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

Análise de polimorfismos em genes relacionados a miRNAs na hanseníase

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Orientador: Prof. Dr. Sidney Emanuel Batista dos Santos

BELÉM – PA

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Tese submetida ao Programa de Pós-Graduação em Genética e Biologia Molécular (PPGBM) da Universidade Federal do Pará (UFPA) como requisito parcial para obtenção do grau de Doutora em Genética e Biologia Molécular.

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“Do or do not, there is no try.”

(Yoda – Star Wars Episode V: The Empire Strikes Back)

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SUMÁRIO

LISTA DE ABREVIATURAS

AIM: Marcadores Informativos de Ancestralidade ou *Ancestry Informative Markers*

BB: Borderline

BL: Borderline Lepromatoso

BT: Borderline Tuberculoide

DNA: Ácido Desoxirribonucleico

EMT: Transição Epitélio Mesenquimal

GIF 0: Grau de Incapacidade Física tipo 0

GIF I: Grau de Incapacidade Física tipo 1

GIF II: Grau de Incapacidade Física tipo 2

GIF: Grau de Incapacidade Física

IFN- γ: Interferon Gama

IL: Interleucina

LL: Polo Lepromatoso

lncRNAs: RNAs longos não codificantes ou *long non-coding RNAs*

M. leprae: *Mycobacterium leprae*

M. lepromatosis: *Mycobacterium lepromatosis*

M. tuberculosis: *Mycobacterium tuberculosis*

MB: Multibacilar

miRNA: microRNA

mRNA: RNA mensageiro

ncRNs: RNAs não codificantes ou *non-coding RNAs*

NK: Células *Natural Killer*

NLRs: Receptores do tipo *Nod-like*

OMS: Organização Mundial da Saúde

PAMPs: Padrões Moléculares Associados à Patogenos

PB: Paucibacilar

PCR: Reação em Cadeia da Polimerase

PGL-1: Glicolipídeo Fenólico 1

piRNA: *piwi-interacting RNAs*

PQT: Polioquimioterapia

Pré-miRNA: Molécula percursora do miRNA maduro

Pri-miRNA: Transcritos primários dos miRNAs

PRRs: Receptores de Reconhecimento de Padrões

qPCR: Reação em Cadeia de Polimerase em Tempo Real

RIG-1-like: Receptores Semelhantes ao Gene I induzíveis por Ácido Retinóico

RISC: *RNA-induced silence complex*

RNA: Ácido Ribonucleico

RR: Reação Reversa

SNP: Polimorfismo de Nucleotídeo Único

TF: Fator de Transcrição

TGF-β: Fator de Crescimento Tumoral Beta

TLRs: Receptores do tipo *Toll-like*

TNF-α: Fator de Necrose Tumoral Alfa

TT: Polo Tuberculoide

UF: Unidade de Federação

UTR: Região Não Codificante ou *Untranslated Region*

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RESUMO

A hanseníase é uma doença infectocontagiosa causada pelo bacilo *Mycobacterium leprae* e que apresenta um espectro contínuo de manifestações clínicas e patológicas. Para fins de tratamento, a OMS propõe a classificação dos pacientes em dois grupos: paucibacilar e multibacilar. O Brasil é o segundo país com a maior taxa de incidência de hanseníase no mundo, sendo o Pará considerado um Estado hiperendêmico. Apesar de ser uma doença intrinsicamente ligada a fatores socioeconômicos, diversas linhas de pesquisa têm evidenciado a importância da genética do hospedeiro como um fator de risco à infecção. Neste trabalho buscamos identificar biomarcadores que possam auxiliar no prognóstico da doença através da investigação de vinte e cinco SNPs em genes codificadores de miRNAs e associados à maquinária de microRNAs em pacientes diagnosticados com hanseníase paucibacilar e multibacilar, sendo o grupo controle indivíduos contactantes saudáveis sem parentesco sanguíneo com os pacientes. Por se tratar de uma população altamente miscigenada, utilizamos marcadores de ancestralidade individual para avaliar a ancestralidade genômica dos participantes. Nossos dados sugerem que o SNP em *pre-miR938* é associado à proteção contra a hanseníase paucibacilar, enquanto polimorfismos em *DROSHA*, *AGO1* e *miR570* são associados à proteção contra o desenvolvimento da hanseníase multibacilar. Em contrapartida, polimorfismos em *pri-let-7a1*, *miR200C* e *miR4513* foram associados ao risco de desenvolvimento da forma paucibacilar da doença. *pri-let-7a1* também foi associado à suscetibilidade da hanseníase *per se*. Outro polimorfismo em *DROSHA* foi associado ao risco de desenvolvimento da hanseníase multibacilar, enquanto *miR146A*, em teste entre pacientes, foi associado a proteção à hanseníase paucibacilar. Este trabalho buscou fornecer novas informações para uma melhor compreensão de como os fatores genéticos influenciam a fisiopatologia da doença, com potencial para encontrar marcadores que possam auxiliar no prognóstico da hanseníase na população amazônica. O outro trabalho presente nesta tese se trata de uma revisão de literatura acerca da importância de RNAs não codificantes em hanseníase. Este tipo de estudo ainda é escasso em hanseníase, e acreditamos que tenha um forte potencial para melhor compreensão do desenvolvimento da doença, além de novas alternativas de tratamento.

PALAVRAS-CHAVE: Hanseníase, Polimorfismos, SNPs, miRNAs, ncRNAs, Ancestralidade Genética, Amazônia Brasileira.

ABSTRACT

Leprosy is an infectious disease caused by the bacillus *Mycobacterium leprae* and presents a continuous spectrum of clinical and pathological manifestations. For treatment, the WHO proposes the classification of patients into two groups: paucibacillary and multibacillary. Brazil is the country with the second highest incidence of leprosy rate in the world, and Pará is considered a hyperendemic state. Despite being a disease intrinsically linked to socioeconomic factors, several lines of research have highlighted the importance of host genetics as a risk factor for infection. In this study, we aimed to identify biomarkers that may assist in disease prognosis by investigating twenty-five SNPs in genes encoding miRNAs and associated with the microRNA machinery in patients diagnosed with paucibacillary and multibacillary leprosy. Due to Brazil's highly admixed population, we used Individual Ancestry Markers to assess the genomic ancestry of the participants. Our data showed that *pre-miR938* is associated with protection against paucibacillary leprosy, whereas *DROSHA*, *AGO1*, and *miR570* are associated with protection against the development of multibacillary leprosy. In contrast, *pri-let-7a1*, *miR200C*, and *miR4513* were associated with the risk of developing the paucibacillary form of the disease. *pri-let-7a1* was also associated with leprosy susceptibility *per se*. Another polymorphism in *DROSHA* was associated with the risk of developing multibacillary leprosy, whereas *miR146A*, in an interpatient trial, was associated with protection from paucibacillary leprosy. With this work, we provide new information for a better understanding of how genetic factors influence the pathophysiology of the disease, with the potential to find markers that may assist in the prognosis of leprosy in the Amazonian population. Our other work is a literature review on the importance of ncRNAs in leprosy. This type of study is still scarce in this disease, and we assume that it has a strong potential to better understand the development of leprosy, as well as new-treatment alternatives.

KEYWORDS: Leprosy, Polymorphisms, SNPs, miRNAs, ncRNAs, Genetic Ancestry, Brazilian Amazon.

1 INTRODUÇÃO

1.1 Breve histórico da hanseníase

A hanseníase é frequentemente descrita como uma doença antiga em muitas culturas (Cole and Singh 2012; Han and Silva 2014). Apesar da idade, não se sabe ao certo quando a hanseníase foi inicialmente reconhecida, já que a doença era frequentemente confundida com outras doenças infecciosas e dermatológicas (Kundakci and Erdem 2019). Os mais antigos escritos que descrevem com acurácia as diferentes formas polares da hanseníase são originados da Índia e China por volta de 600 a 400 antes de Cristo (Cole and Singh 2012). Por isso, acredita-se que a doença tenha surgido nessas regiões e posteriormente migrado para o Oriente Próximo e Médio, antes de se disseminar pela Europa e as Américas através de diversos movimentos sociais como rotas comerciais, migrações e guerras (Cole and Singh 2012; Hegde et al. 2015).

Apesar do grande conhecimento popular acerca da doença, apenas no século XIX foi publicado o que seria conhecido como o primeiro trabalho científico sobre a hanseníase. Escrito pelos pesquisadores noruegueses Daniel Cornelius Danielssen e Carl-Wilhelm Boeck, o tratado denominado *Om Spedalshked* (1847) foi pioneiro em descrever detalhes clínicos e patológicos da enfermidade, além de propor uma classificação baseada em suas características clínicas dominantes, a diferenciando de doenças comuns na época, como sífilis, escorbuto e tuberculose (Bechler 2012; Desikan and Pandya 2017; Kannan 2019).

A identificação do principal agente causador da hanseníase, o bacilo *Mycobacterium leprae*, se deu algumas décadas depois, no ano de 1873, através de pesquisa realizada pelo médico norueguês Gerhard Armauer Hansen (Suzuki et al. 2012; Desikan and Pandya 2017; Donoghue 2019). Nesta época, a população leiga acreditava que a hanseníase era um castigo originado da ira divina, enquanto os estudiosos a viam como uma doença hereditária. Inclusive, Danielssen chegou ao ponto de se autoinocular com material contaminado a fim de provar que a doença não poderia ser transmitida (Bechler 2012; Hegde et al. 2015).

No Brasil, pressupõe-se que a hanseníase tenha se disseminado através dos primeiros colonizadores portugueses e africanos escravizados, sendo os primeiros casos notificados no século XVII, na cidade do Rio de Janeiro (Avelleira et al. 2014). Posteriormente, outras

localidades, como Bahia e Pará, foram identificadas como focos da doença (Eidt 2004). Ações de controle da doença foram tomadas tardiamente dois séculos depois, e se limitaram à construção de asilos e assistência precária aos pacientes (Opromolla 2000; Eidt 2004).

No ano 1912, Emilio Ribas, durante o I Congresso Sul Americano de Dermatologia e Sifiligrafia do Rio, destacou a importância da notificação compulsória e do tratamento baseado no rigor científico de pacientes hansenianos, além do denominado "isolamento humanitário" em hospitais colônias. A partir deste ano a hanseníase foi reconhecida como um problema a ser enfrentado pelas autoridades sanitárias (Monteiro 1987; Eidt 2004).

A internação compulsória de pacientes hansenianos foi abolida em 1954, porém, após décadas de segregação, afastamento de familiares e sem condições de se manterem fora dos muros das instituições de saúde, muitos indivíduos optaram por permanecer nos denominados "leprosários" (Maciel et al. 2003). Durante a década de 60 houve a modificação do nome lepra para hanseníase, a fim de afastar o preconceito e estigma relacionados à doença bíblica (Bernardes and Marques 2015). Desde a década de 80, a Organização Mundial da Saúde (OMS) passou a recomendar a polioquimioterapia (PQT) para controle e cura da doença, além de medidas como diagnóstico precoce, vigilância dos comunicantes, prevenção e tratamento das incapacidades físicas e educação relacionada a saúde (Opromolla 2000; Faria and Santos 2015). Este protocolo permanece até os dias atuais (Ministério da Saúde 2021).

Atualmente, a hanseníase continua sendo um problema de saúde pública no Brasil, perdurando como uma doença negligenciada fortemente condicionada ao contexto de vulnerabilidade social (Cabral-Miranda et al. 2014; Souza et al. 2020; Oliveira et al. 2021). Apesar da hanseníase ser considerada curável, de baixa contagiosidade e cuja a maioria da população possui defesas imunológicas naturais, ainda existem muitas lacunas a serem respondidas, sobretudo do ponto de vista da genética do hospedeiro.

1.2 A hanseníase no mundo atual

A hanseníase é uma doença infectocontagiosa, transmissível e dermatoneurológica de caráter crônico classificada como uma doença negligenciada pela OMS (Pinheiro et al. 2018; WHO 2019a; Lockwood 2019). Atualmente, sabe-se que a hanseníase pode ser causada por *Mycobacterium leprae* ou *Mycobacterium lepromatosis*, que afetam

principalmente nervos periféricos e pele (Han et al. 2008; Han et al. 2012; Bhandari et al. 2021). Embora sejam classificadas como espécies distintas devido diferenças no genoma, ambas as micobactérias são organismos intracelulares obrigatórios e possuem características similares capazes de causar a mesma doença clínica (Han et al. 2008; Bhandari et al. 2021).

Alguns dos principais sintomas da hanseníase são lesões cutâneas com presença de parestesia por acometimento dos nervos periféricos (Naafs and Garbino 2012). A incapacitação permanente é consequência direta do dano neurológico causado pelo bacilo, e ocorre principalmente pela falta de tratamento adequado (Schmitz et al. 2019). As complicações secundárias decorrentes da hanseníase podem causar deformidade, o que estigmatiza socialmente os indivíduos afetados pela doença (Suzuki et al. 2012; Nath et al. 2015).

Baseando-se nos quadros gerados pela doença, a OMS utiliza uma classificação baseada no grau de incapacidade (GIF): grau tipo 0 (GIF 0) quando a força muscular e a sensibilidade do paciente estão preservadas; grau tipo 1 (GIF I) quando há diminuição da força muscular e/ou diminuição de sensibilidade; e grau tipo 2 (GIF II) em casos de pacientes com deformidade visível nas mãos, pés e/ou olhos (Brandsma and Van Brakel 2003; Ministério da Saúde 2017).

Atualmente, a hanseníase é considerada uma doença rara em países industrializados, permanecendo como um problema de saúde pública em países em desenvolvimento (WHO 2022a). Assim, a OMS, visando o controle e erradicação da doença no mundo, possui a Estratégia Global para Hanseníase 2021-2030 (WHO 2021a). Essa estratégia se baseia nos esforços para a interrupção da transmissão da doença e tentativa de eliminação de casos, almejando o conceito de zero hanseníase: zero infecção e doença, zero incapacidade, zero estigma e discriminação (WHO 2021a). Entretanto, apesar das campanhas para a redução da prevalência mundial da Hanseníase realizadas pela OMS, novos casos continuam a ocorrer anualmente e pesquisadores afirmam que os números oficialmente relatados são subestimados e não condizem com a realidade (Cabral-Miranda et al. 2014; Salgado et al. 2018a; Oliveira et al. 2021).

1.3 Epidemiologia

A hanseníase é uma doença que causa impactos sociais e econômicos significativos em diversos países, principalmente países em desenvolvimento (Joshi

2017). A enfermidade é capaz de atingir indivíduos de ambos os sexos e todas as idades, podendo causar deformidades e incapacidades físicas muitas vezes irreversíveis (Secretária de Vigilância em Saúde 2022). De acordo com dados oficiais de 139 países das 6 regiões da OMS, durante o ano de 2020, foram detectados 127.396 novos casos de hanseníase ao redor do mundo (WHO 2022a). Dentre estes países, Índia, Brasil e Indonésia reportaram mais de 10 mil novos casos, o que equivale a aproximadamente 74% dos casos totais da doença (WHO 2022a). A distribuição dos novos casos de hanseníase reportados ao redor do globo em 2020 é apresentada na Figura 1.

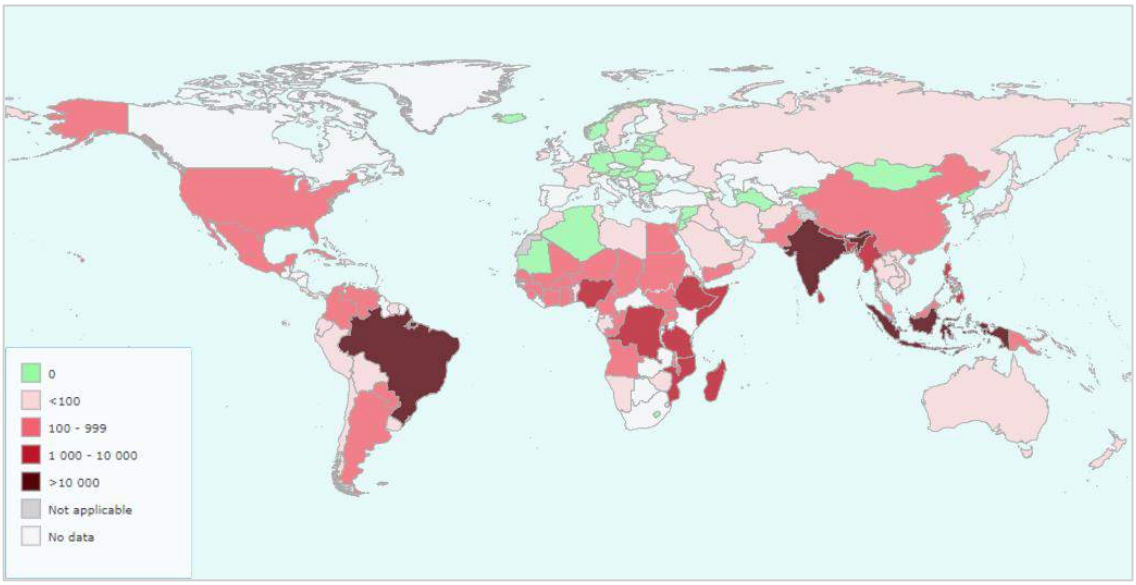

Figura 1. Distribuição geográfica de novos casos de hanseníase no ano de 2020. Fonte: WHO, 2022.

No decorrer do ano de 2020, 8.629 novos casos foram diagnosticados em menores de 15 anos de idade, sendo 878 no Brasil (Secretária de Vigilância em Saúde 2022; WHO 2022a). Em relação ao Grau de Incapacidade Física (GIF), um total de 7.198 novos casos foram diagnosticados com GIF II, sendo Índia e Brasil os únicos países a identificarem mais de 1.000 novos casos com GIF II no momento do diagnóstico (WHO 2022b). Casos novos diagnosticados com GIF II revelam diagnóstico tardio da população, por motivo de maior grau de comprometimento físico provocado pelo patógeno.

No ranking geral da OMS, o Brasil é o segundo país com maior número de novos casos detectados de hanseníase, totalizando 17.979 novos casos no ano de 2020, o que equivale a uma taxa de detecção de 8,46 a cada 100 mil habitantes (Secretária de Vigilância

em Saúde 2022; WHO 2022a). Entre os anos de 2011 a 2020, foram diagnosticados 284.723 novos casos de hanseníase no país, sendo as regiões Centro-Oeste e Norte as áreas mais afetadas no Brasil (Figura 2). É importante salientar que o parâmetro de endemicidade do país mudou de alto para médio nos últimos anos, uma vez que todas as regiões apresentaram redução na taxa de detecção geral da hanseníase (Secretária de Vigilância em Saúde 2022).

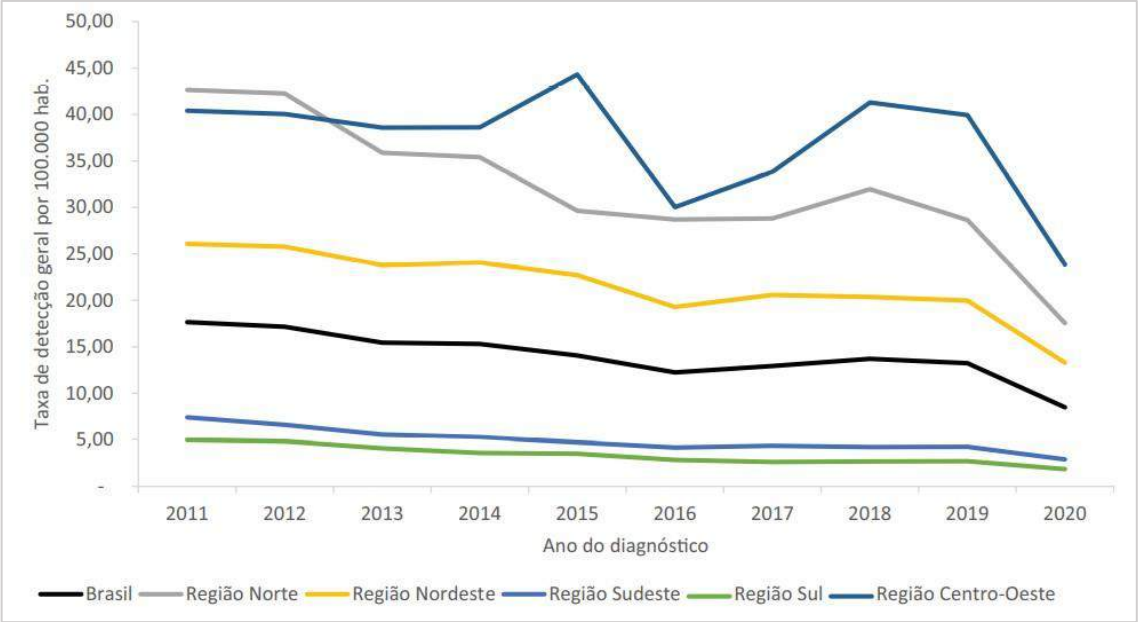

Figura 2. Taxa de detecção geral de novos casos de hanseníase por 100.000 habitantes de acordo com a região. Brasil, 2011 a 2020. Fonte: Sinan/SVS/MS, 2020.

Todavia, o ano de 2020 apresentou uma grande redução na taxa de detecção geral da hanseníase que pode estar relacionada ao menor número de diagnósticos devido sobrecarga dos serviços de saúde e restrições causadas pela pandemia de COVID-19 (Secretária de Vigilância em Saúde 2022). De acordo com a OMS, durante o início da pandemia, onde o mundo se encontrou em estado de emergência, houve interrupção de programas relacionados à hanseníase, levando a uma redução na detecção de novos casos de 37% em 2020 em comparação a 2019 (WHO 2022b).

Na região Norte, o Estado do Pará teve uma taxa de detecção de 9 casos a cada 100.000 habitantes no ano de 2020, sendo o terceiro estado desta região com maior número de casos diagnosticados (Figura 3). Entretanto, esses números são considerados subestimados e estima-se que em áreas altamente endêmicas, como a região amazônica brasileira, a prevalência da hanseníase previamente não diagnosticada na população geral é

em média 10% mais alta do que o número de registrados no país (Barreto et al. 2012; Salgado et al. 2018a; Oliveira et al. 2021).

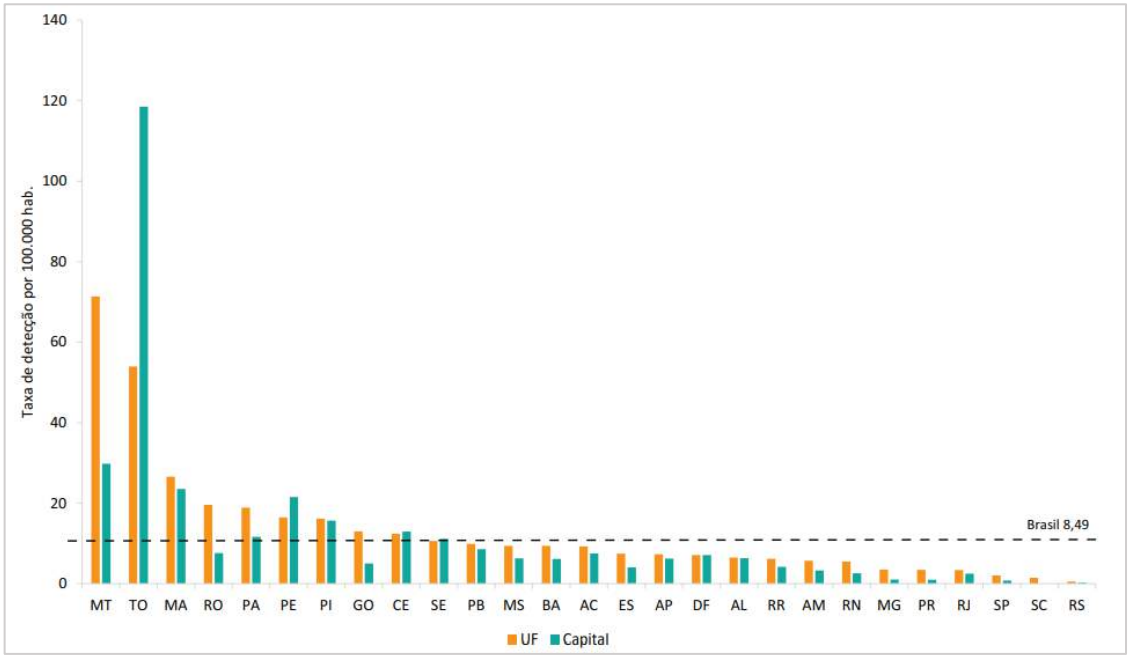

Figura 3. Taxa de detecção geral de novos casos de hanseníase por 100.000 habitantes de acordo com UF e capital de residência. Brasil, 2020. Fonte: Sinan/SVS/MS, 2020.

