yang, D.; Groshen, S.; Pohl, A.; Zhang, W.; Ning, Y.; Manegold, P. C.; El-Khoueiry, A.; et al. Genetic variations in angiogenesis pathway genes associated with clinical outcome in localized gastric adenocarcinoma. *Ann Oncol* **2010**, 21 (1), 78-86. DOI: 10.1093/annonc/mdp280.
43. Eroğlu, A.; Karabıyık, A.; Akar, N. The association of protease activated receptor 1 gene -506 I/D polymorphism with disease-free survival in breast cancer patients. *Ann Surg Oncol* **2012**, 19 (4), 1365-1369. DOI: 10.1245/s10434-011-1969-8.
44. Ajaz, S.; Muneer, R.; Siddiqa, A.; Memon, M. A. Frequencies of TP53 Germline Variations and Identification of Two Novel 3'UTR Variants in a Series of Head and Neck Cancer Cases. *medRxiv* **2021**, 2021.2009.2009.21263314. DOI: 10.1101/2021.09.09.21263314.
45. Denisov, E. V.; Cherdyntseva, N. V.; Litviakov, N. V.; Malinovskaya, E. A.; Babyshkina, N. N.; Belyavskaya, V. A.; Voevoda, M. I. TP53 Gene Polymorphisms in Cancer Risk: The Modulating Effect of Ageing, Ethnicity and TP53 Somatic Abnormalities. In (Ed.), Tumor Suppressor Genes. *IntechOpen*. **2012**, DOI: 10.5772/27768.
46. Ma, L.; Zhou, N. Association between an insertion/deletion polymorphism in IL-1A gene and cancer risk: a meta-analysis. *Onco Targets Ther* **2016**, 9, 1-6. DOI: 10.2147/OTT.S95887.
47. Ma, Q.; Mao, Z.; Du, J.; Liao, S.; Zheng, Y.; Zhi, M.; Zhang, J.; Wang, Y. Association between an insertion/deletion polymorphism in the interleukin-1α gene and the risk of colorectal cancer in a Chinese population. *Int J Biol Markers* **2018**, 1724600818785069. DOI: 10.1177/1724600818785069.
48. Xia, H.; Chen, Y.; Meng, J.; Liang, C. Effect of polymorphism on IL1A to cancer susceptibility: Evidence based on 34,016 subjects. *Artif Cells Nanomed Biotechnol*. **2019**, 47(1): 3138-3152. DOI: 10.1080/21691401.2019.1646750.
49. Kaabi, Y. A.; Mansor, A. S.; Alfagih, A. S.; Hakami, A. M.; Summ, M. A.; Mjery, Y. A.; Alzughbi M. N.; Habibullah, M. M. Frequency of UCP2 45-bp Ins/Del polymorphism in Saudi population from Jazan area and its association with autoimmune hypothyroidism UCP2 45-bp Ins/Del frequency in hypothyroidism. *Int J Health Sci (Qassim)*. **2020**, 14(4): 11-16.
50. Rezapour, S.; Khosroshahi, S. A.; Farajnia, H.; Mohseni, F.; Khoshbaten, M.; Farajnia, S. Association of 45-bp ins/del polymorphism of uncoupling protein 2 (UCP2) and susceptibility to nonalcoholic fatty liver and type 2 diabetes mellitus in North-west of Iran. *BMC Res Notes* **2021**, 14 (1), 169. DOI: 10.1186/s13104-021-05586-9.
51. Say, Y. H. The association of insertions/deletions (INDELs) and variable number tandem repeats (VNTRs) with obesity and its related traits and complications. *J Physiol Anthropol* **2017**, 36 (1), 25. DOI: 10.1186/s40101-017-0142-x.
52. Yu, J.; Shi, L.; Lin, W.; Lu, B.; Zhao, Y. UCP2 promotes proliferation and chemoresistance through regulating the NF-κB/β-catenin axis and mitochondrial ROS in gallbladder cancer. *Biochem Pharmacol* **2020**, 172, 113745. DOI: 10.1016/j.bcp.2019.113745.
53. Esterbauer, H.; Schneitler, C.; Oberkofler, H.; Ebenbichler, C.; Paulweber, B.; Sandhofer, F.; Ladurner, G.; Hell, E.; Strosberg, A. D.; Patsch, J. R.; et al. A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. *Nat Genet* **2001**, 28 (2), 178-183. DOI: 10.1038/88911.
54. Lee, J. H.; Cho, Y. S.; Jung, K. H.; Park, J. W.; Lee, K. H. Genipin enhances the antitumor effect of elesclomol in A549 lung cancer cells by blocking uncoupling protein-2 and stimulating reactive oxygen species production. *Oncol Lett* **2020**, 20 (6), 374. DOI: 10.3892/ol.2020.12237.
55. Su, W. P.; Lo, Y. C.; Yan, J. J.; Liao, I. C.; Tsai, P. J.; Wang, H. C.; Yeh, H. H.; Lin, C. C.; Chen, H. H.; Lai, W. W.; et al. Mitochondrial uncoupling protein 2 regulates the effects of paclitaxel on Stat3 activation and cellular survival in lung cancer cells. *Carcinogenesis* **2012**, 33 (11), 2065-2075. DOI: 10.1093/carcin/bgs253.

CAPÍTULO VI. *UGT1A1* GENE POLYMORPHISM CONTRIBUTES AS A RISK FACTOR FOR LUNG CANCER

Article

UGT1A1 Gene Polymorphism Contributes as a Risk Factor for Lung Cancer: A Pilot Study with Patients from the Amazon

Esdras E. B. Pereira [1], Luciana P. C. Leitão [2], Roberta B. Andrade [1], Antônio A. C. Modesto [1], Bruno M. Fernandes [2], Rommel M. R. Burbano [1,2], Paulo P. Assumpção [2], Marianne R. Fernandes [2,*], João F. Guerreiro [1], Sidney E. B. dos Santos [1,2] and Ney P. C. dos Santos [1,2]

1 Laboratory of Human and Medical Genetics, Institute of Biological Science, Federal University of Pará, Belem 66077-830, Brazil; esdrasedgarbp@gmail.com (E.E.B.P.); robertaborgesandrade@gmail.com (R.B.A.); antonioacm@ufpa.br (A.A.C.M.); rommelburbano@gmail.com (R.M.R.B.); joaofg@ufpa.br (J.F.G.); sidneysantos@ufpa.br (S.E.B.d.S.); npcsantos.ufpa@gmail.com (N.P.C.d.S.)
2 Oncology Research Center, Federal University of Pará, Belem 66073-005, Brazil; colaresluciana@gmail.com (L.P.C.L.); brunomelllo1@gmail.com (B.M.F.); assumpcaopp@gmail.com (P.P.A.)
* Correspondence: fernandesmr@yahoo.com.br

Abstract: Lung cancer is one of the most frequent neoplasms in the world. Because it is a complex disease, its formation occurs in several stages, stemming from interactions between environmental risk factors, such as smoking, and individual genetic susceptibility. Our objective was to investigate associations between a *UGT1A1* gene polymorphism (rs8175347) and lung cancer risk in an Amazonian population. This is a pilot study, case-controlled study, which included 276 individuals with cancer and without cancer. The samples were analyzed for polymorphisms of the *UGT1A1* gene (rs8175347) and genotyped in PCR, followed by fragment analysis in which we applied a previously developed set of informative ancestral markers. We used logistic regression to identify differences in allelic and genotypic frequencies between individuals. Individuals with the TA7 allele have an increased chance of developing lung adenocarcinoma ($p = 0.035$; OR: 2.57), as well as those with related genotypes of reduced or low enzymatic activity: TA6/7, TA5/7, and TA7/7 ($p = 0.048$; OR: 8.41). Individuals with homozygous TA7/7 have an increased chance of developing squamous cell carcinoma of the lung ($p = 0.015$; OR: 4.08). Polymorphism in the *UGT1A1* gene (rs8175347) may contribute as a risk factor for adenocarcinoma and lung squamous cell carcinoma in the population of the Amazon region.

Keywords: genetic polymorphism; *UGT1A1*; biomarker; lung cancer; smoking

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1. Introduction

Lung neoplasms are among the most prevalent types of cancer in the world population, representing 11.4% of all registered cancers, and are responsible for 18% of cancer deaths [1]. This frequency varies between countries, depending on their demographic characteristics, smoking rate, and level of economic development [2]. Lung cancer can be divided into two categories, small cell lung cancer (SCLC), responsible for 15–20% of cases; and non-small cell lung cancer (NSCLC), which represents 80–85% of cases [3,4].

The well-known risk factor for the development of lung cancer is smoking, given the presence of substances contained in tobacco associated with carcinogenesis [5]. However, studies have shown that about half of new cases are in people who have never smoked, or have stopped smoking for many years [6]. This shows that pulmonary carcinogenesis is a complex and gradual process, with complex interactions between environmental risk factors and individual genetic susceptibility [7].

Genetic susceptibility to lung cancer, often modulated by smoking behavior, has been investigated [8]. Disease risk may be associated with genetic variables such as single nucleotide polymorphisms (SNPs) in genes related to metabolism, DNA damage repair,

and cell cycle control [9,10]. This knowledge is important when it comes to lung cancer, and its use can favor the development of population screening tools [11].

The *UGT1A1* gene originates from the UDP-glucuronosyltransferase enzyme, which is active in the glucuronidation metabolic pathway, one of the main biotransformation pathways of xenobiotics [12,13]. This gene has been linked to the development of several types of cancer: colon, breast, and prostate. The UGT1A1 enzyme plays an important role in the detoxification and metabolization of several carcinogens [14,15].

The aim of this study was to investigate possible associations between *UGT1A1* gene polymorphism (rs8175347) and lung cancer risk in an Amazonian population.

2. Materials and Methods

2.1. Ethical Conformity

This pilot study was approved by the Research Ethics Committees of the Oncology Research Center, under protocol 927.808/2014, and by the João de Barros Barreto University Hospital, under protocol number 941.207/2015, both in the city of Belém-Pará, Amazon region of Brazil. All participants signed an informed consent form.

2.2. Case and Control

Participants were recruited from public healthcare centers, of both sexes, not belonging to the same family nucleus, and of the same socioeconomic level. Data and samples were collected from 276 individuals, of which 80 were patients with primary lung cancer (case group), defined and classified in the histopathological examination, and 196 patients were without any type of cancer (control group). Both groups had a survey of demographic and clinical data, which included age, sex, and smoking.

2.3. DNA Extraction and Quantification

Extraction of genomic DNA from peripheral blood leukocytes was conducted using a Mini Spin Plus Kit (P. 250, Biopur, Biometrix) according to the manufacturer's recommendations. DNA concentration and purity were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific NanoDrop 1000; NanoDrop Technologies, Wilmington, DE, USA).

2.4. Genotyping

The *UGT1A1* gene polymorphism (rs8175347) was genotyped by a multiplex PCR reaction followed by capillary electrophoresis. For the amplifications, primers were used: F:5′-CTCTGAAAGTGAACTCCCTGCT-3′; R:5′-AGAGGTTCGCCCTTCCTAT-3′. Analysis of the PCR amplicons was performed from an electrophoresis using the ABI Prism 3130 sequencer and GeneMapper ID v.3.2 software [16]. The genotypes found were: TA6/6 (*UGT1A1**1/*1), TA6/7 (*UGT1A1**1/*28), T5/7 (*UGT1A1**36/*28) and TA7/7 (*UGT1A1**28/*28). In the literature, these genotypes can be grouped by the degree of enzymatic activity of UGT1A1 as: normal (TA6/6), reduced (TA6/7 and TA5/7), and low (TA7/7) enzymatic activity [17].

2.5. Hardy–Weinberg Equilibrium Analysis (HWE)

The allelic and genotypic frequency of the polymorphism was determined by direct counting of alleles, and then Hardy–Weinberg equilibrium (HWE) was calculated using the default parameters of the Arlequin 3.5.1.2 software (Swiss Institute of Bioinformatics, Bern, Switzerland).

2.6. Genetic Ancestrality Analysis

Genotyping was performed to analyze the ancestry of the samples, performed according to Ramos et al. [18], using 61 informative markers of autosomal ancestry in three multiplex PCR reactions. Amplicons were analyzed by electrophoresis using the ABI Prism 3130 sequencer and GeneMapper ID v. 3.2 software (Applied Biosystems, Life Technologies,

Carlsbad, CA, USA). The individual proportions of European, African, and Amerindian genetic ancestry were estimated using Structure v. 2.3.3 software (Stanford University, Stanford, CA, USA), assuming three parental populations [19].

2.7. Statistical Analysis

All statistical analyzes were performed using the SPSS 20.0 software (IBM, Armonk, NY, USA) statistical package. For comparative analysis between the study groups with regard to demographic and clinical variables, Pearson's Chi-square and the Mann–Whitney test were applied. To analyze the association of polymorphisms with risk to lung cancer and the histological types, a logistic regression was performed, estimating the odds ratios (OR) and their 95% confidence intervals (CI). The variables of sex, age, and smoking were controlled in this multivariate analysis, to avoid a confounding association. A significance level of $p < 0.05$ was considered for all statistical analyses.

3. Results

In the results of demographic and clinical analyses, it can be observed that the groups differed in terms of sex, age, and smoking. Most cases of lung cancer were squamous cell carcinoma, followed by large cell carcinoma and adenocarcinoma (Table 1). The ancestry analyses performed revealed that the case and control groups had a similar ancestral genomic profile, with a greater European contribution to both populations (Table 1).

Table 1. Demographic and clinical characteristics of the investigated groups.

Characteristics	Case (n = 80)	Control (n = 196)	p-Value
Sex			
Male	56 (70.0%)	62 (31.6%)	<0.001 [a,*]
Female	24 (30.0%)	134 (68.4%)	
Age (years)			
Median (p25–p75%)	62.0 (55.0–69.0)	69.0 (64.0–76.0)	<0.001 [b,*]
Smoking			
Never smoked	13 (16.33%)	99 (50.5%)	<0.001 [a,*]
Smoker	67 (83.7%)	97 (49.5%)	
Ancestry			
European	47.6 ± 18.0	45.2 ± 17.0	0.334 [b]
Amerindian	30.0 ± 14.1	30.6 ± 14.8	0.888 [b]
African	22.3 ± 12.4	24.2 ± 13.8	0.313 [b]
Histology			
Squamous Cell Carcinoma	41 (51.3%)	-	NA
Large Cell Carcinoma	14 (17.5%)	-	
Adenocarcinoma	12 (15.0%)	-	
Others	13 (16.2%)	-	

NA, not applicable. [a] Chi-square Test; [b] Mann–Whitney Test; * p-value < 0.05.

It was evidenced that the *UGT1A1* gene polymorphism (rs8175347) was present in the HWE (p = 0.340). Four genotypes were presented, TA6/6, TA6/7, TA5/7, and TA7/7, with frequencies of 41%, 45%, 1%, and 13% respectively. As for the allele frequency, three alleles were observed, TA5, TA6, and TA7, with 0.5%, 63.4%, and 36.1%, respectively.

The allele frequencies between the control and lung cancer groups and their histological types are listed in Table 2. It was observed that the TA7 allele was present more frequently in the lung adenocarcinoma group (54.1%). In the analysis for lung adenocarcinoma, it was observed that individuals with the TA7 allele were about 2.5 times more likely to develop the disease (p = 0.035; OR: 2.57; 95% CI: 1.07–6.20).

Table 2. Risk for lung cancer and its histological types, associated with the *UGT1A1* gene polymorphism allele.

Groups	*UGT1A1* (rs8175347) Allelotypes			
	(TA)7	(TA)5 or (TA)6	OR (95% IC)	*p*-Value [a]
Controls	139 (35.5%)	253 (64.5%)	-	-
Lung Cancer	60 (37.5%)	100 (62.5%)	1.24 (0.80–1.93)	0.331
Squamous Cell Carcinoma	35 (42.7%)	47 (57.3%)	1.56 (0.89–2.73)	0.116
Large Cell Carcinoma	5 (17.9%)	23 (82.1%)	0.43 (0.16–1.18)	0.103
Adenocarcinoma	13 (54.1%)	11 (45.9%)	2.57 (1.07–6.20)	0.035 *
Others	7 (26.9%)	19 (73.1%)	0.76 (0.30–1.95)	0.573

OR, odds ratio; CI, confidence interval; [a] Logistic regression adjusted for sex, age, and smoking. * *p*-value < 0.05.

The genotypic frequencies between the control and lung cancer groups and their histological types are listed in Table 3. The TA7/7 genotype was more frequent in the squamous cell carcinoma group (22.0%). Individuals with the TA7/7 genotype were about four times more likely to develop lung squamous cell carcinoma ($p = 0.015$; OR: 4.08; 95% CI: 1.32–12.61).

Table 3. Risk for lung cancer and its histological types, associated with the *UGT1A1* gene polymorphism genotypes.

Groups	*UGT1A1* (rs8175347) Genotypes			
	TA7/7	TA6/6, TA6/7 or TA5/7	OR (95% IC)	*p*-Value [a]
Controls	23 (11.7%)	173 (88.3%)	-	-
Lung Cancer	13 (16.3%)	67 (83.7%)	2.04 (0.85–4.92)	0.110
Squamous Cell Carcinoma	9 (22.0%)	32 (78.0%)	4.08 (1.32–12.61)	0.015 *
Large Cell Carcinoma	1 (7.1%)	13 (92.9%)	0.77 (0.09–6.61)	0.815
Adenocarcinoma	2 (16.7%)	10 (83.3%)	3.19 (0.53–19.05)	0.204
Others	1 (7.7%)	12 (92.3%)	0.73 (0.08–6.40)	0.780

OR, odds ratio; CI, confidence interval; [a] Logistic regression adjusted for sex, age, and smoking. * *p*-value < 0.05.

The genotypic frequencies by the degree of UGT1A1 enzymatic activities, between the control and lung cancer groups and their histological types, are listed in Table 4. Genotypes with reduced or low enzyme activity (TA6/7, TA5/7, TA7/7) were more frequent in the lung adenocarcinoma group (91.7%). Furthermore, individuals with a low or reduced degree of enzyme activity genotypes were about 8 times more likely to develop the disease ($p = 0.048$; OR: 8.41; 95%CI: 1.02–69.55).

Table 4. Risk for lung cancer and its histological types, associated with degree of UGT1A1 enzymatic activities.

Groups	*UGT1A1* (rs8175347) Genotypes by Degree of Enzymatic Activity			
	Low or Reduced	Normal	OR (95%IC)	*p*-Value [a]
Controls	116 (59.2%)	80 (40.8%)	-	-
Lung Cancer	47 (58.8%)	33 (41.2%)	1.09 (0.59–1.99)	0.788
Squamous Cell Carcinoma	26 (63.4%)	15 (36.6%)	1.23 (0.56–2.70)	0.604
Large Cell Carcinoma	4 (28.6%)	10 (71.4%)	0.29 (0.08–0.99)	0.058
Adenocarcinoma	11 (91.7%)	1 (8.3%)	8.41 (1.02–69.55)	0.048 *
Others	6 (46.2%)	7 (53.8%)	0.69 (0.21–2.27)	0.543

OR, odds ratio; CI, confidence interval; [a] Logistic regression adjusted for sex, age, and smoking. * *p*-value < 0.05.

4. Discussion

In the world, lung neoplasms are the second most common cancer diagnosis in both sexes, being more frequent among men with an average age of 70 years, and associated with tobacco consumption [1,20,21]. In Brazil, lung cancer is the third most common cancer among men and the fourth most common cancer among women, and 85% of diagnosed cases are associated with the consumption of tobacco derivatives [22,23]. This is similar to what was observed in our study, where the group of individuals with lung cancer was mainly composed of men aged over 65 years and with a history of smoking.

The prevalence of lung cancer in men is often associated with smoking, as the proportion of men who smoke is higher than that of women. In addition, men are also exposed to carcinogens in some occupational activities, which contributes to the frequency being higher in this group [24–26]. However, these gender differences vary between developed and developing countries, due to differences in tobacco consumption, and exposure to exogenous and endogenous risk factors [1,5].

Regarding age, it is observed that about 34% of lung cancer cases in the world are diagnosed between 65 and 74 years of age, and the elderly population comprises 62% of all cases [1]. Aging favors genomic instability, leading to the accumulation of cells with different molecular aberrations that alter internal homeostasis, increasing susceptibility to carcinogens and, consequently, lung carcinogenesis [27]. According to Schneider et al. (2021) some histological types of lung cancer are caused by the expression of oncogenic drivers, which can be stratified by age, such as adenocarcinoma, revealing the interaction between aging and lung carcinogenesis [28].

In our study, the most prevalent histological type was squamous cell carcinoma (51.7%), followed by large cell carcinoma (17.5%), and adenocarcinoma (15.0%). In Brazil, 86.7% of cases are NSCLC and 13.7% SCLC. Among NSCLC, the most common histological type was adenocarcinoma (50.0%), followed by squamous cell carcinoma (42.1%), and large cell carcinoma (7.9%) [29]. these differences may reflect the reduction in cases of squamous cell carcinoma and the increase in adenocarcinoma in the last 30 years, due to changes in the pattern of tobacco consumption in some regions of the country [30].

Smoking is the well-known risk factor for developing lung cancer [5,6]. Pulmonary carcinogenesis is a complex process, with interactions that modulate the potential risks for the disease [7,31]. Many studies have described how germline variants influence susceptibility to lung cancer, including those linked to smoking [32–34].

In the present study, the analysis of the *UGT1A1* gene polymorphism (rs8175347) showed relevant results. The TA7/7 genotype was present in 13% of the investigated individuals. In Brazil, the frequency of the TA7/7 genotype of this polymorphism occurs in between 3 and 17% of the population, being more frequent among Afrodescendants and less frequent among Amerindians [35].

As for allelic frequency, the TA7 allele was frequent in 36.1% of the individuals studied. This frequency is similar to that observed in the wider world [36], where the distribution of the TA7 allele is 34.9%, in Latin America 31.4%, and in Brazil between 30.0 and 33.0% [37–39]. Studies show that the TA7 allele has a frequency of 42–56% in Afrodescendants, 26–31% in Caucasians, and only 9–16% in Asian populations [40].

In our study, individuals with genotypes related to reduced or low enzymatic activity (TA6/7, TA5/7 and TA7/7) had increased chances ($p = 0.048$; OR: 8.41, 95%CI: 1.07–69.55) for the development of adenocarcinoma of lung. Similar results were observed in the allelic frequency: individuals with the TA7 allele have an increased chance of developing lung adenocarcinoma ($p = 0.035$; OR: 2.57; 95%CI: 1.07–6.20). Furthermore, individuals with the TA7/TA7 genotype have an increased chance of developing squamous cell carcinoma of the lung ($p = 0.015$; OR:4.077; 95%CI:1.318–12.612).

The variant allelic TA7 is characterized by seven thymine-adenine (TA) repeats within the promoter region, unlike the wild-type TA6 allele which has six TA repeats. This extra repeat impairs proper gene transcription [41–43]. This results in a 25–70% reduction in

enzyme activity, depending on the presence of one or two TA7 variant alleles, respectively, which reduce glucuronidation [44–46].

UGT1A1 enzymes were observed in the glucuronidation of estradiol and of a precursor of the potent organic carcinogen benzo(α)pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), which is found in cigarette smoke [40]. There is a correlation between *UGT1A1* genotypes with the expression of UDP-glucuronosyltransferase and glucuronidation activity. Individuals with genotype TA7/7 have a lower level of precursor carcinogen in liv-er microsomes when compared to those with genotypes TA6/7 or TA6/6 [40].

In the present study, smoking was present in 83.7% of patients with lung cancer, similar to that observed in other surveys, where between 80% and 90% of lung cancers are attributed to smoking [47,48]. Tobacco contains dozens of carcinogenic agents that are harmful to humans, who undergo biotransformation through various metabolic pathways [49,50]. Studies show that genetic polymorphisms in genes that encode enzymes involved in the metabolism of tobacco carcinogens, such as UGT1A1, can affect the individual risk of lung cancer [51,52].

The reduction in or low enzymatic activity of UGT1A1 reduces glucuronidation, increasing exposure to the carcinogens present in tobacco, which favor carcinogenesis [42]. Our study observed this association. Individuals with the genotypes of reduced or low enzymatic activity, or with an allele TA7 or with genotype homozygous TA7/7, had a higher chance of some histological types of lung cancer. It is possible that these associations were not observed in all histological types due to the small sample size in some clusters in this pilot study.

This finding corroborates what was observed by Nishikawa et al. (2016) [53] in their study of 194 patients with lung cancer, who found that the genotype homozygous TA7/7 increases the risk of developing lung cancer by approximately five times. They observed that squamous cell carcinoma was predominant in the lung cancer patients with genotype homozygous TA7/7 and smoking history, similar to our study.

This reinforces the findings of other studies that indicate that reducing the activity or expression of UGT1A1 can influence the elimination of precursors to carcinogens [14,48]. This relationship facilitates the understanding of the identification of associations between the TA7 allelic variant with the susceptibility to oxidative damage and, consequently, the increased risk for other types of cancer, such as breast, ovarian, prostate, head and neck, and colorectal cancers, reported in some populations [48,54–57].

In addition to the susceptibility to the development of lung cancer, studies regarding polymorphism in the *UGT1A1* gene (rs8175347) have identified its association with the response to chemotherapy with irinotecan. The reduced enzymatic action of UGT1A1, due to the TA7 variant, may be associated with severe toxicities in patients receiving irinotecan, including patients with lung cancer. This shows this polymorphism is a strong candidate for use in clinical practice as well [57–59].

Importantly, this study is one of the few that has investigated the association between how polymorphism that can alter the length of the *UGT1A1* gene promoter and the risk of lung adenocarcinoma and squamous cell carcinoma. Further epidemiological investigations involving larger groups of individuals should be conducted to confirm the results and determine whether the TA7 variant allele is an isolated risk factor or associated with environmental factors such as smoking.

The validation of these findings may favor, in the future, the screening of individuals with greater susceptibility to developing the disease by facilitating the establishment of personalized preventive measures for early diagnosis, consequently reducing the cost for health services and lowering mortality rates from this malignant neoplasm.

5. Conclusions

The *UGT1A1* gene polymorphism (rs8175347) showed a significant association with lung squamous cell carcinoma and lung adenocarcinoma in the population of the Amazon region. Individuals with homozygous TA7/7 had an increased risk of lung squamous cell

carcinoma. Those with the TA7 allele or with genotypes associated with reduced or low UGT1A1 activity had an increased risk for lung adenocarcinoma.

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References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
2. Bade, B.C.; Dela Cruz, C.S. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. *Clin. Chest Med.* **2020**, *41*, 1–24. [CrossRef] [PubMed]
3. Duma, N.; Santana-Davila, R.; Molina, J.R. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clin. Proc.* **2019**, *94*, 1623–1640. [CrossRef] [PubMed]
4. Saltos, A.; Shafique, M.; Chiappori, A. Update on the Biology, Management, and Treatment of Small Cell Lung Cancer (SCLC). *Front. Oncol.* **2020**, *10*, 1074. [CrossRef]
5. Barta, J.A.; Powell, C.A.; Wisnivesky, J.P. Global Epidemiology of Lung Cancer. *Ann. Glob. Health* **2019**, *85*, 8. [CrossRef]
6. Wang, X.; Ricciuti, B.; Nguyen, T.; Li, X.; Rabin, M.S.; Awad, M.M.; Lin, X.; Johnson, B.E.; Christiani, D.C. Association between Smoking History and Tumor Mutation Burden in Advanced Non-Small Cell Lung Cancer. *Cancer Res.* **2021**, *81*, 2566–2573. [CrossRef]
7. Hu, Z.; Chen, J.; Meng, P.; Li, M. Association between NEAT1 polymorphism and the risk of lung cancer: A protocol for systematic review and meta-analysis. *Medicine* **2021**, *100*, e25478. [CrossRef]
8. Liu, Y.; Xia, J.; McKay, J.; Tsavachidis, S.; Xiao, X.; Spitz, M.R.; Cheng, C.; Byun, J.; Hong, W.; Li, Y.; et al. Rare deleterious germline variants and risk of lung cancer. *NPJ Precis. Oncol.* **2021**, *5*, 12. [CrossRef]
9. Liu, D.; Gao, Y.; Li, L.; Chen, H.; Bai, L.; Qu, Y.; Zhou, B.; Yan, Y.; Zhao, Y. Single nucleotide polymorphisms in breast cancer susceptibility gene 1 are associated with susceptibility to lung cancer. *Oncol. Lett.* **2021**, *21*, 424. [CrossRef]
10. Dang, X.; Zhao, W.; Li, C.; Yang, H.; Li, D.; Zhang, S.; Jin, T. Impact of COL6A4P2 gene polymorphisms on the risk of lung cancer: A case-control study. *PLoS ONE* **2021**, *16*, e0252082. [CrossRef]
11. Lebrett, M.B.; Crosbie, E.J.; Smith, M.J.; Woodward, E.R.; Evans, D.G.; Crosbie, P.A.J. Targeting lung cancer screening to individuals at greatest risk: The role of genetic factors. *J. Med. Genet.* **2021**, *58*, 217–226. [CrossRef] [PubMed]
12. Steventon, G. Uridine diphosphate glucuronosyltransferase 1A1. *Xenobiotica* **2020**, *50*, 64–76. [CrossRef] [PubMed]
13. Sissung, T.M.; Barbier, R.H.; Price, D.K.; Plona, T.M.; Pike, K.M.; Mellott, S.D.; Baugher, R.N.; Whiteley, G.R.; Soppet, D.R.; Venzon, D.; et al. Comparison of Eight Technologies to Determine Genotype at the UGT1A1 (TA). *Int. J. Mol. Sci.* **2020**, *21*, 896. [CrossRef] [PubMed]
14. Bajro, M.H.; Josifovski, T.; Panovski, M.; Jankulovski, N.; Nestorovska, A.K.; Matevska, N.; Petrusevska, N.; Dimovski, A.J. Promoter length polymorphism in UGT1A1 and the risk of sporadic colorectal cancer. *Cancer Genet.* **2012**, *205*, 163–167. [CrossRef]
15. Clendenen, T.; Zeleniuch-Jacquotte, A.; Wirgin, I.; Koenig, K.L.; Afanasyeva, Y.; Lundin, E.; Arslan, A.A.; Axelsson, T.; Försti, A.; Hallmans, G.; et al. Genetic variants in hormone-related genes and risk of breast cancer. *PLoS ONE* **2013**, *8*, e69367. [CrossRef]
16. Pinto, P.; Salgado, C.; Santos, N.P.; Santos, S.; Ribeiro-dos-Santos, Â. Influence of Genetic Ancestry on INDEL Markers of NFKβ1, CASP8, PAR1, IL4 and CYP19A1 Genes in Leprosy Patients. *PLoS Negl. Trop. Dis.* **2015**, *9*, e0004050. [CrossRef]

17. Olatunya, O.S.; Albuquerque, D.M.; Akanbi, G.O.; Aduayi, O.S.; Taiwo, A.B.; Faboya, O.A.; Kayode, T.S.; Leonardo, D.P.; Adekile, A.; Costa, F.F. Uridine diphosphate glucuronosyl transferase 1A (UGT1A1) promoter polymorphism in young patients with sickle cell anaemia: Report of the first cohort study from Nigeria. *BMC Med. Genet.* **2019**, *20*, 160. [CrossRef]
18. Ramos, B.R.; D'Elia, M.P.; Amador, M.A.; Santos, N.P.; Santos, S.E.; da Cruz Castelli, E.; Witkin, S.S.; Miot, H.A.; Miot, L.D.; da Silva, M.G. Neither self-reported ethnicity nor declared family origin are reliable indicators of genomic ancestry. *Genetica* **2016**, *144*, 259–265. [CrossRef]
19. Carvalho, D.C.; Wanderley, A.V.; Amador, M.A.; Fernandes, M.R.; Cavalcante, G.C.; Pantoja, K.B.; Mello, F.A.; de Assumpção, P.P.; Khayat, A.S.; Ribeiro-Dos-Santos, Â.; et al. Amerindian genetic ancestry and INDEL polymorphisms associated with susceptibility of childhood B-cell Leukemia in an admixed population from the Brazilian Amazon. *Leuk. Res.* **2015**, *39*, 1239–1245. [CrossRef]
20. Thandra, K.C.; Barsouk, A.; Saginala, K.; Aluru, J.S. Epidemiology of lung cancer. *Contemp. Oncol.* **2021**, *25*, 45–52. [CrossRef]
21. Rodak, O.; Peris-Díaz, M.D.; Olbromski, M.; Podhorska-Okołów, M.; Dzięgiel, P. Current Landscape of Non-Small Cell Lung Cancer: Epidemiology, Histological Classification, Targeted Therapies, and Immunotherapy. *Cancers* **2021**, *13*, 4705. [CrossRef] [PubMed]
22. Brasil. Ministério da Saúde. Instituto Nacional de Câncer José Alencar Gomes da Silva. Estimativa 2020: Incidência de Câncer no Brasil. Rio de Janeiro: INCA; 2019. Available online: https://www.inca.gov.br/sites/ufu.sti.inca.local/files//media/document//estimativa-2020-incidencia-de-cancer-no-brasil.pdf (accessed on 1 November 2021).
23. Araujo, L.H.; Baldotto, C.; Castro, G.; Katz, A.; Ferreira, C.G.; Mathias, C.; Mascarenhas, E.; Lopes, G.L.; Carvalho, H.; Tabacof, J.; et al. Lung cancer in Brazil. *J. Bras. Pneumol.* **2018**, *44*, 55–64. [CrossRef] [PubMed]
24. Delva, F.; Margery, J.; Laurent, F.; Petitprez, K.; Pairon, J.C.; Group, R.W. Medical follow-up of workers exposed to lung carcinogens: French evidence-based and pragmatic recommendations. *BMC Public Health* **2017**, *17*, 191. [CrossRef] [PubMed]
25. Stapelfeld, C.; Dammann, C.; Maser, E. Sex-specificity in lung cancer risk. *Int. J. Cancer* **2020**, *146*, 2376–2382. [CrossRef]
26. Ragavan, M.; Patel, M.I. The evolving landscape of sex-based differences in lung cancer: A distinct disease in women. *Eur. Respir. Rev.* **2022**, *31*, 210100. [CrossRef]
27. Meiners, S.; Eickelberg, O.; Königshoff, M. Hallmarks of the ageing lung. *Eur. Respir. J.* **2015**, *45*, 807–827. [CrossRef]
28. Schneider, J.L.; Rowe, J.H.; Garcia-de-Alba, C.; Kim, C.F.; Sharpe, A.H.; Haigis, M.C. The aging lung: Physiology, disease, and immunity. *Cell* **2021**, *184*, 1990–2019. [CrossRef]
29. Costa, G.; Thuler, L.C.; Ferreira, C.G. Epidemiological changes in the histological subtypes of 35,018 non-small-cell lung cancer cases in Brazil. *Lung Cancer* **2016**, *97*, 66–72. [CrossRef]
30. Tsukazan, M.T.R.; Vigo, Á.; Silva, V.D.D.; Barrios, C.H.; Rios, J.O.; Pinto, J.A.F. Lung cancer: Changes in histology, gender, and age over the last 30 years in Brazil. *J. Bras. Pneumol.* **2017**, *43*, 363–367. [CrossRef]
31. Sengupta, D.; Banerjee, S.; Mukhopadhyay, P.; Mitra, R.; Chaudhuri, T.; Sarkar, A.; Bhattacharjee, G.; Nath, S.; Roychoudhury, S.; Bhattacharjee, S.; et al. A comprehensive meta-analysis and a case-control study give insights into genetic susceptibility of lung cancer and subgroups. *Sci. Rep.* **2021**, *11*, 14572. [CrossRef]
32. Tlemsani, C.; Takahashi, N.; Pongor, L.; Rajapakse, V.N.; Tyagi, M.; Wen, X.; Fasaye, G.A.; Schmidt, K.T.; Desai, P.; Kim, C.; et al. Whole-exome sequencing reveals germline-mutated small cell lung cancer subtype with favorable response to DNA repair-targeted therapies. *Sci. Transl. Med.* **2021**, *13*, eabc7488. [CrossRef]
33. Yang, I.A.; Holloway, J.W.; Fong, K.M. Genetic susceptibility to lung cancer and co-morbidities. *J. Thorac. Dis.* **2013**, *5* (Suppl. 5), S454–S462. [CrossRef]
34. Gabriel, A.A.G.; Atkins, J.R.; Penha, R.C.C.; Smith-Byrne, K.; Gaborieau, V.; Voegele, C.; Abedi-Ardekani, B.; Milojevic, M.; Olaso, R.; Meyer, V.; et al. Genetic analysis of lung cancer reveals novel susceptibility loci and germline impact on somatic mutation burden. *medRxiv* **2021**. [CrossRef]
35. Marques, S.C.; Ikediobi, O.N. The clinical application of UGT1A1 pharmacogenetic testing: Gene-environment interactions. *Hum. Genom.* **2010**, *4*, 238–249. [CrossRef]
36. Whirl-Carrillo, M.; Huddart, R.; Gong, L.; Sangkuhl, K.; Thorn, C.F.; Whaley, R.; Klein, T.E. An Evidence-Based Framework for Evaluating Pharmacogenomics Knowledge for Personalized Medicine. *Clin. Pharmacol. Ther.* **2021**, *110*, 563–572. [CrossRef] [PubMed]
37. Turatti, L.; Sprinz, E.; Lazzaretti, R.K.; Kuhmmer, R.; Agnes, G.; Silveira, J.M.; Basso, R.P.; Pinheiro, C.A.; Silveira, M.F.; de Almeida, S.; et al. Short communication: UGT1A1*28 variant allele is a predictor of severe hyperbilirubinemia in HIV-infected patients on HAART in southern Brazil. *AIDS Res. Hum. Retrovir.* **2012**, *28*, 1015–1018. [CrossRef]
38. Cavalcante, G.C.; Amador, M.A.; Ribeiro Dos Santos, A.M.; Carvalho, D.C.; Andrade, R.B.; Pereira, E.E.; Fernandes, M.R.; Costa, D.F.; Santos, N.P.; Assumpção, P.P.; et al. Analysis of 12 variants in the development of gastric and colorectal cancers. *World J. Gastroenterol.* **2017**, *23*, 8533–8543. [CrossRef]
39. De Oliveira Almeida, V.C.; Ribeiro, D.D.; Gomes, K.B.; Godard, A.L. Polymorphisms of CYP2C9, VKORC1, MDR1, APOE and UGT1A1 genes and the therapeutic warfarin dose in Brazilian patients with thrombosis: A prospective cohort study. *Mol. Diagn. Ther.* **2014**, *18*, 675–683. [CrossRef]
40. Barbarino, J.M.; Haidar, C.E.; Klein, T.E.; Altman, R.B. PharmGKB summary: Very important pharmacogene information for UGT1A1. *Pharm. Genom.* **2014**, *24*, 177–183. [CrossRef]

41. Fang, J.L.; Lazarus, P. Correlation between the UDP-glucuronosyltransferase (UGT1A1) TATAA box polymorphism and carcinogen detoxification phenotype: Significantly decreased glucuronidating activity against benzo(a)pyrene-7,8-dihydrodiol(-) in liver microsomes from subjects with the UGT1A1*28 variant. *Cancer Epidemiol. Biomark. Prev.* **2004**, *13*, 102–109. [CrossRef]
42. Takano, M.; Sugiyama, T. UGT1A1 polymorphisms in cancer: Impact on irinotecan treatment. *Pharmgenom. Pers. Med.* **2017**, *10*, 61–68. [CrossRef] [PubMed]
43. Patel, P.; Xue, Z.; King, K.S.; Parham, L.; Ford, S.; Lou, Y.; Bakshi, K.K.; Sutton, K.; Margolis, D.; Hughes, A.R.; et al. Evaluation of the effect of UGT1A1 polymorphisms on the pharmacokinetics of oral and long-acting injectable cabotegravir. *J. Antimicrob. Chemother.* **2020**, *75*, 2240–2248. [CrossRef] [PubMed]
44. Belkhir, L.; Seguin-Devaux, C.; Elens, L.; Pauly, C.; Gengler, N.; Schneider, S.; Ruelle, J.; Haufroid, V.; Vandercam, B. Impact of UGT1A1 polymorphisms on Raltegravir and its glucuronide plasma concentrations in a cohort of HIV-1 infected patients. *Sci. Rep.* **2018**, *8*, 7359. [CrossRef] [PubMed]
45. Strassburg, C.P. Pharmacogenetics of Gilbert's syndrome. *Pharmacogenomics* **2008**, *9*, 703–715. [CrossRef]
46. Nelson, R.S.; Seligson, N.D.; Bottiglieri, S.; Carballido, E.; Cueto, A.D.; Imanirad, I.; Levine, R.; Parker, A.S.; Swain, S.M.; Tillman, E.M.; et al. UGT1A1 Guided Cancer Therapy: Review of the Evidence and Considerations for Clinical Implementation. *Cancers* **2021**, *13*, 1566. [CrossRef]
47. Zinser-Sierra, J.W. Tobacco use and lung cancer. *Salud. Publica Mex.* **2019**, *61*, 303–307. [CrossRef]
48. Lim, J.E.; Kimm, H.; Jee, S.H. Combined effects of smoking and bilirubin levels on the risk of lung cancer in Korea: The severance cohort study. *PLoS ONE* **2014**, *9*, e103972. [CrossRef]
49. San Jose, C.; Cabanillas, A.; Benitez, J.; Carrillo, J.A.; Jimenez, M.; Gervasini, G. CYP1A1 gene polymorphisms increase lung cancer risk in a high-incidence region of Spain: A case control study. *BMC Cancer* **2010**, *10*, 463. [CrossRef]
50. Espina, C.; Straif, K.; Friis, S.; Kogevinas, M.; Saracci, R.; Vainio, H.; Schüz, J. European Code against Cancer 4th Edition: Environment, occupation and cancer. *Cancer Epidemiol.* **2015**, *39* (Suppl. 1), S84–S92. [CrossRef]
51. Jin, Y.; Xu, H.; Zhang, C.; Kong, Y.; Hou, Y.; Xu, Y.; Xue, S. Combined effects of cigarette smoking, gene polymorphisms and methylations of tumor suppressor genes on non small cell lung cancer: A hospital-based case-control study in China. *BMC Cancer* **2010**, *10*, 422. [CrossRef]
52. Pliarchopoulou, K.; Voutsinas, G.; Papaxoinis, G.; Florou, K.; Skondra, M.; Kostaki, K.; Roussou, P.; Syrigos, K.; Pectasides, D. Correlation of CYP1A1, GSTP1 and GSTM1 gene polymorphisms and lung cancer risk among smokers. *Oncol. Lett.* **2012**, *3*, 1301–1306. [CrossRef] [PubMed]
53. Nishikawa, Y.; Kanai, M.; Narahara, M.; Tamon, A.; Brown, J.B.; Taneishi, K.; Nakatsui, M.; Okamoto, K.; Uneno, Y.; Yamaguchi, D.; et al. Association between UGT1A1*28*28 genotype and lung cancer in the Japanese population. *Int. J. Clin. Oncol.* **2017**, *22*, 269–273. [CrossRef]
54. Wassenaar, C.A.; Conti, D.V.; Das, S.; Chen, P.; Cook, E.H.; Ratain, M.J.; Benowitz, N.L.; Tyndale, R.F. UGT1A and UGT2B genetic variation alters nicotine and nitrosamine glucuronidation in european and african american smokers. *Cancer Epidemiol. Biomark. Prev.* **2015**, *24*, 94–104. [CrossRef] [PubMed]
55. Girard, H.; Butler, L.M.; Villeneuve, L.; Millikan, R.C.; Sinha, R.; Sandler, R.S.; Guillemette, C. UGT1A1 and UGT1A9 functional variants, meat intake, and colon cancer, among Caucasians and African-Americans. *Mutat. Res.* **2008**, *644*, 56–63. [CrossRef] [PubMed]
56. Lacko, M.; Roelofs, H.M.; Te Morsche, R.H.; Voogd, A.C.; Ophuis, M.B.; Peters, W.H.; Manni, J.J. Genetic polymorphism in the conjugating enzyme UGT1A1 and the risk of head and neck cancer. *Int. J. Cancer* **2010**, *127*, 2815–2821. [CrossRef]
57. Davies, A.M.; Mack, P.C.; Lara, P.N.; Lau, D.H.; Danenberg, K.; Gumerlock, P.H.; Gandara, D.R. Predictive molecular markers: Has the time come for routine use in lung cancer? *J. Natl. Compr. Cancer Netw.* **2004**, *2*, 125–131. [CrossRef]
58. Ikeda, S.; Takabe, K.; Inagaki, M.; Suzuki, K. Expression of phosphorylated Akt in patients with small cell carcinoma of the lung indicates good prognosis. *Pathol. Int.* **2010**, *60*, 714–719. [CrossRef]
59. Huang, Y.; Su, J.; Huang, X.; Lu, D.; Xie, Z.; Yang, S.; Guo, W.; Lv, Z.; Wu, H.; Zhang, X. Detection of UGT1A1*28 Polymorphism Using Fragment Analysis. *Zhongguo Fei Ai Za Zhi* **2017**, *20*, 817–821. [CrossRef]

CAPÍTULO VII. DISCUSSÃO

No mundo, as neoplasias pulmonares são o segundo diagnóstico de câncer mais comum em ambos os sexos, sendo mais frequentes em homens com idade média de 70 anos e associadas ao consumo de tabaco (SUNG *et al*., 2021; THANDRA *et al*., 2021; RODAK *et al*., 2021). No Brasil, o câncer de pulmão é o terceiro câncer mais comum entre os homens e o quarto câncer mais comum entre as mulheres, sendo que 85% dos casos diagnosticados estão associados ao consumo de derivados do tabaco (ARAÚJO *et al*., 2018). Isso é semelhante ao observado em nosso estudo, onde o grupo de indivíduos com câncer de pulmão foi composto principalmente por homens com idade superior a 65 anos e histórico de tabagismo.

A prevalência de câncer de pulmão em homens está frequentemente associada ao tabagismo, pois a proporção de homens que fumam é maior que a de mulheres. Além disso, os homens também estão expostos a carcinógenos em algumas atividades ocupacionais, o que contribui para que a frequência seja maior nesse grupo (DELVA *et al*., 2017; STAPELFELD, DAMMANN, MASER, 2020; RAGAVAN, PATEL, 2022). No entanto, essas diferenças de gênero variam entre países desenvolvidos e em desenvolvimento, devido a diferenças no consumo de tabaco e exposição a fatores de risco exógenos e endógenos (BARTA, POWELL, WISNIVESKY, 2019; SUNG *et al*., 2021).

Em relação à idade, observa-se que cerca de 34% dos casos de câncer de pulmão no mundo são diagnosticados entre 65 e 74 anos, sendo que a população idosa compreende 62% de todos os casos (SUNG *et al*., 2021). O envelhecimento favorece a instabilidade genômica, levando ao acúmulo de células com diferentes aberrações moleculares que alteram a homeostase interna, aumentando a susceptibilidade a carcinógenos e, consequentemente, a carcinogênese pulmonar (MEINERS, EICKELBERG, KÖNIGSHOFF, 2015).

Em nosso estudo, o CPNPC representou 83,7% dos casos, sendo o carcinoma espinocelular o mais prevalente, com 51,7% dos casos, seguido pelo carcinoma de grandes células (17,5%) e adenocarcinoma (15,0%). No Brasil, 86,7% dos casos de neoplasias pulmonares são CPNPC e 13,7% CPPC. Entre os CPNPC, o tipo histológico mais comum é o adenocarcinoma (50,0%), seguido pelo carcinoma espinocelular (42,1%) e carcinoma de grandes células (7,9%) (COSTA, THULER, FERREIRA, 2016). Essas diferenças podem refletir a redução dos casos de carcinoma espinocelular e o aumento do adenocarcinoma nos últimos 30 anos, devido a mudanças no padrão de consumo de tabaco em algumas regiões do país (TSUKAZAN *et al*., 2017).

O tabagismo é um conhecido fator de risco para o desenvolvimento do câncer de pulmão, dada a presença de substâncias contidas no tabaco associadas à carcinogênese

(BARTA, POWELL, WISNIVESKY, 2019). No entanto, estudos mostraram que cerca de metade dos novos casos são em pessoas que nunca fumaram ou que pararam de fumar por muitos anos (WANG *et al*., 2021). Isso mostra que a carcinogênese pulmonar é um processo complexo e gradual, com interações complexas entre fatores de risco ambientais e a susceptibilidade genética individual (HU *et al*., 2021).

Neste estudo, ao avaliar a susceptibilidade individual ao desenvolvimento de CPNPC, podemos observar resultados relevantes em relação aos polimorfismos do gene *NFKB1* (rs28362491), *PAR1* (rs11267092) e *TP53* (rs17878362), os três associados à redução do risco para o desenvolvimento da doença. Já os polimorfismos do gene *IL1A* (rs3783553) e *UCP2* (INDEL 45-pb) estão associados a um maior risco para o desenvolvimento dessa neoplasia.

O gene *NFKB1* expressa o fator de transcrição *NFKB1* (p50/p105), ativado por diversos estímulos intra e extracelulares, favorecendo a repressão da transcrição gênica (CONCETTI, WILSON, 2018; YAZDANI *et al*., 2020). O polimorfismo do *NFKB1* (rs28362491), ocorre na região promotora do gene e está relacionado a uma susceptibilidade a múltiplas doenças associadas à inflamação, imunidade e tumorigênese (DIMITRAKOPOULOS *et al*., 2020; LUO *et al*., 2022).

Em nosso estudo, indivíduos com o genótipo Del/Del do polimorfismo do *NFKB1* (rs28362491) apresentou um efeito protetor ao desenvolvimento do CPNPC. Outros estudos também identificaram associações desse polimorfismo com câncer de pulmão (OLTULU *et al*., 2014; YIN *et al*., 2015). Isso porque o genótipo está associado a menor atividade transcricional e redução da expressão da *NFKB1* (p50/p105), atuando como contra os eventos de tumorigênese (YAZDANI *et al*., 2020; WANG *et al*., 2015).

O mesmo foi observado com o polimorfismo do *PAR1* (rs11267092), onde indivíduos com genótipo Del/Del tiveram menos chances de desenvolver CPNPC. O gene *PAR1* expressa o receptor PAR1, que regula os processos fisiológicos dos sistemas cardiovascular, respiratório, neurológico, inflamação, embriogênese e carcinogênese (RAMOS *et al*, 2016; AMADOR *et al*., 2016).

O polimorfismo *PAR1* (rs11267092) ocorre na região promotora do gene e modula a produção e a atividade do PAR1, influenciando muitos eventos fisiológicos (CASTAÑO-RODRÍGUEZ *et al*., 2012). Esse já foi associado a angiogênese, regulando a liberação de fatores pró-angiogênicos e antiangiogênicos, como o fator de crescimento endotelial vascular (VEGF), endostatina e metaloproteinases de matriz, associados a susceptibilidade e prognóstico de alguns tipos de cânceres (MARTINO *et al*., 2013; OLIVEIRA, 2018). A redução da

expressão do mRNA pelo PAR1 correlaciona-se com a redução das propriedades invasivas de alguns tipos de câncer (DARMOUL *et al*., 3003; LURJE *et al*., 2010). Além disso, observou-se que o alelo Del esteve associado a um melhor prognóstico em alguns tipos de cânceres sólidos, o que corrobora os resultados deste estudo (EROĞLU *et al*., 2012; WANG *et al*., 2015).

Resultado semelhante foi observado no polimorfismo do *TP53* (rs17878362), onde os indivíduos com genótipo Del/Del apresentaram menor risco para CPNPC. O gene *TP53* codifica a proteína p53, que é regulada por múltiplos mecanismos em resposta a uma ampla gama de respostas antiproliferativas (SAGNE *et al.,* 2013). O polimorfismo *TP53* (rs17878362) está associado a diversos tipos de câncer, devido às alterações na expressão gênica e função proteica (HASHEMI *et al*., 2018). Estudos mostram que o genótipo Del/Del desse polimorfismo está associado a níveis mais elevados do mRNA da TP53 e maior capacidade de reparo do DNA do que os genótipos Ins/Del e Ins/Ins, garantindo maior proteção contra eventos de carcinogênese (DENISOV *et al*., 2012; MA, ZHOU, 2016).

Por sua vez, o polimorfismo *IL1A* (rs3783553) apresentou associação com o risco de desenvolvimento de CPNPC. Indivíduos com genótipo Ins/Ins apresentaram duas vezes mais susceptibilidade à neoplasia em relação aos outros genótipos. O gene *IL1A* expressa a interleucina *IL1A*, uma citocina pró-inflamatória produzida por monócitos e macrófagos, liberada em resposta à lesão celular, que pode influenciar na proliferação, angiogênese e invasão tumoral, entre outros eventos carcinogênicos (MA, ZHOU, 2016).

O polimorfismo rs3783553 está associado à regulação dos níveis de expressão de *IL1A*, por interromper um sítio de ligação ao miRNA-122 e miRNA-378, e, portanto, está associado a diversos tipos de neoplasias malignas (MA *et al*., 2018; XIA *et al.*, 2018). Estudos indicam que o genótipo Ins/Ins e o alelo Ins desse polimorfismo também foram associados a um maior risco de desenvolver câncer em geral, corroborando nossos achados (XIA *et al*., 2018).

Neste estudo, o polimorfismo em *UCP2* (INDEL 45-bp) também revelou uma associação de susceptibilidade ao CPNPC. Indivíduos com genótipo Del/Del apresentaram o dobro do risco de desenvolver a doença. O gene UCP2 expressa a proteína desacopladora 2 (UCP2), que atua como transportador de prótons na mitocôndria, envolvida na homeostase energética, termogênese, entre outros fenômenos metabólicos (KAABI *et al*., 2020). Esse gene e suas variantes têm sido associadas a doenças crônicas e alguns tipos de câncer (SAY, 2017; REZAPOUR *et al*., 2021).

O polimorfismo *UCP2* (INDEL 45-bp) pode alterar a estabilidade do mRNA de UCP2 (YU *et al*., 2020). Segundo Esterbauer *et al*. (2001) a meia-vida do mRNA de indivíduos com

o alelo Del é maior do que aqueles que possuem o alelo Ins, o que pode indicar maior expressão. Essa maior expressão pode influenciar na carcinogênese, uma vez que esta mitiga a produção de espécies reativas de oxigênio (EROs), protegendo a célula neoplásica da via apoptótica, o que esclarece os resultados obtidos em nosso estudo (SU *et al*., 2012; LEE *et al*., 2020).

Outro resultado relevante foi o do polimorfismo do gene *UGT1A1* (rs8175347). A associação desse polimorfismo com o CPNPC não foi observada. No entanto, sua relação dos alguns subtipos histológicos foi evidenciada. Em nosso estudo, indivíduos com genótipos relacionados à atividade enzimática reduzida ou baixa (TA6/7, TA5/7 e TA7/7) tiveram oito vezes mais riscos para o desenvolvimento de adenocarcinoma de pulmão. Resultados semelhantes foram observados na frequência alélica: indivíduos com o alelo TA7 têm duas vezes mais chance de desenvolver adenocarcinoma de pulmão. Além disso, indivíduos com o genótipo TA7/TA7 têm quatro vezes mais chance de desenvolver o carcinoma de células escamosas de pulmão.

A variante alélica TA7 é caracterizada por sete repetições de timina-adenina (TA) dentro da região promotora, ao contrário do alelo TA6 de tipo selvagem que tem seis repetições TA. Esta repetição extra prejudica a transcrição gênica adequada (FANG, LAZARUS, 2004; TAKANO, SUGIYAMA, 2017; PATEL *et al.,* 2020). Isso resulta em uma redução de 25 a 70% na atividade da enzima, dependendo da presença de um ou dois alelos variantes de TA7, respectivamente, que reduzem a glucuronidação (STRASSBURG, 2008; BELKHIR, 2018; NELSON *et al*., 2021).

As enzimas UGT1A1 foram observadas na glucuronidação do estradiol e de um precursor do potente carcinógeno orgânico, o benzo(α)pireno-7,8-diidrodiol-9,10-epóxido (BPDE), encontrado na fumaça do cigarro (BARBARINO *et al*., 2014). Existe uma correlação entre os genótipos do *UGT1A1* com a expressão de UDP-glucuronosiltransferase e atividade de glucuronidação. Indivíduos com genótipo TA7/7 apresentam menor nível de precursor cancerígeno nos microssomas hepáticos quando comparados aqueles com genótipos TA6/7 ou TA6/6 (BARBARINO *et al*., 2014).

No presente estudo, o tabagismo esteve presente em 83,7% dos pacientes com câncer de pulmão, semelhante ao observado em outras pesquisas, onde entre 80% e 90% dos cânceres de pulmão são atribuídos ao tabagismo (LIM, KIMM, JEE, 2014; ZINSER-SIERRA, 2019;). O tabaco contém dezenas de agentes cancerígenos prejudiciais aos seres humanos, que sofrem biotransformação através de várias vias metabólicas (SAN JOSE *et al*., 2010; ESPINA *et al*., 2015). Estudos mostram que polimorfismos genéticos em genes que codificam enzimas

envolvidas no metabolismo de carcinógenos do tabaco, como da UGT1A1, podem afetar o risco individual de câncer de pulmão (JIN *et al.*, 2010; PLIARCHOPOULOU *et al.*, 2012).

A redução ou baixa atividade enzimática da UGT1A1 reduz a glucuronidação, aumentando a exposição aos carcinógenos presentes no tabaco, que favorecem a carcinogênese (TAKANO, SUGIYAMA, 2017). Nosso estudo observou essa associação. Indivíduos com os genótipos de atividade enzimática reduzida ou baixa, ou com um alelo TA7 ou com o genótipo homozigoto TA7/7, tiveram maior riscos para alguns tipos histológicos de câncer de pulmão.

Esse achado corrobora o observado por Nishikawa *et al.* (2016) em seu estudo de 194 pacientes com câncer de pulmão, observaram que o genótipo homozigoto TA7/7 aumenta o risco de desenvolver câncer de pulmão em aproximadamente cinco vezes. Eles observaram que o carcinoma de células escamosas foi predominante nos pacientes com câncer de pulmão com genótipo homozigoto TA7/7 e história de tabagismo, semelhante ao nosso estudo.

Isso reforça os achados de outros estudos que indicam que a redução da atividade ou expressão da UGT1A1 pode influenciar a eliminação de precursores de carcinógenos (BAJRO *et al.*, 2012). Essa relação facilita o entendimento da identificação de associações entre a variante alélica TA7 com a susceptibilidade ao dano oxidativo e, consequentemente, o aumento do risco para outros tipos de câncer, como mama, ovário, próstata, cabeça e pescoço e colorretal, relatados em algumas populações (DAVIES *et al.*, 2004; GIRARD *et al.*, 2008; LACKO *et al.*, 2010; WASSENAAR *et al.*, 2015).

Nossos achados reforçam os resultados de outros estudos com outras populações, com um número amostral mais expressivo, mas que também observou uma relação entre esses genes e a carcinogênese. É um dos poucos que investigou a associação dessas seis variantes com o CPNPC e seus tipos histológicos, sendo de extrema importância para a Amazônia, por ser uma região com uma população extremamente miscigenada, única, pouco estudada e com carência de informações de dados epidemiológicos e genéticos sobre a doença.

No futuro, o uso dessas informações como ferramenta de triagem será capaz de identificar indivíduos com maior susceptibilidade ao câncer de pulmão, facilitando o estabelecimento de medidas preventivas e diagnósticos precoces, reduzindo à custo para os serviços de saúde e as taxas de morbimortalidade dessa neoplasia.

CAPÍTULO VIII. CONCLUSÃO

A prevalência do CPNPC foi elevada no grupo estudado, tendo como principais subtipos histológicos o carcinoma de células escamosas e o adenocarcinoma de pulmão, ocorrendo principalmente entre homens, com idade superior aos 60 anos e com histórico de tabagismo.

A ancestralidade genômica não influenciou nas variáveis clínicas e genéticas das populações estudas, não influenciando na susceptibilidade ao câncer de pulmão.

Os seis polimorfismos investigados apresentaram associações significativas com o câncer de pulmão, sendo que cinco deles estiveram ligados a susceptibilidade ao CPNPC e um deles ao carcinoma de células escamosas e ao adenocarcinoma de pulmão.

Os polimorfismos *NFKB1* (rs28362491), *PAR1* (rs11267092) e *TP53* (rs17878362) mostraram-se como fatores de proteção ao desenvolvimento do CPNPC.

Os polimorfismos *IL1A* (rs3783553) e *UCP2* (INDEL 45-pb) mostraram-se como fatores de risco ao desenvolvimento do CPNPC.

O polimorfismo *UGT1A1* (rs8175347) mostrou-se um fator de risco ao desenvolvimento do carcinoma de células escamosas e ao adenocarcinoma de pulmão.

