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TESE DE DOUTORADO

DETERMINANTES IMUNOGENÉTICOS PARA TUBERCULOSE

JUAN MANUEL CUBILLOS ANGULO

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JUAN MANUEL CUBILLOS ANGULO

Tese apresentada ao Curso de Pós-Graduação em Patologia Humana para a obtenção do grau de Doutor

Orientador: Dr. Bruno Bezerril Andrade

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COMISSÃO EXAMINADORA

Dr. José Roberto Lapa e Silva
Professor
UFRJ

Dr. Afrânio Lineu Kritski
Professor
UFRJ

Dr. Marcelo Cordeiro dos Santos
Professor
UEA

Dr. Antônio Ricardo Khouri Cunha
Pesquisador
IGM/ FIOCRUZ

Dr. Bruno de Bezerril Andrade
Pesquisador
IGM/FIOCRUZ

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Dedico esse
trabalho a minha família Helena, Victor, Taryn e
meu filho Manuel Martin

“Não existe
triunfo sem perda, não há vitória sem
sofrimento, não há liberdade sem sacrifício.”

(**John Ronald Reuel Tolkien**)

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RESUMO

Estima-se que aproximadamente um quarto da população global está infectada com *Mycobacterium tuberculoses* (*Mtb*). Polimorfismos genéticos do hospedeiro podem ser importantes na determinação da suscetibilidade à infecção *Mtb*, porém seu papel não é totalmente compreendido. No presente estudo, em uma coorte de contatos próximos de pacientes com TB pulmonar confirmados microbiologicamente atendidos em ambulatório de referência de TB no Rio de Janeiro, identificamos potenciais biomarcadores genéticos de suscetibilidade à infecção (conversão do teste tuberculínico (TST)) por *Mtb* e desenvolvimento de TB ativa, de forma prospectiva e retrospectiva. Ambos os estudos foram realizados em contatos de casos de TB pulmonar confirmados microbiologicamente em laboratórios de referência, no Rio de Janeiro. No estudo prospectivo estudamos diferentes "*single nucleotide polymorphims*" (SNPs) como fatores de risco para conversão do TST e desenvolvimento de TB: *TLR2* (rs5743708), *TLR4* (rs4986791), *TNFA* (rs361525), *IFNG* (rs2430561), *IL1B* (rs1143627). Entre os 526 participantes, 60 tiveram conversão no TST e 44 desenvolveram TB ativa durante o acompanhamento. Na análise de regressão multivariada observou-se que os SNPs em genes *TLR4* (odds ratio [OR]: 62,8, intervalo de confiança de 95% [IC 95%]: 7,5–525,3) e *TNFA* (OR: 4,2, IC 95%: 1,9–9,5) foram independentemente associados à conversão no TST. No segundo estudo retrospectivo, 7 SNPs adicionais foram testados para associação com positividade do TST: genes candidatos *IFI16-PYHIN1-AIM2* (rs1101998, rs1633256, rs866484), *IFIT5* (rs59633641, rs10887959), *IFIT1* (rs304478, rs304498) e *IRF7* (rs11246213). No estudo retrospectivo foram examinados 482 contatos, dos quais 296 contatos apresentaram TST positivo. Em um modelo multivariável, observamos que no modelo recessivo o SNP *PYHIN1-IFI16-AIM2* rs1101998 (OR ajustado [aOR] = 2,90; IC 95% = 1,24-6,78; p = 0,014) e rs1633256 (aOR = 10,1; IC 95% = 2,20-46,28; p = 0,003) foram associados a um risco aumentado de reatividade no TST. Finalmente, realizamos uma revisão sistemática para avaliar a associação entre todos os SNPs relatados de *CD14* e *NOD2* e a ocorrência de TB, e como essa associação pode diferir em populações étnicas distintas. Foram incluídos, 13 estudos que preencheram os critérios de seleção. Destes, 9 foram investigados do gene *CD14* e em 6 foi relatada uma associação significativa entre o alelo T e os genótipos TT do SNP rs2569190 e aumento do risco de TB. Ademais, em 4 estudos foram relatadas relações entre os SNPs do gene *NOD2* e a TB, e destes, foram observadas associações significativas de rs1861759 e rs7194886 com maior risco de TB em uma população chinesa Han em 2 estudos. Os resultados sugerem associações entre polimorfismos dos genes da imunidade e as probabilidades de infecção e/ou adoecimento por *Mtb*. No intuito de promover conhecimento sobre fatores imunogenéticos em TB na presente análise, foram apresentadas associações entre polimorfismos de genes relacionados à imunidade e as probabilidades de infecção e/ou adoecimento por *Mtb*.