1.4 Características clínicas e classificação da hanseníase

A hanseníase apresenta um espectro contínuo de manifestações clínicas e patológicas que podem variar de uma única mancha hipopigmentada a uma ampla envolvendo pele e sistema nervoso periférico e deformidades nos olhos, ossos, músculos e outros tecidos. O bacilo possui preferência pelas partes mais frias do corpo, enquanto partes quentes do corpo, como axilas, virilhas, parte interna das coxas, períneo e couro cabeludo geralmente não são acometidos pela doença e são conhecidos como “zonas imune” (Rajashekar et al. 2009; Nunzi et al. 2022). Estas diferenças em sinais clínicos são resultado da resposta imune do hospedeiro (Rodrigues Júnior et al. 2016; Kundakci and Erdem 2019). Após o diagnóstico, a classificação adequada é de fundamental importância para o tipo de tratamento (Parkash 2009; Ministério da Saúde 2017).

Devido ao seu amplo espectro de manifestações clínicas, a categorização da hanseníase é bastante complexa, existindo diversos diferentes tipos de classificações (Scollard 2004; Maymone et al. 2020). Em 1966, Ridley e Jopling criaram uma classificação

que ainda é considerada essencial para a padronização da pesquisa em hanseníase (Ridley and Jopling 1966). A classificação de Ridley e Jopling utiliza critérios clínicos, patológicos, baciloscópicos e imunológicos que fornecem a base para as várias formas da doença. Nesta classificação são incluídas as formas tuberculoide (TT), tuberculoide borderline (BT), borderline (BB), borderline lepromatoso (BL) e lepromatoso (LL) (Figura 4) (Ridley and Jopling 1966; WHO 2012; Alemu Belachew and Naafs 2019a).

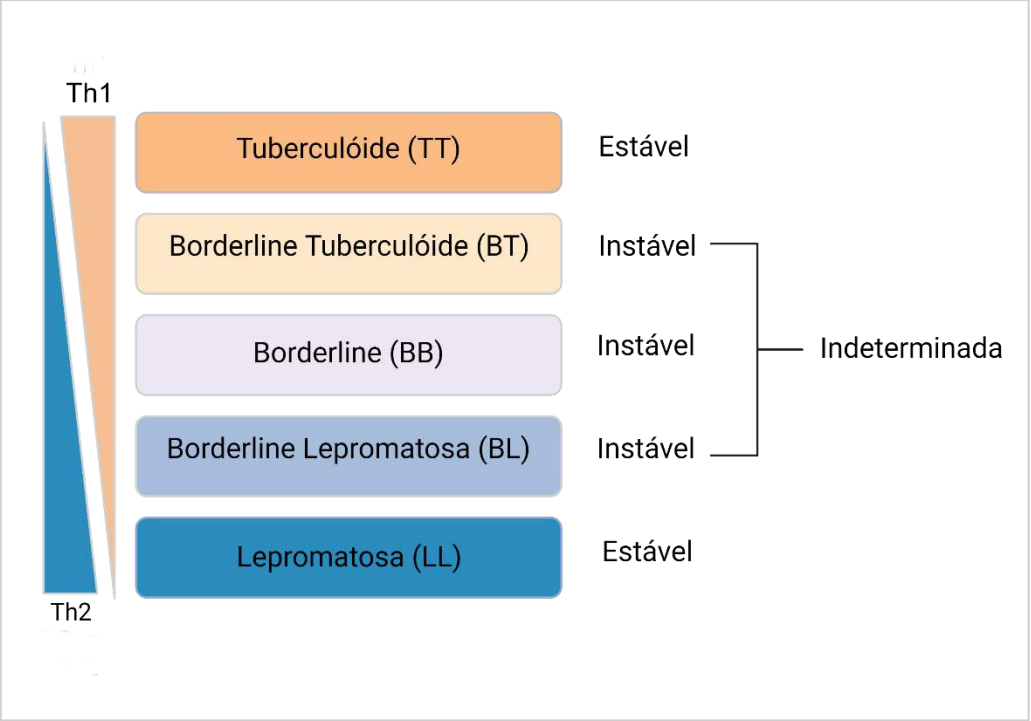

Figura 4. Espectro da hanseníase de acordo com a classificação de Ridley e Joplin. Criado com BioRender.com.

A hanseníase TT é definida pela intensa resposta imune celular do tipo Th1, que induz a produção de IFN-γ e IL-2, que leva a uma forte resposta ao bacilo, contendo a infecção em granulomas. Em lesões TT, macrófagos são ativados e células T CD4+ são o tipo celular predominante. O tipo de resposta imune Th1 é eficiente no combate à infecção por *M. leprae*, consequentemente melhorando o prognóstico do paciente (Misch et al. 2010; Inkeles et al. 2016). A forma TT afeta principalmente os nervos periféricos e pele. Lesões de pele são poucas em número ou solitárias e possuem distribuição assimétrica. Uma lesão tuberculoide típica é um eritema acobreado ou placas levemente arroxeadas com bordas infiltradas e centro hipopigmentado ou normal. A superfície é seca, sem pelos, geralmente escamosa e sem sensibilidade. Os danos nos nervos ocorrem no estágio inicial da hanseníase

TT, mas o número de nervos envolvidos é baixo com pelo menos um (Alemu Belachew and Naafs 2019a; Bhandari et al. 2021).

As formas borderline BT, BB e BL são estágios intermediários, imunológicamente instáveis, caracterizados por lesões neurocutâneas múltiplas e irregulares com aspecto de "queijo suíço" e baciloscopia positiva. Possuem também um aspecto histopatológico diversificado e redução progressiva de resposta celular de BT para as formas BB e BL (Ridley and Jopling 1966; Nath et al. 2015; Alemu Belachew and Naafs 2019a).

Pacientes BT possuem plaquetas discretas sem sensibilidade, semelhantes às dos pacientes com a forma TT. Entretanto, também possuem múltiplas lesões, geralmente mais de cinco ou menos de dez. As lesões costumam ser largas e bem definidas, frequentemente contendo pápulas satélites. Diversos nervos periféricos são assimetricamente aumentados e frequentemente resultam em neuropatia (Tyring et al. 2017; Pavão et al. 2018).

Na hanseníase BB os pacientes apresentam numerosas placas assimétricas. A lesão clássica dessa forma é a lesão dimórfica, que apresenta características de ambas as lesões tuberculoides e lepromatosas. Lesões dimórficas são tipicamente agrupadas em forma de anel (anular) com bordas externas pobremente demarcadas e centros bem definidos. Os pacientes podem apresentar hipertrofia simétrica ou assimétrica dos nervos e/ou neurite (Tyring et al. 2017).

Lesões dimórficas também podem ser encontradas em pacientes com a forma BL. Estes pacientes geralmente possuem lesões simétricas com difusão de pequenas máculas, pápulas e nódulos de diversos tamanhos e formas. A maioria das lesões possuem natureza lepromatosa, mas também há ocorrência de lesões tuberculóides. O índice bacteriano é de 4 ou 5 (Tyring et al. 2017).

O polo lepromatoso (LL) é definido principalmente por pacientes com nódulos e/ou plaquetas e ausência de resposta imune mediada por células contra o bacilo causador da hanseníase (Alemu Belachew and Naafs 2019a). Imunológicamente, a hanseníase lepromatosa é caracterizada pela resposta imune humoral do tipo Th2, que induz a expressão de células T reguladoras, interleucina-4 (IL-4), interleucina-5 (IL-5), Interleucuina-6 (IL-6), Interleucina-8 (IL-8), interleucina-10 (IL-10), formação de anticorpos e ausência de granuloma (Misch et al. 2010). As lesões lepromatosas são caracterizadas por perda de células T CD4+, numerosas células T CD8+ e macrófagos

espumosos (*foamy macrophages*) (Salgame et al. 1991). Devido a presença do bacilo na região intracelular, esse tipo de resposta torna-se incapaz de eliminar o patógeno, levando a um pior prognóstico do paciente (Sehgal 2005; Inkeles et al. 2016).

Os sinais iniciais da hanseníase LL são manchas espalhadas, simétricas, hipopigmentadas que se tornam vermelho acobreadas com beiradas indistintas. Elas são brilhantes e úmidas e caso não sejam examinadas cuidadosamente, podem ser facilmente negligenciadas. Gradualmente, as lesões de pele se espalham pelo corpo, com exceção das áreas de zona imune. Em lesões iniciais não há perda de sensação, mas a sudorese pode ser levemente prejudicada; então as lesões se tornam mais infiltradas, e pápulas, tubérculos e nódulos (lepromas) irão se desenvolver. As lepromas localizadas na face levam a uma infiltração difusa (face leonina) e se localizadas na mucosa nasal podem causar obstrução nasal, sangramento, perfuração e reabsorção da cartilagem nasal (nariz trilobado). Perfuração do palato, perda da úvula, rouquidão devido ao espessamento vocal também pode ser visto nos pacientes (Tyring et al. 2017).

Apesar da predileção de *M. leprae* por nervos periféricos e pele, o bacilo também pode ser encontrado em outros órgãos via sistema linfático e hemático (Desai et al. 1988; Naafs and Garbino 2012). A hanseníase lepromatosa pode causar cegueira devido a infiltração lepromatosa de tecidos próxima dos olhos, infiltração, e atrofia das estruturas do olho e doença inflamatória do segmento anterior (Grzybowski et al. 2015). As lesões destrutivas do osso são formadas diretamente pelo bacilo da hanseníase. A maioria dos ossos das mãos e pés são afetados. Os testículos diminuem de tamanho e ficam mais moles, resultando em atrofia testicular, ginecomastia, esterilidade e impotência (Gunawan et al. 2020; Mohta et al. 2020). A dispersão dos folículos capilares dependentes de andrógeno é interrompida. Glomerulonefrite é reportada em 2% a 23% dos pacientes (Naafs and Noto 2012). Em casos LL, infecções como pneumonia, tuberculose, tétano e insuficiência renal são as principais causas de mortalidade (Ferreira et al. 2019; Ahn et al. 2020).

Para realização de diagnóstico e classificação da hanseníase de acordo com Ridley e Joplin são necessárias instalações médicas capazes de realizar o exame histopatológico da pele e/ou biópsias dos nervos, entretanto, esses testes não estão disponíveis em localidades endêmicas com poucos recursos. Desta forma, a OMS estabeleceu uma classificação simplificada para fins de facilitação do diagnóstico e tratamento de

pacientes (Parkash 2009; Rodrigues Júnior et al. 2016). Esta classificação utiliza o espectro clínico que se estende em dois principais grupos operacionais para guia de tratamento: multibacilar (MB) e paucibacilar (PB), que são categorizados de acordo com o número de lesões.

Na classificação da OMS, pacientes com hanseníase MB possuem acima de cinco lesões, grande quantidade de bacilos *M. leprae* nos macrófagos e nervos, e produção de citocinas anti-inflamatórias, enquanto pacientes acometidos com a forma PB possuem um número de lesões abaixo de cinco, ausência de detecção de bacilo *M. leprae* na baciloscopia e produção local de citocinas inflamatórias (WHO 2019b). A tabela 1 resume os principais sintomas e apresentações clínicas da hanseníase de acordo com a classificação Ridley-Jopley e classificação da OMS.

Tabela 1. Classificação e apresentação clínica da hanseníase. Fonte: Adaptado de Mayone et al., 2020.

Imunidade mediada por células	**Variação de alto (esquerda) para baixo (direita)**				
Classificação de Ridley-Jopling	TT	BT	BB	BL	LL
Classificação OMS	Paucibacilar			Multibacilar	
Descrição da lesão	Máculas bem definidas e placas	Máculas com infiltração e placas	Lesões anulares com bordas indistintas	Lepromas e lesões anulares	Máculas com infiltração, pápulas e nódulos
Número de lesões	Única	Única ou poucas	Várias	Numerosas	Inúmeras
Distribuição	Localizada	Assimétrica	Assimétrica	Simétrica	Simétrica
Superfície da lesão	Seca/Escamosa	Seca	Levemente brilhante	Brilhante	Brilhante
Crescimento de pelo na lesão	Nenhum	Diminuído	Um pouco diminuído	Levemente diminuído	Não afetado
Sensação	Ausente	Ausente	Moderamente diminuída	Levemente diminuída	Não afetado no início; tardiamente difuso de acordo com a progressão
Carga bacilar	0/raramente 1+ (Ausência de bacilos em 100 campos examinados)	1-2+ (Presença de 1-10 bacilos em 100 campos examinados)	2-3+ (Presença de 1-10 bacilos em 10 campos examinados)	3-4+ (Presença de 1-10 bacilos em cada campo examinado)	4-6+ (Presença de 100-1000 bacilos em cada campo examinado)

1.5 Agente Etiológico

O bacilo *M. leprae*, também conhecido como bacilo de Hansen, é uma patógeno intracelular obrigatório ácido-álcool resistente pertencente ao gênero *Mycobaterium* e à família Mycobacteriaceae (Scollard et al. 2006). *M. leprae* possui forma de bastonete, medindo de 1 a 8 µm de comprimento e 0,5 µm de diâmetro, podendo ser observado em microscopia como células individuais ou como um agregado de bacilos denominados *globias* (Figura 5) (Scollard et al. 2006; Reibel et al. 2015, PHIL, 2022). A inaptidão de cultivo de *M. leprae in vitro* é uma das principais especificidades da bactéria e pode ser explicada pelo seu longo tempo de duplicação, que dura em média 14 dias (Suzuki et al. 2012; Reibel et al. 2015).

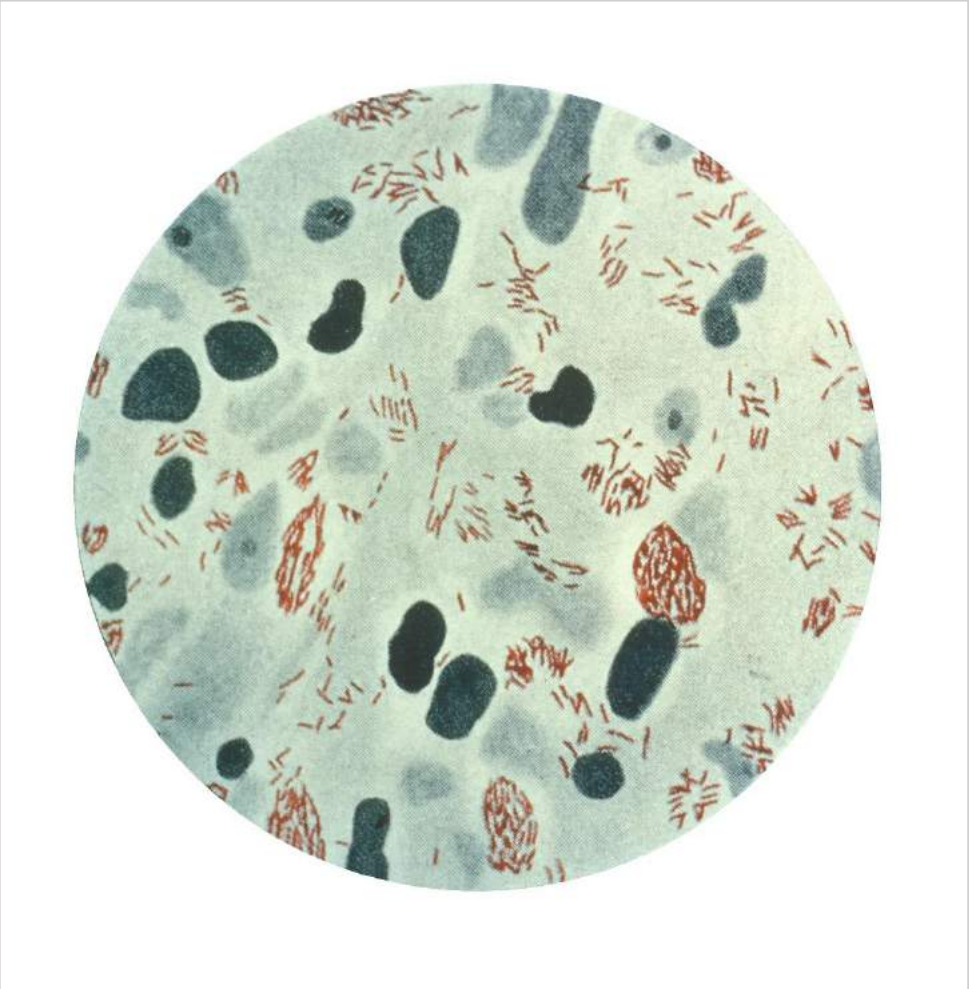

Figura 5. Fotomicrografia de um espécime derivado de uma lesão de pele em paciente com hanseníase lepromatosa revela a presença de diversos bácilos *M. leprae*. Fonte: Public Health Image Library (PHIL), 2022.

M. leprae possui um forte tropismo por células de Schwann (Scollard et al. 2006). A invasão da célula pelo bacilo inicia com a proteína de superfície celular PGL-1 se ligando ao domínio G da cadeia α_2 da laminina-2 que circunda as células de Schawnn (Rambukkana 2004; Chacha et al. 2009). Com o progresso da infecção, *M. leprae* se replica sem restrição, já que o tipo celular infectado não possui mecanismos antimicrobianos (Chavarro-Portillo et al. 2019).

Macrófagos também podem ser infectados por *M. leprae*, onde o bacilo se replica na ausência de uma resposta imune efetiva (Misch et al. 2010). A infecção das células de Schwann e a colonização do nervo pela bactéria provoca diversas alterações patológicas como degeneração dos axônios, desmielinização e fibrose. Estas mudanças induzem danos nos nervos e perda de sensibilidade, que são as características clínicas associadas à hanseníase (Rambukkana 2004; Chavarro-Portillo et al. 2019).

No ano de 2001, Cole e colaboradores publicaram o primeiro trabalho derivado do sequenciamento do genoma de *M. leprae* (Cepa TN), mostrando que menos da metade do genoma contém genes codificadores de proteínas, enquanto o restante é composto por pseudogenes e regiões não codificadoras. Além disso, o genoma de *M. leprae* contém 3.268.203 pares de bases e possui um conteúdo Guanina + Citosina menor que de outras micobactérias, como *Mycobaterium tuberculosis* (Cole et al. 2001).

Análises comparativas entre as sequências genômicas de *M. leprae* e *M. tuberculosis* demonstraram que as deleções de genes em *M. leprae* podem ter resultado na formação dos 1.116 pseudogenes não-funcionais, impactando significativamente no metabolismo da bactéria (Figura 4) (Singh and Cole 2011; Sekar 2017).

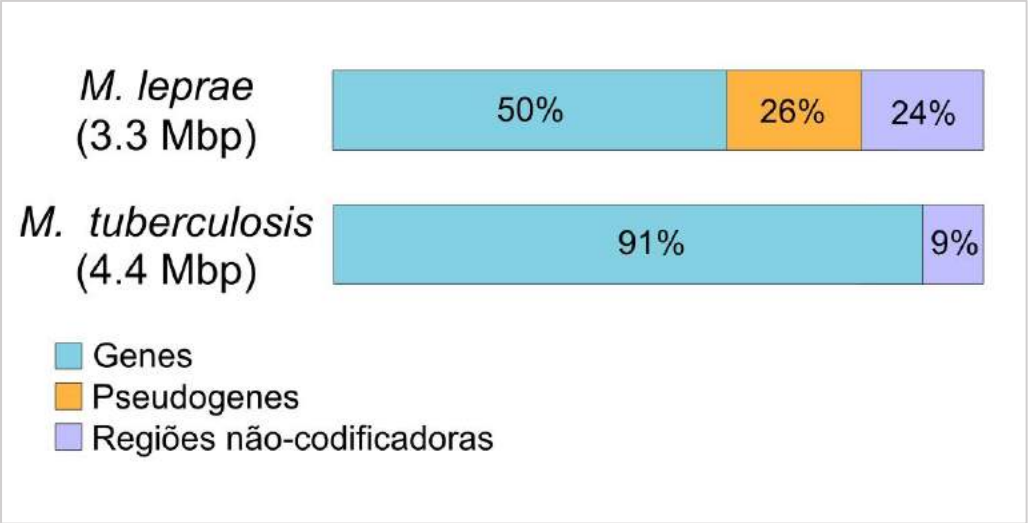

Figura 6. Comparação da porcentagem de genes funcionais, pseudogenes e regiões não-codificadoras nos genomas de *M. leprae* e *M. tuberculosis*. Fonte: Adaptado de Suzuki et al., 2012.

O principal hospedeiro de *M leprae* é o homem e sua transmissão ainda não é totalmente elucidada, porém acredita-se que a rota de transmissão mais comum seja através de contato direto ou aerossóis no contexto de exposição prolongada com indivíduos infectados não tratados, especialmente em casos de pacientes com hanseníase MB (Smith and Aerts 2014).

A transmissão da doença entre populações depende da suscetibilidade natural de indivíduos saudáveis, que é modulada pela imunidade celular e frequência de contato entre indivíduos infectados e saudáveis (Scollard et al. 2006; Fischer et al. 2011; Sarno et al. 2012; Chavarro-Portillo et al. 2019). Outros animais como tatus e algumas espécies de primatas também podem ser infectados e servirem como reservatórios da doença, transmitindo a hanseníase ao homem (Job et al. 1985; Suzuki et al. 2012; Kerr et al. 2015; da Silva et al. 2018).

1.6 Resposta Imunológica

O sistema imune é um aspecto fundamental da hanseníase, e determina a expressão clínica da doença no hospedeiro (Reibel et al. 2015). Essa característica faz com que a hanseníase seja uma doença modelo na medicina clínica para compreensão da defesa do hospedeiro contra patógenos intracelulares (Nath and Chaduvula 2017).

Para sua sobrevivência intracelular, a micobactéria precisa primeiramente escapar dos mecanismos micobactericidas intracelulares. A reposta imune pode ocorrer pela ativação de

padrões moléculares associados à patogenos (PAMPs), que são estruturas microbianas conservadas, com seus respectivos receptores de reconhecimento de padrões (PRRs), presentes na célula do hospedeiro. Após a interação de PAMPs com PRRs, a liberação de sinais intracelulares leva à indução da transcrição de genes importantes para ativação celular ou indução de fagocitose. Os PRRs mais bem descritos são os recptores de lectina-C, receptores NLRs, receptores RIG-1-like e receptores TLRs (Pinheiro et al. 2018).

Os linfócitos T com fenótipo CD4+ possuem atividades imuno-reguladoras que são mediadas por diferentes interleucinas, que geram as subpopulações Th1, Th2, Th17, Th3, Th9. A subpopulação Th1 possui um padrão de resposta mediado principalmente por IL-2, IFN- γ, TNF-α, IL-12 e IL1β, que ativam a resposta imune mediada por células. A IL-2 atua em receptores de linfócitos CD4+ e regula a produção de IL-2, IFN-γ e TNF-α, além de estimular células NK a produzirem IFN-γ, que atuam no estímulo de mecanimos relacionados a fagocitose e produção de TNF-α e IL-12 (Misch et al. 2010; Nath et al. 2015).

De maneira oposta, a subpopulação Th2 produz um padrão de resposta imune mediada por IL-4, IL-5, IL-6,IL-8 IL-10, que são capazes de suprimir a atividade do macrófago e células NK, modificando o padrão da resposta imunológica de resposta imune mediada por células para humoral (Figura 7), levando ao polo lepromatoso da hanseníase (Misch et al. 2010).

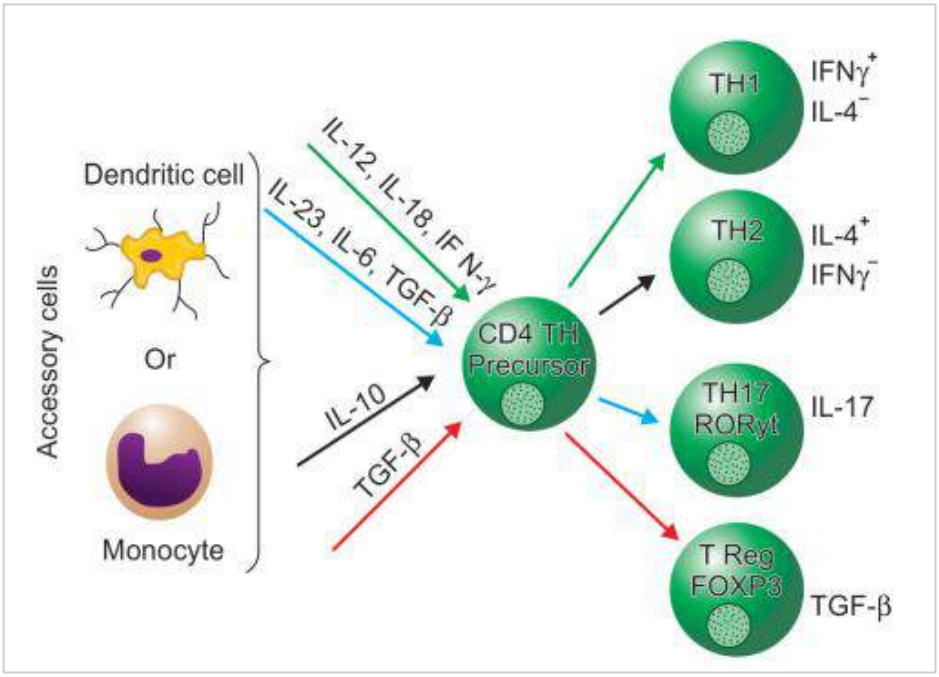

Figura 7. O desenvolvimento de fenótipos Th, células T regulatórias e citocinas relacionadas de uma célula CD4 precursora induzida por antígeno e citocinas liberadas por células

acessórias. INF: Interferon; IL: Interleucina; TGF-β: Fator de crescimento tumoral beta. Fonte: Nath and Chaduvula, 2017.