O presente estudo possui grande relevância para a Amazônia, por possuir uma população extremamente miscigenada, única e com poucas informações relacionadas aos aspectos epidemiológicos e genéticos do câncer de pulmão. Essas informações poderão, no futuro, integrar ferramentas de triagem para identificar indivíduos com maior susceptibilidade a essa neoplasia.

REFERÊNCIAS BIBLIOGRÁFICAS

AFONSO, Max dos Santos. SILVEIRA, Karine dos Santos. SOARES, Michele Pedroso. LOPES, Juciane Francieli. AZEVEDO, Priscila Wittemberg. BRITO, Luziana Cardoso do Nascimento. Déficits funcionais de idosos correlacionados a cada década de vidas. **Revista Inspirar: movimento e saúde**. v. 5, n. 2, p. 1-5, 2013.

ALBERG, Anthony J *et al*. Epidemiology of lung cancer: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. **Chest**. v. 143, n. 5 Suppl, p. e1S-e29S, 2013.

AMADOR, M. A.; CAVALCANTE, G. C.; SANTOS, N. P.; GUSMÃO, L. *et al.* Distribution of allelic and genotypic frequencies of IL1A, IL4, *NFKB1* and PAR1 variants in Native American, African, European and Brazilian populations. **BMC Res Notes**, 9, p. 101, Feb 16 2016.

ARAÚJO, Luiz Henrique. BALDOTTO, Clarissa. CASTRO JR, Gilberto de. KATZ, Artur. FERREIRA, Carlos Gil. MATHIAS, Clarissa. *et al*. Câncer de pulmão no Brasil. **Jornal Brasileiro de Pneumologia**. v. 44, n. 1, p. 55-64, 2018.

AUNAN, Jan R. CHO, William C. SØREIDE, Kjetil. The Biology of Aging and Cancer: A Brief Overview of Shared and Divergent Molecular Hallmarks. **Aging and disease**. v. 8, n. 5, p. 628-642, 2017. doi: 10.14336/AD.2017.0103.

BADE, Brett C. CRUZ, Charles S. Dela. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. **Clinics in Chest Medicine**. v. 41, n. 1, p. 1-24, 2020.

BAJRO, M. H.; JOSIFOVSKI, T.; PANOVSKI, M.; JANKULOVSKI, N. *et al.* Promoter length polymorphism in UGT1A1 and the risk of sporadic colorectal cancer. **Cancer Genet**, 205, n. 4, p. 163-167, Apr 2012.

BARBARINO, J. M.; HAIDAR, C. E.; KLEIN, T. E.; ALTMAN, R. B. PharmGKB summary: very important pharmacogene information for UGT1A1. **Pharmacogenet Genomics**, 24, n. 3, p. 177-183, Mar 2014.

LIM, J. E.; KIMM, H.; JEE, S. H. Combined effects of smoking and bilirubin levels on the risk of lung cancer in Korea: the severance cohort study. **PLoS One**, 9, n. 8, p. e103972, 2014.

BARTA, J. A.; POWELL, C. A.; WISNIVESKY, J. P. Global Epidemiology of Lung Cancer. **Ann Glob Health**, 85, n. 1, 01 22 2019.

BELKHIR, L.; SEGUIN-DEVAUX, C.; ELENS, L.; PAULY, C. *et al.* Impact of UGT1A1 polymorphisms on Raltegravir and its glucuronide plasma concentrations in a cohort of HIV-1 infected patients. **Sci Rep**, 8, n. 1, p. 7359, 05 09 2018.

BERBEN, Lieze. FLORIS, Giuseppe. WILDIERS, Hans. HATSE, Sigrid. Cancer and Aging: Two Tightly Interconnected Biological Processes. **Cancers** v. 13, n. 6, p. 1-20, 2021.

BERNARDI, Fabiola Del Carlo. BERNARDI, Marcela Del Carlo. TAKAGAKI, Teresa. COELHO, Sheila Aparecida. DOLHNIKOFF, Siqueira,I Marisa. Lung cancer biopsy: Can diagnosis be changed after immunohistochemistry when the H&E-Based morphology corresponds to a specific tumor subtype?. **Clinics**, v. 73, n. e361, 2018.

BERNARDI, Fabíola Del Carlo. SILVA, Luiz Fernando Ferraz da. DOLHNIKOFF, Marisa. Pulmões. *In*. Brasileiro Filho, Geraldo. **Bogliolo, patologia**. 9. ed. Rio de Janeiro: Guanabara Koogan, 2016.

BERNICKER, Eric H. ALLEN, Timothy Craig. CAGLE, Phillip T. Update on emerging biomarkers in lung cancer. **Journal of Thoracic Disease**. v. 11, n. 1 suppl, p. S81-S88, 2019. doi: 10.21037/jtd.2019.01.46.

BERNSTEIN, Carol. PRASAD, Anil R. NFONSAM, Valentine. BERNSTEIN, Harris. DNA Damage, DNA Repair and Cancer. *In*: CHEN, Clark. **New Research Directions in DNA Repair**. Intech, 2013. 672p.

BOUKHENOUNA, Samia. WILSON, Mark A. BAHMED, Karim. KOSMIDER, Beata. Reactive Oxygen Species in Chronic Obstructive Pulmonary Disease. **Oxidative Medicine and Cellular Longevity**, v. 2018, n. 5730395, p. 1-9, 2018. doi: 10.1155/2018/5730395

BUDOVSKY, Arie. CRAIG, Thomas. WANG, Jingwei. TACUTU Robi. CSORDAS, Attila. LOURENC, Joana. *et al.* LongevityMap: a database of human genetic variants associated with longevity. **Trends in Genetics**. v. 29, n. 10, p. 559-560, 2013. doi: 10.1016/j.tig.2013.08.003.

BÜRKLE, Alexander. VILLANUEVA, María Moreno. BERNHARD, Jürgen. BLASCO, María. ZONDAG, Gerben. HOEIJMAKERS, Jan H.J. *et al.* MARK-AGE biomarkers of ageing. **Mechanisms of Ageing and Development**. v. 151, p. 2-12, 2015. doi: 10.1016/j.mad.2015.03.006.

CALVO, I. OLIVAR, J. MARTÍNEZ, E. RICO, A. DÍAZ, J. GIMENA, M. MNA®Mini Nutritional Assessment as a nutritional screening tool for hospitalized older adults; rationales and feasibility. **Nutricion Hospitalaria**, v. 27, n. 5, p. 1619-1625, 2012. doi: 10.3305/nh.2012.27.5.5888.

CARVALHO, Darlen C. WANDERLEY, Alayde V. AMADOR, Marcos A.T. FERNANDES, Marianne R. CAVALCANTE, Giovanna C. PANTOJA, Karla B.C.C. *et al.* Amerindian genetic ancestry and INDEL polymorphisms associatedwith susceptibility of childhood B-cell Leukemia in an admixedpopulation from the Brazilian **Leukemia Research**. v. 15, S0145-2126, 2015. doi: 10.1016/j.leukres.2015.08.008.

CASTAÑO-RODRÍGUEZ, N.; KAAKOUSH, N. O.; GOH, K. L.; FOCK, K. M. *et al.* PAR-1 polymorphisms and risk of Helicobacter pylori-related gastric cancer in a Chinese population. **Anticancer Res**, 32, n. 9, p. 3715-3721, Sep 2012.

CEFALU, Charles A. Theories and Mechanisms of Aging. **Clinics in Geriatric Medicine**. v. 27, n. 4, p. 491-506, 2011. doi: 10.1007/978-3-319-66878-9_2.

CHAMMAS, Roger. Biologia do Câncer: uma Breve Introdução. In: HOFF, Paulo Marcelo Gehm. KATZ, Artur. CHAMMAS, Roger. NOVIS, Yana Sarkis. ODONE FILHO, Vicente. **Tratado de Oncologia**. Rio de Janeiro: Atheneu, 2012. 2862p.

CHENG, Ting-Yuan David. CRAMB, Susanna M. BAADE, Peter D. YOULDEN, Danny R. NWOGU, Chukwumere. REID, Mary E. The International Epidemiology of Lung Cancer: Latest Trends, Disparities, and Tumor Characteristics. **Journal of Thoracic Oncology**. v. 11, n. 10, p. 1653-71, 2016. doi: 10.1016/j.jtho.2016.05.021.

CHUDGAR, Neel P. BUCCIARELLI, Peter R. JEFFRIES, Elizabeth M. RIZK, Nabil P. PARK, Bernard J. ADUSUMILLI, Prasad S. *et al*. Results of the National Lung Cancer Screening Trial: Where Are We Now? **Thorac Surg Clin.** v. 25, n. 2, p. 145-153, 2015. doi: 10.1016/j.thorsurg.2014.11.002.

CONCETTI, J.; WILSON, C. L. *NFKB1* and Cancer: Friend or Foe? **Cells**, 7, n. 9, Sep 07 2018.

CORRÊA, Gefter Thiago Batista. BANDEIRA, Gabriela Alencar. CAVALCANTI, Bruna Gonçalves. SANTOS, Francis Balduíno Guimarães. *et al.* Analysis of ECOG performance status in head and neck squamous cell carcinoma patients: association with sociodemographical and clinical factors, and overall survival. **Journal Supportive Care in Cancer**, v. 20, n. 11, p. 2679-2685, 2012.

COSTA, G.; THULER, L. C.; FERREIRA, C. G. Epidemiological changes in the histological subtypes of 35,018 non-small-cell lung cancer cases in Brazil. **Lung Cancer**, 97, p. 66-72, 07 2016.

CRAIG, Thomas. SMELICK, Chris. TACUTU, Robi. WUTTKE, Daniel. WOOD, Shona H. STANLEY, Henry. *et al.* The Digital Ageing Atlas: integrating the diversity of age-related changes into a unified resource. **Nucleic Acids Research**. v. 43, p. d873-d878, 2015. doi: 10.1093/nar/gku843.

CRUZ, Charles S. Dela. TANOUE, Lynn T. MATTHAY, Richard A. Lung Cancer: Epidemiology, Etiology, and Prevention. **Clinics in chest medicine.** v. 32, n. 4, p. 605–644 2011. doi: 10.1016/j.ccm.2011.09.001.

DARMOUL, D.; GRATIO, V.; DEVAUD, H.; LEHY, T. *et al.* Aberrant expression and activation of the thrombin receptor protease-activated receptor-1 induces cell proliferation and motility in human colon cancer cells. **Am J Pathol**, 162, n. 5, p. 1503-1513, May 2003.

DAVIES, A. M.; MACK, P. C.; LARA, P. N.; LAU, D. H. *et al.* Predictive molecular markers: has the time come for routine use in lung cancer? **J Natl Compr Canc Netw**, 2, n. 2, p. 125-131, Mar 2004.

DELVA, F.; MARGERY, J.; LAURENT, F.; PETITPREZ, K. *et al.* Medical follow-up of workers exposed to lung carcinogens: French evidence-based and pragmatic recommendations. **BMC Public Health**, 17, n. 1, p. 191, 02 14 2017.

DENISOV, E. V.; CHERDYNTSEVA, N. V.; LITVIAKOV, N. V.; MALINOVSKAYA, E. A. et al. TP53 Gene Polymorphisms in Cancer Risk: The Modulating Effect of Ageing, Ethnicity and TP53 Somatic Abnormalities. In (Ed.), Tumor Suppressor Genes. IntechOpen. 2012, DOI: 10.5772/27768.

DIDKOWSKA, Joanna. WOJCIECHOWSK, Urszula. MAŃCZUK, Marta. ŁOBASZEWSKI, Jakub. Lung cancer epidemiology: contemporary and future challenges worldwide. Lung cancer epidemiology: contemporary and future challenges worldwide. **Annals of Translational Medicine**, v. 4, n. 8, p. 150, 2016. doi: 10.21037/atm.2016.03.11.

DIMITRAKOPOULOS, F. D.; KOTTOROU, A. E.; KALOFONOU, M.; KALOFONOS, H. P. The Fire Within: NF-κB Involvement in Non-Small Cell Lung Cancer. **Cancer Res**, 80, n. 19, p. 4025-4036, 10 01 2020.

DUMA, Narjust. DAVILA, Rafael Santana. MOLINA, Julian R. Non-Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. **Mayo Clinic Proceedings**. v. 94, n. 8, p. 1623-1640, 2019. doi: 10.1016/j.mayocp.2019.01.013.

Eastern Cooperative Oncology Group - ECOG. **ECOG Performance Status**, 27 jul. 2006. Disponível em: <http://ecog.dfci.harvard.edu/general/perf_stat.html>. Acesso em: 18 mar. 2013.

EROĞLU, A.; KARABIYIK, A.; AKAR, N. The association of protease activated receptor 1 gene -506 I/D polymorphism with disease-free survival in breast cancer patients. **Ann Surg Oncol**, 19, n. 4, p. 1365-1369, Apr 2012.

ESPINA, C.; STRAIF, K.; FRIIS, S.; KOGEVINAS, M. *et al.* European Code against Cancer 4th Edition: Environment, occupation and cancer. **Cancer Epidemiol**, 39 Suppl 1, p. S84-92, Dec 2015.

ESTERBAUER, H.; SCHNEITLER, C.; OBERKOFLER, H.; EBENBICHLER, C. *et al.* A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. **Nat Genet**, 28, n. 2, p. 178-183, Jun 2001.

FALANDRY, Claire. BONNEFOY, Marc. FREYER, Gilles. GILSON, Eric. Biology of Cancer and Aging: A Complex Association With Cellular Senescence. **Journal of Clinical Oncology**. v. 32, p. 1-8, Jul. 2014. doi: 10.1200/JCO.2014.55.1432.

FANE, Mitchell. WEERARATNA, Ashani T. How the ageing microenvironment influences tumour progression. **Nature Reviews Cancer**. v. 20, n.2, p. 89-106, 2020. doi: 10.1038/s41568-019-0222-9.

FANG, J. L.; LAZARUS, P. Correlation between the UDP-glucuronosyltransferase (UGT1A1) TATAA box polymorphism and carcinogen detoxification phenotype: significantly decreased glucuronidating activity against benzo(a)pyrene-7,8-dihydrodiol(-) in liver microsomes from subjects with the UGT1A1*28 variant. **Cancer Epidemiol Biomarkers Prev**, 13, n. 1, p. 102-109, Jan 2004.

FIGUEIREDO, Carolina S. ASSIS, Marcella G. SILVA, Silvia L. A. DIAS, Rosângela C. MANCINI, Marisa C. Functional and cognitive changes in community-dwelling elderly: Longitudinal study. **Brazilian Journal of Physical Therapy**, v. 17, n. 3, p. 297-306. 2013.

GEIB, Guilherme. ROITHMANN, Sérgio. Câncer de Pulmão Não Pequenas Células. *In*: HOFF, Paulo Marcelo Gehm. *et al.* **Tratado de Oncologia**. São Paulo: Editora Atheneu, 2013.

GIGLIO, Auro Del. KARNAKIS, Theodora. TODARO, Juliana. Carcinogênese e o Processo do Envelhecimento. *In*: GIGLIO, Auro Del. KALIS, Rafael. KARNAKIS, Theodora. JACOB FILHO, Wilson. **Oncogeriatria: uma abordagem multidisciplinar**. Barueri: Manole, 2012. p. 143-149.

GIRARD, H.; BUTLER, L. M.; VILLENEUVE, L.; MILLIKAN, R. C. *et al.* UGT1A1 and UGT1A9 functional variants, meat intake, and colon cancer, among Caucasians and African-Americans. **Mutat Res**, 644, n. 1-2, p. 56-63, Sep 26 2008.

GISBERGEN, M.W. van. VOETS, A.M. STARMANS, M.H.W. COO, I.F.M. de. YADAK, R. HOFFMANN, R.F. *et al.* How do changes in the mtDNA and mitochondrial dysfunction influence cancer and cancer therapy? Challenges, opportunities and models. **Mutation Research**. v. 764, p. 16-30, 2015.

GOECKENJAN, G. *et al.* Prevention, Diagnosis, Therapy, and Follow-up of Lung Cancer Interdisciplinary Guideline of the German Respiratory Society and the German Cancer Society. **Pneumologie**. v. 65, n. 1, p. 39-59, 2011.

GOOZNER, Merrill. A Tale of Two Countries: Lung Cancer Care in Brazil and China. **Journal of the National Cancer Institute**. v. 104, n. 21, p. 1621-1623, 2012.

GROOT, Patricia M. de. WU, Carol C. CARTER, Brett W. MUNDEN, Reginald F. The epidemiology of lung cancer. **Translational Lung Cancer Research**. v. 7, n. 3, p. 220–233, 2018.

GRUNDY, Anne. RICHARDSON, Harriet. BURSTYN, Igor. LOHRISCH, Caroline. GUPTA, Sandip K Sen. LAI, Agnes S. LEE, Derrick. SPINELLI, John J. ARONSON, Kristan J. Increased risk of breast cancer associated with long-term shift work in Canada. **Occupational and Environmental Medicine**, v. 70, n. 12, p. 831-838, 2013.

HALL, William H. RAMACHANDRAN, Ramanathan. NARAYAN, Samir. JANI, Ashesh B. VIJAYAKUMAR, Srinivasan. An electronic application for rapidly calculating Charlson comorbidity score. **Biomed Center - BMC Cancer**, v. 94, n. 4, p. 1-8, 2004.

HAMMERSCHMIDT, Stefan. WIRTZ, Hubert. Lung Cancer: Current Diagnosis and Treatment. **Archive of Deutsches Ärzteblatt International.** v. 106, n. 49, p. 809-818, 2009.

HANAHAN, Douglas. Hallmarks of Cancer: New Dimensions. **Cancer Discovery**. v. 12, n. 1, p. 31-46, 2022.

HANAHAN, Douglas. WEINBERG, Robert A. Hallmarks of Cancer: The Next Generation. **Cell**. v. 144, n. 5, p. 646-674, Mar. 2011.

HASHEMI, M.; BAHARI, G.; SARHADI, S.; ESKANDARI, E. *et al.* 4-bp insertion/deletion (rs3783553) polymorphism within the 3'UTR of IL1A contributes to the risk of prostate cancer in a sample of Iranian population. **J Cell Biochem**, 119, n. 3, p. 2627-2635, 03 2018.

HECHT, Stephen S. Lung carcinogenesis by tobacco smoke. **International Journal of Cancer**. v. 131, n. 12, p. 2724–2732, 2012.

HOWLADER N, NOONE A. M., KRAPCHO M., MILLER D., BISHOP K., ALTEKRUSE S.F., *et al*. SEER Cancer Statistics Review, 1975-2013, **National Cancer Institute**. Bethesda, 2015. Disponível em: <http://seer.cancer.gov/csr/1975_2013/> Acesso em: 15 abr. 2017.

HU, Z.; CHEN, J.; MENG, P.; LI, M. Association between NEAT1 polymorphism and the risk of lung cancer: A protocol for systematic review and meta-analysis. **Medicine (Baltimore)**, 100, n. 16, p. e25478, Apr 23 2021.

HÜHNE, Rolf. THALHEIM, Torsten. SÜHNEL, Jürgen. AgeFactDB--the JenAge Ageing Factor Database--towards data integration in ageing research. **Nucleic Acids Research**. v. 42, p. D892-896.

Instituto Nacional de Câncer (INCA) José Alencar Gomes da Silva. Coordenação Geral de Ações Estratégicas. Coordenação de Educação. **ABC do câncer: abordagens básicas para o controle do câncer**. Rio de Janeiro: INCA, 2012. 129p.

Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA). Coordenação de Prevenção e Vigilância. **Estimativa 2018: incidência de câncer no Brasil**. Rio de Janeiro: INCA, 2017. 120p.

Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA). Coordenação de Prevenção e Vigilância. **Estimativa 2020: incidência de câncer no Brasil**. Rio de Janeiro: INCA, 2019. 120p.

Instituto Nacional do Câncer (INCA). Coordenação de Prevenção e Vigilância. **Estimativa 2016: Incidência de Câncer no Brasil**. Rio de Janeiro: INCA, 2015. 122p.

JIN, Y.; XU, H.; ZHANG, C.; KONG, Y. *et al.* Combined effects of cigarette smoking, gene polymorphisms and methylations of tumor suppressor genes on non small cell lung cancer: a hospital-based case-control study in China. **BMC Cancer**, 10, p. 422, Aug 12 2010.

JIN, Yongtang. XU, Heyun. ZHANG, Chunye. KONG, Yunming. HOU, Yong. XU, Yingchun. XUE, Shaoli. Combined effects of cigarette smoking, gene polymorphisms and methylations of tumor suppressor genes on non small cell lung cancer: a hospital-based case-control study in China. **BMC Cancer**. v. 10, n. 422, p. 1-9, 2010.

JONES, Gavin S. BALDWIN, David R. Recent advances in the management of lung cancer. **Clinical Medicine**, v. 18, n. s2, p. s41–s46, 2018. doi: 10.7861/clinmedicine.18-2s-s41.

JUNG, Sungmo. PARK, Misung. KIM, Won Jin. KIM, Chang Oh. Coagulopathy Caused by Concurrent Ciprofloxacin and Warfarin Use: What Other Factors Induce Coagulopathy? **Journal of the Korean Geriatrics Society**, v. 17, n. 1, p. 47-54, 2013. doi: 10.4235/jkgs.2013.17.1.47.

KAABI, Y. A.; MANSOR, A. S.; ALFAGIH, A. S.; HAKAMI, A. M. *et al.* Frequency of UCP2 45-bp Ins/Del polymorphism in Saudi population from Jazan area and its association with autoimmune hypothyroidism. **Int J Health Sci (Qassim)**, 14, n. 4, p. 11-16, 2020 Jul-Aug 2020.

KIM, Christopher. BASSIG, Bryan A. SEOW, Wei Jie. HU, Wei. PURDUE, Mark P. SHU, Xiao-Ou. *et al*. Pooled analysis of mitochondrial DNA copy number and lung cancer risk in three prospective studies. **Cancer Epidemiology, Biomarkers & Prevention**. v. 23, n. 12, p. 2977-2980, 2014.

KNUST, Renata Erthal. PORTELA, Margareth Crisóstomo. PEREIRA, Claudia Cristina de Aguiar. **Estimativa dos custos diretos da assistência oncologia do câncer de pulmão não pequenas células avançado em um hospital público de referência.** [Dissertação] Escola Nacional de Saúde Pública Sergio Arouca, Rio de Janeiro, 2015.

KUMAR, Vinay; ABBAS, Abul K.; ASTER, Jon C. **Patologia Básica**. 9. ed. Rio de Janeiro: Elsevier, 2013.

LATIMER, Kelly M. MOTT, Timothy F. Lung cancer: diagnosis, treatment principles, and screening. **American Family Physician**. v. 91, n. 4, p. 250-256. 2015.

LATORRE-PELLICER, Ana. MORENO-LOSHUERTOS, Raquel; LECHUGA-VIECO, Ana Victoria. SÁNCHEZ-CABO, Fátima. TORROJA, Carlos. ACÍN-PÉREZ, Rebeca *et al*. Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. **Nature**. v. 535, n. 7613, p. 561-565.

LEE, J. H.; CHO, Y. S.; JUNG, K. H.; PARK, J. W. *et al.* Genipin enhances the antitumor effect of elesclomol in A549 lung cancer cells by blocking uncoupling protein-2 and stimulating reactive oxygen species production. **Oncol Lett**, 20, n. 6, p. 374, Dec 2020.

LEE, Pyng. LEUNG, Chi Chiu. RESTREPO, Marcos I. TAKAHASHI, Kazuhisa. SONG, Yuanlin. PORCEL, José M. Year in review 2015: Lung cancer, pleural diseases, respiratory infections, bronchiectasis and tuberculosis, bronchoscopic intervention and imaging**.** **Respirology**. v. 21, n. 5, p. 961-967, 2016.

LIM, Wanyin. RIDGE, Carole A. NICHOLSON, Andrew G. MIRSADRAEE, Saeed. The 8 th lung cancer TNM classification and clinical staging system: review of the changes and clinical implications. **Quantitative Imaging in Medicine and Surgery**. v. 8, n. 7, p. 709-718, 2018.

LOPEZ-OTIN, Carlos. BLASCO, Maria A. PARTRIDGE, Linda. SERRANO, Manuel. KROEMER, Guido. The Hallmarks of Aging. **Cell**. v. 153, n. 6, p. 1194-1217, 2013. doi: 10.1016/j.cell.2013.05.039.

LOWERY, E. M., BRUBAKER, A. L., KUHLMANN, E., KOVACS, E. J. The aging lung. **Clinical interventions in aging**, v. 8, p. 1489-1496, 2013.

LUO, J. Y.; LIU, F.; ZHANG, T.; TIAN, T. *et al.* Association of *NFKB1* gene rs28362491 mutation with the occurrence of major adverse cardiovascular events. **BMC Cardiovasc Disord**, 22, n. 1, p. 313, 07 13 2022.

LURJE, G.; HUSAIN, H.; POWER, D. G.; YANG, D. *et al.* Genetic variations in angiogenesis pathway genes associated with clinical outcome in localized gastric adenocarcinoma. **Ann Oncol**, 21, n. 1, p. 78-86, Jan 2010.

MA, L.; ZHOU, N. Association between an insertion/deletion polymorphism in *IL1A* gene and cancer risk: a meta-analysis. **Onco Targets Ther**, 9, p. 1-6, 2016.

MA, Q.; MAO, Z.; DU, J.; LIAO, S. *et al.* Association between an insertion/deletion polymorphism in the interleukin-1α gene and the risk of colorectal cancer in a Chinese population. **Int J Biol Markers**, p. 1724600818785069, Jul 01 2018.

MACHADO, Karime Kalil. LEAL, Alessandro Igor Cavalcanti. Prevenção Primária e Secundária do Câncer. *In*: KATZ, Artur. CHAMMAS, Roger. ODONE FILHO, Vicente. NOVIS, Yana Sarkis. **Tratado de Oncologia**. São Paulo: Editora Atheneu, 2013. p. 873-901.

MAGALHÃES, João Pedro de. How ageing processes influence cancer. **Nature Reviews Cancer**, v. 13, n. 5, p. 357-365, 2013.

MALIK, Prabhat Singh. RAINA, Vinod. Lung cancer: Prevalent trends & emerging concepts. **Indian Journal of Medical Research**. v. 14, n. 1, p. 5–7, 2015.

MARCHENA-GOMEZ, Joaquin. ACOSTA-MERIDA, Maria Asuncion. HEMMERSBACH-MILLER, Marion. CONDE-MARTEL, Alicia. ROQUE-CASTELLANO, Cristina. HERNANDEZ-ROMERO, Juan. The Age-Adjusted Charlson Comorbidity Index as an Outcome Predictor of Patients with Acute Mesenteric Ischemia. **Annals of Vascular Surgery**. v. 23, n. 4, p. 458-464, jul. 2009.

MARTINO, M.; HAITEL, A.; SCHATZL, G.; KLATTE, T. The protease activated receptor 1 gene variation IVSn -14 A>T is associated with distant metastasis and cancer specific survival in renal cell carcinoma. **J Urol**, 190, n. 4, p. 1392-1397, Oct 2013.

MATHIAS, Clarissa. UGALDE, Paula A. ROSA, Arthur Accioly. *In*: HOFF, Paulo Marcelo Gehm. *et al.* **Tratado de Oncologia**. São Paulo: Editora Atheneu, 2013.

MAVROGONATOU, Eleni. PRATSINIS, Harris. KLETSAS, Dimitris. The role of senescence in cancer development. **Seminars in Cancer Biology**. v. 62, p. 182-191, 2020.

MEINERS, Silke. EICKELBERG, Oliver. KÖNIGSHOFF, Melanie. Hallmarks of the ageing lung. **European Respiratory Journal**, v. 45, n. 3, p. 807-827, 2015.

MELO, Lúcia Nunes Pereira. SAINTRAIN, Maria Vieira de Lima. Perfil epidemiológico de mulheres idosas atendidas no "grupo de apoio à prevenção da incapacidade funcional". **Revista Brasileira em Promoção a Saúde**, p. 22, n. 4, p. 251-258, 2009.

Ministério da Saúde. Sistema Único de Saúde. Sistema de Informações Hospitalares (SIH). **Morbidade hospitalar do SUS: Cidade de Belém, Estado do Pará.** Disponível em: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sih/cnv/nipa.def. Acesso em: 24 jan. 2021.

Ministério da Saúde. Sistema Único de Saúde. Sistema de Informações sobre Mortalidade (SIM). **Mortalidade: Cidade de Belém, Estado do Pará.** Disponível em: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sim/cnv/obt-10pa.def. Acesso em: 24 mar. 2021.

MORAES, Edgar Nunes de. Saúde do Idoso. **Atenção à saúde do Idoso: Aspectos Conceituais.** Brasília: Organização Pan-Americana da Saúde, 2012.

NELSON, R. S.; SELIGSON, N. D.; BOTTIGLIERI, S.; CARBALLIDO, E. *et al.* Guided Cancer Therapy: Review of the Evidence and Considerations for Clinical Implementation. **Cancers (Basel)**, 13, n. 7, Mar 29 2021.

NISHIKAWA, Y.; KANAI, M.; NARAHARA, M.; TAMON, A. *et al.* Association between UGT1A1*28*28 genotype and lung cancer in the Japanese population. **Int J Clin Oncol**, 22, n. 2, p. 269-273, Apr 2017.

NOGUEIRA, Fernanda de Pinho. GARCIA, Rosamaria Rodrigues. Evolução da Capacidade Funcional de Mulheres Institucionalizadas e não-institucionalizadas. **Revista Brasileira de Ciências da Saúde**, v. 6, n. 18, p. 56-68. 2008.

OLIVEIRA, Déborah Cristina de. NERI, Anita Liberalesso. D'ELBOUX, Maria José. Variáveis relacionadas à expectativa de suporte para o cuidado de idosos residentes na comunidade. **Revista Latino Americana de Enfermagem**. v. 21, n. 3, p. 1-8, mai/jun. 2013.

OLIVEIRA, Polyanna Carôzo de. Suscetibilidade genética ao câncer hereditário e espotádico na população de Monte Santo-BA. 2018. 82 f. Tese (Doutorado em Biotecnologia em Saúde e Medicina Investigativa) - Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, 2018.

OLTULU, Y. M.; COSKUNPINAR, E.; OZKAN, G.; AYNACI, E. *et al.* Investigation of NF-κB1 and NF-κBIA gene polymorphism in non-small cell lung cancer. **Biomed Res Int**, 2014, p. 530381, 2014.

PALLIS, Athanasios G. HATSE, Sigrid. BROUWERS, Barbara. PAWELEC, Graham. FALANDRY, Claire. WEDDING, Ulrich. *et al*. Evaluating the physiological reserves of older patients with cancer: The value of potential biomarkers of aging? **Journal Geriatric Oncology**, v. 5, n. 2, p. 204-2018, 2014.

PARKIN, M. TYCZYNSKI, J.E. BOFFETTA, P. SAMET, J. SHIELDS, P. CAPORASO, N. Lung cancer epidemiology and etiology. In: TRAVIS W.D., BRAMBILLA E., MULLER-HERMELINK H.K., HARRIS C.C. et al. World Health Organization Classification of Tumours. **Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart.** Lyon: IARC Press, 2004.

PATEL, P.; XUE, Z.; KING, K. S.; PARHAM, L. *et al.* Evaluation of the effect of UGT1A1 polymorphisms on the pharmacokinetics of oral and long-acting injectable cabotegravir. **J Antimicrob Chemother**, 75, n. 8, p. 2240-2248, 08 01 2020.

PATZ, Edward F. PINSKY, Paul. GATSONIS, Constantine. SICK, JoRean D. KRAMER, Barnett S. TAMMEMÄGI, Martin C. *et al.* Overdiagnosis in Low-Dose Computed Tomography Screening for Lung Cancer. **JAMA Internal Medicine.** v. 174, n. 2, p. 269-274, 2014.

PERKINSA, Anthony J., KROENKE, Kurt. UNUTZER, Jurgen. KATON, Wayne. WILLIAMS, John W. HOPE, Carol. CALLAHANA, Christopher M. Common comorbidity scales were similar in their ability to predict health care costs and mortality. **Journal of Clinical Epidemiology**, v. 57, p. 1040-1048, 2004.

PLIARCHOPOULOU, K.; VOUTSINAS, G.; PAPAXOINIS, G.; FLOROU, K. *et al.* Correlation of CYP1A1, GSTP1 and GSTM1 gene polymorphisms and lung cancer risk among smokers. **Oncol Lett**, 3, n. 6, p. 1301-1306, Jun 2012.

POPPER, Helmut H. Progression and metastasis of lung câncer. **Cancer and Metastasis Reviews**. v. 35, n. 1, p. 75-91, 2016.

PRESLEY, Carolyn J. REYNOLDS, Craig H. LANGER, Corey J. MORE, Show. Caring for the Older Population With Advanced Lung Cancer. **American Society of Clinical Oncology Educational.** n. 37, p. 587-596.

QUARESMA, Ivone Rosário Neves. O significado que os idosos atribuem aos cuidados de enfermagem domiciliários. [dissertação]. Ovar: **Instituto de Ciências Biomédicas Abel Salazar**, Universidade do Porto; 2008.

RAGAVAN, M.; PATEL, M. I. The evolving landscape of sex-based differences in lung cancer: a distinct disease in women. **Eur Respir Rev**, 31, n. 163, Mar 31 2022.

RAMOS, B. R.; D'ELIA, M. P.; AMADOR, M. A.; SANTOS, N. P. *et al.* Neither self-reported ethnicity nor declared family origin are reliable indicators of genomic ancestry. **Genetica**, 144, n. 3, p. 259-265, Jun 2016.

REZAPOUR, S.; KHOSROSHAHI, S. A.; FARAJNIA, H.; MOHSENI, F. *et al.* Association of 45-bp ins/del polymorphism of uncoupling protein 2 (UCP2) and susceptibility to nonalcoholic fatty liver and type 2 diabetes mellitus in North-west of Iran. **BMC Res Notes**, 14, n. 1, p. 169, May 06 2021.

RODAK, O.; PERIS-DÍAZ, M. D.; OLBROMSKI, M.; PODHORSKA-OKOŁÓW, M. *et al.* Current Landscape of Non-Small Cell Lung Cancer: Epidemiology, Histological Classification, Targeted Therapies, and Immunotherapy. **Cancers (Basel)**, 13, n. 18, Sep 20 2021.

RODRIGUES, Natália Oliveira. NERI, Anita Liberalesso. Vulnerabilidade social, individual e programática em idosos da comunidade: dados do estudo FIBRA, Campinas, SP, Brasil. **Revista Ciência e Saúde Coletiva**, v. 17, n. 8, p. 2129-2139, 2012.

SALTOS, Andreas. SHAFIQUE, Michael. CHIAPPORI, Alberto. Update on the Biology, Management, and Treatment of Small Cell Lung Cancer (SCLC). **Frontiers in Oncology**, v. 10, n. 1074, 2020.

SAN JOSE, C.; CABANILLAS, A.; BENITEZ, J.; CARRILLO, J. A. *et al.* CYP1A1 gene polymorphisms increase lung cancer risk in a high-incidence region of Spain: a case control study. **BMC Cancer**, 10, p. 463, Aug 30 2010.

SANTOS, N.P. RIBEIRO, Rodrigues E.M. SANTOS, A.K. Ribeiro dos. PEREIRA, R. GUSMÃO, L. AMORIM, A. GUERREIRO, J.F., *et al*. Assessing individual interethnicadmixture and population substructure using a 48-insertion-deletion (INSEL)ancestry-informative marker (AIM) panel**, Human Mutation**. v. 31, p. 184-190, 2010.

SAY, Y. H. The association of insertions/deletions (INDELs) and variable number tandem repeats (VNTRs) with obesity and its related traits and complications. **J Physiol Anthropol**, 36, n. 1, p. 25, Jun 14 2017.

SERRANO, Manuel. BLASCO, Maria A. Cancer and ageing: convergent and divergent mechanisms. **Nature Reviews - Molecular Cell Biology**. v. 8, n. 9, p. 715-722, 2007.

SHOKOLENKO, Inna N. WILSON, Glenn L. ALEXEYEV, Mikhail F. Aging: A mitochondrial DNA perspective, critical analysis and an update. **World Journal of Experimental Medicine.** v. 20, n. 4, p. 46-57, 2014.

SIDDIQUI, Faraz. VAQAR, Sarosh. SIDDIQUI, Abdul H. **Lung Cancer**. 2021 In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022.

STAPELFELD, C.; DAMMANN, C.; MASER, E. Sex-specificity in lung cancer risk. **Int J Cancer**, 146, n. 9, p. 2376-2382, 05 01 2020.

STRASSBURG, C. P. Pharmacogenetics of Gilbert's syndrome. **Pharmacogenomics**, 9, n. 6, p. 703-715, Jun 2008.

SU, W. P.; LO, Y. C.; YAN, J. J.; LIAO, I. C. *et al.* Mitochondrial uncoupling protein 2 regulates the effects of paclitaxel on Stat3 activation and cellular survival in lung cancer cells. **Carcinogenesis**, 33, n. 11, p. 2065-2075, Nov 2012.

SUNG, H.; FERLAY, J.; SIEGEL, R. L.; LAVERSANNE, M. *et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. **CA Cancer J Clin**, 71, n. 3, p. 209-249, 05 2021.

SUNG, Hyuna. FERLAY, Jacques. SIEGEL, Rebecca L. LAVERSANNE, Mathieu. SOERJOMATARAM, Isabelle. JEMAL, Ahmedin. BRAY, Freddie. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. **CA: A Cancer Journal for Clinicians**. v. 71, n. 3, p. 209-249, 2021.

TACUTU, Robi. CRAIG, Thomas. BUDOVSKY, Arie. WUTTKE, Daniel. LEHMANN, Gilad. TARANUKHA, Dmitri. *et al*. Human Ageing Genomic Resources: Integrated databases and tools for the biology and genetics of ageing. **Nucleic Acids Research**, v. 41, p. d1027–d1033, 2013.

TAKANO, M.; SUGIYAMA, T. UGT1A1 polymorphisms in cancer: impact on irinotecan treatment. **Pharmgenomics Pers Med**, 10, p. 61-68, 2017.

TEIXEIRA, Ilka Nicéia D'Aquino Oliveira. GUARIENTO, Maria Elena. Biologia do envelhecimento: teorias, mecanismos e perspectivas. **Revista Ciência e Saúde Coletiva**. v. 15, n. 6, p. 2845-2857, 2010.

THANDRA, K. C.; BARSOUK, A.; SAGINALA, K.; ALURU, J. S. Epidemiology of lung cancer. **Contemp Oncol (Pozn)**, 25, n. 1, p. 45-52, 2021.

TSAO, Anne S. SCAGLIOTTI, Giorgio V. BUNN JR., Paul A. CARBONE, David P. WARREN, Graham W. et al. Scientific Advances in Lung Cancer 2015. **Journal of Thoracic Oncology**. v. 11, n. 5, p. 613-638, 2016.

TSUKAZAN, M. T. R.; VIGO, Á.; SILVA, V. D. D.; BARRIOS, C. H. *et al.* Lung cancer: changes in histology, gender, and age over the last 30 years in Brazil. **J Bras Pneumol**, 43, n. 5, p. 363-367, 2017 Sep-Oct 2017.

URREA, R. Serrano. MESEGUER, M.J. Garcia. Malnutrition in an Elderly Population without Cognitive Impairment Living in Nursing Homes in Spain: Study of Prevalence Using the Mini Nutritional Assessment Test. **Gerontology**, v. 59, n. 6, p. 490-498, 2013.

WANG, X.; RICCIUTI, B.; NGUYEN, T.; LI, X. *et al.* Association between Smoking History and Tumor Mutation Burden in Advanced Non-Small Cell Lung Cancer. **Cancer Res**, 81, n. 9, p. 2566-2573, May 01 2021.

WANG, Yue. CHEN, Libo. PAN, Liming. XUE, Jinru. Yu, Haixiang. The association between *NFKB1*-94ins/del ATTG polymorphism and non-small cell lung cancer risk in a Chinese Han population. **International Journal of Clinical and Experimental Medicine**. v. 8, n. 5, p. 8153–8157, 2015.

WASSENAAR, C. A.; CONTI, D. V.; DAS, S.; CHEN, P. *et al.* UGT1A and UGT2B genetic variation alters nicotine and nitrosamine glucuronidation in european and african american smokers. **Cancer Epidemiol Biomarkers Prev**, 24, n. 1, p. 94-104, Jan 2015.

World Health Organization (WHO). International Agency for Research on Cancer (IARC). **GLOBOCAN 2020: Estimated number of deaths and new cases in 2020, lung cnacer, both sexes, all ages.** Graph production: IARC/WHO, 2020. Disponível em: <http://gco.iarc.fr/today>. Acesso em: Fev. 2021.

World Health Organization (WHO). International Agency for Research on Cancer (IARC). **GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012**. v1.0. WHO/IARC, 2013

WU, Song. ZHU, Wei. THOMPSON, Patricia. HANNUN, Yusuf A. Evaluating intrinsic and non-intrinsic cancer risk factors. **Nature Communications**. v, 9, n. 3490, 2018.

XIA, H.; CHEN, Y.; MENG, J.; LIANG, C. Effect of polymorphism on. **Artif Cells Nanomed Biotechnol**, 47, n. 1, p. 3138-3152, Dec 2019.

YANG, Ian A. HOLLOWAY, John W. FONG, Kwun M. Genetic susceptibility to lung cancer and co-morbidities. **Journal of Thoracic Disease**, v. 5, s. 5, s. 454-S462, 2013.

YAZDANI, Z.; MOUSAVI, Z.; GHASEMIMEHR, N.; KALANTARY KHANDANY, B. *et al.* Differential regulatory effects of chemotherapeutic protocol on CCL3_CCL4_CCL5/CCR5 axes in acute myeloid leukemia patients with monocytic lineage. **Life Sci**, 240, p. 117071, Jan 01 2020.

YIN, J.; YIN, M.; VOGEL, U.; WU, Y. *et al. NFKB1* common variants and PPP1R13L and CD3EAP in relation to lung cancer risk in a Chinese population. **Gene**, 567, n. 1, p. 31-35, Aug 01 2015.

YOUNES, R. N. ABRÃO, F. C. Câncer de pulmão: epidemiologia e prevenção. *In*: ANTUNES, R. C. P. PERDICARIS, A. A. M. GOMES, R. **Prevenção do câncer**. 2ed. Barueri: Manole, 2015. p. 149-170.

YOUSAF-KHAN, Uraujh. HOREWEG, Nanda. AALST, Carlijn van der. HAAF, Kevin ten. OUDKERK, Mathijs. KONING, Harry de. Baseline Characteristics and Mortality Outcomes of Control Group Participants and Eligible Non-Responders in the NELSON Lung Cancer Screening Study. **Journal of Thoracic Oncology**.v. 10, n.5, p. 747–753, 2015.

YU, J.; SHI, L.; LIN, W.; LU, B. *et al.* UCP2 promotes proliferation and chemoresistance through regulating the NF-κB/β-catenin axis and mitochondrial ROS in gallbladder cancer. **Biochem Pharmacol**, 172, p. 113745, 02 2020a.

YUN, C. M.; SANG, X.Y. Role of proteinase-activated receptor-1 gene polymorphisms in susceptibility to chronic obstructive pulmonary disease. **Genetics and Molecular Research**, v. 14, n. 4, p. 13215-13220, 2015.

ZHANG, H.; WANG, M.; SHI, T.; SHEN, L. *et al.* Genetic polymorphisms of PAI-1 and PAR-1 are associated with acute normal tissue toxicity in Chinese rectal cancer patients treated with pelvic radiotherapy. **Onco Targets Ther**, 8, p. 2291-2301, 2015.

ZHENG, Min. Classification and Pathology of Lung Cancer. **Surgical Oncology Clinics of North America**. v. 25, n. 3, p. 447-468, 2016.

ZHONG, Rong. LIU, Li. ZOU, Li. ZHU, Yaowu. CHEN, Wei. ZHU, Beibei. *et al.* Genetic Variations in TERT-CLPTM1L Locus Are Associated With Risk of Lung Cancer in Chinese Population. **Molecular Carcinogenesis**. v. 52, n. 1, p. 118-126, 2013.

ZINGER, Adar. CHO, William C. BEN-YEHUDA, Arie. Cancer and Aging - the Inflammatory Connection. **Aging and Disease**. v. 8, n. 5, p. 611-627, 2017.

ZINSER-SIERRA, J. W. [Tobacco use and lung cancer]. **Salud Publica Mex**, 61, n. 3, p. 303-307, 2019 May-Jun 2019.

ZUKIN, Mauro. DIENSTMANN, Rodrigo. ARAÚJO, Luiz Henrique de Lima. Biologia Molecular do Câncer de Pulmão. *In*: HOFF, Paulo Marcelo Gehm. *et al.* **Tratado de Oncologia**. São Paulo: Editora Atheneu, 2013.

APÊNDICE A

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

CORRELAÇÃO ENTRE A CAPACIDADE FUNCIONAL E BIOMARCADORES DO ENVELHECIMENTO EM PACIENTES COM CÂNCER

I) Você está sendo convidado(a) a participar da pesquisa intitulada "Correlação entre a Capacidade Funcional e Biomarcadores do Envelhecimento em Idosos com Câncer", que será realizado no Hospital Universitário João de Barros Barreto, no período de agosto de 2015 a agosto de 2020. O objetivo do estudo é identificar a associação entre a capacidade funcional e biomarcadores do envelhecimento em pacientes com câncer. O estudo auxiliará a predizer a saúde do paciente de forma ampla, embasar estratégias de prevenção, facilitar a gestão e a elaboração de futuros planos de cuidados necessários ao envelhecimento natural ou por doenças.
II) Para alcançar os objetivos do estudo, será aplicada uma ficha de avaliação, a avaliação geriatria ampla, que analisa as características da capacidade funcional do idoso. Além disso, será realizada a coleta de sangue periférico, para medir os marcadores do envelhecimento em seu material genético. Essas informações estão sendo fornecidas para sua participação voluntária neste estudo e você tem a garantia do recebimento de uma via deste documento.
III) A presente pesquisa implica em riscos para os participantes, os quais podem ter danos morais e éticos, em virtude de algumas informações requeridas no protocolo de pesquisa. Para impedir tal ocorrência os questionários serão identificados por números e não por nomes, pois as informações relatadas no protocolo serão de uso exclusivamente científico.
IV) Na coleta do sangue periférico, podendo causar uma leve dor na hora e uma pequena mancha roxa que desaparecerá após alguns dias da coleta, mas espera-se que nada disso aconteça. Para evitá-los a realização dessa etapa será realizada por um profissional treinado, que tomará as devidas medidas de segurança. Esse material será devidamente armazenado no Núcleo de Pesquisa em Oncologia, para posterior análise relacionada exclusivamente ao presente estudo. Após a análise, o material restante será devidamente descartado, conforme normas vigentes de órgãos técnicos competentes, respeitando-se a confidencialidade dos voluntários da pesquisa.
V) Os resultados desta pesquisa trarão benefícios para os participantes, pois irão melhorar a qualidade de vida destes a partir do conhecimento sobre a capacidade funcional, podendo melhor conduzir o possível problema encontrado.
VI) Você tem garantida a liberdade de retirada de consentimento a qualquer momento e deixar de participar do estudo, sem qualquer prejuízo à continuidade da sua assistência na instituição.
VII) Os voluntários da pesquisa terão o direito de serem mantidos atualizados sobre os resultados parciais da pesquisa, que sejam de conhecimento dos pesquisadores.
VIII) Despesas e compensações: não há despesas pessoais para o voluntário em qualquer fase do estudo, incluindo exames e consultas. Também não há compensação financeira relacionada a sua participação. Se existir qualquer despesa adicional, ela será absorvida pelo orçamento da pesquisa.
IX) Em caso de qualquer dano pessoal, diretamente causado pelos procedimentos propostos neste estudo (nexo causal comprovado), o participante tem direito a tratamento médico, bem como as indenizações legalmente estabelecidas.
X) Acredito ter sido suficientemente informado a respeito das informações que li e/ou que foram lidas para mim, descrevendo a pesquisa intitulada "Correlação entre a Capacidade Funcional e Biomarcadores do Envelhecimento em Pacientes com Câncer".
Eu, discuti com Esdras Edgar Batista Pereira, sobre minha decisão em participar nesse estudo. Ficaram claros para mim quais são os propósitos do estudo, os procedimentos a serem realizados, seus riscos, as garantias de confidencialidade e de esclarecimento permanente.

______________________________________ Belém, ______/______/________

Assinatura do Sujeito/representante responsável

______________________________________ Belém, ______/______/_________

Assinatura do sujeito que colheu o TCLE
(Somente para o responsável do projeto)

Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste paciente ou representante legal para a participação neste estudo.

____

ASSINATURA DO PESQUISADOR RESPONSÁVEL
Nome: Esdras Edgar Batista Pereira
End: Conj. Cidade Nova 4, Tv. We 34, n. 72
Telefone: (91) 98520-0709
CRM-PA: 15.424 / CREFITO-PA: 194091.1.F

APÊNDICE B

FICHA DE AVALIAÇÃO CLÍNICA EPIDEMIOLÓGICA

DATA DA AVALIAÇÃO: _____/_____/________	MATRICULA:	LEITO:	ID:

I. DADOS SOCIO-DEMOGRAFICOS

NOME:	SEXO: ( )M ( )F	IDADE:	DATA DE NASC.: _____/_____/______
END.:	BAIRRO:	CIDADE:	TELEFONE:
COR: ( ) Negro ( ) Pardo ( ) Branco	ESTADO CIVIL: ( ) Casado ( ) Solteiro ( ) Viúvo ( ) Separado		
ESCOLARIDADE: ( ) Analfabeto ( )1 - 4 anos ( ) 5 – 8 anos ( ) > 8 anos	PROFISSÃO:	OCUPAÇÃO ATUAL:	

II. DADOS CLÍNICOS-EPIDEMILÓGICOS

DOENÇAS DIAGNÓSTICADAS:		TIH:
PRINCIPAIS QUEIXAS:		
TIPO DE CÂNCER:	HISTOPATOLÓGICO:	ESTADIAMENTO (TMN):

SINAIS VITAIS	PA: mmHg	FC: bpm	FR: irpm	T: ºC	SPO2: %	EVA:

III. AVALIAÇÃO DA CAPACIDADE FUNCIONAL GLOBAL

PERFOMACE STATUS *(Eastern Cooperative Oncology Group)*		TABAGISMO	ETILISMO	EXERCICIO FÍSICO
0	Atividade normal em relação ao que realizava antes da doença	Nunca Fumou ( )	Nunca Bebeu ( )	Sedentário ( )
1	Enfrenta sintomas da doença, mas deambula e mantém atividades diárias.	Fumante ( )	Etilista Social ( )	Caminhada ( )
2	Consegue ficar fora do leito mais de 50% do dia e realizar algum a atividade.	Ex-fumante ( )	Etilista ( )	Musculação ( )
3	Restrito ao leito mais de 50% do dia, dependente de cuidados relativos.	Tempo:	Ex-Etilista ( )	Hidroginástica ( )
4	Acamado, preso ao leito, dependente de cuidado contínuo.	Nº Cig./dia:	Tempo:	Tempo:

ATIVIDADES BÁSICAS DE VIDA DIÁRIA *(Índice de Katz)*

1 - Faz sozinho, habitualmente e corretamente atividade considerada / 2 - Ajuda não humana / 2 - Ajuda humana / 3 - O idoso não faz a atividade considerada.

FUNÇÃO		1	2	3
Banhar-se				
Vestir-se				
Uso do Banheiro				
Transferir-se				
Continência	Micção			
	Evacuação			
Alimentar-se				
	ESCORE TOTAL:			

ATIVIDADES INSTRUMENTAIS DE VIDA DIÁRIA *(Escala de Lawton e Brody)*

3 – Sem Ajuda / 2 – Com ajuda parcial / 1 – Incapaz

FUNÇÃO	3	2	1
Preparo das refeições / subir escadas			
Utilização da medicação			
Fazer compras			
Administração das Finanças			
Utilização do telefone			
Cuidados com o lar			
Lava e passar roupa / cuidar do jardim			
Pequenos trabalhos domésticos			
Utilização de transporte			
ESCORE TOTAL:			

ATIVIDADES AVANÇADAS DE VIDA DIÁRIA

Eu gostaria de saber qual a sua relação com as seguintes atividades?	NUNCA FEZ (1)	PAROU (2)	FAZ (3)
1. Fazer visita na casa de outras pessoas			
2. Receber visitas em sua casa			
3. Ir a igreja ou templo para rituais religiosos ou atividades sociais ligadas a religião			
4. Participa de centro de convivência, Universidade da Terceira Idade ou algum curso			
5. Participa de reuniões sociais, festas ou bailes			
6. Participa de eventos culturais: concertos, espetáculos, exposição, peças teatrais ou cinema			
7. Dirigir automóvel			
8. Fazer viagem de um dia para fora da cidade			
9. Fazer viagem de duração mais longa para fora da cidade ou pais			
10. Fazer trabalho voluntário			
11. Fazer trabalho remunerado			
12. Participa de diretorias, clubes, escolas, sindicatos ou desenvolve atividades políticas.			

IV. AVALIAÇÃO DOS SISTEMAS FUNCIONAIS

AVALIAÇÃO DA MOBILIDADE

DOR:	DINAMOMETRIA (kgf)	
MMSS ( ) EVA:________ MMII ( ) EVA:________ TRONCO ( ) EVA:________	Direita: 1ª(_________) / 2ª(_________) / 3ª(_________) Esquerda: 1ª(_________) / 2ª(_________) / 3ª(_________)	LEVE / MODERADA / INTENSA 0 1 2 3 4 5 6 7 8 9 10

TRANSFERENCIAS: Decúbito: ( ) Sem Ajuda ( ) Com ajuda parcial ( ) Incapaz
Sedestação: ( ) Sem Ajuda ( ) Com ajuda parcial ( ) Incapaz
Bipedestação: ( ) Sem Ajuda ( ) Com ajuda parcial ( ) Incapaz

MARCHA: ( ) Sozinho ( ) Ajuda ocasional ( )Ajuda frequente ( )Muleta ou bengala ( ) Andador ( ) Cadeira de rodas ( ) Acamado

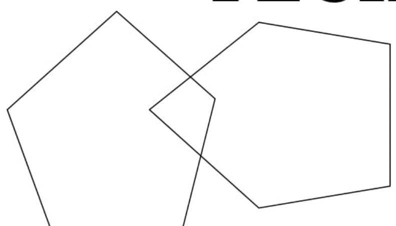
EQUILIBRIO			
1	Equilíbrio sentado	Escorrega	0
		Equilibrado	1
2	Levantar	Incapaz	0
		Utiliza os braços como apoio	1
		Levanta-se sem apoiar os braços	2
3	Tentativas para levantar	Incapaz	0
		Mais de uma tentativa	1
		Tentativa única	2
4	Assim que levanta	Desequilibrado	0
		Estável mas utiliza suporte	1
		Estável sem suporte	2
5	Equilíbrio em pé	Desequilibrado	0
		Pés afastado (base de sustentação) > 12 cm	1
		Sem suporte e base estreita	2
6	Teste dos três campos	Começa a cair	0
		Garra ou balança (braços)	1
		Equilibrado	2
7	Olhos fechados	Desequilibrado, instável	0
		Equilibrado	1
8	Girando 360º	Passos descontínuos	0
		Instável (desequilíbrios)	1
		Estável (equilibrado)	2
9	Sentado	Inseguro (erra a distância, cai na cadeira)	0
		Utiliza os braços ou movimentação abrupta	1
		Seguro, movimentação suave	2
		ESCORE EQUILIBRIO:	

MARCHA			
10	Inicio da marcha	Hesitação ou varias tentativas para iniciar	0
		Sem hesitação	1
11	Comprimento e altura dos passos	a) Pé direito	
		Não ultrapassa o pé esquerdo	0
		Ultrapassa o pé esquerdo	1
		Não sai completamente do chão	0
		Sai completamente do chão	1
		b) Pé esquerdo	
		Não ultrapassa o pé direito	0
		Ultrapassa o pé direito	1
		Não sai completamente do chão	0
		Sai completamente do chão	1
12	Simetria dos passos	Passos diferentes	0
		Passos semelhantes	1
13	Continuidade dos passos	Paradas ou passos descontínuos	0
		Passos contínuos	1
14	Direção	Desvio Nítido	0
		Desvio leve ou moderado ou uso de apoio	1
		Linha reta sem apoio (bengala ou andador)	2
15	Tronco	Balanço grave ou uso de apoio	0
		Flexão dos joelhos ou dorso ou abertura dos braços enquanto anda	1
		Sem flexão, balanço, não usa os braços ou apoio	2
16	Distância dos tornozelos	Tornozelos Separados	0
		Tornozelos quase se tocam enquanto anda	1
		ESCORE MARCHA:	
		ESCORE TOTAL:	

TIME UP AND GOTEST _______/

AVALIAÇÃO COGNITIVA *(MEEM)*		
Orientação Temporal (05 pontos)	Ano	
	Mês	
	Dia do mês	
	Dia da semana	
	Semestre/Hora aproximada	
Orientação Espacial (05 pontos)	*Estado*	
	Cidade	
	Bairro ou nome de rua próxima	
	Local geral	
	Andar ou local específico: em que local nós estamos	
Registro (3 pontos)	*Repetir: GELO, LEÃO e PLANTA*	
Atenção e Cálculo (5 pontos)	*Subtrair 100 – 7 = 93 – 7 = 86 – 7 = 79 – 7 = 72 – 7 = 65*	
	MUNDO=ODNUM	
Memória de Evocação (3 pontos)	Quais os três objetos perguntados anteriormente?	
Nomear dois objetos (2 pontos)	*Relógio e caneta*	
Repetir (1 ponto)	*"NEM AQUI, NEM ALI, NEM LÁ"*	
Comando de estágios (3 pontos)	*"Apanhe esta folha de papel com a mão direita, dobre-a ao meio e coloque-a no chão"*	
Escrever uma frase completa (1 ponto)	"Escreva alguma frase que tenha começo, meio e fim"	
Ler e executar (1 ponto)	*FECHE SEUS OLHOS*	
Copiar diagrama (1 ponto)	*Copiar dois pentágonos com interseção*	
	ESCORE TOTAL:	

FECHE SEUS OLHOS



AVALIAÇÃO DO HUMOR *(Escala de Depressão Geriátrica de Yesavage)*

Você está basicamente satisfeito com sua vida?	( )Sim ( )**Não**
Você se aborrece com frequência?	( )**Sim** ( )Não
Você se sente um inútil nas atuais circunstâncias?	( )**Sim** ( )Não
Você prefere ficar em casa a sair e fazer coisas novas?	( )**Sim** ( )Não
Você sente que sua situação não tem saída?	( )**Sim** ( )Não

Você tem medo que algum mal vá lhe acontecer?	( )**Sim** ( )Não
Você acha que sua situação é sem esperanças?	( )**Sim** ( )Não
Você acha maravilhoso estar vivo?	( )Sim ( )**Não**
Você sente que sua vida está vazia?	( )**Sim** ( )Não
Você sente que a maioria das pessoas está melhor que você?	( )**Sim** ( )Não
Você se sente com mais problemas de memória do que a maioria?	( )**Sim** ( )Não
Você deixou muitos de seus interesses e atividades?	( )**Sim** ( )Não
Você se sente de bom humor a maior parte do tempo?	( )Sim ( )**Não**
Você se sente cheio de energia?	( )Sim ( )**Não**
Você se sente feliz a maior parte do tempo?	( )Sim ( )**Não**
ESCORE TOTAL:	

V. AVALIAÇÃO DA QUALIDADE DE VIDA *(WHOQOL-BREF)*

		PERGUNTAS	1	2	3	4	5
G	1	Como você avaliaria sua qualidade de vida?					
	2	Quão satisfeito(a) você está com a sua saúde?					

VI. AVALIAÇÃO NUTRICIONAL *(MAN-MINIAVALIAÇÃO NUTRICIONAL)*

Sexo: Idade: Peso, kg: Altura, cm: Data:

Responda à secção "triagem", preenchendo as caixas com os números adequados. Some os números da secção "triagem".
Se a pontuação obtida for igual ou menor que 11, continue o preenchimento do questionário para obter a pontuação indicadora de desnutrição.