Palavras chave: *Mycobacterium tuberculosis*. Polimorfismo de nucleotídeo único. Teste Cutâneo da Tuberculina. CD14. NOD2. Receptores Toll-Like. Fator de Necrose Tumoral.

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ABSTRACT

Mycobacterium tuberculosis (*Mtb*) infection affects approximately a quarter of the global population. Host genetic polymorphisms may be important in determining susceptibility to Mtb infection, but their role is not fully understood. In a first study, different SNPs were tested as risk factors for tuberculin skin test (TST)conversion and development of Tuberculosis (TB): *TLR2* (rs5743708), *TLR4* (rs4986791), *TNFA* (rs361525), *IFNG* (rs2430561), *IL1B* (rs1143627). In a second study, seven additional SNPs were tested for association with TT positivity: candidate genes *IFI16-PYHIN1-AIM2* (rs1101998, rs1633256, rs866484), *IFIT5* (rs59633641, rs10887959), *IFIT1* (rs304478, rs730449.8), and *IRF7* (rs11246213). Both studies were conducted on contacts of microbiologically confirmed pulmonary TB cases in reference laboratories. Finally, we performed a systematic review to assess the association between *CD14* and *NOD2* reported polymorphisms and *Mtb* diseases, and how this association might differ in distinct ethnic populations. In the prospective study, among the 526 participants, 60 had a conversion to TT, and 44 developed active TB during follow-up. Multivariate regression analysis demonstrated that SNPs in *TLR4* genes (odds ratio [OR]: 62, 8, 95% confidence interval [95% CI: 7.5–525.3) and *TNFA* (OR: 4.2, 95% CI: 1.9–9.5) were independently associated with TT conversion. In the retrospective studio outside 482 contacts were examined, of which 296 contacts had positive TT. In a multivariate model, we observed in the recessive model that *PYHIN1-IFI16-AIM2* rs1101998 (adjusted OR [aOR] = 2.90; 95% CI = 1.24-6.78; p = 0.014) and rs1633256 (aOR = 10, 1; 95% CI = 2.20-46.28; p = 0.003) were associated with an increased risk of TT positivity. In the systematic review, thirteen studies met the selection criteria. Of these, nine investigated *CD14* SNPs and six reported a significant association between the T allele and the TT genotypes of SNP rs2569190 and increased risk of *Mtb* disease. In addition, four studies reported data finding the relationship between *NOD2* SNPs and Mtb disease risk, with two reporting significant associations of rs1861759 and rs7194886 and increased risk of *Mtb* disease in a Han Chinese population. The results suggest associations between immunity-related genes polymorphisms and the probabilities of *Mtb* infection. This study contributes to the understanding of associations between immunity-related genes polymorphisms and the probabilities of *Mtb* infection.

Keywords: Mycobacterium tuberculosis. Single nucleotide polymorphism. Tuberculin skin test. CD14. NOD2. Toll-like receptor. Tumor necrosis factor.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

TB	Tuberculose
Mtb	*Mycobacterium tuberculosis*
COVID 19	Doença do coronavírus de 2019
HIV	Vírus da imunodeficiência humana
LTBI	Infecção Latente por Tuberculose
TNF-α	Fator de necrose tumoral
TST	Teste tuberculínico
PCR	Reação em cadeia da polimerase
IGRAs	Testes de liberação de interferon-γ
ONU	Assembleia Geral das Nações Unidas
TB-DR	Tuberculoses droga resistente
TB-MDR	Tuberculose Multidroga Resistente
NK	Células natural killer
IFN-γ	Interferon-gama
CD1b	Cluster de diferenciação 1b
ATS	*American Thoracic Society*
LES	Lúpus eritematoso sistêmico
H3K4	Histona H3 lisina 4
miRNAs	Micros RNAs
SNPs	Polimorfismos de nucleotídeo único
GWAS	Estudos de associação ampla do genoma
TLRs	Receptores *Toll-like*
PRRs	Receptores de reconhecimento de padrões
VDR	Receptor da vitamina D
IRFs	Fator regulador de interferon
NOD2	*Nucleotide Binding Oligomerization Domain Containing 2*
CD14	*Cluster Differentiation antigen 14*