Em adição às subpopulações CD4+ mais estudadas, os subconjuntos de células regulatórias Th17 e Treg (CD25+FOXP3) foram inicialmente descobertas em modelos de autoimunidade e posteriormente estudadas em doenças infecciosas causadas por micobactérias (Torrado and Cooper 2010; Saini et al. 2013; Saini et al. 2016). As células CD4+ Th17 possuem importante função no processo inflamatório através da indução das interleucinas IL-17A, IL-17F e IL-22, que levam à inflamação e destruição do tecido. Uma maior quantidade de células CD4+ Th17+ vêm sendo associadas ao polo TT em lesões e estudos de expressão gênica utilizando sangue periferico. Esta população pode constituir a resposta imune inicial do bacilo *M. leprae*, antes da polarização entre Th1 e Th2 (Saini et al. 2013; Nath and Chaduvula 2017).

Outro mecanismo conhecido é o de células T reguladoras que representam uma subpopulação de linfócitos T caracterizados pela expressão da molécula CD25+ e do fator nuclear FOXP3, que estão envolvidos na regulação da resposta imune (Fontenot et al. 2003; Melo and Carvalho 2009). Estudos demonstraram que ocorre um aumento de células Treg FOXP3+ em pacientes com hanseníase LL, quando comparados com pacientes com hanseníase TT (Saini et al. 2013; Kumar et al. 2013). Estas células secretam TGF-β ou IL-10 que podem ser responsáveis por uma função supressora (Saini et al. 2013; Nath and Chaduvula 2017).

Uma esquematização de como as características imunológicas são relacionadas aos diversos espectros da hanseníase, de acordo com a classificação de Ridley-Jopling, é demonstrada na Figura 8.

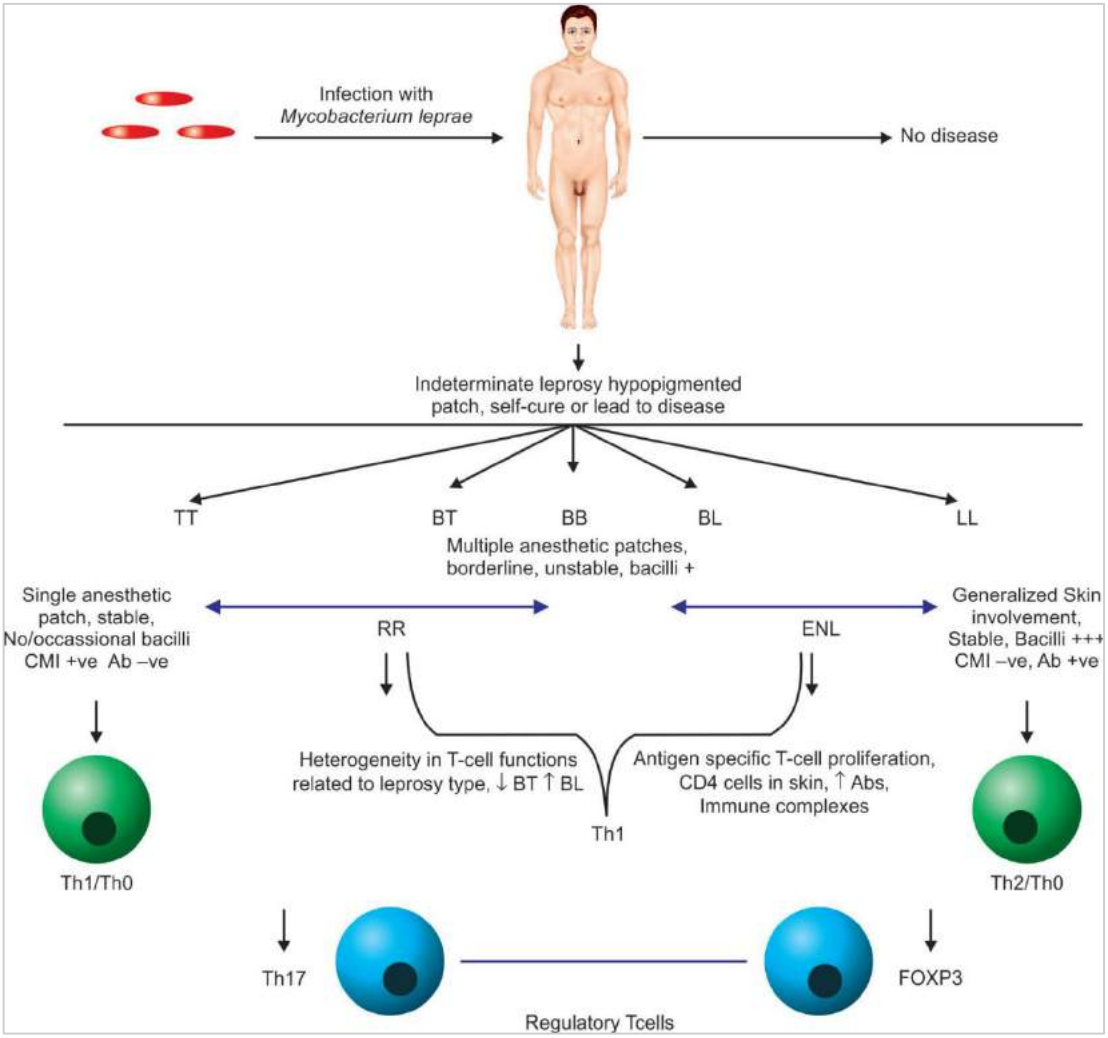

Figura 8. Características imunológicas do espectro da hanseníase de acordo com a classificação Ridley-Jopling, reações da doença e sua relação com a carga bacilar. Fonte: Nath and Chaduvula, 2017.

1.7 Aspectos genéticos relacionados à hanseníase

1.7.1 A GENÉTICA DO HOSPEDEIRO

A genética do hospedeiro desempenha um papel crucial na variabilidade da suscetibilidade de diversas doenças infectocontagiosas, dentre elas a hanseníase (Alemu Belachew and Naafs 2019b; Fava et al. 2019). A genética como um importante fator de risco à infecção da hanseníase foi incialmente evidenciada através de estudo com gêmeos realizado por Chakravartti and Vogel (1973). Desde então, diferentes abordagens em estudos genéticos têm

sido aplicadas à hanseníase (Miller et al. 2004; Mira et al. 2004; Anoosheh et al. 2011; Ali et al. 2013; Shinde et al. 2013; Pinto et al. 2015; Camargo et al. 2018; Fava et al. 2019).

Além disso, fatores genéticos do hospedeiro também estão envolvidos na diversidade de manifestações clínicas da hanseníase, que podem variar da resistência inata a *M. leprae* ao controle do tipo e extensão da resposta imune do hospedeiro à infecção (Fava et al. 2019). A interação entre a resposta imune e imunopatogenicidade do hospedeiro em resposta a *M. leprae* envolve interações complexas entre uma variedade de células expressando diferentes efetores e moléculas regulatórias (Rani 2017). Desta forma, biomarcadores podem representar o *status* do sistema imune do hospedeiro e identificar padrões de suscetibilidade para a hanseníase (Geluk 2018).

Recentemente, um novo tópico para compreensão do controle da expressão genica e interação gene-ambiente, denominado epigenética, surgiu e forneceu maiores informações a respeito da interação do hospedeiro com agentes infecciosos (Ferreira et al. 2017). A epigenética pode ser definida como o estudo de moléculas e mecanismos capazes de modificar a regulação e a expressão genética, sem alterar a sequência genômica (Cavalli and Heard 2019a). Alguns dos principais mecanismos relacionados à regulação epigenética são os RNAs não codificantes (ncRNAs) (Chuang and Jones 2007; Henikoff and Smith 2015; Cavalli and Heard 2019a; Zhang et al. 2020).

Os ncRNAs são moléculas de RNA que não codificam proteínas, como os mRNAs (Li and Liu 2019; Li 2021). Os ncRNAs possuem função estrutural, funcional e regulatória, representando cerca de 70% do genoma humano (ENCODE Project Consortium 2004; ENCODE Project Consortium 2012) . Estes RNAs podem interagir com DNA, RNA e proteínas, funcionando como silenciadores e mediadores em sítios transcricionais específicos e relacionando-se com diversos processos fisiológicos e patológicos (Syn et al. 2016; Wei et al. 2017; Zhang et al. 2020; Li 2021).

Atualmente, muitos estudos evidenciam que a desregulação na transcrição e maturação de ncRNAs podem ser associados à suscetibilidade de diversas doenças, sendo os microRNAs (miRNAs) os ncRNAs mais estudados na atualidade (Calin et al. 2004; KAZEMZADEH et al. 2015; Anastasiadou et al. 2018; Sallam Tamer et al. 2018; Yan et al. 2018; Schulte et al. 2019; Grillone et al. 2020; Sinigaglia et al. 2020).

Os miRNAs são pequenas sequências não codificadoras de 17 a 25 nucleotídeos que possuem a função de regular a expressão gênica (Felekkis et al. 2010). Estudos têm demonstrado que os miRNAs exercem um importante papel como reguladores da resposta imune inata e adaptativa no desenvolvimento de doenças micobacterianas (Mehta and Liu 2014; Cezar-de-Mello et al. 2014; Jorge et al. 2017; Agarwal et al. 2019).

A expressão e síntese de miRNAs são regulados por genes processadores de miRNA. Inicialmente, miRNAs primários (pri-miRNAs) são transcritos pela RNA polimerase II dentro do núcleo. Então, DROSHA e seu cofator DGCR8 clivam os pri-miRNAs, formando os pré-miRNAs (Bartel 2004; Bhaskaran and Mohan 2014; Ha and Kim 2014). Após transporte por XPO5, os miRNAs precursores entram no citoplasma e são processados pelo complexo DICER, tornando-se miRNAs maduros. Assim, o complexo RISC incorpora a fita de miRNA, mediando o silenciamento do gene alvo (Figura 9) (Chuang and Jones 2007; Bhaskaran and Mohan 2014; Yuan and Weidhaas 2019).

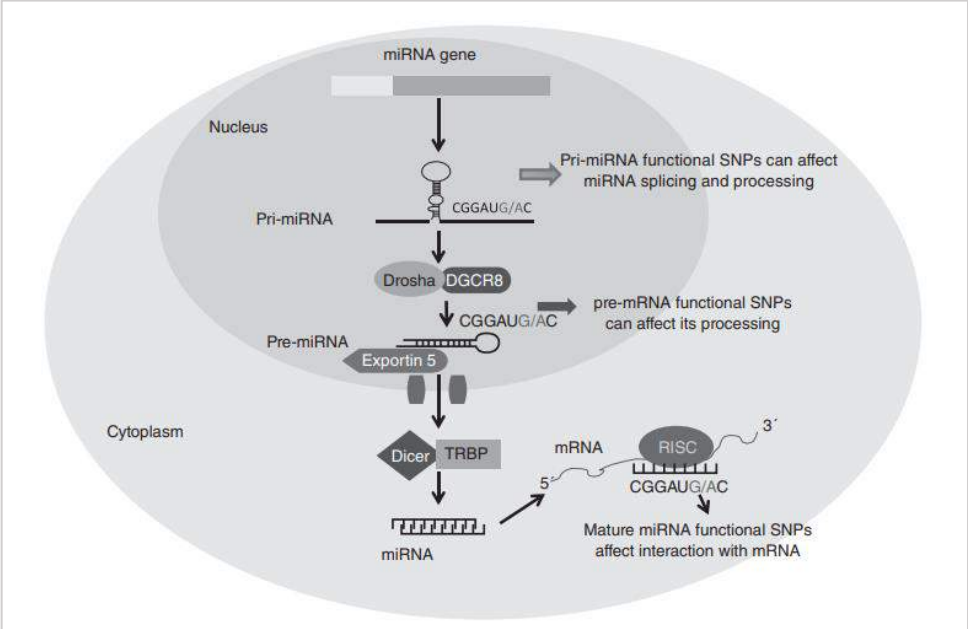

Figura 9. Via de processamento de miRNAs e efeito funcionais de SNPs em genes de miRNAs localizados em pri-miRNA, pré-miRNA e miRNA maduro. Fonte: Ramírez-Bello e Jiménez-Morales, 2017.

Polimorfismos de nucleotídeo único (SNPs) em genes processadores de miRNAs podem ser responsáveis por modificações no *splicing*, processamento, alteração em sítios de ligação a proteína, podendo causar interferências na interação miRNA-mRNA,

diferenciação da expressão gênica, estrutura e função (Figura 9) (Duan et al. 2007; Ju et al. 2015; Xu et al. 2018). Estima-se que o genoma humano contenha mais do que 38 milhões de SNPs, e que estas variantes sejam importantes contribuintes para a compreensão de diversas doenças, podendo ser utilizadas também como marcadores moléculares (Shastry 2009; Ramírez-Bello and Jiménez-Morales 2017; Chhichholiya et al. 2021). Milhares desses SNPs existem em sequências de miRNA e em suas regiões de flaqueamento, e pressupõe-se que cerca de 40% dos pré-miRNAs contenham pelo menos um SNP (Jin and Lee 2013).

Grande parte do genoma humano é composto por genes não codificantes, onde são transcritos os miRNAs e demais ncRNAs (Elkon and Agami 2017). Variantes genéticas em estrutura de pri-, pré- ou em RNAs maduros podem afetar seu processamento, atividade e função (Ramírez-Bello and Jiménez-Morales 2017). Considerando as importantes funções dos miRNAs em diversos processos fisiológicos, essas variantes podem ser consideradas como um potencial alvo de estudo em diversas doenças (Kupcinskas et al. 2014; Ferreira et al. 2017; Khanizadeh et al. 2019).

1.7.2 Ancestralidade Genética

Nos últimos anos, estudos genômicos de grande escala, como 1000 GENOMES e HapMap, têm evidenciado a existência de uma ampla gama de variação genética entre as populações humanas, assim mostrou-se que frequências alélicas variam entre populações etnicamente distintas (Morton 2008; Siva 2008; Gonzaga-Jauregui et al. 2012). Essas variações são resultantes de deriva genética, adaptação e demais fatores seletivos (Pena et al. 2011), e podem levar estudos de associação genética a resultados errôneos, principalmente quando se trata de populações miscigenadas, como a brasileira (Santos et al. 2010; Pereira et al. 2019).

A população brasileira é uma das mais heterogêneas do mundo, resultado de séculos de miscigenação entres três principais grupos continentais: europeus, africanos e nativo americanos (de Souza et al. 2019). A história da miscingenação do país tem início no século XVI, com a chegada dos europeus ao território brasileiro. Inicialmente, a miscingenação ocorreu principalmente entre homens europeus e mulheres nativas, o que contribiu para redução drástica dos povos originários devido doenças e conflitos. Os africanos foram introduzidos no país através do trafico escravo (1452-1870), vindos principalmente da

Guiné, Congo, Angola, Moçambique e Nigéria. Durante o período da colonização mais de 500.000 portugueses chegaram ao Brasil. Posteriormente outros povos europeus, como italianos, espanhóis e alemães também embarcaram no país. No século XX, também houve grande exodo de povos asiáticos e do oriente médio vindos principalmente do Japão e Síria, respectivamente (Pena et al. 2011; de Souza et al. 2019; Pena et al. 2020).

A variação no processo de colonização e ocupação das regiões brasileiras criou uma grande diversificação de mistura genética em todo o país (Pena et al. 2020). Devido a isto, diversos estudos têm avaliado as diferentes contribuições entre a ancestralidade genômica em regiões do Brasil e em diversos tipos de doenças (Benedet et al. 2012; Carvalho et al. 2015; Leal et al. 2020; Leturiondo et al. 2020).

Para fins de controle da ancestralidade genômica, estudos recomendam o uso de Marcadores Informativos de Ancestralidade (AIM), que são ferramentas moléculares que permitem a discriminação de populações parentais e identificação de subestruturação em populações miscingenadas (Enoch et al. 2006; Santos et al. 2010; Andrade et al. 2018). Estes marcadores são de grande importância em estudos de genética de populações, uma vez que detectam os efeitos da substruturação populacional em estudos de associação caso-controle (Santos et al. 2010).

Em trabalho realizado por Santos e colaboradores, um painel de 48 AIM mostrou-se capaz de identificar um alto grau de subestruturação população na região Norte e outras populações do país através da medição da proporção dos grupos parentais africano, nativo americano e europeu (Santos et al. 2010). Posteriormente, este painel foi ampliado para 61 AIM, sendo capaz de estimar com acurácia contribuições individuais e globais em populações mistas, e adicionalmente identificando a contribuição asiática (Andrade et al. 2018). Nos últimos anos este painel de marcadores de ancestralidade vem sendo aplicado com sucesso e utilizado em diversos estudos genéticos, muitos deles na região amazônica (Benedet et al. 2012; Pinto et al. 2015; Carvalho et al. 2015; Rolim et al. 2016; Lopes et al. 2017; Leal et al. 2020; Cavalcante et al. 2020; Leal et al. 2022; Porchera et al. 2022).

1.8 Justificativa

Apesar dos esforços relacionados à erradicação da doença, a hanseníase permanece um problema de saúde pública no Brasil, com uma alta taxa de incidência na região Norte. Durante o período de 2016 a 2020 foram notificados 11.890 novos casos da doença no Pará, classificando o Estado como hiperendêmico e indicando a necessidade de atenção especial à hanseníase (Secretária de Vigilância em Saúde 2022).

A transmissão da hanseníase requer um longo período de contato com pacientes e, uma vez instalada a doença, as manifestações clínicas estão intrinsicamente relacionadas a resposta imune deste novo hospedeiro. Nos anos recentes, diversas investigações têm procurado identificar biomarcadores moléculares capazes de auxiliar tanto no diagnóstico precoce da doença como na identificação dos diferentes polos de manifestação clínica dessa doença.

Neste estudo investigamos a influência de vinte e cinco SNPs, sendo vinte e uma variantes em genes de miRNAs (*miR20b/miR-17-5P*: rs3660; *miR300*: rs12894467; *miR423*: rs6505162; *mir604*: rs2368392; *pre-miR938*: rs2505901; *miR605*: rs2043556; *miR100*: rs1834306; *miR219A1*: rs213210; *miR453*: rs56103835; *miR196A2*: rs11614913; *pri-let-7a-1*: rs1073997; *miR146A*: rs2910164; *miR570*: rs4143815; *miR200B*: rs9660710; *miR26-A1*: rs7372209; *miR200C*: rs12904; *miR4513*: rs2168518; *miR219-1*: rs107822; *miR149*: rs2292832; *miR2053*: rs10505168; *miR499*: rs3746444) e quatro variantes em genes codificadores de proteínas relacionadas a maquinária de miRNAs (*AGO1*: rs636832 e *DROSHA*: rs639174, rs3805500 rs10035440).

Os vinte e cinco SNPs em genes de miRNAs deste trabalho foram selecionados com base em estudos de associação encontrados no banco de dados *National Library of Medicine* (www.ncbi.nlm.nih.gov/). Os polimorfismos e miRNAs transcritos pelos genes de miRNAs contribuem para diversos processos biológicos, como regulação de vias relacionadas a apoptose e resposta inflamatória.

Assim, este trabalho buscou compreender melhor os fatores genéticos que podem estar relacionados à resposta imune e mecanismos patofisiológicos envolvidos no desenvolvimento da hanseníase e suas formas clínicas paucibacilar e multibacilar.

2 OBJETIVOS

2.1 Objetivo Geral

O presente trabalho tem como objetivo investigar a influência de polimorfismos genéticos em genes codificadores de miRNA e relacionados à maquinaria de miRNA na suscetibilidade à hanseníase em uma população miscigenada da Amazônia Brasileira.

2.2 Objetivos Específicos:

a) investigar a ancestralidade genética em indivíduos acometidos com hanseníase e grupo controle de contactantes saudáveis;
b) avaliar se a ancestralidade pode contribuir como fator de risco para a hanseníase *per se* e suas formas clínicas paucibacilar e multibacilar;
c) aplicar um painel de genes precursores de miRNAs em amostras de pacientes com hanseníase paucibacilar e multibacilar;
d) investigar a associação dos SNPs em genes precursores de miRNAs na suscetibilidade a hanseníase paucibacilar e multibacilar.

3 CAPÍTULO I

Este capítulo é referente ao manuscrito intitulado "**Association between SNPs in microRNAs and microRNAs-machinery genes with susceptibility of leprosy in the Amazon population**" aceito para publicação na edição especial "*Cellular and Molécular Mechanisms in Mycobacterial Infection 2.0*" do periódico científico *International Journal of Molécular Sciences* (Fator de Impacto: 6.208).

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Association between SNPs in microRNAs and microRNAs-Machinery Genes with Susceptibility of Leprosy in the Amazon Population

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Abstract: Leprosy is a chronic neurodermatological disease caused by the bacillus *Mycobacterium leprae*. Recent studies show that SNPs in genes related to miRNAs have been associated with several diseases in different populations. This study aimed to evaluate the association of twenty-five SNPs in genes encoding miRNAs related to biological processes and immune response with susceptibility to leprosy and its polar forms paucibacillary and multibacillary in the Brazilian Amazon. A total of 114 leprosy patients and 71 household contacts were included in this study. Genotyping was performed using TaqMan Open Array Genotyping. Ancestry-informative markers were used to estimate individual proportions of case and control groups. The SNP rs2505901 (*pre-miR938*) was associated with protection against the development of paucibacillary leprosy, while the SNPs rs639174 (*DROSHA*), rs636832 (*AGO1*), and rs4143815 (*miR570*) were associated with protection against the development of multibacillary leprosy. In contrast, the SNPs rs10739971 (*pri-let-7a1*), rs12904 (*miR200C*), and rs2168518 (*miR4513*) are associated with the development of the paucibacillary leprosy. The rs10739971 (*pri-let-7a1*) polymorphism was associated with the development of leprosy, while rs2910164 (*miR146A*) and rs10035440 (*DROSHA*) was significantly associated with an increased risk of developing multibacillary leprosy.

Keywords: leprosy; SNPs; biomarkers; mycobacteria; host-pathogen interaction; Amazon; genetics population

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1. Introduction

Leprosy is a chronic infectious and contagious dermatoneurological disease caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis*. The bacillus *M. leprae*, the most common causative agent of leprosy, is an intracellular pathogen that has a tropism for macrophages and Schwann cells [1,2]. The skin and peripheral nerves are the main areas affected by the disease, which can lead to sensory loss and muscle atrophy in face, hands and feet, with consequent disability if left untreated.

Despite being considered eradicated in many countries, leprosy remains a major public health problem in countries such as India, Brazil, and Indonesia. In 2020, the World Health Organization (WHO) recorded 202,162 new cases of leprosy in the world, and of the 29,936 cases reported in the Americas region, the equivalent of 93% (27,864) occurred in Brazil [3]. The Pará state, located in the Brazilian Amazon region, is classified

as hyperendemic for leprosy according to the National Notifiable Diseases Information System (SINAN) in 2019 [4].

The variability of clinical manifestations of leprosy is intrinsically linked to the host's immune response against *M. leprae*. depending on the action of the cell-mediated immune response or humoral immune response [5–7]. One of the main leprosy classification systems, the Ridley–Jopling classification, is based on immunological, pathological, and clinical criteria categorized into five groups: tuberculoid (TT), borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL), and lepromatous (LL) [6]. The WHO has an operational classification that uses the number of skin and nerve lesions: paucibacillary (PB) leprosy for patients with lesions equal to or below five, and multibacillary leprosy (MB) for patients with lesions above five [8].

The development of leprosy is associated with several factors such as socioeconomic status, time of exposure to the bacillus, and genetic characteristics related to the host [9–12]. Recently, the study of miRNAs has advanced to several infectious diseases, including leprosy [13–15]. MiRNAs are small non-coding RNA molecules capable of regulating the gene expression of mRNA target molecules, having an important role in the regulation of the innate and adaptive immune response [16,17]. Single-nucleotide polymorphisms (SNPs) in genes related to miRNAs play a significant role in the generation, processing, and function of these molecules, and contribute to multiple phenotypes and diseases [18–21].

In recent years, genetic variants in miRNA genes and machinery related to miRNA processing have been associated with cancer [18,22–24] and infectious diseases [13,25–27] including mycobacterial diseases such as tuberculosis and leprosy in different populations. MiRNAs are involved in several important biological processes, such as modulation of the immune and adaptive response against pathogens [28,29]. They can target hundreds of mRNAs and regulate them, which implicates them in cell proliferation, differentiation, apoptosis, and pathways of the immune response [25,30,31]. SNPs in miRNA genes can alter the transcription of the primary miRNA transcript and the interaction of miRNA with mRNA, affecting the expression of certain genes [32].