Triagem

A Nos últimos três meses houve diminuição da ingesta alimentar devido a perda de apetite, problemas digestivos ou dificuldade para mastigar ou deglutir?
0 = diminuição grave da ingesta
1 = diminuição moderada da ingesta
2 = sem diminuição da ingesta ☐

B Perda de peso nos últimos 3 meses
0 = superior a três quilos
1 = não sabe informar
2 = entre um e três quilos
3 = sem perda de peso ☐

C Mobilidade
0 = restrito ao leito ou à cadeira de rodas
1 = deambula mas não é capaz de sair de casa
2 = normal ☐

D Passou por algum *stress* psicológico ou doença aguda nos últimos três meses?
0 = sim 2 = não ☐

E Problemas neuropsicológicos
0 = demência ou depressão graves
1 = demência ligeira
2 = sem problemas psicológicos ☐

F Índice de Massa Corporal (IMC = peso[kg] / estatura [m²])
0 = IMC < 19
1 = 19 ≤ IMC < 21
2 = 21 ≤ IMC < 23
3 = IMC ≥ 23 ☐

Pontuação da Triagem (subtotal, máximo de 14 pontos) ☐☐
12-14 pontos: estado nutricional normal
8-11 pontos: sob risco de desnutrição
0-7 pontos: desnutrido
Para uma avaliação mas detalhada,continue com as perguntas G-R

Avaliação global

G O doente vive na sua própria casa (não em instituição geriátrica ou hospital)
1 = sim 0 = não ☐

H Utiliza mais de três medicamentos diferentes por dia?
0 = sim 1 = não ☐

I Lesões de pele ou escaras?
0 = sim 1 = não ☐

J Quantas refeições faz por dia?
0 = uma refeição
1 = duas refeições
2 = três refeições ☐

K O doente consome:
- pelo menos uma porção diária de leite ou derivados (leite, queijo, iogurte)? sim ☐ não ☐
- duas ou mais porções semanais de leguminosas ou ovos? sim ☐ não ☐
- carne, peixe ou aves todos os dias? sim ☐ não ☐

0.0 = nenhuma ou uma resposta «sim»
0.5 = duas respostas «sim»
1.0 = três respostas «sim» ☐.☐

L O doente consome duas ou mais porções diárias de fruta ou produtos hortícolas?
0 = não 1 = sim ☐

M Quantos copos de líquidos (água, sumo, café, chá, leite) o doente consome por dia?
0.0 = menos de três copos
0.5 = três a cinco copos
1.0 = mais de cinco copos ☐.☐

N Modo de se alimentar
0 = não é capaz de se alimentar sozinho
1 = alimenta-se sozinho, porém com dificuldade
2 = alimenta-se sozinho sem dificuldade ☐

O O doente acredita ter algum problema nutricional?
0 = acredita estar desnutrido
1 = não sabe dizer
2 = acredita não ter um problema nutricional ☐

P Em comparação com outras pessoas da mesma idade, como considera o doente a sua própria saúde?
0.0 = pior
0.5 = não sabe
1.0 = igual
2.0 = melhor ☐.☐

Q Perímetro braquial (PB) em cm
0.0 = PB < 21
0.5 = 21 ≤ PB ≤ 22
1.0 = PB > 22 ☐.☐

R Perímetro da perna (PP) em cm
0 = PP < 31
1 = PP ≥ 31 ☐

Avaliação global (máximo 16 pontos) ☐☐.☐
Pontuação da triagem ☐☐.☐
Pontuação total (máximo 30 pontos) ☐☐.☐

VII. RISCO DE ULCERA POR PRESSÃO

Escala de Avaliação de Risco de Norton

Condição Física	Estado Mental	Atividade	Mobilidade	Incontinência	ESCORE TOTAL
Boa (4) □	Alerta (4) □	Deambulante (4) □	Plena (4) □	Ausente (4) □	
Regular (3) □	Apático (3) □	Caminha com Ajuda (3) □	Limitada (3) □	Ocasional (3) □	
Ruim (2) □	Confuso (2) □	Limitado à Cadeira (2) □	Muito Limitada (2) □	Urinária (2) □	
Muito Ruim (1) □	Estupor (1) □	Acamado (1) □	Imóvel (1) □	Dupla (1) □	

Escala de Avaliação de Risco de Gosnell

Nutrição	Estado Mental	Atividade	Mobilidade	Continência	ESCORE TOTAL
Boa (1) □	Alerta (1) □	Deambulante (1) □	Completa (1) □	Controlada (1) □	
Regular (2) □	Apático (2) □	Caminha com Ajuda (2) □	Limitada (2) □	Usualmente Controlada (2) □	
Pobre (3) □	Confuso (3) □	Limitado à Cadeira (3) □	Muito Limitada (3) □	Minimamente Controlada (3) □	
	Estupor (4) □	Acamado (4) □	Imóvel (4) □	Ausemte (4) □	

VIII. RISCO DE TROMBOSE

Escala de Risco CHADS2

ICC (1) □ Hipertensão (1) □ Idade ≥75 anos (1) □ Diabetes (1) □ AVC ou AIT (2) □	ESCORE TOTAL	

Escala de Risco CHADS2-VASC

ICC (1) □ Hipertensão (1) □ Idade ≥75 anos (1) □ Diabetes (1) □ AVC ou AIT (2) □ Doença Vascular (IAM/DAP/Placa Aórtica) (1) □ Idade 65-74 anos (1) □ Sexo Feminino (1) □	ESCORE TOTAL	

Escala de Risco QTHROMBOSIS

1. Idade ( ) 2. Sexo: Masculino □ Feminino □ 3. Etnia ( ) 4. Você Foi admitido no Hospital nos ultimos 6 meses? Sim □ Não □

5. Tabagismo: Nunca Fumou □ Ex-fumante □ Fumante Leve (menos de 10 cigarros) □ Fumante Moderado (10-19 cigarros) □ Fumante Pesados (10-19 cigarros) □

6. Você teve algum dos seguintes eventos? 6.1. Cirugia de Varizes □ 6.2. DRC (estágio 3 ou 4) □ 6.3. Câncer □ 6.4. ICC □ 6.5. DPOC □ 6.6. Doença de Chrons □

	ESCORE DO RISCO P/ ANO	1	2	3	4	5
7. Somente para Mulheres: Fazendo uso de Terapia de Reposição Homonal □ Fazendo uso de Tamoxifeno □						
8. Antropometria: Peso: (kg) Altura: (cm) IMC: (kg/m^2)						

IX. RISCO DE QUEDAS

Escala de Avaliação do Risco de Quedas de Morse

1. Histórico de quedas nessa internação ou nos últimos 3 meses	Não (0) □ Sim (25) □	2. Diagnósticos Secundários	Não (0) □ Sim (15) □
3. Ajuda para caminhar	Nenhuma/ajuda de enfermeiros/acamado/cadeira de rodas (0) □ Muletas /bengalas (15) □ Apoia-se no mobiliário para andar (25) □		
4. Terapia Intravenosa	Não (0) □ Sim (20) □	5. Postura no andar e na transferência	Normal/acamado/imovel (0) □ Debilitado (10) □ Dependent de Ajuda (20) □
6. Estado Mental	Consciente das suas capacidades (0) □ Esquece suas limitações (15) □	ESCORE TOTAL	

X. RISCO DE FRATURAS

Escala de Risco QFRACTURE®

1. Idade ( ) 2. Sexo: Masculino □ Feminino □ 3. Etnia ( ) 4. Diabetes Mellitus: Nenhum □ Tipo 1 □ Tipo 2 □

5. Tabagismo: Nunca Fumou □ Ex-fumante □ Fumante Leve (menos de 10 cigarros) □ Fumante Moderado (10-19 cigarros) □ Fumante Pesados (10-19 cigarros) □

6. Etilismo: < 1 Unid. por dia □ 1-2 Unid. por dia □ 3-6 Unid. por dia □ 7-9 Unid. por dia □ > 9 Unid. por dia □

7. Algum dos seus pais apresentou osteoporose/fratura de quadril? Sim □ Não □	17. DRC (Estágio 3 ou 4)? Sim □ Não □	18. AR ou LES? Sim □ Não □
8. Você mora em alguma casa de Enfermagem ou Cuidados? Sim □ Não □	19. Doença Endocrina? Sim □ Não □	20. D. Chrons? Sim □ Não □
9. Você teve algum tipo de fratura (quadril, ombro ou punho)? Sim □ Não □	21. Epilepsia ou Toma Anticovulsivante? Sim □ Não □	
10. História de Queda? Sim □ Não □ / 11. Demência? Sim □ Não □	22. Toma antidepressivo? Sim □ Não □	25. Antropometria: IMC: (kg/m^2)
12. Câncer? Sim □ Não □ / 13. Asma ou DPOC? Sim □ Não □	23. Toma esteroides? Sim □ Não □	Peso: (kg) Altura: (cm)
14. Ataque cardíaco, Angina, AVC ou TIA? Sim □ Não □	24. Toma Estrogeno/TRH? Sim □ Não □	1 2 3 4 5 6 7 8 9 0
15. DHC? Sim □ Não □ / 16. Parkinson? Sim □ Não □	ESCORE DO RISCO P/ ANO	

XI. RISCO DE ATAQUE CARDÍACO E ACIDENTE VASCULAR ENCEFÁLICO

Escala de QRISK®2

1. Idade ( ) 2. Sexo: Masculino □ Feminino □ 3. Etnia ( ) 4. Diabetes Mellitus: Nenhum □ Tipo 1 □ Tipo 2 □

5. Tabagismo: Nunca Fumou □ Ex-fumante □ Fumante Leve (menos de 10 cigarros) □ Fumante Moderado (10-19 cigarros) □ Fumante Pesados (10-19 cigarros) □

6. Angina ou ataque cardíaco em um parente de 1º grau <60? Sim □ Não □	10. Artrite reumatóide? Sim □ Não □				
7. Doença renal crônica (estágio 4 ou 5)? Sim □ Não □	11. Relação colesterol/HDL:	13. Peso: (kg) Altura: (cm)			
8. Fibrilação atrial? Sim □ Não □	12 PAS: mmHg	QRISK2	Controle	OR	QRISK2
9. Encontra-se no tratamento da pressão arterial? Sim □ Não □	ESCORE TOTAL				

Escala de QRISK®3

1. Idade ( ) 2. Sexo: Masculino □ Feminino □ 3. Etnia ( ) 4. Diabetes Mellitus: Nenhum □ Tipo 1 □ Tipo 2 □

5. Tabagismo: Nunca Fumou □ Ex-fumante □ Fumante Leve (menos de 10 cigarros) □ Fumante Moderado (10-19 cigarros) □ Fumante Pesados (10-19 cigarros) □

6. Angina ou ataque cardíaco em um parente de 1º grau <60? Sim □ Não □	14. Faz uso de medicação antipsicótica atípica? Sim □ Não □				
7. Doença renal crônica (estágio 4 ou 5)? Sim □ Não □	15. Faz uso de medicação esteróide regularmente? Sim □ Não □				
8. Fibrilação atrial? Sim □ Não □	16. Dignóstico ou tratamento para disfunção erétil? Sim □ Não □				
9. Encontra-se no tratamento da pressão arterial? Sim □ Não □	17. Relação colesterol/HDL:	18. PAS: mmHg			
10. Você possui enxaqueca? Sim □ Não □	19. DP de pelo menos 2 medidas da PAS: mmHg				
11. Artrite reumatóide? Sim □ Não □	20: Antropometria: Peso: (kg) Altura: (cm) IMC: (kg/m^2)				
12. Lupus eritematoso sistémico (LES) Sim □ Não □	ESCORE TOTAL	QRISK3	Controle	OR	QRISK3
13. Doença mental grave? Sim □ Não □					

XII. RISCO DE ACIDENTE VASCULAR CEREBRAL

Escala QSTROKE®

1. Idade ( ) 2. Sexo: Masculino □ Feminino □ 3. Etnia ( ) 4. Diabetes Mellitus: Nenhum □ Tipo 1 □ Tipo 2 □

5. Tabagismo: Nunca Fumou □ Ex-fumante □ Fumante Leve (menos de 10 cigarros) □ Fumante Moderado (10-19 cigarros) □ Fumante Pesados (10-19 cigarros) □

6. Fibrilação atrial? Sim □ Não □	7. ICC? Sim □ Não □	8. Ataque Cardiaco ou Agina? Sim □ Não □	9. Artrite reumatóide? Sim □ Não □
10. Doença renal crônica (estágio 4 ou 5)? Sim □ Não □	11. Angina ou ataque cardíaco em um parente de 1º grau <60? Sim □ Não □		

12. Encontra-se no tratamento para HAS? Sim □ Não □	13. Doença cardíaca valvular? Sim □ Não □	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	ESCORE TOTAL										

XIII. AVALIAÇÃO DA DEPENDÊNCIA FÍSICA

Escala de Barthel

1. Alimentação	Dependente (0) □ Semidependente (5) □ Independente (10) □	7. Uso do Sanitário	Dependente (0) □ Semidependente (5) □ Independente (10) □
2. Banho	Dependente (0) □ Independente (5) □	8. Uso da Escada	Dependente (0) □ Semidependente (5) □ Independente (10) □
3. Higiene	Dependente (0) □ Independente (5) □	9. Incontin. Urinária	Incontinência (0) □ Semicontinência (5) □ Continência (10) □
4. Vestir-se	Dependente (0) □ Semidependente (5) □ Independente (10) □	10. Incontin. Fecal	Incontinência (0) □ Semicontinência (5) □ Continência (10) □
5. Transferência	Dependente (0) □ Muita Ajuda (5) □ Pouca Ajuda (10) □ Independente (15) □	**ESCORE TOTAL**	
6. Mobilidade	Imóvel (0) □ Cadeira de Rodas (5) □ Semidependente (10) □ Independente (15) □		

Escala de incapacidade funcional da Cruz Vermelha Espanhola

Grau 0 □	Vale -se totalmente por si mesmo. Caminha normalmente.
Grau 1 □	Realiza suficientemente as Atividades da Vida Diária (AVD). Apresenta algumas dificuldades para locomoções complicadas.
Grau 2 □	Apresenta algumas dificuldades nas AVD, necessitando de apoio ocasional. Caminha com ajuda de bengala ou similar.
Grau 3 □	Apresenta graves dificuldades nas AVD, necessitam de apoio em quase todas. Caminha com muita dificuldade ajudado por pelo menos uma pessoa.
Grau 4 □	Impossível realizar, sem ajuda, qualquer das AVD. Capaz de caminhar com extraordinária dificuldade, ajudado por pelo menos duas pessoas.
Grau 5 □	Imobilizado na cama ou sofá, necessitando de cuidados contínuos.

Escala MIF - Medida de Independência Funcional

Categorias	1	2	3	4	5	6	7	**Categorias**	1	2	3	4	5	6	7
1. Alimentação								10. Banheiro							
2. Auto cuidado								11. Banho chuveiro / banheira							
3. Banhar-se								12. Andar / cadeira de rodas							
4. Vestir tronco superior								13. Escadas							
5. Vestit tronco inferior								14. Compreensão							
6. Higiene intima								15. Expressão							
7. Controle vesical								16. Interação Social							
8. Controle intestinal								17. Resolver problemas							
9. Cama / cadeira / cadeira de rodas								18. Memória							
											ESCORE TOTAL				

XIV. AVALIAÇÃO DO RISCO DE FRAGILIDADE DO IDOSO

Protocolo de Identificação do Idoso Vulnerável (VES-13)

1. Idade <75anos (0) □ 75-84anos (1) □ ≥85anos (3) □	2. Estado geral de Saúde: Excelente/Muito Boa/Boa (0) □ Ruim/Muito Ruim (1) □
3. Em média, quanta dificuldade você tem para fazer as seguintes atividades físicas:	**Nível de Dificuldade** *(considerar no máximo 2 pontos)*
3a. Dificuldade para curvar-se, agachar ou ajoelhar-se	Nenhuma / Pouca / Média (0) □ Muita / Incapaz de Fazer (1) □
3b. Dificuldade para levantar ou carregar objetos com peso aproximado de 5 quilos?	Nenhuma / Pouca / Média (0) □ Muita / Incapaz de Fazer (1) □
3c. Dificuldade para elevar ou estender os braços acima do nível do ombro?	Nenhuma / Pouca / Média (0) □ Muita / Incapaz de Fazer (1) □
3d. Dificuldade para escrever ou manusear e segurar pequenos objetos?	Nenhuma / Pouca / Média (0) □ Muita / Incapaz de Fazer (1) □
3e. Dificuldade para andar 400 metros (aproximadamente quatro quarteirões)?	Nenhuma / Pouca / Média (0) □ Muita / Incapaz de Fazer (1) □
3f. Dificuldade para fazer serviço doméstico pesado (esfregar o chão ou limpar janelas)?	Nenhuma / Pouca / Média (0) □ Muita / Incapaz de Fazer (1) □
4. Por causa de sua saúde ou condição física, você tem alguma dificuldade para:	**Nível de Dificuldade** *(considerar no máximo 4 pontos)*
4a. Fazer compras de itens pessoais (itens de higiene e medicamentos)? ( ) Não	( ) Sim→ Você recebe ajuda para fazer compras? → Sim (4) □ Não (0) □ ( ) Não faço compras → por causa de sua saúde? → Sim (4) □ Não (0) □
4b. Lidar com dinheiro (como controlar suas despesas ou pagar contas)? ( ) Não	( ) Sim→ Você recebe ajuda para lidar c/ dinheiro?→ Sim (4) □ Não (0) □ ( ) Não lido c/ dinheiro → por causa de sua saúde? → Sim (4) □ Não (0) □
4c. Atravessar o quarto andando? é permitido o uso de bengala ou andador! ( ) Não	( ) Sim→ Você recebe ajuda para andar? → Sim (4) □ Não (0) □ ( ) Não ando → por causa de sua saúde? → Sim (4) □ Não (0) □
4d. Realizar tarefas domésticas leves (como lavar louça ou fazer limpeza leve)? ( ) Não	( ) Sim→ Você recebe ajuda p/ tarefas domésticas?→ Sim (4) □ Não (0) □ ( ) Não faço essas tarefas → p/ causa de sua saúde?→ Sim (4) □ Não (0) □
4e. Tomar banho de chuveiro ou banheira? ( ) Não	( ) Sim→ Você recebe ajuda p/ tomar banho neles?→ Sim (4) □ Não (0) □ ( ) Não tomo banho neles→ p/ causa de sua saúde?→ Sim (4) □ Não (0) □
	ESCORE TOTAL

Estadiamento funcional (Functional Assessment Staging — FAST)

Estágio	**Caracteristicas**	**Estágio**	**Caracteristicas**
1 □	Nenhuma dificuldade objetiva ou subjetiva	6E □	Incontinência fecal
2 □	Queixas de esquecimento (locais/objetos). Dificuldades subjetivas no trabalho.	7A □	Capacidade de falar limitada a meia dúzia de palavras ou menos, no curso médio de um dia.
3 □	Decréscimo do funcionamento no trabalho, evidente para os colegas. Dificuldade nas viagens para novas localidades.	7B □	Capacidade de falar limitada a uma única palavra inteligível no curso médio de um dia.
4 □	Decréscimo na habilidade de execução de tarefas complexas, manejo de finanças pessoais, execução de compras, etc.	7C □	Capacidade de deambulação perdida
5 □	Requer assistência na escolha de trajes adequados	7D □	Perda da capacidade de se sentar sem assistência

6A ☐	Dificuldade em vestir-se adequadamente	7E ☐	Perda da capacidade de sorrir
6B ☐	Incapaz de banhar-se adequadamente, podendo ter medo do banho.	7F ☐	Perda da capacidade de levantar a cabeça
6C ☐	Incapacidade de manuseio da toalete	7G ☐	Postura fletida
6D ☐	Incontinência urinária	**CLASSIFICAÇÃO**	

Índice De Vulnerabilidade Clinico Funcional (IVCF-20)

1. Idade	60-74 anos (0) ☐ 75-84 anos (1) ☐ ≥85 anos (3) ☐	2. Auto-percepção de Saúde:	Excelente/Muito Boa/Boa (0) ☐ Ruim/Muito Ruim (1) ☐

3. Por causa da sua saúde/condição física, deixou de fazer compras?	Sim (4) ☐ Não ou não faz compras por outros motivos que não a saúde (0) ☐
4. Por causa da sua saúde/condição física, deixou de controlar seu dinheiro?	Sim (4) ☐ Não ou não controla por outros motivos que não a saúde (0) ☐
5. Por causa da sua saúde/condição física, deixou de fazer trabalhos domésticos?	Sim (4) ☐ Não ou não faz esses por outros motivos que não a saúde (0) ☐

6. Por causa da sua saúde/condição física, deixou de tomar banho sozinho?	Sim (6) ☐ Não (0) ☐	14. Você tem alguma das 4 condições abaixo?
7. Algum familiar ou amigo falou que você está ficando esquecido?	Sim (1) ☐ Não (0) ☐	- Perda de peso não intencional de 4,5kg ou 5% do peso corporal no ultimo ano ou 6kg nos últimos 6 meses ou 3 kg no último mês ( )
8. Este esquecimento está piorando nos últimos meses	Sim (1) ☐ Não (0) ☐	
9. Este esquecimento está impedindo a realização de alguma atividade do cotidiano?	Sim (2) ☐ Não (0) ☐	
10. No ultimo mês, você ficou com desânimo, tristeza ou desesperança?	Sim (2) ☐ Não (0) ☐	- Teste da velocidade da marcha (4m) > 5 s ( )
11. No último mês, você perdeu o interesse ou prazer em atividades antes prazerosas?	Sim (2) ☐ Não (0) ☐	- Circunferencia da Panturrilha < 31 cm ( )
12. Você é incapaz de elevar os braços acima do nível do ombro?	Sim (1) ☐ Não (0) ☐	- IMC < 22kg/m^2 ( )
13. Você é incapaz de manusear pequenos objetos?	Sim (1) ☐ Não (0) ☐	Sim (2) ☐ Não (0) ☐
15. Você tem dificuldade para caminhar capaz de impedir a realização das AVD?	Sim (2) ☐ Não (0) ☐	20. Você tem alguma das 3 condições abaixo?
16. Você teve duas ou mais quedas no último ano?	Sim (2) ☐ Não (0) ☐	- Possui ≥ 5 doenças crônicas ( )
17. Você perde urina ou fezes sem querer em algum momento?	Sim (2) ☐ Não (0) ☐	- Uso regular de ≥ 5 medicamentos diferentes / dia ( )
18. Você tem problemas de visão capazes de impedir a realização das AVD?	Sim (2) ☐ Não (0) ☐	- Internação recente nos últimos 6 meses ( )
19. Você tem problemas de audição capazes de impedir a realização das AVD?	Sim (2) ☐ Não (0) ☐	Sim (2) ☐ Não (0) ☐
		ESCORE TOTAL

XV. AVALIAÇÃO DE PERFORMANCE PARA CUIDADOS PALIATIVOS

Índice de Desempenho de Karnofsky

100% ☐	Normal; ausência de queixas, sem evidencias de doença	**50%** ☐	Requer considerável assistência e frequentes cuidados médicos.
90% ☐	Capaz de realizar atividades normais; sinais e sintomas mínimos de doença.	**40%** ☐	Incapacitado; requer cuidados especiais e assistência, auto-cuidado limitado. Permanece mais de 50% do horário vigil sentado ou deitado.
80% ☐	Atividade normal com esforço; alguns sinais ou sintomas de doença. Incapacidade para grande esforço físico, consegue deambular.	**30%** ☐	Severamente incapacitado, indicado hospitalização, embora a morte não seja iminente.
70% ☐	Não requer assistência para cuidados pessoais, mas é incapaz de realizar atividades normais como tarefas caseiras e trabalhos ativos.	**20%** ☐	Muito doente, necessário internação hospitalar e tratamento de suporte. Completamente incapaz de realizar autocuidado. Confinado à cama.
60% ☐	Requer assistência ocasional, mas consegue realizar a maioria dos seus cuidados pessoais.	**10%** ☐	Moribundo; processo de morte progredindo rapidamente.

Escala de Desepenho Paliativo - *Palliative Performance Scale* (PPS)

%	**Deambulação**	**Atividade e vivência de doença**	**Autocuidado**	**Ingesta**	**Nível de Consciência**
100% ☐	Completa	Atividade normal e trabalho. Sem evidencia de doenças.	Completo	Normal	Completa
90% ☐	Completa	Atividade normal e trabalho. Sem evidencia de doenças.	Completo	Normal	Completa
80% ☐	Completa	Atividade normal e trabalho. Sem evidencia de doenças.	Completo	Normal ou Reduzida	Completa
70% ☐	Reduzida	Incapaz para o trabalho. Doença significativa	Completo	Normal ou Reduzida	Completa
60% ☐	Reduzida	Incapaz para hobbies/ trabalho doméstico. Doença significativa	Assistência Ocasional	Normal ou Reduzida	Completa ou períodos de confusão
50% ☐	Maior parte sentado ou deitado	Incapaz para qualquer trabalho. Doença extensa	Assistência Consideravel	Normal ou Reduzida	Completa ou períodos de confusão
40% ☐	Maior parte acamado	Incapaz para a maioria das atividade. Doença extensa.	Assistência Quase Completa	Normal ou Reduzida	Completa ou sonolência. +/-. Confusão
30% ☐	Totalmente acamado	Incapaz para qualquer atividade. Doença extensa.	Dependência completa	Normal ou Reduzida	Completa ou sonolência. +/-. Confusão
20% ☐	Totalmente acamado	Incapaz para qualquer atividade. Doença extensa.	Dependência completa	Mínima a pequenos goles	Completa ou sonolência. +/-. Confusão
10% ☐	Totalmente acamado	Incapaz para qualquer atividade. Doença extensa.	Dependência completa	Cuidados com a boca	Sonolência ou coma. +/-. Confusão

Escala Palliative Care Screening Tool8

Critério número 1 - Doenças de base – Dois pontos para cada subitem:		**Critério número 3** - Condição funcional do paciente – de 0 a quatro pontos	
1. Câncer – metástase ou recidivas	☐	12. Paciente Independente (totalmente independente) 0 ponto	☐
2. DPOC avançada – repetidas exacerbações	☐	13. Paciente Semidependente (restrito em atividade física extenuante) 1 pontos	☐
3. Sequela de AVC – decréscimo de função motora ≥50%	☐	14. Paciente Semidependente (ABVD preservada, AIVD comprometida) 2 pontos	☐
4. Insuficiência renal grave – clearance de creatinina <10 ml/min	☐	15. Paciente Dependente (incapacitado, mais de 50% do tempo deitado) 3 pontos	☐
5. Doença cardíaca grave – ICC com FE do ventrículo esquerdo <25%, miocardiopatia e insuficiência coronariana significativa	☐	15. Paciente Dependente (totalmente dependente, acamado) 4 pontos	☐
6. Outras doenças limitantes à vida do paciente	☐	**Critério número 4** - Condições pessoais do paciente – um ponto para cada subitem:	
Critério número 2 - Doenças associadas – um ponto para cada subitem:		16. Necessidade de ajuda para decisões complexas de tratamento e questões psicológicas ou espirituais não definidas	☐
7. Doença hepática	☐		
8. Doença renal moderada – clearance de creatinina < 60 ml/min	☐	17. Histórico de internações recentes em serviços de emergência	☐
9. DPOC moderada – quadro clínico estável	☐	18. Hospitalizações frequentes por descompensação da doença de base	☐
10. ICC moderada – quadro clínico estável	☐	19. . Internações prolongadas em Unidades de Terapia Intensiva (UTI) ou paciente já internado em UTI com mau prognóstico	☐
11. Outras doenças associadas – o conjunto delas vale 1 ponto	☐	**ESCORE TOTAL**	

XVI. QCÂNCER – Questionário de Risco ao Câncer do ClinRisk		*somente para as mulheres
1. TABAGISMO	**2. ETILISMO**	**3. HISTÓRICO MÉDICO PESSOAL E FAMILIAR**
Não fumante □ Ex-fumante □ Fumante Leve (< 10 anos) □ Fumante Moderado (10-19 anos) □ Fumante Inveterado (>20 anos) □	Não etilista □ <1 unidade por dia □ 1-2 unidades por dia □ >3 unidades por dia □	Você tem história de câncer gastrointestinal na família? Sim □ Não □ Você tem história de câncer de próstata na família? Sim □ Não □ Você tem história de câncer de ovário na família?* Sim □ Não □ Você tem história de câncer de mama na família?* Sim □ Não □ Você tem diabetes tipo 2? Sim □ Não □ Você tem hiperplasia ou pólipo endometrial?* Sim □ Não □ Você tem pancreatite crônica? Sim □ Não □ Você tem DPOC? Sim □ Não □

4. QUEIXAS ATUAIS			
Você tem perda de apetite?	Sim □ Não □	Você tem suor noturno?	Sim □ Não □
Você tem perda de peso involuntária?	Sim □ Não □	Você tem tromboembolismo venoso?	Sim □ Não □
Você tem dor abdominal?	Sim □ Não □	Você tem nódulo testicular?	Sim □ Não □
Você tem inchaço abdominal?	Sim □ Não □	Você tem dor testicular?	Sim □ Não □
Você tem vontade de engolir?	Sim □ Não □	Você tem sangramento pós-menopausa?*	Sim □ Não □
Você tem azia ou má digestão?	Sim □ Não □	Você tem sangramento menstrual irregular?*	Sim □ Não □
Você tem sangramento retal?	Sim □ Não □	Você tem sangramento vaginal depois do sexo?*	Sim □ Não □
Você tem sangue quando você vomita?	Sim □ Não □	Você tem um nódulo na mama?*	Sim □ Não □
Você tem sangue quando você tosse?	Sim □ Não □	Você tem retração da mama ou descarga de mamilo?*	Sim □ Não □
Você tem sangue na urina?	Sim □ Não □	Você tem dor no peito?*	Sim □ Não □
Você tem caroço no pescoço?	Sim □ Não □		

5. MOTIVOS DE CONSULTAS MÉDICAS NO ÚLTIMO ANO			
Mudança de hábito intestinal?	Sim □ Não □	Frequência urinária?	Sim □ Não □
Prisão de ventre?	Sim □ Não □	Noctúria (urinar a noite)?	Sim □ Não □
Tosse?	Sim □ Não □	Impotência? somente para os homens	Sim □ Não □
Contusões inexplicáveis?	Sim □ Não □	**ALTURA (m)** / **PESO (kg)** / **IMC (kg/m²)**	
Anemia (hemoglobina <11g / dL)?	Sim □ Não □		
Retenção urinária?	Sim □ Não □		

HISTÓRICO DE CÂNCER NA FAMÍLIA: 1. Sua mãe, seu pai ou seus irmãos/irmãs, incluindo meio-irmãos, tem ou tiveram câncer? Sim □ Não □

2. Se sim, qual tipo de câncer?

XVII. QUESTÕES SOBRE O TABAGISMO

1. Já fumou mais de 100 cigarros ao longo da vida? Sim □ Não □ 2. Com quantos anos começou a fumar?_____ 3. Com quantos anos parou?_____

4. Fuma atualmente? Sim □ Não □ 5. Se não, com que idade fumou pela última vez? ______ 6. Quantos cigarros fuma ou fumava por dia?______

7. Que tipo de derivado do tabaco você fuma(va)? Cigarro □ Cachimbo □ Mastiga Tabaco □ Cigarro Artesanal □ Charuto □ Narguilé □ Outros □

8. Convive com pessoas que fumam no mesmo ambiente que você? Sim □ Não □ 9. Se sim, onde? Casa □ Trabalho □ Automóvel □ Outros □

TESTE DE FAGERSTRON para os fumantes

1. Quanto tempo após acordar você fuma seu primeiro cigarro? Dentro de 5 min. □ Entre 6 e 30 min. □ Entre 31 e 60 min. □ Após 60 min. □

2, Você acha difícil não fumar em lugares proibidos como igrejas, bibliotecas, cinemas, etc? Sim □ Não □

3. Qual o cigarro do dia que traz mais satisfação? O primeiro da manhã □ Outros □ 4. Quantos cigarros você fuma por dia?

5. Você fuma frequentemente pela manhã? Sim □ Não □ 6. Você fuma, mesmo doente, quando precisa ficar de cama? Sim □ Não □

XVIII. QUESTÕES SOBRE O ETILISMO

1. Já consumiu bebidas alcoólicas? Sim □ Não □ 2. Com quantos anos começou a beber?_____ 3. Com quantos anos parou de beber?_____

4. Bebe atualmente? Sim □ Não □ 5. Se não, com que idade bebeu pela última vez? ______

6. Com que frequência você consome bebida alcoólica?
1 vez por dia □ >1 vez por dia □ 1 vez por semana □ 1-2 vezes por semana □ 2-3 vezes no mês □ Somente em ocasiões especiais □ Nunca □

XIX. QUESTÕES SOBRE ATIVIDADE FÍSICA – Questionário IPAQ

1. Em quantos dias da última semana você **CAMINHOU** por pelo menos 10 minutos contínuos? dias _____ por SEMANA Nenhum

2. Nos dias em que você **CAMINHOU** por pelo menos 10 minutos contínuos, quanto tempo no total você gastou caminhando por dia? ______h_____min

3. Em quantos dias da última semana, você realizou atividades **MODERADAS** (são aquelas que precisam de algum esforço físico e que fazem respirar um pouco mais forte que o normal) por pelo menos 10 minutos contínuos? dias _____ por SEMANA Nenhum

4. Nos dias em que você fez essas atividades **MODERADAS** (são aquelas que precisam de algum esforço físico e que fazem respirar um pouco mais forte que o normal) por pelo menos 10 minutos contínuos, quanto tempo no total você gastou fazendo essas atividades por dia? ______h_____min

5. Em quantos dias da última semana, você realizou atividades **VIGOROSAS** (são aquelas que precisam de algum esforço físico e que fazem respirar muito mais forte que o normal) por pelo menos 10 minutos contínuos? ? dias _____ por SEMANA Nenhum

6. Nos dias em que você fez essas atividades **VIGOROSAS** (são aquelas que precisam de algum esforço físico e que fazem respirar muito mais forte que o normal) por pelo menos 10 minutos contínuos quanto tempo no total você gastou fazendo essas atividades por dia? ______h_____min

7. Quanto tempo no total você gasta **SENTADO** durante um dia de semana? ______h _____min

8. Quanto tempo no total você gasta **SENTADO** durante o final de semana? ______h _____min

XX. QUESTÕES SOBRE SINDROME METABÓLICA

1. Circunferência Abdominal: cm	2. Triglicerídeos: mg/dL	3. HDL Colesterol: mg/dL	4. Glicemia Jejum: mg/dL
5. Circunferência do Quadril: cm	6. Índice Cintura-Quadril:	6. Pressão Arterial: mmHg	

ANEXO A

NÚCLEO DE PESQUISA EM
ONCOLOGIA DA
UNIVERSIDADE FEDERAL DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Correlação entre a Capacidade Funcional e Biomarcadores do Envelhecimento em idosos com câncer.

Pesquisador: Esdras Edgar Batista Pereira

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 2

CAAE: 37386214.3.0000.5634

Instituição Proponente: Núcleo de Pesquisa em Oncologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 927.808

Data da Relatoria: 29/12/2014

Apresentação do Projeto:

A pesquisa se propõe a realizar um estudo transversal analítico comparativo, que será desenvolvido a partir da aplicação da Avaliação Geriátrica Ampla e da analise de biomarcadores do envelhecimento. A amostra da pesquisa será composta por pacientes idosos assistidos pelo Hospital Universitário João de Barros Barreto, formando dois grupos 2 grupos: Grupo com Câncer (GCC) e Grupo sem Câncer (GSC). A avaliação geriátrica ampla incluirá a avaliação sociodemográficos, avaliação clínica e epidemiológica, avaliação da capacidade funcional global, avaliação dos sistemas funcionais, avaliação nutricional e avaliação da qualidade de vida. A mensuração de biomarcadores do envelhecimento será realizada utilizando a amostra do sangue periférico do paciente, para mensuração do comprimento dos telômeros e da expressão da proteína p16INK4a.

Objetivo da Pesquisa:

Objetivo Primário:

Identificar a associação entre a capacidade funcional e biomarcadores do envelhecimento em idosos com câncer.

Endereço: Rua dos Mundurucus, 4457
Bairro: GUAMA **CEP:** 66.073-000
UF: PA **Município:** BELEM
Telefone: (91)3201-6776 **E-mail:** nao.informado@cep.com.br

ANEXO B

HOSPITAL UNIVERSITÁRIO
JOÃO DE BARROS BARRETO -
UFPA

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Correlação entre a Capacidade Funcional e Biomarcadores do Envelhecimento em idosos com câncer.

Pesquisador: Esdras Edgar Batista Pereira

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 1

CAAE: 37386214.3.3001.0017

Instituição Proponente: Núcleo de Pesquisa em Oncologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 941.207

Data da Relatoria: 26/01/2015

Apresentação do Projeto:

A pesquisa se propõe a realizar um estudo transversal analítico comparativo, que será desenvolvido a partir da aplicação da Avaliação Geriátrica Ampla e da analise de biomarcadores do envelhecimento. A amostra da pesquisa será composta por pacientes idosos assistidos pelo Hospital Universitário João de Barros Barreto, formando dois grupos 2 grupos: Grupo com Câncer (GCC) e Grupo sem Câncer (GSC).A avaliação geriátrica ampla incluirá a avaliação sociodemográficos, avaliação clínica e epidemiológica, avaliação da capacidade funcional global, avaliação dos sistemas funcionais, avaliação nutricional e avaliação da qualidade de vida. A mensuração de biomarcadores do envelhecimento será realizada utilizando a amostra do sangue periférico do paciente, para mensuração do comprimento dos telômeros e da expressão da proteína p16INK4a.

Objetivo da Pesquisa:

Identificar a associação entre a capacidade funcional e biomarcadores do envelhecimento em idosos com câncer.

Objetivo Secundário:

Endereço: RUA DOS MUNDURUCUS 4487
Bairro: GUAMA **CEP:** 66.073-000
UF: PA **Município:** BELEM
Telefone: (91)3201-6754 **Fax:** (91)3201-6663 **E-mail:** cephujbb@yahoo.com.br

ANEXO C

PROTOCOLO PARA EXTRAÇÃO DO DNA

PROTOCOLO 1

- **Extração de DNA genômico de 200 µL de sangue total humano ou de mamífero**
- **Extração de DNA genômico de 30 µL de creme leucocitário (buffy coat)**

IMPORTANTE: Aliquotar a quantidade de Tampão de Eluição necessária para o número de amostras e colocar no termomixer a 56°C.

1) Transferir 200 µL de sangue total ou 30 µL de buffy coat dentro de um microtubo de 1,5 mL (não fornecido). Para amostras com volumes menores de 200 µL, completar o volume com PBS 1X ou água (ultrapura, bidestilada ou de injeção).

2) Adicionar 200 µL de Tampão de Lise A e 20 µL de Proteinase K. Homogeneizar a amostra no vortex ou por pipetagem repetitiva.

3) Incubar o microtubo de 1,5 mL por 15 minutos a 56°C, enquanto estiver em agitação contínua no termomixer.

Obs.: Caso não tenha um equipamento com agitação, homogeneizar a amostra cinco vezes no vortex durante a lise.

4) Adicionar 400 µL de Tampão de Ligação B6 e homogeneizar a amostra no vortex ou por pipetagem repetitiva. Transferir toda a mistura para o Tubo Spin RTA e incubar por 1 minuto.

5) Centrifugar por 2 minutos a 13.000 x g. Descartar o tubo inferior com o filtrado e colocar o tubo-filtro em um Tubo de Coleta RTA.

6) Adicionar 500 µL de Tampão de Lavagem I e centrifugar por 1 minuto a 13.000 x g. Descartar o filtrado e colocar o tubo-filtro no mesmo Tubo de Coleta RTA.

7) Adicionar 800 µL de Tampão de Lavagem II e centrifugar por 1 minuto a 13.000 x g. Descartar o filtrado e colocar o tubo-filtro no mesmo Tubo de Coleta RTA.

8) Centrifugar por 4 minutos na velocidade máxima para eliminar completamente o etanol.

9) Colocar o tubo-filtro em um Tubo de Eluição 1,5 mL devidamente identificado. Adicionar 200 µL de Tampão de Eluição pré-aquecido. Incubar a temperatura ambiente por 1 minuto.

Obs.: Destacar a tampa do tubo-filtro antes da centrifugação e colocá-lo novamente no correspondente Tubo de Eluição 1,5 mL.

10) Centrifugar 8.000 x g por 1 minuto. Descartar o tubo-filtro e armazenar a amostra de DNA para futuros testes.

ANEXO D
PRODUÇÕES DO DISCENTE NO PERÍODO DO DOUTORADO

ANEXO I - Analysis of 12 variants in the development of gastric and colorectal cancers.

ANEXO II - Effect of genetic ancestry to the risk of susceptibility to gastric cancer in a mixed population of the Brazilian Amazon.

ANEXO III - Pulmonary hypertension in hospitalized patients with chronic end-stage kidney disease at a referral hospital in nephrology in Pará State, Brazil.

ANEXO IV - Functional Capacity Assessment in Oncogeriatric.

ANEXO V - Polymorphisms in the *CYP2A6* and *ABCC4* genes are associated with a protective effect on chronic myeloid leukemia in the Brazilian Amazon population.

ANEXO VI - Influence of Polymorphism on the *NFKB1* Gene (rs28362491) on the Susceptibility to Sarcopenia in the Elderly of the Brazilian Amazon.

ANEXO VII - Identification of Genomic Variants Associated with the Risk of Acute Lymphoblastic Leukemia in Native Americans from Brazilian Amazonia.

ANEXO VIII - The Role of *SLC22A1* and Genomic Ancestry on Toxicity during Treatment in Children with Acute Lymphoblastic Leukemia of the Amazon Region.

ANEXO IX - Exome Evaluation of Autism-Associated Genes in Amazon American Populations.

ANEXO X - Association between *TP53, PAR1* and *CCR5* gene polymorphisms and non-small cell lung câncer.

ANEXO XI - Identification of pharmacogenomic variants associated with oncology treatments in Brazilian Amazonian Amerindians.

ANEXO XII - Investigation of the and genes in patients who developed fatal fluoropyrimidine-associated toxicity in Northern Brazil.

ANEXO XIII - MUC family influence on acute lymphoblastic leukemia in Native American populations from Brazilian Amazon.

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ORIGINAL ARTICLE

Retrospective Study

Analysis of 12 variants in the development of gastric and colorectal cancers

Giovanna C Cavalcante, Marcos AT Amador, André M Ribeiro dos Santos, Darlen C Carvalho, Roberta B Andrade, Esdras EB Pereira, Marianne R Fernandes, Danielle F Costa, Ney PC Santos, Paulo P Assumpção, Ândrea Ribeiro dos Santos, Sidney Santos

Giovanna C Cavalcante, Marcos AT Amador, André M Ribeiro dos Santos, Darlen C Carvalho, Roberta B Andrade, Ney PC Santos, Ândrea Ribeiro dos Santos, Sidney Santos, Laboratório de Genética Humana e Médica, Universidade Federal do Pará, Belém 66075-970, Brazil

Giovanna C Cavalcante, Darlen C Carvalho, Roberta B Andrade, Esdras EB Pereira, Marianne R Fernandes, Danielle F Costa, Ney PC Santos, Paulo P Assumpção, Ândrea Ribeiro dos Santos, Sidney Santos, Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém 66073-005, Brazil

ORCID number: Giovanna C Cavalcante (0000-0001-9814-4819); Marcos AT Amador (0000-0003-0477-4751); André M Ribeiro dos Santos (0000-0002-7005-3576); Darlen C Carvalho (0000-00022716-860X); Roberta B Andrade (0000- 0001-7745-1799); Esdras EB Pereira (0000-0003-4830-4233); Marianne R Fernandes (0000-0002-1396-3442); Danielle F Costa (0000-0002-7735-7732); Ney PC Santos (0000-0001-8087-7037); Paulo P Assumpção (0000-0003-3846-8445); Ândrea Ribeiro dos Santos (0000-0001-7001-1483); Sidney Santos (0000-0002-9417-8622).

Author contributions: Cavalcante GC and Amador MAT performed the laboratory experiments; Cavalcante GC, Carvalho DC and Andrade RB drafted the manuscript; Pereira EEB, Fernandes MR and Costa DF provided the samples for the study; Cavalcante GC, Ribeiro dos Santos AM and Santos S performed the data analysis; Santos S reviewed the statistical methods of the study; Santos NPC, Assumpção PP and Ribeiro dos Santos Â made substantial contributions to the study design and the manuscript; Cavalcante GC and Santos S designed the study and wrote the final version of the paper.

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Informed consent statement: All participants provided their informed consent prior to study inclusion.

Conflict-of-interest statement: The authors declare no conflict of interests in this study.

Data sharing statement: No additional data are available.

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Correspondence to: Sidney Santos, PhD, Professor, Laboratório de Genética Humana e Médica, Universidade Federal do Pará, Rua Augusto Correa 01, Belém 66075-970, Brazil. sidneysantos@ufpa.br
Telephone: +55-91-32017843
Fax: +55-91-32017843

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Abstract

AIM

To evaluate the relation between 12 polymorphisms and the development of gastric cancer (GC) and colorectal cancer (CRC).

METHODS
In this study, we included 125 individuals with GC diagnosis, 66 individuals with CRC diagnosis and 475 cancer-free individuals. All participants resided in the North region of Brazil and authorized the use of their samples. The 12 polymorphisms (in *CASP8*, *CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *MDM2*, *NFKB1*, *PAR1*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1* genes) were genotyped in a single PCR for each individual, followed by fragment analysis. To avoid misinterpretation due to population substructure, we applied a previously developed set of 61 ancestry-informative markers that can also be genotyped by multiplex PCR. The statistical analyses were performed in Structure v.2.3.4, R environment and SPSS v.20.

RESULTS
After statistical analyses with the control of confounding factors, such as genetic ancestry, three markers (rs79071878 in *IL4*, *rs3730485* in *MDM2* and *rs28362491* in *NFKB1*) were positively associated with the development of GC. One of these markers (rs28362491) and the marker in the *UGT1A1* gene (rs8175347) were positively associated with the development of CRC. Therefore, we investigated whether the joint presence of the deleterious alleles of each marker could affect the development of cancer and we obtained positive results in all analyses. Carriers of the combination of alleles RP1 + DEL (rs79071878 and rs28361491, respectively) are at 10-times greater risk of developing GC than carriers of other combinations. Similarly, carriers of the combination of DEL + RARE (rs283628 and rs8175347) are at about 12-times greater risk of developing CRC than carriers of other combinations.

CONCLUSION
These findings are important for the comprehension of gastric and CRC development, particularly in highly admixed populations, such as the Brazilian population.

Key words: Inflammatory processes; Immune response; Genomic and cellular stability; Gastric cancer; Colorectal cancer; Amazon

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Core tip: Gastric cancer and colorectal cancer (CRC) are among the most incident and aggressive types of cancer in Brazil, especially in the Amazon region. Alterations in genes involved in pathways of immune responses, inflammatory processes or genomic and cellular stability may generate cellular imbalances and lead to tumorigenesis. Therefore, it is vital to understand the effect of different alleles in the development of gastric and CRC, which could contribute to the early detection of these types of cancer, increasing the survival chances of the patient.

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INTRODUCTION

Cancer is one of the main causes of death worldwide[1]. In Brazil, it is considered a severe problem of public health, and in the North region of this country gastric cancer (GC) and colorectal cancer (CRC) are among the three most incident and aggressive types of cancer[2].

Carcinogenesis is a multifactorial process. Gastritis and colitis have been related to the development of GC[3,4] and CRC[5,6], respectively, but they are not determinant. Infection by *Helicobacter pylori*, one of the most common human infectious agents, is also very important for the development of gastritis and GC[7]. However, it should not be considered the only cause for development of this type of cancer[8]. Genetics also play a major role in the carcinogenesis, and there is much to be discovered regarding this subject.

Genes involved in important pathways, such as inflammatory processes, metabolism of carcinogens, cell stability and hormonal pathways, are possible susceptibility factors to cancer[9-14]. Alterations in these genes may generate imbalances in such pathways and trigger tumor development. In this study, we investigated the following 12 polymorphisms of important genes of these pathways: *CASP8* (rs3834129), *CYP2E1* (96 bp-deletion), *CYP19A1* (rs11575899), *IL1A* (rs3783553), *IL4* (rs79071878), *MDM2* (rs3730485), *NFKB1* (rs28362491), *PAR1* (rs11267092), *TP53* (rs17878362), *TYMS* (rs16430), *UGT1A1* (rs8175347) and *XRCC1* (rs3213239).

These genes and polymorphisms have been studied in association with various types of cancer in different populations, *e.g*, breast cancer[15-19], bladder cancer[20], endometrial cancer[21], acute lymphoblastic leukemia[22], chronic lymphoblastic leukemia[23], oral carcinoma[24,25], lung cancer[26], nasopharyngeal cancer[27], thyroid cancer[28], hepatocellular carcinoma[29], GC[30-39] and CRC[40-50]. Therefore, these markers were chosen based on the importance of each gene as a potential influencing factor in the susceptibility of tumor development. All are functional polymorphisms that correspond to insertion/deletion (INDEL) of small DNA fragments and can be analyzed in a single multiplex PCR, which makes it a cheap and accessible methodology that could be used in different laboratories worldwide.

Thus, the aim of this work was to investigate the association between 12 polymorphisms in genes related to pathways of immune/inflammatory response (*CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *NFKB1* and *PAR1*) and cellular or genomic stability (*CASP8*, *MDM2*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1*) and the development of GC

Table 1 Technical characteristics of the studied markers

Gene	ID	Type	Length, bp	Primers	Amplicon, bp
CASP8	rs3834129	INDEL	6	F-5'CTCTTCAATGCTTCCTTGAGGT3' R-5'CTGCATGCCAGGAGCTAAGTAT3'	249-255
CYP2E1	-	INDEL	96	F-5'TGTCCCAATACAGTCACCTCTTT3' R-5'GGCTTTTATTTGTTTTGCATCTG3'	303-399
CYP19A1	rs11575899	INDEL	3	F-5'TGCATGAGAAAGGCATCATATT3' R-5'AAAAGGCACATTCATAGACAAAAA3'	122-125
IL1A	rs3783553	INDEL	4	F-5'TGGTCCAAGTTGTGCTTATCC3' R-5'ACAGTGGTCTCATGGTTGTCA3'	230-234
IL4	rs79071878	VNTR	70	F-5'AGGGTCAGTCTGGCTACTGTGT3' R-5'CAAATCTGTTCACCTCAACTGC3'	147/217/287
MDM2	rs3730485	INDEL	40	F-5'GGAAGTTTCCTTTCTGGTAGGC3' R-5'TTTGATGCGGTCTCATAAATTG3'	192-232
NFKB1	rs28362491	INDEL	4	F-5'TATGGACCGCATGACTCTATCA3' R-5'GGCTCTGGCATCCTAGCAG3'	366-370
PAR1	rs11267092	INDEL	13	F-5'AAAACTGAACTTTGCCGGTGT3' R-5'GGGCCTAGAAGTCCAAATGAG3'	265-277
TP53	rs17878362	INDEL	16	F-5'GGGACTGACTTTCTGCTCTTGT3' R-5'GGGACTGTAGATGGGTGAAAAG3'	148-164
TYMS	rs16430	INDEL	6	F-5'ATCCAAACCAGAATACAGCACA3' R-5'CTCAAATCTGAGGGAGCTGAGT3'	213-219
UGT1A1	rs8175347	VNTR	2	F-5'CTCTGAAAGTGAACTCCCTGCT3' R-5'AGAGGTTCGCCCTCTCCTAT3'	133/135/137/139
XRCC1	rs3213239	INDEL	4	F-5'GAACCAGAATCCAAAAGTGACC3' R-5'AGGGGAAGAGAGAGAAGGAGAG3'	243-247

F: Forward; INDEL: Insertion/deletion; R: Reverse; VNTR: Variable number tandem repeat.

and CRC in a population in Northern Brazil. In addition, we investigated the influence of genetic ancestry in the development of these types of cancer in the studied population.

MATERIALS AND METHODS

Samples

In this study, we included three groups: (1) 125 individuals with GC diagnosis; (2) 66 individuals with CRC diagnosis; and (3) 475 cancer-free individuals that were considered the control group. The cancer-free individuals did not have personal or familial histories of any kind of cancer and they did not show any symptoms or signs of cancer. All participants resided in Belém, which is a city located in the Northern region of Brazil, and signed an informed consent, with approval by the Committee for Research Ethics of Hospital João de Barros Barreto under Protocol No. CAAE 25865714.6.0000.0017.

DNA Extraction and Quantification

Samples of peripheral blood were collected from all individuals of the study and the DNA extraction was performed accordingly[51]. DNA quantification was performed with NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, United States).

Genotyping

Samples were then submitted to multiplex PCR and fragment analysis in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, United States) according to the protocol described[22]. Technical characteristics of the studied markers are presented in Table 1. Due to the high level of genetic admixture in the studied population, we applied a panel of 61 ancestry-informative markers to avoid misinterpretations caused by population substructure, as described[52,53].

Statistical Analyses

Statistical analyses were conducted with different programs. Ancestry analyses were performed in Structure v.2.3.4[54], and tests concerning the genotyping analyses (Student's *t*-test, Pearson's χ^2 test, Mann-Whitney test and logistic regression) were performed in R[55] and in SPSS v.20.0 (IBM Corp., Armonk, NY, United States).

The genotype distribution was assessed as established by Hardy-Weinberg equilibrium (HWE), with post-test correction by the Bonferroni method for multiple tests. P-value $\leqslant$ 0.05 was considered statistically significant.

RESULTS

All population distributions were according to HWE (P > 0.004) for the analyzed polymorphisms, with the exception of the *IL4* marker in the control group. The observed deviation seems to be due to a significant increase of heterozygotes in this population (P = 0.0003).

We also investigated the possible confounding factors of age, sex and genetic ancestry. Table 2 shows these results. When considered statistically significant in the comparison between groups (GC patients *vs*

Table 2 Demographic data for patient and control groups

Variable	GC	CRC	Control	*P*-value	
				GC *vs* Control	CRC *vs* Control
n	120	64	475	-	-
Age, yr[1]	57.02 ± 1.29	52.84 ± 1.90	55.59 ± 0.91	0.522	0.294
Sex, % of male/female	55.0/45.0	45.3/54.70	34.7/65.3	0.000[3]	0.098
European ancestry[2]	0.42 ± 0.01	0.53 ± 0.02	0.47 ± 0.01	0.002[3]	0.003[3]
African ancestry[2]	0.26 ± 0.01	0.20 ± 0.01	0.23 ± 0.01	0.071	0.016[3]
Amerindian ancestry[2]	0.32 ± 0.01	0.27 ± 0.02	0.30 ± 0.01	0.114	0.100

[1]Values are expressed as mean ± SD. Significance was obtained by Student's *t*-test; [2]Values are expressed as mean ± SD. Significance was obtained by Mann-Whitney test; [3]Statistically significant. CRC: Colorectal cancer; GC: Gastric cancer.

cancer-free individuals, and CRC patients *vs* cancer-free individuals; $P \leq 0.05$), such characteristics were controlled in the logistic regression that assessed whether there are significant differences in the following tests: (1) carriers of INS/INS genotype *vs* carriers of other genotypes (INS/DEL + DEL/DEL); (2) carriers of DEL/DEL genotype *vs* carriers of other genotypes (INS/DEL + INS/INS); and (3) additive effect of the alleles (joint presence of the significant alleles from tests Ⅰ and Ⅱ).

In the analyses with GC patients, positive associations were observed for the markers rs79071878 (*IL4* gene), rs3730485 (*MDM2* gene) and rs28362491 (*NFKB1* gene) after correction of confounding factors for this group (sex and European ancestry) (Table 3). For rs79071878, carriers of the RP1/RP1 genotype have approximately 3-fold increased chances of developing GC than carriers of other genotypes (RP1/RP1 + RP1/RP2) [$P = 0.002$; odds ratio (OR) = 2.857; 95% confidence interval (CI) = 1.490-5.479]. For rs3730485, INS/INS genotype shows a protection effect for the development of GC in comparison with different genotypes ($P = 0.021$; OR = 0.409; 95%CI: 0.192-0.872). For rs28362491, carriers of the DEL/DEL genotype have more chances of developing GC than carriers of the other genotypes ($P = 0.006$; OR = 2.918; 95%CI: 1.352-6.298).

In the analyses with CRC patients, markers rs28362491 (*NFKB1* gene) and rs8175347 (*UGT1A1* gene) showed positive association after the correction of confounding factors (European and African ancestries) (Table 4). Similar to the result for GC, carriers of the DEL/DEL genotype for rs28362491 should present more chances of developing CRC in comparison to carriers of other genotypes ($P = 0.006$; OR = 3.732; 95%CI: 1.451-9.599). For rs8175347, which has multiple alleles (*1, *28, *36 and *37), our results show that 8% of the CRC patients and 0.6% of the cancer-free individuals carry at least one of the rare alleles (*36 and *37). Comparing both groups, we observed that such allele presence could lead to almost 13-fold increased chances of developing CRC ($P = 0.001$; OR = 12.849; 95%CI: 2.906-56.817).

In addition, we analyzed whether the joint presence of the alleles that were statistically significant when in homozygosis (RP1 allele of rs79071878, INS allele of rs3730485, DEL allele of rs28362491 and *36 and *37 alleles in rs8175347) may affect the development of GC and CRC. After controlling for the confounding factors, we obtained statistically significant results for both GC ($P = 0.004311$) and CRC ($P = 3.52 \times 10^{-6}$) analyses. These findings are shown in Figure 1 for GC and in Figure 2 for CRC.

We highlight some positive associations of these alleles due to the absence of neutral effect (logOR = 0 or OR = 1) in the 95%CI for GC [IL4(RP1): OR = 3.068, 95%CI: 1.036-9.088; NFKB1(DEL): OR = 3.414, 95%CI: 1.347-8.654; IL4(RP1) + NFKB1(DEL): OR = 10.475, 95%CI: 4.845-22.624); IL4(RP1) + NFKB1(DEL) + MDM2(INS): OR = 4.437, 95%CI: 2.948-6.686] and CRC [NFKB1(DEL): OR = 2.552, 95%CI: 2.014-3.238; NFKB1(DEL) + UGT1A1(RARE): OR = 11.929, 95%CI: 1.732-82.187)].

DISCUSSION

In the HWE analysis for the *IL4* marker in the control group, the large amount of heterozygotes could be explained either by selective advantage of the heterozygote or by an intense or continuous process of admixture between populations with different genetic backgrounds. Allele frequencies for this marker vary greatly between the three main populations that contributed to the formation of the Brazilian population; the frequency of the RP2 allele has been described as 0.74 among Europeans, 0.23 among Amerindians and 0.42 among Africans[56]. Due to the recent formation of the Brazilian population, we believe that the admixture process is more fitted to explain the observed disequilibrium.