SUMÁRIO

1 **INTRODUÇÃO**

1.1 EPIDEMIOLOGIA DA INFECÇÃO POR MYCOBACTERIUM TUBERCULOSIS (*Mtb*)

A tuberculose (TB) é uma doença infecciosa de proporções pandêmicas com mais de 1 bilhão de pessoas mortas nos últimos 200 anos (PAULSON, 2013). Hoje, a TB é uma das princiPAIs causas de problemas de saúde, uma das 10 princiPAIs causas de morte no mundo e continua sendo uma das princiPAIs causa de morte humana por um único agente infeccioso (WHO, 2020). Por volta de 10,0 milhões de pessoas adoeceram de TB em 2019 causando cerca de 1.4 milhão neste mesmo ano (WHO, 2020). Embora a incidência global de tuberculose tenha diminuído lentamente durante os últimos anos, no continente americano, especificamente no Brasil observou-se uma tendência de aumento nos últimos anos (DHEDA; BARRY *et al.*, 2016). Porém, os efeitos da pandemia do SARS-CoV-2 (covid-19) afetaram o diagnóstico de novos casos da doença. Nas Américas observou-se uma queda entre 15% e 20%, em 2020 e neste mesmo ano, no Brasil, foram menores os casos de TB notificados, correspondendo a uma queda de 10,9% comparado aos casos registrados em 2019 (RIBEIRO; TELLES *et al.*, 2021). Existem dados limitados sobre o risco de doença grave ou mortalidade em pacientes com TB e COVID-19, mas uma revisão sistemática e meta-análise para avaliar si a TB está associada a um risco aumentado de doença grave e morte em pacientes com covid-19 (GAO; LIU *et al.*, 2021). Esta conseguiu confirmar em seis estudos da china onde se mostram que existe uma associação de um aumento de 2,10 vezes no risco de doença grave por Covid-19 (GAO; LIU *et al.*, 2021).

O risco de exposição ao *Mtb* pode afetar qualquer pessoa em qualquer lugar e depende de comportamentos sociais e de risco. A maioria das pessoas que desenvolvem a doença são adultos, com mais casos em homens do que em mulheres (HARGREAVES; BOCCIA *et al.*, 2011). Um dos maiores riscos de exposição é o morar ou trabalhar em ambientes com superlotação e uma ventilação reduzida (BAKER; DAS *et al.*, 2008). A tuberculose é uma doença relacionada à pobreza, já que a desnutrição aumenta a suscetibilidade e se há visto que nas pessoas de baixa renda o diagnóstico é retrasado o que aumenta o tempo de exposição a um paciente com TB infecciosa (ERLINGER; STRACKER *et al.*, 2019).

Além dos fatores socioeconômicos, outros fatores de risco para TB também já foram amplamente descritos. Entre estes, destaca-se o vírus da imunodeficiência humana (HIV) que é um dos determinantes de alta prevalência do desenvolvimento de TB ativa já que pacientes infectados pelo HIV apresentam maior risco de desenvolver TB em comparação com pessoas