This study investigated the association of twenty-five genetic variants in miRNAs and miRNA machinery-related genes (*DROSHA* and *AGO1*) with leprosy susceptibility in a population from the Amazon region, northern Brazil.

2. Results

2.1. Clinical and Demographic Characteristics of the Groups Studied

Clinical and demographic data such as age, sex, and genetic ancestry of the case and control groups were compared to verify possible confounding factors (Table 1). We did not observe statistically significant differences in the variables age and sex, although our data demonstrate that the mean age of patients was higher in the case group, and men were more frequent among the patient group, while women were more frequent in the control group. The population structure showed that the mean frequency of European ancestry was higher in the group of patients ($p = 0.007$), while the mean frequency of African ancestry was higher in the group of healthy individuals ($p = 0.012$) (Table 1).

Table 1 also summarizes the clinical and demographic characteristics of leprosy patients grouped according to paucibacillary (PB) and multibacillary (MB) clinical forms. We observed a significant difference between age, with a higher mean among MB patients, and gender, with a greater number of male patients in the MB group and a greater number of female patients in PB. This result corroborates with several other studies on different populations and WHO data [3,4,7,13]. Concerning the age between patients from different leprosy groups, the difference is expected due to the longer time to develop MB leprosy. When comparing the clinical forms of leprosy, we did not observe any statistical differences regarding the proportions of genetic ancestry (Table 1).

Table 1. Demographic and clinical characteristics of the sample of subjects.

Variables	Case (n = 114)	Control (n = 71)	*p*-Value
Age, years [1]	39.62 ± 17	36.92 ± 18	0.324
Sex, % male/female [2]	62 (54.4)/52 (45.6)	41 (57.7)/30 (42.3)	0.146
genetic ancestry [3]			
European	0.640 ± 0.218	0.571 ± 0.251	**0.007**
Amerindian	0.199 ± 0.161	0.203 ± 0.166	0.882
African	0.161 ± 0.153	0.226 ± 0.203	**0.012**
Variables	**PB (n = 56)**	**MB (n = 58)**	
Age, years [1]	36.34 ± 15	42.96 ± 19	**0.040**
Sex, % of male/female [2]	19 (34.0)/37 (66.0)	43 (74.2)/15 (25.8)	**0.001**
genetic ancestry [3]			
European	0.668 ± 0.190	0.652 ± 0.220	0.841
Amerindian	0.211 ± 0.170	0.181 ± 0.150	0.320
African	0.121 ± 0.130	0.167 ± 0.110	0.132

[1] Values are expressed as mean ± SE (Standard Error of Mean), Student's t-test; [2] Values are expressed as distribution percentages, chi-squared test; [3] Mann–Whitney test; The data are shown as mean ± standard deviation.

2.2. Analysis of Association of miRNA and DROSHA Precursor Genes with Leprosy Susceptibility

Twenty-five SNPs were analyzed, and twenty-one were in Hardy–Weinberg Equilibrium in the case and control groups (p-value > 0.05); therefore, they were included in the genotypic association analyses, while the markers that presented HWE deviation in one of the studied groups were excluded from further analyses.

Table 2 summarizes the significant results found in this study according to the genotypic associations between the case group of leprosy patients versus healthy control group, paucibacillary leprosy patients versus control, multibacillary leprosy patients versus control, and finally, patients grouped according to clinical form PB versus MB.

Table 2. Overview and comparison of the main results found according to the genotype associations between all the different groups studied. The values in bold show comparisons with a significant association.

Gene	Model	Case vs. Control	PB vs. Control	MB vs. Control	PB vs. MB
pre-miR938 (rs2505901)	TT vs. TC + CC	**<0.01** [1] **↓0.40 (0.20–0.81)** [2]	**<0.01** [1] **↓0.31 (0.12–0.75)** [2]	0.07 [1] 0.49 (0.22–1.08) [2]	0.18 [1] 1.90 (0.74–4.90) [2]
	TT + CT vs. CC	**0.03** [1] **↓0.41 (0.18–0.95)** [2]	**0.01** [1] **↓0.31 (0.12–0.84)** [2]	0.21 [1] 0.56 (0.22–1.42) [2]	0.07 [1] 2.56 (0.89–7.38) [2]
DROSHA (rs639174)	CC vs. CT + TT	**0.02** [1] **↓0.45 (0.23–0. 89)** [2]	0.08 [1] 0.50 (0.22–1.12) [2]	**0.03** [1] **↓0.43 (0.19–0.98)** [2]	0.73 [1] 0.85 (0.32–2.22) [2]
DROSHA (rs10035440)	TT vs. CT + CC	**0.03** [1] **↑2.04 (1.03–4.02)** [2]	0.32 [1] 1.50 (0.66–3.39) [2]	**0.01** [1] **↑2.88 (1.22–6.79)** [2]	0.63 [1] 0.80 (0.31–2.05) [2]
AGO1 (rs636832)	GG vs. GA + AA	**0.01** [1] **↓0.45 (0.23–0.88)** [2]	0.11 [1] 0.53 (0.24–1.16) [2]	**0.04** [1] **↓0.45 (0.21–0.99)** [2]	0.52 [1] 1.33 (0.55–3.18) [2]
pri-let-7a1 (rs10739971)	GG vs. GA + AA	**0.02** [1] **↑4.66 (1.17–18.6)** [2]	**<0.01** [1] **↑13.3 (1.82–90.0)** [2]	0.23 [1] 2.54 (0.53–12.3) [2]	**<0.01** [1] **↑5.21 (1.59–7.86)** [2]
	GG + GA vs. AA	**0.04** [1] **↑5.09 (0.95–7.45)** [2]	0.09 [1] 6.55 (0.53–8.90) [2]	0.13 [1] 3.95 (0.58–27.1) [2]	0.55 [1] 2.68 (0.11–6.46) [2]
miR146A (rs2910164)	GG + GC vs. CC	0.66 [1] 1.31 (0.22–2.59) [2]	0.52 [1] 0.65 (0.18–2.43) [2]	0.66 [1] 4.26 (0.47–38.5) [2]	**0.04** [1] **↓0.14 (0.01–1.36)** [2]
miR570 (rs4143815)	GG vs. GC + CC	0.73 [1] 1.14 (0.73–2.73) [2]	0.82 [1] 1.10 (0.49–2.47) [2]	**0.03** [1] **↓0.45 (0.21–0.96)** [2]	0.17 [1] 1.79 (0.77–4.17) [2]
miR200C (rs12904)	GG vs. GA + AA	**0.01** [1] **↑2.77 (1.25–6.11)** [2]	**<0.01** [1] **↑3.42 (1.36–8.57)** [2]	0.06 [1] 2.28 (0.93–5.58) [2]	0.26 [1] 1.66 (0.68–4.06) [2]
miR4513 (rs2168518)	GG + GA vs. AA	0.38 [1] 1.68 (0.53–5.34) [2]	**0.03** [1] **↑7.65 (0.83–70.5)** [2]	0.87 [1] 0.91 (0.27–3.03) [2]	0.09 [1] 5.37 (0.56–9.89) [2]

[1] p-value; [2] Odds Ratio and 95%CI; ↑ increased leprosy risk; ↓ decreased leprosy risk.

In the association analysis between leprosy patients and healthy individuals, we found six statistically significant markers for the risk of developing leprosy in the population studied: rs2505901 (*pre-mir938*), rs639174 (*DROSHA*), rs636832 (*AGO1*), rs10739971 (*pri-let -7a1*), rs12904 (*miR200C*) and rs10035440 (*DROSHA*) (Table 2). The complete results can be viewed in Supplementary Table S1.

According to the results, the SNP rs2505901 (*pre-mir938*) was associated with a decreased risk of leprosy in both the dominant ($p \leq 0.01$; OR = 0.40; 95%CI = 0.20–0.81) and recessive model ($p = 0.03$ OR = 0.41; 95%CI = 0.18–0.95), showing that the CC and CT genotype can protect against the disease. SNPs rs639174 (*DROSHA*) and rs636832 (*AGO1*) were also associated with decreased risk of leprosy in the dominant model ($p = 0.02$; OR = 0.45; 95%CI = 0.23–0.89 and $p = 0.01$; OR = 0.45; 95%CI = 0.23–0.89, respectively).

The marker rs10739971 (*pri-let-7a1*) was associated with increased risk of leprosy in analyzes using the dominant ($p = 0.02$; OR = 4.66; 95%CI = 1.17–18.6) and recessive model ($p = 0.04$; OR = 5.09;95%CI = 0.95–7.45). Likewise, SNPs rs12904 (*miR200C*) and rs10035440 (*DROSHA*) showed a positive association with the risk of disease development in the dominant model ($p = 0.01$; OR = 2.77; 95%CI = 1.25–6.11 and $p = 0.03$; OR = 2.04; 95%CI = 1.03–4.02, respectively).

Comparing leprosy patients grouped according to the paucibacillary clinical form (PB) with the control group, we found a significant association in SNPs rs2505901 (*pre-miR938*), rs10739971 (*pri-let-7a1*), rs12904 (*miR200C*) and rs2168518 (*miR4513*) (Table 2). The marker rs2505901 (*pre-miR938*) was associated with a decreased risk of PB leprosy in recessive ($p \leq 0.01$; OR = 0.31; 95%CI = 0.12–0.75) and dominant models ($p = 0.01$; OR = 0.31; 95%CI = 0.12–0.84). On the other hand, SNPs rs10739971 (*pri-let-7a1*) and rs12904 (*miR200C*) were associated with increased risk of paucibacillary leprosy in a dominant model ($p \leq 0.01$; OR = 13.3; 95%CI = 1.82–90.0 and $p \leq 0.01$; OR = 3.24; 95%CI = 1.36–8.57) and rs2168518 (*miR4513*) in recessive model ($p = 0.03$; OR = 7.68; 95%CI = 0.83–70.5) (Supplementary Table S2).

Comparing genotypes of patients grouped according to multibacillary (MB) clinical form with the control group, SNPs rs639174 (*DROSHA*), rs636832 (*AGO1*) and rs4143815 (*miR570*) were associated with a reduced risk of MB leprosy using a dominant model ($p = 0.03$; OR = 0.43; 95%CI = 0.19–0.98, $p = 0.04$; OR = 0.45; 95%CI = 0.21–0.99 and $p = 0.03$; OR = 0.45; 95%CI = 0.21–0.96, respectively) (Table 2). On the other hand, SNP rs10035440 (*DROSHA*) was associated with increased disease risk in a dominant model ($p = 0.01$; OR = 2.88; 95%CI = 1.22–6.79). The complete data can be viewed in Supplementary Table S3.

In a genotype comparison analysis between PB and MB patients, only SNP rs10739971 (*pri-let-7a1*) showed an association with increased risk for leprosy in the PB form in a dominant model ($p = 0.01$; OR = 5.21; 95%CI = 1.59–7.86) (Table 2). While the rs2910164 SNP (*miR146A*) was associated with a decreased risk of leprosy in the PB form in a recessive model ($p = 0.04$; OR = 0.14; 95%CI = 0.01–1.36) (Supplementary Table S4).

3. Discussion

It is known that the exact mode of leprosy transmission is still not well understood; however, continuous exposure may result in an increased risk of infection, and genetic factors play important roles in the host immune response against *Mycobacterium leprae* [7,9,10]. Thus, using contacts in genetic association studies in leprosy will tell us more adequately whether the polymorphisms studied may be an important factor in disease susceptibility [33]. In this study, we chose to use only household contacts with negative anti-PGL-1 and PCR as the healthy control group. In Pará State, the location in the Brazilian Amazon where the study was conducted, there is no adequate data on hot spots, and the technical data from the Brazilian Ministry of Health recognizes the entire geographical extent as a region of leprosy hyperendemicity, with an annual prevalence rate of ≥ 20 cases per 10,000 inhabitants [4]. Therefore, the population groups included in this study live in a region of high endemicity.

The Brazilian population is highly mixed in terms of the genetic contribution of different continental groups, being composed mainly of Europeans, Amerindians, and Africans.

In case–control association studies, genetic ancestry has high relevance in mixed populations, since it can influence the genotypic distribution, due to population stratification. Our data show that the contribution of different ethnic groups in the genetic composition of the Amazonian population can influence the risk of developing leprosy, with the European ethnic contribution being greater in the group of patients and the African ethnic contribution, in the opposite way, having a higher frequency in the control group. This result corroborates with a study carried out by Pinto et al., (2015), it was found that the increase in the European interethnic contribution increases the risk of developing leprosy, while the increase in the African contribution decreases the risk of developing the disease in the Amazonian population [34]. Another study, conducted with patients infected with *M. tuberculosis*, also demonstrated the great importance of genetic ancestry in the development of mycobacterial diseases among Amazonian populations [35].

Currently, there are few studies on the association of polymorphisms in miRNA genes and factors that act via miRNA machinery in leprosy and infectious diseases in general in the Amazon region. In our work, the TT/CT genotypes of the SNP rs2505901 (*pre-miR938*) were associated with protection against the development of leprosy per se and in the PB form. SNP-like variants of the *pre-miR938* gene have been associated with changes in miR938 biogenesis and stability [36]. According to genotype expression data from the GTEx portal, rs2505901 TT/CT (*pre-miR938*) has decreased expression about the CC genotype, showing that the TT/CT genotypes downregulate miR938 expression [37]. MiR938 is associated with regulatory pathways related to cell survival and apoptosis [36,38].

Furthermore, the inflammatory cytokines IL-6 and IL-17A are potential targets of miR938. IL-6 is a cytokine with a pleiotropic activity that acts in the acute inflammatory response and activation of Th17 lymphocytes, also inhibiting pro-inflammatory and immunosuppressive T cells [39]. While IL-17A is produced by CD4+ Th17 cells, it is involved in neutrophilia, inflammation, tissue destruction, and repair through the control of regulatory molecules (programmed death-1/programmed death ligand-1) and is also related to reverse reaction (RR) episodes in leprosy patients [40–42]. In a study carried out by Sadhu et al., the frequency of Th17 cells (CD4, CD45RO, IL-17) was significantly higher in BT/TT patients [43]. Santos et al., found higher concentrations of IL-17A in lesions from TT patients and serum from PB patients when compared to LL and MB patients, respectively, associating Th17 cells with the inflammatory response in PB patients [44].

The rs639174 variant (*DROSHA*) is an intronic SNP with a recognized role in transcriptional regulation, and in our study, the CC genotype of this polymorphism, in a dominant model, was associated with protection against leprosy per se and MB. Interestingly, the TT genotype of the SNP rs10035440 (*DROSHA*), which also plays an important role in the splicing and transcriptional regulation of the *DROSHA* gene [45], was associated with the risk of developing leprosy per se and MB in the dominant model. Investigating the effect of these polymorphisms on the GTEx portal, the CC genotype of rs639174 (*DROSHA*) can decrease gene expression compared to other genotypes, while the TT genotype of rs10035440 (*DROSHA*), conversely, can increase gene expression [37].

The *DROSHA* gene encodes a type III RNase and a subunit of the eponymous microprocessor complex, which catalyzes the initial processing step of pri-miRNAs, producing pre-miRNAs [46]. Previous studies have associated rs10035440 (*DROSHA*) with the high risk of developing tuberculosis in the Amazonian population [47].

The genetic variant rs636832 (*AGO1*) was associated with protection against the development of MB leprosy. This SNP is located in the intron region of the gene, so the proposed mechanism of effect for this variant is its potential influence on mRNA splicing or via the activities of intronic regulatory elements [48,49]. The *AGO1* gene encodes a member of the Argonaute family of proteins, which can associate with small RNAs and play an important role in RNA interference and silencing and transcriptional regulation of target genes. AGO1 inhibits cell proliferation by inducing apoptosis, in addition to regulating genes that influence cell cycle growth, survival, and progression [22,50,51].

Our results showed that the SNP rs10739971 (*pri-let-7a1*), in a dominant model, was shown to be associated with the development of leprosy per se and the PB form, with an association between groups of patients (PB versus MB), with increased risk to the PB form. The miRNAs let-7 family plays important roles in several biological processes, including inflammation, immunity, cell proliferation, and differentiation [52,53]. In a miRNoma expression analysis, hsa-let-7f-5p was downregulated in LP in lesions and blood of leprosy patients [54]. In another study performed by Kumar et al., the miRNA profile of macrophages infected with Mycobacterium tuberculosis showed downregulation of miR-let-7f [55]. This microRNA targets the A20 protein, an inhibitor of the NF- kB pathway, so let-7f expression decreases and A20 increases with the progression of *M. tuberculosis* infection in mice [56]. Wambier et al., report that PB leprosy patients, who present greater immune reactivity against *M. leprae*, exhibited less NF- kB activation when compared to MB patients [57].

Our data demonstrate that the rs2910164 (*miR146A*) polymorphism was associated with decreased risk of PB leprosy and consequent susceptibility to risk of MB leprosy. This genetic variant is located in the middle of a stem hairpin, suggesting that these SNPs in pre -miRNAs can alter the secondary structure conformation, and consequently alter the expression of mature miRNA [58,59]. According to a study by Shen et al., this variation from G to C in miR146A resulted in an elevated expression of the mature miRNA when compared to the common allele [59]. The SNP rs2910164 (*miR146A*) has also been associated with leprosy in a population in southeastern Brazil [60]. Regarding mycobacterial diseases, Li et al., reported that the rs2910164 variant (*miR146A*) plays different roles in two distinct ethnic populations, with the G allele increasing the risk of pulmonary tuberculosis in the Tibetan population, while the C allele increases the risk of the disease. in a Han population [61]. However, in a study carried out with the Iranian population, there was no significant association between rs2910164 (*miR146A*) and the risk of tuberculosis [62].

In our study, we suggest the rs4143815 variant (*miR570*) is associated with protection against MB leprosy and, according to the expression database (GTEx), decreases miR570 expression [36]. MiR570 was initially identified in airway epithelial cells, involved in the regulation of the inflammatory response [63]. In a study carried out by Roff et al., it was observed that miR570 can increase the expression of CCL4 and CCL5 and, at the same time, inhibit the expression of CCL2 after a strong inflammatory stimulus, indicating a complex system of direct and indirect regulation [64]. Chemokines play an important role in granuloma formation in diseases caused by mycobacteria. CCL2 is a chemokine capable of recruiting monocytes, memory T cells, and dendritic cells to sites of tissue injury and infection, suggesting maintenance of granuloma integrity in asymptomatic patients. Several studies report that the varying levels of chemokines such as CCL2 can influence the predisposition and severity of leprosy [33].

The rs12904 variant (*miR200C*), in a dominant model, was associated with leprosy per se and PB form. The miR200c gene encodes a member of the miR200 family, which is known to play an important role in epithelial–mesenchymal transition (EMT) [65–67]. EMT is a biological process responsible for causing polarized epithelial cells that interact with the basement membrane to lose their intracellular adhesion and acquire a mesenchymal cell phenotype, increasing the capacity for migration, invasion, resistance to apoptosis, and inducing fibrosis [68–70]. According to a study by Salgado et al., miRNAs ZEB1/2, which are transcriptional repressors of the miR200 family, were downregulated in LL patients. SOX miRNAs were upregulated in LP, indicating that ZEB1/2 may be a regulator of SOX2 expression [54].

The rs2168518 polymorphism (*miR4513*), in a recessive model, is associated with the development of the PB form of leprosy. miR-4513 is related to cell proliferation, invasion, and EMT and was recently reported to be overexpressed in cancer cell lines [71–74]. According to work carried out by Xu et al., downregulation of miR-4513 inhibited cell proliferation, migration, and invasion, and at the same time promoted apoptosis [75]. Some polymorphisms in the miR-4513 seed sequences are critical regions for target binding

specificity of miRNAs [76]. The rs2168518 variant (*miR4513*) is an example of a polymorphism that is inserted in the seed region of hsa-mir-4513, affecting the regulation of this miRNA. Previous studies have reported the association of this variant with fasting glucose, lipid traits [73], disease risk hitchhiker [75,77], lung adenocarcinoma [78] and age-related macular degeneration [79].

There are very few works in the literature that address the role of miRNAs in relation to leprosy. This is the first study to report how SNPs in miRNA genes and miRNA processing-related machinery may be associated with a predisposition to the development of leprosy and its different clinical forms, PB and MB, especially in an understudied population, such as admixed Brazilian Amazonian population. In the present study, we demonstrated a strong association with the risk of developing leprosy and its different forms in genetic variants in the miRNA genes *pre-miR938*, *miR570*, *pri-let-7a1*, *miR200C*, *miR4513*, *miR146A* and variants in machinery-related genes of *DROSHA* miRNAs and *AGO1* in the Amazonian population. The findings may provide valuable information for a better understanding of how genetic factors influence the pathophysiology of the disease, with the potential to find predictive markers of the development of leprosy in this population.

4. Materials and Methods

4.1. Sampling

The case group consisted of 114 individuals diagnosed with leprosy in the Dr. Marcello Cândia Reference Unit in Sanitary Dermatology of the State of Pará (URE). The populations attended in this reference medical clinical are in a condition of socioeconomic vulnerability. Patients were grouped according to clinical form, totaling 56 patients with paucibacillary leprosy (BT and TT: PB) and 58 with multibacillary form (BL and LL: MB). For the control group, 71 samples were included from individuals who lived or had close contact with leprosy patients, had no clinical signs of leprosy after being examined by experienced leprologists, and were negative for qPCR and anti-PGL-I IgM serology. All the individuals included in the study were residents of Pará, a state located in the Amazon region of Brazil and a hyperendemic leprosy area [4]. Thus, the individuals in the control group are exposed to the same environmental conditions and bacterial load, are intra- or peridomestic contacts of leprosy patients and are not related to the case group of the present study. Individuals were informed about the research and signed a consent form. This study complies with the Declaration of Helsinki and was approved by the Research Ethics Committee of the Institute of Health Sciences of the Federal University of Pará (CAAE 26765414.0.0000.0018 CEP-ICS/UFPA). All analyzed data were anonymized to protect the privacy of participants.

4.2. SNPs Selection

Twenty-five SNPs were selected based on association studies found in the PubMed database (www.ncbi.nlm.nih.gov/, accessed on 1 January 2020). These polymorphisms contribute to biological processes and immune responses in infectious diseases. Supplementary Table S5 summarizes the main features related to the polymorphisms investigated in the present work.

4.3. DNA Extraction and Quantification

For each individual participating in the research, a blood sample was collected in 10mL tubes of the K2-EDTA type (Beckton Dickinson, Franklin Lakes, NJ, USA). The genetic material was extracted from the peripheral blood of the patients and the control group using the phenol-chloroform method, based on. DNA quantification was performed using NanoDrop 1000 Spectrophotometer equipment (NanoDrop Technologies, Wilmington, DE, USA).

4.4. Genotyping and Quality Control of Investigated SNPs

SNP Genotyping was performed by allelic discrimination using TaqMan technology. OpenArray Genotyping, with a panel of 32 custom assays on the QuantStudio ™ 12K Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) according to

the manufacturer's recommended protocol. The Taqman software Genotyper (Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze plate data and genotype reading accuracy, in addition to genotyping quality control.

4.5. Ancestry Informative Markers

A panel of 61 ancestry informative markers (AIM) previously developed and expanded by our research group was used in this study, according to established protocols [80,81]. The AIMs panel is composed of specific ancestry markers capable of estimating individual and population genetic contributions to control possible ancestry influences.

4.6. Statistical Analysis

Genetic ancestry inference based on the AIM panel was performed in Structure software version 2.3.4 (Pritchard Lab, Stanford University, CA, USA) [82,83]. Differences in demographic and clinical characteristics such as age, sex, and ancestry analysis were compared using Student's t-test, chi-squared and Mann–Whitney tests, respectively. Hardy–Weinberg equilibrium (HWE) and logistic regression analyses between leprosy genotypes and risk and its clinical forms PB and MB were performed by the SNPassoc version 2.0-11 package (Gonzáles et al., Barcelona, ESP) [84] with covariate adjustment. All statistical analyzes were performed using the statistical program R version 4.1.0 (Ross Ihaka & Robert Gentleman, Auckland, NZ) [85]. Values of $p \leq 0.05$ were considered statistically significant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms231810628/s1.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Table S1. Genotypic distribution of the investigated polymorphisms for leprosy patients in comparison to control group to markers associated with susceptibility to leprosy.