In the analysis for GC, we observed a positive association between the *IL4* marker (rs79071878) and the development of this type of cancer. This polymorphism is a 70-bp variable number tandem repeat located in an intron of *IL4*, which is an interleukin involved in inflammatory pathways. We did not find other studies relating to this polymorphism and GC, but the increased risk of the development of bladder cancer among the carriers of RP1 allele has been previously described[14,57]. Recently, we reported

Table 3 Genotypic and allelic distributions of the investigated polymorphisms for patients with gastric cancer in comparison to control group

Genotype	GC	Control	*P* value[1]	OR (95%CI)[1]	Genotype	GC	Control	*P* value[1]	OR (95%CI)[1]
CASP8	120	475			RP2/RP2	18 (15.1)	154 (32.5)	0.189	0.673 (0.372-1.216)
DEL/DEL	11 (9.2)	90 (19.0)	0.650	0.892 (0.545-1.461)	Allele RP1	0.54	0.41		
INS/DEL	70 (58.3)	230 (48.4)			Allele RP2	0.46	0.59		
INS/INS	39 (32.5)	155 (32.6)	0.080	1.936 (0.924-4.058)	*NFKB1*	120	473		
Allele DEL	0.38	0.43			DEL/DEL	34 (28.3)	117 (24.7)	0.006[2]	2.918 (1.352-6.298)
Allele INS	0.62	0.57			INS/DEL	71 (59.2)	246 (52.0)		
MDM2	120	475			INS/INS	15 (12.5)	110 (23.3)	0.88	0.959 (0.5662-1.610)
DEL/DEL	13 (10.8)	33 (6.9)	0.199	1.365 (0.849-2.192)	Allele DEL	0.58	0.51		
INS/DEL	46 (38.3)	168 (35.4)			Allele INS	0.42	0.49		
INS/INS	61 (50.9)	274 (57.7)	0.021[2]	0.409 (0.192-0.872)	*PAR1*	113	473		
Allele DEL	0.30	0.25			DEL/DEL	66 (58.4)	273 (57.7)	0.068	0.482 (0.221-1.054)
Allele INS	0.70	0.75			INS/DEL	36 (31.9)	169 (35.7)		
TP53	120	475			INS/INS	11 (9.7)	31 (6.6)	0.949	0.984 (0.601-1.610)
DEL/DEL	91 (75.8)	350 (73.7)	0.999	138214253.0 (0.000)	Allele DEL	0.74	0.76		
INS/DEL	27 (22.5)	116 (24.4)			Allele INS	0.26	0.24		
INS/INS	2 (1.7)	9 (1.9)	0.247	0.708 (0.395-1.270)	*CYP2E1*	116	475		
Allele DEL	0.87	0.86			DEL/DEL	94 (81.0)	398 (83.8)	0.999	276187721.0 (0.000)
Allele INS	0.13	0.14			INS/DEL	21 (18.1)	73 (15.4)		
TYMS	120	475			INS/INS	1 (0.9)	4 (0.8)	0.574	1.193 (0.644-2.212)
DEL/DEL	16 (13.3)	65 (13.7)	0.409	1.231 (0.752-2.015)	Allele DEL	0.90	0.91		
INS/DEL	53 (44.2)	224 (47.2)			Allele INS	0.10	0.09		
INS/INS	51 (42.5)	186 (39.2)	0.867	1.060 (0.536-2.096)	*CYP19A1*	120	475		
Allele DEL	0.35	0.37			DEL/DEL	18 (15.0)	76 (16.0)	0.654	1.127 (0.669-1.897)
Allele INS	0.65	0.63			INS/DEL	67 (55.8)	248 (52.2)		
XRCC1	119	474			INS/INS	35 (29.2)	151 (31.8)	0.415	1.334 (0.667-2.671)
DEL/DEL	10 (8.4)	35 (7.4)	0.346	1.257 (0.781-2.021)	Allele DEL	0.43	0.42		
INS/DEL	48 (40.3)	179 (37.8)			Allele INS	0.57	0.58		
INS/INS	61 (51.3)	260 (54.8)	0.396	0.697 (0.303-1.604)	*UGT1A1*	120	464		
Allele DEL	0.29	0.26			*1/*1	49 (40.8)	206 (44.5)	0.792	1.109 (0.515-2.386)
Allele INS	0.71	0.74			*1/*28	57 (47.5)	209 (45.0)		
IL1A	120	475			*28/*28	12 (10.0)	46 (9.9)	0.445	1.205 (0.746-1.946)
DEL/DEL	17 (14.2)	86 (18.1)	0.626	0.882 (0.522-1.460)	*36/*1	2 (1.7)	3 (0.6)		
INS/DEL	63 (52.5)	246 (51.8)			*36/*37	0 (0.0)	0 (0.0)	0.585	1.941 (0.180-20.973)
INS/INS	40 (33.3)	143 (30.1)	0.143	1.705 (0.835-3.482)	*1/*37	0 (0.0)	0 (0.0)		
Allele DEL	0.40	0.44			Allele *36	0.01	0.01		
Allele INS	0.60	0.56			Allele *1	0.65	0.67		
IL4	119	474			Allele *28	0.34	0.32		
RP1/RP1	28 (23.6)	69 (14.5)	0.002[2]	2.857 (1.490-5.479)	Allele *37	0.00	0.00		
RP1/RP2	73 (61.3)	251 (53.0)							

Data for GC and Control columns are presented as *n* or *n* (%). [1]Analysis of combined genotypes (INS/INS *vs* others, or DEL/DEL *vs* others) with adjusted values for confounding factors (sex and European ancestry) in logistic regression; [2]Statistically significant. GC: Gastric cancer.

that the frequency of the RP1 allele of rs79071878 is higher in the North of Brazil (0.414) than in the other regions of the country (mean = 0.233), probably due to the elevated frequency of this marker in Amerindian populations[56]. Data have revealed that the highest incidence of GC in Brazil occurs in the North region. The apparent overlap between the greater incidence of GC and the elevated frequency of RP1 (rs78071878) in the North region of Brazil seems to corroborate the results that indicate that the carriers of homozygous RP1 allele have greater chances of developing GC than the carriers of other genotypes, possibly due to the close relation of this type of cancer with increased inflammation. More studies involving this polymorphism in different admixed populations in this country are recommended.

As for the polymorphism in the *MDM2* gene (rs3730485), we observed that the carriers of INS/INS genotype have less chances of developing GC than carriers of the other genotypes of this marker. To the best of our knowledge, there are no other studies reporting the positive association of this polymorphism and GC development, but the DEL allele has been shown to be associated with increased risk of developing various types of cancer, *e.g*, hepatocellular carcinoma[29], breast cancer[58], prostate cancer[59] and colon cancer[60] in different populations. *MDM2* is an oncogene responsible for the regulation of *TP53* expression[61]. The INS allele of rs3730485 may reduce the activity of *MDM2*, possibly increasing the activity of the tumor suppressor *TP53* and then reducing the chances of developing cancer.

In the current study, we observed an association of the DEL/DEL genotype of the polymorphism in *NFKB1* (rs28362491) with increased chances of developing both GC and CRC. This is an INDEL polymorphism that is

Table 4 Genotypic and allelic distributions of the investigated polymorphisms for patients with colorectal cancer in comparison to control group

Genotype	CRC	Control	*P* value[1]	OR (95%CI)[a]	Genotype	CRC	Control	*P*-value[1]	OR (95%CI)[1]
CASP8	63	475			RP2/RP2	16 (25.4)	154 (32.5)	0.871	1.068 (0.482-2.368)
DEL/DEL	13 (20.6)	90 (19.0)	0.676	0.888 (0.508-1.552)	Allele RP1	0.44	0.41		
INS/DEL	28 (44.4)	230 (48.4)			Allele RP2	0.56	0.59		
INS/INS	22 (35.0)	155 (32.6)	0.939	0.974 (0.503-1.887)	*NFKB1*	63	473		
Allele DEL	0.43	0.43			DEL/DEL	16 (25.4)	117 (24.7)	0.006[2]	3.732 (1.451-9.599)
Allele INS	0.57	0.57			INS/DEL	42 (66.7)	246 (52.0)		
MDM2	64	475			INS/INS	5 (7.9)	110 (23.3)	0.829	0.935 (0.508-1.723)
DEL/DEL	7 (10.9)	33 (6.9)	0.412	1.166 (0.143-9.487)	Allele DEL	0.60	0.51		
INS/DEL	25 (39.1)	168 (35.4)			Allele INS	0.40	0.49		
INS/INS	32 (50.0)	274 (57.7)	0.986	0.995 (0.546-1.811)	*PAR1*	63	473		
Allele DEL	0.30	0.25			DEL/DEL	37 (58.7)	273 (57.7)	0.464	0.704 (0.275-1.801)
Allele INS	0.70	0.75			INS/DEL	20 (31.8)	169 (35.7)		
TP53	64	475			INS/INS	6 (9.5)	31 (6.6)	0.813	0.937 (0.546-1.608)
DEL/DEL	47 (73.4)	350 (73.7)	0.886	1.166 (0.143-9.487)	Allele DEL	0.75	0.76		
INS/DEL	16 (25.0)	116 (24.4)			Allele INS	0.25	0.24		
INS/INS	1 (1.6)	9 (1.9)	0.986	0.995 (0.546-1.811)	*CYP2E1*	62	475		
Allele DEL	0.86	0.86			DEL/DEL	56 (90.3)	398 (83.8)	0.999	189364591.0 (0.000)
Allele INS	0.14	0.14			INS/DEL	6 (9.7)	73 (15.4)		
TYMS	63	475			INS/INS	0 (0.0)	4 (0.8)	0.351	0.655 (0.269-1.593)
DEL/DEL	11 (17.5)	65 (13.7)	0.304	1.342 (0.765-2.354)	Allele DEL	0.95	0.91		
INS/DEL	31 (49.2)	224 (47.2)			Allele INS	0.05	0.09		
INS/INS	21 (33.3)	186 (39.2)	0.429	0.751 (0.369-1.526)	*CYP19A1*	64	475		
Allele DEL	0.42	0.37			DEL/DEL	7 (10.9)	76 (16.0)	0.297	0.747 (0.431-1.293)
Allele INS	0.58	0.63			INS/DEL	33 (51.6)	248 (52.2)		
XRCC1	64	474			INS/INS	24 (37.5)	151 (31.8)	0.313	1.532 (0.669-3.508)
DEL/DEL	4 (6.2)	35 (7.4)	0.771	1.082 (0.637-1.838)	Allele DEL	0.37	0.42		
INS/DEL	27 (42.2)	179 (37.8)			Allele INS	0.63	0.58		
INS/INS	33 (51.6)	260 (54.8)	0.445	1.528 (0.515-4.535)	*UGT1A1*	63	464		
Allele DEL	0.27	0.26			*1/*1	20 (31.7)	206 (44.5)	0.098	0.541 (0.262-1.120)
Allele INS	0.73	0.74			*1/*28	32 (50.8)	209 (45.0)		
IL1A	64	475			*28/*28	6 (9.5)	46 (9.9)	0.370	1.282 (0.745-2.205)
DEL/DEL	10 (15.6)	86 (18.1)	0.657	0.880 (0.500-1.548)	*36/*1	3 (4.8)	3 (0.6)		
INS/DEL	33 (51.6)	246 (51.8)			*36/*37	1 (1.6)	0 (0.0)	0.001[2]	12.849 (2.906-56.817)
INS/INS	21 (32.8)	143 (30.1)	0.610	1.208 (0.584-2.368)	*1/*37	1 (1.6)	0 (0.0)		
Allele DEL	0.41	0.44			Allele *36	0.03	0.01		
Allele INS	0.59	0.56			Allele *1	0.60	0.67		
IL4	63	474			Allele *28	0.35	0.32		
RP1/RP1	8 (12.7)	69 (14.5)	0.195	1.493 (0.814-2.740)	Allele *37	0.02	0.00		
RP1/RP2	39 (61.9)	251 (53.0)							

Data for CRC and Control columns are presented as *n* or *n* (%). [1]Analysis of combined genotypes (INS/INS *vs* others, or DEL/DEL *vs* others) with adjusted values for confounding factors (European and African ancestries) in logistic regression; [2]Statistically significant. CRC: Colorectal cancer; INDEL: Insertion/deletion.

located in the promoter region of the gene, which is highly involved in inflammatory pathways. The DEL/DEL genotype has been previously associated with an increased risk of developing GC in a Japanese population[37] and bladder cancer in a Chinese population[62]. In addition, the DEL allele of this polymorphism has been related to the development of ulcerative colitis and *H. pylori* infection[63,64], which can increase the risk of CRC and GC. Regarding the INS/INS genotype, it has been associated with decreased development risk of ovarian cancer[65] and with increased risk of developing melanoma[66], while the DEL/DEL genotype has also been associated with reduced risk of developing other types of cancer[67]. Previous studies have suggested that the effects of rs28362491 on the risk of carcinogenesis may be ethnic- and cancer type-specific, as described by two meta-analyses involving Asian and Caucasian populations[68,69].

The *UGT1A1* gene is involved in hepatic detoxification and metabolization of different substances. The studied marker in this gene (rs8175347) has four possible alleles [*36 (5 repeats), *1 (6 repeats), *28 (7 repeats) and *37 (8 repeats)]. Allele *1 is considered the wild-type and the most common allele, *28 is the second most common allele and *36 and *37 are considered rare alleles. In this study, we observed that the presence of at least one of the rare alleles of this polymorphism appears to increase the chances of developing CRC by 13-times. In the literature, some studies show that alleles *36 and *37 are absent or extremely rare in different populations[70,71], but there are no studies relating the association of these alleles with the development of CRC. Although little is known about *36 and *37 alleles, it

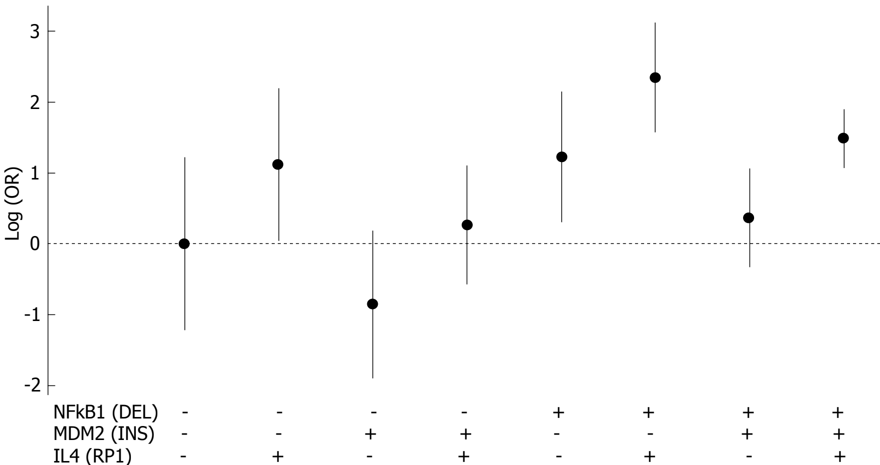

Figure 1 Analysis of the joint presence of three alleles regarding gastric cancer development. DEL allele of rs28362491 is represented by NFKB1 (DEL), INS allele of rs3730485 is represented by MDM2 (INS) and RP1 allele of rs79071878 is represented by IL4(RP1). All possible combinations were considered. Allele presence is represented by (+) and allele absence is represented by (-). DEL: Deletion; GC: Gastric cancer; INS: Insertion.

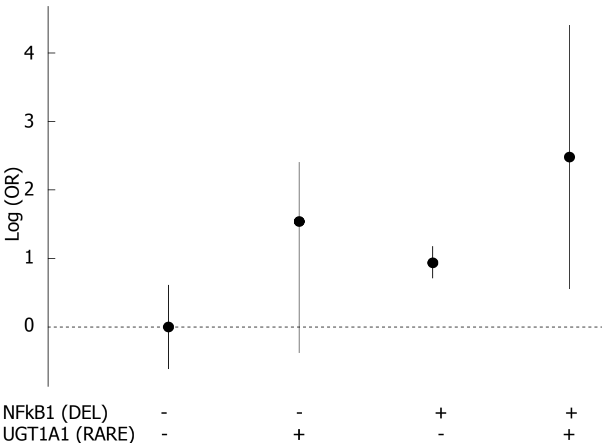

Figure 2 Analysis of the joint presence of two alleles regarding colorectal cancer development. DEL allele of rs28362491 is represented by NFKB1 (DEL) and *36 and *37 alleles in rs8175347 are represented by UGT1A1 (RARE). All possible combinations were considered. Allele presence is represented by (+) and allele absence is represented by (-). CRC: Colorectal cancer; DEL: Deletion.

is possible that the presence of such alleles could lead to a decreased activity of the *UGT1A1* gene, inducing the carcinogenesis process. We understand that the sample size of CRC patients may have influenced the observed result in this study, but we believe that our findings indicate the need to expand the investigation to a great number of patients from other Brazilian admixed populations, considering the important increase rate we observed.

In addition, we investigated the joint presence of the alleles that were statistically significant in homozygosis in the analyses discussed above. This is important because the interaction of alleles in different loci could lead to an increased effect in the carcinogenesis. Recently, this kind of additive effect has been reported for multiple types of cancer in different populations[72,74], but there is a lack of this type of study involving GC and CRC in the Brazilian population. To the best of our knowledge, this is the first study using this approach for these types of cancer in a Brazilian population.

The analyses of combined effect showed statistical significance for both types of cancer, presenting some interesting results. Among these, it is notable that: (1) individuals carrying both RP1 (*IL4* marker) and DEL (*NFKB1* marker) alleles have more than 10-fold increased chances of developing GC than carriers of the other alleles; and (2) individuals carrying the DEL allele (*NFKB1* marker) and at least one of the rare alleles *36 and *37 (*UGT1A1* marker) have almost 12-fold increased chances of developing CRC than carriers of other alleles of these markers. These results reinforce the importance of knowing which markers may play a role in cancer development.

In conclusion, we investigated 12 polymorphisms in genes with functions in inflammatory pathways, immune response or cellular and genomic stability (*i.e. CASP8*, *CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *MDM2*, *NFKB1*, *PAR1*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1*) regarding the development of GC and CRC. Our findings indicate that some of these markers may be related to the development of GC and CRC. Moreover, the interaction between such polymorphisms may increase the risk of developing these types of cancer. These results contribute to a greater knowledge of possible risk factors in the development of GC and CRC.

ARTICLE HIGHLIGHTS

Research background

Our research group, located in the North region of Brazil, has been working with population genetics for many years. More recently, we have designed a set of 12 markers that are able to be genotyped in a single multiplex PCR and capillary electrophoresis, which is faster than Sanger sequencing and cheaper than real-time PCR. All markers in this set are in genes related to different pathways (*e.g.* inflammatory and immune response, and cellular and genomic stability). We have previously investigated not only the association of this set with the development of different diseases (*i.e.* acute lymphoblastic leukemia and leprosy), but also the distribution of these markers in individuals from the five regions of Brazil (North, Northeast, Midwest, Southeast and South) and in individuals representative of the main parental populations of this country

(Europeans, Africans and Native Americans). However, we believe it also is important to investigate the association of this set with the development of other types of cancer, such as gastric cancer (GC) and colorectal cancer (CRC).

Research motivation

GC and CRC are two of the most incident and aggressive types of malignant neoplasms in Brazil. A notable aspect of the Brazilian population is that it is highly admixed and, then, it is important not to extrapolate results from one region to another. For instance, these types of cancer are particularly frequent in the North region of Brazil. In general, most cases of GC and CRC are diagnosed in advanced stages and the death rate related to these types of cancer is high. To help early diagnosis, many research groups worldwide have been working to identify biomarkers able to detect increased risk of developing such types of cancer. Considering the high incidence of GC and CRC in the North region, we believe that it is important to study such neoplasms in this region.

Research objectives

In this study, we analyzed the association of 12 polymorphisms in genes involved in inflammatory pathways, immune response or cellular and genomic stability (namely, *CASP8*, *CYP2E1*, *CYP19A1*, *IL1A*, *IL4*, *MDM2*, *NFKB1*, *PAR1*, *TP53*, *TYMS*, *UGT1A1* and *XRCC1*) regarding GC and CRC development in a population from the North region of Brazil. Understanding the distribution of these markers in the studied population helps to improve the knowledge of the different factors that lead to cancer development.

Research methods

We collected blood samples from the participants (125 GC patients, 66 CRC patients and 475 cancer-free individuals), from which we extracted the DNA using a phenol-chloroform-based method. The studied 12-polymorphism set can be genotyped through amplification in a single multiplex PCR, followed by capillary electrophoresis. The different statistical analyses were performed in Structure v.2.3.4 and SPSS v.20 programs, and the R language. We analyzed the allelic and genotypic distribution of these markers, as well as the combined effect of the statistically significant alleles. The latter approach is not a common approach for studying GC and CRC. In fact, to the best of our knowledge, this is the first study using this kind of approach for these types of cancer in the Brazilian population. It gave us interesting results.

Research results

After performing the statistical analyses with correction of confounding factors, we observed positive associations between the markers rs79071878 (*IL4* gene), rs3730485 (*MDM2* gene) and rs28362491 (*NFKB1* gene) and GC development, as well as between the markers rs28362491 (*NFKB1* gene) and rs8175347 (*UGT1A1* gene) and CRC development. When we analyzed the combined effect of the alleles of the statistically significant genotypes of each marker (RP1 allele of rs79071878, INS allele of rs3730485, DEL allele of rs28362491 and *36 and *37 alleles in rs8175347), we obtained statistically significant results for both types of cancer. From these results, we highlight that: (1) individuals carrying both RP1 (IL4 marker) and DEL (NFKB1 marker) alleles have more than 10-fold increased chances of developing GC than carriers of the other alleles; and (2) individuals carrying the DEL allele (NFKB1 marker) and at least one of the rare alleles *36 and *37 (UGT1A1 marker) have almost 12-fold increased chances of developing CRC than carriers of other alleles of these markers. Our results reinforce the importance of knowing the role that different markers play in the development of cancer, which may contribute to the early detection of GC and CRC.

Research conclusions

In this study, we observed that the individual or joint presence of some alleles of the 12 polymorphisms of the set may affect the development of GC (RP1 allele of rs79071878, INS allele of rs3730485 and DEL allele of rs28362491) and/or CRC (DEL allele of rs28362491 and *36 and *37 alleles in rs8175347) in a population from the North region of Brazil. To the best of our knowledge, this is the first time it has been reported, and it supports the notion that more attention should be given to these polymorphisms in relation to the development of GC and CRC. Considering the results we obtained, we recommend that the individual and the joint presence of these markers should be further investigated in the other regions of Brazil, due to the high levels of admixture in this country, and in other types of cancer.

Research perspectives

Although there have been many advances in the complex field of oncogenetics, there is still a lot remaining to be discovered. The present study investigated 12 polymorphisms, some of them not frequently studied, and showed statistically significant association between four of these markers and the development of GC and CRC in a population from the North region of Brazil. It shows the importance of studying different polymorphisms in important genes, some of which may be involved not only in the development of GC and CRC but also of other types of malignant neoplasms. In addition, our study reinforces the notion of investigating different types of cancer in genetically admixed populations, such as the Brazilian population.

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REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 **INCA - Instituto Nacional de Câncer José Alencar Gomes da Silva**. Estimativa 2016: Incidência de Câncer no Brasil. Rio de Janeiro: INCA, 126p, 2015

3 **Watari J**, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, Miwa H, Lim KJ, Das KM. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *World J Gastroenterol* 2014; **20**: 5461-5473 [PMID: 24833876 DOI: 10.3748/wjg.v20.i18.5461]

4 **Yoon SB**, Park JM, Lim CH, Cho YK, Choi MG. Effect of Helicobacter pylori eradication on metachronous gastric cancer after endoscopic resection of gastric tumors: a meta-analysis. *Helicobacter* 2014; **19**: 243-248 [PMID: 25056262 DOI: 10.1111/hel.12146]

5 **Yashiro M**. Ulcerative colitis-associated colorectal cancer. *World J Gastroenterol* 2014; **20**: 16389-16397 [PMID: 25469007 DOI: 10.3748/wjg.v20.i44.16389]

6 **Herszényi L**, Barabás L, Miheller P, Tulassay Z. Colorectal cancer in patients with inflammatory bowel disease: the true impact of the risk. *Dig Dis* 2015; **33**: 52-57 [PMID: 25531497 DOI: 10.1159/000368447]

7 **Wang F**, Meng W, Wang B, Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. *Cancer Lett* 2014; **345**: 196-202 [PMID: 23981572 DOI: 10.1016/j.canlet.2013.08.016]

8 **Khatoon J**, Rai RP, Prasad KN. Role of Helicobacter pylori in gastric cancer: Updates. *World J Gastrointest Oncol* 2016; **8**: 147-158 [PMID: 26909129 DOI: 10.4251/wjgo.v8.i2.147]

9 **Vannucci L**, Stepankova R, Grobarova V, Kozakova H, Rossmann P, Klimesova K, Benson V, Sima P, Fiserova A, Tlaskalova-Hogenova H. Colorectal carcinoma: Importance of colonic environment for anti-cancer response and systemic immunity. *J Immunotoxicol* 2009; **6**: 217-226 [PMID: 19908940 DOI: 10.3109/15476910903334343]

10 **Mantovani A**. Molecular pathways linking inflammation and cancer. *Curr Mol Med* 2010; **10**: 369-373 [PMID: 20455855]

11 **Del Prete A**, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A. Molecular pathways in cancer-related inflammation. *Biochem Med* (Zagreb) 2011; **21**: 264-275 [PMID: 22420240]

12 **Lin CW**, Hsieh YS, Hsin CH, Su CW, Lin CH, Wei LH, Yang SF, Chien MH. Effects of NFKB1 and NFKBIA gene polymorphisms on susceptibility to environmental factors and the clinicopathologic development of oral cancer. *PLoS One* 2012; **7**: e35078 [PMID: 22509384 DOI: 10.1371/journal.pone.0035078]

13 **Janakiram NB**, Rao CV. The role of inflammation in colon cancer.

Adv Exp Med Biol 2014; **816**: 25-52 [PMID: 24818718 DOI: 10.1007/978-3-0348-0837-8_2]

14 **Elingarami S**, Liu H, Kalinjuma AV, Hu W, Li S, He N. Polymorphisms in NEIL-2, APE-1, CYP2E1 and MDM2 Genes are Independent Predictors of Gastric Cancer Risk in a Northern Jiangsu Population (China). *J Nanosci Nanotechnol* 2015; **15**: 4815-4828 [PMID: 26373042]

15 **Ramalhinho AC**, Fonseca-Moutinho JA, Breitenfeld Granadeiro LA. Positive association of polymorphisms in estrogen biosynthesis gene, CYP19A1, and metabolism, GST, in breast cancer susceptibility. *DNA Cell Biol* 2012; **31**: 1100-1106 [PMID: 22300440 DOI: 10.1089/dna.2011.1538]

16 **Guan X**, Liu H, Ju J, Li Y, Li P, Wang LE, Brewster AM, Buchholz TA, Arun BK, Wei Q, Liu Z. Genetic variant rs16430 6bp > 0bp at the microRNA-binding site in TYMS and risk of sporadic breast cancer risk in non-Hispanic white women aged ≤ 55 years. *Mol Carcinog* 2015; **54**: 281-290 [PMID: 24166930 DOI: 10.1002/mc.22097]

17 **Pineda B**, García-Pérez MÁ, Cano A, Lluch A, Eroles P. Associations between aromatase CYP19 rs10046 polymorphism and breast cancer risk: from a case-control to a meta-analysis of 20,098 subjects. *PLoS One* 2013; **8**: e53902 [PMID: 23342035 DOI: 10.1371/journal.pone.0053902]

18 **Hashemi M**, Omrani M, Eskandari-Nasab E, Hasani SS, Mashhadi MA, Taheri M. A 40-bp insertion/deletion polymorphism of Murine Double Minute2 (MDM2) increased the risk of breast cancer in Zahedan, Southeast Iran. *Iran Biomed J* 2014; **18**: 245-249 [PMID: 25326024]

19 **Kuhlmann JD**, Bankfalvi A, Schmid KW, Callies R, Kimmig R, Wimberger P, Siffert W, Bachmann HS. Prognostic relevance of caspase 8 -652 6N InsDel and Asp302His polymorphisms for breast cancer. *BMC Cancer* 2016; **16**: 618 [PMID: 27507139 DOI: 10.1186/s12885-016-2662-x]

20 **Ahirwar D**, Kesarwani P, Manchanda PK, Mandhani A, Mittal RD. Anti- and proinflammatory cytokine gene polymorphism and genetic predisposition: association with smoking, tumor stage and grade, and bacillus Calmette-Guérin immunotherapy in bladder cancer. *Cancer Genet Cytogenet* 2008; **184**: 1-8 [PMID: 18558283 DOI: 10.1016/j.cancergencyto.2008.02.015]

21 **Olson SH**, Orlow I, Bayuga S, Sima C, Bandera EV, Pulick K, Faulkner S, Tommasi D, Egan D, Roy P, Wilcox H, Asya A, Modica I, Asad H, Soslow R, Zauber AG. Variants in hormone biosynthesis genes and risk of endometrial cancer. *Cancer Causes Control* 2008; **19**: 955-963 [PMID: 18437511 DOI: 10.1007/s10552-008-9160-7]

22 **Carvalho DC**, Wanderley AV, Amador MA, Fernandes MR, Cavalcante GC, Pantoja KB, Mello FA, de Assumpção PP, Khayat AS, Ribeiro-Dos-Santos Â, Santos S, Dos Santos NP. Amerindian genetic ancestry and INDEL polymorphisms associated with susceptibility of childhood B-cell Leukemia in an admixed population from the Brazilian Amazon. *Leuk Res* 2015 [PMID: 26321572 DOI: 10.1016/j.leukres.2015.08.008]

23 **Karakosta M**, Kalotychou V, Kostakis A, Pantelias G, Rombos I, Kouraklis G, Manola KN. UGT1A1*28 polymorphism in chronic lymphocytic leukemia: the first investigation of the polymorphism in disease susceptibility and its specific cytogenetic abnormalities. *Acta Haematol* 2014; **132**: 59-67 [PMID: 24458221 DOI: 10.1159/000355714]

24 **Yang CM**, Chen HC, Hou YY, Lee MC, Liou HH, Huang SJ, Yen LM, Eng DM, Hsieh YD, Ger LP. A high IL-4 production diplotype is associated with an increased risk but better prognosis of oral and pharyngeal carcinomas. *Arch Oral Biol* 2014; **59**: 35-46 [PMID: 24169152 DOI: 10.1016/j.archoralbio.2013.09.010]

25 **Tang YI**, Liu Y, Zhao W, Yu T, Yu H. Caspase-8 polymorphisms and risk of oral squamous cell carcinoma. *Exp Ther Med* 2015; **10**: 2267-2276 [PMID: 26668627 DOI: 10.3892/etm.2015.2832]

26 **Ji GH**, Li M, Cui Y, Wang JF. The relationship of CASP 8 polymorphism and cancer susceptibility: a meta-analysis. *Cell Mol Biol* (Noisy-le-grand) 2014; **60**: 20-28 [PMID: 25553350]

27 **Yang ZH**, Dai Q, Zhong L, Zhang X, Guo QX, Li SN. Association of IL-1 polymorphisms and IL-1 serum levels with susceptibility to nasopharyngeal carcinoma. *Mol Carcinog* 2011; **50**: 208-214 [PMID: 21154765 DOI: 10.1002/mc.20706]

28 **Santoro AB**, Vargens DD, Barros Filho Mde C, Bulzico DA, Kowalski LP, Meirelles RM, Paula DP, Neves RR, Pessoa CN, Struchine CJ, Suarez-Kurtz G. Effect of UGT1A1, UGT1A3, DIO1 and DIO2 polymorphisms on L-thyroxine doses required for TSH suppression in patients with differentiated thyroid cancer. *Br J Clin Pharmacol* 2014; **78**: 1067-1075 [PMID: 24910925 DOI: 10.1111/bcp.12437]

29 **Dong D**, Gao X, Zhu Z, Yu Q, Bian S, Gao Y. A 40-bp insertion/deletion polymorphism in the constitutive promoter of MDM2 confers risk for hepatocellular carcinoma in a Chinese population. *Gene* 2012; **497**: 66-70 [PMID: 22285926 DOI: 10.1016/j.gene.2012.01.004]

30 **Lurje G**, Husain H, Power DG, Yang D, Groshen S, Pohl A, Zhang W, Ning Y, Manegold PC, El-Khoueiry A, Iqbal S, Tang LH, Shah MA, Lenz HJ. Genetic variations in angiogenesis pathway genes associated with clinical outcome in localized gastric adenocarcinoma. *Ann Oncol* 2010; **21**: 78-86 [PMID: 19622587 DOI: 10.1093/annonc/mdp280]

31 **Malik MA**, Sharma K, Goel S, Zargar SA, Mittal B. Association of TP53 intron 3, 16 bp duplication polymorphism with esophageal and gastric cancer susceptibility in Kashmir Valley. *Oncol Res* 2011; **19**: 165-169 [PMID: 21473292]

32 **Pan XF**, Xie Y, Loh M, Yang SJ, Wen YY, Tian Z, Huang H, Lan H, Chen F, Soong R, Yang CX. Polymorphisms of XRCC1 and ADPRT genes and risk of noncardia gastric cancer in a Chinese population: a case-control study. *Asian Pac J Cancer Prev* 2012; **13**: 5637-5642 [PMID: 23317230]

33 **Fujimoto D**, Hirono Y, Goi T, Katayama K, Matsukawa S, Yamaguchi A. The activation of proteinase-activated receptor-1 (PAR1) promotes gastric cancer cell alteration of cellular morphology related to cell motility and invasion. *Int J Oncol* 2013; **42**: 565-573 [PMID: 23242308 DOI: 10.3892/ijo.2012.1738]

34 **Qiao W**, Wang T, Zhang L, Tang Q, Wang D, Sun H. Association study of single nucleotide polymorphisms in XRCC1 gene with the risk of gastric cancer in Chinese population. *Int J Biol Sci* 2013; **9**: 753-758 [PMID: 23983608 DOI: 10.7150/ijbs.6783]

35 **Shen R**, Liu H, Wen J, Liu Z, Wang LE, Wang Q, Tan D, Ajani JA, Wei Q. Genetic polymorphisms in the microRNA binding-sites of the thymidylate synthase gene predict risk and survival in gastric cancer. *Mol Carcinog* 2015; **54**: 880-888 [PMID: 24756984 DOI: 10.1002/mc.22160]

36 **Zeng XF**, Li J, Li SB. A functional polymorphism in IL-1A gene is associated with a reduced risk of gastric cancer. *Tumour Biol* 2014; **35**: 265-268 [PMID: 23900673 DOI: 10.1007/s13277-013-1034-2]

37 **Arisawa T**, Tahara T, Shiroeda H, Yamada K, Nomura T, Yamada H, Hayashi R, Matsunaga K, Otsuka T, Nakamura M, Shimasaki T, Toshikuni N, Kawada N, Shibata T. Functional promoter polymorphisms of NFKB1 influence susceptibility to the diffuse type of gastric cancer. *Oncol Rep* 2013; **30**: 3013-3019 [PMID: 24101096 DOI: 10.3892/or.2013.2768]

38 **Ghoshal U**, Tripathi S, Kumar S, Mittal B, Chourasia D, Kumari N, Krishnani N, Ghoshal UC. Genetic polymorphism of cytochrome P450 (CYP) 1A1, CYP1A2, and CYP2E1 genes modulate susceptibility to gastric cancer in patients with Helicobacter pylori infection. *Gastric Cancer* 2014; **17**: 226-234 [PMID: 23686565 DOI: 10.1007/s10120-013-0269-3]

39 **Hua T**, Qinsheng W, Xuxia W, Shuguang Z, Ming Q, Zhenxiong L, Jingjie W. Nuclear factor-kappa B1 is associated with gastric cancer in a Chinese population. *Medicine* (Baltimore) 2014; **93**: e279 [PMID: 25526460 DOI: 10.1097/MD.0000000000000279]

40 **Yin G**, Morita M, Ohnaka K, Toyomura K, Hamajima N, Mizoue T, Ueki T, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Yasunami Y, Maekawa T, Takenaka K, Ichimiya H, Terasaka R. Genetic polymorphisms of XRCC1, alcohol consumption, and the risk of colorectal cancer in Japan. *J Epidemiol* 2012; **22**: 64-71 [PMID: 22186158]

41 **Tian Z**, Li YL, Liu JG. XRCC1 Arg399Gln polymorphism contributes to increased risk of colorectal cancer in Chinese population. *Mol Biol Rep* 2013; **40**: 4147-4151 [PMID: 23712778 DOI: 10.1007/s11033-012-2463-5]

42 **Le Marchand L**, Donlon T, Seifried A, Wilkens LR. Red meat intake, CYP2E1 genetic polymorphisms, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1019-1024 [PMID: 12376502]

43 **Morita M**, Tabata S, Tajima O, Yin G, Abe H, Kono S. Genetic polymorphisms of CYP2E1 and risk of colorectal adenomas in the Self Defense Forces Health Study. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 1800-1807 [PMID: 18628434 DOI: 10.1158/1055-9965]

44 **Morita M**, Le Marchand L, Kono S, Yin G, Toyomura K, Nagano J, Mizoue T, Mibu R, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Maekawa T, Yasunami Y, Takenaka K, Ichimiya H, Imaizumi N. Genetic polymorphisms of CYP2E1 and risk of colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 235-241 [PMID: 19124503 DOI: 10.1158/1055-9965]

45 **Andersen V**, Christensen J, Overvad K, Tjønneland A, Vogel U. Polymorphisms in NFkB, PXR, LXR and risk of colorectal cancer in a prospective study of Danes. *BMC Cancer* 2010; **10**: 484 [PMID: 20836841 DOI: 10.1186/1471-2407-10-484]

46 **Sameer AS**, Nissar S, Qadri Q, Alam S, Baba SM, Siddiqi MA. Role of CYP2E1 genotypes in susceptibility to colorectal cancer in the Kashmiri population. *Hum Genomics* 2011; **5**: 530-537 [PMID: 22155602]

47 **Silva TD**, Felipe AV, Pimenta CA, Barão K, Forones NM. CYP2E1 RsaI and 96-bp insertion genetic polymorphisms associated with risk for colorectal cancer. *Genet Mol Res* 2012; **11**: 3138-3145 [PMID: 23007992 DOI: 10.4238/2012.September.3.2]

48 **Jiang O**, Zhou R, Wu D, Liu Y, Wu W, Cheng N. CYP2E1 polymorphisms and colorectal cancer risk: a HuGE systematic review and meta-analysis. *Tumour Biol* 2013; **34**: 1215-1224 [PMID: 23355335 DOI: 10.1007/s13277-013-0664-8]

49 **Mohd Suzairi MS**, Tan SC, Ahmad Aizat AA, Mohd Aminudin M, Siti Nurfatimah MS, Andee ZD, Ankathil R. The functional -94 insertion/deletion ATTG polymorphism in the promoter region of NFKB1 gene increases the risk of sporadic colorectal cancer. *Cancer Epidemiol* 2013; **37**: 634-638 [PMID: 23806437 DOI: 10.1016/j.canep.2013.05.007]

50 **Kopp TI**, Andersen V, Tjonneland A, Vogel U. Polymorphisms in NFKB1 and TLR4 and interaction with dietary and life style factors in relation to colorectal cancer in a Danish prospective case-cohort study. *PLoS One* 2015; **10**: e0116394 [PMID: 25705893 DOI: 10.1371/journal.pone.0116394]

51 **Sambrook J**, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. 2nd ed. New York: Cold Springer Harbor, 1989: 1626

52 **Santos NP**, Ribeiro-Rodrigues EM, Ribeiro-Dos-Santos AK, Pereira R, Gusmão L, Amorim A, Guerreiro JF, Zago MA, Matte C, Hutz MH, Santos SE. Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. *Hum Mutat* 2010; **31**: 184-190 [PMID: 19953531 DOI: 10.1002/humu.21159]

53 **Ramos BR**, D'Elia MP, Amador MA, Santos NP, Santos SE, da Cruz Castelli E, Witkin SS, Miot HA, Miot LD, da Silva MG. Neither self-reported ethnicity nor declared family origin are reliable indicators of genomic ancestry. *Genetica* 2016; **144**: 259-265 [PMID: 26984822 DOI: 10.1007/s10709-016-9894-1]

54 **Pritchard JK**, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000; **155**: 945-959 [PMID: 10835412]

55 **R Development Core Team.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2008. URL: http://www.R-project.org

56 **Amador MA**, Cavalcante GC, Santos NP, Gusmão L, Guerreiro JF, Ribeiro-dos-Santos Â, Santos S. Distribution of allelic and genotypic frequencies of IL1A, IL4, NFKB1 and PAR1 variants in Native American, African, European and Brazilian populations. *BMC Res Notes* 2016; **9**: 101 [PMID: 26879815 DOI: 10.1186/s13104-016-1906-9]

57 **Jia Y**, Xie X, Shi X, Li S. Associations of common IL-4 gene polymorphisms with cancer risk: A meta-analysis. *Mol Med Rep* 2017; **16**: 1927-1945 [PMID: 28656227 DOI: 10.3892/mmr.2017.6822]

58 **Gallegos-Arreola MP**, Márquez-Rosales MG, Sánchez-Corona J, Figuera LE, Zúñiga-González G, Puebla-Pérez AM, Delgado-Saucedo JI, Montoya-Fuentes H. Association of the Del1518 Promoter (rs3730485) Polymorphism in the MDM2 Gene with Breast Cancer in a Mexican Population. *Ann Clin Lab Sci* 2017; **47**: 291-297 [PMID: 28667029]

59 **Hashemi M**, Amininia S, Ebrahimi M, Simforoosh N, Basiri A, Ziaee SAM, Narouie B, Sotoudeh M, Mollakouchekian MJ, Rezghi Maleki E, Hanafi-Bojd H, Rezaei M, Bahari G, Taheri M, Ghavami S. Association between polymorphisms in TP53 and MDM2 genes and susceptibility to prostate cancer. *Oncol Lett* 2017; **13**: 2483-2489 [PMID: 28454424 DOI: 10.3892/ol.2017.5739]

60 **Gansmo LB**, Vatten L, Romundstad P, Hveem K, Ryan BM, Harris CC, Knappskog S, Lønning PE. Associations between the MDM2 promoter P1 polymorphism del1518 (rs3730485) and incidence of cancer of the breast, lung, colon and prostate. *Oncotarget* 2016; **7**: 28637-28646 [PMID: 27081698 DOI: 10.18632/oncotarget.8705]

61 **Ma Y**, Bian J, Cao H. MDM2 SNP309 rs2279744 polymorphism and gastric cancer risk: a meta-analysis. *PLoS One* 2013; **8**: e56918 [PMID: 23451111 DOI: 10.1371/journal.pone.0056918]

62 **Li P**, Gu J, Yang X, Cai H, Tao J, Yang X, Lu Q, Wang Z, Yin C, Gu M. Functional promoter -94 ins/del ATTG polymorphism in NFKB1 gene is associated with bladder cancer risk in a Chinese population. *PLoS One* 2013; **8**: e71604 [PMID: 23977085 DOI: 10.1371/journal.pone.0071604]

63 **Yang X**, Li P, Tao J, Qin C, Cao Q, Gu J, Deng X, Wang J, Liu X, Wang Z, Wu B, Gu M, Lu Q, Yin C. Association between NFKB1 -94ins/del ATTG Promoter Polymorphism and Cancer Susceptibility: An Updated Meta-Analysis. *Int J Genomics* 2014; **2014**: 612972 [PMID: 24895544 DOI: 10.1155/2014/612972]

64 **Karban AS**, Okazaki T, Panhuysen CI, Gallegos T, Potter JJ, Bailey-Wilson JE, Silverberg MS, Duerr RH, Cho JH, Gregersen PK, Wu Y, Achkar JP, Dassopoulos T, Mezey E, Bayless TM, Nouvet FJ, Brant SR. Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. *Hum Mol Genet* 2004; **13**: 35-45 [PMID: 14613970 DOI: 10.1093/hmg/ddh008]

65 **Chen LP**, Cai PS, Liang HB. Association of the genetic polymorphisms of NFKB1 with susceptibility to ovarian cancer. *Genet Mol Res* 2015; **14**: 8273-8282 [PMID: 26345753 DOI: 10.4238/2015]

66 **Escobar GF**, Arraes JA, Bakos L, Ashton-Prolla P, Giugliani R, Callegari-Jacques SM, Santos S, Bakos RM. Polymorphisms in CYP19A1 and NFKB1 genes are associated with cutaneous melanoma risk in southern Brazilian patients. *Melanoma Res* 2016; **26**: 348-353 [PMID: 27145040 DOI: 10.1097/CMR.0000000000000267]

67 **Fu W**, Zhuo ZJ, Chen YC, Zhu J, Zhao Z, Jia W, Hu JH, Fu K, Zhu SB, He J, Liu GC. NFKB1 -94insertion/deletion ATTG polymorphism and cancer risk: Evidence from 50 case-control studies. *Oncotarget* 2017; **8**: 9806-9822 [PMID: 28039461 DOI: 10.18632/oncotarget.14190]

68 **de Martino M**, Haitel A, Schatzl G, Klingler HC, Klatte T. The CASP8 -652 6N insertion/deletion promoter polymorphism is associated with renal cell carcinoma risk and metastasis. *J Urol* 2013; **190**: 717-722 [PMID: 23313206 DOI: 10.1016/j.juro.2013.01.008]

69 **Xu L**, Huang S, Chen W, Song Z, Cai S. NFKB1 -94 insertion/deletion polymorphism and cancer risk: a meta-analysis. *Tumour Biol* 2014; **35**: 5181-5187 [PMID: 24532467 DOI: 10.1007/s13277-014-1672-z]

70 **Horsfall LJ**, Zeitlyn D, Tarekegn A, Bekele E, Thomas MG, Bradman N, Swallow DM. Prevalence of clinically relevant UGT1A alleles and haplotypes in African populations. *Ann Hum Genet* 2011; **75**: 236-246 [PMID: 21309756 DOI: 10.1111/j.1469-1809.2010.00638.x]

71 **Alkharfy KM**, Alghamdi AM, Bagulb KM, Al-Jenoobi FI, Al-Mohizea AM, Al-Muhsen S, Halwani R, Parvez MK, Al-Dosari MS. Distribution of selected gene polymorphisms of UGT1A1 in a Saudi population. *Arch Med Sci* 2013; **9**: 731-738 [PMID: 24049537 DOI: 10.5114/aoms.2013.37012]

72 **Jamhiri I**, Saadat I, Omidvari S. Genetic polymorphisms of superoxide dismutase-1 A251G and catalase C-262T with the risk of colorectal cancer. *Mol Biol Res Commun* 2017; **6**: 85-90 [PMID: 28775994]

73 **Sangalli A**, Orlandi E, Poli A, Maurichi A, Santinami M, Nicolis M, Ferronato S, Malerba G, Rodolfo M, Gomez Lira M. Sex-specific effect of RNASEL rs486907 and miR-146a rs2910164 polymorphisms' interaction as a susceptibility factor for melanoma skin cancer. *Melanoma Res* 2017; **27**: 309-314 [PMID: 28654546 DOI: 10.1097/CMR.0000000000000360]

74 **Zhao MM**, Zhang Y, Shen L, Ren YW, Li XL, Yin ZH, Zhou BS. Genetic variations in TERT-CLPTM1L genes and risk of lung cancer in a Chinese population. *Asian Pac J Cancer Prev* 2014; **15**: 2809-2813 [PMID: 24761905 DOI: 10.3892/ol.2017.6289]

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SHORT REPORT

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Effect of genetic ancestry to the risk of susceptibility to gastric cancer in a mixed population of the Brazilian Amazon

Ellen Moreno da Silva[1,2,4*†], Marianne Fernandes Rodrigues[1,2†], Darlen Cardoso de Carvalho[1,2], Luciana Pereira Colares Leitao[1], Giovanna Chaves Cavalcante[2], Esdras Edgar Batista Pereira[1], Antônio André Conde Modesto[1,2], João Farias Guerreiro[2], Paulo Pimentel de Assumpção[1,2,3], Sidney Emanuel Batista dos Santos[1,2] and Ney Pereira Carneiro dos Santos[1,2]

Abstract

Background: Global literature describes differences in the incidence of gastric cancer among populations. For instance, Europeans have lower incidence rates of gastric cancer in relation to Latin and Asian populations, particularly Korean and Japanese populations. However, only a few studies have been able to verify the occurrence of gastric cancer in admixed populations with high interethnic degree mix, such as the Brazilian Amazon region.

Results: We observed an increase in European ancestry in the control group compared to the case group (47% vs. 41%). Using increments of 10%, compared to categorical distribution of European ancestry in the sample, we found a difference in the contribution between cases and controls ($p = 0.03$). Multiple logistic regression was performed to determine the influence of European ancestry in susceptibility to gastric cancer in the sample. According to the adopted model, for each 10% increase in European ancestry, there is a 20% decrease chance of developing gastric cancer ($P = 0.0121$; $OR = 0.81$; 95% CI 0.54–0.83).

Conclusion: Overall, the results suggest that a greater contribution of European ancestry can be a protective factor for the development of gastric cancer in the studied Amazon population. It can help to establish protocols able to predict susceptibility to gastric cancer in admixed populations.

Background

Stomach cancer, also known as gastric cancer, is the third leading cause of cancer death worldwide [1]. The incidence of stomach cancer is the sixth highest in the world [2] and is among the highest incidences in Latin America [3].

The literature reports differences in the incidence of gastric cancer among populations. For example, Europeans have lower rates of gastric cancer incidence when compared to Latin and Asian populations, especially Japanese and Korean [4, 5]. These differences in incidence can be attributed to risk factors associated with lifestyle, such as diet, obesity, stress and physical inactivity. In addition, other studies suggest that the lower incidence of gastric cancer in the European population may be related to lower frequency of genes associated with gastric cancer susceptibility [5, 6].

In this context, it is conceivable that heterogeneity among populations and different genetic ancestries can contribute to the varying levels of susceptibility to cancer development. This is especially important in admixed populations, such as the Brazilian population, which is known to be one of the most heterogeneous populations in the world, with contributions from three main parental groups: Amerindian, European and African [7–9].

*Correspondence: Ellenmoreno.biomed@gmail.com
†Ellen Moreno da Silva and Marianne Fernandes Rodrigues contributed equally to this work
[4] Instituto de Ciências Biológicas, Laboratório de Genética Humana e Médica, Cidade Universitária Prof. José da Silveira Netto, Rua Augusto Corrêa, 01, BOX: 8615, Belém, PA CEP 66.075-970, Brazil
Full list of author information is available at the end of the article

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Literature demonstrates the influence of genetic ancestry and the risk of developing various types of cancer, such as breast [10–12], colorectal [13], leukemia [14] and ovarian cancer [15]. More specifically, there is a work that explores the influence of genetic ancestry in the risk of developing gastric cancer among Brazilian populations [12].

Therefore, the objective of this study is to investigate the influence of genetic ancestry in susceptibility to gastric cancer in a population of the Amazon region with a high degree of interethnic admixture.

Methods

Cases and control

The participants of the research were chosen based on a case–control study. Participants from both case and control groups were recruited from free services in public institutions, were from the same socioeconomic level, and belonged to the same geographic area.

The case group constituted of 137 individuals that were diagnosed with gastric adenocarcinoma, attended by the Unified Health System (SUS), in a public hospital that is reference in the treatment of this kind of neoplasm (Hospital Universitário João de Barros Barreto, Pará, Brazil). Most patients involved in the study (80%) presented advanced tumor staging—grade III and IV according to Borrmann classification.

The control group constituted of 262 cancer-free individuals, attended in the Laboratório de Exercício Resistido e de Saúde (LERES), from a public university of the region (Universidade Estadual do Pará, Pará, Brazil).

Ethical approval

The protocol used in the study was approved by the Ethics Committee of the University Hospital João de Barros Barreto (Protocol Number 3505/2004). All patients in the present study signed a consent form.

Analysis of genetic ancestry

The analysis of genetic ancestry was performed using a panel of 48 ancestry informative markers (AIM) developed by Santos et al. [16]. Amplification was performed using three multiplex PCR reactions with 16 markers each. PCR separation and analysis was performed by capillary electrophoresis using the ABI PRISM 3130 sequencing and GeneMapper ID Software v3.2. Individual proportions of ancestries of Europeans, Africans and Amerindians were estimated using the STRUCTURE software v2.3.3, with three parental populations (European, African and Amerindian).

Parental populations involved individuals that were representative of three great ancestral groups: 222 Amerindian from nine tribes in the Brazilian Amazon (Tiriyó, Waiãpi, Zoé, Urubu-Kaapor, Awa-Guajá, Parakanã, Wai Wai, Gavião and Zoró), 211 African (Angola, Mozambique, Congo Republic, Cameroon and Ivory Coast) and 268 European (Portugal and Spain). More details on these populations can be found in [17].

Statistical analysis

All statistical analyses were performed using the statistical program SPSS v.20.0 (SPSS, Chicago, IL. USA). Chi squared test in pairs was applied for group comparisons of categorical variables (sex), while Student's t-test was used for the analysis of quantitative variables (age). For comparisons of ancestry among the samples, we used the Mann–Whitney test. Multiple logistic regression analyses were performed to estimate odds ratios (ORs) and 95% confidence intervals (CIs). In these analyses, variables considered confounding factors (age, sex) were corrected. All statistical tests were two-tailed and based on $P < 0.05$ probability to be significant.

Results

We analyzed 137 patients with gastric cancer and 262 cancer-free individuals. Table 1 shows the demographic characteristics of these groups. The case group showed a predominance of men while the control group showed a predominance of women. The results were statistically significant between the case and control groups regarding the following variables: age ($P < 0.001$), sex ($P < 0.001$) and European ancestry ($P = 0.001$).

According to the ancestry analyses conducted in this study, the ethnic composition of the case group was 41% European, 26% African and 33% Amerindian. By comparison, the control group's ancestry contributions was 47% European, 23% African and 30% Amerindian (Table 1). The results revealed that there was a higher contribution of European ancestry in the control group compared to the case group (47% vs. 41%). Using increments of 10%, we compared the categorical distribution of

Table 1 Demographic variables for patients with gastric cancer and the control group

Variable	Case	Control	*P*
Age, years[a]	52.81 ± 18.89	46.80 ± 24.48	< 0.001
Sex (women/men)	34/103	156/106	< 0.001
Genetic ancestry[b]			
European ancestry	0.415 ± 0.143	0.466 ± 0.158	0.001
African ancestry	0.259 ± 0.140	0.232 ± 0.117	0.089
Amerindian ancestry	0.326 ± 0.142	0.302 ± 0.137	0.079

[a] Values are as expressed as mean (± SD = standard deviation). Significance determined by Student's *t*-test

[b] Values are as expressed as mean ± SD. Significance determined by Mann–Whitney test

European ancestry in the sample and found a difference in this ancestry contribution between cases and controls (P = 0.03) (Table 2).

Multiple logistic regressions were performed to determine the influence of European ancestry in the susceptibility to gastric cancer in the sample (Table 3). According to the adopted model, for each 10% increase in European ancestry there is a 20% lower chance of developing gastric cancer (P = 0.0121; OR = 0.81; 95% CI 0.68–0.95).

Table 3 Odds ratio (OR) and 95% confidence intervals (CIs) in the logistic regression model of European ancestry

Genetic ancestry	Case mean	Control mean	OR[a]	95% CI[a]	*P*[a]
European ancestry	0.415 ± 0.143	0.466 ± 0.158	0.807	0.682–0.953	0.0121

[a] Logistic regression adjusted for sex and age. Odds-ratio of the analysis of 10% ethnicity increase

Discussion

In our analyses, the case group presented a greater proportion of men (75%), while in the control group, the proportion was greater among women (60%). The incidence of gastric cancer, as well as other gastrointestinal neoplasms, was greater in men than in women. This finding has been observed in different populations worldwide [18–22]. The justification for this disparity in incidence between men and women goes beyond the explanation of sex differences for exposure to known risk factors. Many studies have suggested a protective effect of estrogen in the development of gastric cancer [22, 23].

To better comprehend the sex difference in the susceptibility to gastric cancer, we performed new statistical tests, which included isolated analyses for men (Additional file 1) and for women (Additional file 2). In both men and women, the European ancestry was the most prevalent, being more frequent in the control group than in the case group.

In the analyses involving only men (Additional file 1), European ancestry was statistically different between case and control (P = 0.009). Logistic regression analysis showed a reduction of 94% in the chances of developing gastric cancer (P = 0.004; OR = 0.059; 95% CI 0.008–0.414).

In the analyses involving only women (Additional file 2), there was no statistically significant difference of European ancestry between case and control groups (P = 0.052). We believe that the absence of significance may be due to the low sample number of women in the case group (N = 34), which may not allow reliable statistical associations.

Table 2 Categorical distribution of European ancestry in patients with gastric cancer in the comparison with the control group

Genetic ancestry (%)	Case no. (%)	Control no. (%)
European ancestry		
10–20	8 (6)	14 (5.3)
20–30	24 (17.5)	32 (12.2)
30–40	30 (21.8)	40 (15.2)
40–50	38 (27.7)	60 (23)
50–60	21 (15.3)	61 (23.2)
> 60	16 (11.6)	55 (21)
P	0.03	

Brazilian population is one of the most heterogeneous populations worldwide and it is formed by an admixture of Amerindians, Europeans and Africans. The admixture process occurred through different means in the Brazilian geographic regions. Our sample presented a variety in its composition, with a higher prevalence of European ancestry, followed by Amerindian and African ancestries in both case and control groups (Fig. 1). Previous data have demonstrated that the European ancestry is predominant in four regions in Brazil—North (68.8%), Northeast (60.1%), Southeast (74.2%) and South (79.5%). The African proportion was highest in the Northeast (30.3%) and the Amerindian proportion was highest in the North (19.4%) [16, 24].

Our results demonstrated that European ancestry is more represented in the control group than it is in the case group (Table 1) and that genetic ancestry modifies the risk of developing gastric cancer. The 10% increase in

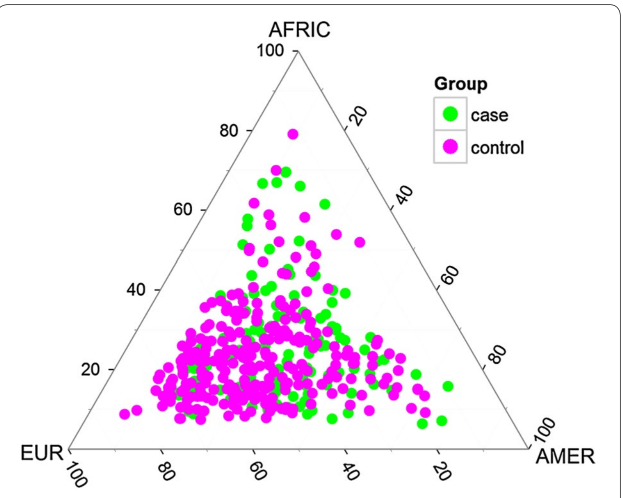

Fig. 1 Individual interethnic mixture representation. Cancer patients are represented by points in green and control subjects by dots in purple, their locations in the graph correspond to the mixing ratio. The mixture is estimated by comparison with populations of parent individuals represented in the vertices of the triangle: European, Amerindian and African

European ancestry was associated with decreased risk of developing gastric cancer (Table 3). There are only a few works in literature on the association of genetic ancestry and gastric cancer susceptibility [12, 25].

The work by [12] has investigated the susceptibility to gastric and breast cancer together, in a different population from the North region of Brazil, and found a protection association between European ancestry and the development of these neoplasms. Each increase of 10% in the European ancestry was inversely correlated to the risk of developing cancer (OR = 0.186), corroborating with our results.

A work developed by [25] has investigated the contribution of genetic ancestry in the risk of developing gastric cancer in an admixed population from Lima (Peru). European ancestry was correlated to a negative effect in the development of gastric cancer, supporting our results.

In addition, other studies have analyzed the association of genetic ancestry and cancers around the world. For example, African ancestry was associated with colorectal cancer [13] and with prostate cancer [26]. Amerindian ancestry was associated with the development of acute lymphoblastic leukemia (ALL) [27].

Our results showed that genetic ancestry may modify the risk of developing gastric cancer. European ancestry was related to a reduction in the chances of developing this neoplasm. Further studies must be carried out to identify genetic polymorphisms that are more frequent in populations with high European ancestry and that may confer a protective effect to this ancestry regarding the development of gastric cancer.

Conclusion

Our results corroborate with studies that suggest that the lower incidence of gastric cancer in the European population may be related to lower frequency of alleles related to high susceptibility of developing gastric cancer [5, 6]. Therefore, a higher European ancestry contribution may be considered a protection factor to gastric cancer in the studied Amazon population. More studies are needed to confirm such results, but this work presented significant findings that contribute to a greater knowledge of the influence of genetic ancestry in the development of gastric cancer.

Additional files

Additional file 1. Demographic variables for men in both groups (gastric cancer and control). To better comprehend the sex difference in the susceptibility to gastric cancer, we performed new statistical tests, which included isolated analyses for men.

Additional file 2. Demographic variables for women in both groups (gastric cancer and control). To better comprehend the sex difference in the susceptibility to gastric cancer, we performed new statistical tests, which included isolated analyses for women.

Abbreviations

ALL: acute lymphoblastic leukemia; PCR: polymerase chain reaction; OR: odds-ratio.

Authors' contributions

All the authors listed, contributed significantly with the preparation of the article: EMS and MFR conducted the survey, data analysis, and wrote the manuscript; DCC, GCC, LCL, EEBP, AACM, JFG and PPA analyzed the data, performed statistical analysis and edited the manuscript; SEBS and NPCS reviewed the statistical analysis, edited and approved the manuscript. All authors read and approved the final manuscript.

Author details

[1] Núcleo de Pesquisas em Oncologia, Hospital Universitário João de Barros Barreto, Universidade Federal do Pará, Belém, Brazil. [2] Laboratório de Genética Humana e Médica, Universidade Federal do Pará, Belém, PA, Brazil. [3] Hospital Universitário João de Barros Barreto, Universidade Federal do Pará, Belém, PA, Brazil. [4] Instituto de Ciências Biológicas, Laboratório de Genética Humana e Médica, Cidade Universitária Prof. José da Silveira Netto, Rua Augusto Corrêa, 01, BOX: 8615, Belém, PA CEP 66.075-970, Brazil.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional files.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The protocol used in the study was approved by the Ethics Committee of the University Hospital João de Barros Barreto (Protocol Number 3505/2004). All patients in the present study signed a consent form for the collection of biological samples and clinical records of medical records.