sem HIV (BELL; NOURSADEGHI, 2018). Adicionalmente, a tuberculose pode agravar a doença HIV-1 e AIDS devido ao aumento da replicação viral que pode contribuir para a progressão da doença por HIV-1 (BELL; NOURSADEGHI, 2018). Se estima que em 2019, 208.000 das mortes totais de tuberculoses se apresentaram entre pessoas HIV-positivas (WHO, 2020). A coinfecção por *Mtb* e HIV-1 potencializam um ao outro, aumentando o risco de TB ativa, progressão da doença por HIV-1 acelerando a deterioração das funções imunológicas (BRUCHFELD; CORREIA-NEVES *et al.*, 2015). O risco de TB ativa aumenta para 2 a 5 vezes em indivíduos infectados pelo HIV-1 durante as fases iniciais e crônicas da infecção (BELL; NOURSADEGHI, 2018). Quando o HIV-1 progride e causa imunodeficiência grave, o risco de TB aumenta para pelo menos 20 vezes mais do que na população geral (BELL; NOURSADEGHI, 2018). Em ambientes de alta carga, a coinfecção por HIV aumenta a susceptibilidade a infecção primaria ou reinfecção e a ativação da TB nos pacientes com TB latente (BRUCHFELD; CORREIA-NEVES *et al.*, 2015).

Um dos maiores problemas com a TB é que pode ser reativada pela infecção latente de TB (LTBI) (SHEA; KAMMERER *et al.*, 2014). A maioria da TB ativa resulta da reativação da LTBI, e também pode ocorrer se o sistema imunológico do indivíduo tem alguma deficiência e não pode mais conter a bactéria latente (CHEE; REVES *et al.*, 2018). Os inibidores do fator de necrose tumoral alfa (TNF-α) estão associados ao aumento do risco de reativação da LTBI, uma vez que o ressurgimento da doença foi registrado em pacientes com terapias direcionadas ao anti-TNF-α para o tratamento de diferentes doenças como artrite reumatoide (NOGUEIRA; WARREN *et al.*, 2021). Recentemente um meta-análises demostrou que a prevalência global de LTBI não é mais um terço da população mundial, mas está perto de um quarto, (COHEN; MATHIASEN *et al.*, 2019). Se estima que 56 milhões de pessoas correm alto risco de desenvolver tuberculose devido a uma reinfecção recente (HOUBEN; DODD, 2016) e existe uma taxa geral de reativação de TB entre pessoas com LTBI de aproximadamente 0,084 casos por 100 pessoas-ano (SHEA; KAMMERER *et al.*, 2014). O rastreamento de contatos de pacientes com TB é uma das estratégias mais importantes para interromper a transmissão e posterior desenvolvimento da TB visto que ajuda a identificar esses contatos com LTBI (DE AGUIAR; DA SILVA VIEIRA *et al.*, 2020).

Atualmente, não existe um padrão-ouro para o diagnóstico de LTBI dado que a quantidade de *Mtb* é pequena nestes pacientes, fazendo com que o diagnóstico dependa principalmente da reação imunológica do hospedeiro, e não da própria bactéria (AI; RUAN *et al.*, 2016). Existem dois testes de triagem disponíveis atualmente para LTBI: o teste tuberculínico (TST) e os testes de liberação de interferon-γ (IGRAs, incluindo o

QuantiFERON-TB Gold e o teste T-SPOT.TB) (AI; RUAN *et al.*, 2016).

As duas diferentes formas de infecção da TB (TB ativa e LTBI) foram analisadas, e de fato se indica que não são estados binários como se pensava e agora são vistos como um espectro dinâmico (SOUSA; SARAIVA, 2019). Os estudos em modelos animais e humanos sugerem um curso mais complexo, onde pessoas com LTBI mantem uma relação com o Mtb através da regulação do sistema imunológico inato e adquirido e a disponibilidade de nutrientes 30021818 (DRAIN; BAJEMA *et al.*, 2018). Por este curso da infecção é que existe a TB subclínica, que é uma TB que não causa sintomas clínicos relacionados a TB mas causa anormalidades que podem ser confirmadas bacteriologicamente ou por reação em cadeia da polimerase (PCR) (FRASCELLA; RICHARDS *et al.*, 2021). A TB incipiente é um tipo de doença onde é provável que o paciente evoluirá para TB ativa na ausência de profilaxia; o interessante é que a infecção ainda não induziu anormalidades radiográficas, sintomas clínicos ou evidências microbiológicas consistentes com doença de TB ativa (DRAIN; BAJEMA *et al.*, 2018).