Gene	Genotype	Case (%)	Control (%)	*p*-value	Model	OR (95%CI)
miR300	rs12894467	111	68			
	TT	44 (39.6)	30 (44.1)	0.76	TT vs TC + CC	1.10(0.49-2.45)
	CT	45 (40.5)	26 (38.2)			
	CC	22 (19.8)	12 (17.6)	0.82	TT + TC vs CC	1.10(0.49-2.45)
miR423	rs6505162	93	59			
	AA	27 (29.0)	15 (25.4)	0.71	AA vs AC + CC	1.15(0.40-1.86)
	AC	47 (50.5)	32 (54.2)			
	CC	19 (20.4)	12 (20.3)	0.87	AA + AC vs CC	1.07(0.45-2.53)
mir604	rs2368392	83	54			
	AA	42 (50.6)	24 (44.4)	0.48	AA vs AG + GG	0.77(0.36-1.61)
	AG	40 (48.2)	29 (53.7)			
	GG	1 (1.2)	1 (1.9)	0.69	AA + AG vs GG	0.57(0.03-1.67)
pre-miR938	rs2505901	109	68			
	TT	26 (23.9)	27 (39.7)	**<0.01**	TT vs TC + CC	**0.40(0.20-0.81)**
	CT	53 (48.6)	30 (44.1)			
	CC	30 (27.5)	11 (16.2)	**0.03**	TT + CT vs CC	**0.41(0.18-0.95)**

miR100	rs1834306	93	58			
	AA	44 (47.3)	25 (43.1)	0.60	AA vs AG + GG	0.83(0.42-1.66)
	AG	36 (38.7)	26 (44.8)			
	GG	13 (14.0)	7 (12.1)	0.99	AA + AG vs GG	1.01(0.36-2.77)
miR219A1	rs213210	140	70			
	AA	91 (81.2)	58 (84.1)	0.60	AA vs AG + GG	1.25(0.54-2.86)
	AG	21 (18.8)	9 (13.0)			
	GG	0 (0.0)	2 (2.9)	0.14	AA + AG vs GG	0.00 (0.00)
DROSHA	rs639174	101	64			
	CC	32 (31.7)	30 (46.9)	**0.02**	CC vs CT + TT	**0.45(0.23-0.89)**
	CT	51 (50.5)	28 (43.8)			
	TT	18 (17.8)	6 (9.4)	0.16	CC + CT vs TT	2.00(0.73-5.51)
miR453	rs56103835	109	69			
	TT	60 (55.0)	32 (46.4)	0.42	TT vs CT + CC	0.77(0.41-1.45)
	CT	38 (34.9)	30 (43.5)			
	CC	11 (10.1)	7 (10.1)	0.96	TT + CT vs CC	0.98(0.35-2.72)
miR196A2	rs11614913	101	66			
	CC	61 (60.4)	31 (47.0)	0.08	CC vs CT + TT	0.56(0.29-1.08)

	CT	31 (30.7)	30 (45.5)			
	TT	9 (8.9)	5 (7.6)	0.64	CC + CT vs TT	1.32(0.40-4.35)
AGO	rs636832	102	66			
	GG	46 (45.1)	39 (59.1)	**0.01**	GG vs GA + AA	**0.45(0.23-0.88)**
	GA	43 (42.2)	21 (31.8)			
	AA	13 (12.7)	6 (9.1)	0.42	GG + GA vs AA	0.66(0.23-1.86)
pri-let-7a1	rs10739971	30	37			
	GG	15 (50.0)	6 (22.2)	**0.02**	GG vs GA + AA	**4.66(1.17-18.66)**
	GA	12 (40.0)	14 (51.9)			
	AA	3 (10.0)	7 (25.9)	**0.04**	GG + GA vs AA	**5.09(0.95-7.45)**
miR146A	rs2910164	108	69			
	GG	47 (43.5)	29 (42.0)	0.67	GG vs GC + CC	0.87(0.46-1.65)
	GC	54 (50.0)	35 (50.7)			
	CC	7 (6.5)	5 (7.2)	0.66	GG + GC vs CC	1.31(0.22-2.59)
miR570	rs4143815	111	69			
	GG	62 (55.9)	46 (66.7)	0.73	GG vs GC + CC	1.41(0.73-2.73)
	GC	38 (34.2)	19 (27.5)			
	CC	11 (9.9)	4 (5.8)	0.49	GG + GC vs CC	1.64(0.49-5.50)
miR200B	rs9660710	103	67			

	CC	79 (76.7)	55 (82.1)	0.62	CC vs CA + AA	1.22 (0.54-2.79)
	CA	22 (21.4)	12 (17.9)			
	AA	2 (1.9)	0 (0.0)	0.14	CC + CA vs AA	1.00 (0.00)
miR26-A1	rs7372209	98	62			
	CC	44 (44.9)	33 (53.2)	0.32	CC vs CT + TT	1.40(0.72-2.72)
	CT	43 (43.9)	23 (37.1)			
	TT	11 (11.2)	6 (9.7)	0.41	CC + CT vs TT	1.59(0.51-4.94)
miR200C	rs12904	111	69			
	GG	36 (32.4)	11 (15.9)	**0.01**	GG vs AG + AA	**2.77(1.25-6.11)**
	AG	53 (47.7)	45 (65.2)			
	AA	22 (19.8)	13 (18.8)	0.92	GG + AG vs AA	1.04(0.43-2.15)
DROSHA	rs10035440	110	69			
	TT	80 (72.7)	40 (58.0)	**0.03**	TT vs CT + CC	**2.04(1.03-4.02)**
	CT	29 (26.4)	27 (39.1)			
	CC	1 (0.9)	2 (2.9)	0.27	TT + CT vs CC	3.82(0.32-3.10)
miR4513	rs2168518	112	69			
	GG	51 (45.5)	43 (62.3)	0.19	GG vs GA + AA	1.56(0.80-3.04)
	GA	53 (47.3)	20 (29.0)			
	AA	8 (7.1)	6 (8.7)	0.38	GG + AG vs AA	1.68(0.53-5.34)

miR219-1	rs107822	95	64			
	CC	58 (61.1)	37 (57.8)	0.73	CC vs CT + TT	0.89(0.46-1.74)
	CT	28 (29.5)	23 (35.9)			
	TT	9 (9.5)	4 (6.2)	0.52	CC + CT vs TT	1.49(0.42-1.55)
miR2053	rs10505168	105	66			
	TT	45 (42.9)	25 (37.9)	0.66	TT vs TC + CC	0.87(0.45-1.67)
	TC	45 (42.9)	29 (43.9)			
	CC	15 (14.3)	12 (18.2)	0.63	TT + CT vs CC	0.81(0.34-1.90)
miR499	rs3746444	109	69			
	GG	79 (72.5)	53 (76.8)	0.62	GG vs GA + AA	1.20(0.57-2.50)
	GA	26 (23.9)	14 (20.3)			
	AA	4 (3.7)	2 (2.9)	0.61	GG + GA vs AA	1.62(0.24-10.69)

*Sample number; *P*-value, OR and 95%CI were obtained with logistic regression adjusted for genetic ancestry.

Table S2. Genotypic distribution of the investigated polymorphisms for leprosy patients grouped in clinical form PB in comparison to the control group.

Gene	Genotype	PB (%)	Control (%)	*p*-value	Model	OR (95%CI)
miR300	rs12894467	55	68			
	TT	18 (32.7)	30 (44.1)	0.22	TT vs TC + CC	1.63(0.74-3.59)
	TC	24 (43.6)	26 (38.2)			
	CC	13 (23.6)	12 (17.6)	0.48	TT + TC vs CC	1.39(0.55-3.50)
	Alelo T	0.44	0.53			
	Alelo C	0.56	0.46			
miR423	rs6505162	49	59			
	AA	12 (24.5)	15 (25.4)	0.79	AA vs AC + CC	1.13(0.45-2.83)
	AC	26 (53.1)	32 (54.2)			
	CC	11 (22.4)	12 (20.3)	0.42	AA + AC vs CC	1.14(0.42-3.08)
	Alelo A	0.38	0.39			
	Alelo C	0.62	0.61			
mir604	rs2368392	43	54			
	AA	21 (48.8)	24 (44.4)	0.64	AA vs AG + GG	0.81(0.33-2.00)
	AG	22 (51.2)	29 (53.7)			
	GG	0 (0.0)	1 (1.9)	0.23	AA + AG vs GG	0.00(0.00)
	Alelo A	0.74	0.71			

	Alelo G	0.26	0.29			
pre-miR938	rs2505901	53	68			
	TT	11 (20.8)	27 (39.7)	**<0.01**	TT vs TC + CC	**0.31(0.12-0.75)**
	TC	25 (47.2)	30 (44.1)			
	CC	17 (32.1)	11 (16.2)	**0.01**	TT + CT vs CC	**0.31(0.12-0.84)**
	Alelo T	0.44	0.62			
	Alelo C	0.56	0.38			
miR100	rs1834306	48	58			
	AA	24 (50.0)	25 (43.1)	0.42	AA vs AG + GG	0.72(0.32-1.63)
	AG	17 (35.4)	26 (44.8)			
	GG	7 (14.6)	7 (12.1)	0.77	AA + AG vs GG	1.19(0.37-3.87)
	Alelo A	0.68	0.65			
	Alelo G	0.32	0.35			
miR219A1	rs213210	55	69			
	AA	42 (76.4)	58 (84.1)	0.38	AA + AG vs GG	1.52(0.59-3.94)
	AG	13 (23.6)	9 (13.0)			
	GG	0 (0.00)	2 (2.9)	0.06	AA vs AG + GG	0.00 (0.00)
	Alelo A	0.88	0.90			
	Alelo G	0.12	0.10			

DROSHA	rs639174	50	64			
	CC	16 (32.0)	30 (46.9)	0.08	CC vs CT + TT	0.50(0.22-1.12)
	CT	27 (54.0)	28 (43.8)			
	TT	7 (14.0)	6 (9.4)	0.60	CC + CT vs TT	0.72(0.21-2.31)
	Alelo C	0.59	0.69			
	Alelo T	0.41	0.31			
miR453	rs56103835	54	69			
	TT	30 (55.6)	32 (46.4)	0.52	TT vs CT + CC	0.78(0.36-1.67)
	CT	20 (37.0)	30 (43.5)			
	CC	4 (7.4)	7 (10.1)	0.53	TT + CT vs CC	0.66(0.17-2.53)
	Alelo T	0.74	0.68			
	Alelo C	0.26	0.32			
miR196A2	rs11614913	52	66			
	CC	32 (61.5)	31 (47.0)	0.12	CC vs CT + TT	0.53(0.24-1.19)
	CT	14 (26.9)	30 (45.5)			
	TT	6 (11.5)	5 (7.6)	0.24	CC + CT vs TT	2.29(0.56-9.30)
	Alelo C	0.75	0.70			
	Alelo T	0.25	0.30			
AGO	rs636832	52	66			

	GG	24 (46.2)	39 (59.1)	0.11	GG vs GA + AA	0.53(0.24-1.16)
	GA	20 (38.5)	21 (31.8)			
	AA	8 (15.4)	6 (9.1)	0.28	GG + GA vs AA	0.53(0.16-1.73)
	Alelo G	0.65	0.75			
	Alelo A	0.35	0.25			
pri-let-7a1	rs10739971	13	27			
	GG	9 (69.2)	6 (22.2)	**<0.01**	GG vs GA + AA	**13.3(1.82-90.0)**
	GA	3 (23.1)	14 (51.9)			
	AA	1 (7.7)	7 (25.9)	0.09	GG + GA vs AA	6.55(0.53-8.90)
	Alelo G	0.81	0.48			
	Alelo A	0.19	0.52			
miR146A	rs2910164	52	69			
	GG	23 (44.2)	29 (42.0)	0.74	GG vs GC + CC	1.14(0.40-1.92)
	GC	23 (44.2)	35 (50.7)			
	CC	6 (11.5)	5 (7.2)	0.52	GG + GC vs CC	0.65(0.18-2.43)
	Alelo G	0.66	0.67			
	Alelo C	0.34	0.33			
miR570	rs4143815	54	69			
	GG	35 (64.8)	46 (66.7)	0.82	GG vs GC + CC	1.10(0.49-2.47)

	GC	17 (31.5)	19 (27.5)			
	CC	2 (3.7)	4 (5.8)	0.32	GG + GC vs CC	2.38(0.41-13.01)
	Alelo G	0.80	0.80			
	Alelo C	0.20	0.20			
miR200B	rs9660710	55	67			
	CC	42 (76.4)	55 (82.1)	0.36	CC vs CA + AA	1.27 (0.48-3.37)
	CA	11 (20.0)	12 (17.9)			
	AA	2 (3.6)	0 (0.00)	0.27	CC + CA vs AA	0.00 (0.00)
	Alelo C	0.86	0.90			
	Alelo A	0.14	0.10			
miR26-A1	rs7372209	49	62			
	CC	19 (38.8)	33 (53.2)	0.16	CC vs CT + TT	1.78(0.79-3.99)
	CT	24 (49.0)	23 (37.1)			
	TT	6 (12.2)	6 (9.7)	0.59	CC + CT vs TT	1.42(0.39-5.14)
	Alelo C	0.63	0.71			
	Alelo T	0.37	0.28			
miR200C	rs12904	54	69			
	GG	20 (37.0)	11 (15.9)	**< 0.01**	GG vs GA + AA	**3.42(1.36-8.57)**
	GA	25 (46.3)	45 (65.2)			

	AA	9 (16.7)	13 (18.8)	0.53	GG + GA vs AA	1.39(0.50-3.89)
	Alelo G	0.60	0.48			
	Alelo A	0.30	0.52			
DROSHA	rs10035440	54	69			
	TT	36 (66.7)	40 (58.0)	0.32	TT vs CT + CC	1.50(0.66-3.39)
	CT	17 (31.5)	27 (39.1)			
	CC	1 (1.9)	2 (2.9)	0.66	TT + CT vs CC	1.73(0.14-7.12)
	Alelo T	0.82	0.77			
	Alelo C	0.18	0.23			
miR4513	rs2168518	55	69			
	GG	26 (47.3)	43 (62.3)	0.40	GG vs GA + AA	0.71(0.32-1.58)
	GA	28 (50.9)	20 (29.0)			
	AA	1 (1.8)	6 (8.7)	**0.03**	GG + AG vs AA	**7.65(0.83-70.5)**
	Alelo G	0.73	0.77			
	Alelo A	0.27	0.23			
miR219-1	rs107822	49	64			
	CC	30 (61.2)	37 (57.8)	0.77	CC vs CT + TT	0.89(0.40-1.99)
	CT	12 (24.5)	23 (35.9)			
	TT	7 (14.3)	4 (6.2)	0.18	CC + CT vs TT	2.50(0.63-9.84)

	Alelo C	0.73	0.76			
	Alelo T	0.26	0.24			
miR2053	rs10505168	52	66			
	TT	27 (51.9)	25 (37.9)	0.21	TT vs TC + CC	0.61(0.28-1.33)
	TC	19 (36.5)	29 (43.9)			
	CC	6 (11.5)	12 (18.2)	0.55	TT + CT vs CC	0.72(0.24-2.19)
	Alelo T	0.70	0.60			
	Alelo C	0.30	0.40			
miR499	rs3746444	52	69			
	GG	37 (71.2)	53 (76.8)	0.36	GG vs GA + AA	1.51(0.62-3.69)
	GA	12 (23.1)	14 (20.3)			
	AA	3 (5.8)	2 (2.9)	0.27	GG + GA vs AA	3.15(0.37-27.04)
	Alelo G	0.83	0.87			
	Alelo A	0.17	0.13			

*Sample number; *P*-values, OR and 95%CI were obtained with logistic regression adjusted for genetic ancestry.

Table S3. Genotypic distribution of the investigated polymorphisms for leprosy patients grouped in clinical form MB in comparison to the control group.

ID	Genotype	MB (%)	Control (%)	*p*-value	Model	OR (95%CI)
miR300	rs12894467	54	68			
	TT	26 (46.4)	30 (44.1)	0.66	TT vs TC +	1.02(0.40-1.)

					CC	
	CT	21 (37.5)	26 (38.2)			
	CC	9 (16.1)	12 (17.6)	0.79	TT + TC vs CC	1.12(0.33-2.32)
	Alelo T	0.67	0.63			
	Alelo C	0.33	0.37			
miR423	rs6505162	44	59			
	AA	15 (34.1)	15 (25.4)	0.40	AA vs AC + CC	0.69(0.29-1.65)
	AC	21 (47.7)	32 (54.2)			
	CC	8 (18.2)	12 (20.3)	0.96	AA + AC vs CC	0.97(0.35-2.73)
	Alelo A	0.58	0.53			
	Alelo C	0.42	0.47			
mir604	rs2368392	40	54			
	AA	21 (52.5)	24 (44.4)	0.50	AA vs AG + GG	0.74(0.30-1.81)
	AG	18 (45.0)	29 (53.7)			
	GG	1 (2.5)	1 (1.9)	0.79	AA + AG vs GG	1.46(0.29-1.74)
	Alelo A	0.75	0.71			
	Alelo G	0.25	0.29			
pre-miR938	rs2505901	56	68			
	TT	15 (26.8)	27 (39.7)	0.07	TT vs TC + CC	0.49(0.22-1.08)

	TC	28 (50.0)	30 (44.1)			
	CC	13 (23.2)	11 (16.2)	0.21	TT + CT vs CC	0.56(0.22-1.42)
	Alelo T	0.52	0.62			
	Alelo C	0.48	0.38			
miR100	rs1834306	45	58			
	AA	20 (44.4)	25 (43.1)	0.87	AA vs AG + GG	0.94(0.41-2.12)
	AG	19 (42.2)	26 (44.8)			
	GG	6 (13.3)	7 (12.1)	0.84	AA + AG vs GG	0.88(0.26-2.98)
	Alelo A	0.66		0.65		
	Alelo G	0.34		0.35		
miR219A1	rs213210	57	69			
	AA	49 (86.0)	58 (84.1)	0.78	AA vs AG + GG	0.87(0.31-2.41)
	AG	8 (14.0)	9 (13.0)			
	GG	0 (0.00)	2 (2.9)	0.09	AA vs AG vs GG	0.00 (0.00)
	Alelo A	0.93	0.90			
	Alelo G	0.07	0.10			
DROSHA	rs639174	51	64			
	CC	16 (31.4)	30 (46.9)	**0.03**	CC vs CT + TT	**0.43(0.19-0.98)**
	CT	24 (47.1)	28 (43.8)			

	TT	11 (21.6)	6 (9.4)	0.08	CC + CT vs TT	0.39(0.13-1.18)
	Alelo C	0.55	0.69			
	Alelo T	0.45	0.31			
miR453	rs56103835	55	69			
	TT	30 (54.5)	32 (46.4)	0.47	TT vs CT + CC	0.77(0.37-1.59)
	CT	18 (32.7)	30 (43.5)			
	CC	7 (12.7)	7 (10.1)	0.64	TT + CT vs CC	1.30(0.42-1.43)
	Alelo T	0.71	0.68			
	Alelo C	0.29	0.32			
miR196A2	rs11614913	49	66			
	CC	29 (59.2)	31 (47.0)	0.15	CC vs CT + TT	1.39(0.29-2.03)
	CT	17 (34.7)	30 (45.5)			
	TT	3 (6.1)	5 (7.6)	0.71	CC + CT vs TT	1.32(0.40-4.35)
	Alelo C	0.76	0.70			
	Alelo T	0.24	0.30			
AGO	rs636832	50	66			
	GG	22 (44.0)	39 (59.1)	**0.04**	GG vs GA + AA	**0.45(0.21-0.99)**
	GA	23 (46.0)	21 (31.8)			
	AA	5 (10.0)	6 (9.1)	0.98	GG + GA vs AA	1.01(0.28-3.61)

	Alelo G	0.67	0.75			
	Alelo A	0.33	0.25			
pri-let-7a1	rs10739971	17	27			
	GG	6 (35.3)	6 (22.2)	0.23	GG vs GA + AA	2.54(0.53-12.3)
	GA	9 (52.9)	14 (51.9)			
	AA	2 (11.8)	7 (25.9)	0.13	GG + GA vs AA	3.95(0.58-27.1)
	Alelo G	0.62	0.48			
	Alelo A	0.38	0.52			
miR146A	rs2910164	56	69			
	GG	24 (42.9)	29 (42.0)	0.74	GG vs GC + CC	1.110.53-2.30)
	GC	31 (55.4)	35 (50.7)			
	CC	1 (1.8)	5 (7.2)	0.66	GG + GC vs CC	4.26(0.47-38.5)
	Alelo G	0.71	0.67			
	Alelo C	0.29	0.33			
miR570	rs4143815	57	69			
	GG	27 (47.4)	46 (66.7)	**0.03**	GG vs GC + CC	**0.45(0.21-0.96)**
	GC	21 (36.8)	19 (27.5)			
	CC	9 (15.8)	4 (5.8)	0.08	GG + GC vs CC	0.34(0.10-1.28)
	Alelo G	0.66	0.80			

	Alelo C	0.34	0.20			
miR26-A1	rs7372209	49	62			
	CC	25 (51.0)	33 (53.2)	0.85	CC vs CT + TT	1.40(0.72-2.72)
	CT	19 (38.8)	23 (37.1)			
	TT	5 (10.2)	6 (9.7)	0.66	CC + CT vs TT	1.59(0.51-4.94)
	Alelo C	0.70	0.71			
	Alelo T	0.30	0.29			
miR200C	rs12904	57	69			
	GG	16 (28.1)	11 (15.9)	0.06	GG vs GA + AA	2.28(0.93-5.58)
	GA	28 (49.1)	45 (65.2)			
	AA	13 (22.8)	13 (18.8)	0.73	GG + GA vs AA	0.86(0.35-2.11)
	Alelo G	0.52	0.48			
	Alelo A	0.48	0.52			
DROSHA	rs10035440	56	69			
	TT	44 (78.6)	40 (58.0)	**0.01**	TT vs CT + CC	**2.88(1.22-6.79)**
	CT	12 (21.4)	27 (39.1)			
	CC	0 (0.0)	2 (2.9)	0.11	TT + CT vs CC	0.00(0.00)
	Alelo T	0.89	0.77			
	Alelo C	0.11	0.24			

miR4513	rs2168518	57	69			
	GG	25 (43.9)	43 (62.3)	0.17	GG vs GA + AA	0.57(0.26-1.27)
	GA	25 (43.9)	20 (29.0)			
	AA	7 (12.3)	6 (8.7)	0.87	GG + AG vs AA	0.91(0.27-3.03)
	Alelo G	0.66	0.77			
	Alelo A	0.34	0.23			
miR219-1	rs107822	46	64			
	CC	28 (60.9)	37 (57.8)	0.59	CC vs CT + TT	0.89(0.46-1.74)
	CT	16 (34.8)	23 (35.9)			
	TT	2 (4.3)	4 (6.2)	0.54	CC + CT vs TT	1.49(0.42-5.25)
	Alelo C	0.78	0.76			
	Alelo T	0.22	0.24			
miR2053	rs10505168	53	66			
	TT	18 (34.0)	25 (37.9)	0.53	TT vs TC + CC	0.87(0.34-1.90)
	TC	26 (49.1)	29 (43.9)			
	CC	9 (17.0)	12 (18.2)	0.96	TT + CT vs CC	0.81(0.34-1.86)
	Alelo T	0.58	0.59			
	Alelo C	0.42	0.41			
miR499	rs3746444	57	69			

	GG	42 (73.7)	53 (76.8)	0.80	GG vs GA + AA	1.20(0.57-2.50)
	GA	14 (24.6)	14 (20.3)			
	AA	1 (1.8)	2 (2.9)	0.84	GG + GA vs AA	1.62(0.24-10.69)
	Alelo G	0.86	0.87			
	Alelo A	0.14	0.13			

*Sample number; *P*-values, OR and 95%CI were obtained with logistic regression adjusted for genetic ancestry.

Table S4. Genotypic distribution of the investigated polymorphisms between leprosy patients grouped according clinical form MB and PB.