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References

1. International Agency for Research on Cancer—IARC. International Agency for Research on Cancer: Biennial Cancer Report. IARC; 2013. http://governance.iarc.fr/. Accessed 20 Jan 2015.
2. Globocan. Estimated cancer incidence, mortality, prevalence and disability-adjusted life years (DALYs) worldwide in 2008. International agency for research of cancer—World Health Organization; 2013. http://globocan.iarc.fr/. Accessed 25 Jan 2015.
3. Bonequi P, Meneses-González F, Correa P, Rabkin CS, Camargo MC. Risk factors for gastric cancer in latin-america: a meta-analysis. Cancer Causes Control. 2014;24(2):217–31.
4. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D, Bray F. Cancer Incidence and Mortality

Patterns in Europe: estimates for 40 countries in 2012. Eur J Cancer. 2013;49(6):1374–403.
5. Saeki N, Sakamoto H, Yoshida T. Mucin 1 gene (muc1) and gastric-cancer susceptibility. Int J Mol Sci. 2014;15(5):7958–73.
6. Kim J, Kim Y, Lee K. Ethnic differences in gastric cancer genetic susceptibility: allele flips of interleukin gene. World J Gastroenterol. 2014;20(16):4558–65.
7. Salzano F, Sans M. Interethnic admixture and the evolution of Latin American population. Genet Mol Biol. 2014;37(Suppl 1):151–70.
8. Henderson BE, Lee NH, Seewaldt V, Shen H. The influence of race and ethnicity on the biology of cancer. Nat Rev Cancer. 2012;12(9):648–53.
9. Wang S, Ray N, Rojas W, Parra MV, Bedoya G, Gallo C, Poletti G, Mazzotti G, Hill K, Hurtado AM, Camrena B, Nicolini H, Klitz W, Barrantes R, Molina JA, Freimer NB, Bortolini MC, Salzano FM, Petzl-Erler ML, Tsuneto LT, Dipierri JE, Alfaro EL, Bailliet G, Bianchi NO, Llop E, Rothhammer F, Excoffier L, Ruiz-Linares A. Geographic patterns of genome admixture in Latin American Mestizos. PLoS Genet. 2008;4(3):e1000037.
10. Fejerman L, Hu D, Huntsman S, John EM, Stern MC, Haiman CA, Pérez-Stable EJ, Ziv E. Genetic ancestry and the risk of mortality among US Latinas with breast cancer. Cancer Res. 2013;73(24):7243–53.
11. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006;295(21):2492–502.
12. Vieira PC, Burbano RM, Fernandes DC, Montenegro RC, Dos Santos SE, Sortica VA, Assumpção PP, Ribeiro-Dos-Santos ÂK, Carvalho AA, Dos Santos NP. Population stratification effect on cancer susceptibility in an admixed population from Brazilian Amazon. Anticancer Res. 2015;35(4):2009–14.
13. Hernandez-Suarez G, Sanabria MC, Serrano M, Herran OF, Perez J, Plata JL, Zabaleta J, Tenesa A. Genetic ancestry is associated with colorectal adenomas and adenocarcinomas in Latino populations. Eur J Hum Genet. 2014;22(10):1208–16.
14. Carvalho DC, Wanderley AV, Amador MA, Fernandes MR, Cavalcante GC, Pantoja KB, Mello FA, de Assumpção PP, Khayat AS, Ribeiro-Dos-Santos Â, Santos S, Dos Santos NP. Amerindian genetic ancestry and INDEL polymorphisms associated with susceptibility of childhood B-Cell Leukemia in an admixed population from the Brazilian Amazon. Leuk Res. 2015. https://doi.org/10.1016/j.leukres.2015.08.008.
15. Raska P, Iversen E, Chen A, Chen Z, Fridley BL, Permuth-Wey J, Tsai YY, Vierkant RA, Goode EL, Risch H, Schildkraut JM, Sellers TA, Barnholtz-Sloan J. European american stratification in ovarian cancer case control data: the utility of genome-wide data for inferring ancestry. PLoS ONE. 2012;7(5):e35235.
16. Santos NP, Ribeiro-Rodrigues EM, Ribeiro-Dos-Santos AK, Pereira R, Gusmão L, Amorim A, Guerreiro JF, Zago MA, Matte C, Hutz MH, Santos SE. Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. Hum Mutat. 2010;31(2):184–90.
17. Amador MA, Cavalcante GC, Santos NP, Gusmão L, Guerreiro JF, Ribeiro-dos-Santos Â, Santos S. Distribution of allelic and genotypic frequencies of IL1A, IL4, NFKB1 and PAR1 variants in Native American, African, European and Brazilian populations. BMC Res Notes. 2016. https://doi.org/10.1186/s13104-016-1906-9.
18. Nelson HD, Humphrey LL, Nygren P, Teutsch SM, Allan JD. Postmenopausal hormone replacement therapy: scientific review. JAMA. 2002;288(7):872–81.
19. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer Incidence in five Continents. In: IARC Scientific Publication No. 155. International Agency for Research on Cancer; 2002. http://www.iarc.fr/en/publications/pdfs-online/epi/sp155/. Accessed 23 Feb 2017.
20. Chlebowski RT, Wactawski-Wende J, Ritenbaugh C, Hubbell FA, Ascensao J, Rodabough RJ, Rosenberg CA, Taylor VM, Harris R, Chen C, Adams-Campbell LL, White E, Women's Health Initiative Investigators. Estrogen plus progestin and colorectal cancer in postmenopausal women. New Engl J Med. 2004;350(10):991–1004.
21. Chandanos E, Lagergren J. Oestrogen and the enigmatic male predominance of gastric cancer. Eur J Cancer. 2008;44(16):2397–403.
22. Nikfarjam Z, Massoudi T, Salehi M, Salehi M, Khoshroo F. Demographic survey of 4000 patients with 10 common cancers in north eastern Iran over the past three decades. Asian Pac J Cancer Prev. 2014;15(23):10193–8.
23. Jukic Z, Radulovic P, Stojković R, Mijic A, Grah J, Kruslin B, Ferencic Z, Fucic A. Gender difference in distribution of estrogen and androgen receptors in intestinal-type gastric cancer. Anticancer Res. 2017;37(1):197–202.
24. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy Fde S, Kohlrausch F, Magno LA, Montenegro RC, Moraes MO, de Moraes ME, de Moraes MR, Ojopi EB, Perini JA, Racciopi C, Ribeiro-Dos-Santos AK, Rios-Santos F, Romano-Silva MA, Sortica VA, Suarez-Kurtz G. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS ONE. 2011. https://doi.org/10.1371/journal.pone.0017063.
25. Pereira L, Zamudio R, Soares-Souza G, Herrera P, Cabrera L, Hooper CC, Schneider S. Socioeconomic and nutritional factors account for the association of gastric cancer with Amerindian ancestry in a Latin American admixed population. PLoS ONE. 2012;7(8):41200.
26. Yamoah K, Johnson MH, Choeurng V, Faisal FA, Yousefi K, Haddad Z, Ross AE, Alshalafa M, Den R, Lal P, Feldman M, Dicker AP, Klein EA, Davicioni E, Rebbeck TR, Schaeffer EM. Novel biomarker signature that may predict aggressive disease in African American men with prostate cancer. J Clin Oncol. 2015;33(25):2789–96.
27. Yang JJ, Cheng C, Devidas M, Cao X, Fan Y, Campana D, Yang W, Neale G, Cox NJ, Scheet P, Borowitz MJ, Winick NJ, Martin PL, Willman CL, Bowman WP, Camitta BM, Carroll A, Reaman GH, Carroll WL, Loh M, Hunger SP, Pui CH, Evans WE, Relling MV. Ancestry and pharmacogenomics of relapse in acute lymphoblastic leukemia. Nat Genet. 2011;43(3):237–41.

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Hipertensão pulmonar em pacientes com doença renal crônica terminal internados em um hospital de referência em nefrologia do estado do Pará, Brasil

Pulmonary hypertension in hospitalized patients with chronic end-stage kidney disease at a referral hospital in nephrology in Pará State, Brazil

Hivis da Costa Sousa[1], Joelson Felipe da Silva Oliveira[1], Brenda Brandão Moreira[2], Esdras Edgar Batista Pereira[2], José Tadeu Colares Monteiro[1,3]

[1] *Fundação Hospital de Clínicas Gaspar Vianna, Belém, Pará, Brasil*
[2] *Universidade Federal do Pará, Belém, Pará, Brasil*
[3] *Centro Universitário do Pará, Curso de Medicina, Belém, Pará, Brasil*

RESUMO

OBJETIVOS: Verificar a existência de hipertensão arterial pulmonar (HAP) entre os pacientes portadores de doença renal crônica (DRC) em hemodiálise e identificar seus perfis clínicos. MATERIAIS E MÉTODOS: Estudo retrospectivo, realizado por meio da análise de prontuários de pacientes internados em um hospital de referência em nefrologia do estado do Pará, Brasil, de janeiro a dezembro de 2014. Pela aplicação de um questionário, foram analisados idade, gênero, comorbidades, motivo da internação, tipo de acesso vascular e informações do ecocardiograma Doppler transtorácico, como a medida da pressão sistólica da artéria pulmonar (PSAP), considerando-se valores ≥ 35 mmHg sugestivos de HAP. RESULTADOS: Dos 101 pacientes investigados, 21,8% apresentavam sinais sugestivos de HAP. Desses, 63,6% pertenciam ao gênero masculino, com média de idade de 60,36 ± 12,35 anos. Constatou-se média de PSAP de 51,13 ± 11,40 mmHg e a maioria das internações foram motivadas por uremia (45,5%) e síndrome coronariana aguda (13,6%). Entre as comorbidades, destacaram-se a hipertensão arterial sistêmica (95,5%) e a diabetes mellitus (50,0%). A maioria dos pacientes usava um acesso vascular de curta permanência do tipo Shilley (95,5%) para hemodiálise, e o desfecho mais reportado foi o óbito (36,4%). CONCLUSÃO: O rastreamento de HAP por ecocardiograma pode ser útil para a avaliação cardiopulmonar entre os pacientes urêmicos, havendo necessidade de estudos prospectivos para melhor esclarecer a relação entre HAP e a terapia de hemodiálise em portadores de DRC.

Palavras-chave: Doença Renal Crônica; Hemodiálise; Hipertensão Pulmonar.

ABSTRACT

OBJECTIVES: To verify the existence of pulmonary arterial hypertension (PAH) among patients with chronic kidney disease (CKD) undergoing hemodialysis, and to identify their clinical profiles. MATERIALS AND METHODS: A retrospective study was carried out by analyzing the medical records of patients admitted to a referral hospital in nephrology in Pará State, Brazil, from January to December 2014. Variables as age, gender, comorbidities, reason for hospitalization, type of vascular access were obtained by a questionnaire and analyzed. In addition, transthoracic Doppler echocardiography information, such as pulmonary artery systolic pressure (PASP), with values ≥ 35 mmHg suggesting PAH, was considered. RESULTS: From the total of 101 patients investigated, 21.8% presented signs suggestive of PAH, and 63.6% of those were male with mean age of 60.36 ± 12.35 years. Mean PASP was 51.13 ± 11.40 mmHg and the majority of hospitalizations were due to uremia (45.5%) and acute coronary syndrome (13.6%). Among the main comorbidities identified, systemic arterial hypertension (95.5%) and diabetes mellitus (50.0%) stand out. Most of patients used the Shilley vascular access for hemodialysis (95.5%), and the most reported outcome was death (36.4%). CONCLUSION: Screening for PAH by echocardiography may be useful for cardiopulmonary evaluation among uremic patients, and prospective studies are needed to clarify the relationship between PAH and hemodialysis therapy in patients with CKD.

Keywords: Chronic Kidney Disease; Hemodialysis; Pulmonary Hypertension.

Correspondência / Correspondence:
José Tadeu Colares Monteiro
Centro Universitário do Pará
Av. Almirante Barroso, 3775. Bairro: Souza. CEP: 66613-903 – Belém, Pará, Brasil – Tel.: +55 (91) 3205-9000 / 98934-6998
E-mail: tadeucolares@hotmail.com

INTRODUÇÃO

A doença renal crônica (DRC) terminal representa a falência da excreção renal e da função endócrino-metabólica dos rins, podendo ocasionar diversas repercussões sistêmicas. O número de pacientes portadores de DRC vem aumentando. De 1994 a 2004, houve um incremento de 24.000 para mais de 59.000 pacientes dialíticos no Brasil, que é o terceiro país em número de pacientes portadores de DRC[1,2].

A prevalência de hipertensão arterial pulmonar (HAP), em pacientes com DRC, é difícil de ser estimada, pois os dados epidemiológicos são raros e baseiam-se, principalmente, em informações retrospectivas e/ou pequenos estudos com limitações metodológicas. Contudo, a prevalência de HAP varia de 9% a 39% em indivíduos com DRC em estágio 5, de 18,8% a 68,8% em pacientes hemodialisados e de 0% a 42% em pacientes em terapia de diálise peritoneal[3].

O diagnóstico de HAP exige, de início, a suspeita clínica baseada nos sintomas e exame físico, seguida de uma investigação abrangente clínico-diagnóstica que permita confirmar a hipótese, com posterior cumprimento dos critérios hemodinâmicos para auxiliar a descrever a etiologia, a gravidade funcional e hemodinâmica da condição. Para tal, as perícias em cardiologia, imaginologia e medicina respiratória são imprescindíveis e mostram a importância da interdisciplinaridade[4].

O ecocardiograma bidimensional transtorácico com Doppler é o método não invasivo mais sensível na investigação e rastreamento da HAP, onde a pressão sistólica da artéria pulmonar (PSAP) é estimada pela medida do jato de regurgitação tricúspide, que se correlaciona positivamente com a pressão média da artéria pulmonar medida de forma invasiva. Uma PSAP > 35 mmHg, medida por cateterismo cardíaco direito, corresponde à pressão média da artéria pulmonar > 25 mmHg. Portanto, valores de PSAP ≥ 35 mmHg, medidos pelo ecocardiograma, sugerem HAP, assim como aumento de câmaras direitas, insuficiência tricúspide, movimento paradoxal do septo interventricular e diminuição da complacência do ventrículo esquerdo[3,5]. O ecocardiograma deve ser sempre realizado em caso de suspeita de HAP e pode ser usado para inferir o diagnóstico em doentes nos quais diferentes medidas ecocardiográficas estejam consistentes com o diagnóstico[6]. Esse exame, isoladamente, não é suficiente para apoiar uma decisão de tratamento, sendo necessária, por vezes, a confirmação diagnóstica por meio do cateterismo cardíaco direito[7].

Segundo as diretrizes para o diagnóstico e tratamento da hipertensão pulmonar da Sociedade Europeia de Cardiologia e da Sociedade Europeia Respiratória, uma classificação clínica da HAP destina-se a qualificar múltiplas condições clínicas em grupos de 1 a 5, conforme semelhanças clínicas, achados patológicos, características hemodinâmicas e estratégia de tratamento, podendo ser atualizada e/ou modificada se novos dados estiverem disponíveis sobre as características citadas ou quando entidades clínicas adicionais forem consideradas[7].

Desse modo, no grupo 5 da classificação internacional de HAP estão reunidas as causas associadas a mecanismos pouco claros e/ou multifatoriais, onde está inclusa a DRC em fase terminal com ou sem hemodiálise[4]. Diversos fatores têm sido sugeridos como contribuintes para o desenvolvimento da HAP na DRC em fase terminal, e, devido à multiplicidade de possíveis mecanismos, a HAP na DRC pertence a esse grupo[8].

A associação da HAP à DRC é complexa, de etiologia multifatorial, podendo ser induzida e/ou agravada por distúrbios do ventrículo esquerdo, bem como pela presença de fatores de risco típicos para DRC, como idade avançada, tempo de doença, sobrecarga de volume intravascular, distúrbios do sono, exposição a membranas de diálise, alterações no metabolismo do cálcio e fósforo, disfunção endotelial, calcificação vascular e anemia grave[9].

Pacientes renais crônicos produzem elevados níveis de substâncias vasoconstritoras, como endotelina-1 e angiotensina II[10], associados a valores baixos de substâncias vasodilatadoras, como óxido nítrico e prostaciclina[11], resultando em um aumento no tônus dos vasos pulmonares e consequente hipertensão pulmonar.

A HAP pode levar ao aumento dos níveis de citocinas e de fatores de crescimento (fibroblásticos – FGF, derivado das plaquetas – PDGF e transformante-β – TGF-β), bem como ativação concomitante da enzima conversora de angiotensina, tendo como consequência a proliferação anormal de células musculares lisas, fibrose e trombose arterial, sendo essas características patológicas comuns de progressão da doença[12].

No Brasil, os custos com terapia renal substitutiva consomem uma parcela significativa do orçamento destinado à saúde pública e ainda maior quando se avalia a complexa interação da DRC com o risco aumentado de eventos sistêmicos[13]. Desse modo, este estudo teve por objetivos identificar a existência de HAP entre os pacientes portadores de DRC em hemodiálise admitidos em um hospital de referência em nefrologia, e, a partir dos dados obtidos, verificar o perfil clínico dos pacientes.

MATERIAIS E MÉTODOS

Estudo retrospectivo, realizado de fevereiro a dezembro de 2016, por meio da análise da ficha cadastral de 150 pacientes portadores de DRC em hemodiálise, internados na Fundação Hospital de Clínicas Gaspar Vianna (FHCGV), em Belém, estado do Pará, Brasil, no período de janeiro a dezembro de 2014. Foi utilizado um questionário com as seguintes informações: idade, gênero, comorbidades, motivo da internação, início do tratamento de hemodiálise e tipo de acesso vascular. Para a análise sugestiva de HAP,

foram consideradas as informações dos laudos dos ecocardiogramas Doppler transtorácicos e a medida da PSAP com valores ≥ 35 mmHg.

Foram incluídos na amostra todos os pacientes portadores de DRC em terapia renal substitutiva de hemodiálise internados na FHCGV e que realizaram pelo menos um ecocardiograma Doppler transtorácico no período do estudo, de ambos os gêneros, com faixa etária ≥ 18 anos.

Os critérios de exclusão foram: paciente com faixa etária < 18 anos e aqueles sabidamente portadores de doenças pulmonares crônicas, neoplasias, colagenoses, grávidas ou em condições que pudessem incluí-los em outros grupos de HAP.

Foi realizada uma análise descritiva dos dados referentes à caracterização da amostra, utilizando frequência absoluta, porcentagens, média e desvio padrão (DP). As variáveis quantitativas foram submetidas ao teste Kolmogorov-Smirnov, para a análise da distribuição de normalidade; e a obtenção de um p-valor menor que 0,05 indicou a aplicação de testes não paramétricos. A análise específica do grupo de pacientes com HAP foi realizada pelos testes binominal e qui-quadrado para as variáveis não paramétricas, e o teste T de Student para as variáveis paramétricas. Os dados foram reunidos em um grupo com e outro sem HAP. As variáveis gênero, faixa etária, comorbidade, motivo da internação e acesso vascular foram avaliadas pelo teste qui-quadrado. Para a análise de risco das variáveis investigadas, sobre a predição ao desenvolvimento da HAP, foi realizada uma regressão logística. Todas as análises estatísticas foram feitas utilizando-se o pacote estatístico do *software* SPSS v20.0, respeitando-se o nível de significância de 5% (p ≤ 0,05).

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos da FHCGV, sob o parecer nº 49443515.0.0000.0016, em 18 de fevereiro de 2016.

RESULTADOS

Foram analisados os dados de 101 pacientes, dos quais 22 (21,8%) apresentavam sinais sugestivos de HAP pela medida indireta (ecocardiograma Doppler transtorácico) da PSAP. A tabela 1 mostra as análises comparativas entre os grupos estudados.

No grupo com HAP, houve prevalência do gênero masculino (63,6%). A faixa etária isoladamente mostrou-se pouco significativa; a idade variou de 29 a 75 anos e a média foi de 60,36 ± 12,35 anos. As comorbidades mais prevalentes encontradas nos pacientes desse grupo foram a hipertensão arterial sistêmica (HAS) com 95,5% e a diabetes mellitus com 50,0%. Já a insuficiência cardíaca congestiva (ICC), encontrada em 36,4% dos portadores de HAP, e a insuficiência coronariana obstrutiva (ICO), em 27,3%, são importantes fatores predisponentes à HAP.

Tabela 1 – Análise das variáveis clínico-epidemiológicas dos pacientes portadores de DRC, com e sem HAP, internados na FHCGV em Belém, estado do Pará, Brasil, 2014

Variáveis	Grupo com HAP		Grupo sem HAP		p*
	N = 22	%	N = 79	%	
Gênero					
Masculino	14	63,6	52	65,8	0,849
Feminino	8	36,4	27	34,2	
Faixa etária					
< 60 anos	11	50,0	25	31,6	0,112
≥ 60 anos	11	50,0	54	68,4	
Comorbidade					
Hipertensão arterial sistêmica	21†	95,5	60	75,9	0,042†,‡
Diabetes mellitus	11	50,0	41	51,9	0,875
Insuficiência cardíaca congestiva	8	36,4	20	25,3	0,306
Insuficiência coronariana obstrutiva	6	27,3	9	11,4	0,064
Motivo de internação					
Uremia	10	45,5	33	41,8	0,757
Síndrome coronariana aguda	3	13,6	18	22,8	0,350
Pneumonia	2	9,1	11	13,9	0,449
Insuficiência cardíaca congestiva	2	9,1	7	8,9	0,973
Outros	5	22,7	10	12,6	0,240
Acesso vascular					
Cateter tipo Shilley	21	95,5	74	93,7	
Cateter tipo Permicath	–	–	1	1,3	0,745
Fístula arteriovenosa	1	4,5	4	5,0	

Fonte: Serviço de Nefrologia da FHCGV, 2014.

Sinal convencional utilizado: – Dado numérico igual a zero, não resultante de arredondamento; * Teste qui-quadrado; † p < 0,001 em análise univariada, teste binominal; ‡ p ≤ 0,05.

Na regressão logística bivariada, a variável HAS foi a única que demonstrou significância estatística, onde as chances da presença de HAP aumentam em nove vezes se associada à HAS (p = 0,041; OR = 9,736). Além disso, a presença de HAS e ICO aumentam em quatro vezes as chances de desenvolver HAP (p = 0,028; OR = 4,384); e a associação entre idade ≥ 60 anos, HAS e ICO aumentam em nove vezes as chances de desenvolvimento de HAP (p = 0,010; OR = 9,344) (Tabela 2).

Quanto à análise clínica, 54,6% apresentaram HAP considerada leve, 22,7% moderada e 22,7% grave. A média e o DP das medidas de PSAP foi de 51,13 ± 11,40 mmHg, valor esse estatisticamente significativo com p < 0,001 (Tabela 3).

Sobre o desfecho dos pacientes em hemodiálise com provável HAP, 36,4% foram a óbito durante a internação hospitalar, 31,8% seguiram o tratamento de modo conservador e 31,8% se mantiveram em hemodiálise em regime ambulatorial (Tabela 3). O desfecho clínico do grupo sem HAP não foi investigado.

Tabela 2 – Predição do risco de HAP em pacientes portadores de DRC internados na FHCGV, em Belém, estado do Pará, Brasil, 2014

Variáveis	Grupo com HAP		Grupo sem HAP		p	OR (IC95%)
	N = 22	%	N = 79	%		
Gênero masculino	14	63,6	52	65,8	0,554	0,721 (0,244–2,131)
Idade ≥ 60 anos	11	50,0	54	68,4	0,055	0,357 (0,125–1,023)
HAS	21	95,5	60	75,9	0,041*	9,736 (1,101–86,123)
DM	11	50,0	41	51,9	0,464	0,667 (0,225–1,972)
ICC	8	36,4	20	25,3	0,535	1,427 (0,464–4,384)
ICO	6	27,3	9	11,4	0,135	2,687 (0,736–9,814)
HAS + DM	11	50,0	39	49,4	0,645	1,269 (0,460–3,503)
HAS + ICC	8	36,4	14	17,7	0,120	2,396 (0,796–7,212)
HAS + ICO	6	27,3	6	7,6	0,028*	4,384 (1,177–16,336)
Idade ≥ 60 anos + HAS + DM	5	22,7	29	36,7	0,872	0,891 (0,218–3,647)
Idade ≥ 60 anos + HAS + ICC	3	13,6	11	13,9	0,678	1,388 (0,294–6,550)
Idade ≥ 60 anos + HAS + ICO	4	18,2	3	3,8	0,010*	9,344 (1,688–51,743)

Fonte: Serviço de Nefrologia da FHCGV, 2014.

HAP: Hipertensão arterial pulmonar; HAS: Hipertensão arterial sistêmica; DM: Diabetes mellitus; ICC: Insuficiência cardíaca congestiva; ICO: Insuficiência coronariana obstrutiva; * p ≤ 0,05.

Tabela 3 – Análise clínica do grupo de pacientes com DRC e HAP internados na FHCGV, e Belém, estado do Pará, Brasil, 2014

Variáveis	Grupo com HAP		p
	N = 22	%	
Hipertensão arterial pulmonar*,†			
Leve	12	54,6	
Moderada	5	22,7	0,108
Grave	5	22,7	
PSAP – Média ± DP (mmHg)	51,13 ± 11,40		<0,001‡
Comorbidade§			
Hipertensão arterial sistêmica	21	95,5	<0,001‡
Diabetes mellitus	11	50,0	1,000
Insuficiência cardíaca congestiva	8	36,4	0,285
Insuficiência coronariana obstrutiva	6	27,3	0,052
Desfecho clínico*			
Tratamento conservador	7	31,8	
Hemodiálise	7	31,8	0,091
Óbito	8	36,4	

Fonte: Serviço de Nefrologia da FHCGV, 2014.

HAP: Hipertensão arterial pulmonar; PSAP: Pressão sistólica da artéria pulmonar; DP: Desvio padrão; * Teste qui-quadrado; † Teste T de Student; ‡ p ≤ 0,05; § Teste binominal.

DISCUSSÃO

A HAP é uma doença cuja fisiopatologia envolve diversas condições clínicas e pode ser um fator complicador para a maioria das doenças cardiovasculares e respiratórias[4]. São tidos como prováveis mecanismos, pelos quais a HAP se relaciona com a DRC, a congestão venosa, a diminuição do débito cardíaco e a ativação neuro-hormonal, além do aumento da sinalização de TGF-β e dos níveis de citocinas circulantes[14].

Na avaliação dos pacientes com DRC em hemodiálise que realizaram ecocardiograma Doppler transtorácico durante o período do estudo, identificou-se que 21,8% apresentaram o exame com níveis de PSAP ≥ 35 mmHg, sugestivo de HAP. Ainda são poucos os dados publicados referentes à prevalência de HAP a nível global. A menor estimativa de prevalência de HAP é de 15 casos/1 milhão da população adulta. Na Europa, a prevalência está na faixa de 15–60 casos/1 milhão da população adulta ao ano, conforme dados da Sociedade Europeia de Cardiologia e Sociedade Europeia Respiratória[6].

Quanto à prevalência de HAP em pacientes com DRC em hemodiálise, um estudo realizado por Alhamad et al.[8] apresentou prevalência semelhante ao encontrado neste estudo, pois, entre 12 doentes renais crônicos terminais, 21,8% tinham HAP (medidas pelo ecocardiograma), embora só tenham sido consideradas medidas de PSAP ≥ 40 mmHg. Outros estudos também encontraram prevalência de HAP maior que a do presente estudo, como os de Zhao et al.[15] (38,5% em 70 pacientes de Chengdu, na China), Etemadi et al.[16] (41,1% em 34 pacientes de Tabriz, no Irã) e Mukhtar et al.[17] (56% em 80 pacientes de Karachi, no Paquistão).

Neste estudo, 63,6% dos pacientes com sinais de HAP eram do gênero masculino, sem relação com a faixa etária, com média de PSAP de 51,13 ± 11,40 mmHg, em sua maioria internados por uremia (45,5%) e síndrome coronariana aguda (13,6%). Entre as principais comorbidades identificadas, destacam-se a HAS (95,5%) e a diabetes mellitus (50,0%). Estudos como o de Etemadi et al.[16] revelaram média de PSAP de 37,5 (35–45) mmHg, sem variações quanto ao gênero, com acesso vascular por meio de fístula arteriovenosa e um tempo de início da terapia de hemodiálise considerável, em média 102 semanas. Os estudos de Mukhtar et al.[17] e Mousavi et al.[18] revelaram predomínio de HAP em pacientes do gênero feminino (67% e 59,4%, respectivamente) e valor médio de PSAP de 38,5 ± 19,17 e 39,58 ± 13,27 mmHg, respectivamente.

Para Galiè et al.[4], a HAP pode ocorrer em diferentes configurações, dependendo das condições clínicas associadas, com apresentação de forma esporádica, sem qualquer história familiar ou fator desencadeante evidente. Alguns estudos, como o de Yigla et al.[19], descreveram a presença da fístula arteriovenosa como um dos principais mecanismos que podem explicar a HAP em pacientes submetidos à hemodiálise, por aumentar o retorno venoso ao lado direito do coração, resultando em um aumento do débito de saída do ventrículo direito e sobrecarga da vasculatura. No entanto, essa informação é questionável, tendo em vista que o pulmão normal apresenta complacência natural, fato não observado no pulmão de pacientes com DRC, o que leva a pensar que outros mecanismos fisiopatológicos podem levar à diminuição da complacência da vasculatura pulmonar e consequente hipertensão desse leito de vasos[12,20].

O tipo de acesso vascular para hemodiálise mais utilizado foi o cateter de curta permanência tipo Shilley (95,5%) e apenas 4,5% por meio de fístula arteriovenosa. Por outro lado, em estudos como os de Mukhtar et al.[17] e Di Lullo et al.[21], a HAP estava presente em 60% e 20% dos pacientes em uso de fístula arteriovenosa, respectivamente. Portanto, a associação de tipos de acesso com o desenvolvimento de HAP é variável.

É importante ressaltar que há poucos estudos correlacionando a HAP com a HAS ou a quadros de insuficiência cardíaca. Em 2016, um estudo analisou as tendências relacionadas à HAP em adultos, nos Estados Unidos, e demonstrou que a hipertensão arterial (5,1–17,1%), a diabetes (4,6–7,8%) e a doença coronariana (15,6–22,3%) são comorbidades frequentemente associadas. A insuficiência cardíaca congestiva (40,7–56,1%) e a doença renal aguda (5,9–20,1%) e crônica (1,1–16,4%) também aparecem como fatores importantes[22].

CONCLUSÃO

O perfil dos pacientes com HAP e DRC em hemodiálise, atendidos no Serviço de Nefrologia da FHCGV, mostrou que essa condição clínica é frequente no sexo masculino, sem correlação com a idade, com comorbidade mais relevante à HAS e à diabetes mellitus, seguidas de ICC e ICO, onde a HAP foi classificada como leve, a maioria das internações foram motivadas por uremia e síndrome coronariana aguda e o acesso vascular de curta permanência do tipo Shilley foi o mais utilizado para as hemodiálises. A caracterização clínica é importante para que estudos prospectivos ofereçam melhor compreensão entre a HAP e a terapia de hemodiálise em portadores de DRC.

CONFLITOS DE INTERESSE

Os autores declaram que não houve conflitos de interesse.

CONTRIBUIÇÃO DOS AUTORES

Todos os autores contribuíram com a idealização do estudo, a análise e a interpretação dos dados e com a redação do manuscrito, aprovando a versão final publicada. Declaram-se responsáveis pelo conteúdo integral do artigo, garantindo sua precisão e integridade.

REFERÊNCIAS

1 Dhaun N, Goddard J, Webb DJ. The endothelin system and its antagonism in chronic kidney disease. J Am Soc Nephrol. 2006 Apr;17(4):943-55.

2 Romão Junior JE. Doença renal crônica: definição, epidemiologia e classificação. J Bras Nefrol. 2004 ago;26(3 supl 1):1-3.

3 Bolignano D, Rastelli S, Agarwal R, Fliser D, Massy Z, Ortiz A, et al. Pulmonary hypertension in CKD. Am J Kidney Dis. 2013 Apr;61(4):612-22.

4 Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Respir J. 2015;46:903-75.

5 Hernandez-Suarez DF, Menendez FRL, Palm D, Lopez-Candales A. Left ventricular diastolic function assessment of a heterogeneous cohort of pulmonary arterial hypertension patients. J Clin Med Res. 2017 Apr;9(4):353-9.

6 Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography. J Am Soc Echocardiogr. 2010 Jul;23(7):685-713.

7 Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2015 Mar;16(3):233-70.

8 Alhamad EH, Al-Ghonaim M, Alfaleh HF, Cal JP, Said N. Pulmonary hypertension in end-stage renal disease and post renal transplantation patients. J Thorac Dis. 2014 Jun;6(6):606-16.

9 Yang QM, Bao XR. Pulmonary hypertension in patients with stage 1-3 chronic kidney disease. Genet Mol Res. 2014 Jul;13(3):5695-703.

10 Yigla M, Nakhoul F, Sabag A, Tov N, Gorevich B, Abassi Z, et al. Pulmonary hypertension in patients with end-stage renal disease. Chest. 2003 May;123(5):1577-82.

11 Chester AH, Yacoub MH. The role of endothelin-1 in pulmonary arterial hypertension. Glob Cardiol Sci Pract. 2014 Jun;2014(2):62-78.

12 Di Lullo L, Floccari F, Rivera R, Barbera V, Granata A, Otranto G, et al. Pulmonary hypertension and right heart failure in chronic kidney disease: new challenge for 21st-century cardionephrologists. Cardiorenal Med. 2013 Jul;3(2):96-103.

13 Gouveia DSS, Bignelli AT, Hokazono SR, Danucalov I, Siemens TA, Meyer F, et al. Análise do impacto econômico entre as modalidades de terapia renal substitutiva. Braz J Nephrol. 2017;39(2): 162-71.

14 Nickel NP, O'Leary JM, Brittain EL, Fessel JP, Zamanian RT, West JD, et al. Kidney dysfunction in patients with pulmonary arterial hypertension. Pulm Circ. 2017 Mar;7(1):38-54.

15 Zhao LJ, Huang SM, Liang T, Tang H. Pulmonary hypertension and right ventricular dysfunction in hemodialysis patients. Eur Rev Med Pharmacol Sci. 2014;18(21):3267-73.

16 Etemadi J, Zolfaghari H, Firoozi R, Ardalan MR, Toufan M, Shoja MM, et al. Unexplained pulmonary hypertension in peritoneal dialysis and hemodialysis patients. Rev Port Pneumol. 2012 Jan-Feb;18(1):10-4.

17 Mukhtar KN, Mohkumuddin S, Mahmood SN. Frequency of pulmonary hypertension in hemodialysis patients. Pak J Med Sci. 2014 Nov-Dec;30(6):1319-22.

18 Mousavi SA, Mahdavi-Mazdeh M, Yahyazadeh H, Azadi M, Rahimzadeh N, Yoosefnejad H, et al. Pulmonary hypertension and predisposing factors in patients receiving hemodialysis. Iran J Kidney Dis. 2008 Jan;2(1):29-33.

19 Yigla M, Banderski R, Azzam ZS, Reisner SA, Nakhoul F. Arterio-venous access in end-stage renal disease patients and pulmonary hypertension. Ther Adv Respir Dis. 2008 Apr;2(2):49-53.

20 Bianchi PDA, Barreto SSM, Thomé FS, Klein AB. Repercussão da hemodiálise na função pulmonar de pacientes com doença renal crônica terminal. J Bras Nefrol. 2009;31(1):25-31.

21 Di Lullo L, Floccari F, Polito P. Right ventricular diastolic function in dialysis patients could be affected by vascular access. Nephron Clin Pract. 2011;118(3):c257-61.

22 Anand V, Roy SS, Archer SL, Weir EK, Garg SK, Duval S, et al. Trends and outcomes of pulmonary arterial hypertension-related hospitalizations in the United States: analysis of the nationwide inpatient sample database from 2001 through 2012. JAMA Cardiol. 2016 Dec;1(9):1021-9.

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Mini Review

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Functional Capacity Assessment in Oncogeriatric

Esdras Edgar Batista Pereira*, Ney Pereira Carneiro dos Santos

Faculty of Medicine, Federal University of Pará, Brazil

***Corresponding author:** Esdras Edgar Batista Pereira, Faculty of Medicine, Federal University of Pará, Brazil

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Mini Review

In the last decades, the number of elderly people in the world population has increased, a conquest of humanity, through improvements in health, education and economics, among other factors that contribute to a greater longevity [1,2]. The increase in the life expectancy of the population occurs together with the increase in the prevalence of chronic and degenerative diseases, which rise from 60 years, among them cancer [3,4]. According to the International Agency for Research on Cancer (IARC), the world had an incidence of about 11 million cancer cases in 2002, where 45% of cases were people 65 years of age or older. This incidence increased to 14 million in 2012 and the elderly represent 47% of these cases [5]. By 2050, projections estimate that three out of five cases of cancer will occur among the elderly [6]. This information reinforces the need for integrated action between two areas of health, oncology and geriatrics, called geriatric oncology or oncogeriatric, capable of understanding and treating the elderly oncology, taking into account their specificities [7]. This new approach evidences global functionality as a health predictor. Its identification can help in the prediction of tolerance to cancer treatment, as well as structure conducts directed to this age group [8].

Global functionality is the starting point for assessing the health of the elderly, which is measured by functional capacity, defined as the ability to manage one's own life or take care of oneself. These criteria encompass the integrated and harmonious functioning of daily life activities with functional systems such as cognition, mood, mobility and communication [9].This research includes the application of Comprehensive Geriatric Assessment, a multidimensional assessment tool where the various problems of the elderly are discovered, described and explained [10]. It is a research tool used by geriatricians and gerontologists to evaluate the general health of the elderly. It is one of the pillars of geriatric care, as part of an interdisciplinary and multidimensional diagnostic process [11-13]. The evaluation of these domains early identifies problems of a biopsychosocial nature, through scales and standardized instruments, that help in the elaboration and management of more effective, efficient and safe care plans, reducing the possible iatrogenics, which can potentiate the functional impairment of the oncological elderly [14].

With this evaluation, the so-called geriatric syndromes, which are clinical conditions involving multiple systems, can be described as common, such as cancer, despite their different presentations, such as cognitive impairment, postural instability, incontinence, immobility, etc. Therefore, knowledge of the particularities of aging is necessary for interventions that prioritize the health of the elderly with cancer [15,16]. Studies point to the benefits related to the evaluation of functionality in elderly care services, showing that this practice is associated with lower health spending, reduction in the number and length of hospitalizations, reduction of institutionalization, improvement of quality of life, lower frequency of polypharmacy, improvement in performance in daily life activities and reduction of mortality rates [17].The clinical parameters contained in the functional capacity assessment allow the global visualization of the oncological elderly, which in some way interfere with the quality of life of this population. The approximation between these clinical parameters makes possible the identification of remediable problems that imply in the prevention of risk factors, treatment, prognosis and rehabilitation in the health of the elderly oncology.

The instruments present in the broad geriatric evaluation have ideal characteristics for a population screening: non-invasive methodology, with relatively easy and quick and inexpensive applicability. This demonstrates its application in clinical practice in oncogeriatric. Thus, understanding the aging process broadly, considering the instruments of clinical and functional domain contained in the broad geriatric evaluation, facilitates the understanding of the determinants of health of the elderly population with cancer, having the applicability of predicting the

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health of the elderly in an interdisciplinary way, to base prevention strategies, to facilitate the management and elaboration of future care plans necessary for the elderly oncology.

References

1. United Nations (2011) Security, economic power and independence in aging. Report on the State of World Population 2011. New York: United Nations Population Fund (UNFPA).
2. World Health Organization (2012) The Demographics of Ageing. Global brief for World Health Day 2012. Genebra.
3. Hansen EO, Tavares STO, Cândido SA, Pimenta FAP, Moraes EM, et al. (2011) International classification of functionality, disease and medical prognosis in elderly patients. Rev. méd. Minas Gerais 21 (1): 55-60.
4. Campolina AG, Adami F, Santos JLF, Lebrão ML (2013) The health transition and changes in the healthy life expectancy of the elderly population: possible impacts of the prevention of chronic diseases. Reports in Public Health 29 (6): 1217-1229.
5. International Agency for Research on Cancer (IARC). World Health Organization (2013) GLOBOCAN 2012: Cancer Incidence, Mortality and Prevalence Worldwide.
6. Bray F, Moller B (2006) Predicting the future burden of cancer. Nature 6 (1): 63-74.
7. Assis CMRB, Melo HMA, Melo EMA, Kitner D, Costa Júnior JI (2011) Geriatric oncology: concepts, trends and challenges. Geriatr. Gerontol 5 (2): 106-111.
8. Cerullo FC, Ferrini G, Ciaburri A, Scambia M, Bernabei G, et al. (2011) Misure di funzione fisica in oncogeriatria. Giornale Di Gerontologia 5 (9): 265-272.
9. Moraes EN (2012) Health Care of the Elderly: Conceptual Aspects. 1 ed. Brasília (DF): Pan American Health Organization - Representation Brazil, P: 98.
10. Karnakis T (2011) Oncogeriatric: a review of the broad geriatric assessment in cancer patients. RBM 68(2): 8-12.
11. Ellis G, Whitehead MA, Robinson D. O'neill D, Langhorne P (2011) Comprehensive geriatric assessment for older adults admitted to hospital: meta-analysis of randomized controlled trials. British Medical Journal.
12. Parks RM, Lakshmanan R, Winterbottom L, Morgan DA, Cox K, et al. (2012) Comprehensive geriatric assessment for older women with early breast cancer – a systematic review of literature. World Journal of Surgical Oncology 88 (10): 1-9.
13. Mohile SG, Magnuson A (2013) Comprehensive Geriatric Assessment in Oncology. Interdisciplinary Topics in Gerontology Journal 38: 85-103.
14. Welsh TJ, Gordon AL, Gladman JR (2013) Comprehensive geriatric assessment – a guide for the non-specialist. International Journal of Clinical Practice, 68: 3.
15. Moraes EM, Marino MCA, Santos RR (2010) Major geriatric syndromes. Rev Med Minas Gerais 20 (1): 54-66.
16. Limpawattana P, Sawanyawisuth K, Soonpornrai S, Huangthaisong W (2011) Prevalence and recognition of geriatric syndromes in an outpatient clinic at a tertiary care hospital of Thailand. Asian Biomedicine Journal 5 (4): 493-497.
17. Nishinaga M (2007) Comprehensive Geriatric Assessment and Team Intervention. Japan Med Assoc J 50 (6): 461-466.

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ORIGINAL ARTICLE

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Polymorphisms in the CYP2A6 and ABCC4 genes are associated with a protective effect on chronic myeloid leukemia in the Brazilian Amazon population

Natasha Monte[1] | **Karla B. C. C. Pantoja**[1] | **Juliana C. G. Rodrigues**[1] | **Darlen C. de Carvalho**[1] | **Tereza C. B. Azevedo**[2] | **Esdras E. B. Pereira**[1] | **Paulo P. de Assumpção**[1] | **Sidney E. B. dos Santos**[1] | **Marianne R. Fernandes**[1,2] | **Ney P. C. dos Santos**[1]

[1]Núcleo de Pesquisas em Oncologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Hospital Universitário João de Barros Barreto, Unidade de Alta Complexidade em Oncologia, Belém, Brazil

[2]Departamento de Oncohematologia, Hospital Ophir Loyola, Belém, Brazil

Correspondence
Ney P. C dos Santos, Núcleo de Pesquisas em Oncologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Hospital Universitário João de Barros Barreto, Unidade de Alta Complexidade em Oncologia, 4487, Belém, PA, Brazil.
Email: npcsantos.ufpa@gmail.com

Abstract

Background: Susceptibility to Chronic Myeloid Leukemia (CML) may be modulated by genetic variables. However, the majority of previous investigations have focused on genetically homogeneous populations, resulting in a lack of evidence on how genetic factors may influence the development of CML in miscegenated populations. We analyzed 30 polymorphisms in genes related to DNA repair, folate metabolism, transmembrane transport, xenobiotic metabolism, and pyrimidine synthesis in relation to their potential role in the susceptibility of the individual to CML.

Methods: This case-control study included 126 healthy individuals and 143 patients diagnosed with CML from the admixed population of the Brazilian Amazon. The samples were genotyped by real-time PCR and the genetic ancestry analysis was based on a panel of 61 ancestry informative markers.

Results: The results indicated a protective effect against the development of CML in carriers of the C allele of the rs28399433 (*CYP2A6*) gene and the CC genotype of the rs3742106 (*ABCC4*) gene.

Conclusion: Our findings suggest that the rs3742106 (*ABCC4*) and rs28399433 (*CYP2A6*) polymorphisms may modulate susceptibility to CML in a population of the Brazilian Amazon region.

KEYWORDS

ABCC4, ancestry, chronic myeloid leukemia, *CYP2A6*, genetic susceptibility

1 | INTRODUCTION

The carcinogenesis of Chronic Myeloid Leukemia (CML) is complex and multifactorial (Li et al., 2014). The principal genetic hallmark of CML is the *BCR-ABL1* oncogene, originated by the t(9;22) translocation, which fuses *ABL1* on chromosome 9q34 to *BCR* on chromosome 22q11 (Langabeer, 2013). This encodes the chimeric protein BCR-ABL, a constitutively active tyrosine kinase that drives the pathogenesis of CML (Egan & Radich, 2016).

The etiology of CML is still not completely elucidated. Some studies have reported that environmental factors may be involved

The authors Natasha Monte and Karla B C C Pantoja share the first authorship.
[Correction added on July 2, 2021, after first Online publication: The first authorship footnote has been included.]

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in the development of the disease, but this is not conclusive (Bispo et al., 2020; Musselman et al., 2013). The importance of the expression of *BCR-ABL* in the onset and progression of CML is well established, however, although other genes may also be involved in its development. For example, Ferri et al., (2019) found an association with CML risk in the case of the A allele of the rs12573787 polymorphism of the phosphatase and tensin homolog gene (*PTEN*), a tumor suppressor gene involved in the modulation of cell proliferation and apoptosis.

To better understand the genetic factors involved in the etiology of CML, we investigated 30 polymorphisms in genes of the carcinogenic metabolism involved in susceptibility to different types of cancer, including CML (Campa et al., 2012; Özten et al., 2012; Weich et al., 2016). These genes encode DNA repair proteins (Coskunpinar et al., 2015; Damineni et al., 2014), transmembrane carriers (Campa et al., 2012; Pereira et al., 2016), and endogenous and xenobiotic-metabolizing enzymes (He et al., 2014; Islam et al., 2013; Zhao et al., 2016).

The genetic variability of the target population is an important consideration for susceptibility studies, given that groups with distinct ancestry may present substantial differences in their allelic frequencies. Given this, the findings on a specific population may not be applicable to other groups that have a distinct genetic composition (Carvalho et al., 2015; Yu & Chen, 2012;). One clear example here is the Brazilian population, which is one of the most genetically heterogeneous groups found anywhere in the world, with principal contributions from three parental groups: Europeans, Amerindians, and Africans (Carvalho et al., 2015). The study of highly admixed populations is paramount to the comprehension of the influence of genetic polymorphisms on the predisposition of the individual to develop complex diseases, such as CML.

Given these considerations, the present study evaluated the association between the polymorphisms of key genes (in the transport and metabolism pathways) and susceptibility to CML in a population with a high degree of genetic admixture from the Brazilian Amazon. The key genes evaluated in this study and their OMIM access numbers are: FPGS (136510), ABCC2 (601107), ABCC4 (605250), ABCB1 (171050), ABCG2 (603756), SLC29A1 (602193), SLC22A7 (604995), DPYD (612779), CYP2A6 (122720), UMPS (613891), MTHFR (607093), GGH (601509), RRM1 (180410), and TP53 (191170).

2 | MATERIAL AND METHODS

2.1 | Ethical compliance

The present study was approved by the Research Ethics Committees of the Oncology Research Nucleus, under protocol number 3.354.571/2019, and the Ophir Loyola Hospital, under protocol number 1.575.920/2016. All the participants signed a statement of informed consent.

2.2 | Case and controls

The participants in the present study were selected based on a retrospective case-control study design. Data and samples were collected from 269 individuals, of which, 143 were patients diagnosed with CML (case group) and 126 were cancer-free individuals (control group), both from the city of Belém, located in the Amazon region of Brazil.

The CML patients had been treated for a minimum of 5 years and a maximum of 15 years, and they all had well-documented laboratory and clinical data. These patients were being treated in the onco-hematology sector of the Ophir Loyola Hospital in Belém (Pará, Brazil). The control group consisted of elderly individuals (ages of between 60 and 75 years) with no clinical reports of any type of cancer. Some of these individuals had been diagnosed with chronic conditions, such as hypertension or diabetes.

2.3 | Selection of markers

Thirty Single Nucleotide Polymorphisms (SNPs) of 14 genes were chosen through a search of two databases, *The Human Gene Mutation Database* (www.hgmd.cf.ac.uk/) and *PharmGKB* (www.pharmgkb.org/). The markers were selected based on two principal criteria: (1) polymorphisms involved in pivotal intracellular metabolic activities, and (2) previous reports of the marker in associative studies found in the *Pubmed* (www.ncbi.nlm.nih.gov/) database, which identify the marker as a potential predictor of susceptibility to different types of cancer. The polymorphisms presented in Table 1 met these prerequisites, and were thus selected for the analyses presented here.

2.4 | Extraction and quantification of the DNA

The DNA was extracted using the commercial Axy Prep™ Blood Genomic DNA Miniprep kit (Axygen Biotechnology), according to the manufacture's recommendations. The concentration and purity of the DNA were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific NanoDrop 1000; NanoDrop Technologies).

2.5 | Genotyping

The polymorphisms were genotyped by allelic discrimination using the TaqMan OpenArray Genotyping technology, which was run in a QuantStudio™ 12K Flex Real-Time PCR system (Applied Biosystems, Life Technologies), according to the manufacture's protocol.

TABLE 1 Polymorphisms chosen after applying the criteria of selection of markers

Gene	Pathway	Reference sequence (RefSeq)	SNP
TP53	DNA repair	NG_017013.2	rs1042522
RRM1	DNA repair	NG_027992.2	rs1042927
RRM1			rs12806698
MTHFR	Folate metabolism	NG_013351.1	rs1801131
MTHFR			rs1801133
GGH	Folate metabolism	NG_028126.1	rs3758149
FPGS	Folate metabolism	NG_023245.1	rs4451422
ABCG2	Transmembrane transporter	NG_032067.2	rs2231142
ABCB1	Transmembrane transporter	NG_011513.1	rs1045642
ABCB1			rs1128503
ABCC2	Transmembrane transporter	NG_011798.2	rs717620
ABCC4	Transmembrane transporter	NG_050651.2	rs4148551
ABCC4			rs3741206
ABCC4			rs9524885
SLC29A1	Transmembrane transporter	NG_042893.1	rs747199
SLC29A1			rs760370
SLC22A7	Transmembrane transporter	NC_000006.12	rs2270860
SLC22A7			rs4149178
DPYD	Xenobiotic-metabolizing	NG_008807.2	rs17116806
DPYD			rs1801159
DPYD			rs1801265
DPYD			rs3918290
DPYD			rs4970722
DPYD			rs55886062
DPYD			rs67376798
DPYD			rs17376848
DPYD-AS1	Xenobiotic-metabolizing	NC_000001.11	rs1760217
CYP2A6	Xenobiotic-metabolizing	NG_008377.1	rs28399433
CYP2A6			rs8192726
UMPS	Pyrimidine synthesis	NG_017037.1	rs1801019

2.6 | Quality control

The polymorphisms that were not in Hardy-Weinberg equilibrium or had at least 15% of missing genotypes were excluded from subsequent statistical analyses. Of the 30 markers selected initially, then only 13 polymorphisms met all the criteria for analysis (see Table S1 in the Appendix).

2.7 | Analysis of genetic ancestry

Genetic ancestry was analyzed according to Ramos et al., (2016), using 61 autosomal ancestry informative markers in three multiplex PCR reactions. The amplicons were analyzed by electrophoresis using the ABI Prism 3130 sequencer and the Gene Mapper ID v.3.2 software. The individual proportions of European, African, and Amerindian genetic ancestry were estimated using STRUCTURE v.2.3.3, assuming the contribution of three parental populations.

2.8 | Statistical analysis

The statistical analyses were run in RStudio v.3.6.1 (SNPassoc library). Differences in the categorical variable (sex) were tested using Pearson's Chi-square, while the quantitative variable (mean age) was evaluated using Student's *t*. The ancestry indices were compared between case and control groups using the Mann-Whitney test. Multiple logistic regressions were used to assess possible associations between the polymorphisms and susceptibility to CML, by estimating the odds ratios (ORs) and their 95% confidence intervals (CIs).

Sex and age were controlled for in this multivariate analysis, to avoid confounding associations. A significance level of $p < 0.05$ was considered for all the statistical analyses.

3 | RESULTS

3.1 | Description of the study population

The results of the epidemiological analyses are presented in Table 2. Women predominated in the control group, accounting for 66.7% of all individuals, whereas men dominated the CML patients, with 63.7% of the total. There was a significant difference ($p < 0.001$) between the case and control groups in the distribution of the sexes. The mean age of the case group (47.5 years) was also significantly lower ($p < 0.001$) than that of the control group (66.0 years). Given these differences between the two groups, both age and sex were controlled for in the subsequent analyses, in order to minimize their interference in the assessment of the genetic data.

The analysis of the genetic ancestry of the participants revealed a mostly European ancestry in both groups, being 46.3% in the case group and 44.2% in the control group, followed by Amerindian (31.0% in patients and 30.5% in the control individuals), and African ancestry (22.6% in the case group and 25.4% in the control). There was no significant variation ($p > 0.05$) in the ancestral makeup of the two groups.

3.2 | Genotype distribution and risk estimates

Table 3 shows the distribution of the genotypes that varied significantly between the case and control groups, in in addition to their ORs, with regard to the potential susceptibility to CML. Significant differences were found in the cases of the rs28399433 polymorphism of the *CYP2A6* gene and the rs3742106 polymorphism of the *ABCC4* gene.

Individuals with the C allele of the rs28399433 polymorphism of the *CYP2A6* gene, either homozygously or heterozygously, presented a significant protective effect against the development of CML ($p = 0.008$; OR = 0.33; 95% CI = 0.14–0.78). Similarly, carriers of the CC genotype of the rs3742106 variant of the *ABCC4* gene also presented a significantly reduced susceptibility to CML ($p = 0.020$; OR = 0.30; 95% CI = 0.10–0.88), in comparison with the other genotypes.

None of the other polymorphisms presented any significant variation between case and control groups. The distribution of the genotypes in the case and control groups is shown in the Appendix (Table S2).

TABLE 2 Epidemiological data of the CML patients and the control group

Variable	CML (%)	Control (%)	*p*-value
Total	143	126	
Age (years)	47.41 ± 14.93	66.02 ± 4.45	<0.001
Sex (%)			
Men	79 (63.7)	42 (33.3)	<0.001
Women	45 (36.3)	84 (66.7)	
Ancestry mean			
European	0.463 ± 0.149	0.442 ± 0.161	0.268
Amerindian	0.310 ± 0.138	0.305 ± 0.147	0.572
African	0.226 ± 0.097	0.254 ± 0.147	0.517

4 | DISCUSSION

Carcinogenesis is a multistep process that involves both genetic and environmental risk factors. The presence of genetic polymorphisms in pivotal genes may affect the structure, function, stability or folding of the encoded proteins, which may initiate a carcinogenic event (Frikha et al., 2020; Zhang & Zhu, 2020). The present study evaluated the possible association of SNPs in genes of key cellular pathways, such as the transport of substances across cell membranes, DNA repair, the folate and xenobiotic metabolisms, and pyrimidine synthesis, with a predisposition to the development of CML.

Over the years, these polymorphisms have been associated with different cancers and other complex diseases. The *RRM1* gene, for example, has been related to a high degree of sensitivity to oncological chemotherapy (Yang et al., 2019; Zhao et al., 2012) and has been identified as a predictive factor in gemcitabine therapy in oncology patients (Jordheim et al., 2011). The *ABCG2* gene has also been associated with resistance to chemotherapy in the treatment of many types of cancer (Amawi et al., 2019; Robey et al., 2018). Polymorphisms of the *CYP* gene family are also known to modulate susceptibility to a number of different complex diseases (Daly, 2015). Up to now, however, there has been little research into possible associations between these variants and susceptibility to CML.

The results of the present study indicated that carriers of the C allele (whether homozygous or heterozygous) of the rs28399433 polymorphism of the *CYP2A6* gene are significantly less susceptible to the development of CML in comparison with the AA genotype. Previous studies have shown that the rs28399433 polymorphism, which is common in Asian populations, is located in the TATA box region, and is responsible for reducing gene transcription (Rodriguez-Antona et al., 2010).

TABLE 3 Odds Ratios and genotype distribution of the statistically significant polymorphisms in the CML patients and the control group

Genotype	Control (%)	CML (%)	*p*-value	OR (95% CI)[a]
CYP2A6[b] rs28399433				
AA	107 (66.5)	95 (83.3)	0.008	AC + CC vs. AA[c]: 0.33 (0.14–0.78)
AC	45 (28.0)	17 (14.9)		
CC	9 (5.6)	2 (1.8)		
Allele A	107 (66.5)	95 (83.3)		
Allele C	54 (33.5)	19 (16.7)		
ABCC4[e] rs3742106				
AA	43 (29.7)	43 (37.1)	0.020	CC vs. AA + AC[d]: 0.30 (0.10–0.88)
AC	71 (49.0)	55 (47.4)		
CC	31 (21.4)	18 (15.5)		
Allele A	114 (78.6)	98 (84.5)		
Allele C	31 (21.4)	18 (15.5)		

[a]Multiple logistic regression adjusted by age and gender.
[b]*CYP2A6* RefSeq: NG_008377.1.
[c]Heterozygous genotype + mutant homozygous genotype *vs*. Wild homozygous genotype.
[d]Mutant homozygous genotype *vs*. Wild homozygous genotype + heterozygous genotype.
[e]*ABCC4* RefSeq: NG_050651.2.

The only previous study that has linked this variant to predisposition to cancer was that of Ezzeldin et al., (2018), who attempted to establish a relationship between this polymorphism and the risk of lung cancer in the population of Egypt. However, this variant occurred at a prohibitively low frequency in the study group (3.7%) to support the statistical analyses.

The association between other polymorphisms of the *CYP2A6* gene and cancer has also been evaluated in a number of other studies. For example, Song et al., (2009) found that the deletion of the entire *CYP2A6* gene (CYP2A6*4) resulted in a reduced risk of bladder cancer in Chinese smokers, while Coskunpinar et al., (2015) found that this same variant was linked to a decreased risk of lung cancer in a population from Bangladesh.

The present study also showed that the homozygous CC genotype of the rs3742106 polymorphism of the *ABCC4* gene confers a decreased susceptibility to the development of CML. It is important to note here that *ABCC4*, also known as the multi-drug resistance-associated protein 4, is an important member of the ATP-binding cassette transporter family, and is responsible for transporting a variety of endogenous and exogenous organic anions, of varying composition, out of the cell (Wen et al., 2015). Given its chemotherapeutic drug efflux capacity, *ABCC4* has been studied extensively in relation to drug resistance, in various types of cancer cells. Previous research has also shown that this gene influences the biology of the cancer cell.

For example, Zhao et al., (2014) found a high level of expression of ABCC4 in different lung cancer cell lines. In this study, the authors reported that the suppression of the expression of the ABCC4 messenger RNA (mRNA) resulted in a decrease in cell proliferation, probably due to the ability of ABCC4 to transport the molecules involved in cell signaling. Corroborating these findings, Chen et al., (2017) demonstrated in cell culture that the presence of the T allele of the rs3742106 polymorphism affects the regulatory role of the mRNA and thus decreases the expression of the ABCC4 protein.

A high level of ABCC4 expression has also been observed in aggressive primary neuroblastoma (Murray et al., 2017), the blast cells of adult patients with acute myeloid leukemia, and in acute childhood lymphoblastic leukemia (Copsel et al., 2011; Mesrian Tanha et al., 2017). Pereira et al., (2014) also found an association between other variants of the *ABCC4* gene, which were not evaluated in the present study, and colorectal carcinogenesis. Despite the existence of previous studies linking polymorphisms of the *ABCC4* gene with certain types of cancer, there has been no research on the relationship between the rs3742106 variant and a predisposition to neoplasms or other diseases.

As shown in the present study, the proteins expressed by these genes can be modulated by SNPs, which modifies their function. In the case of the *ABBC4* gene, this would affect the cell's efflux, while in the *CYP2A6* gene, the effect would be on the metabolizing activity of potential carcinogens (Ezzeldin et al., 2018; Rodriguez-Antona et al., 2010), which would contribute to the carcinogenic process.

In addition to the variation in the genotype frequencies of the study polymorphisms and their association with the risk

of CML, significant differences were also found in the mean age and the sexes of the case and control groups, but not in their ancestral makeup.