No ano de 2019, trinta países foram responsáveis por 21% da carga total de TB no mundo, e o Brasil permanece entres estes com uma incidência de TB de 39-53 casos por 100.000 habitantes (WHO, 2020). A maior parte da população Brasileira vive em áreas urbanas (mais do 85%) onde a incidência de TB supera a taxa nacional e o número de casos de LTBI também aumenta (REBEIRO; COHEN *et al.*, 2020). Em uma pesquisa com uma amostra representativa da população do conhecimento sobre TB e LTBI foi alto, mas este aumento do conhecimento foi associado a maiores taxas de estigma o que poderia limitar os esforços de prevenção da TB (REBEIRO; COHEN *et al.*, 2020).

O Brasil junto à China e à Índia, deram passos firmes para liderar a agenda de pesquisa da TB, em 2015. O Brasil estabeleceu sua Estratégia Nacional de Pesquisa em TB (PAI, 2018), resultando em ser um dos poucos países com alta carga que possuem diretrizes nacionais para o controle do LTBI. Essas diretrizes, são fornecidas em um documento de política separado e específico, baseado em um estudo que fez os levantamentos das políticas dos Programas Nacionais de TB em países com alta carga (FAUST; RUHWALD *et al.*, 2020).

Em 26 de setembro de 2018, a Assembleia Geral das Nações Unidas (ONU) realizou a primeira Reunião de Alto Nível sobre a luta contra a tuberculose (ONU, 2019). Nesta reunião, líderes de todos os Estados-Membros da ONU se comprometeram a acabar com a epidemia de tuberculose até 2030 e foi realizada uma declaração política listando marcos tangíveis e específicos de cada país a serem alcançados até 2022 (ONU, 2019). Umas das metas seria tratar 3.5 milhões de crianças para a TB, mas apenas o 30% das metas foram atingidas (CHAKAYA;

KHAN *et al.*, 2021). Os principais avanços foram: 1. Aumento na capacidade de diagnóstico de Tuberculoses resistente (TBR) (MONEDERO-RECUERO; GEGIA *et al.*, 2021); 2. Novos regimes de terapia preventiva (CHAKAYA; KHAN *et al.*, 2021); 3. O desenvolvimento de novos regimes orais para Tuberculose Multirresistente (TBMR) (CHAKAYA; KHAN *et al.*, 2021).

Em 2020, a pandemia COVID-19 desalojou a TB da principal causa de mortalidade em doenças infecciosas em todo o mundo (CHAKAYA; KHAN *et al.*, 2021). De acordo com as estimativas, as primeiras ondas da pandemia COVID-19 podem ter aumentado a mortalidade por TB em 13% devido ao desvio de instalações e recursos humanos e econômicos para seu tratamento (MONEDERO-RECUERO; GEGIA *et al.*, 2021). Notavelmente, os esforços globais de controle da TB não estavam em andamento, mesmo antes do advento da pandemia COVID-19 (CHAKAYA; KHAN *et al.*, 2021). É crucial que os programas mundiais do controle da TB melhorem as fraquezas sistêmicas existentes para poder conseguir cumprir as metas ambiciosas durante os próximos anos; a pandemia do COVID-19 e sua rápida disseminação desvendou as fraquezas intrínsecas dos sistemas de saúde e se identificou a necessidade de criar uma base de saúde forte sobre a qual construir programas específicos para doenças como a TB.

1.2 IMUNOLOGIA DA INFECÇÃO POR MTB

1.2.1 **Complexo Mycobacterium tuberculosis**

O gênero Mycobacterium compreende mais de 170 espécies e se se originou há mais de 150 milhões de anos, a maioria de estas espécies são organismos ambientais (GAGNEUX, 2018). O *Mtb* é parte do complexo Mycobacterium tuberculoses, onde também estão inclusas outras espécies sendo um grupo de bacilos álcool-ácido resistentes intimamente relacionados (>99% de similaridade na sequência de nucleotídeos) (ACHTMAN, 2008). Tradicionalmente, todas as espécies do gênero Mycobacterium foram divididas em espécies de crescimento rápido e de crescimento lento, sendo o complexo Mycobacterium parte de este último grupo (GAGNEUX, 2018). A Maioria dos casos de TB é atribuída ao *Mtb* ou ao organismo intimamente relacionado o *Mycobacterium africanum*; uma minoria dos casos é devida a membros zoonóticos do complexo Mycobacterium tuberculosis, como *Mycobacterium bovis* ou *Mycobacterium caprae* (PAI; BEHR *et al.*, 2016). Além disso, várias das chamadas micobactérias não tuberculosas podem causar doenças em indivíduos imunocomprometidos

(GAGNEUX, 2018).