ID	Genotype	PB (%)	MB (%)	*p*-value	Model	OR (95%CI)
miR300	rs12894467	55	53			
	TT	18 (32.7)	25 (47.2)	0.09	TT vs CT + CC	0.48(0.20-1.14)
	CT	24 (43.6)	20 (37.7)			
	CC	13 (23.6)	8 (15.1)	0.08	TT + CT vs CC	0.39(0.13-1.16)
	Alelo T	0.54	0.66			
	Alelo C	0.46	0.34			
miR423	rs6505162	49	41			
	AA	12 (24.5)	15 (36.6)	0.46	AA vs AC + CC	0.69(0.26-1.87)
	AC	26 (53.1)	18 (43.9)			
	CC	11 (22.4)	8 (19.5)	0.99	AA + AC vs CC	1.00(0.33-3.08)
	Alelo A	0.51	0.59			

	Alelo C	0.49	0.41			
mir604	rs2368392	43	37			
	AA	21 (48.8)	19 (51.4)	0.52	AA vs AG + GG	0.73(0.28-1.94)
	AG	22 (51.2)	17 (45.9)			
	GG	0 (0.0)	1 (2.7)	0.14	AA + AG vs GG	0.00(0.00)
	Alelo A	0.74	0.74			
	Alelo G	0.26	0.26			
pre-miR938	rs2505901	53	53			
	TT	17 (32.1)	12 (22.6)	0.18	TT vs CT + CC	1.90(0.74-4.90)
	CT	25 (47.2)	27 (50.9)			
	CC	11 (20.8)	14 (26.4)	0.07	TT + CT vs CC	2.56(0.89-7.38)
	Alelo T	0.56	0.48			
	Alelo C	0.44	0.52			
miR100	rs1834306	48	42			
	AA	24 (50.0)	18 (42.9)	0.45	AA vs AG + GG	1.41(0.57-3.45)
	AG	17 (35.4)	19 (45.2)			
	GG	7 (14.6)	5 (11.9)	0.65	AA + AG vs GG	0.74(0.20-2.76)
	Alelo A	0.68	0.65			
	Alelo G	0.32	0.35			
DROSHA	rs639174	50	48			
	CC	16 (32.0)	14 (29.2)	0.73	CC vs CT + TT	0.85(0.32-2.22)
	CT	27 (54.0)	23 (47.9)			

	TT	7 (14.0)	11 (22.9)	0.85	CC + CT vs TT	1.11(0.35-3.51)
	Alelo C	0.59	0.53			
	Alelo T	0.41	0.47			
miR453	rs56103835	54	52			
	TT	30 (55.6)	28 (53.8)	0.69	TT vs CT + CC	0.84(0.36-1.96)
	CT	20 (37.0)	17 (32.7)			
	CC	4 (7.4)	7 (13.5)	0.27	TT + CT vs CC	2.16(0.53-8.86)
	Alelo T	0.74	0.70			
	Alelo C	0.26	0.30			
miR196A2	rs11614913	52	46			
	CC	32 (61.5)	27 (58.7)	0.59	CC vs CT + TT	1.27(0.52-3.08)
	CT	14 (26.9)	16 (34.8)			
	TT	6 (11.5)	3 (6.5)	0.73	CC + CT vs TT	1.27(0.52-3.08)
	Alelo C	0.75	0.76			
	Alelo T	0.25	0.24			
AGO	rs636832	52	47			
	GG	24 (46.2)	20 (42.6)	0.52	GG vs GA + AA	1.33(0.55-3.18)
	GA	20 (38.5)	22 (46.8)			
	AA	8 (15.4)	5 (10.6)	0.97	GG + GA vs AA	1.02(0.27-3.80)
	Alelo G	0.65	0.66			
	Alelo A	0.35	0.34			
pri-let-7a1	rs10739971	13	16			

	GG	9 (69.2)	6 (37.5)	**0.01**	GG vs GA + AA	**5.21(1.59-7.86)**
	GA	3 (23.1)	9 (56.2)			
	AA	1 (7.7)	1 (6.2)	0.55	GG + GA vs AA	2.68(0.11-6.46)
	Alelo G	0.81	0.65			
	Alelo A	0.19	0.36			
miR146A	rs2910164	52	53			
	GG	23 (44.2)	24 (45.3)	0.25	GG vs GC + CC	0.60(0.24-1.46)
	GC	23 (44.2)	28 (52.8)			
	CC	6 (11.5)	1 (1.9)	**0.04**	GG + GC vs CC	**0.14(0.01-1.36)**
	Alelo G	0.66	0.72			
	Alelo C	0.34	0.28			
miR570	rs4143815	54	54			
	GG	35 (64.8)	26 (48.1)	0.17	GG vs GC + CC	1.79(0.77-4.17)
	GC	17 (31.5)	20 (37.0)			
	CC	2 (3.7)	8 (14.8)	0.28	GG + GC vs CC	2.38(0.44-12.79)
	Alelo G	0.80	0.66			
	Alelo C	0.20	0.34			
miR200B	rs9660710	55	45			
	CC	42 (76.4)	35 (77.8)	0.98	CC vs CA + AA	1.01 (0.35-2.91)
	CA	11 (20.0)	10 (22.2)			
	AA	2 (3.6)	0 (0.0)	0.10	CC + CA vs AA	0.00 (0.00)

	Alelo C	0.86	0.88			
	Alelo A	0.14	0.11			
miR26-A1	rs7372209	49	46			
	CC	19 (38.8)	23 (50.0)	0.10	CC vs CT + TT	0.47(0.19-1.18)
	CT	24 (49.0)	18 (39.1)			
	TT	6 (12.2)	5 (10.9)	0.52	CC + CT vs TT	0.64(0.17-2.50)
	Alelo C	0.63	0.70			
	Alelo T	0.37	0.30			
miR200C	rs12904	54	54			
	GG	20 (37.0)	15 (27.8)	0.26	GG vs AG + AA	1.66(0.68-4.06)
	AG	25 (46.3)	26 (48.1)			
	AA	9 (16.7)	13 (24.1)	0.41	GG + AG vs AA	1.54(0.54-4.37)
	Alelo G	0.60	0.52			
	Alelo A	0.40	0.48			
DROSHA	rs10035440	54	53			
	TT	36 (66.7)	41 (77.4)	0.63	TT vs CT + CC	0.80(0.31-2.05)
	CT	17 (31.5)	12 (22.60			
	CC	1 (1.9)	0 (0.0)	0.47	TT + CT vs CC	0.00(0.00)
	Alelo T	0.82	0.89			
	Alelo C	0.17	0.11			
miR4513	rs2168518	55	54			
	GG	26 (47.3)	23 (42.6)	0.70	GG vs GA + AA	1.56(0.80-3.04)

	GA	28 (50.9)	25 (40.3)			
	AA	1 (1.8)	6 (11.1)	0.09	GG + GA vs AA	5.37(0.56-9.89)
	Alelo G	0.73	0.66			
	Alelo A	0.27	0.34			
miR219-1	rs107822	49	43			
	CC	30 (61.2)	26 (60.5)	0.83	CC vs CT + TT	0.91(0.36-2.30)
	CT	12 (24.5)	15 (34.9)			
	TT	7 (14.3)	2 (4.7)	0.07	CC + CT vs TT	0.23(0.04-1.31)
	Alelo C	0.73	0.78			
	Alelo T	0.27	0.22			
miR2053	rs10505168	52	50			
	TT	27(51.9)	17 (34.0)	0.06	TT vs TC + CC	2.31(0.95-5.61)
	TC	19 (36.5)	24 (48.0)			
	CC	6 (11.5)	9 (18.0)	0.51	TT + TC vs CC	1.50(0.45-5.06)
	Alelo T	0.70	0.58			
	Alelo C	0.30	0.42			
miR499	rs3746444	52	54			
	GG	37 (71.2)	40 (74.1)	0.97	GG vs GA + AA	1.01(0.39-2.61)
	GA	12 (23.1)	13 (24.1)			
	AA	3 (5.8)	1 (1.9)	0.25	GG + GA vs AA	0.25(0.02-3.05)
	Alelo G	0.82	0.86			
	Alelo A	0.17	0.14			

*Sample number; *P*-values, OR and 95%CI were obtained with logistic regression adjusted for age and sex.

Table S5. Characterization of the polymorphisms studied.

Gene	ID	Alleles	Function	MAF*
miR20b/miR-17-5P	rs3660	C>G	3 Prime UTR Variant	48%
miR300	rs12894467	C>T	Non-Coding Transcript exon variant	39%
miR423	rs6505162	C>A	Non-Coding Transcript variant	50%
mir604	rs2368392	G>A	Non-Coding Transcript Variant	32%
pre-miR938	rs2505901	C>T	Intron Variant	40%
miR605	rs2043556	T>C	Non Coding Transcript Variant	25%
miR100	rs1834306	A>G	Intron Variant	45%
miR219A1	rs213210	A>G	Regulatory Region Variant	17%
DROSHA	rs639174	C>T	Intron Variant	46%
miR453	rs56103835	T> C	Non-Coding Transcript Exon Variant	30%
miR196A2	rs11614913	C>T	Non-Coding Transcript Exon Variant	33%
AGO1	rs636832	A>G	Intron Variant	36%
pri-let-7a-1	rs10739971	G>A	Intron Variant	26%
miR146A	rs2910164	C>G	Mature miRNA Variant	29%
miR570	rs4143815	G>C	3 prime UTR ariant	28%
miR200B	rs9660710	A>C	Regulatory Region Variant	10%
miR26-A1	rs7372209	C>T	Intron Variant	20%
miR200C	rs12904	G> A	3 Prime UTR Variant	49%
DROSHA	rs3805500	G>A	Intron Variant	49%
DROSHA	rs10035440	T>C	Intron Variant	15%
miR4513	rs2168518	G> A	Non-Coding Transcript Exon Variant	24%
miR219-1	rs107822	C>T	TF Binding Site	37%
miR149	rs2292832	T>C	Intron Variant	38%
miR2053	rs10505168	C>T	Non Coding Transcript Variant	39%

miR499	rs3746444	A>G	Mature miRNA Variant	18%

*Minor Allele Frequency according 1000 Genomes Project.

4 CAPÍTULO II

Este capítulo é referente ao manuscrito intitulado "**ncRNAs: an unexplored cellular defense mechanism in leprosy**" em preparação para submissão na revista científica *Frontiers in Immunology* (IF: 8.786).

ncRNAs: an unexplored cellular defense mechanism in leprosy

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Abstract

Leprosy is an infectious disease caused primarily by the obligate intracellular parasite *Mycobacterium leprae*. Despite being considered eradicated in many countries, leprosy remains a health problem in developing countries. In addition to stigmatization, individuals affected by leprosy can suffer nerve impairment that leads to physical disability when the disease is not treated correctly. Leprosy is known to be a complex disease in which socio-environmental factors, immune response, and host genetics act together in the development of the disease. A new field of study called epigenetics has recently shown that the immune response and other mechanisms related to infectious diseases can be influenced by non-coding RNAs. In this review, we summarize the main advances related to non-coding RNAs in leprosy, discussing the main perspectives on this new approach for understanding disease pathophysiology and searching for molécular markers. In our opinion, studies on non-coding RNAs in leprosy are promising and deserve greater attention from scholars in this field.

1 Introduction

Leprosy (also known as Hansen's disease) is a dermatoneurological disease that progresses to deformities and incapacities if not diagnosed and treated correctly (1). The causative agent of leprosy, *Mycobacterium leprae*, is known for a tropism for the upper respiratory tract, skin macrophages and Schwann cells (SCs) of peripheral nerves, conferring neurodermatological manifestations, including hypopigmented or erythematous cutaneous patches, sensorimotor loss and thickened peripheral nerves (1,2,3).

The Leprosy is a disease that remains quite stigmatized, constituting a serious public health problem in countries like India, Brazil and Indonesia, where there is still a high prevalence rate (4). It is important to emphasize that according to WHO data, during the year 2020 there was a decrease in the detection of new cases of the disease by about 37% compared to the previous year due to the pandemic of COVID-19 (5). As a consequence of the pandemic, leprosy detection and treatment have been seriously affected due to staff shortages, suspension of activities in communities, delayed drug supply, etc. This disruption may result in an increase in cases and individuals with grade 2 disability (G2D) (5,6).

The clinical manifestations of leprosy are presented as a spectrum, being intrinsically related to the host's immune response against *M. leprae* (7,8). Based on clinical, pathological, bacilloscopic and immunological criteria, leprosy can be classified as: tuberculoid (TT), borderline tuberculoid (BT), borderline borderline (BB), borderline lepromatous (BL) and lepromatous (LL) (9). Another type of classification, currently used by the World Health Organization, is based on the number of lesions: patients with up to five lesions are classified as paucibacillary (PB) and, patients with over five lesions, as multibacillary (MB) (10).

Importantly, it is still not entirely clear how immune response initiates in leprosy infection. We know that, at the TT pole, the infection is limited by a strong cell-mediated immunity, guaranteed by Th_1 $CD4^+$ cells, that secretes interleukin 2 (IL-2), and interferon (IFN)-γ that enhance macrophages and natural killers (NKs) microbicidal activities; therefore, in TT lesions, a granuloma formation and a low number of bacterial are observed (11–13). As for LL lesions, the cell-mediated immunity is absent, giving place to Th2 antibody response, that produces IL-4 and IL-5 in abundant levels, which are known to

activate B cells to switch antibodies (11). However, the humoral immunity against *M. leprae* is ineffective, allowing the bacilli to multiply in large number and disseminate the disease (11,12).

It has been estimated that almost 5% of the individuals exposed to *M. leprae* are infected, of which only 20% actually develop leprosy (14,15). The explanation for such variability can be addressed to different reasons, including environmental factors, divergence in pathogen burden and human genetic susceptibility (3). In the global literature, there is evidence from a wide variety of studies supporting that host genes play an important role in susceptibility to leprosy in its different clinical forms, ranging from a classic twin study in the mid-twentieth century (16) to more recent Genome-Wide Association Studies (GWAS) (17,18), which may elucidate leprosy pathology with the investigation of different immune-related genes (3,7,11).

Currently, it is known that the immune response and other related mechanisms may be influenced not only by genetic factors but also by epigenetic regulation, which includes the activity of non-coding RNAs (ncRNAs) (19). *M. leprae* alter host cell functionality to their own advantage to promote survival and generate a suitable environment for replication within the host cell by modifying host epigenome (20,21). In the last few years, it has been demonstrated that ncRNAs are broadly involved in the activation or suppression of the expression of distinct gene sets related to leprosy phenotype, which directed to novel knowledge on the role of ncRNAs in immunity generation and disease progression, although much remains to be discovered. Here, we review the recent advances in understanding ncRNAs-mediated regulation on leprosy physiopathology and we discuss their importance as potential biomarkers for this disease.

2 Epigenetics

Epigenetics can be defined as the study of molecules and mechanisms capable of modifying regulation and gene expression, without altering the genomic sequence (19). The main mechanisms related to epigenetic regulation are DNA methylation (22), histone modifications (23) and, as previously mentioned, ncRNAs (24,25). These host mechanisms can become excellent tools for pathogens, providing persistent infections by downregulation

of the immune response through bacterial factors capable of altering various cell signaling pathways (26–28).

DNA methylation and histone modification are processes related to chromatin. The methylation involves chemical modifications through the addition of methyl groups within specific sequences rich in guanine-cytosine, which alter the three-dimensional structure of the DNA strand molecule, inhibiting or allowing access to certain regions by other molecules (29,30). Histone modifications may occur through two main mechanisms: modifications that directly influence the general structure of chromatin and modifications that regulate the binding of effector molecules (31). The histone octamers are components of the nucleosome and also play an important role in the epigenetic control of the genome (29).

The ncRNAs are RNA molecules that may have structural, functional, and regulatory roles, representing about 70% of the genome. The ncRNAs can interact with DNA, RNA, and proteins, and generally function as cis-action silencers and trans-action mediators for specific transcriptional sites and post-transcriptional processes, nuclear organization, RNA processing, and transposon suppression through sequence complementarity (25).

Based on size, biogenesis and structure, ncRNAs are grouped into three major groups: long non-coding RNAs (lncRNAs >200 nt), mid-size RNAs (20-300 nt) and short ncRNAs (<200 nt) (32). Currently, there is increasing evidence that deregulation in the transcription and maturation of ncRNAs, incorrect interaction with target mRNAs and mutations in the processing mechanism can increase the risk of neurological, cardiovascular diseases and tumorigenesis (33–38). However, there are still few studies on the association of ncRNAs with infectious diseases, such as lncRNAs, microRNAs (miRNAs) e piwi-interacting RNAs (piRNAs) in leprosy (**Figure 1**) (39–42). Therefore, in the following sessions, we will focus on these classes of ncRNAs and their relation to leprosy. **Table 1** presents their main characteristics.

Figure 1. Timeline of studies involving ncRNAs and leprosy. It is observed that these investigations are still quite limited.

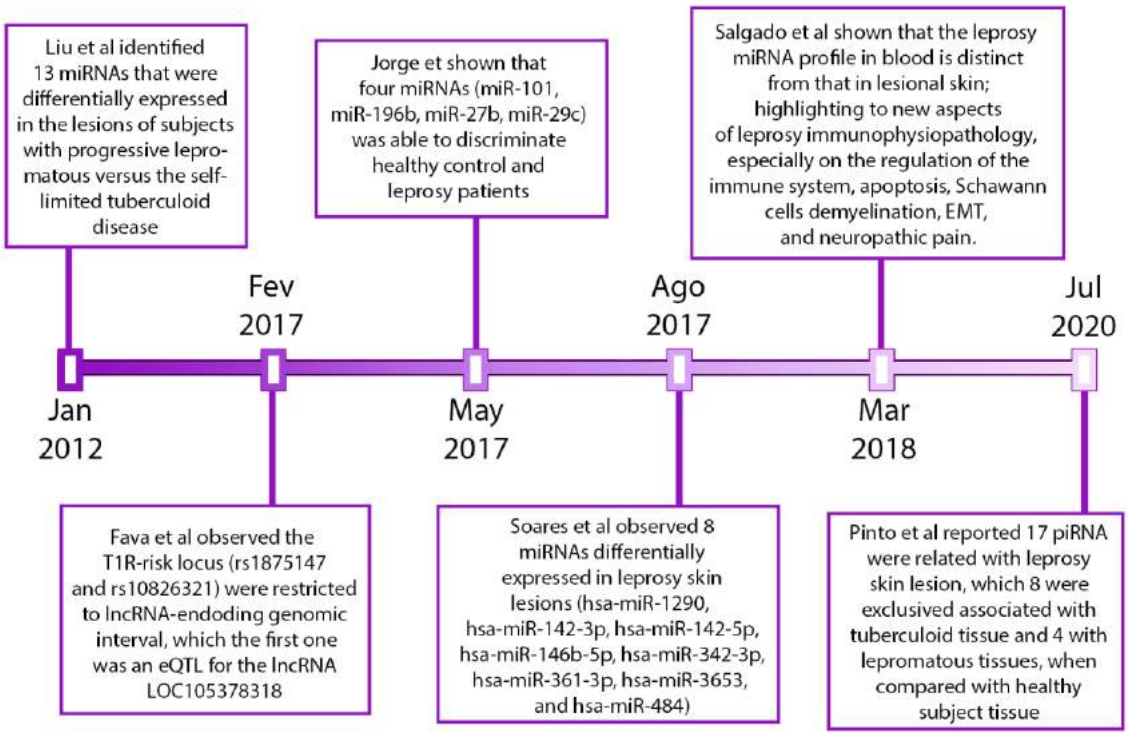

Table 1. Characteristics of the main classes of ncRNAs.

Class	Length (nt)	Functions	References
LncRNAs	> 200	Regulation of gene expression in the transcriptional and post-transcriptional levels, chromatin remodeling, miRNA sponge	(43,44)
miRNAs	19-24	RNA and gene silencing, gene modulation at the post-transcriptional level	(45,46)
piRNAs	26-31	Epigenetic and post-transcriptional gene silencing	(41,47,48)

3 LncRNAs

Long non-coding RNAs (lncRNAs) are functionally heterogeneous molecules that present a length of at least 200 nt, a lack of protein-coding potential, usually a poly-A tail and that may be spliced, similar to mRNAs (49). They are classified according to the relative location to protein-coding genes as: sense lncRNA, antisense lncRNA, bidirectional lncRNA, intron lncRNA, intergenic lncRNA and enhancer lncRNA (50).

LncRNAs have been associated with several functions and represent the largest class of ncRNAs. Contrary to short ncRNAs, which are generally attributed to gene regulation,

the mechanistic role of lncRNAs is highly diverse, increasing their functional complexity (32). At the epigenetic level, lncRNAs can regulate DNA methylation, alter methylation, acetylation, or ubiquitination of histones and reconstruct a chromatin or alter its conformation (51). It has been demonstrated that lncRNAs play important roles in stem cell maintenance and differentiation, X-chromosome inactivation, imprinting, maintenance of nuclear architecture, cell autophagy, cell proliferation, apoptosis and embryonic development (49,52)

In the immune system, lncRNAs exhibit dynamic expression in cell type-, developmental stage-, and context-specific manners to coordinate several aspects of immune function (53). The majority of lncRNAs that are associated with infection diseases have been shown to dysregulate expression in different tissues and cells. Several studies have found differentially expressed host lncRNAs in various bacterial infections such as *Escherichia coli*, *Salmonella*, *Campylobacter concisus* and *Mycobacterium tuberculosis* (54–56,56,57), indicating that lncRNAs can be used as molécular markers of infection associated with pathogenic bacteria.

To date, the only study that has directly related lncRNAs and leprosy was done by Fava and collaborators (58). In this genome wide association study, the authors identified a lncRNA as risk factor for pathological inflammatory responses in leprosy. Two risk variants for leprosy type-1 reactions (T1R) (rs1875147 and rs10826321) are represented by two isoforms of a novel lncRNA, one encoded by the *ENSG00000235140* (also known as *RP11-135D11.2*) gene and the other encoded by the uncharacterized *LOC105378318*. These two variants are located at 6.5 kb and 8.7 kb, respectively, upstream of the transcription start site of the *ENSG00000235140* gene and present a risk factor for T1R, also known as reversal reactions. Leprosy T1R are pathological inflammatory responses that affect a sub-group of leprosy patients and result in peripheral nerve damage. These episodic events contribute to host immune-mediated tissue damage and, consequently, disability in leprosy (58).

Another study (59), using learning machine to predict leprosy progression amongst household contacts of leprosy patients, identified a group of genes among which three lncRNAs were found (*ENSG00000283633*, *ENSG00000266538* and *ENSG00000279227*), however, these lnc-RNAs, along with other pseudogenes were not included for validation of the RNA-Seq signature due the lack of commercially available probes for RTqPCR (59).

Currently, research on lncRNAs in leprosy and other infectious immunity is just beginning when compared to studies on other ncRNAs, such as miRNAs, in this field. One of the major challenges in identifying lncRNAs relates to difficulty in discriminating their pleiotropic functions, as well as understanding the mechanisms by which they interact with other molecules, such as proteins, miRNAs, mRNAs and circRNAs. Similarly, the interaction of lncRNAs with the immune system has not yet been fully elucidated, being needed more experimental and clinical studies to offer novel approaches for better diagnosis and therapy in the future.

4 miRNAs

Among the variety of ncRNAs, the most frequently studied are miRNAs. Discovered as non- coding and post-transcriptional regulators in eukaryotes in the early 1990s (60), miRNAs are small, endogenous, stable, and highly conserved among species. MiRNAs are predicted to control the activity of approximately 30% of all protein-coding genes in the human genome (61), and have been shown to participate in the regulation of thousands of genes (42). They are among the most important regulatory molecules of an organism and participate in a variety of biological processes that include the modulation of the immune response during infections (45,62–65).

However, studies that associate the expression of miRNAs in infectious diseases are still insufficient, especially related to diseases caused by mycobacteria (46,64,66). It is known that these microorganisms have various routes of infection and can cause diverse immune response based on the cells that are likely infected (67) For example, the main cell types directly infected by the mycobacteria are macrophages, which are crucial modulators of innate and adaptive immune responses, leading to different immune responses by deregulation of host miRNAs (68,69).

Macrophages act in the front line of host defense and are a major target of these pathogens (70,71). In leprosy, the infection of macrophages results in the recognition by TLR-4 (72) and TLR-2/1 (73), and subsequent direction of adaptive immune responses. Two miRNAs that target *PRKCE* gene (hsa-miR-1-3p and hsa-miR-31-5p), which in turn is involved in TLR-4 signaling in mycobacterial infections (Hestvik et al., 2003; Aksoy et al., 2004), showed low expression in leprosy tissues of both TT and LL poles through miRNA sequencing (74). On the other hand, Liu and colleagues (8), using microarray analysis,

demonstrated that in LL patients, the vitamin D-dependent antimicrobial effects of TLR-2/1 are directly inhibited by the overexpression of hsa-miR-21. This miRNA negatively regulates the expression of *IL1B* and *CYP27B1* genes, activates downstream of TLR-2/1 signaling, and plays a role in the induction of vitamin D immunomodulatory activities. This leads to an indirect increase in the production of IL-10, which leads to the subsequent inhibition of the antimicrobial peptides CAMP and DEFB4A (8).

The persistence of *M. leprae* infection depends on the type of the host immune response and macrophages present in the skin lesions. Macrophages have high plasticity and can polarize to different phenotypes, including M1 (pro-inflammatory profile) and M2 (anti-inflammatory profile), with mostly homeostatic and phagocytic functions (75). In the skin lesions of LL patients, miR-34a-5p and miR-326 were upregulated, causing the inhibition of *NOTCH1/2* expression and preventing the differentiation of monocytes to M1 macrophages (74). Thus, the populations of M2 macrophages were found to be predominant in LL lesions, indicating that the types of macrophages present in lesions are associated with clinical spectrum of leprosy.

To initiate the adaptive response, the interaction between adhesion molecule 1 (CADM1) expressed on the surface of dendritic cells and the molecule associated with MHC class I restricted T cells (CRTAM) coordinates the immunological synapse required for T cell receptor (TCR) activation and polarization in subpopulations of CD4+ T cells (76). MiRNAs can control the bridge between innate and adaptive immune signaling mechanisms and regulatory networks that determine which subpopulations of Th cells will be activated during leprosy (77,78). The miR-15a-5 and miR-16-5p, that control the interaction between *CADM1* and *CRTAM*, have been shown to be downregulated in all groups of leprosy patients (74). However, the downregulation of miR-181a is associated with the progression of leprosy to BL and LL forms (79). This miRNA is particularly important for TCR signaling for regulating SHP2, a phosphatase that reduces TCR expression and, as a consequence, SHP2 overexpression compromises TCR signaling and specificity of T cells against *M. leprae* (79).