These findings indicate that CML occurs more frequently in men than in women, which is consistent with the findings of two studies in Bangladesh and Pakistan (Bhatti et al., 2012; Mottalib et al., 2014). It remains unclear, however, while there is a higher frequency of CML in men, with different studies suggesting social, behavioral, or even biological factors (Bortolheiro & Chiattone, 2008). Radivoyevitch et al., (2014) raised the hypothesis that men have more target cells at risk of developing CML than women.

The mean ages of the two groups were significantly different ($p < 0.001$; case = 47.41 ± 14.93 years; control = 66.02 ± 4.45 years). As the incidence of CML is known to increase with age, peaking between 55 and 60 years of age, the control group was selected specifically to include individuals of an older age.

5 | CONCLUSIONS

The results of the present study demonstrated that the rs28399433 (CYP2A6) and the rs3742106 (ABCC4) polymorphisms are associated with a protective effect against the development of CML in a highly miscegenated population from the Brazilian Amazon. This is the first study to associate genetic polymorphisms with a susceptibility to CML in an admixed population from the Brazilian Amazon region, which has a unique genetic background and may thus deviate from the patterns found in more genetically homogeneous populations.

The present study is the first to show an association between polymorphisms of the ABCC4 and CYP2A6 genes with a decreased predisposition for the development of CML. The findings of this study may provide important insights into the genetic predisposition of individuals to develop CML, although further research will be required to provide a more conclusive interpretation of the observed patterns.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Natasha Monte https://orcid.org/0000-0003-4061-5045
Karla B. C. C. Pantoja https://orcid.org/0000-0002-1278-3722
Ney P. C. dos Santos https://orcid.org/0000-0001-8087-7037

REFERENCES

Amawi, H., Sim, H. M., Tiwari, A. K., Ambudkar, S. V., & Shukla, S. (2019). ABC transporter-mediated multidrug-resistant cancer. *Advances in Experimental Medicine and Biology*, *1141*, 549–580. https://doi.org/10.1007/978-981-13-7647-4_12

Bhatti, F. A., Ahmed, S., & Ali, N. (2012). Clinical and hematological features of 335 patients of chronic myelogenous leukemia diagnosed at single centre in Northern Pakistan. *Clinical Medicine. Blood Disorders*, *5*, CMBD.S10578. https://doi.org/10.4137/cmbd.s10578

Bispo, J., Pinheiro, P. S., & Kobetz, E. K. (2020). Epidemiology and etiology of leukemia and lymphoma. *Cold Spring Harbor Perspectives in Medicine*, *10*(6), a034819. https://doi.org/10.1101/cshperspect.a034819

Bortolheiro, T. C., & Chiattone, C. S. (2008). Leucemia Mielóide Crônica: história natural e classificação. *Revista Brasileira De Hematologia E Hemoterapia*, *30*(Suppl. 1), 3–7. https://doi.org/10.1590/S1516-84842008000700003

Campa, D., Butterbach, K., Slager, S. L., Skibola, C. F., de Sanjosé, S., Benavente, Y., Becker, N., Foretova, L., Maynadie, M., Cocco, P., Staines, A., Kaaks, R., Boffetta, P., Brennan, P., Conde, L., Bracci, P. M., Caporaso, N. E., Strom, S. S., Camp, N. J., Cerhan, J. R., … Nieters, A. (2012). A comprehensive study of polymorphisms in the ABCB1, ABCC2, ABCG2, NR1I2 genes and lymphoma risk. *International Journal of Cancer*, *131*(4), 803–812. https://doi.org/10.1002/ijc.26436

Carvalho, D. C., Wanderley, A. V., Amador, M. A. T., Fernandes, M. R., Cavalcante, G. C., Pantoja, K. B. C. C., Mello, F. A. R., de Assumpção, P. P., Khayat, A. S., Ribeiro-dos-Santos, Â., Santos, S., & dos Santos, N. P. C. (2015). Amerindian genetic ancestry and INDEL polymorphisms associated with susceptibility of childhood B-cell Leukemia in an admixed population from the Brazilian Amazon. *Leukemia Research*, *39*(11), 1239–1245. https://doi.org/10.1016/j.leukres.2015.08.008

Chen, Q., Meng, F., Wang, L., Mao, Y., Zhou, H., Hua, D., Zhang, H., & Wang, W. (2017). A polymorphism in ABCC4 is related to efficacy of 5-FU/capecitabine-based chemotherapy in colorectal cancer patients. *Scientific Reports*, *7*(1), 7059. https://doi.org/10.1038/s41598-017-07491-3

Copsel, S., Garcia, C., Diez, F., Vermeulem, M., Baldi, A., Bianciotti, L. G., Russel, F. G., Shayo, C., & Davio, C. (2011). Multidrug resistance protein 4 (MRP4/ABCC4) regulates cAMP cellular levels and controls human leukemia cell proliferation and differentiation. *The Journal of Biological Chemistry*, *286*(9), 6979–6988. https://doi.org/10.1074/jbc.M110.166868

Coskunpinar, E., Yildiz, P., Aynaci, E., Turna, A., Musteri Oltulu, Y., Hekimoglu, E., Isbir, T., & Yaylim, I. (2015). Investigation of

some DNA repair genes association in non small cell lung cancer. *Cellular and Molecular Biology*, *61*(8), 57–62.

Daly, A. K. (2015). Polymorphic variants of cytochrome p450: relevance to cancer and other diseases. *Advances in Pharmacology*, *74*, 85–111. https://doi.org/10.1016/bs.apha.2015.03.001

Damineni, S., Rao, V. R., Kumar, S., Ravuri, R. R., Kagitha, S., Dunna, N. R., Digumarthi, R., & Satti, V. (2014). Germline mutations of TP53 gene in breast cancer. *Tumour Biology*, *35*(9), 9219–9227. https://doi.org/10.1007/s13277-014-2176-6

Egan, D., & Radich, J. (2016). Making the diagnosis, the tools, and risk stratification: More than just BCR-ABL. *Best Practice & Research Clinical Haematology*, *29*(3), 252–263. https://doi.org/10.1016/j.beha.2016.10.015

Ezzeldin, N., El-Lebedy, D., Darwish, A., El Bastawisy, A., Abd Elaziz, S. H., Hassan, M. M., & Saad-Hussein, A. (2018). Association of genetic polymorphisms CYP2A6*2 rs1801272 and CYP2A6*9 rs28399433 with tobacco-induced lung Cancer: case-control study in an Egyptian population. *BMC Cancer*, *18*(1), 525. https://doi.org/10.1186/s12885-018-4342-5

Ferri, C., Weich, N., Gutiérrez, L., De Brasi, C., Bengió, M. R., Zapata, P., Fundia, A., & Larripa, I. (2019). Single nucleotide polymorphism in PTEN-Long gene: A risk factor in chronic myeloid leukemia. *Gene*, *694*, 71–75. https://doi.org/10.1016/j.gene.2019.01.038

Frikha, R., Turki, F., Frikha, F., Elloumi, M., & Rebai, T. (2020). Involvement of MTHFR rs1801133 in the susceptibility of acute lymphoblastic leukemia: A preliminary study. *Journal of Pediatric Hematology/Oncology*. Advance online publication. https://doi.org/10.1097/MPH.0000000000001970

He, H., He, G., Wang, T., Cai, J., Wang, Y., Zheng, X., Dong, Y., & Lu, J. (2014). Methylenetetrahydrofolate reductase gene polymorphisms contribute to acute myeloid leukemia and chronic myeloid leukemia susceptibilities: evidence from meta-analyses. *Cancer Epidemiology*, *38*(5), 471–478. https://doi.org/10.1016/j.canep.2014.07.002

Islam, M. S., Ahmed, M. U., Sayeed, M. S., Maruf, A. A., Mostofa, A. G., Hussain, S. M., Kabir, Y., Daly, A. K., & Hasnat, A. (2013). Lung cancer risk in relation to nicotinic acetylcholine receptor, CYP2A6 and CYP1A1 genotypes in the Bangladeshi population. *Clinica Chimica Acta*, *416*, 11–19. https://doi.org/10.1016/j.cca.2012.11.011

Jordheim, L. P., Sève, P., Trédan, O., & Dumontet, C. (2011). The ribonucleotide reductase large subunit (RRM1) as a predictive factor in patients with cancer. *The Lancet. Oncology*, *12*(7), 693–702. https://doi.org/10.1016/S1470-2045(10)70244-8

Langabeer, S. E. (2013). Is the BCR-ABL1 transcript type in chronic myeloid leukaemia relevant? *Medical Oncology (Northwood, London, England)*, *30*(2), 508. https://doi.org/10.1007/s12032-013-0508-9

Li, B., Zhang, J., Wang, L., Li, Y., Jin, J., Ai, L., Li, C., Li, Z., & Mao, S. (2014). MTHFR genetic polymorphisms may contribute to the risk of chronic myelogenous leukemia in adults: a meta-analysis of 12 genetic association studies. *Tumour Biology*, *35*(5), 4233–4245. https://doi.org/10.1007/s13277-013-1554-9

Mesrian Tanha, H., Rahgozar, S., & Mojtabavi Naeini, M. (2017). ABCC4 functional SNP in the 3' splice acceptor site of exon 8 (G912T) is associated with unfavorable clinical outcome in children with acute lymphoblastic leukemia. *Cancer Chemotherapy and Pharmacology*, *80*(1), 109–117. https://doi.org/10.1007/s00280-017-3340-7

Mottalib, M. A., Sultana, T. A., Khalil, M. I., Gan, S. H., Islam, M. S., Choudhury, S., & Hossain, M. A. (2014). Phase distribution of chronic myeloid leukemia in Bangladesh. *BMC Research Notes*, *7*, 142. https://doi.org/10.1186/1756-0500-7-142

Murray, J., Valli, E., Yu, D., Truong, A. M., Gifford, A. J., Eden, G. L., Gamble, L. D., Hanssen, K. M., Flemming, C. L., Tan, A., Tivnan, A., Allan, S., Saletta, F., Cheung, L., Ruhle, M., Schuetz, J. D., Henderson, M. J., Byrne, J. A., Norris, M. D., … Fletcher, J. I. (2017). Suppression of the ATP-binding cassette transporter ABCC4 impairs neuroblastoma tumour growth and sensitises to irinotecan in vivo. *European Journal of Cancer*, *83*, 132–141. https://doi.org/10.1016/j.ejca.2017.06.024

Musselman, J. R., Blair, C. K., Cerhan, J. R., Nguyen, P., Hirsch, B., & Ross, J. A. (2013). Risk of adult acute and chronic myeloid leukemia with cigarette smoking and cessation. *Cancer Epidemiology*, *37*(4), 410–416. https://doi.org/10.1016/j.canep.2013.03.012

Özten, N., Sunguroğlu, A., & Bosland, M. C. (2012). Variations in glutathione-S-transferase genes influence risk of chronic myeloid leukemia. *Hematological Oncology*, *30*(3), 150–155. https://doi.org/10.1002/hon.1018

Pereira, C., Queirós, S., Galaghar, A., Sousa, H., Marcos-Pinto, R., Pimentel-Nunes, P., Brandão, C., Medeiros, R., & Dinis-Ribeiro, M. (2016). Influence of genetic polymorphisms in prostaglandin E2 pathway (COX-2/HPGD/SLCO2A1/ABCC4) on the risk for colorectal adenoma development and recurrence after polypectomy. *Clinical and Translational Gastroenterology*, *7*(9), e191. https://doi.org/10.1038/ctg.2016.47

Pereira, C., Queirós, S., Galaghar, A., Sousa, H., Pimentel-Nunes, P., Brandão, C., Moreira-Dias, L., Medeiros, R., & Dinis-Ribeiro, M. (2014). Genetic variability in key genes in prostaglandin E2 pathway (COX-2, HPGD, ABCC4 and SLCO2A1) and their involvement in colorectal cancer development. *PLoS One*, *9*(4), e92000. https://doi.org/10.1371/journal.pone.0092000

Radivoyevitch, T., Jankovic, G. M., Tiu, R. V., Saunthararajah, Y., Jackson, R. C., Hlatky, L. R., Gale, R. P., & Sachs, R. K. (2014). Sex differences in the incidence of chronic myeloid leukemia. *Radiation and Environmental Biophysics*, *53*(1), 55–63. https://doi.org/10.1007/s00411-013-0507-4

Ramos, B. R., Mendes, N. D., Tanikawa, A. A., Amador, M. A., dos Santos, N. P., dos Santos, S. E., Castelli, E. C., Witkin, S. S., & da Silva, M. G. (2016). Ancestry informative markers and selected single nucleotide polymorphisms in immunoregulatory genes on preterm labor and preterm premature rupture of membranes: a case control study. *BMC Pregnancy and childBirth*, *16*, 30. https://doi.org/10.1186/s12884-016-0823-1

Robey, R. W., Pluchino, K. M., Hall, M. D., Fojo, A. T., Bates, S. E., & Gottesman, M. M. (2018). Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nature Reviews. Cancer*, *18*(7), 452–464. https://doi.org/10.1038/s41568-018-0005-8

Rodriguez-Antona, C., Gomez, A., Karlgren, M., Sim, S. C., & Ingelman-Sundberg, M. (2010). Molecular genetics and epigenetics of the cytochrome P450 gene family and its relevance for cancer risk and treatment. *Human Genetics*, *127*(1), 1–17. https://doi.org/10.1007/s00439-009-0748-0

Song, D. K., Xing, D. L., Zhang, L. R., Li, Z. X., Liu, J., & Qiao, B. P. (2009). Association of NAT2, GSTM1, GSTT1, CYP2A6, and CYP2A13 gene polymorphisms with susceptibility and clinicopathologic characteristics of bladder cancer in Central China. *Cancer Detection and Prevention*, *32*(5–6), 416–423. https://doi.org/10.1016/j.cdp.2009.02.003

Weich, N., Ferri, C., Moiraghi, B., Bengió, R., Giere, I., Pavlovsky, C., Larripa, I., & Fundia, A. (2016). TP53 codon 72 polymorphism

predicts chronic myeloid leukemia susceptibility and treatment outcome. *Blood Cells, Molecules, and Diseases*, *59*, 129–133. https://doi.org/10.1016/j.bcmd.2016.05.007

Wen, J., Luo, J., Huang, W., Tang, J., Zhou, H., & Zhang, W. (2015). The pharmacological and physiological role of multidrug-resistant protein 4. *The Journal of Pharmacology and Experimental Therapeutics*, *354*(3), 358–375. https://doi.org/10.1124/jpet.115.225656

Yang, Z., Fu, B., Zhou, L., Xu, J., Hao, P., & Fang, Z. (2019). RRM1 predicts clinical outcome of high-and intermediate-risk non-muscle-invasive bladder cancer patients treated with intravesical gemcitabine monotherapy. *BMC Urology*, *19*(1), 69. https://doi.org/10.1186/s12894-019-0497-x

Yu, L., & Chen, J. (2012). Association of MTHFR Ala222Val (rs1801133) polymorphism and breast cancer susceptibility: An update meta-analysis based on 51 research studies. *Diagnostic Pathology*, *7*, 171. https://doi.org/10.1186/1746-1596-7-171

Zhang, W. J., & Zhu, Z. M. (2020). Association between the rs3751143 polymorphism of P2RX7 gene and chronic lymphocytic leukemia: A meta-analysis. *Purinergic Signalling*, *16*(4), 479–484. https://doi.org/10.1007/s11302-020-09737-8

Zhao, L. P., Xue, C., Zhang, J. W., Hu, Z. H., Zhao, Y. Y., Zhang, J., Huang, Y., Zhao, H. Y., & Zhang, L. (2012). Expression of RRM1 and its association with resistancy to gemcitabine-based chemotherapy in advanced nasopharyngeal carcinoma. *Chinese Journal of Cancer*, *31*(10), 476–483. https://doi.org/10.5732/cjc.012.10029

Zhao, X. Q., Cao, W. J., Yang, H. P., Yang, X. W., Tang, P., Sun, L., & Gao, X. (2016). DPYD gene polymorphisms are associated with risk and chemotherapy prognosis in pediatric patients with acute lymphoblastic leukemia. *Tumour Biology*, *37*(8), 10393–10402. https://doi.org/10.1007/s13277-016-4908-2

Zhao, X., Guo, Y., Yue, W., Zhang, L., Gu, M., & Wang, Y. (2014). ABCC4 is required for cell proliferation and tumorigenesis in non-small cell lung cancer. *OncoTargets and Therapy*, *7*, 343–351. https://doi.org/10.2147/OTT.S5602

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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Article

Influence of Polymorphism on the *NFkB1* Gene (rs28362491) on the Susceptibility to Sarcopenia in the Elderly of the Brazilian Amazon

Esdras E. B. Pereira [1], Darlen C. de Carvalho [2], Luciana P. C. Leitão [2], Juliana C. G. Rodrigues [2], Antônio A. C. Modesto [1], Evitom C. de Sousa [3], Sidney E. B. dos Santos [1,2], Marianne R. Fernandes [2] and Ney P. C. dos Santos [1,2,*]

1 Laboratory of Human and Medical Genetics, Institute of Biological Science, Federal University of Pará, Belém 66077-830, Pará, Brazil; esdrasedgarbp@gmail.com (E.E.B.P.); antonioacm@ufpa.br (A.A.C.M.); sidneysantos@ufpa.br (S.E.B.d.S.)

2 Oncology Research Center, Federal University of Pará, Belém 66073-005, Pará, Brazil; darlen.c.cavalho@gmail.com (D.C.d.C.); colaresluciana@gmail.com (L.P.C.L.); julianacgrodrigues@gmail.com (J.C.G.R.); fernandesmr@yahoo.com.br (M.R.F.)

3 Resistance Exercise and Health Laboratory, State University of Pará, Belém 66095-049, Pará, Brazil; evitomuepa@gmail.com

* Correspondence: npcsantos.ufpa@gmail.com

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Abstract: Background: Sarcopenia is a disease characterized by progressive reduction in muscle mass and strength or function. Although it is known that sarcopenia may be associated with environmental factors, studies suggest the identification of genes related to skeletal muscle maintenance that explain the susceptibility to the disease. Objective: To analyze the influence of *NFkB1* gene polymorphism on susceptibility to sarcopenia in the elderly. Methods: This is a case-control study, which included 219 elderly people, 74 elderly people with sarcopenia, and 145 without sarcopenia. Samples were analyzed for *NFkB1* gene polymorphism (rs28362491), genotyped in PCR, and followed by fragment analysis. To avoid misinterpretation due to population substructure, we applied a previously developed set of 61 informative ancestral markers that were genotyped by multiplex PCR. We used logistic regression to identify differences in genotypic frequencies between elderly people with and without sarcopenia. Results: It was observed that the *NFkB1* gene polymorphism presented frequencies of 24%, 50%, and 26% for the genotype DEL/DEL, DEL/INS, and INS/INS, respectively. Furthermore, elderly individuals with the INS/INS genotype had increased chances (p = 0.010; OR:2.943; 95%CI:1.301–6.654) for the development of sarcopenia. Conclusion: The INDEL polymorphism of the *NFkB1* gene (rs28362491) may influence the susceptibility to sarcopenia in the elderly in elderly people in the Amazon.

Keywords: skeletal muscle; genetic biomarkers; *NFkB1*; sarcopenia

1. Introduction

Sarcopenia is a progressive degenerative disease characterized by loss of skeletal muscle mass and reduced function [1]. Worldwide, this disease is common between 9.9% and 40.4% of people over 60 years, depending on the population investigated and the criteria used [2]. In Brazil, this prevalence is 17% among the elderly [3]. The clinical consequences of sarcopenia include the risk of falls, fractures, loss of function, frailty, and increased mortality, generating significant personal and socioeconomic costs, and it is considered a public health problem that needs to be identified early [4–6].

The pathophysiology of sarcopenia is complex and can be attributed to a variety of factors, including oxidative stress, inflammation, apoptosis, and mitochondrial dysregulation [7]. The disease is also associated with environmental factors, such as physical activity and diet, and may have specific genetic influences, related to skeletal muscle maintenance,

which may explain the variability of inter-individual characteristics related to muscle mass and strength [8].

This has motivated studies that suggest the importance of determining the genetic factors that are associated with aging and that can modulate skeletal muscle phenotypes in the elderly and the unfavorable genotypes associated with accelerated sarcopenia [9]. These would be biomarkers, possibly used in screening, diagnosis, staging, and sarcopenia prognosis [10–12].

Most studies involving genetic susceptibility to sarcopenia explore genes for hormones, receptors, cytokines, growth factors, structural factors, and metabolism [10,11,13]. Despite this, little progress has been made in identifying the specific genetic influences on sarcopenia. Furthermore, there are few studies that aim to predict the risk of sarcopenia based on single nucleotide polymorphisms (SNPs) [8]. Thus, further studies in different populations are needed to understand the genetic influence on sarcopenia.

A common element of these pathways is nuclear factor kappa B (NFkB), a family of pleiotropic transcription factors, NFkB1 (p50/p105), NFkB2 (p52/p100), RelA (p65), c-Rel and Rel B, which regulate other myogenic transcription factors such as MyoD, MURF-1, and MAXbx involved in the development of sarcopenia [14–16].

The *NFkB1* gene is located on chromosome 4q24 and consists of 24 exons [16]. The *NFkB1* gene polymorphism (rs28362491) occurs between activator protein 1 (AP-1) and the κB binding site and has been associated with diseases of aging, such as inflammatory diseases, believed to also influence longevity [17–20]. Therefore, the aim of this study was to investigate possible associations between *NFkB1* gene polymorphism (rs28362491) and susceptibility to sarcopenia in the elderly people in the Amazon.

2. Methods

2.1. Ethical Conformity

The study was approved by the Research Ethics Committees of the Oncology Research Center, under protocol 927.808/2014, and by the João de Barros Barreto University Hospital, under protocol number 941.207/2015. All participants signed an informed consent form.

2.2. Case and Control

Participants were randomly selected from two public health care centers in the city of Belém do Pará, in the Brazilian Amazon, in a retrospective case-control study design. The 219 individuals were 60 years old or older, of both sexes, not belonging to the same family nucleus, and of the same socioeconomic level. Two groups were structured: 74 patients with sarcopenia (Case Group) and 145 patients without any type of sarcopenia (Control Group).

2.3. Sarcopenia Assessment

The sarcopenia diagnosis was based on the EWGSOP algorithm, following two criteria: mass reduction and physical performance [21]. The equation by Lee et al. [22] was used to estimate total muscle mass (MMT): MMT = $(0.244 \times \text{body weight}) + (7.8 \times \text{height}) + (6.6 \times \text{gender}) - (0.098 \times \text{age}) + (\text{race} - 3.3)$. The number 0 was assigned for women and 1 for men, -1.2 for Asians, 1.4 for blacks, and 0 for whites. The race variable was based on the predominance of genomic ancestry. This equation was validated for the Brazilian population using DEXA as the "gold standard". The equation was fitted by dividing by height squared, creating a total muscle mass index (TMMI). The cutoff point used to determine reduced muscle mass was ≤ 6.75 kg/m^2 for women and ≤ 10.75 kg/m^2 for men. Physical performance was assessed by mobility, using the 6-m Gait Speed Test (TVM6) and the cutoff point of <0.8 m/s indicating participants with low physical performance [1,21].

2.4. Clinical Assessment

The clinical assessment consisted of scales and quantitative tests. The clinical characterization of the sample included sex, age, lifestyle (alcoholism, smoking, and sedentary lifestyle), present comorbidities, nutritional status, and a screening questionnaire for sar-

copenia (SARC-CalF) and anthropometry. The presence of comorbidities was assessed using the Charlson Comorbidity Index (ICC) [23]. Nutritional status was assessed by the Mini Nutritional Assessment (MAN) [24]. The SARC-CalF questionnaire consists of 6 items questioning strength, help to walk, stand up from a chair, climbing stairs, falls, and calf perimeter [25]. Anthropometry includes measurement of weight (kg), height (m), body mass index (kg/m^2), and calf perimeter (cm).

2.5. DNA Extraction and Quantification

Extraction of genomic DNA from peripheral blood leukocytes was conducted using a Mini Spin Plus Kit (P.250, Biopur, Biometrix) according to the manufacturer's recommendations. DNA concentration and purity were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific NanoDrop 1000; NanoDrop Technologies).

2.6. Genotyping

The *NFkB1* gene polymorphism (rs28362491) was genotyped by a multiplex PCR reaction followed by capillary electrophoresis. For the amplifications, 0.5 µL of the QIAGEN Multiplex PCR Kit (QIAGEN), 1.0 µL of Q-solution, 1.0 µL of Primer Mix, 2.0 µL of water, and 20 ng of DNA were used. PCR was performed following the following protocol: an initial denaturation at 95 °C for 15 min, 35 cycles at 94 °C for 45 s, 60 °C for 90 s, and 72 °C for 60 s, followed by a final extension of 70 °C for 30 min. Analysis of the PCR amplicons was performed from an electrophoresis using the ABI Prism 3130 sequencer and GeneMapper ID v.3.2 software.

2.7. Hardy–Weinberg Equilibrium Analysis (HWE)

The allelic and genotypic frequency of the polymorphism was determined by direct counting of alleles, and then Hardy–Weinberg equilibrium (HWE) was calculated using Arlequin 3.5.1.2 software. The individual proportions of European, African, and Amerindian genetic ancestry were estimated using Structure 2.3.3 software. It was evidenced that the polymorphism was present in the HWE.

2.8. Genetic Ancestrality Analysis

Genotyping was performed to analyze the ancestry of the samples, performed according to Ramos et al. [26], using 61 informative markers of autosomal ancestry in three multiplex PCR reactions. Amplicons were analyzed by electrophoresis using the ABI Prism 3130 sequencer and GeneMapper ID v.3.2 software. The individual proportions of European, African, and Amerindian genetic ancestry were estimated using Structure v.2.3.3 software, assuming three parental populations.

2.9. Statistical Analysis

For the comparative analysis between the studied groups, Pearson's Chi-Square Test was applied, for categorical variables, and the Mann–Whitney test for non-parametric quantitative variables. To analyze the risk of polymorphisms on sarcopenia, a logistic regression controlled by the covariates age, comorbidity, sedentary lifestyle and nutritional status was performed. All statistical analyses were performed using the statistical package of the SPSS 20.0 software, respecting the significance level of 5% (p-value ≤ 0.05).

3. Results

A prevalence of sarcopenia of 34% of the elderly evaluated was observed, being more frequent in women (56.8%), aged over 70 years (56.8%), with a greater number of comorbidities, with a history of sedentary lifestyle (90.5%), and possessing a predominantly European ancestry. It was evident that the demographic and clinical characteristics between the groups differed significantly for age ($p = 0.014$), comorbidities ($p < 0.0001$), sedentary lifestyle ($p < 0.0001$), nutritional status ($p < 0.0001$), and the SARC-CalF score ($p < 0.0001$) (Table 1).

Table 1. Demographic and clinical characteristics of the investigated groups.

Characteristics	Case (n = 74)	Control (n = 145)	p-Value
Sex			
Male	32 (43.2%)	51 (35.2%)	0.2440 [a]
Female	42 (56.8%)	94 (64.8%)	
Age			
≤70 years	32 (43.2%)	88 (60.7%)	0.0140 [a,*]
>70 years	42 (56.8%)	57 (39.3%)	
Charlson Index (Score)			
Median (p25%–p75%)	5.0 (4.0–7.0)	3.0 (2.0–5.0)	<0.0001 [b,*]
Lifestyle			
Smoking	46 (62.2%)	74 (51.0%)	0.1180 [a]
Alcoholism	34 (45.9%)	74 (51.0%)	0.4760 [a]
Sedentary lifestyle	67 (90.5%)	50 (34.5%)	<0.0001 [a,*]
Nutritional Status (NMA)			
Normal	49 (66.2%)	139 (95.9%)	<0.0001 [a,*]
Malnourished	25 (33.8%)	6 (4.1%)	
SARC-CalF (Score)			
Median (p25%–p75%)	16.0 (12.0–18.0)	10.0 (0.0–10.0)	<0.0001 [b,*]
Ancestry			
European	0.451 ± 0.178	0.456 ± 0.170	0.9530 [b]
Amerindian	0.316 ± 0.151	0.299 ± 0.149	0.4200 [b]
African	0.232 ± 0.123	0.244 ± 0.146	0.8470 [b]

NMA, Nutritional Mini-Assessment; SARC-CalF, Screening Questionnaire for Sarcopenia; p, percentile. [a] Pearson's Chi-Square test; [b] Mann–Whitney test; * p-value ≤ 0.05.

Table 2 shows information regarding anthropometry, total muscle mass index, and functional mobility. In anthropometry, it could be observed that the elderly group with sarcopenia was lighter in weight ($p < 0.0001$), with lower BMI ($p < 0.0001$), and lower calf circumference ($p < 0.0001$). In addition, this group also had a lower total muscle mass index ($p < 0.0001$) and lower gait speed ($p < 0.0001$).

Table 2. Anthropometry, total muscle mass index, and functional mobility of the investigated groups.

Variables	Case (n = 74)	Control (n = 145)	p-Value [a]
Anthropometry			
Weight (kg)	50.99 ± 11.31	65.99 ± 11.89	<0.0001 *
Height (m)	1.58 ± 0.09	1.59 ± 0.09	0.8890
BMI (kg/m^2)	20.31 ± 4.52	26.08 ± 4.57	<0.0001 *
Calf Circumference (cm)	27.92 ± 2.09	34.26 ± 3.00	<0.0001 *
Total Muscle Mass Index (kg/m^2)			
Mean (±sd)	7.17 ± 1.69	8.50 ± 1.59	<0.0001 *
Functional Mobility			
Gait Speed (m/s)	0.30 ± 0.19	0.60 ± 0.32	<0.0001 *

BMI, body mass index; sd, standard deviation. [a] Mann–Whitney test; * p-value ≤ 0.05.

It was evidenced that polymorphism was present in the HWE (p-value 0.955). The results of the logistic regression analysis showed that the INS/INS genotype of the *NFkB1* polymorphism (rs28362491) increased the risk of sarcopenia compared to the other genotypes (OR: 2.943; 95% CI: 1.301–6.654). As for allele frequency, the INS allele of this polymorphism increased the risk of sarcopenia compared to the DEL allele (OR: 1.932; 95%CI: 1.187–3.145) (Table 3).

Comparisons of total muscle mass index (TMMI), calf circumference, and gait speed among the three NFKB1 genotypes (rs28362491) shown in Table 4 show that the calf circumference was significantly smaller among the elderly with the INS/INS genotype (p-value: 0.013). The total muscle mass index and gait speed were also lower in the elderly with the INS/INS genotype; however, this difference was not significant (Table 4).

Table 3. Genotypic distribution of the *NFkB1* gene polymorphism (rs28362491) in the investigated groups.

Genotype	Case (n = 74)	Control (n = 145)	p-Value [a]	OR (95% CI)
***NFkB1* (rs28362491)**				
DEL/DEL	15 (20.3%)	37 (25.5%)	0.010 *	INS/INS vs. Others: 2.943 (1.301–6.654)
DEL/INS	30 (40.5%)	79 (54.5%)		
INS/INS	29 (39.2%)	29 (20.0%)		
Allele DEL	60 (40.5%)	153 (52.8%)	0.008 *	1.932 (1.187–3.145)
Allele INS	88 (59.5%)	137 (47.2%)		

INS/INS, insertion/insertion; DEL/INS, deletion/insertion; DEL/DEL, deletion/deletion; OR, odds ratio; CI, confidence interval; [a] logistic regression adjusted for age, comorbidities, sedentary lifestyle, and nutritional status; * p-value $\leq$ 0.05.

Table 4. Comparison of total muscle mass index, calf circumference, and gait speed among the NFkB1 genotypes (rs28362491) of the investigated groups.

Variables	*NFkB1* Genotypes (rs28362491)				INS/INS vs. Others p-Value [a]
	DEL/DEL (n = 52)	DEL/INS (n = 109)	INS/INS (n = 58)	DEL/DEL+DEL/INS (n = 161)	
TMMI (kg/m^2)					
Mean (±sd)	8.39 ± 1.58	8.00 ± 1.77	7.84 ± 1.82	8.14 ± 1.71	0.227
Calf Circumference (cm)					
Mean (±sd)	31.98 ± 3.09	32.71 ± 4.23	31.05 ± 2.29	32.48 ± 3.91	0.013 *
Gait Speed (m/s)					
Mean (±sd)	0.43 ± 0.27	0.55 ± 0.34	0.46 ± 0.30	0.51 ± 0.33	0.269

TMMI, total muscle mass index; INS/INS, insertion/insertion; DEL/INS, deletion/insertion; DEL/DEL, deletion/deletion; sd, standard deviation; [a] Mann–Whitney test; * p-value $\leq$ 0.05.

4. Discussion

Sarcopenia is a common syndrome among the elderly, and its prevalence varies significantly worldwide [27,28]. In the present study, in the Amazon region, the prevalence of sarcopenia was 34%, similar to a study carried out in the southern region of Brazil, which identified a prevalence of 33.3% among the elderly [29]. However, both are superior to what was observed by Diz et al. [3], who identified in their review an average prevalence of 17% of sarcopenia among elderly Brazilians. These distinctions must be analyzed with caution, due to the use of different methods for assessing sarcopenia between studies and differences in the characteristics of the populations investigated.

In this study, most elderly people with sarcopenia were over 70 years of age (56.8%) and were female (56.8%). According to Cruz-Jentoft et al. [21], the frequency of sarcopenia varies from 5 to 13% in the elderly aged between 60 and 70 years and increases to 11–50% for those with more advanced age, which may be associated with the identification of a higher prevalence of sarcopenia in this population.

As in the rest of the world, in Brazil the life expectancy of the female population is greater than that of men, causing them to experience more geriatric syndromes, which may justify sarcopenia being more prevalent in this group [3,30,31]. Furthermore, the emergence of sarcopenia in women seems to be associated with menopause, which causes hormonal changes, which modify the maintenance of muscle tissue [32]. This reduction in sex hormones is more accelerated among women than men [27].

Susceptibility to sarcopenia includes constitutional risk factors and lifestyle and health conditions. Among the constitutional factors are female gender and genetic propensity [33]. Genetic influence explains a significant fraction of the inter-individual variability of sarcopenia, which is driven by genes involved in pathways of metabolism, DNA damage repair, oxidative stress, inflammatory and immune responses, and programmed cell death [34].

In the present study, the analysis of the *NFkB1* gene polymorphism (rs28362491) showed relevant results. The polymorphism presented frequencies of 24%, 50%, and 26% for the genotype DEL/DEL, DEL/INS, and INS/INS, respectively. Elderly individuals with the INS/INS genotype had increased chances (p = 0.010; OR: 2.943; 95%CI: 1.301–6.654)

for the development of sarcopenia. Therefore, individuals homozygous for this insertion in the *NFkB1* gene have an approximately three-fold increased risk for the development of sarcopenia. In addition, the elderly with this genotype had smaller calf circumference (p = 0.013), which also indicates reduced muscle mass.

Oxidative stress, immune response and chronic inflammation influence the maintenance of skeletal muscle, affecting the balance between protein synthesis and breakdown, inducing muscle wasting [14]. The INS/INS genotype of the rs28362491 polymorphism of the *NFkB1* gene has been related to the risk of developing inflammatory diseases associated with altered immune response [16]. The likely mechanism that explains this association may be related to the increased expression and activity of NFkB1, where the INS allele is supposedly associated with increased promoter activity and increased expression of NFkB mRNA [34].

The increased activity of NFkB1 promotes the expression of proteins of the ubiquitin–proteasome system, involved in the breakdown of muscle proteins, in the increased expression of molecules related to inflammation, and in the influence of the myogenic differentiation process, which is necessary for the regeneration of skeletal muscles [35,36]. These pathways, when activated, favor chronic inflammation, sarcopenia, and the frailty syndrome [37–39].

This shows that sarcopenia is certainly related to genetic and environmental mechanisms, which manifest as atrophy, reduced endurance, and loss of muscle strength, which culminate in functional disabilities [40]. This is because joint movement needs preserved muscle strength to maintain gait, limb movements, and actions against gravity, such as sitting and standing, necessary for activities of daily living [41,42].

We believe that the results of association of the *NFKB1* gene polymorphism (rs28362491) with sarcopenia observed in the present study can also be found in other Brazilian regions, respecting the constitutional characteristics of populations between regions. According to Souza et al. [43] the proportions of European, African, and Amerindian genomic ancestry are 68.1%, 19.6%, and 11.6%, respectively, while in the states of the Amazon region, these respective proportions are 52.6%, 19.8%, and 27.7%, revealing that the Amazon region has a greater contribution from Amerindian genomic ancestry, similar to what was observed in the present study.

In the world, as it is a germline structural variant, similar results of this association of *NFKB1* gene polymorphism (rs28362491) with sarcopenia can be found, considering that this polymorphism has already been described in non-Brazilian populations [17–20]. There are already studies showing the association of this gene with the muscle [14–16]. However, our study is the first to show an association of this polymorphism with sarcopenia.

The NFkB1 pathway is not only activated in aging, but directly contributes to age-related pathologies such as sarcopenia. Knowledge of genetic polymorphisms, present in this and other pathways, is important in translational research for sarcopenia, as they provide information for the identification of susceptibility, risk stratification, and potential therapeutic targets, whether by pharmacological or non-pharmacological measures, which aim to maintain or increase the mass, strength, and function of the skeletal muscle, thus preserving the functional capacities and independence of the elderly.

5. Conclusions

In conclusion, the *NFkB1* gene variant (rs28362491) was associated with the risk of developing sarcopenia in elderly people in the Amazon. Elderly people with the INS/INS genotype were almost three times more susceptible to developing the disease. This indicates its potential additional use in screening for sarcopenia in the elderly population, which may facilitate the targeting of prevention, control, and treatment strategies for the disease.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Conflicts of Interest: The authors declare that they have no competing interests.

References

1. Cruz-Jentoft, A.J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyère, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A.; et al. Sarcopenia: Revised European consensus on definition and diagnosis. *Age Ageing* **2019**, *48*, 16–31. [CrossRef]
2. Mayhew, A.J.; Amog, K.; Phillips, S.; Parise, G.; McNicholas, P.D.; de Souza, R.J.; Thabane, L.; Raina, P. The prevalence of sarcopenia in community-dwelling older adults, an exploration of differences between studies and within definitions: A systematic review and meta-analyses. *Age Ageing* **2018**, *48*, 48–56. [CrossRef]
3. Diz, J.B.M.; Leopoldino, A.A.O.; Moreira, B.D.S.; Henschke, N.; Dias, R.C.; Pereira, L.S.M.; Oliveira, V.C. Prevalence of sarcopenia in older Brazilians: A systematic review and meta-analysis. *Geriatr. Gerontol. Int.* **2016**, *17*, 5–16. [CrossRef]
4. Hirschfeld, H.P.; Kinsella, R.; Duque, G. Osteosarcopenia: Where bone, muscle, and fat collide. *Osteoporos. Int.* **2017**, *28*, 2781–2790. [CrossRef]
5. Falcon, L.J.; Harris-Love, M.O. Sarcopenia and the New ICD-10-CM Code: Screening, Staging, and Diagnosis Considerations. *Fed. Pract.* **2017**, *34*, 24–32. [CrossRef]
6. Tosato, M.; Marzetti, E.; Cesari, M.; Savera, G.; Miller, R.R.; Bernabei, R.; Landi, F.; Calvani, R. Measurement of muscle mass in sarcopenia: From imaging to biochemical markers. *Aging Clin. Exp. Res.* **2017**, *29*, 19–27. [CrossRef]
7. Yao, X.; Yang, L.; Li, M.; Xiao, H. Relationship of vitamin D receptor gene polymorphism with sarcopenia and muscle traits based on propensity score matching. *J. Clin. Lab. Anal.* **2020**, *34*, e23485. [CrossRef] [PubMed]
8. Urzi, F.; Pokorny, B.; Buzan, E. Pilot Study on Genetic Associations with Age-Related Sarcopenia. *Front. Genet.* **2021**, *11*, 615238. [CrossRef] [PubMed]
9. Tacutu, R.; Craig, T.; Budovsky, A.; Wuttke, D.; Lehmann, G.; Taranukha, D.; Costa, J.; Fraifeld, V.E.; de Magalhaes, J.P. Human Ageing Genomic Resources: Integrated databases and tools for the biology and genetics of ageing. *Nucleic Acids Res.* **2012**, *41*, D1027–D1033. [CrossRef] [PubMed]
10. Roth, S.M. Genetic aspects of skeletal muscle strength and mass with relevance to sarcopenia. *Bonekey Rep.* **2012**, *1*, 58. [CrossRef]
11. Tan, L.-J.; Liu, S.-L.; Lei, S.-F.; Papasian, C.J.; Deng, H.-W. Molecular genetic studies of gene identification for sarcopenia. *Qual. Life Res.* **2011**, *131*, 1–31. [CrossRef] [PubMed]
12. Calvani, R.; Marini, F.; Cesari, M.; Tosato, M.; Picca, A.; Anker, S.D.; Von Haehling, S.; Miller, R.R.; Bernabei, R.; Landi, F.; et al. Biomarkers for physical frailty and sarcopenia. *Aging Clin. Exp. Res.* **2017**, *29*, 29–34. [CrossRef] [PubMed]
13. Karasik, D.; Cohen-Zinder, M. The genetic pleiotropy of musculoskeletal aging. *Front. Physiol.* **2012**, *3*, 303. [CrossRef] [PubMed]
14. Meng, S.-J.; Yu, L.-J. Oxidative Stress, Molecular Inflammation and Sarcopenia. *Int. J. Mol. Sci.* **2010**, *11*, 1509–1526. [CrossRef] [PubMed]
15. Patel, H.P.; Al-Shanti, N.; Davies, L.C.; Barton, S.; Grounds, M.; Tellam, R.; Stewart, C.; Cooper, C.; Sayer, A.A. Lean Mass, Muscle Strength and Gene Expression in Community Dwelling Older Men: Findings from the Hertfordshire Sarcopenia Study (HSS). *Calcif. Tissue Int.* **2014**, *95*, 308–316. [CrossRef]
16. Yenmis, G.; Oner, T.; Cam, C.; Koc, A.; Kucuk, O.S.; Yakicier, M.C.; Dizman, D.; Sultuybek, G.K. Association ofNFKB1 and NFKBIAPolymorphisms in Relation to Susceptibility of Behçet's Disease. *Scand. J. Immunol.* **2014**, *81*, 81–86. [CrossRef] [PubMed]

17. Yang, Y.-N.; Zhang, J.-Y.; Ma, Y.-T.; Xie, X.; Li, X.-M.; Liu, F.; Chen, B.-D.; Dong, X.-H.; Zheng, Y.-Y.; Pan, S.; et al. −94 ATTG Insertion/Deletion Polymorphism of theNFKB1Gene Is Associated with Coronary Artery Disease in Han and Uygur Women in China. *Genet. Test. Mol. Biomark.* **2014**, *18*, 430–438. [CrossRef]
18. Chen, L.; Cai, P.; Liang, H. Association of the genetic polymorphisms of NFKB1 with susceptibility to ovarian cancer. *Genet. Mol. Res.* **2015**, *14*, 8273–8282. [CrossRef]
19. Lai, H.-M.; Li, X.-M.; Yang, Y.-N.; Ma, Y.-T.; Xu, R.; Pan, S.; Zhai, H.; Liu, F.; Chen, B.-D.; Zhao, Q. Genetic Variation in NFKB1 and NFKBIA and Susceptibility to Coronary Artery Disease in a Chinese Uygur Population. *PLoS ONE* **2015**, *10*, e0129144. [CrossRef]
20. Wang, Y.; Chen, L.; Pan, L.; Xue, J.; Yu, H. The association between NFKB1-94ins/del ATTG polymorphism and non-small cell lung cancer risk in a Chinese Han population. *Int. J. Clin. Exp. Med.* **2015**, *8*, 8153–8157.
21. Cruz-Jentoft, A.J.; Baeyens, J.P.; Bauer, J.M.; Boirie, Y.; Cederholm, T.; Landi, F.; Martin, F.C.; Michel, J.-P.; Rolland, Y.; Schneider, S.; et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **2010**, *39*, 412–423. [CrossRef] [PubMed]
22. Lee, R.C.; Wang, Z.; Heo, M.; Ross, R.; Janssen, I.; Heymsfield, S.B. Total-body skeletal muscle mass: Development and cross-validation of anthropometric prediction models. *Am. J. Clin. Nutr.* **2000**, *72*, 796–803. [CrossRef] [PubMed]
23. Marchena-Gómez, J.; Acosta-Merida, M.A.; Hemmersbach-Miller, M.; Conde-Martel, A.; Roque-Castellano, C.; Hernandez-Romero, J. The Age-Adjusted Charlson Comorbidity Index as an Outcome Predictor of Patients with Acute Mesenteric Ischemia. *Ann. Vasc. Surg.* **2009**, *23*, 458–464. [CrossRef] [PubMed]
24. Serrano-Urrea, R.; Garcia-Meseguer, M.J. Malnutrition in an elderly population without cognitive impairment living in nursing homes in Spain: Study of prevalence using the Mini Nutritional Assessment test. *Gerontology* **2013**, *59*, 490–498. [CrossRef]
25. Bahat, G.; Oren, M.M.; Yilmaz, O.; Kiliç, C.; Aydin, K.; Karan, M.A. Comparing SARC-F with SARC-CalF to Screen Sarcopenia in Community Living Older Adults. *J. Nutr. Health Aging* **2018**, *22*, 1034–1038. [CrossRef] [PubMed]
26. Ramos, B.R.D.A.; D'Elia, M.P.B.; Amador, M.A.T.; Santos, N.P.C.; Santos, S.E.B.; Castelli, E.D.C.; Witkin, S.S.; Miot, H.A.; Miot, L.D.B.; da Silva, M.G. Neither self-reported ethnicity nor declared family origin are reliable indicators of genomic ancestry. *Genetica* **2016**, *144*, 259–265. [CrossRef]
27. Shafiee, G.; Keshtkar, A.; Soltani, A.; Ahadi, Z.; Larijani, B.; Heshmat, R. Prevalence of sarcopenia in the world: A systematic review and meta- analysis of general population studies. *J. Diabetes Metab. Disord.* **2017**, *16*, 1–10. [CrossRef] [PubMed]
28. Dhillon, R.J.; Hasni, S. Pathogenesis and Management of Sarcopenia. *Clin. Geriatr. Med.* **2017**, *33*, 17–26. [CrossRef]
29. Pelegrini, A.; Mazo, G.Z.; Pinto, A.D.A.; Benedetti, T.R.B.; Silva, D.A.S.; Petroski, E.L. Sarcopenia: Prevalence and associated factors among elderly from a Brazilian capital. *Fisioter. Mov.* **2018**, *31*, e003102. [CrossRef]
30. Moreira, V.G.; Perez, M.; Lourenço, R.A. Prevalence of sarcopenia and its associated factors: The impact of muscle mass, gait speed, and handgrip strength reference values on reported frequencies. *Clinics* **2019**, *74*, e477. [CrossRef]
31. Alodhayani, A.A. Sex-specific differences in the prevalence of sarcopenia among pre-frail community-dwelling older adults in Saudi Arabia. *Saudi J. Biol. Sci.* **2021**, *28*, 4005–4009. [CrossRef] [PubMed]
32. Geraci, A.; Calvani, R.; Ferri, E.; Marzetti, E.; Arosio, B.; Cesari, M. Sarcopenia and Menopause: The Role of Estradiol. *Front. Endocrinol.* **2021**, *12*, 682012. [CrossRef] [PubMed]
33. Hairi, N.N.; Bulgiba, A.; Hiong, T.G.; Mudla, I. Sarcopenia in Older People. In *Geriatrics Craig Atwood*; Atwood, C., Ed.; IntechOpen: Rijeka, Croatia, 2012; pp. 29–40. [CrossRef]
34. Kolovou, G.; Kolovou, V.; Vasiliadis, I.; Giannakopoulou, V.; Mihas, C.; Bilianou, H.; Kollia, A.; Papadopoulou, E.; Marvaki, A.; Goumas, G.; et al. The Frequency of 4 Common Gene Polymorphisms in Nonagenarians, Centenarians, and Average Life Span Individuals. *Angiology* **2013**, *65*, 210–215. [CrossRef] [PubMed]
35. Romanick, M.; Thompson, L.V.; Brown-Borg, H.M. Murine models of atrophy, cachexia, and sarcopenia in skeletal muscle. *Biochim. Biophys. Acta* **2013**, *1832*, 1410–1420. [CrossRef]
36. Li, H.; Malhotra, S.; Kumar, A. Nuclear factor-kappa B signaling in skeletal muscle atrophy. *J. Mol. Med.* **2008**, *86*, 1113–1126. [CrossRef] [PubMed]
37. Ghosh, S.; Lertwattanarak, R.; Garduño, J.D.J.; Galeana, J.J.; Li, J.; Zamarripa, F.; Lancaster, J.L.; Mohan, S.; Hussey, S.; Musi, N. Elevated Muscle TLR4 Expression and Metabolic Endotoxemia in Human Aging. *J. Gerontol. Ser. A Boil. Sci. Med. Sci.* **2014**, *70*, 232–246. [CrossRef] [PubMed]
38. Thalacker-Mercer, A.E.; Dell'Italia, L.J.; Cui, X.; Cross, J.M.; Bamman, M.M. Differential genomic responses in old vs. young humans despite similar levels of modest muscle damage after resistance loading. *Physiol. Genom.* **2010**, *40*, 141–149. [CrossRef]
39. Buford, T.W.; Cooke, M.B.; Manini, T.M.; Leeuwenburgh, C.; Willoughby, D.S. Effects of Age and Sedentary Lifestyle on Skeletal Muscle NF- B Signaling in Men. *J. Gerontol. Ser. A Boil. Sci. Med. Sci.* **2010**, *65*, 532–537. [CrossRef]
40. Di Iorio, A.; Abate, M.; Di Renzo, D.; Russolillo, A.; Battaglini, C.; Ripari, P.; Saggini, R.; Paganelli, R.; Abate, G. Sarcopenia: Age-Related Skeletal Muscle Changes from Determinants to Physical Disability. *Int. J. Immunopathol. Pharmacol.* **2006**, *19*, 703–719. [CrossRef]
41. Abate, M.; Di Iorio, A.; Di Renzo, D.; Paganelli, R.; Saggini, R.; Abate, G. Frailty in the elderly: The physical dimension. *Eura Medicophys.* **2007**, *43*, 407–415.

42. Dietzel, R.; Felsenberg, D.; Armbrecht, G. Mechanography performance tests and their association with sarcopenia, falls and impairment in the activities of daily living—A pilot cross-sectional study in 293 older adults. *J. Musculoskelet. Neuronal Interact.* **2015**, *15*, 249–256. [PubMed]
43. De Souza, A.M.; Resende, S.S.; De Sousa, T.N.; De Brito, C.F.A. A systematic scoping review of the genetic ancestry of the Brazilian population. *Genet. Mol. Biol.* **2019**, *42*, 495–508. [CrossRef] [PubMed]

Article

Identification of Genomic Variants Associated with the Risk of Acute Lymphoblastic Leukemia in Native Americans from Brazilian Amazonia

Luciana P. C. Leitão [1,2,†], Darlen C. de Carvalho [1,†], Juliana C. G. Rodrigues [1], Marianne R. Fernandes [1], Alayde V. Wanderley [3], Lui W. M. S. Vinagre [1], Natasha M. da Silva [1], Lucas F. Pastana [1], Laura P. A. Gellen [1], Matheus C. E. Assunção [1], Sweny S. M. Fernandes [3], Esdras E. B. Pereira [4,5], André M. Ribeiro-Dos-Santos [4], João F. Guerreiro [4], Ândrea Ribeiro-dos-Santos [4], Paulo P. de Assumpção [1], Sidney E. B. dos Santos [4] and Ney P. C. dos Santos [1,*]

[1] Oncology Research Nucleus, Universidade Federal do Pará, Belém 66073-005, PA, Brazil; colaresluciana@gmail.com (L.P.C.L.); darlen.c.carvalho@gmail.com (D.C.d.C.); julianacgrodrigues@gmail.com (J.C.G.R.); fernandesmr@yahoo.com.br (M.R.F.); luivinagre@gmail.com (L.W.M.S.V.); ntshmonte@gmail.com (N.M.d.S.); lucas.favacho.celular@gmail.com (L.F.P.); laura.patricia.agellen@hotmail.com (L.P.A.G.); matheus.assuncao@icb.ufpa.br (M.C.E.A.); assumpcaopp@gmail.com (P.P.d.A.)
[2] Faculdade de Ciências Médicas do Pará (FACIMPA), Marabá 68508-030, PA, Brazil
[3] Pediatrics Department, Ophir Loyola Hospital, Belém 66063-240, PA, Brazil; alaydevieira@yahoo.com.br (A.V.W.); swenymf@gmail.com (S.S.M.F.)
[4] Human and Medical Genetics Laboratory, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém 66075-110, PA, Brazil; esdrasedgarbp@gmail.com (E.E.B.P.); andremrsantos@gmail.com (A.M.R.-D.-S.); joao.guerreiro53@gmail.com (J.F.G.); akely@ufpa.br (Â.R.-d.-S.); sidneysantos@ufpa.br (S.E.B.d.S.)
[5] Instituto Tocantinense Presidente Antônio Carlos (ITPAC), Abaetetuba 68440-000, PA, Brazil
* Correspondence: npcsantos.ufpa@gmail.com; Tel.: +55-(91)-3201-6778
† These authors contributed equally to this work.

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Abstract: A number of genomic variants related to native American ancestry may be associated with an increased risk of developing Acute Lymphoblastic Leukemia (ALL), which means that Latin American and hispanic populations from the New World may be relatively susceptible to this disease. However, there has not yet been any comprehensive investigation of the variants associated with susceptibility to ALL in traditional Amerindian populations from Brazilian Amazonia. We investigated the exomes of the 18 principal genes associated with susceptibility to ALL in samples of 64 Amerindians from this region, including cancer-free individuals and patients with ALL. We compared the findings with the data on populations representing five continents available in the 1000 Genomes database. The variation in the allele frequencies found between the different groups was evaluated using Fisher's exact test. The analyses of the exomes of the Brazilian Amerindians identified 125 variants, seven of which were new. The comparison of the allele frequencies between the two Amerindian groups analyzed in the present study (ALL patients vs. cancer-free individuals) identified six variants (rs11515, rs2765997, rs1053454, rs8068981, rs3764342, and rs2304465) that may be associated with susceptibility to ALL. These findings contribute to the identification of genetic variants that represent a potential risk for ALL in Amazonian Amerindian populations and might favor precision oncology measures.

Keywords: Acute Lymphoblastic Leukemia; Amerindian populations; genetic susceptibility

1. Introduction

Acute Lymphoblastic Leukemia (ALL) is the most common cancer in children and is the principal cause of childhood mortality due to malignant disease [1,2]. The genetic etiology of ALL is driven by an ample diversity of alterations of the pathways responsible for the regulation of the cell cycle in the lymphoid precursors of the B and T cell lines, which

include chromosomal translocations, mutations, and aneuploidy [3–5]. In recent years, Genome-Wide Association Studies (GWASs) have identified a number of loci associated with the risk of developing ALL, including the *ARID5B*, *IKZF1*, *PIP4K2A*, *CEBPE*, *GATA3*, *BMI*, and *CDKN2A* genes [6–11].

Most GWASs addressing ALL susceptibility have focused on homogeneous populations in regions such as Europe or North America, whereas the highest incidence of childhood ALL is found in populations with a major component of native American ancestry, such as the Latin American and hispanic populations of the New World [9,12–14]. These populations are highly admixed and, like the population of Brazil, are primarily descended from European, African, and native American ancestors [15,16], and the high incidence of ALL observed in Latin American and hispanic populations has been attributed [9,12,13,17] to genetic risk factors related to native American ancestry. Despite the evidence that genetic variants related to native American ancestry may influence the incidence of childhood ALL, no data are available on the distribution of these variants in traditional Amerindian populations.

Given this, in the present study, we investigated the genetic variants potentially involved in the etiology of ALL in traditional Amerindian populations from Brazilian Amazonia. For this, we used New Generation Sequencing (NGS) to define the exomes of 17 genes associated with susceptibility to ALL in samples obtained from indigenous groups that inhabit Brazilian Amazonia, in both ALL patients and cancer-free individuals. The variants encountered in this initial analysis were compared with the data available on populations representing five different continents, which were obtained from the 1000 Genomes database.

2. Materials and Methods

2.1. Ethics, Consent, and Permissions

The present study was approved by Brazilian National Committee on Research Ethics—CONEP (identified by No 1062/2006 and 123/1998). All participants signed a free-informed consent as well as the tribe leaders and when necessary, a translator explained the project and the importance of the research. Their materials were collected according to the Declaration of Helsinki.

2.2. Study Population

The study sample included 59 cancer-free Amerindians and five individuals diagnosed with ALL. All the participants were members of isolated ethnic groups located in Brazilian Amazonia (Table S1). The genomic Amerindian ancestry of all these individuals was quantified and found to be at least 64% in all cases. The Amerindians with ALL were diagnosed and treated in two public hospitals specialized in the treatment of childhood cancer, the Ophir Loyola Hospital and the Octavio Lobo Childhood Oncology Hospital, both located in the city of Belém, in Pará state, northern Brazil. The clinical and demographic data on these patients are presented in Table S2.

Data were obtained from the 1000 Genomes Project database (available at https://www.1000genomes.org, accessed on: 20 February 2021) to provide comparisons with ethnic groups from other continents. This sample included 661 individuals from Africa (AFR), 503 from Europe (EUR), 347 from the Americas (AMR), 504 from East Asia (EAS), and 489 from South Asia (SAS).

2.3. Selection of the Genes

A total of 17 genes were selected for the present study (see Table S3). The genes were selected based on a search of the literature available in the NCBI and Ensembl databases, and on GWASs or studies of other genetic markers associated with the risk of ALL. More details are described in Table S4.

2.4. Extraction of the DNA and Preparation of the Exomes

Samples of 5 mL of peripheral blood were collected from each of the participants of the study. The genetic material was extracted from these blood samples using the Roche Applied Science DNA extraction kit (Roche, Penzberg, Germany) following the manufacturer's instructions, and it was quantified using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The exome libraries were prepared using the commercial Nextera Rapid Capture Exome kit (Illumina, San Diego, CA, EUA) and the SureSelect Human All Exon V6 kit (Agilent, Santa Clara, CA, USA), with the manufacturer's protocol being followed in both cases. The sequencing reactions were run in the NextSeq 500® platform (Illumina®, San Diego, CA, USA) using the NextSeq 500 high-output v2 300 cycle kit (Illumina®, San Diego, CA, USA).

2.5. Bioinformatic Analysis

The bioinformatic analyses followed the approach described by Ribeiro-Dos-Santos et al. [18] and Rodrigues et al. [19]. For this, the sequences were first filtered to eliminate low-quality reads, and then mapped and aligned with the reference genome (GRCh38) using BWA v.0.7. The alignment was then processed to remove duplicate sequences, recalibrate the mapping quality, and finalize local realignment. The results were processed in GATK v.3.2 to identify the reference genome variants. The Viewer of Variants (ViVa®, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil) software was used to analyze the annotations of the variants. The variants were annotated in three databases—SnpEff v.4.3.T, Ensembl Variant Effect Predictor (Ensembl version 99), and ClinVar (v.2018–10). The SIFT (v.6.2.1), PolyPhen-2 (v.2.2), LRT (November, 2009), Mutation Assessor (v.3.0), Mutation Taster (v.2.0), FATHMM (v.2.3), PROVEAN (v.1.1.3), MetaSVM (v.1.0), M-CAP (v.1.4), and FATHMM-MKL databases were used for the in silico prediction of pathogenicity.

2.6. Statistical Analysis

For the statistical analyses, the cancer-free Amerindians were assigned to the Native (NAT) group, while the ALL patients were in the ALL_NAT (Native with ALL) group. All the analyses were run in the R v.3.5.1 program. The differences in the allelic frequencies between the NAT and ALL_NAT were evaluated using Fisher's exact test, which was also applied to the comparisons with the continental populations. A $p \leq 0.05$ significance level was considered for all the analyses.

3. Results

The analysis of the exomes of the 64 Amerindians investigated in the present study revealed the presence of 125 variants, seven of which were new. The SnpEff software [20] was used to annotate and predict the effects of these variants. This procedure classifies the impact of the variants in four categories: (i) modifier (no evidence of impact), (ii) low (no apparent alteration of protein function), (iii) moderate (some alteration of protein function), and (iv) high (high level of impact on protein function). The 118 variants identified in the 17 genes investigated in the present study are described in Table S3. Four of the new variants were classified as modifiers (Table 1), two as low effect, and one as moderate. The majority of these new variants were found in the ALL_NAT group, at frequencies of less than 0.1%.

As some of the variants were not covered adequately in one or other of the groups (NAT or ALL_NAT), they were excluded from the comparison of allele frequencies. This left 64 variants that were included in the analysis of association. The frequencies of these variants are compared between groups in Table 2.