A tuberculose é uma doença que tem atormentado a humanidade ao longo da história, iniciando na pré-história (DANIEL, 2006). Os estudos genômicos no complexo Mycobacterium tuberculosis demonstraram vários milhares de polimorfismos o que mostra uma deriva genética acumulada que pode ser associada a padrões de migração nos humanos (PAI; BEHR *et al.*, 2016). O complexo Mycobacterium tuberculosis faz parte de uma espécie antiga que infectou hominídeos desde suas origens e inicialmente consistia em um grupo altamente diverso de organismos, acredita-se que este grupo de organismos tenha evoluído nos últimos 15.000-20.000 anos (ACHTMAN, 2008).

A visão atual é que o complexo Mycobacterium tuberculosis emergiu como um patógeno profissional de um Mycobacterium ambiental por meio de uma adaptação gradual a um meio intracelular (GAGNEUX, 2018). Uma das hipóteses da origem é que tinham a capacidade de sobrevier em protozoários tipo amebas, que se alimentavam de bactérias ambientais (GAGNEUX, 2018). Isto poderia explicar a capacidade que tem atualmente o *Mtb* de infectar e se multiplicar nos macrófagos dos mamíferos uma das caraterísticas mais importantes dentro de este grupo (GAGNEUX, 2018).

1.2.2 **infecção do *mtb***

Existe uma coevolução entre a interação do patógeno (*Mtb*) e o hospedeiro (*homo sapiens*) que tem levado milhares de anos. O sucesso da infecção, sobrevivência e como se espalha o *Mtb* se evidencia nas estimativas de que um quarto da população global é sensibilizada ao *Mtb* (SCRIBA; COUSSENS *et al.*, 2017). A transmissão de *Mtb* ocorre após a inalação de gotículas aerossolizadas contendo bactérias vivas, chegando a os pulmões (SIA; RENGARAJAN, 2019). Para que esta transmissão seja bem-sucedida existem uma grande variedade de condições como a proximidade e duração do contato com um indivíduo portador de TB ativa e as diferentes condições que possam gerar algum risco de imunocompetência no indivíduo infectado com *Mtb* (SIA; RENGARAJAN, 2019).

Uma vez inalado, o *Mtb* viaja da traqueia para os pulmões, onde é fagocitado por macrófagos alveolares, nos quais é internalizado nos fagossomos e, em seguida, nos fagolisossomos (PIETERS, 2008) (**figura 1**). Apesar disso, o *Mtb* tem a capacidade de bloquear a acidificação ou interromper a maturação do fagossomo para sobreviver, permitindo que o *Mtb* se replique dentro dos macrófagos, o tipo de célula mais competente para matar micróbios intracelulares (EHRT; SCHNAPPINGER, 2009). Macrófagos e outras células imunes agregam-

se para formar o granuloma (PAGAN; RAMAKRISHNAN, 2018). A hipóxia é um tipo de estresse a qual são expostos os bacilos do *Mtb*, promovendo a dormência das micobactérias (DUTTA; KARAKOUSIS, 2014). Este estado dormente do *Mtb* resulta na capacidade de parecer silencioso para o sistema imunológico e poder persistir nos tecidos do hospedeiro por meses ou mesmo anos, sem causar tuberculose, e resultando em infecção crônica assintomática em até 90% das pessoas infectadas com LTBI (CARDONA; RUIZ-MANZANO, 2004). Por outro lado, 5% das pessoas infectadas desenvolverão TB ativa, enquanto outras poderão eliminar o patógeno (CARDONA; RUIZ-MANZANO, 2004). Nestes pacientes com TB ativa, a presença de colônias de *Mtb* e os sintomas não aparecem simultaneamente, mas provavelmente se desenvolvem com o tempo (TURNER; CHIU *et al.*, 2017). É interessante que os indivíduos com doença cavitária e alta carga bacilar tenham maior probabilidade de liberar *Mtb* no meio ambiente, está claro a partir de estudos epidemiológicos que nem todos esses pacientes são infecciosos (TURNER; CHIU *et al.*, 2017).