The regulatory network of miRNAs that operate in differentiation for TCD4+ Th1, TCD4+ Th2 and TCD4+ Th17 cells are still far from being fully comprehended. When overexpressed, miR-125b regulates genes involved in the differentiation of naive TCD4+

cells (e.g., *IFNG*, *IL2RB*, *IL10RA* and *PRDM1*); whereas it is downregulated in leprosy patients, it does not seem to prevent the differentiation of TCD4+ in the subpopulations of Th cells (74). It has also been previously demonstrated *in vitro* that the overexpression of miR-155 in TCD4+ cells promote differentiation into Th1 cells, while the its downregulation appears to activate Th2 cells (80), but, in LL patients, miR-155 was upregulated (74). In a previous study, miR-155 was associated with the repression of SHIP1 and the transcriptional repressor BTB and CNC homology 1 (Bach1), which results in increased activation of Akt (serine/threonine protein kinase B) and favors the survival of mycobacteria inside cells (81,82). However, these results are still quite controversial, since studies show that miR-155 is also able to promote differentiation into Th17 cells through regulation of the *JARID2* gene (83–85).

The Th17 subset is usually found in patients with unstable leprosy, where there is absence of Th1 and Th2 polarization, and is also associated with T1R leprosy reactions (86,87). Similar to miR-155, the miRNA hsa-miR-326, which is reported to be an important inductor of differentiation of Th17 cells (88,89) was also found upregulated in LL (74). On the other hand, some studies report the importance of miR-326 as a regulator of apoptosis in neuronal cells, able to inhibit JNK and MAPK signaling pathways when overexpressed (90,91). From an immunological point of view, apoptosis is a form of programmed cell death (PCD) that functions as a defense against infections, involving the death of infected macrophages (92). Modulation of apoptosis can influence the course of infection, allowing intracellular pathogens to survive, and may be an important mechanism in the development of the various clinical forms of leprosy, as they are influenced by the bacillary load (93,94).

Several miRNAs that target Caspase-8 (CASP-8) inductor are upregulated in the two leprosy extreme poles. Apoptosis-related genes like *MYC* (miR-34a-5p and miR-155-5p), *FAS* (miR-196b-5p and miR-146a-5p) and *FADD* (miR-155-5p and miR-146a-5p) are upregulated only in LL patients, while miR-126-3p, miR-15a-5p, miR-20a-5p and miR-16-5p, that target *BCL-2*, an antiapoptotic gene, and miR-193a-3p and miR-133a-3p, that target *MCL1*, a member of BCL-2 family, were downregulated (74). In LL lesions, miRNAs that target the pro-apoptotic gene *YAP1* (miR-200a-3p and miR-375) and miRNAs that target *AKT1* (miR-199a-3p and miR-708-5p) were all downregulated. AKT1 is a known suppressor of the apoptotic machinery, including *YAP1* and *FOX03*. *TP53* also presented its miRNAs (IAmiR-200a-3p and miR-375) downregulated in LL lesions, but it has its functions blocked

through activation of RBL1/2, that are activated after inhibition of *FOX03*, besides, *AKT1* also stimulates *MDM4*, a regulator of *TP53*. Therefore, *M. leprae* can impact macrophages miRNAs expression levels, thus altering apoptosis to save itself from intracellular killing (74).

In addition to understanding about the immunopathophysiology of leprosy, some studies have also sought biomarker miRNAs that are accessible and reliable. Jorge et al (95) studied sets of miRNAs differentially expressed by low-density array in skin lesions of LL and TT patients, with the expression levels of seven miRNAs (miR-125b, miR-196b, miR-27b, miR-29c, miR-425-5p, miR-502-3p, and miR-92a), and among these four miRNAs were selected (miR-101, miR-196b, miR-27b and miR-29c) from evaluation by their ability to distinguish between group of patients and control in the first stage, and group of LL and TT in a second stage. The combination of miRNAs miR-101, miR-196b, miR-27b and miR-29c show 80% sensitivity and 91% specificity (AUC 87%) in discriminating patients with leprosy, and was also able to discriminate between TT and LL with 83% sensitivity and 80% specificity (AUC 83%).

In another study carried out by Soares and collaborators (96), comparing clinical forms (TT, BT, BB., BL, LL) and a control group, twenty miRNAs were found differentially expressed, while in polar forms (TT and LL) only one miRNA was identified (hsa-mir181a). The hsa-mir181a miRNA upregulated across the spectrum of leprosy and reaction forms, with greater expression of the polar forms TT and LL, which may indicate importance in the pathophysiological process of the disease (96). It is known that hsa-mir-181a is capable of regulating the sensitivity of T cells, allowing mature T cells to recognize antagonists as agonists (97,98). The higher expression of hsa-mir-181a is correlated with greater sensitivity of T cells in immature T cells, suggesting that this miRNA acts as a rheostat of intrinsic sensitivity to the antigen (96,98). Of the sixty-four miRNAs, eight were validated, seven of which were upregulated (hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-146b-5p, hsa-miR-342-3p, hsa-miR-361 -3p, hsa-miR-3653, and hsa-miR-484) and one were downregulated (hsa-miR-1290) (96).

Among ncRNAs, miRNAs are the most studied in leprosy, with studies aiming at basic knowledge of how these molecules act in the immune response and pathophysiology of the disease, aiming at new therapeutic targets aimed at the interaction between bacillus /

host and accessible biomarkers. However, many of the miRNAs found require validation and functional analysis to assess their roles in the pathogenesis of leprosy.

5 piRNAs

PIWI-interacting RNAs (piRNAs) represent the most abundant and diverse group of sncRNAs (99), with more than 30,000 piRNAs molecules identified in the human genome (100). Several genomic loci, defined as piRNA clusters, can transcribe pri-piRNA sequences (101), that unlike miRNA and siRNA, are processed through Dicer-independent mechanisms, to mature piRNAs (102). Structurally, piRNAs are single-stranded molecules, with 24-32 nt in length and bear 2′-O-methyl-modified 3′ termini, these sncRNAs interact with PIWI subfamily Argonaute (AGO) proteins to form the piRNA-induced silencing complex (piRISC) (103).

The involvement of piRNAs in the innate and adaptative immune response is not a well-established concept as for miRNAs, and few studies with controversial results have pointed out a possible relationship between the expression of piRNAs and the response to viral infections (104). Despite this, the pioneer work by Pinto et al. (41) focused on the global changes in the piRNA expression profile (piRNome) of leprosy skin lesions, and detected 337 piRNAs in human skin, of which five piRNAs (piR-hsa-28634, piR-hsa-1580, piR-hsa-27007, piR-hsa- 21131, piR-hsa-12454) were differentially expressed (DE) in leprosy tissues when compared with healthy tissues (HS) (TT + LL vs HS).

Other piRNAs were exclusively DE to the different leprosy poles: eight DE piRNAs (piR-hsa-23327, piR-hsa-23655, piR-hsa-2153, piR-hsa-12790, piR-hsa-31280, piR-hsa-28394, piR-hsa-27283, piR-hsa-23289) were found only in TT and three DE piRNAs (piR-hsa-23919, piR-hsa-26131, piR-hsa-15215) were found only in LL leprosy (41). This indicates that piRNA expression profiles can distinguish leprosy tissue from a non-leprosy tissue, even more, can distinguish leprosy between its TT and LL poles, therefore, these piRNAs are attractive biomarkers for leprosy.

Under normal physiological conditions, the piRNAs are produced at stable levels (Story et al., 2019), however, in leprosy skin lesions all but one of DE piRNAs (piR-hsa-27283) are downregulated, emphasizing epigenetic alterations in leprosy (Pinto et al., 2020). Considering that, piR-hsa-27283, was the only piRNA upregulated, it may be useful as a risk

biomarker of leprosy, more specifically, for TT patients, since it was DE for this pole, and as leprosy poles have different immunological and molécular features, it is suggested that piR-hsa-27283 functions can have a significant impact on progression to TT leprosy. On the other hand, its role in leprosy is unclear, as it may has multiple gene targets, thus making its analysis difficult (41).

To better clarify the functional and mechanistic features of piRNAs in leprosy context, the authors built a piRNA-gene regulatory network, based on DE piRNAs putative target genes, which revealed that, in general, DE piRNAs regulate genes involved in leprosy-related processes, such as, programmed cell death (*i.e.* apoptosis, autophagic cell death), immune response, neural and epidermal regeneration (41).

Lines of evidences suggest that immunological response generated during *M. leprae*, induces the apoptosis of infected cells, mainly by the secretion of pro-apoptotic cytokines, such as TNF and IFN, which are markedly dominant in TT lesions (105). However, synergistically with miRNAs, the piRNAs regulate anti-apoptotic pathways, via CARF activation, an inhibitor of caspase-dependent apoptosis, allowing a favorable condition to bacterial survival (41).

An important question is if piRNAs are downregulated in leprosy biopsies and considering that SCs of peripheral nerves possess genomic plasticity and high regenerate capacity guaranteed by SOX10 and ERBB gene family, why in leprosy the regrowth of axons and SCs is inhibited? (106,107). Recently, Masaki et al. (108) demonstrated that the leprosy bacterium hijacks this regenerative property and turn-off myelination-associated genes by DNA methylation of *SOX10* promotor region, a regulator of SCs differentiation and myelination, thus, the cells remain in an undifferentiated stage and become capable to migrate to other tissues, which facilitated the spread of *M. leprae* to other niches, such as skeletal and smooth muscle, and also contribute to granuloma formation that subsequently release of *M. leprae*-laden macrophages.

Another important mechanism for SCs regeneration is the recruitment of pro-regenerative macrophages. The pro-regenerative macrophages function to clear axonal and myelin debris, persisting in nerve microenvironment to guide remyelination and SC differentiation after nerve injury, which mechanism are regulated by growth arrest specific 6 (GAS6) and IL-6 (109). It is proposed that the downregulation of piRNAs (piR-hsa-12454,

piR-hsa-1580, piR-hsa-2153, piR-hsa-23289) that target IL6R to culminate in the expression of this receptor in pro-regenerative macrophages and active the IL-6, a well-known neuropathic biomarker in leprosy patients (41). The IL-6 in turn, stimulates pro-regenerative macrophages to produce GAS6 that promotes better SCs remyelination within the injured nerve (109).

Unfortunately, the work of Pinto and collaborators (41) is the only one study available in the specialized literature that aimed to understand the involvement of piRNAs pathway in leprosy. Clearly, there is still a gap in this regard, but as above mentioned these data are important to understand the epigenetic control of genes that participate in leprosy immunophysiopathology. It is believed that future studies may reveal mechanisms to reactivate neural regeneration genes, in this case, the expression of piRNAs should also be modulated to avoid inhibiting regeneration.

6 Perspectives

Noncoding RNA are implicated in a wide variety of human diseases including infectious diseases. Recently, the use of RNA technology against COVID-19 has caused a "boom" in the study of ncRNAs as transcription modulators. NcRNAs have also been identified as important novel regulators of infectious disease risk factors and cell functions and are thus important candidates to improve diagnostics assessment. Beyond their application in diagnostic, ncRNA can also be the targets or tools of novel therapeutic strategies.

In recent years we have seen the growing number of studies related to ncRNAs, however there are still few studies related to leprosy. There is a lot to improve our understanding of leprosy pathophysiology. We provide an update on recent developments and perspectives for diagnostic use of ncRNAs in leprosy diseases and new therapeutic targets in different forms of leprosy infection.

7 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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5 DISCUSSÃO GERAL

A hanseníase é uma doença infectocontagiosa causada principalmente pelo bacilo *M. leprae*, que resulta da exposição prolongada ao patógeno, variáveis socioambientais e fatores genéticos do hospedeiro (Adams and Krahenbuhl 2003; Alter et al. 2011; Bhandari et al. 2021). No Brasil, a região amazônica conta com os mais elevados índices de incidência de hanseníase e estima-se que estes números estejam subnotificados (Barreto et al. 2012; Salgado et al. 2018a). Apesar da elevada incidência da hanseníase na população brasileira, ainda muito pouco é conhecido sobre fatores genéticos que possam influenciar na susceptibilidade e no desenvolvimento clínico desta doença.

Atualmente, ainda são escassas as investigações que tentam associar polimorfismos em genes de miRNA com a predisposição e o desenvolvimento de doenças infecciosas e, especialmente, com a hanseníase. Os miRNAs têm função regulatória e atuam nos mais diferentes processos biológicos, incluindo os da resposta imune (Jinnin 2014; Ha and Kim 2014; Schulte et al. 2019; Wang et al. 2021). Nós partimos da hipótese de que mutações presentes em genes miRNAs podem modificar significativamente a sua função regulatória, alterando, então, a expressão de genes relacionados a processos biológicos importantes, especialmente aqueles relacionados com a resposta imune.

Desta forma, a presente tese buscou investigar a associação de polimorfismos em genes de miRNA, *DROSHA* e *AGO1* com a suscetibilidade à hanseníase e ao desenvolvimento clínico da doença, em uma amostra da população da Amazônia brasileira. Adicionalmente, investigamos marcadores de ancestralidade genética na amostra investigada de forma a mensurar como a ancestralidade pode afetar na hanseníase *per se* e suas formas clínicas. Este projeto originou dois artigos, presentes no Capítulo I e Capítulo II deste documento.

No Capítulo I investigamos e analisamos a associação de vinte e cinco SNPs em genes de miRNA e relacionados à maquinaria de miRNAs com a suscetibilidade à hanseníase. A população amazônica estudada é considerada altamente miscigenada, formada pela mistura interétnica de três principais grupos parentais: europeus, africanos e nativo americanos (Santos et al. 2010; Manta et al. 2013; Bhaskar et al. 2017). Nossos resultados demonstram que há diferenças significativas na composição étnica das amostras investigadas. As diferenças se referem a maior contribuição de europeus no grupo de

pacientes do que no de controles (64% versus 57% de contribuição de ancestralidade europeia entre pacientes e controles) e menor contribuição de africanos entre os pacientes da amostra (16% versus 20% de contribuição de ancestralidade africana entre pacientes e controles). Quando comparamos apenas os pacientes não foi observada diferenças significativas na ancestralidade genética de pacientes com diferentes formas clínicas da hanseníase.

Nossos dados são diferentes daqueles descritos por Leturiondo *et al.* (2020) em uma amostra de pacientes de hanseníase e controles da população brasileira do estado do Amazonas, na região Norte do país. No entanto, os dados obtidos em ambas as investigações não são comparáveis na medida em que a investigação conduzida por Leturiondo e colaboradores, os grupos de pacientes e controles foram pareados por idade, sexo e ancestralidade genética. De forma diferenciada, nossa investigação empregou amostra de controles escolhida entre aqueles indivíduos contactantes dos pacientes, pareados por sexo e idade e, em função da forma de escolha, pareados também pelo nível socioeconômico.

No que pese a ancestralidade de pacientes e controles, nossos resultados são semelhantes ao descritos por Pinto e colaboradores (2015) com dados da mesma população amazônica aqui investigada (população do estado do Pará). Apesar de ambas as investigações serem conduzidas na mesma região as amostras são diferentes, principalmente no que se refere aos controles da amostra. Na investigação de Pinto e colaboradores os controles foram recrutados entre indivíduos sadios da população da mesma área geográfica. Na presente investigação os controles foram recrutados entre indivíduos sem sinais clínicos de hanseníase e de contato direto e permanente com os pacientes de forma que dividem o mesmo nível socioeconômicos deles.

De forma resumida, em ambas as investigações conduzidas na população do estado do Pará a contribuição de ancestralidade europeia é significantemente maior e a contribuição da ancestralidade africana é significativamente menor entre pacientes do que entre controles da população. Mais ainda, em ambas as investigações não foi possível detectar diferenças significativas de ancestralidade em portadores de formas paucibacilar e multibacilar.

Em relação aos polimorfismos estudados neste trabalho, nossos dados sugerem que o SNP rs2505901 (*pre-miR938*) é associado à proteção contra o desenvolvimento da hanseníase paucibacilar, enquanto os SNPs rs639174 (*DROSHA),* rs636832 (*AGO1*) e

rs4143815 (*miR570)* são associados à proteção contra o desenvolvimento da hanseníase multibacilar. Em contrapartida, os SNPs rs10739971 (*pri-let-7a1) e* rs12904 (*miR200C),* em modelo dominante, e rs2168518 *(miR4513),* em modelo recessivo, mostraram ser associados ao desenvolvimento da forma paucibacilar da doença. No caso de *pri-let-7a1*, a associação ocorre tanto em comparação aos indivíduos saudáveis quanto em comparação entre pacientes. Em modelo recessivo, rs10739971 (*pri-let-7a1)* foi associado ao desenvolvimento da hanseníase. O marcador rs10035440 (*DROSHA*) foi significativamente associado ao aumento de risco de desenvolvimento da hanseníase multibacilar, enquanto rs2910164 (*miR146A*), em teste entre pacientes, foi associado á diminuição de risco de hanseníase paucibacilar e consequente suscetibilidade ao risco de hanseníase multibacilar.

MiR938 é associado a vias regulatórias relacionadas à sobrevivência celular e apoptose (Wu et al. 2017; de Souza et al. 2020). Ademais, as citocinas inflamatórias IL-16 e IL-17A são potenciais alvos do miR938 (Mi et al. 2011). IL-6 é uma citocina de atividade pleiotrópica que atua na resposta inflamatória aguda e ativação de linfócitos Th17, também inibindo células T pró-inflamatórias e imunossupressoras (Tanaka et al. 2014). IL-17A é produzida por células CD4+ Th17, e está envolvida em neutrofilia, inflamação, destruição e reparo de tecidos através de controle de moléculas reguladoras (*programmed death-1/programmed death ligand-1*) (Sadhu and Mitra 2018), sendo também relacionada a episódios de reação reversa (RR) em pacientes hansenianos (Saini et al. 2013; Sales-Marques et al. 2017).

Variantes do tipo SNP do gene *pre-miR938* têm sido associadas a modificações na biogênese e estabilidade de miR938 (Mi et al. 2011). Em nosso estudo os genótipos TT/TC foram associados à proteção em pacientes com hanseníase paucibacilar. De acordo com dados de expressão de genótipos do portal GTEX, rs2505901 TT/CT (*pre-miR938*) possuem expressão diminuída em relação ao genótipo CC, mostrando que os genótipos TT/CT regularam negativamente a expressão do miR938 (Carithers et al. 2015).

Em estudo realizado por Sadhu e colaboradores, a frequência de células Th17 (CD4, CD45RO, IL-17) foi significativamente mais alta em pacientes BT/TT (Sadhu et al. 2016). Santos e colaboradores, encontrou concentrações mais altas de IL-17A em lesões de pacientes TT e soro de pacientes PB quando comparados a pacientes LL e MB, respectivamente, associando células Th17 a resposta inflamatória em pacientes PB (Santos et al. 2017). Em estudo prévios, a variante rs2505901 (*pre-mir938*) foi associada à redução

de risco de desenvolvimento de leucemia linfoblástica aguda (ALL) na população amazônica (de Souza et al. 2020), ao aumento de risco de doença Hirschsprung em crianças chinesas (Zhong et al. 2021) e suscetibilidade ao câncer gástrico em população japonesa (Arisawa et al. 2012).

O gene *DROSHA* codifica uma RNase tipo III e subunidade do complexo de microprocessadores de mesmo nome, que catalisa a etapa inicial de processamento de pri-miRNAs, produzindo pré-miRNAs (Lee and Shin 2017). A variante rs639174 (*DROSHA*) é um SNP intrônico com um reconhecido papel na regulação transcricional (López-López et al. 2014), e em nosso estudo o genótipo CC deste polimorfismo, em modelo dominante, foi associado à proteção contra hanseníase MB. Curiosamente, o genótipo TT do SNP rs10035440 (*DROSHA*), que também possui importante papel no *splicing* e regulação da transcrição do gene *DROSHA* foi associado ao risco de desenvolvimento da hanseníase MB em modelo dominante. Investigando o efeito destes polimorfismos no portal GTEX, o genótipo CC de rs639174 (*DROSHA*) é capaz de diminuir a expressão do gene em comparação aos outros genótipos (Carithers et al. 2015). Enquanto o genótipo TT de rs10035440 (*DROSHA*), de maneira inversa, é capaz de aumentar a expressão do gene (Carithers et al. 2015).

Estudos prévios associaram rs639174 (*DROSHA*) a recorrência de câncer de cabeça e pescoço (Zhang et al. 2010), enquanto rs10035440 (*DROSHA*) foi associado ao alto risco de desenvolvimento de tuberculose em população amazônica (Leal et al. 2022). Outros polimorfismos no gene *DROSHA* foram associados a ALL (López-López et al. 2014; Gutierrez-Camino et al. 2014; de Souza et al. 2020) e câncer de mama (Khan et al. 2014).

O gene *AGO1*, assim como *DROSHA*, também é relacionado a maquinaria de miRNAs. Este gene codifica um membro da família de proteínas argonautas, que são capazes de se associar a pequenos RNAs e possuem papel importante na interferência e silenciamento de RNAs e regulação transcricional de genes-alvo (Liu et al. 2018; Müller et al. 2020). AGO1 inibe a proliferação de células através da indução de apoptose, além de regular genes que influenciam no crescimento, sobrevivência e progressão do ciclo celular (Parisi et al. 2011; Huang et al. 2013; Kim et al. 2019).

A variante genética rs636832 (*AGO1*) encontra-se localizada na região íntron do gene, assim o mecanismo proposto para efeito dessa variante é sua potencial influência em *splicing* de

mRNAs ou via atividades de elementos reguladores intrônicos (Cooper 2010; Dobrijević et al. 2020). Em estudo realizado por Kim e colaboradores, o genótipo GG do polimorfismo rs636832 (*AGO1*), em modelo recessivo, foi associado à suscetibilidade à perda de gravidez recorrente em mulheres coreanas (Kim et al. 2019). A variante também é associada ao risco de algumas doenças, como câncer de pulmão (Kim et al. 2010) e metástase linfática em pacientes de câncer gástrico (Song et al. 2017) em população coreana, câncer de mama em mulheres do Mediterrâneo (Fawzy et al. 2020), doença de Basedow-Graves em população japonesa (Tokiyoshi et al. 2018), e hepatite B crônica (Shang et al. 2014), depressão (He et al. 2012) e falha recorrente de implantação embrionária (Ryu et al. 2019) em população chinesa.

O miR570 foi inicialmente identificado em células epiteliais das vias aéreas, envolvido na regulação da resposta inflamatória (Stellato et al. 2011). Em nosso estudo sugerimos que o genótipo GG do SNP rs4143815 (*miR570)*, que de acordo com o banco de dados GTEX, diminui a expressão de *miR570* (Carithers et al. 2015), pode estar associado a proteção contra a hanseníase MB.

Roff e colaboradores, observaram que miR570 é capaz de aumentar a expressão CCL4 e CCL5 e, ao mesmo tempo, inibir a expressão de CCL2 após forte estímulo inflamatório, indicando um complexo sistema de regulação direta e indireta (Roff et al. 2014). As quimiocinas desempenham importante papel na formação do granuloma em doenças causadas por micobactérias (Qiu et al. 2001; Roach et al. 2002). A quimiocina CCL4 possui diversos efeitos em vários tipos de células imune, funcionando como um quimioatraente para monócitos capaz de inibir ativação de células T (Geluk et al. 2012). Enquanto CCL5 possui importante papel na co-estimulação da proliferação de células T e ativação de células T localizadas em lesões inflamatórias (Bacon et al. 1995; Vesosky et al. 2010).

CCL2 é uma quimocina capaz de recrutar monócitos, células T de memória e células dendríticas para locais de lesão e infecção tecidual, sugerindo manutenção na integridade do granuloma em pacientes assintomáticos (Parkash 2009). De acordo com estudo de Queiroz e colaboradores, realizado com pacientes de hanseníase PB, MB e contatos domiciliares saudáveis, os níveis sorológicos de CCL2 estão aumentados nos contactantes, mas não entre os grupos de pacientes (Queiroz et al. 2021). Outro estudo mostra que a redução de CCL2

induzida por *M. leprae* na hanseníase MB, em conjunto com a redução de TNF, pode resultar na disseminação da doença (Hasan et al. 2006).

Variantes no gene *miR570* foram associadas ao risco de adenocarcinoma gástrico em população chinesa (Wang et al. 2013), proteção contra câncer de mama em mulheres do sudeste do Irã (Karami et al. 2020), associação com infecção por HTLV-1 e carga pró-viral em doadores de sangue assintomáticos em população iraniana (Hezave et al. 2022), risco e prognóstico de carcinoma hepatocelular em população chinesa da etnia Han (Xie et al. 2018) e suscetibilidade a diabetes tipo 1 (Qian et al. 2018).