Table 1. The new gene variants identified in the Amerindian population investigated in the present study.

Chromosome	Chromosomal Position	Gene	Impact	Reference Allele	Group
chr7	50368329	*IKZF1*	MODIFIER	C	ALL_NAT
chr16	78108386	*WWOX*	MODIFIER	T	ALL_NAT
chr16	78386853	*WWOX*	LOW	A	NAT
chr17	39832190	*IKZF3*	MODIFIER	T	NAT
chr17	39765793	*IKZF3*	MODERATE	C	ALL_NAT
chr17	39868422	*ZPBP2*	MODIFIER	C	ALL_NAT
chr17	39765799	*IKZF3*	LOW	C	ALL_NAT

Table 2. Comparisons of the frequencies of the alleles investigated in the present study between the two Amerindian groups, that is, ALL patients (ALL_NAT) and cancer-free individuals (NAT).

		Frequency in Group:		
dbSNP	**Gene**	**ALL_NAT**	**NAT**	*** *p***
rs773061413	*ARID5B*	0.20	0	0.0781
rs513349	*BAK1*	0.80	0.56	0.3868
rs11515	*CDKN2A*	0.60	1	**0.0050**
rs3088440	*CDKN2A*	0.50	0.56	1
rs974336	*CDKN2B*	0.25	0	0.0781
rs2302901	*ELK3*	0	0.03	1
rs422628	*GATA3*	0.75	0.97	0.2197
rs2305479	*GSDMB*	0.30	0.43	1
rs2305480	*GSDMB*	0.30	0.43	1
rs11078928	*GSDMB*	0.30	0.43	1
rs12450091	*GSDMB*	0.20	0.26	1
rs11078927	*GSDMB*	0.30	0.42	1
rs8068981	*IGF2BP1*	0.60	0	**0.0002**
rs62078405	*IGF2BP1*	0.20	0	0.0781
rs2289637	*IGF2BP1*	0.20	0.06	0.3434
rs10899750	*IKZF1*	0.50	0.67	0.6572
rs61731355	*IKZF1*	0.20	0.31	1
rs12669559	*IKZF1*	0.10	0.28	1
rs907092	*IKZF3*	0.20	0.04	0.2197
rs3824810	*LHPP*	0.60	0.57	1
rs3824809	*LHPP*	0.60	0.71	0.6287
rs6597801	*LHPP*	0.70	0.88	0.1907
rs943192	*PIP4K2A*	0.80	0.98	0.1513
rs2765997	*PIP4K2A*	0.50	0.96	**0.0043**
rs1132816	*PIP4K2A*	0.10	0.01	0.1774
rs61731109	*PIP4K2A*	0.10	0.01	0.1774
rs1053454	*PIP4K2A*	0.90	0	$\mathbf{7.264 \times 10^{-7}}$
rs2447919	*USP7*	0.13	0.31	1
rs11551182	*USP7*	0.30	0.40	1
rs139138924	*USP7*	1	1	1
rs2304465	*USP7*	0.40	0	**0.0050**
rs1382390	*USP7*	1.00	1	1
rs2447918	*USP7*	0.16	0	0.0781
rs117832776	*WWOX*	0.20	0	0.0781
rs383362	*WWOX*	0.30	0.16	0.3052
rs77897021	*WWOX*	0.10	0.08	0.4545
rs12934985	*WWOX*	0.10	0.21	1
rs3764342	*WWOX*	0.40	0	**0.0050**
rs200461412	*WWOX*	0.20	0.03	0.2197
rs140060332	*WWOX*	0.20	0	0.0781
rs4130513	*WWOX*	0.30	0.09	0.1551
rs11545029	*WWOX*	0.10	0.08	0.4545

Table 2. *Cont.*

		Frequency in Group:		
dbSNP	**Gene**	**ALL_NAT**	**NAT**	*** *p***
rs8050128	*WWOX*	0.75	0.41	0.1590
rs75559202	*WWOX*	0.10	0.09	0.4545
rs146481440	*WWOX*	0.20	0.06	0.3434
rs12446823	*WWOX*	0.10	0.01	0.1774
rs2288034	*WWOX*	0.60	0.32	0.3292
rs144601717	*WWOX*	0.10	0.01	0.1774
rs2303190	*WWOX*	0	0.22	0.5739
rs3764340	*WWOX*	0.20	0.09	0.3990
rs2303191	*WWOX*	0.80	0.98	0.1513
rs2288035	*WWOX*	0.10	0.08	0.4545
rs8048830	*WWOX*	0.25	0.01	0.1513
rs67493355	*WWOX*	0.25	0.16	1
rs202093359	*WWOX*	0.20	0.08	0.3990
rs11545028	*WWOX*	0.10	0.22	1
rs2288033	*WWOX*	0.40	0.25	0.5917
rs7199110	*WWOX*	0.60	0.40	0.6423
rs555396422	*WWOX*	0.10	0	0.0923
rs384216	*WWOX*	0	0	1
rs75027016	*ZPBP2*	0.30	0.15	0.2662
rs11557467	*ZPBP2*	0.30	0.43	1
rs11557466	*ZPBP2*	0.30	0.42	1
rs10852935	*ZPBP2*	0.30	0.43	1

* Fisher's exact test.

The analyses revealed significant differences between the two groups in six variants of five genes: *PIP4K2A* (variants rs2765997 and rs1053454), *CDKN2A* (rs11515), *IGF2BP1* (rs8068981), *USP7* (rs2304465), and *WWOX* (rs3764342). The allele frequencies of these six variants recorded in the ALL_NAT group were also compared with those recorded in the 1000 Genomes Project for the five continental populations (AFR, AMR, EAS, EUR, and SAS). The comparisons are shown in Table 3 and the *p* values, in Table 4.

The analyses revealed significant differences between the Amerindian group (ALL_NAT) and all the continental populations (AFR, AMR, EAS, EUR and SAS) in the frequency of the rs11515 variant of the *CDKN2A* gene. The frequency of the rs8068981 variant of the IGF2BP1 gene was also significantly different from that of the EAS population, while the frequency of the rs2765997 variant of the *PIP4K2A* gene was significantly different from those of the AFR and EAS populations.

Table 3. Allele frequencies of the six key variants identified in the present study in the ALL_NAT populations (Table 2) and the five continental populations from the 1000 Genomes Project (AFR, AMR, EAS, EUR, and SAS).

dbSNP	**Gene**	**Frequency in the Population:**					
		ALL_NAT	**AFR**	**AMR**	**EAS**	**EUR**	**SAS**
rs11515	*CDKN2A*	0.60	0.16	0.08	0.02	0.15	0.05
rs8068981	*IGF2BP1*	0.60	0.57	0.68	0.95	0.71	0.82
rs2765997	*PIP4K2A*	0.50	0.18	0.20	0.05	0.37	0.37
rs1053454	*PIP4K2A*	0.90	0.85	0.81	0.65	0.64	0.78
rs2304465	*USP7*	0.40	0.66	0.58	0.70	0.59	0.49
rs3764342	*WWOX*	0.40	0.55	0.14	0.30	0.11	0.32

Table 4. The p values recorded for the pairwise comparisons (ALL_NAT vs. AFR, AMR, EAS, EUR or SAS) of the allele frequencies of the six key variants identified in the present study (see Table 3).

dbSNP	Gene	Frequency ALL_NAT	*p* for the ALL_NAT Population Versus:				
			AFR	AMR	EAS	EUR	SAS
rs11515	*CDKN2A*	0.67	**0.0349**	**0.0050**	**0.0001**	**0.0267**	**0.0015**
rs8068981	*IGF2BP1*	0.60	1	0.6577	**0.0263**	0.6283	0.2302
rs2765997	*PIP4K2A*	0.50	**0.0452**	0.0579	**0.0015**	0.3627	0.3663
rs1053454	*PIP4K2A*	0.90	1	0.5880	0.1702	0.1655	0.5911
rs2304465	*USP7*	0.40	0.3447	0.6535	0.1602	0.6536	1
rs3764342	*WWOX*	0.40	0.6626	0.1595	0.6411	0.0960	0.6550

4. Discussion

The incidence of ALL is relatively high in populations with a high degree of native American ancestry, such as Latin American and Hispanic populations, which has been attributed, in part, to the contribution of molecular markers associated with a high risk of ALL in these populations [9,12–14,21]. Despite this known association, no genomic data are available on the susceptibility to ALL of traditional Amerindian populations from Brazilian Amazonia. In the present study, we investigated the exomes of 17 of the principal genes associated with susceptibility to ALL in samples of indigenous Amazonian populations, including ALL patients and cancer-free individuals. It would be interesting to demonstrate whether the results observed for the markers investigated here in Amerindian populations would demonstrate the same profile in a larger cohort of leukemic patients from the same ethnic group. However, samples from indigenous patients with ALL are difficult to obtain, given the rarity of the disease and due to the fact that these populations inhabit remote and difficult-to-access rural regions, which reflects on the difficulty of care and clinical follow-up of these patients.

During the study, we identified seven new variants in four genes (*IKZF1*, *IKZF3*, *WWOX*, and *ZPBP2*), most of which were present in the ALL_NAT group, that is, Amerindian ALL patients. These genes are known to have alleles associated with some level of risk in the etiology of ALL in different populations [17,22–24]. Given this, we believe that the new variants identified in the present study should be investigated further as potential risk factors for the incidence of ALL in Amerindian populations.

We also identified six variants of five genes that we associated with susceptibility to ALL (*CDKN2A*_rs11515, *PIP4K2A*_rs2765997 and rs1053454, *IGF2BP1*_rs8068981, *WWOX*_ rs3764342, and *USP7*_rs2304465) given the significant differences in the frequencies recorded in the two Amerindian groups (ALL patients and cancer-free individuals), and between the ALL group and populations of other groups from around the world.

The *CDKN2A* gene plays an important role in leukemogenesis. The rs11515 variant is located in the 3′-untranslated region (UTR) of the *CDKN2A* gene, and is known to be associated with a number of different types of cancer [25], including breast cancer [26], glioblastoma [27], melanoma [28], and colorectal cancer [29]. However, no data are available on the possible influence of this variant on the risk of developing ALL. In the present study, we recorded a high frequency of this variant in the Amerindian population, which was significantly higher than that recorded in other populations around the world. This indicates that research on this variant should be prioritized for the identification of its potential role in the etiology of ALL in this population.

A number of previous studies of genetic polymorphisms in the *PIP4K2A* gene have identified an association with susceptibility to ALL [30,31]. We identified an association with ALL in two variants (rs2765997 and rs105334534) of the *PIP4K2A* gene, given that both were more frequent in the Amerindian population, in comparison with the other continental populations, which may reflect a potential correlation with the risk of ALL.

The *IGF2BP1* protein is expressed in a number of different types of cancer, including leukemia. In a recent study based on in vivo and in vitro analyses, Elcheva et al. [32]

found evidence of a significant correlation between *IGF2BP1* and the aggressiveness of leukemia, through the persistence of tumorigenicity by increasing critical transcriptional and metabolic regulators. These authors also emphasized that the *IGF2BP1* gene is often positively regulated in many types of malignant disease, and is not expressed in most normal tissue, which means that it is a potentially important target for anticancer therapy. The results of the present study indicate that the rs8068981 variant of the *IGF2BP1* gene is present at similar frequencies in the Amerindians with ALL and the European population, but contrasts with the Asian populations.

We also identified in the Amerindian population a positive association with the rs3764342 variant of the *WWOX* gene, which has a suppressor role in solid cancers, with the loss of function resulting in alterations in the adhesion of the cancerous cells to the extra-cellular matrix, which affects cell migration and metastasis. A number of studies have contributed to the description of the role of this gene in leukemic malignancies. Luo et al. [33] found that the expression of the *WWOX* mRNA and protein is significantly reduced or absent in cases of leukemia and their cell lines in comparison with the controls, which is consistent with the findings of Cui et al. [34], who evaluated patients diagnosed with different types of leukemia.

In the present study, the rs2304465 variant of the *USP7* gene was also more frequent in Amerindian patients. Jin et al. [35] found an association between *USP7* and a lack of ubiquitination and the stabilization of the *NOTCH1* oncogene, which contributes to the control of the cell growth of the T cells in lymphoblastic leukemia.

5. Conclusions

The present study is the first to investigate the exome of genes involved in the etiology of Acute Lymphoblastic Leukemia (ALL) in Amerindian populations from the Amazon region. The results of the study provide important genetic data related to the etiology of ALL in such population in which genetic investigations are scarce. The study also contributes to the identification of variants of potential risk involved in the etiology of ALL in Amerindian populations, as well as in other Brazilian populations formed through a high level of admixture with this indigenous group.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jpm12060856/s1, Table S1. Names and the number of individuals in each population group analyzed in the present study; Table S2. Epidemiological and clinical characteristics of the patients included in the study; Table S3. Description of the variants found in the Amerindian population investigated in the present study; Table S4 [36–48]. Description of the studies selected to choose the genes studied.

Author Contributions: All the authors contributed significantly to the preparation of the manuscript: L.P.C.L. and D.C.d.C. collected and analyzed the data and wrote the text; M.R.F., A.V.W., L.W.M.S.V., N.M.d.S., L.F.P., L.P.A.G., M.C.E.A., S.S.M.F., E.E.B.P., A.M.R.-D.-S. and J.C.G.R. collected and analyzed the data, ran the statistical analyses, and revised the manuscript; J.F.G., Â.R.-d.-S., P.P.d.A., S.E.B.d.S. and N.P.C.d.S. reviewed the statistical analyses, and edited and approved the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Brazilian National Commission for Ethics in Research (CONEP) and by the Research Ethics Committee of the UFPA Tropical Medicine Center, under authorization CAAE-20654313.6.0000.5172.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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References

1. Pui, C.-H.; Carroll, W.L.; Meshinchi, S.; Arceci, R.J. Biology, Risk Stratification, and Therapy of Pediatric Acute Leukemias: An Update. *J. Clin. Oncol.* **2011**, *29*, 551–565. [CrossRef] [PubMed]
2. Bagnasco, F.; Caruso, S.; Andreano, A.; Valsecchi, M.G.; Jankovic, M.; Biondi, A.; Miligi, L.; Casella, C.; Terenziani, M.; Massimino, M.; et al. Late Mortality and Causes of Death among 5-Year Survivors of Childhood Cancer Diagnosed in the Period 1960–1999 and Registered in the Italian Off-Therapy Registry. *Eur. J. Cancer* **2019**, *110*, 86–97. [CrossRef] [PubMed]
3. Hunger, S.P.; Mullighan, C.G. Acute Lymphoblastic Leukemia in Children. *N. Engl. J. Med.* **2015**, *373*, 1541–1552. [CrossRef] [PubMed]
4. Mullighan, C.G.; Goorha, S.; Radtke, I.; Miller, C.B.; Coustan-Smith, E.; Dalton, J.D.; Girtman, K.; Mathew, S.; Ma, J.; Pounds, S.B.; et al. Genome-Wide Analysis of Genetic Alterations in Acute Lymphoblastic Leukaemia. *Nature* **2007**, *446*, 758–764. [CrossRef] [PubMed]
5. Coccaro, N.; Anelli, L.; Zagaria, A.; Specchia, G.; Albano, F. Next-Generation Sequencing in Acute Lymphoblastic Leukemia. *Int. J. Mol. Sci.* **2019**, *20*, 2929. [CrossRef]
6. Papaemmanuil, E.; Hosking, F.J.; Vijayakrishnan, J.; Price, A.; Olver, B.; Sheridan, E.; Kinsey, S.E.; Lightfoot, T.; Roman, E.; Irving, J.A.E.; et al. Loci on 7p12.2, 10q21.2 and 14q11.2 Are Associated with Risk of Childhood Acute Lymphoblastic Leukemia. *Nat. Genet.* **2009**, *41*, 1006–1010. [CrossRef]
7. Treviño, L.R.; Yang, W.; French, D.; Hunger, S.P.; Carroll, W.L.; Devidas, M.; Willman, C.; Neale, G.; Downing, J.; Raimondi, S.C.; et al. Germline Genomic Variants Associated with Childhood Acute Lymphoblastic Leukemia. *Nat. Genet.* **2009**, *41*, 1001–1005. [CrossRef]
8. Perez-Andreu, V.; Roberts, K.G.; Harvey, R.C.; Yang, W.; Cheng, C.; Pei, D.; Xu, H.; Gastier-Foster, J.; Shuyu, E.; Lim, J.Y.-S.; et al. Inherited GATA3 Variants Are Associated with Ph-like Childhood Acute Lymphoblastic Leukemia and Risk of Relapse. *Nat. Genet.* **2013**, *45*, 1494–1498. [CrossRef]
9. Xu, H.; Yang, W.; Perez-Andreu, V.; Devidas, M.; Fan, Y.; Cheng, C.; Pei, D.; Scheet, P.; Burchard, E.G.; Eng, C.; et al. Novel Susceptibility Variants at 10p12.31-12.2 for Childhood Acute Lymphoblastic Leukemia in Ethnically Diverse Populations. *JNCI J. Natl. Cancer Inst.* **2013**, *105*, 733–742. [CrossRef]
10. Moriyama, T.; Relling, M.V.; Yang, J.J. Inherited Genetic Variation in Childhood Acute Lymphoblastic Leukemia. *Blood* **2015**, *125*, 3988–3995. [CrossRef]
11. Vijayakrishnan, J.; Studd, J.; Broderick, P.; Kinnersley, B.; Holroyd, A.; Law, P.J.; Kumar, R.; Allan, J.M.; Harrison, C.J.; Moorman, A.V.; et al. Genome-Wide Association Study Identifies Susceptibility Loci for B-Cell Childhood Acute Lymphoblastic Leukemia. *Nat. Commun.* **2018**, *9*, 1340. [CrossRef] [PubMed]
12. Yamamoto, J.F.; Goodman, M.T. Patterns of Leukemia Incidence in the United States by Subtype and Demographic Characteristics, 1997–2002. *Cancer Causes Control.* **2008**, *19*, 379–390. [CrossRef] [PubMed]
13. Walsh, K.M.; Chokkalingam, A.P.; Hsu, L.-I.; Metayer, C.; de Smith, A.J.; Jacobs, D.I.; Dahl, G.V.; Loh, M.L.; Smirnov, I.V.; Bartley, K.; et al. Associations between Genome-Wide Native American Ancestry, Known Risk Alleles and B-Cell ALL Risk in Hispanic Children. *Leukemia* **2013**, *27*, 2416–2419. [CrossRef] [PubMed]
14. Quiroz, E.; Aldoss, I.; Pullarkat, V.; Rego, E.; Marcucci, G.; Douer, D. The Emerging Story of Acute Lymphoblastic Leukemia among the Latin American Population—Biological and Clinical Implications. *Blood Rev.* **2019**, *33*, 98–105. [CrossRef] [PubMed]
15. Santos, N.P.C.; Ribeiro-Rodrigues, E.M.; Ribeiro-dos-Santos, Â.K.C.; Pereira, R.; Gusmão, L.; Amorim, A.; Guerreiro, J.F.; Zago, M.A.; Matte, C.; Hutz, M.H.; et al. Assessing Individual Interethnic Admixture and Population Substructure Using a 48-Insertion-Deletion (INSEL) Ancestry-Informative Marker (AIM) Panel. *Hum. Mutat.* **2010**, *31*, 184–190. [CrossRef]
16. Secolin, R.; Mas-Sandoval, A.; Arauna, L.R.; Torres, F.R.; de Araujo, T.K.; Santos, M.L.; Rocha, C.S.; Carvalho, B.S.; Cendes, F.; Lopes-Cendes, I.; et al. Distribution of Local Ancestry and Evidence of Adaptation in Admixed Populations. *Sci. Rep.* **2019**, *9*, 13900. [CrossRef]
17. Wiemels, J.L.; Walsh, K.M.; de Smith, A.J.; Metayer, C.; Gonseth, S.; Hansen, H.M.; Francis, S.S.; Ojha, J.; Smirnov, I.; Barcellos, L.; et al. GWAS in Childhood Acute Lymphoblastic Leukemia Reveals Novel Genetic Associations at Chromosomes 17q12 and 8q24.21. *Nat. Commun.* **2018**, *9*, 286. [CrossRef]

18. Ribeiro-Dos-Santos, A.M.; Vidal, A.F.; Vinasco-Sandoval, T.; Guerreiro, J.; Santos, S.; Ribeiro-Dos-Santos, Â.; de Souza, S.J. Exome Sequencing of Native Populations From the Amazon Reveals Patterns on the Peopling of South America. *Front. Genet.* **2020**, *11*, 548507. [CrossRef]
19. Rodrigues, J.C.G.; de Souza, T.P.; Pastana, L.F.; Ribeiro dos Santos, A.M.; Fernandes, M.R.; Pinto, P.; Wanderley, A.V.; de Souza, S.J.; Kroll, J.E.; Pereira, A.L.; et al. Identification of NUDT15 Gene Variants in Amazonian Amerindians and Admixed Individuals from Northern Brazil. *PLoS ONE* **2020**, *15*, e0231651. [CrossRef]
20. Cingolani, P.; Platts, A.; Wang, L.L.; Coon, M.; Nguyen, T.; Wang, L.; Land, S.J.; Lu, X.; Ruden, D.M. A Program for Annotating and Predicting the Effects of Single Nucleotide Polymorphisms, SnpEff. *Fly* **2012**, *6*, 80–92. [CrossRef]
21. Shoag, J.M.; Barredo, J.C.; Lossos, I.S.; Pinheiro, P.S. Acute Lymphoblastic Leukemia Mortality in Hispanic Americans. *Leuk. Lymphoma* **2020**, *61*, 2674–2681. [CrossRef] [PubMed]
22. Churchman, M.L.; Qian, M.; te Kronnie, G.; Zhang, R.; Yang, W.; Zhang, H.; Lana, T.; Tedrick, P.; Baskin, R.; Verbist, K.; et al. Germline Genetic IKZF1 Variation and Predisposition to Childhood Acute Lymphoblastic Leukemia. *Cancer Cell* **2018**, *33*, 937–948.e8. [CrossRef] [PubMed]
23. Hong, Y.; Wang, Q.; Song, Y.L.; Liu, G.M.; Yang, X.; Yan, M.X. Relationship between IKZF3 Gene Single Nucleotide Polymorphisms and Childhood Acute Lymphoblastic Leukemia. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* **2021**, *29*, 690–695. [CrossRef] [PubMed]
24. Shi, Y.; Du, M.; Fang, Y.; Tong, N.; Zhai, X.; Sheng, X.; Li, Z.; Xue, Y.; Li, J.; Chu, H.; et al. Identification of a Novel Susceptibility Locus at 16q23.1 Associated with Childhood Acute Lymphoblastic Leukemia in Han Chinese. *Hum. Mol. Genet.* **2016**, *25*, 2873–2880. [CrossRef] [PubMed]
25. Dong, Y.; Wang, X.; Yang, Y.-W.; Liu, Y.-J. The Effects of CDKN2A Rs3731249, Rs11515, and Rs3088440 Polymorphisms on Cancer Risk. *Cell. Mol. Biol.* **2017**, *63*, 40. [CrossRef] [PubMed]
26. Royds, J.A.; Pilbrow, A.P.; Ahn, A.; Morrin, H.R.; Frampton, C.; Russell, I.A.; Moravec, C.S.; Sweet, W.E.; Tang, W.H.W.; Currie, M.J.; et al. The Rs11515 Polymorphism Is More Frequent and Associated with Aggressive Breast Tumors with Increased ANRIL and Decreased P16INK4a Expression. *Front. Oncol.* **2016**, *5*, 306. [CrossRef]
27. Sibin, M.K.; Dhananjaya, I.B.; Narasingarao, K.V.L.; Harshitha, S.M.; Jeru-Manoj, M.; Chetan, G.K. Two Gene Polymorphisms (Rs4977756 and Rs11515) in CDKN2A/B and Glioma Risk in South Indian Population. *Meta Gene* **2016**, *9*, 215–218. [CrossRef]
28. Sauroja, I.; Smeds, J.; Vlaykova, T.; Kumar, R.; Talve, L.; Hahka-Kemppinen, M.; Punnonen, K.; Jansèn, C.T.; Hemminki, K.; Pyrhönen, S. Analysis of G1/S Checkpoint Regulators in Metastatic Melanoma. *Genes Chromosomes Cancer* **2000**, *28*, 404–414. [CrossRef]
29. McCloud, J.; Sivakumar, R.; Greenhough, A.; Elder, J.; Jones, P.; Deakin, M.; Elder, J.; Fryer, A.; Hoban, P. P16INK4a Polymorphism: Associations with Tumour Progression in Patients with Sporadic Colorectal Cancer. *Int. J. Oncol.* **2004**, *25*, 1447–1452. [CrossRef]
30. Liao, F.; Yin, D.; Zhang, Y.; Hou, Q.; Zheng, Z.; Yang, L.; Shu, Y.; Xu, H.; Li, Y. Association Between PIP4K2A Polymorphisms and Acute Lymphoblastic Leukemia Susceptibility. *Medicine* **2016**, *95*, e3542. [CrossRef]
31. Zhou, X.; Liao, F.; Zhang, J.; Qin, Y.; Xu, H.; Ding, Z.; Zhang, Y.; Zhang, F. Association of the Independent Polymorphisms in CDKN2A with Susceptibility of Acute Lymphoblastic Leukemia. *Biosci. Rep.* **2018**, *38*. [CrossRef] [PubMed]
32. Elcheva, I.A.; Wood, T.; Chiarolanzio, K.; Chim, B.; Wong, M.; Singh, V.; Gowda, C.P.; Lu, Q.; Hafner, M.; Dovat, S.; et al. RNA-Binding Protein IGF2BP1 Maintains Leukemia Stem Cell Properties by Regulating HOXB4, MYB, and ALDH1A1. *Leukemia* **2020**, *34*, 1354–1363. [CrossRef] [PubMed]
33. Luo, L.; Chen, Y.; Cheng, X.; Lin, Y.; Fu, X.; Li, D.; Cui, Z.; Lin, D. Reduced Expression of the WW Domain-Containing Oxidoreductase in Human Hematopoietic Malignancies. *Oncol. Lett.* **2016**, *11*, 4083–4088. [CrossRef]
34. Cui, Z.; Lin, D.; Cheng, F.; Luo, L.; Kong, L.; Xu, J.; Hu, J.; Lan, F. The Role of the WWOX Gene in Leukemia and Its Mechanisms of Action. *Oncol. Rep.* **2013**, *29*, 2154–2162. [CrossRef]
35. Jin, Q.; Martinez, C.A.; Arcipowski, K.M.; Zhu, Y.; Gutierrez-Diaz, B.T.; Wang, K.K.; Johnson, M.R.; Volk, A.G.; Wang, F.; Wu, J.; et al. USP7 Cooperates with NOTCH1 to Drive the Oncogenic Transcriptional Program in T-Cell Leukemia. *Clin. Cancer Res.* **2019**, *25*, 222–239. [CrossRef] [PubMed]
36. Hao, Q.; Cao, M.; Zhang, C.; Yin, D.; Wang, Y.; Ye, Y.; Zhao, S.; Yang, Y.; Chen, K.-L.; Ying, B.; et al. Age-related differences of genetic susceptibility to patients with acute lymphoblastic leukemia. *Aging* **2021**, *13*, 12456–12465. [CrossRef] [PubMed]
37. Perez-Andreu, V.; Roberts, K.G.; Xu, H.; Smith, C.; Zhang, H.; Yang, W.; Harvey, R.C.; Payne-Turner, D.; Devidas, M.; Cheng, I.-M.; et al. A genome-wide association study of susceptibility to acute lymphoblastic leukemia in adolescents and young adults. *Blood* **2015**, *125*, 680–686. [CrossRef]
38. Urayama, K.Y.; Takagi, M.; Kawaguchi, T.; Matsuo, K.; Tanaka, Y.; Ayukawa, Y.; Arakawa, Y.; Hasegawa, D.; Yuza, Y.; Kaneko, T.; et al. Regional evaluation of childhood acute lymphoblastic leukemia genetic susceptibility loci among Japanese. *Sci. Rep.* **2018**, *8*, 789. [CrossRef]
39. Semmes, E.C.; Vijayakrishnan, J.; Zhang, C.; Hurst, J.H.; Houlston, R.S.; Walsh, K.M. Leveraging Genome and Phenome-Wide Association Studies to Investigate Genetic Risk of Acute Lymphoblastic Leukemia. *Cancer Epidemiol. Biomarkers Prev.* **2020**, *29*, 1606–1614. [CrossRef]
40. Vijayakrishnan, J.; Qian, M.; Studd, J.B.; Yang, W.; Kinnersley, B.; Law, P.J.; Broderick, P.; Raetz, E.A.; Allan, J.; Pui, C.-H.; et al. Identification of four novel associations for B-cell acute lymphoblastic leukaemia risk. *Nat. Commun.* **2019**, *10*, 1–9. [CrossRef]

41. Brown, A.L.; de Smith, A.J.; Gant, V.U.; Yang, W.; Scheurer, M.E.; Walsh, K.M.; Chernus, J.M.; Kallsen, N.A.; Peyton, S.A.; Davies, G.E.; et al. Inherited genetic susceptibility to acute lymphoblastic leukemia in Down syndrome. *Blood* **2019**, *134*, 1227–1237. [CrossRef] [PubMed]
42. Sherborne, A.; Hosking, F.; Prasad, R.; Kumar, R.; Koehler, R.; Vijayakrishnan, J.; Papaemmanuil, E.; Bartram, C.R.; Stanulla, M.; Schrappe, M.; et al. Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. *Nat. Genet.* **2010**, *42*, 492–494. [CrossRef]
43. Hungate, E.A.; Vora, S.R.; Gamazon, E.; Moriyama, T.; Best, T.; Hulur, I.; Lee, Y.; Evans, T.-J.; Ellinghaus, E.; Stanulla, M.; et al. A variant at 9p21.3 functionally implicates CDKN2B in paediatric B-cell precursor acute lymphoblastic leukaemia aetiology. *Nat. Commun.* **2016**, *7*, 10635. [CrossRef]
44. Liao, F.; Ye, Y.; Yin, D.; Qin, Y.; Zhao, J.; Zhang, W.; Zhang, Y.; Deng, Z.; Wang, Y.; Ying, B.; et al. Validations of Top and Novel Susceptibility Variants in All-Age Chinese Patients With Acute Lymphoblastic Leukemia. *Front. Genet.* **2020**, *11*, 1004. [CrossRef] [PubMed]
45. Al-Absi, B.; Razif, M.F.M.M.F.; Noor, S.M.S.M.; Saif-Ali, R.; Aqlan, M.; Salem, S.D.S.D.; Ahmed, R.H.R.H.; Muniandy, S. Contributions of IKZF1, DDC, CDKN2A, CEBPE, and LMO1 Gene Polymorphisms to Acute Lymphoblastic Leukemia in a Yemeni Population. *Genet. Test. Mol. Biomark.* **2017**, *21*, 592–599. [CrossRef] [PubMed]
46. Vijayakrishnan, J.; Kumar, R.; Henrion, M.Y.R.; Moorman, A.V.; Rachakonda, P.S.; Hosen, I.; Filho, M.I.D.S.; Holroyd, A.; E Dobbins, S.; Koehler, R.; et al. A genome-wide association study identifies risk loci for childhood acute lymphoblastic leukemia at 10q26.13 and 12q23.1. *Leukemia* **2016**, *31*, 573–579. [CrossRef]
47. Zhang, H.; Liu, A.P.-Y.; Devidas, M.; Lee, S.H.; Cao, X.; Pei, D.; Borowitz, M.; Wood, B.; Gastier-Foster, J.M.; Dai, Y.; et al. Association of GATA3 Polymorphisms With Minimal Residual Disease and Relapse Risk in Childhood Acute Lymphoblastic Leukemia. JNCI: J. *Natl. Cancer Inst.* **2020**, *113*, 408–417. [CrossRef]
48. Qian, M.; Zhao, X.; Devidas, M.; Yang, W.; Gocho, Y.; Smith, C.; Gastier-Foster, J.M.; Li, Y.; Xu, H.; Zhang, S.; et al. Genome-Wide Association Study of Susceptibility Loci for T-Cell Acute Lymphoblastic Leukemia in Children. JNCI: J. *Natl. Cancer Inst.* **2019**, *111*, 1350–1357. [CrossRef]

Article

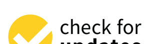
The Role of *SLC22A1* and Genomic Ancestry on Toxicity during Treatment in Children with Acute Lymphoblastic Leukemia of the Amazon Region

Sweny de S. M. Fernandes [1], Luciana P. C. Leitão [1], Amanda de N. Cohen-Paes [1], Laura P. A. Gellen [1], Lucas F. Pastana [1], Darlen C. de Carvalho [1], Antônio A. C. Modesto [1,2], Ana C. A. da Costa [1], Alayde V. Wanderley [1], Carlos H. V. de Lima [1], Esdras E. B. Pereira [2], Marianne R. Fernandes [1,*], Rommel M. R. Burbano [1,2], Paulo P. de Assumpção [1], Sidney E. B. dos Santos [1,2] and Ney P. C. dos Santos [1,2]

[1] Oncology Research Center, Federal University of Pará, Belém 66073, PA, Brazil; swenymf@gmail.com (S.d.S.M.F.); colaresluciana@gmail.com (L.P.C.L.); acohencastro@gmail.com (A.d.N.C.-P.); laura.patricia.agellen@hotmail.com (L.P.A.G.); lucas.pastana@ics.ufpa.br (L.F.P.); darlen.c.carvalho@gmail.com (D.C.d.C.); antonioacm@ufpa.br (A.A.C.M.); carolinecostaodonto19@gmail.com (A.C.A.d.C.); alaydevieira@yahoo.com.br (A.V.W.); carloshvl@hotmail.com (C.H.V.d.L.); rommelburbano@gmail.com (R.M.R.B.); assumpcaopp@gmail.com (P.P.d.A.); sidneysantos@ufpa.br (S.E.B.d.S.); npcsantos.ufpa@gmail.com (N.P.C.d.S.)

[2] Laboratory of Human and Medical Genetics, Institute of Biological Science, Federal University of Pará, Belém 66077-830, PA, Brazil; esdrasedgarbp@gmail.com

* Correspondence: fernandesmr@yahoo.com.br



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Abstract: In Brazil, Acute lymphoid leukemia (ALL) is the leading cause of cancer deaths in children and adolescents. Treatment toxicity is one of the reasons for stopping chemotherapy. Amerindian genomic ancestry is an important factor for this event due to fluctuations in frequencies of genetic variants, as in the *NUDT15* and *SLC22A1* genes, which make up the pharmacokinetic and pharmacodynamic pathways of chemotherapy. This study aimed to investigate possible associations between *NUDT15* (rs1272632214) and *SLC22A1* (rs202220802) gene polymorphism and genomic ancestry as a risk of treatment toxicities in patients with childhood ALL in the Amazon region of Brazil. The studied population consisted of 51 patients with a recent diagnosis of ALL when experiencing induction therapy relative to the BFM 2009 protocol. Our results evidenced a significant association of risk of severe infectious toxicity for the variant of the *SLC22A1* gene (OR: 3.18, $p = 0.031$). Genetic ancestry analyses demonstrated that patients who had a high contribution of African ancestry had a significant protective effect for the development of toxicity (OR: 0.174; $p = 0.010$), possibly due to risk effects of the Amerindian contribution. Our results indicate that mixed populations with a high degree of African ancestry have a lower risk of developing general toxicity during induction therapy for ALL. In addition, individuals with the *SLC22A1* variant have a higher risk of developing severe infectious toxicity while undergoing the same therapy.

Keywords: acute lymphoid leukemia; severe toxicity; ancestry; *NUDT1*; SLC22A1

1. Introduction

Representing 75% of acute leukemias and approximately 35% of all malignant neoplasms in childhood, ALL is a type of cancer that mostly affects children worldwide [1,2]. In Brazil, this type of leukemia is the leading cause of cancer deaths in children and adolescents aged 0–19 years [3]. The survival rate of ALL is approximately 80% when using multiagent chemotherapy regimens [4]. Despite this, treatment toxicity is one of the main reasons for interrupting or discontinuing chemotherapy, which can affect the quality of life of patients not only during but also after its completion [1,5].

An investigation carried out in a miscegenated population with high ancestry of Amazonian Amerindians, treated with the protocol of European Group Berlin-Frankfurt-Münster (BFM) for ALL, showed that 65.3% of this population presents grade 3 and 4 toxicity [6], a frequency higher than that found in other world populations submitted to the same protocol (26%) [7]. Thus, Amerindian genomic ancestry could be an important factor for the high rates of toxicity reported in the Amazon region in patients undergoing treatment for ALL. Other studies have shown even worse survival rates among Hispanic ALL patients when compared to Europeans, Americans, or Asians [8]. Therefore, the different drug responses related to ethnic differences can be explained, in part, by fluctuations in the frequencies of important functional gene variants that make up the pharmacokinetic and pharmacodynamic pathway of chemotherapeutic agents [9–11].

Thus, a number of studies have been looking for genetic markers that can help adjust doses of anti-leukemic drugs in order to optimize clinical outcomes and avoid the occurrence of toxicities [12–14]. Some of these genes are present in the Absorption, Distribution, Metabolism, and Excretion (ADME) pathways of drugs, such as NUDT15 and the SLAC22A1 transporter. The mutational frequency of the *NUDT15* gene varies widely among different continental populations. In Northern Brazil, a study reported that *NUDT15* variants alter the metabolization profile of drugs used in the standard ALL protocol [15]. In the case of the *SLC22A1* gene, it is also known as organic cationic transporter 1 (*OCT1*, encoded by the *SLC22A1* gene) and is part of a family that plays an important role in drug–drug interactions (DDI); several polymorphisms of this gene have been associated with alterations in drug availability, response, and toxicity [16].

The aim of this study was to investigate possible associations between NUDT15 (rs1272632214) and SLC22A1 (rs202220802) gene polymorphism and genomic ancestry as a risk of treatment toxicities in patients with childhood ALL in the Amazon region of Brazil.

2. Methods

2.1. Ethical Aspects

The protocol used in this study was approved by the Research Ethics Committee of the Health Sciences Institute of the Federal University of Pará, under protocol number 119.649/2012. Individuals under 18 years of age and their guardians were duly informed about the research. Volunteers agreed to participate in the study by signing the Free and Informed Assent Term and their guardians signed the Free and Informed Consent Term.

2.2. Study Populations

This is a prospective study, which included 51 patients with a recent diagnosis of ALL by immunophenotyping and treated at a referral center for pediatric cancer treatment (Otávio Lobo Hospital), in Belém of Pará, in the Amazon Region of Brazil. The patients included in the research were between 1 and 18 years of age; had nonrecurrence; were without comorbidities or other types of cancer; and had morphological, immunophenotypic, and, when available, molecular diagnoses. All patients who did not meet this inclusion criteria were excluded from the study.

2.3. Induction Therapy Protocol for ALL

Included patients underwent induction therapy for ALL with the BFM 2009 protocol [17]. During induction therapy, which lasts for a total of 64 days, patients undergo phase 1 corticosteroids for 36 days; four doses of vincristine; 2 doses of doxorubicin (in low-risk and intermediate-risk patients) or 4 doses of doxorubicin (in high-risk patients); and l-asparaginase (8 doses), and during the second phase of cyclophosphamide induction (two doses), patients received 16 doses of cytarabine and 28 consecutive days of mercaptopurine [17].

2.4. Assessment and Classification of Toxicity

Laboratory tests, including transaminases and blood count, were computed in a table at three times during the induction phase: on the first day of treatment, prior to the use of any medication; before the second induction phase, before the use of mercaptopurine but as a patient was already sensitized to other drugs; and after the second induction phase. Adverse events such as anorexia, colitis, diarrhea, dyspepsia, mucositis, nausea, vomiting, neutropenia, and documented or undocumented infection were computed, and numerical stratification was applied to events according to the CTC-NCI guide (Common Toxicity Criteria). After collecting information, adverse effects were classified according to the degree, by considering CTC-NCI: mild/moderate (number 0, 1, and 2) and severe (3 and 4) [18].

2.5. DNA Extraction and Quantification

DNA was extracted by the conventional method with phenol-chloroform according to Sambrook [19]. Samples were quantified in the NanoDrop ND-1000 equipment (Thermo Scientific NanoDrop Products, Wilmington, DE, USA).

2.6. Selection of Polymorphisms

Potential genetic markers were selected from a previous survey in the literature [15,16]. Thus, we obtained 2 polymorphisms, *SLC22A1* (rs202220802) and *NUDT15* (rs1272632214), related to susceptibility, toxicity, and response in the treatment of ALL.

2.7. Genotyping of Polymorphisms

Genotyping of single nucleotide polymorphisms was performed by allelic discrimination using the TaqMan OpenArray Genotyping technology, in the QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) according to the protocol recommended by Applied Biosystem. The TaqMan Genotyper software was used to analyze plaque data and genotype reading accuracy, in addition to genotyping quality control.

2.8. Genomic Ancestry

Analysis was performed using a panel of 61 autosomal informative ancestry markers according to Ramos et al. [20]. Two multiplex PCRs were performed, followed by electrophoresis in the sequencer in ABI Prism 3130 (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and analysis in the GeneMapper ID v.3.2 program (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). The individual proportions of European, African, and Amerindian genetic ancestry were estimated using Structure v.2.3.3 software.

2.9. Statistical Analysis

A descriptive analysis of the data referring to the characterization of the sample was carried out. Quantitative variables were first submitted to the Kolmogorov–Smirnov test to analyze the distribution of normality. The individual proportions of European, African, and Amerindian genetic ancestry were estimated using Structure 2.3.3 software.

For the comparative analysis between the study groups regarding the variables for characterizing the samples, the Chi-square test was applied for categorical variables and the Mann–Whitney test for continuous variables. In order to analyze the association of *SLC22A1* and *NUDT15* gene polymorphisms with the risk of general toxicity in ALL, logistic regression was performed, controlled by the African ancestry variable. In order to analyze the association of *SLC22A1* and *NUDT15* gene polymorphisms with the risk of severe infectious toxicity in ALL, an age-controlled logistic regression was performed.

All statistical analyses were performed using the statistical package of the SPSS 20.0 software while respecting the significance level of 5% (p value ≤ 0.05).

3. Results

In the present study, overall toxicity occurred in 47.1% (95%CI: 33.3–62.7) among ALL patients. In the analysis of demographic and epidemiological characteristics, it can be observed that the groups were similar in terms of gender, age, and ALL subtype. However, they differed significantly in the distribution of African ancestry. These results suggested a loss in the contribution of African ancestry (*p* value: 0.029) in the toxic group compared to the nontoxic group (Table 1).

Table 1. Demographic and epidemiological characteristics, according to the occurrence of general toxicity, of patients with ALL of the Amazon Region.

Variables	General Toxicity		*p* Value
	Yes (n. 27)	No (n. 24)	
Gender			
Female	9 (33.3%)	13 (54.2%)	0.134 [a]
Male	18 (66.7%)	11 (45.8%)	
Age (years)			
Average (±SD)	7.19 (±4.34)	6.96 (±4.43)	0.776 [b]
ALL Sub-type			
ALL B	23 (37.0%)	19 (16.7%)	0.804 [a]
ALL T	4 (14.8%)	5 (20.8%)	
Ancestry			
European	0.494 (±0.520)	0.496 (±0.120)	0.439 [b]
Amerindian	0.342 (±0.149)	0.324 (±0.123)	0.970 [b]
African	0.143 (±0.060)	0.179 (±0.057)	0.029 [b,*]
Toxicity type			
Gastrointestinal			
Low	9 (33.3%)	NA	NA
Moderate	8 (29.6%)	NA	
High	0 (0.0%)	NA	
Hepatic			
Low	2 (7.4%)	NA	NA
Moderate	21 (77.7%)	NA	
High	3 (11.1%)	NA	
Infectious			
Low	0 (0.0%)	NA	NA
Moderate	11 (40.7%)	NA	
High	14 (51.8%)	NA	
Mortality			
Yes	6 (22.2%)	9 (37.5%)	0.375 [a]
No	21 (77.8%)	15 (62.5%)	

ALL, acute lymphoid leukemia. SD, standard deviation. [a] Chi-square test. [b] Mann–Whitney test. * p value ≤ 0.05. NA, not applicable.

Regarding the type of toxicity observed, 62.9% of patients with general toxicity had symptoms of gastrointestinal toxicity, 96.2% had liver toxicity, 88.8% were classified as moderate or severe hepatotoxicity, 92.5% had infectious toxicity, and 53.8% were classified as severe infectious toxicity (Table 1).

The occurrence of deaths among the investigated patients was 29.4% (95%CI: 17.6–41.2%). Regarding the occurrence of deaths due to general toxicity between the groups of ALL patients, it can be observed that there was no significant difference in the occurrence of deaths between the groups (*p* value: 0.375) (Table 1).

The influence of the distribution of African ancestry on the susceptibility to the development of general toxicity in patients with ALL was evaluated using a logistic regression model (Figure 1). The analysis showed that African ancestry in the range between 10% and 25% demonstrated protection for the development of general toxicity in patients with ALL.

However, only a 20% contribution showed a significant protective effect, 0.17 (OR: 0.174; 95%CI: 0.04–0.65; *p* value: 0.010).

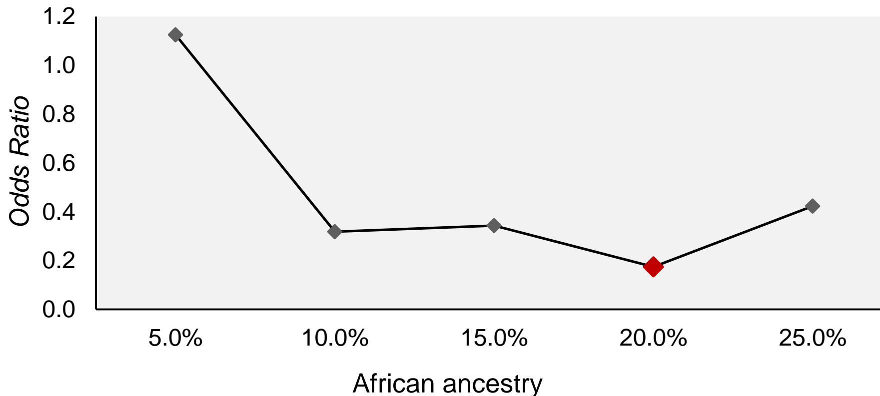

Figure 1. Variation in odds ratios recorded for different percentages of African ancestry as a protective factor for general toxicity in patients with ALL of the Amazon Region.

This protective effect of African ancestry may have occurred in counterpoint to the risk effect shown by the Amerindian contribution, as the increase in African ancestry is offset by Amerindian ancestry, and vice versa. Logistic regression model analysis did not reveal a significant effect on overall toxicity in ALL patients. However, the analysis allowed us to observe a potential risk effect of Amerindian ancestry in the range between 45% and 55% for general toxicity in patients with ALL, reaching the peak risk in 50% of this ancestry (OR: 1.93; 95%CI: 0.30–12.16; *p* value: 0.483) (Figure 2).

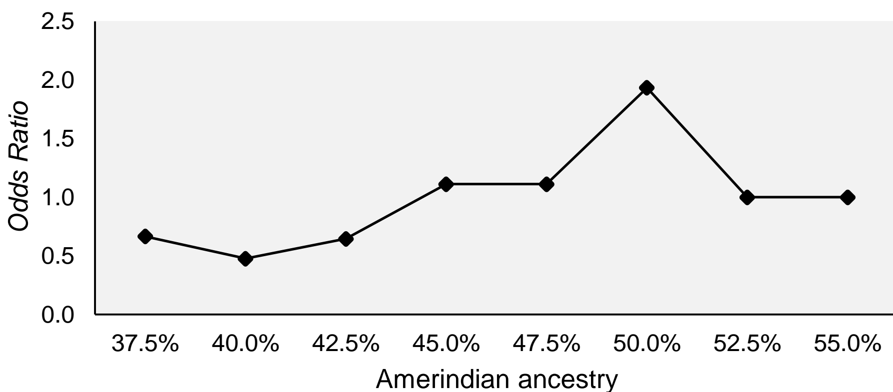

Figure 2. Variation in the odds ratios recorded for different percentages of Amerindian ancestry as a risk factor for general toxicity in patients with ALL of the Amazon Region.

The influence of gene variants SLC22A1 (rs202220802) and NUDT15 (rs1272632214) for the development of general toxicity in patients with ALL was evaluated using the multivariate logistic regression model, controlled by African ancestry. This analysis showed that none of the genotypes and none of the alleles of both polymorphisms showed significant effects on the occurrence of general toxicity in patients with ALL (Table 2).

The influence of gene variants SLC22A1 (rs202220802) and NUDT15 (rs1272632214) on the development of severe infectious toxicity in patients with ALL was also evaluated using the age-controlled logistic regression model. This analysis showed that, for the NUDT gene variant (rs1272632214), none of the genotypes and none of the alleles showed significant effects on the occurrence of severe infectious toxicity in patients with ALL (Table 3). However, for the SLC gene variant (rs202220802), it was possible to observe that homozygous and heterozygous genotypes with deletions were more frequent in the group with severe infectious toxicity. In addition, the deletion allele was also significantly more frequent in the group with severe infectious toxicity, demonstrating a risk effect

for this event and increasing the chance of its occurrence by about three times (OR: 3.18; 95%CI: 1.11–9.11, *p* value: 0.031) (Table 3).

Table 2. Comparative analysis of the variation in polymorphisms of the *SLC22A1* (rs202220802) and *NUDT15* (rs1272632214) genes, by the occurrence of general toxicity, in patients with ALL of the Amazon Region.

Polymorphisms	General Toxicity		*p* Value [a]	*p* Value [b]	OR (95%IC)
	Yes (n. 27)	No (n. 24)			
SLC22A1					
n-n	5 (18.5%)	9 (37.5%)	0.112	0.230	0.43 (0.11–1.69)
n-ins	6 (22.2%)	2 (8.3%)		0.209	3.64 (0.51–20.63)
n-del	9 (33.3%)	6 (25.0%)		0.435	1.70 (0.45–6.51)
del-ins	1 (3.7%)	5 (20.8%)		0.072	0.12 (0.01–1.20)
del-del	5 (18.5%)	1 (4.2%)		0.196	4.68 (0.45–48.62)
ins-ins	1 (3.7%)	1 (4.2%)		0.635	0.50 (0.03–8.77)
Allele n	25 (46.3%)	26 (54.2%)	0.578	0.737	0.86 (0.37–2.01)
Allele ins	9 (16.7%)	9 (18.8%)		0.502	0.68 (2.31–2.05)
Allele del	20 (37.0%)	13 (27.1%)		0.366	1.52 (0.61–3.77)
NUDT15					
Del-Del	20 (76.9%)	22 (91.7%)	0.155	0.321	0.39 (0.06–2.64)
Ins-Del	6 (23.1%)	2 (8.3%)			
Allele Del	46 (88.5%)	46 (95.8%)	0.175	0.347	2.32 (0.40–13.55)
Allele Ins	6 (11.5%)	2 (4.2%)			

[a] Chi-square test. [b] Multivariate logistic regression adjusts for African ancestry. OR, odds ratio. CI, confidence interval.

Table 3. Comparative analysis of the variation in polymorphisms of the *SLC22A1* (rs202220802) and *NUDT15* (rs1272632214) genes, by the occurrence of severe infectious toxicity in patients with ALL of the Amazon Region.

Polymorphisms	Severe Infectious Toxicity		*p* Value [a]	*p* Value [b]	OR (95%IC)
	Yes (n. 14)	No (n. 37)			
SLC22A1					
n-n	3 (21.4%)	11 (29.7%)	0.304	0.597	0.66 (0.15–3.02)
n-ins	2 (14.3%)	6 (16.2%)		0.216	0.27 (0.03–2.13)
n-del	6 (42.9%)	9 (24.3%)		0.194	2.80 (0.69–11.32)
del-ins	0 (0.0%)	6 (16.2%)		0.999	0.00 (0.00–0.00)
del-del	3 (21.4%)	3 (8.1%)		0.085	6.17 (0.78–49.11)
ins-ins	0 (0.0%)	2 (5.4%)		0.999	0.00 (0.00–0.00)
Allele n	14 (50.0%)	37 (50.0%)	0.155	0.715	1.19 (1.06–1.33)
Allele ins	2 (7.1%)	16 (21.6%)		0.049*	0.19 (0.04–0.99)
Allele del	12 (42.9%)	21 (28.4%)		0.031*	3.18 (1.11–9.11)
NUDT15					
Del-Del	10 (76.9%)	32 (86.5%)	0.418	0.333	0.43 (0.08–2.33)
Ins-Del	3 (23.1%)	5 (13.5%)			
Allele Del	23 (88.5%)	69 (93.2%)	0.432	0.356	2.11 (0.43–10.35)
Allele Ins	3 (11.5%)	5 (6.8%)			

[a] Chi-square test. [b] Age-adjusted multivariate logistic regression. OR, odds ratio. IC, confidence interval. * p-value ≤ 0.05.

4. Discussion

Currently, studies on new treatments for ALL are not only aimed at discovering more efficient antineoplastics but also seek to minimize the toxic effects of these medications. Given the intensification of treatment in the last three decades, the chance of death related to the toxicity of the therapy can be compared to chance of relapse in low-risk patients [21]. There are several symptoms that can occur during ALL treatment associated with the unwanted pharmacological effect of the medications. In the literature, the occurrence of mainly

gastrointestinal and hematological effects during treatment for ALL is evidenced and is mainly associated with genetic variations in genes such as *NUDT15*, *TPMT*, and *PDE4D* causing more severe toxicities in certain ethnic groups such as the Amerindians [15,22,23].

Infections and febrile neutropenia, which are changes resulting from hematological toxicity and which are considered infectious toxicities, can impact the response and effectiveness of treatment. The results are in accordance with the data found in the literature, which describes an increase in hematological events associated with the genetic variability of certain populations [24–27].

Genetic variants in *NUDT15*, which characterize a loss or severe decrease in its function, can lead patients treated with thiopurines to an excessive activation of these drugs, causing serious adverse effects, such as hematopoietic toxicities in malignant conditions such as ALL [8]. These variants are already present in the guidelines of the Consortium for the Implementation of Clinical Pharmacogenetics (CPIC) as an alternative to prevent toxicity in these patients [28]. A study recently published by our research group demonstrated that the NUDT15*2 and NUDT15*4 haplotypes of *NUDT15* are present in high frequencies in Amerindian and mixed populations of Northern Brazil when compared to other continental populations, which implies the alteration of the metabolization profile of these individuals when treated with the standard regimen for ALL [15]. In this study, however, no association of the *NUDT15* gene with any of the toxicities investigated here was found.

SCL22A1 gene encodes hOCT1 (Human Organic Cation Transporter 1), a genetically variable transporter that is strongly expressed in the epithelial barriers and sinusoidal membrane of the human liver [29]. hOCT1 plays a role in the pharmacodynamics and pharmacokinetics of anticancer, antiviral, anti-inflammatory, and antiemetic drugs, as well as drugs used in the treatment of neurological diseases [29,30]. Variants that cause complete loss of hOCT1 activity have already been identified, and on average 1 in 11 Europeans or Americans have a reduction in hepatic drug absorption because they have poor hOCT1 transporters [31–33]. Studies carried out on the treatment of different types of leukemias showed results indicating that variants in the *SLC22A1* gene have different treatment toxicities or clinical responses [29,34]. Our study demonstrated that the rs202220802 polymorphism of the *SLC22A1* gene, with the deletion allele being a risk factor, had approximately three times the risk of suffering severe hematotoxicity (OR: 3.18; 95%CI: 1.11–9.11, p value: 0.031).

Our results showed a protective factor for toxicity to ALL treatment in patients above 20% of African ancestry. A study by Yao et al. [35] also pointed to a reduction in the risk of toxicities related to the treatment of ALL in patients aged 1–18 years who had a high degree of African genomic ancestry, particularly related to fractures and osteonecrosis. This same study presented data relating the worst treatment outcomes to the high degree of Native American ancestry, which has already been described in different studies [8,11] and which are associated with our findings, further demonstrating a potential risk for the development of severe treatment toxicities in patients with a range of up to 50% of Amerindian ancestry. The degree of Amerindian ancestry associated with the potential risk of developing toxicities may be related to the genomic ancestral opposition to African ancestry, which confers a potential for protection. However, we cannot exclude the possibility that environmental, sociocultural, and dietary differences play a role in the observed toxicities.

The important role of ancestry and polymorphism in the *SLC22A1* gene in toxicity that occurs in the treatment of ALL was evidenced in the present study. However, more studies are needed to minimize some of the limitations presented, such as the increase in participants; the inclusion of other clinical factors; sociocultural and nutritional particularities that may be related to toxicity; and addressing other metabolic pathways that are possibly associated with toxicity in the treatment of ALL.