A família de miRnas *let-7* desempenha importantes funções em diversos processos biológicos, entre eles inflamação, imunidade, proliferação e diferenciação celular (Lee et al. 2016; Jiang 2018). Alguns estudos também apontam os membros da família *let-7* como supressores tumorais em diversos tipos de câncer (Biamonte et al., 2019; Chirshev et al., 2019; Kumar et al., 2015; Lee and Dtta, 2007). O polimorfismo rs10739971 em *pri-let-7a-1* foi associado á suscetibilidade de câncer gástrico em populações chinesas (Xu et al. 2014; Li et al. 2016), e alto de risco de tuberculose pulmonar em população da região amazônica (Leal et al. 2022).

Em estudo de Salgado e colaboradores, relacionado a análise de expressão de miRnoma de lesões e sangue de pacientes com hanseníase, *hsa-let-7f-5p* foi hiporregulado em LP (Salgado et al. 2018b). Em estudo prévio realizado por Kumar e colaboradores, o perfil de miRNA de macrófagos infectados com *Mycobacterium tuberculosis* mostrou regulação negativa de miR-let-7f (Kumar et al. 2015). Esse microRNA tem como alvo A20, um inibidor da via NF-kB, assim a expressão de let-7f diminui e A20 aumenta com a progressão da infecção por *M. tuberculosis* em camundongos (Kumar et al. 2015). Wamber e colaboradores, relata que pacientes hansenianos PB, que apresentam maior reatividade imune contra *M. leprae,* exibiram menor ativação de NF-kB quando comparados a pacientes MB (Wambier et al. 2014).

O gene *miR200c* codifica um membro da família miR200, que é conhecida por desempenhar importante papel na transição epitélio-mesenquimal (EMT) (Gregory et al. 2008; Byun et al. 2019; Roth and Moorehead 2021). A EMT é um processo biológico responsável por fazer com que as células epiteliais polarizadas que interagem com a membrana basal percam sua adesão intracelular e adquiram fenótipo de célula mesenquimal, aumentando a capacidade de

migração, invasão, resistência à apoptose e induzindo fibrose (Kalluri 2009; Kalluri and Weinberg 2009; Lamouille et al. 2014). ZEB1 e ZEB2 são dois repressores transcricionais de E-caderina que são alvos diretos da família miR200, consequentemente inibindo EMT (Park et al. 2008; Brabletz and Brabletz 2010; Roth and Moorehead 2021).

De acordo com estudo de Salgado e colaboradores, miRNAs ZEB1/2 foram regulados negativamente em pacientes LL (Salgado et al. 2018b).Os miRnas de *SOX* foram hiper-regulados em LP, indicando que ZEB1/2 pode ser um regulador da expressão de *SOX2* (Salgado et al. 2018b). Estudos prévios com a variante rs12904 (*miR200C*) relatam suscetibilidade a câncer colorretal em população iraniana (Simonian et al. 2019) e chinesa (Mao et al. 2013), câncer gástrico (Li et al. 2014) e fator de proteção contra risco de acidente vascular cerebral isquêmico (Zeng et al. 2019).

O miR-4513 é um miRNA relacionado a proliferação celular, invasão, EMT e recentemente relatado como hiperexpresso em linhagens de células cancerosas (Ghanbari et al. 2014; Li et al. 2019; Xu et al. 2019; Ding et al. 2019; Kiel et al. 2021). De acordo com trabalho realizado por Xu e colaboradores, a regulação negativa de miR-4513 inibiu a proliferação celular, migração, invasão, e ao mesmo tempo promoveu apoptose (Xu et al. 2019).

Alguns polimorfismos na sequência *seed* de miR-4513 vêm sendo associados a diversas doenças humanas (Ghanbari et al. 2014; Ding et al. 2019; Kiel et al. 2021). Sequências *seed* são regiões críticas para especificidade de ligação aos alvos dos miRNAs (Stavast and Erkeland 2019). Quando maduros, os miRNAs são pequenos RNAs não codificantes de mais ou menos 22 nucleotídeos de comprimento. Após processamento e integração ao RISC, eles estão prontos para seu papel na regulação pós-transcricional, orientando o complexo aos seus transcritos alvo específicos. O reconhecimento e ligação ao mRNA alvo ocorre via pareamento de base complementar, no qual a região *seed*, que abrange os nucleotídeos 2-7 da região final 5' do miRNA, é de fundamental importância (Agarwal et al. 2015; Chipman and Pasquinelli 2019; Stavast and Erkeland 2019).

A variante rs2168518 (*miR4513*) é um exemplo de polimorfismo que está inserido na região *seed* de hsa-mir-4513, afetando a regulação deste miRNA. Estudos prévios relatam a associação desta variante com glicemia de jejum, traços lipídicos (Ghanbari et al. 2014), risco de doença caronariana (Ghanbari et al. 2014; Li et al. 2015; Mir et al.

2019), adenocarcinoma pulmonar (Zhang et al. 2019a) e degeneração macular relacionada à idade (Ghanbari et al. 2017).

O miR-146a é conhecido por seu importante papel na regulação de processos inflamatórios, além de afetar diretamente a proliferação celular e apoptose (Lu et al. 2010; Zhao et al. 2011; Boldin et al. 2011; Qiu et al. 2020). Este miRNA é altamente expresso em células Treg, sendo induzido após a ativação de células efetoras e células mieloides (Lu et al. 2010; Rusca and Monticelli 2011; Qiu et al. 2020). A hanseníase lepromatosa é tipicamente associada ao aumento de células Treg, que também apresentam maior expressão de IL-10 e CTL4-4 (Froes et al. 2022). Chaves e colaboradores, demonstraram que Tregs foram mais frequentes no grupo MB, e estas células também coexpressaram altas quantidades de PD1 e PDL-2, indicando que as Tregs podem induzir a apoptose de células efetoras e simultaneamente prevenir sua própria apoptose (Chaves et al. 2018).

A variante genética rs2910164 (*miR146A*) está localizada no meio de um *stem hairpin*, sugerindo que este SNPs em pré-miRNAs pode alterar a conformação da estrutura secundária, e consequentemente alterar a expressão do miRNA maduro (Shen et al. 2008; Harnprasopwat et al. 2010; Li et al. 2011). Shen e colaboradores, mostraram que essa variação de G para C em miR146A resultou em uma expressão elevada do miRNA maduro quando comparado a alelo comum (Shen et al. 2008).

Em estudo realizado por Cezar-de-Mello *et al*., o SNP rs2910164 (*miR146A*) foi associado a hanseníase em população do sudeste brasileiro, entretanto, não foi observada diferenças significativas entre pacientes PB e MB (Cezar-de-Mello et al. 2014). Em relação a doenças micobacterianas, Li e colaboradores, relataram que a variante rs2910164 (*miR146A*) desempenha papeis diferentes em duas populações étnicas distintas, com o alelo G aumentando o risco de tuberculose pulmonar em população tibetana, enquanto o alelo C aumenta o risco da doença em população da etnia Han (Li et al. 2011). Entretanto, em estudo realizado com a população iraniana, não houve associação significativa entre rs2910164 (*miR146A*) e risco de tuberculose (Naderi et al. 2015).

Nos últimos anos, a variante rs2910164 (*miR146A*) vem sendo associada a diversas doenças como o desenvolvimento de leishmaniose cutânea (de Mesquita et al. 2021), associação com doença de Behçet (Kamal et al. 2021; Shan et al. 2021), artrite inflamatória (Wang and Pan 2019), colite ulcerativa (Li et al. 2018), psoríase (Gong et al. 2019), ALL

(Zou et al. 2020), câncer de mama em população pasquitanesa (Ahmad et al. 2019), infecção crônica por vírus da hepatite B (HBV) em população iraniana (Khanizadeh et al. 2019), aumento de incidência de preeclampsia em pacientes com diabetes gestacional (Abo-Elmatty and Mehanna 2019), e alto risco de carcinoma hepatocellular (Dong et al. 2017).

O Capítulo II se refere a uma revisão de literatura sobre o que se sabe atualmente o papel de RNAs não codificantes (ncRNAs) na hanseníase. Os ncRNAs surgiram como importantes reguladores da expressão gênica em diversos tipos células, incluindo aquelas ligadas a resposta imune (Tamgue et al. 2021). A expressão dos ncRNAs é alterada em diferentes níveis fisiológicos e estão intrinsicamente ligados a uma variedade de doenças humanas, incluindo doenças infecciosas mycobacterianas (Schulte et al. 2019; Fathizadeh et al. 2020; Tamgue et al. 2021). Recentemente, o uso da tecnologia de RNA contra o COVID-19 causou um aumento considerável nos estudos de ncRNAs como moduladores de transcrição (Zhang et al. 2021; Schoenmaker et al. 2021; Bivona et al. 2021). NcRNAs também foram identificados como importantes novos reguladores de fatores de risco de doenças infecciosas e, portanto, são importantes candidatos a biomarcadores e alvo de novas estratégias terapêuticas (Acuña et al. 2020; Zhang et al. 2021).

Apesar do aumento crescente do estudo de ncRNAs nos últimos anos, ainda são escassos estudos relacionando estes com a hanseníase. Em nossa busca de literatura apenas encontramos trabalhos com hanseníanse e lncRNAs, piRNAs e miRNAs, sendo os miRNAs com maior número de estudos. Este artigo foi pensado a partir da constatação de que ainda existem poucos artigos de revisão sobre ncRNAs em hansensíase e suas diferentes formas clínicas. Este é um campo que ainda precisa ser vastamente explorado, especialmente no contexto atual.

6 CONCLUSÃO

Existem diversos trabalhos que abordam a genética do hospedeiro e sua relação com a hanseníase, mas ainda há muito o que ser descoberto sobre essa doença milenar e negligenciada. Aqui buscamos esclarecer alguns dos mecanismos moléculares relacionados à suscetibilidade desta doença, oferecendo resultados inéditos que ressaltam a importância do estudo de variantes em genes codificadores de miRNAs.

Nesta tese, também enfatizamos a necessidade de maiores estudos acerca de ncRNAs em hanseníase, para que possamos compreender melhor a patogênese e fisiopatologia da doença e qual o papel dessas moléculas no controle da resposta imune contra o patógeno *M. leprae*. O estudo de ncRNAs vem crescendo continuamente nos últimos anos e ainda é pouco explorado na hanseníase, apesar de seu grande potencial como biomarcador e na confecção de vacinas.

Por fim, estes achados podem fornecer informações valiosas para uma melhor compreensão de como os fatores genéticos do hospedeiro influenciam em variados aspectos da doença, com potencial para encontrar marcadores preditivos do desenvolvimento da hanseníase na população amazônica brasileira.

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ANEXOS: ARTIGOS PUBLICADOS PELA DISCENTE DURANTE O PERÍODO DO DOUTORADO

ANEXO I - Cavalcante GC, Schaan AP, Cabral GF, Santana-da-Silva MN, Pinto P, Vidal AF and Ribeiro-dos-Santos Â (2019) **A Cell's Fate: An Overview of the Molécular Biology and Genetics of Apoptosis. International Journal of Molécular Sciences** 20:4133. doi: 10.3390/ijms20174133

ANEXO II – Leal DF da VB, Santana da Silva MN, Fernandes DCR de O, Rodrigues JCG, Barros MC da C, Pinto PD do C, Pastana LF, Silva CA da, Fernandes MR, Assumpção PP de et al. (2020) **Amerindian genetic ancestry as a risk factor for tuberculosis in an amazonian population.** PLoS One 15:e0236033. doi: 10.1371/journal.pone.0236033

ANEXO III – Silva CA, Fernandes DCRO, Braga ACO, Cavalcante GC, Sortica VA, Hutz MH, Leal DFVB, Fernades MR, Santana-da-Silva MN, Valente SEL et al. (2020) **Investigation of genetic susceptibility to Mycobacterium tuberculosis (VDR and IL10 genes) in a population with a high level of substructure in the Brazilian Amazon region.** International Journal of Infectious Diseases 98:447–453. doi: 10.1016/j.ijid.2020.06.090

ANEXO IV – Leal DF da VB, Santana da Silva MN, Pastana LF, Fernandes MR, Athayde A do SC de, Fernandes Porchera DCR, Silva CA da, Modesto AAC, De Assumpcão PP, Santos SEB dos et al. (2022) **Genetic Variants of MicroRNA and DROSHA Genes in Association With the Risk of Tuberculosis in the Amazon Population.** Frontiers in Genetics 13: e850058. doi: 10.3389/fgene.2022.850058

ANEXO V - Porchera DCRF, Leal DFVB, Braga ACO, Pinto PDC, Santana da Silva MN, Bezerra Santos LC, Braga da Silva CH, da Costa GE, Barros MC da C, Athayde A do SC de et al. (2022) **Association of the rs4646994 in ACE gene with susceptibility to tuberculosis in a region of the Brazilian Amazon.** Translational Medicine Communications 7:10. doi: 10.1186/s41231-022-00116-6

Artigos publicados durante o período do Doutorado

ANEXO I

International Journal of *Molecular Sciences*

MDPI

Review

A Cell's Fate: An Overview of the Molecular Biology and Genetics of Apoptosis

Giovanna C. Cavalcante [1], Ana Paula Schaan [1], Gleyce Fonseca Cabral [1], Mayara Natália Santana-da-Silva [1], Pablo Pinto [1,2], Amanda F. Vidal [1,2] and Ândrea Ribeiro-dos-Santos [1,2,*]

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Abstract: Apoptosis is one of the main types of regulated cell death, a complex process that can be triggered by external or internal stimuli, which activate the extrinsic or the intrinsic pathway, respectively. Among various factors involved in apoptosis, several genes and their interactive networks are crucial regulators of the outcomes of each apoptotic phase. Furthermore, mitochondria are key players in determining the way by which cells will react to internal stress stimuli, thus being the main contributor of the intrinsic pathway, in addition to providing energy for the whole process. Other factors that have been reported as important players of this intricate molecular network are miRNAs, which regulate the genes involved in the apoptotic process. Imbalance in any of these mechanisms can lead to the development of several illnesses, hence, an overall understanding of these processes is essential for the comprehension of such situations. Although apoptosis has been widely studied, the current literature lacks an updated and more general overview on this subject. Therefore, here, we review and discuss the mechanisms of apoptosis, highlighting the roles of genes, miRNAs, and mitochondria involved in this type of cell death.

Keywords: regulated cell death; apoptosis; mitochondria; miRNAs; genetics

1. Introduction

The mechanisms underlying cell death and survival have a great impact on maintaining cellular balance, such that their deregulation may lead to the development of various diseases, such as multiple types of cancer and neurodegenerative disorders [1,2]. The classifications about cell death modalities depend mainly on morphological and structural details of individual tissues and cells [3]. Among the different types of cell death, apoptosis stands out as one of the most widely studied in the past years. Still, only a few articles provide a general and descriptive overview of apoptosis.

Apoptosis is a normal mechanism that can occur at any stage of the individual's development or upon cell damage, and is marked by the following characteristics: Protein cleavage (occurring mainly by the activation of cysteine proteases known as caspases), nuclear DNA breakdown, and apoptotic cell recognition by phagocytic cells [4]. These processes are responses to internal (intrinsic or mitochondrial) or external (extrinsic pathway or death receptors, DR) stimuli to the cell, converging in the final stage, known as the apoptotic execution phase (Figure 1).

There are many intricate aspects to both apoptotic pathways and the numerous molecules involved in their mechanisms. The study of the apoptotic pathway has shown to be a great approach in the search for new anticancer therapy, since promising compounds that trigger apoptosis are often non-toxic to healthy cells [5].

ANEXO II

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Investigation of genetic susceptibility to *Mycobacterium tuberculosis* (*VDR* and *IL10* genes) in a population with a high level of substructure in the Brazilian Amazon region

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ABSTRACT

Objectives: Tuberculosis (TB) is an infectious and contagious disease that has been very influential in human history and presents high rates of mortality. The objective of this study was to investigate the association of *VDR*, *IL10*, and *SLC11A1* gene polymorphisms with susceptibility to the presence of *Mycobacterium tuberculosis* infection.
Methods: A total of 135 patients with confirmed TB and 141 healthy individuals were included in the analysis. Blood samples were collected for DNA extraction. Genotyping of the polymorphisms in the *VDR* and *IL10* genes was performed by real-time PCR, and genotyping of the polymorphisms in the *SLC11A1* gene by conventional PCR, followed by visualization in polyacrylamide gel. The genomic ancestry was obtained using an autosomal panel with 48 insertion/deletion ancestry-informative markers.
Results: Polymorphisms TaqI (TT, $p = 0.004$), FokI (CC and CC + CT, $p = 0.012$ and $p = 0.003$, respectively), and BsmI (GG, $p = 0.008$) in the *VDR* gene, as well as A-592C (GC + AG, $p = 0.001$) in the *IL10* gene, were significantly associated with susceptibility to TB. In addition, high production of *VDR* combined with low production of *IL10* showed protection for the TB group ($p = 0.035$).
Conclusions: The *VDR* polymorphisms may confer an increased risk and the *IL10* haplotype may be a protection factor for the presence of *M. tuberculosis* infection in the Brazilian population.

1. Introduction

Tuberculosis (TB) is an infectious and contagious disease caused by *Mycobacterium tuberculosis* infection. One-fourth of the global population has a latent TB infection and 5–15% of people infected with *M. tuberculosis* bacteria develop the clinical disease. According to the World Health Organization (WHO), 10 million new TB cases and 1.3 million TB deaths among HIV-negative people were reported in 2017 (WHO, 2019).

TB may be considered a polygenic and multifactorial disease that results from a complex interaction between the host's genome, pathogen characteristics, and environmental conditions, including social and economic factors (Möller et al., 2010). However, the occurrence of TB at different rates among ethnicities and families suggests that human genetic variability is an important factor in the host response to *M. tuberculosis* infection and in regulating susceptibility and progression of the disease (Naranbhai, 2016; Kinnear et al., 2017; Turner et al., 2017; Abel et al., 2018).

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ANEXO III

PLOS ONE

RESEARCH ARTICLE

Amerindian genetic ancestry as a risk factor for tuberculosis in an amazonian population

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Abstract

In recent years, the incidence of tuberculosis (TB) has declined worldwide, although this disease still occurs at relatively high rates in Amerindian populations. This suggests that the genetic ancestry of Amerindians may be an important factor in the development of infections, and may account for at least some of the variation in infection rates in the different populations. The present study investigated the potential influence of Amerindian genetic ancestry on susceptibility to tuberculosis in an Amazon population. The study included 280 patients diagnosed with tuberculosis and 138 asymptomatic hospital employees with no history of TB, but who were in contact with bacterially active TB patients. Ancestry analysis was run on a set of 61 Ancestry-Informative Markers to estimate European, African, and Amerindian genetic ancestry using STRUCTURE v2.2. The TB group had significantly higher Amerindian ancestry in comparison with the control group, and significantly lower European ancestry. Amerindian ancestry in the 20–60% range was found to be the principal risk factor for increased susceptibility to TB. The results of the study indicate that Amerindian ancestry is an important risk factor for susceptibility to TB in the admixed population of the Brazilian Amazon region.

Introduction

Tuberculosis (TB) is an infectious disease caused by the *Mycobacterium tuberculosis* bacillus (MTB). In 2018, 10 million incident cases of TB were registered, resulting in 1.2 million TB deaths, a number higher than that caused by Human Immunodeficiency Virus [1,2]. While the incidence of TB and the total number of deaths, worldwide, have declined slightly in recent years, TB continues to be a major endemic concern for the amerindian people of the Brazilian Amazon [3,4].

In amerindian population tend to have much higher rates of TB in comparison with the general (non-indigenous) population [5–7]. This disparity has been attributed to multiple factors, including environmental and socioeconomic variables, co-infections with other

ANEXO IV

frontiers in Genetics

ORIGINAL RESEARCH
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Genetic Variants of MicroRNA and DROSHA Genes in Association With the Risk of Tuberculosis in the Amazon Population

Diana Feio da Veiga Borges Leal[1], *Mayara Natália Santana da Silva*[2], *Lucas Favacho Pastana*[1], *Marianne Rodrigues Fernandes*[1]*, *Aidalucy do Socorro Costa de Athayde*[1], *Débora Christina Ricardo Fernandes Porchera*[1], *Cleonardo Augusto da Silva*[1], *Antônio André Conde Modesto*[1], *Paulo Pimentel De Assumpcão*[1], *Sidney Emanuel Batista dos Santos*[1,2] and *Ney Pereira Carneiro dos Santos*[1]

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Tuberculosis (TB) is a chronic infection caused by *Mycobacterium tuberculosis* (Mtb) with high incidence and mortality. Studies reported that host genetic variants might be associated with the risk of tuberculosis. The aim of this study was to perform an association study between 26 single nucleotide polymorphisms (SNPs) and tuberculosis and evaluate whether these SNPs may confer risk factors to tuberculosis in the Amazon population. There were 52 males and 126 females, with total of 178 healthy controls. Genotyping was performed using TaqMan Open Array Genotyping. Ancestry-informative markers were used to estimate the ancestral proportions of the individuals in the case and control groups. The results indicated that the SNPs rs10035440 (DROSHA), rs7372209 (miR26-a1), rs1834306 (miR100), rs4919510 (miR608), and rs10739971 (pri-let-7a-1) were significantly associated with high risk and rs3746444 (miR499) and rs6505162 (miR423), with low risk of developing tuberculosis in the Amazon population. Our study concluded that seven miRNA polymorphisms were associated with tuberculosis. Our study contributes to a better understanding of TB pathogenesis and may promote the development of new diagnostic tools against *M. tuberculosis* infection.

Keywords: genetic variants, microRNA, drosha, tuberculosis, risk factor

1 INTRODUCTION

Tuberculosis (TB) is a chronic infection caused by *Mycobacterium tuberculosis* (Mtb), and it remains one of the most serious infections with high incidence and mortality, mostly in developing countries. According to the World Health Organization (WHO) report, there were an estimated number of 10 million new TB cases and 1.4 million deaths worldwide in 2019 (WHO 2020).

Early detection and appropriate treatment are the most important control strategy for TB. However, there is a low rate of TB cases detected and officially notified. In addition, smear microscopy and culture are the "gold standards" for the diagnosis of TB; however, faster and

ANEXO V

Porchera *et al.*
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Translational Medicine Communications

RESEARCH Open Access

Association of the rs4646994 in *ACE* gene with susceptibility to tuberculosis in a region of the Brazilian Amazon

Débora C. R. F. Porchera[1,2], Diana F. V. B. Leal[2], Ana C. O. Braga[2], Pablo D. C. Pinto[1,2], Mayara N. Santana da Silva[1], Lucas C. Bezerra Santos[1], Cintia H. Braga da Silva[1], Giovana E. da Costa[2], Maria Clara da C. Barros[2], Aidalucy do S. C. de Athayde[2], Amanda de N Cohen-Paes[2], Cleonardo A. da Silva[2], Paulo P. de Assumpção[2], Ândrea K. C. Ribeiro-dos-Santos[1,2], Sidney E. B. dos Santos[1,2], Marianne R. Fernandes[2] and Ney P. C. dos Santos[1,2*]

Abstract

Background: Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* and represents an important global public health issue. Single-nucleotide polymorphisms and INDELs are common genetic variations that can be located in genes associated with immune response and, therefore, they may have direct implications over the phenotype of susceptibility to infections like tuberculosis. This study aimed to investigate the association between the 17 genetic polymorphisms and susceptibility to tuberculosis in a Brazilian population.

Methods: This case-control study enrolled 283 individuals with active tuberculosis and 145 health care workers. Four INDELs and 13 single nucleotide polymorphisms and were genotyped using Multiplex PCR method and TaqMan SNP Genotyping Assays. Group comparisons for categorical variables were performed using the chi-squared test, whilst the t-Student test was used to analyze the continuous variables. Multiple logistic regression analyses were performed to estimate the odds ratio (OR) with 95% confidence intervals (CI). Deviation from Hardy-Weinberg equilibrium was assessed using chi-squared tests with Bonferroni correction. The results were analyzed comparing the genotypic distributions adopting the dominant model and the estimated values of p corrected for multiple tests through FDR (False Discovery Rate) test.

Results: The HWE test confirmed that the genotypic frequencies for polymorphisms were balanced. The frequency of Del allele was 73 and 75%, in cases and controls respectively. Frequency of Del allele was significantly higher in the control group than TB group. The homozygous Del/Del genotype was present in 51.6% of cases and 58.6% of controls. The rare Ins/Ins genotype was present in only 7.6% of controls and 6% of cases. The *ACE* Del/Del genotype was significantly higher in the cases than in controls revealing significant protection for TB in the domain model ($OR = 0.465$; $p < 0.005$).

Conclusions: The Del/Del genotype of the rs4646994 in *ACE* gene was associated with susceptibility to tuberculosis. The identification of genetic variants responsible for susceptibility to tuberculosis will allow the development of new

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