5. Conclusions

In conclusion, the *SLC22A1* gene variant (rs202220802) was associated with a potential risk of developing severe infectious toxicity in patients with ALL. In addition, African ancestry demonstrated protection for the development of general toxicity in patients with ALL. These results are important for stimulating new genomic studies that can identify genetic variants with high frequency or those that are exclusive to populations with high miscegenation degrees that can explain the predisposition of these patients to severe toxicities.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Research Ethics Committee of the Health Sciences Institute of the Federal University of Pará, under protocol number 119.649/2012. All participants signed an informed consent form.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

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References

1. Pui, C.H.; Cheng, C.; Leung, W.; Rai, S.N.; Rivera, G.K.; Sandlund, J.T.; Ribeiro, R.C.; Relling, M.V.; Kun, L.E.; Evans, W.E.; et al. Extended follow-up of long-term survivors of childhood acute lymphoblastic leukemia. *N. Engl. J. Med.* **2003**, *349*, 640–649. [CrossRef]
2. Howlader, N.; Noone, A.M.; Krapcho, M.; Miller, D.; Bishop, K.; Kosary, C.L.; Yu, M.; Ruhl, J.; Tatalovich, Z.; Mariotto, A.; et al. (Eds.) *SEER Cancer Statistics Review, 1975–2014*; National Cancer Institute, Bethesda: Rockville, MD, USA. Available online: https://seer.cancer.gov/archive/csr/1975_2014/ (accessed on 1 November 2021).
3. Ministério da Saúde; Instituto Nacional de Câncer José Alencar Gomes da Silva. *Estimativa 2020: Incidência de Câncer No Brasil.*; INCA: Rio de Janeiro, Brazil, 2019. Available online: https://www.inca.gov.br/sites/ufu.sti.inca.local/files//media/document//estimativa-2020-incidencia-de-cancer-no-brasil.pdf (accessed on 1 November 2021).
4. Kishi, S.; Cheng, C.; French, D.; Pei, D.; Das, S.; Cook, E.H.; Hijiya, N.; Rizzari, C.; Rosner, G.L.; Frudakis, T.; et al. Ancestry and pharmacogenetics of antileukemic drug toxicity. *Blood* **2007**, *109*, 4151–4157. [CrossRef] [PubMed]
5. Meadows, A.T.; Robison, L.L.; Neglia, J.P.; Sather, H.; Hammond, D. Potential long-term toxic effects in children treated for acute lymphoblastic leukemia. *N. Engl. J. Med.* **1989**, *321*, 1830–1831. [CrossRef] [PubMed]
6. Carvalho, D.C.; Wanderley, A.V.; Amador, M.A.; Fernandes, M.R.; Cavalcante, G.C.; Pantoja, K.B.; Mello, F.A.; de Assumpção, P.P.; Khayat, A.S.; Ribeiro-Dos-Santos, Â.; et al. Amerindian genetic ancestry and INDEL polymorphisms associated with susceptibility of childhood B-cell Leukemia in an admixed population from the Brazilian Amazon. *Leuk. Res.* **2015**, *39*, 1239–1245. [CrossRef] [PubMed]
7. Stary, J.; Zimmermann, M.; Campbell, M.; Castillo, L.; Dibar, E.; Donska, S.; Gonzalez, A.; Izraeli, S.; Janic, D.; Jazbec, J.; et al. Intensive chemotherapy for childhood acute lymphoblastic leukemia: Results of the randomized intercontinental trial ALL IC-BFM 2002. *J. Clin. Oncol.* **2014**, *32*, 174–184. [CrossRef]
8. Yang, J.J.; Cheng, C.; Devidas, M.; Cao, X.; Fan, Y.; Campana, D.; Yang, W.; Neale, G.; Cox, N.J.; Scheet, P.; et al. Ancestry and pharmacogenomics of relapse in acute lymphoblastic leukemia. *Nat. Genet.* **2011**, *43*, 237–241. [CrossRef]
9. Li, J.; Lou, H.; Yang, X.; Lu, D.; Li, S.; Jin, L.; Pan, X.; Yang, W.; Song, M.; Mamatyusupu, D.; et al. Genetic architectures of ADME genes in five Eurasian admixed populations and implications for drug safety and efficacy. *J. Med. Genet.* **2014**, *51*, 614–622. [CrossRef]

10. Hareedy, M.S.; El Desoky, E.S.; Woillard, J.B.; Thabet, R.H.; Ali, A.M.; Marquet, P.; Picard, N. Genetic variants in 6-mercaptopurine pathway as potential factors of hematological toxicity in acute lymphoblastic leukemia patients. *Pharmacogenomics* **2015**, *16*, 1119–1134. [CrossRef]
11. de Carvalho, D.C.; Wanderley, A.V.; Dos Santos, A.M.R.; Moreira, F.C.; de Sá, R.B.A.; Fernandes, M.R.; Modesto, A.A.C.; de Souza, T.P.; Cohen-Paes, A.; Leitão, L.P.C.; et al. Characterization of pharmacogenetic markers related to Acute Lymphoblastic Leukemia toxicity in Amazonian native Americans population. *Sci. Rep.* **2020**, *10*, 10292. [CrossRef]
12. Lange, B.J.; Bostrom, B.C.; Cherlow, J.M.; Sensel, M.G.; La, M.K.; Rackoff, W.; Heerema, N.A.; Wimmer, R.S.; Trigg, M.E.; Sather, H.N.; et al. Double-delayed intensification improves event-free survival for children with intermediate-risk acute lymphoblastic leukemia: A report from the Children's Cancer Group. *Blood* **2002**, *99*, 825–833. [CrossRef]
13. Pui, C.H.; Robison, L.L.; Look, A.T. Acute lymphoblastic leukaemia. *Lancet* **2008**, *371*, 1030–1043. [CrossRef]
14. Lopez-Lopez, E.; Gutierrez-Camino, A.; Bilbao-Aldaiturriaga, N.; Pombar-Gomez, M.; Martin-Guerrero, I.; Garcia-Orad, A. Pharmacogenetics of childhood acute lymphoblastic leukemia. *Pharmacogenomics* **2014**, *15*, 1383–1398. [CrossRef] [PubMed]
15. Rodrigues, J.C.G.; Souza, T.P.; Pastana, L.F.; Ribeiro Dos Santos, A.M.; Fernandes, M.R.; Pinto, P.; Wanderley, A.V.; Souza, S.J.; Kroll, J.E.; Pereira, A.L.; et al. Identification of NUDT15 gene variants in Amazonian Amerindians and admixed individuals from northern Brazil. *PLoS ONE* **2020**, *15*, e0231651. [CrossRef] [PubMed]
16. Goswami, S.; Gong, L.; Giacomini, K.; Altman, R.B. PharmGKB summary: Very important pharmacogene information for SLC22A1. *Pharmacogenet. Genom.* **2014**, *24*, 324–328. [CrossRef] [PubMed]
17. ALL IC-BFM 2009 A Randomized Trial of the I-BFM-SG for the Management of Childhood Non-B Acute Lymphoblastic Leukemia. Final Version of Therapy Protocol from August-14-2009. Available online: https://www.bialaczka.org/wp-content/uploads/2016/10/ALLIC_BFM_2009.pdf (accessed on 25 November 2021).
18. Saad, E.D.; Hoff, P.M.; Carnelós, R.P.; Katz, A.; Novis, Y.A.S.; Pietrocola, M.; Hamerschlak, N.; Tabacof, J.; Gansl, R.C.; Simon, S.D. Critérios comuns de toxicidade do Instituto Nacional de Câncer dos Estados Unidos. *Rev. Bras. Cancerol.* **2002**, *48*, 63–96. [CrossRef]
19. Sambrook, J.; Fritsch, E.F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 1989.
20. Ramos, B.R.D.A.; D'Elia, M.P.B.; Amador, M.A.T.; Santos, N.P.C.; Santos, S.E.B.; Castelli, E.D.C.; Witkin, S.S.; Miot, H.A.; Miot, L.D.B.; da Silva, M.G. Neither self-reported ethnicity nor declared family origin are reliable indicators of genomic ancestry. *Genetica* **2016**, *144*, 259–265. [CrossRef]
21. Schmiegelow, K.; Attarbaschi, A.; Barzilai, S.; Escherich, G.; Frandsen, T.L.; Halsey, C.; Hough, R.; Jeha, S.; Kato, M.; Liang, D.C.; et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: A Delphi consensus. *Lancet Oncol.* **2016**, *17*, e231–e239. [CrossRef]
22. Özdemir, Z.; Kar, Y.; Turhan, A.; Bör, Ö. Assessment of Hematological Toxicity in Children with Acute Lymphoblastic Leukemia, Receiving Treatment with ALL IC-BFM 2009 Protocol. *OALib J.* **2017**, *4*, 78829. [CrossRef]
23. Suarez-Kurtz, G.; Brisson, G.D.; Hutz, M.H.; Petzl-Erler, M.L.; Salzano, F.M. NUDT15 Polymorphism in Native American Populations of Brazil. *Clin. Pharmacol. Ther.* **2019**, *105*, 1321–1322. [CrossRef]
24. de Carvalho, D.C.; Wanderley, A.V.; dos Santos, A.M.R.; Fernandes, M.R.; Cohen Lima de Castro, A.N.; Leitão, L.P.C.; de Carvalho, J.A.N.; de Souza, T.P.; Khayat, A.S.; dos Santos, S.E.B.; et al. Pharmacogenomics and variations in the risk of toxicity during the consolidation/maintenance phases of the treatment of pediatric B-cell leukemia patients from an admixed population in the Brazilian Amazon. *Leuk. Res.* **2018**, *74*, 10–13. [CrossRef]
25. Moradveisi, B.; Muwakkit, S.; Zamani, F.; Ghaderi, E.; Mohammadi, E.; Zgheib, N.K. ITPA, TPMT, and NUDT15 Genetic Polymorphisms Predict 6-Mercaptopurine Toxicity in Middle Eastern Children With Acute Lymphoblastic Leukemia. *Front. Pharmacol.* **2019**, *10*, 916. [CrossRef] [PubMed]
26. Wahlund, M.; Nilsson, A.; Kahlin, A.Z.; Broliden, K.; Myrberg, I.H.; Appell, M.L.; Berggren, A. The Role of TPMT, ITPA, and NUDT15 Variants during Mercaptopurine Treatment of Swedish Pediatric Patients with Acute Lymphoblastic Leukemia. *J. Pediatr.* **2020**, *216*, 150–157.e151. [CrossRef] [PubMed]
27. Buaboonnam, J.; Sripatanatadasakul, P.; Treesucon, A.; Glomglao, W.; Siraprapapat, P.; Narkbunnam, N.; Vathana, N.; Takpradit, C.; Phuakpet, K.; Pongtanakul, B.; et al. Effect of NUDT15 on incidence of neutropenia in children with acute lymphoblastic leukemia. *Pediatr. Int.* **2019**, *61*, 754–758. [CrossRef] [PubMed]
28. Relling, M.V.; Schwab, M.; Whirl-Carrillo, M.; Suarez-Kurtz, G.; Pui, C.H.; Stein, C.M.; Moyer, A.M.; Evans, W.E.; Klein, T.E.; Antillon-Klussmann, F.G.; et al. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. *Clin. Pharmacol. Ther.* **2019**, *105*, 1095–1105. [CrossRef]
29. Arimany-Nardi, C.; Koepsell, H.; Pastor-Anglada, M. Role of SLC22A1 polymorphic variants in drug disposition, therapeutic responses, and drug-drug interactions. *Pharm. J.* **2015**, *15*, 473–487. [CrossRef]
30. Yee, S.W.; Brackman, D.J.; Ennis, E.A.; Sugiyama, Y.; Kamdem, L.K.; Blanchard, R.; Galetin, A.; Zhang, L.; Giacomini, K.M. Influence of Transporter Polymorphisms on Drug Disposition and Response: A Perspective From the International Transporter Consortium. *Clin. Pharmacol. Ther.* **2018**, *104*, 803–817. [CrossRef]
31. Kerb, R.; Brinkmann, U.; Chatskaia, N.; Gorbunov, D.; Gorboulev, V.; Mornhinweg, E.; Keil, A.; Eichelbaum, M.; Koepsell, H. Identification of genetic variations of the human organic cation transporter hOCT1 and their functional consequences. *Pharmacogenetics* **2002**, *12*, 591–595. [CrossRef]

32. Shu, Y.; Leabman, M.K.; Feng, B.; Mangravite, L.M.; Huang, C.C.; Stryke, D.; Kawamoto, M.; Johns, S.J.; DeYoung, J.; Carlson, E.; et al. Evolutionary conservation predicts function of variants of the human organic cation transporter, OCT1. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5902–5907. [CrossRef]
33. Tzvetkov, M.V.; Matthaei, J.; Pojar, S.; Faltraco, F.; Vogler, S.; Prukop, T.; Seitz, T.; Brockmöller, J. Increased Systemic Exposure and Stronger Cardiovascular and Metabolic Adverse Reactions to Fenoterol in Individuals with Heritable OCT1 Deficiency. *Clin. Pharmacol. Ther.* **2018**, *103*, 868–878. [CrossRef]
34. Cargnin, S.; Ravegnini, G.; Soverini, S.; Angelini, S.; Terrazzino, S. Impact of SLC22A1 and CYP3A5 genotypes on imatinib response in chronic myeloid leukemia: A systematic review and meta-analysis. *Pharmacol. Res.* **2018**, *131*, 244–254. [CrossRef]
35. Yao, S.; Zhu, Q.; Cole, P.D.; Stevenson, K.; Harris, M.H.; Schultz, E.; Kahn, J.M.; Ladas, E.J.; Athale, U.H.; Clavell, L.A.; et al. Genetic ancestry and skeletal toxicities among childhood acute lymphoblastic leukemia patients in the DFCI 05-001 cohort. *Blood Adv.* **2021**, *5*, 451–458. [CrossRef] [PubMed]

Article

Exome Evaluation of Autism-Associated Genes in Amazon American Populations

Giovana E. da Costa [1], Giordane L. Fernandes [1], Juliana C. G. Rodrigues [1], Diana F. da V. B. Leal [1], Lucas F. Pastana [1], Esdras E. B. Pereira [2], Paulo P. Assumpção [2], Rommel M. R. Burbano [1,2], Sidney E. B. dos Santos [1,2], João F. Guerreiro [2], Marianne R. Fernandes [1,*] and Ney P. C. dos Santos [1,2]

[1] Núcleo de Pesquisas em Oncologia, Universidade Federal do Pará, Belém 66075-110, Brazil; giovanaescribano@hotmail.com (G.E.d.C.); giordanelages@hotmail.com (G.L.F.); julianacgrodrigues@gmail.com (J.C.G.R.); dianafeio@hotmail.com (D.F.d.V.B.L.); lucas.pastana@ics.ufpa.br (L.F.P.); rommelburbano@gmail.com (R.M.R.B.); sidneysantos@ufpa.br (S.E.B.d.S.); npcsantos.ufpa@gmail.com (N.P.C.d.S.)

[2] Laboratório de Genética Humana e Médica, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém 66075-110, Brazil; esdrasedgarbp@gmail.com (E.E.B.P.); assumpcaopp@gmail.com (P.P.A.); joao.guerreiro53@gmail.com (J.F.G.)

* Correspondence: fernandesmr@yahoo.com.br

Abstract: Autism spectrum disorder is a neurodevelopmental disorder, affecting one in 160 children worldwide. The causes of autism are still poorly understood, but research shows the relevance of genetic factors in its pathophysiology, including the *CHD8*, *SCN2A*, *FOXP1* and *SYNGAP1* genes. Information about the genetic influence on various diseases, including autism, in the Amerindian population from Amazon, is still scarce. We investigated 35 variants of the *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* gene in Amazonian Amerindians in comparison with publicly available population frequencies from the 1000 Genomes Project database. Our study identified 16 variants in the Amerindian population of the Amazon with frequencies significantly different from the other populations. Among them, the *SCN2A* (rs17183814, rs75109281, and rs150453735), *FOXP1* (rs56850311 and rs939845), and *SYNGAP1* (rs9394145 and rs115441992) variants presented higher frequency than all other populations analyzed. In addition, nine variants were found with lower frequency among the Amerindians: *CHD8* (rs35057134 and rs10467770), *SCN2A* (rs3769951, rs2304014, rs1838846, and rs7593568), *FOXP1* (rs112773801 and rs56850311), and *SYNGAP1* (rs453590). These data show the unique genetic profile of the indigenous population of the Brazilian Amazon. Knowledge of these variants can help to understand the pathophysiology and diagnosis of autism among Amerindians, Brazilians, and in admixed populations that have contributions from this ethnic group.

Keywords: autism; susceptibility; genetic; Amerindians

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1. Introduction

Autism spectrum disorder (ASD) is one of the main neuropsychiatric conditions and is characterized by different behavioral manifestations, including deficits in social communication and interaction, repetitive patterns of behavior, interests, and performance in specific activities [1]. The World Health Organization estimates that one in every 160 children is identified with ASD, being approximately 70 million people with ASD worldwide. In Brazil, it is estimated that 2 million people have ASD (1%) [2,3].

According to the Brazilian Institute of Geography and Statistics (IBGE), the Amerindian population is estimated at 896,917 individuals, representing 0.47% of the Brazilian population [4]. Studies about ASD in Amerindian populations are rare. In other countries, such as Australia, Amerindian Australians with the autism spectrum are twice as likely to have a severe or profound form of ASD and may have worse long-term outcomes compared to

non-Amerindian Australians with the same condition. It was reported that Amerindians had less support and access to services in health and medicines [5].

ASD was discovered by Kanner in 1943 in the U.S. and by Asperger in 1944 in Vienna [6,7]. Currently, the causes of autism are still not well understood, although environmental, non-genetic, and genetic factors contribute to the disease. Bai et al. evaluated the contribution of various genetic and non-genetic factors to ASD risk. Researchers estimated heritability with maternal effects and shared and nonshared environments on ASD risk, including more than 2 million individuals from 5 countries. This study results reported significant evidence that most of the risk for ASD came from genetic factors [8,9].

In the largest genetic sequencing study of autism spectrum disorder (ASD) to date, researchers identified 102 genes related to the risk of ASD. The study enrolled 35,584 participant samples, including nearly 11,986 with ASD. Allelic variations in the 102 genes were related to susceptibility to neurodevelopmental disorders, such as ASD, and were able to differentiate this condition from other general neurodevelopmental disorders [10]. However, the impact of genetic factors associated with ASD in the Amazonian Amerindian is still unknown.

This is the first genetic study based on single-nucleotide polymorphisms (SNPs) associated with ASD in Amerindians from the Brazilian Amazon. This study characterizes the molecular profile of four of 102 genes related to ASD from a study by Satterstrom et al. [10] by analyzing the exome of Amerindian individuals from the Brazilian Amazon. The objective was to describe SNPs that may explain the predisposition to the development of ASD in Amazonian Amerindian and compare them with the worldwide population.

2. Materials and Methods

2.1. Study and Reference Populations

The Indigenous group (IND) was composed of non-related 64 Amerindians which represent 12 Amazonian ethnic groups of Northern Brazil: (i) Asurini do Xingu (N = 5), (ii) Arara (N = 7), (iii) Araweté (N = 6), (iv) Asurini from Tocantins (N = 16), (v) Awa-Guajá (N = 8), (vi) Kayapó/Xikrin (N = 2), (vii) Zo'é (N = 5), (viii) Wajãpi (N = 10), (ix) Karipuna (N = 1), (x) Phurere (N = 1), (xi) Munduruku (N = 1), and (xii) Juruna (N = 2).

All participants of the study and their ethnic group leaders signed a free-informed consent. The study was approved by the National Research Ethics Committee (CONEP) and the Research Ethics Committee of the Tropical Medicine Center of the Federal University of Pará, under CAAE number 20654313.6.0000.5172. The period for recruiting participants was from September 2017 to December 2018.

The results were compared with genomic data from populations from other countries available in the phase 3 version of the 1000 Genomes Database [11]. These populations included 661 Africans (AFR), 347 Americans (AMR), 504 from East Asia (EAS), 503 from Europe (EUR), and 489 from South Asia (SAS).

2.2. Extraction of the DNA and Preparation of the Exome Library

DNA extraction was performed using the phenol-chloroform method described by Sambrook et al. [12]. The quantification and integrity of the genetic material were analyzed by a Nanodrop-8000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE, USA) and 2% agarose gel electrophoresis, respectively.

Exome libraries were prepared using the Nextera Rapid Capture Exome (Illumina®, San Diego, CA, USA) and SureSelect Human All Exon V6 (Agilent) kits. The sequencing reactions were performed on the NextSeq 500® platform (Illumina®, San Diego, CA, USA) using the NextSeq 500 High-output v2 300 cycle kit (Illumina®, San Diego, CA, USA).

2.3. Bioinformatics Analysis

Bioinformatics analysis was performed as previously described by Rodrigues et al. [13].

2.4. Statistical Analysis

Allele frequencies of the IND populations were obtained by gene counting compared to the other study populations (AFR, EUR, AMR, EAS, and SAS). Fisher's test was used to compare frequency differences between populations. A p-value < 0.05 was considered statistically significant. Interpopulation variability of polymorphisms was assessed using the Wright fixation index (FST). Data analyses were performed using RStudio version 3.5.1.

2.5. Selection of Genes and Variants

The selection of genes was based in the results pointed out in the study of Satterstrom et al. [10]. Four of these genes, *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1*, were classified as risk genes with the lowest rates of "false discovery rate (FDR)" and "family-wise error Rate (FWER)".

The SNP inclusion criteria were: (i) minimum of 10 reeds of coverage (fastx_tools v.0.13 http://hannonlab.cshl.edu/fastx_toolkit/, accessed on October 2021); (ii) variant impact: modifier, moderate or high (SNPeff classification (https://pcingola.github.io/SnpEff/, accessed on October 2021); and (iii) allelic and genomic frequency in worldwide populations (http://www.1000genomes.org, accessed on October 2021).

3. Results

A total of 59 genetics variants were identified in *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* (Supplementary Table S1). Thirty-five of 59 variants met the SNP inclusion criteria. Eight variants were identified in the *CHD8* gene, eleven in *SCN2A*, twelve in *FOXP1*, and four in *SYNGAP1* in the individuals analyzed. Table 1 shows characteristics of these variants, including their reference number, chromosome region, nucleotide exchange, impact predicted by the SNPeff software, and the allele frequency referring to the indigenous group (IND) and the five continental populations present in the 1000 Genomes Program (AFR, AMR, EAS, EUR, and SAS) [11]. Among the selected polymorphisms, thirty have predicted impact as a modifier and five as moderate. Twenty-eight are from the intronic region, five from the CDS region, and two from the 3′UTR region. The frequencies of 35 variants were compared with different population groups (Table 2).

Among the 35 variants, 16 variants showed frequencies among Amerindians significantly different from all other populations. Seven variants with greater frequency among Amerindians: *SCN2A* (rs17183814, rs75109281, and rs150453735), *FOXP1* (rs939845 and rs2037474), and *SYNGAP1* (rs115441992 and rs9394145). Nine variants with lower frequency among the Amerindians: *CHD8* (rs35057134 and rs10467770), *SCN2A* (rs3769951, rs2304014, rs1838846, and rs7593568), *FOXP1* (rs112773801 and rs56850311), and *SYNGAP1* (rs453590).

The EUR and SAS populations stand out as those with the most variants with significant differences for the Amerindian population ($p < 0.05$). For the EUR population, five were in the *CHD8* gene (rs35057134, rs10467770, rs111776414, rs1998332, and rs149307240), nine in the *SCN2A* gene (rs17183814, rs75109281, rs3769951, rs28472553, rs139906774, rs2304014, rs150453735, rs1838846, and rs7593568), seven in the *FOXP1* gene (rs112773801, rs58847217, rs72960080, rs13068094, rs56850311, rs939845, and rs2037474), and four in *SYNGAP1* (rs76557362, rs453590, rs115441992, and rs9394145).

For SAS, four were in the *CHD8* gene (rs35057134, rs10467770, rs1998332, and rs149307240), nine were in the *SCN2A* gene (rs17183814, rs75109281, rs3769951, rs28472553, rs139906774, rs2304014, rs150453735, rs1838846, and rs7593568), eight in *FOXP1* (rs112773801, rs58847217, rs72960080, rs13068094, rs56850311, rs939845, rs2037474, and rs15101125) and four in *SYNGAP1* (rs76557362, rs453590, rs115441992, and rs9394145).

In relation to the AFR population, five polymorphisms were found to be significantly divergent in the *CHD8* gene (rs35057134, rs10467770, rs57764234, rs111776414, and rs149307240), eight in the *SCN2A* gene (rs17183814, rs75109281, rs3769951, rs2304014, rs150453735, rs1867864, rs1838846, and rs7593568), four in *FOXP1* (rs112773801, rs56850311, rs939845, and rs2037474), and four in the *SYNGAP1* gene (rs76557362, rs453590, rs115441992,

and rs9394145), adding up to a total of twenty-one significantly different variants of the IND population.

The AMR population presented twenty-two statistically different polymorphisms in relation to the IND population: three in the *CHD8* gene (rs35057134, rs10467770, and rs1998332), nine in the *SCN2A* gene (rs17183814, rs75109281, rs3769951, rs28472553, rs139906774, rs2304014, rs150453735, rs1838846, and rs7593568), six in the *FOXP1* gene (rs112773801, rs72960080, rs13068094, rs568503111, rs939845, and rs2037474), and four in *SYNGAP1* (rs76557362, rs453590, rs115441992, and rs9394145).

Table 1. Description of variants in the *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* genes in the Indigenous group and continental populations (African, American, East Asia, European, and South Asia) described in the 1000 Genomes Project.

Gene	SNP ID	Region	Alleles	Impact Predicted by SNPeff	IND	AFR	AMR	EAS	EUR	SAS
CHD8	rs35057134	Intronic	GA > G	Modifier	0.0143	0.2250	0.2070	0.3480	0.2740	0.2550
CHD8	rs80311097	Intronic	C > A	Modifier	0.0000	0.0610	0.0010	0.0000	0.0010	0.0000
CHD8	rs10467770	CDS	C > T	Moderate	0.0781	0.2240	0.1900	0.3450	0.2450	0.2490
CHD8	rs111250264	CDS	G > A	Moderate	0.0086	0.0050	0.0000	0.0000	0.0000	0.0000
CHD8	rs57764234	Intronic	C > T	Modifier	0.0246	0.3160	0.0290	0.0000	0.0210	0.0050
CHD8	rs111776414	Intronic	G > GA	Modifier	0.0417	0.1610	0.0120	0.0010	0.0020	0.0110
CHD8	rs1998332	Intronic	G > A	Modifier	0.6172	0.5730	0.7780	0.8880	0.9060	0.9170
CHD8	rs149307240	CDS	C > T	Moderate	0.0259	0.0000	0.0160	0.0000	0.0010	0.0000
SCN2A	rs17183814	CDS	G > A	Moderate	0.2500	0.0210	0.0820	0.1380	0.0570	0.1420
SCN2A	rs75109281	Intronic	C > T	Modifier	0.0833	0.0120	0.0030	0.0000	0.0000	0.0000
SCN2A	rs3769951	Intronic	C > T	Modifier	0.0135	0.1640	0.2480	0.2610	0.2920	0.3310
SCN2A	rs28472553	Intronic	A > C	Modifier	0.0833	0.0290	0.0030	0.0000	0.0010	0.0000
SCN2A	rs139906774	Intronic	G > GA	Modifier	0.0000	0.0520	0.3000	0.3420	0.2420	0.1860
SCN2A	rs2304014	Intronic	T > A	Modifier	0.0270	0.2280	0.1330	0.1410	0.1760	0.1390
SCN2A	rs6432821	Intronic	T > C	Modifier	1.0000	0.9520	0.9970	1.0000	1.0000	0.9990
SCN2A	rs150453735	Intronic	C > T	Modifier	0.1852	0.0020	0.0530	0.0000	0.0000	0.0000
SCN2A	rs1867864	Intronic	C > T	Modifier	0.4453	0.6130	0.4600	0.3430	0.5640	0.4870
SCN2A	rs1838846	Intronic	A > G	Modifier	0.0000	0.7930	0.7940	0.7430	0.8300	0.6980
SCN2A	rs7593568	Intronic	A > G	Modifier	0.0000	0.7950	0.7950	0.7430	0.8300	0.6950
FOXP1	rs1435680522	3UTR	GT > G	Modifier	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
FOXP1	rs112773801	3UTR	G > GT	Modifier	0.0167	0.4240	0.1860	0.4510	0.1340	0.3290
FOXP1	rs58847217	Intronic	T > C	Modifier	0.0278	0.1010	0.0040	0.0000	0.0000	0.0000
FOXP1	rs76145927	CDS	T > C	Moderate	0.0000	0.0000	0.0060	0.0390	0.0030	0.0000
FOXP1	rs72960080	Intronic	T > C	Modifier	0.0833	0.1190	0.0040	0.0000	0.0000	0.0000
FOXP1	rs13068094	Intronic	C > T	Modifier	0.0833	0.1040	0.5400	0.0550	0.5750	0.2820
FOXP1	rs7638391	Intronic	G > T	Modifier	1.0000	0.9970	0.9650	1.0000	0.9230	0.9780
FOXP1	rs56850311	Intronic	A > T	Modifier	0.0000	0.3960	0.2520	0.1060	0.2890	0.2230
FOXP1	rs7639736	Intronic	C > A	Modifier	0.0000	0.0760	0.0560	0.0730	0.0130	0.0200
FOXP1	rs939845	Intronic	A > G	Modifier	0.3984	0.1630	0.2250	0.1110	0.0640	0.0440
FOXP1	rs2037474	Intronic	A > G	Modifier	0.5156	0.2720	0.3430	0.4360	0.1360	0.2450
FOXP1	rs151011253	Intronic	T > TA	Modifier	0.0139	0.0850	0.0560	0.0310	0.0540	0.0960
SYNGAP1	rs76557362	Intronic	C > T	Modifier	0.0833	0.2530	0.0130	0.0000	0.0000	0.0000
SYNGAP1	rs453590	Intronic	C > T	Modifier	0.0000	0.2700	0.4060	0.6410	0.3860	0.5430
SYNGAP1	rs115441992	Intronic	C > T	Modifier	0.0833	0.0140	0.0090	0.0000	0.0130	0.0020
SYNGAP1	rs9394145	Intronic	C > T	Modifier	0.5078	0.0130	0.3240	0.2500	0.3150	0.3220

IND. Indigenous; AFR. African; AMR. American, EAS. East Asia; EUR. European; SAS. South Asia; SAS. CDS. coding sequence.

Table 2. Comparison between the allelic frequency of the Indigenous population and continental populations (African, American, East Asia, European, and South Asia) described in the database of 1000 Genomes Project.

Gene	DbSNP	IND vs. AFR *	IND vs. AMR *	IND vs. EAS *	IND vs. EUR *	IND vs. SAS *
CHD8	rs35057134	**5.98×10^{-6}**	**2.74×10^{-5}**	**5.31×10^{-10}**	**1.39×10^{-7}**	**7.66×10^{-7}**
CHD8	rs80311097	0.24887	0.28751	0.21283	0.21318	0.21826
CHD8	rs10467770	**0.00572**	**0.03028**	**2.97×10^{-6}**	**0.00220**	**0.00138**
CHD8	rs111250264	0.30959	0.28751	0.21283	0.21318	0.21826
CHD8	rs57764234	**6.46×10^{-8}**	1.00000	**0.03481**	0.64915	0.06785
CHD8	rs111776414	**0.01566**	0.07929	**0.00504**	**0.00507**	0.05415
CHD8	rs1998332	0.50781	**0.01151**	**4.40×10^{-7}**	**3.06×10^{-8}**	**6.23×10^{-9}**
CHD8	rs149307240	**0.02173**	0.36125	**0.03481**	**0.03493**	**0.03665**
SCN2A	rs17183814	**1.19×10^{-10}**	**0.00026**	**0.02585**	**5.70×10^{-6}**	**0.04046**
SCN2A	rs75109281	**0.00339**	**0.00042**	**8.63×10^{-5}**	**8.70×10^{-5}**	**9.84×10^{-5}**
SCN2A	rs3769951	**0.00037**	**1.60×10^{-6}**	**4.39×10^{-7}**	**3.69×10^{-8}**	**2.16×10^{-9}**
SCN2A	rs28472553	0.05210	**0.00042**	**8.63×10^{-5}**	**8.70×10^{-5}**	**9.84×10^{-5}**
SCN2A	rs139906774	0.35386	**3.54×10^{-8}**	**6.13×10^{-10}**	**1.54×10^{-6}**	**0.00011**
SCN2A	rs2304014	**3.85×10^{-5}**	**0.01857**	**0.00941**	**0.00166**	**0.01459**
SCN2A	rs6432821	0.10268	1.00000	1.00000	1.00000	1.00000
SCN2A	rs150453735	**9.91×10^{-13}**	**0.00066**	**1.86×10^{-11}**	**1.90×10^{-11}**	**2.57×10^{-11}**
SCN2A	rs1867864	**0.01590**	1.00000	0.09628	0.10907	0.69027
SCN2A	rs1838846	**8.09×10^{-38}**	**3.83×10^{-35}**	**5.39×10^{-32}**	**1.00×10^{-40}**	**2.50×10^{-28}**
SCN2A	rs7593568	**5.55×10^{-38}**	**3.83×10^{-35}**	**5.39×10^{-32}**	**1.00×10^{-40}**	**3.54×10^{-28}**
FOXP1	rs1435680522	0.16887	0.28751	0.21283	0.21318	0.21826
FOXP1	rs112773801	**2.15×10^{-13}**	**0.00013**	**3.53×10^{-14}**	**0.00344**	**2.18×10^{-9}**
FOXP1	rs58847217	0.07415	0.06453	**0.03481**	**0.03493**	**0.03665**
FOXP1	rs76145927	0.16887	0.39900	0.49517	0.30231	0.21827

IND. Indigenous; AFR. African; AMR. American, EAS. East Asia; EUR. European; SAS. South Asia; SAS. CDS. coding sequence. *. Fisher's exact test.

The EAS population showed six statistically different variants in the *CHD8* gene (rs35057134, rs10467770, rs57764234, rs111776414, rs1998332, and rs149307240), nine in the *SCN2A* gene (rs17183814, rs75109281 rs3769951, rs28472553, rs139906774, rs2304014, rs150453735, rs1838846, and rs7593568), five in the *FOXP1* gene (rs112773801, rs58847217, rs72960080, rs56850311, and rs939845), and four in the *SYNGAP1* gene (rs76557362, rs453590, rs115441992, and rs9394145), summing twenty-four polymorphisms.

The rs35057134 (*CHD8*) had a low frequency in the Amerindian group, with differences greater than 20% of that found in the world population, as well as the rs10467770 (*CHD8*), rs3769951 (*SCN2A*), rs2304014 (*SCN2A*), and rs112773801 (*FOXP1*). Otherwise, the rs17183814 (*SCN2A*) variant presented higher frequencies in the Amerindian population, in contrast to those found in the world populations, except for EAS. This frequency pattern is also observed in the rs9394145 (*FOXP1*), which shows higher frequencies in Amazonian Amerindians.

Multidimensional scale analysis (MDS), using FST values (Supplementary Table S2) for the 35 variants in the *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* genes revealed the existence of four major groups (Figure 1): The African population (AFR) is completely isolated, showing greater genetic diversity, as well as the American population (AMR); European (EUR), East Asian (EAS), and South Asian (SAS) populations clustered in the lower center; and the Indigenous group (IND) in the lower left corner. This analysis reported that the Amazonian population distances itself from other world populations concerning the variants analyzed for ASD susceptibility. The populations diverged significantly from African populations and showed greater proximity with populations from South and East Asia, compared to populations of European and, even, Latin American peoples.

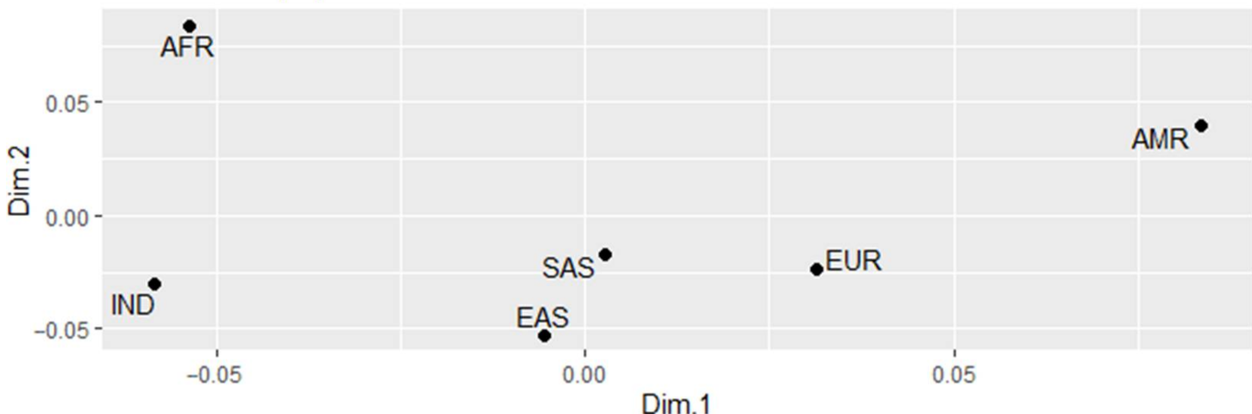

Figure 1. Multidimensional scale graph illustrating the ethnic populations grouping according to the genetic profile of the 35 variants in the *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* genes.

4. Discussion

Previous evidence suggests ASD is modulated by genetic factors, such as SNPs. However, it is unclear which genes or SNPs contribute significantly to autism. A large genetic sequencing report showed 102 genes associated with the risk of autism [10]. In this study, we selected four genes from this previous study. We identified and characterized candidate SNPs in these selected genes associated with ASD, which have not been studied in Amazonian Amerindians. We also compared these data with worldwide populations. We hypothesize that SNPs in *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* genes could predispose an individual to ASD, especially those with a greater contribution of Amerindian ancestry.

The influence of ancestry difference in the autism spectrum disorder is limited. Population-based studies of the prevalence of autism spectrum disorder (ASD) in the United States have reported no differences among selected racial and ethnic groups, however without analyzing other ethnicities, such as native people [14].

There is still a lack of research investigating this issue, especially in Brazil. The Brazilian population has an admixture population characterized by a tetra-hybrid ancestry with European, African, American, and Asian composition [15]. Besides few genetic studies related to ASD in Brazil, there are no studies on this subject in Amerindians.

A previous study by Shochet et al. [16] had shown that Indigenous with ASD people living in remote areas had limited access to healthcare services. This is due to cultural and linguistic differences that are potential barriers to the diagnosis and treatment of this condition among the Amerindian population. Besides, some clinical features, such as avoiding eye contact and social communication, were not considered problematic in Amerindian cultures [17].

Current studies showed heritability of ASD was estimated to be approximately 50 to 80%, indicating that the variation in ASD occurrence in the population was mostly owing to inherited genetic influences [8,18]. Satterstrom and collaborators have identified 102 ASD risk genes in a large-scale genetic analysis to date. These genes, including *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1*, regulate the development and function of the human brain [10].

The present study is the first to investigate the *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* genes in Amazonian Amerindians and highly admixed population in the Amazon region of Brazil with a major Amerindian component. The Amerindian ancestral contribution in the Brazilian population is 17%, except in the Amazon region which increases up to approximately 30%. In this area, the Amerindian ancestry population has the highest contribution in the country [18,19].

Besides the *SCN2A*, *FOXP1*, and *SYNGAP1* genes, *CHD8* variants are among the most replicated and common findings in ASD genetic studies. They are associated with the most

common form of autism spectrum disorder, classic autism, along with macrocephaly, distinct dysmorphic facial features, and gastrointestinal disturbance [20,21]. Genetic variants in the *SCN2A* gene are also important in ASD; they can play a significant role in psychiatric disorders. They were associated with childhood seizures, epileptic encephalopathy, epileptic syndromes, as well as intellectual disability, and ASD without epilepsy [22,23].

The *FOXP1* gene has been implicated in neurodevelopmental disorders, such as ASD, and the *FOXP1* syndrome, in individuals with the presence of autistic spectrum disorder traits, intellectual disability, language impairment, and psychiatric characteristics [24,25]. In addition, the *SYNGAP1* gene is associated with several neurodevelopmental disorders, including non-syndromic intellectual disability and ASD, with symptoms that include encephalopathy, epilepsy, hypotonia, stereotyped behaviors, and aggression [23].

Of 59 variants found in the exome analysis made of the *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* genes, 35 variants could potentially be associated with the development of autistic spectrum disorder. Among the investigated variants, five of them had a moderate impact. They were all classified as CDS (coding sequence) and 30 variants had a modifier impact, 28 were intronic, and 2 were in the 3′UTR region.

In the present study, we compared the genetic variability of Amerindian populations from the Amazon region with five populations from the 1000 Genomes Project [11]. Our results about the comparison between ethnic groups revealed that the AFR group were isolated, with the greatest genetic difference from the AMR. This finding is consistent with the history of human populations in the world, in which the Amerindian and African groups represent the extremes of the evolutionary process [26].

Still, regarding the comparative results between ethnic groups, the lowest values of genetic differences with Amazonian Amerindians were observed in the population of East Asia (FST value = 0.00219). This result corroborates the hypothesis of the "Bering Strait", an extension of land that joined Northeast Asia and North America [27].

The distancing of the IND and AMR groups was not expected (FST value = 0.07114); however, this analysis was only evaluated in the variants found for the investigated genes. The sample of the American population of the 1000 Genomes Project includes several countries in Latin America, such as Mexico, Peru, Colombia, and Puerto Rico, countries with Amerindian ancestral contributions that are heterogeneous among them, due to the different historical aspects of their formations and their degree of genetic mixing, which can explain the distance we found [11,28–30].

The identification of genetic variants associated with autism in the Amerindian population may favor the development of specific screening and diagnosis tools for this population, as well as for the Brazilian population and admixed populations, which have an important contribution of Amerindian ancestry in their constitution.

5. Conclusions

Our study was the first to investigate genes associated with autism in the Amazonian Amerindian, an understudied population that has a unique genetic profile. Our findings identify and characterized ASD-related SNPs, which could facilitate early disease testing and diagnosis, as well as early intervention in the Amerindian population and admixture populations with high contribution of Amerindian ancestry. This study may help better understand the biological mechanisms involved in the development of autism.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes13020368/s1. Table S1: Description of all variants in the *CHD8*, *SCN2A*, *FOXP1*, and *SYNGAP1* genes found in the Amerindian individuals (IND) Table S2: Pairwise FST among Amerindians (IND) and the five continental populations from the 1000 Genomes Project.

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administration, R.M.R.B., P.P.A., J.F.G. and N.P.C.d.S.; funding acquisition, J.F.G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the guidelines of the Declaration of Helsinki, and approved by Ethics Committee of the by the National Committee for Ethics in Research (CONEP) and the Research Ethics Committee of the UFPA Tropical Medicine Center (CAAE number: 20654313.6.0000.5172).

Informed Consent Statement: All participants of the study and their ethnic group leaders signed a free-informed consent.

Data Availability Statement: The dataset used in this study is publicly available. The name of the repository and accession number(s) can be found at https://doi.org/10.6084/m9.figshare.18822272, accessed on October 2021.

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References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed.; American Psychiatric Association: Washington, DC, USA, 2013.
2. Transtorno Do Espectro Autista—OPAS/OMS | Organização Pan-Americana da Saúde. Available online: https://www.paho.org/pt/topicos/transtorno-do-espectro-autista (accessed on 1 September 2021).
3. Elsabbagh, M.; Divan, G.; Koh, Y.-J.; Kim, Y.S.; Kauchali, S.; Marcín, C.; Montiel-Nava, C.; Patel, V.; Paula, C.S.; Wang, C.; et al. Global Prevalence of Autism and Other Pervasive Developmental Disorders. *Autism Res.* **2012**, *5*, 160–179. [CrossRef] [PubMed]
4. Bailey, B.; Arciuli, J. Indigenous Australians with Autism: A Scoping Review. *Autism* **2020**, *24*, 1031–1046. [CrossRef] [PubMed]
5. Baron-Cohen, S. Leo Kanner, Hans Asperger, and the Discovery of Autism. *Lancet* **2015**, *386*, 1329–1330. [CrossRef]
6. Kanner, L. Autistic Disturbances of Affective Contact. *Nerv. Child* **1943**, *2*, 217–250.
7. Silberman, S.; Sacks, O. *NeuroTribes the Legacy of Autism and How to Think Smarter about People Who Think Differently*; Allen & Unwin: London, UK, 2015.
8. Bai, D.; Yip, B.H.K.; Windham, G.C.; Sourander, A.; Francis, R.; Yoffe, R.; Glasson, E.; Mahjani, B.; Suominen, A.; Leonard, H.; et al. Association of Genetic and Environmental Factors with Autism in a 5-Country Cohort. *JAMA Psychiatry* **2019**, *76*, 1035–1043. [CrossRef] [PubMed]
9. Sharma, S.R.; Gonda, X.; Tarazi, F.I. Autism Spectrum Disorder: Classification, Diagnosis and Therapy. *Pharmacol. Ther.* **2018**, *190*, 91–104. [CrossRef]
10. Satterstrom, F.K.; Kosmicki, J.A.; Wang, J.; Breen, M.S.; De Rubeis, S.; An, J.-Y.; Peng, M.; Collins, R.; Grove, J.; Klei, L.; et al. Large-Scale Exome Sequencing Study Implicates Both Developmental and Functional Changes in the Neurobiology of Autism. *Cell* **2020**, *180*, 568–584.e23. [CrossRef]
11. The 1000 Genomes Project Consortium. A Global Reference for Human Genetic Variation. *Nature* **2015**, *526*, 68–74. [CrossRef]
12. Sambrook, J.; Fritsch, E.F.; Maniatis, T. *Molecular Cloning. 1*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 1989.
13. Rodrigues, J.C.G.; Fernandes, M.R.; Guerreiro, J.F.; da Silva, A.L.D.C.; Ribeiro-Dos-Santos, Â.; Santos, S.; Santos, N.P.C.D. Polymorphisms of ADME-Related Genes and Their Implications for Drug Safety and Efficacy in Amazonian Amerindians. *Sci. Rep.* **2019**, *9*, 7201. [CrossRef]
14. Maenner, M.J. Prevalence of Autism Spectrum Disorder among Children Aged 8 Years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. MMWR. *Surveill. Summ.* **2020**, *69*, 1–12. [CrossRef]
15. da Silva Montenegro, E.M.; Costa, C.S.; Campos, G.; Scliar, M.; de Almeida, T.F.; Zachi, E.C.; Silva, I.M.W.; Chan, A.J.S.; Zarrei, M.; Lourenço, N.C.V.; et al. Meta-Analyses Support Previous and Novel Autism Candidate Genes: Outcomes of an Unexplored Brazilian Cohort. *Autism Res. Off. J. Int. Soc. Autism Res.* **2020**, *13*, 199–206. [CrossRef] [PubMed]

16. Shochet, I.M.; Orr, J.A.; Kelly, R.L.; Wurfl, A.M.; Saggers, B.R.; Carrington, S.B. Psychosocial Resources Developed and Trialled for Indigenous People with Autism Spectrum Disorder and Their Caregivers: A Systematic Review and Catalogue. *Int. J. Equity Health* **2020**, *19*, 1–19. [CrossRef] [PubMed]
17. Sandin, S.; Lichtenstein, P.; Kuja-Halkola, R.; Larsson, H.; Hultman, C.M.; Reichenberg, A. The Familial Risk of Autism. *JAMA* **2014**, *311*, 1770. [CrossRef] [PubMed]
18. Santos, N.P.C.; Ribeiro-Rodrigues, E.M.; Ribeiro-dos-Santos, Â.K.C.; Pereira, R.; Gusmão, L.; Amorim, A.; Guerreiro, J.F.; Zago, M.A.; Matte, C.; Hutz, M.H.; et al. Assessing Individual Interethnic Admixture and Population Substructure Using a 48-Insertion-Deletion (INSEL) Ancestry-Informative Marker (AIM) Panel. *Hum. Mutat.* **2010**, *31*, 184–190. [CrossRef] [PubMed]
19. Rodrigues de Moura, R.; Coelho, A.V.C.; de Queiroz Balbino, V.; Crovella, S.; Brandão, L.A.C. Meta-Analysis of Brazilian Genetic Admixture and Comparison with Other Latin America Countries. *Am. J. Hum. Biol.* **2015**, *27*, 674–680. [CrossRef] [PubMed]
20. Alotaibi, M.; Ramzan, K. A de Novo Variant of CHD8 in a Patient with Autism Spectrum Disorder. *Discoveries* **2020**, *8*, e107. [CrossRef]
21. Hoffmann, A.; Spengler, D. Chromatin Remodeler CHD8 in Autism and Brain Development. *J. Clin. Med.* **2021**, *10*, 366. [CrossRef]
22. Tavassoli, T.; Kolevzon, A.; Wang, A.T.; Curchack-Lichtin, J.; Halpern, D.; Schwartz, L.; Soffes, S.; Bush, L.; Grodberg, D.; Cai, G.; et al. De Novo SCN2A Splice Site Mutation in a Boy with Autism Spectrum Disorder. *BMC Med. Genet.* **2014**, *15*, 1–8. [CrossRef]
23. Varghese, M.; Keshav, N.; Jacot-Descombes, S.; Warda, T.; Wicinski, B.; Dickstein, D.L.; Harony-Nicolas, H.; De Rubeis, S.; Drapeau, E.; Buxbaum, J.D.; et al. Autism Spectrum Disorder: Neuropathology and Animal Models. *Acta Neuropathol.* **2017**, *134*, 537–566. [CrossRef]
24. Bowers, J.M.; Konopka, G. The Role of the FOXP Family of Transcription Factors in ASD. *Dis. Mark.* **2012**, *33*, 251–260. [CrossRef]
25. Siper, P.M.; De Rubeis, S.; Trelles, M.d.P.; Durkin, A.; Di Marino, D.; Muratet, F.; Frank, Y.; Lozano, R.; Eichler, E.E.; Kelly, M.; et al. Prospective Investigation of FOXP1 Syndrome. *Mol. Autism* **2017**, *8*, 1–16. [CrossRef] [PubMed]
26. Skoglund, P.; Reich, D. A Genomic View of the Peopling of the Americas. *Curr. Opin. Genet. Dev.* **2016**, *41*, 27–35. [CrossRef] [PubMed]
27. Hoffecker, J.F.; Elias, S.A.; O'Rourke, D.H.; Scott, G.R.; Bigelow, N.H. Beringia and the Global Dispersal of Modern Humans. *Evol. Anthropol. Issues News Rev.* **2016**, *25*, 64–78. [CrossRef] [PubMed]
28. Reich, D.; Patterson, N.; Campbell, D.; Tandon, A.; Mazieres, S.; Ray, N.; Parra, M.V.; Rojas, W.; Duque, C.; Mesa, N.; et al. Reconstructing Native American Population History. *Nature* **2012**, *488*, 370–374. [CrossRef]
29. Gravel, S.; Zakharia, F.; Moreno-Estrada, A.; Byrnes, J.K.; Muzzio, M.; Rodriguez-Flores, J.L.; Kenny, E.E.; Gignoux, C.R.; Maples, B.K.; Guiblet, W.; et al. Reconstructing Native American Migrations from Whole-Genome and Whole-Exome Data. *PLoS Genet.* **2013**, *9*, e1004023. [CrossRef]
30. Rodrigues, J.C.G.; Souza, T.P.D.; Pastana, L.F.; Ribeiro Dos Santos, A.M.; Fernandes, M.R.; Pinto, P.; Wanderley, A.V.; Souza, S.J.D.; Kroll, J.E.; Pereira, A.L.; et al. Identification of NUDT15 Gene Variants in Amazonian Amerindians and Admixed Individuals from Northern Brazil. *PLoS ONE* **2020**, *15*, e0231651. [CrossRef]

e20538 **Publication Only**

Association between TP53, PAR1 and CCR5 gene polymorphisms and non-small cell lung cancer susceptibility in the Amazon.

Esdras Edgar Batista Pereira, Williams Fernandes Barra, Marianne Rodrigues Fernades, Juliana Carla Gomes Rodrigues, Antônio André Conde Modesto, Bruno Melo Fernandes, Lucas Favacho Pastana, Rommel Mario Rodriguez Burbano, Paulo Pimentel Assumpção, João Farias Guerreiro, Sidney Emanuel Batista dos Santos, Ney Pereira Carneiro dos Santos; Laboratório de Genética Humana e Médica–Universidade Federal do Pará, Belém, Pará, Brazil; Universidade Federal do Para - Nucleo de Pesquisas em Oncologia, Belem, Brazil; Núcleo De Pesquisas Em Oncologia - Universidade Federal Do Pará, Belém, Pará, Brazil

Background: Lung cancer is one of the most frequent neoplasms in the world, representing 11.4% of all registered cancers, and is responsible for 18% of cancer deaths. It is divided into two categories, small cell lung cancer, responsible for 15% of cases; and non-small cell lung cancer (NSCLC), which represents 85% of cases. The most well-known risk factor for the development of lung cancer is smoking, due to substances contained in tobacco associated with inflammation and carcinogenesis. Pulmonary carcinogenesis is a complex and gradual process, with synergistic and complex interactions between environmental risk factors and individual genetic susceptibility. The aim of this study was to investigate possible associations between *TP53*, *PAR1*, and *CCR5* gene polymorphism for susceptibility to NSCLC in the Amazon. **Methods:** This is a pilot, case-control study, which included 263 subjects, 67 patients with NSCLC and 196 healthy subjects. The samples were analyzed for *TP53* (rs17880560), *PAR1* (rs11267092), and *CCR5* (rs333) gene polymorphism, genotyped in PCR, followed by fragment analysis. To avoid misinterpretation due to population substructure, we applied a previously developed set of 61 informative ancestral markers that were genotyped by multiplex PCR. We used logistic regression to identify differences in genotypic frequencies between individuals with and without lung cancer. **Results:** We observed that some genotypes were associated with protection for NSCLC: *TP53* gene LED/DEL genotype (p = 0.041, OR: 0.510, 95%CI: 0.267-0.974); DEL/DEL genotype of the *PAR1* gene (p = 0.023, OR: 0.471, 95%CI: 0.247-0.971); and also the INS/INS genotype of the *CCR5* gene (p = 0.033, OR: 0.331, 95%CI: 0.120-0.917). **Conclusions:** *TP53* (rs17880560), *PAR1* (rs11267092) and *CCR5* (rs333) gene variants were significantly associated with NSCLC in patients from the Amazon. The validation of these findings may favor, in the future, the screening of individuals, facilitating the institution of preventive measures personalized, early diagnosis, consequently reducing the cost for health services and mortality from this malignant neoplasm. Keywords: Genetic Polymorphism; TP53; PAR1; CCR5; Biomarker; Non-Small Cell Lung Cancer. Research Sponsor: UFPA.

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e15082 **Publication Only**

Identification of pharmacogenomic variants associated with oncology treatments in Brazilian Amazonian Amerindians.

Juliana Carla Gomes Rodrigues, Marianne Rodrigues Fernades, Williams Fernandes Barra, Esdras Edgar Batista Pereira, André Ribeiro-dos-Santos, Paulo Pimentel Assumpção, João Farias Guerreiro, Andrea Ribeiro-dos-Santos, Sidney Emanuel Batista dos Santos, Ney Pereira Carneiro dos Santos; Núcleo De Pesquisas Em Oncologia - Universidade Federal Do Pará, Belém, Pará, Brazil; Universidade Federal do Para - Nucleo de Pesquisas em Oncologia, Belem, Brazil; Laboratório de Genética Humana e Médica–Universidade Federal do Pará, Belém, Pará, Brazil; Universidade Federal do Pará - Núcleo de Pesquisas em Oncologia, Belém, Brazil

Background: Adverse drug reactions are an important cause of morbidity and mortality commonly found in different therapeutic regimens, particularly in oncologic. About 20-30% of these reactions are due to the individual genetic variability of patients. Pharmacogenomics focuses on the identification of genetic variants that influence drug efficacy, response, and/or toxicity by changes in pharmacokinetics/ pharmacodynamics. Pharmacogenomic investigations are known to have a population bias. There is a gap in the accumulated about pharmacogenomic variants in poorly studied populations, such as the Amerindian population from the Brazilian Amazon region. The objective of this work is to describe the pharmacogenetic variability regarding 160 pharmacogenes involved in pharmacokinetic processes and biological pathways of different therapies, including oncology treatments, based on data obtained through complete exome sequencing of 64 individuals from different Amerindian groups of the Brazilian Amazon. **Methods:** The present study was approved by the National Committee for Ethics in Research and the Research Ethics Committee of the UFPA Tropical Medicine Center, under CAAE number 20654313.6.0000.5172. All participants signed a free-informed consent. The DNA extraction was performed using phenol-chloroform. Libraries were prepared using the Nextera Rapid Capture Exome and SureSelect Human All Exon V6 kits. Bioinformatic analysis was performed by ViVa software. **Results:** Our results show a total of 3,311 variants found in the study subjects. Of this, 167 are exclusive variants found in the Amerindians. Among these new variants, we found a non-synonymous coding variant in the *DPYD* gene with an allelic frequency of 3%, and variants with high allelic frequencies in intronic regions, which may regulate gene expression: *MTHFR* (5.56%), *TYMS* (11.5%) *GSTT1* (54.1%), and three variants in the *CYP2D6* gene with frequencies of 54%, 62.3%, and 65.6%. The *DPYD* is a pivotal gene involved with the efficacy of fluoropyrimidine-based treatment; the *MTHFR* and *TYMS* genes also participate in the biological pathways of these drugs, responsible for variation in response rates. The *GSTT1* is a gene associated with platinum-based treatments, such as carboplatin and cisplatin. And, finally, *CYP2D6* is one of the main pharmacogene described, involved in several drug schemes, including tamoxifen and gefitinib, used to treat breast and lung cancer, respectively. **Conclusions:** Understanding the diversity of genetic markers in Amazonian Amerindian is crucial to the implementation of pharmacogenomic-guided oncology treatment protocols, since pharmacogenomic data validated in other ancestral groups may not be fully applicable in these populations due to their unique genetic profile. Keywords: Exome, Native American populations, fluoropyrimidine, platinum-based drugs, tamoxifen, gefitinib. Research Sponsor: UFPA.

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e15532 **Publication Only**

Investigation of the *DPYD, TYMS, ENOSF1, MTHFR, CDA, CES2,* and *UGT1A1* genes in patients who developed fatal fluoropyrimidine-associated toxicity in Northern Brazil.

Marianne R. Fernandes, Danielle Feio da Costa, Williams Fernandes Barra, Juliana Carla Gomes Rodrigues, Millene Arruda Bechara Gonçalves, Raiza Martins Fontoura, Francisco Cezar Aquino de Moraes, Esdras Edgar Batista Pereira, Rommel Mario Rodriguez Burbano, Paulo Pimentel Assumpção, Ana Latorre-Pellice, Olalla Maroñas Amigo, Raquel Cruz Guerrero, Sidney Emanuel Batista dos Santos, Ángel María Carracedo Álvarez, Ney Pereira Carneiro dos Santos; HUJBB, Belem, Brazil; Núcleo De Pesquisas Em Oncologia - Universidade Federal Do Pará, Belém, Pará, Brazil; Universidade Federal do Para - Nucleo de Pesquisas em Oncologia, Belem, Brazil; Laboratório de Genética Humana e Médica–Universidade Federal do Pará, Belém, Pará, Brazil; Unit of Clinical Genetics and Functional Genomics, Department of Pharmacology-Physiology, School of Medicine, University of Zaragoza, Zaragoza, Spain; Grupo de Medicina Xenómica, Centro Nacional de Genotipado (CEGEN-PRB3-ISCIII), Universidade de Santiago de Compostela, Santiago De Compostela, Spain; Fundación Pública Galega de Medicina Xenómica-CIBERER-IDIS, Santiago De Compostela, Spain

Background: Fluoropyrimidine-based treatments are still considered challenging due to the wide variability in efficacy and toxicity rates presented by patients. This variability can be explained, partially, by individual genetic differences that contribute significantly to the response of the drug, with 30% of patients experiencing severe toxicity related to dihydropyrimidine dehydrogenase (DPD) deficiency and 5-Fluorouracil (5-FU) metabolism. **Methods:** This study analyzed the exome of the main genes involved in the fluoropyrimidine metabolism pathway (*DPYD, TYMS / ENOSF1, MTHFR, CDA, CES2,* and *UGT1A1*) in seven patient who developed fatal 5-FU toxicity diagnosed with gastric or colorectal adenocarcinoma and underwent fluoropyrimidine-based cancer treatment. The genotyping service was carried out at CEGEN-PRB3-ISCIII; it is supported by grant PT17/0019, of the PE I+D+i 2013-2016, funded by ISCIII and ERDF. **Results:** A total of 74 genetic polymorphisms were identified, 25 of which stand out due to their potential role in the safety of 5-FU administration. Eight variants are exclusive of the studied population (two found in the *DPYD* gene, two in the *MHFR*, one in the *CES2*, and three in the *UGT1A1* gene) and 17 have already been associated with fluoropyrimidine efficacy and/or toxicity events: 10 of them are located in the *DPYD* gene (rs1801159, rs2297595, rs72728438, rs17376848, rs1801160, rs1801265, rs22447512, rs5568, rs56038477, rs56276561, and rs56293913), two in the intergenic region of the *TYMS/ENOSF1* genes (rs11280056 and rs699517), two in the *MTHFR* gene (rs1801131 and rs1801133) and three in the CDA gene (rs1048977, rs2072671, and rs3215400). **Conclusions:** The current study has allowed us to obtain a global profile of genetic variants in the pharmacokinetic and pharmacodynamic pathways of fluoropyrimidines in patients with high genetic miscegenation in the northern region of Brazil who have evolved to death due to the fatal toxicity resulting from 5-FU-based therapies. Keywords: Pharmacogenomics; Exome; 5-Fluorouracil; Drug toxicity; Brazil. Research Sponsor: None.

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e19025 **Publication Only**

MUC family influence on acute lymphoblastic leukemia in Native American populations from Brazilian Amazon.

Angélica Leite de Alcântara, Lucas Favacho Pastana, Laura Patrícia Albarello Gellen, Giovana Miranda Vieira, Elizabeth Ayres Fragoso Dobbin, Thays Amâncio Silva, Esdras Edgar Batista Pereira, Williams Fernandes Barra, Juliana Carla Gomes Rodrigues, Marianne Rodrigues Fernades, Paulo Pimentel Assumpção, João Farias Guerreiro, Sidney Emanuel Batista dos Santos, Ney Pereira Carneiro dos Santos; Núcleo De Pesquisas Em Oncologia - Universidade Federal Do Pará, Belém, Pará, Brazil; Laboratório de Genética Humana e Médica–Universidade Federal do Pará, Belém, Pará, Brazil; Universidade Federal do Para - Nucleo de Pesquisas em Oncologia, Belem, Brazil

Background: The MUC family includes several genes previously associated with carcinogenesis and it is associated with solid neoplasms such as those of the gastrointestinal tract. As far as we are aware, there are no studies relating MUC genes to acute lymphoblastic leukemia (ALL), and this type of cancer is more frequent in the Amerindian population, which has been understudied. Therefore, the present work aimed to investigate the MUC family exome in Amerindian individuals from Brazilian Amazon, in a sample containing healthy Native Americans (NAM) and indigenous with ALL, comparing the frequency of polymorphisms between these two groups. **Methods:** The population was formed by 64 Amerindians from the Brazilian Amazon from 12 different ethnic groups, 5 of whom were diagnosed with ALL. We analyzed 16 genes from the MUC family (MUC1, MUC2, MUC3A, MUC4, MUC5AC, MUC5B, MUC6, MUC7, MUC12, MUC13, MUC15, MUC16, MUC17, MUC19, MUC20, MUC21) and found a total of 1858 variants. **Results:** After the quality filter, only 743 variants remained. Among them, we compared the frequency of each polymorphism in the LLA vs NAM group, which led to 77 variants with a significant difference, and, among these, we excluded those with LOW impact, resulting in 63. The 63 polymorphisms were distributed in 9 genes, concentrated especially in MUC 19 (n = 30) and MUC 3A (n = 18). Finally, 11 new variants were found in the NAM population. **Conclusions:** This is the first work with a sample of native americans with cancer, a population which is susceptible to ALL, but remains understudied. The MUC family seems to have an influence on the development of ALL in the Amerindian population, and especially MUC19 and MUC3A are shown as possible hotspots. In addition, the 11 new variants found point to the need to have their clinical impact analyzed. Keywords: MUC family; ALL; Susceptibility; Native American Populations; Brazil. Research Sponsor: UFPA.

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