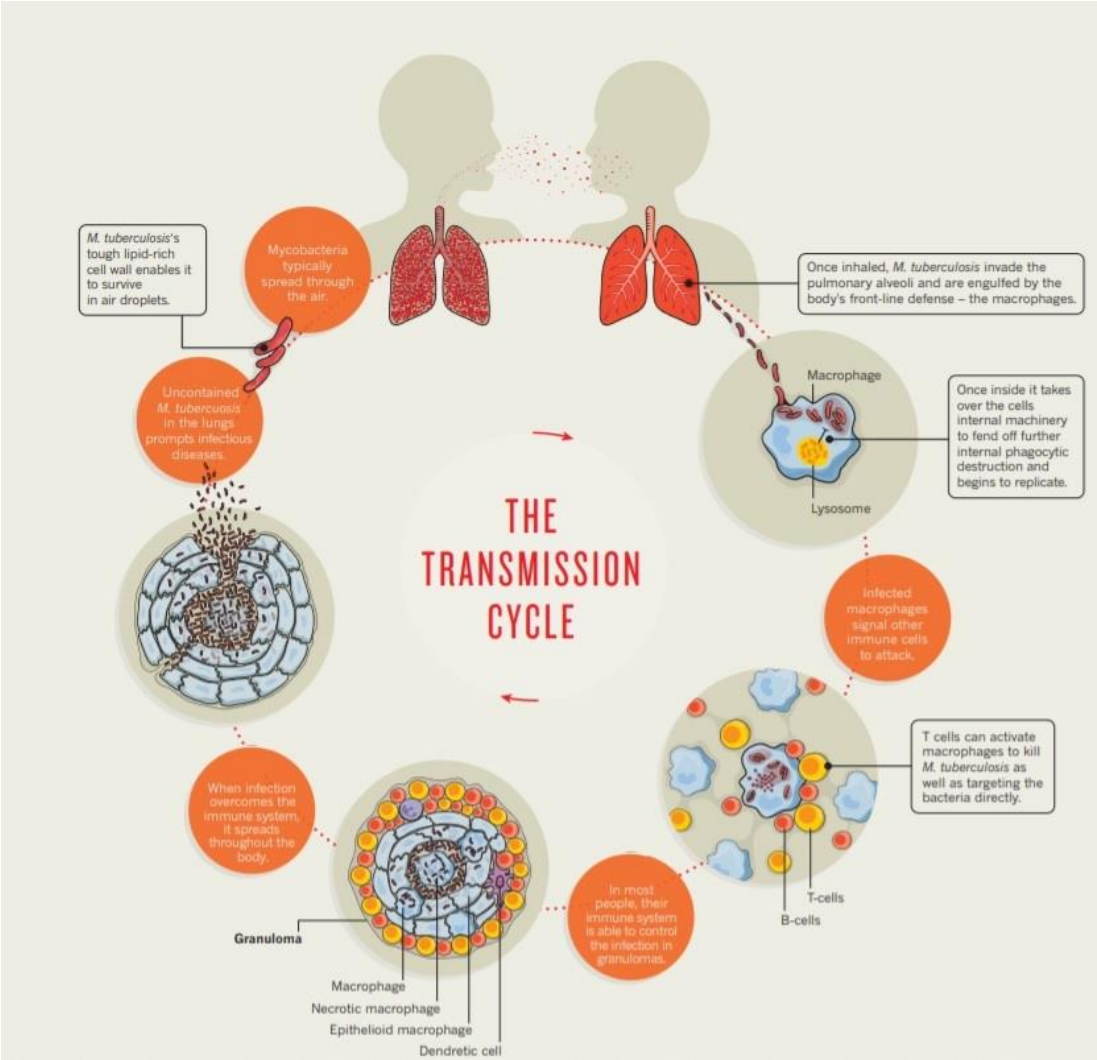

Figura 1 - Ciclo biológico do *Mycobacterium tuberculosis*
Fonte: (PAULSON, 2013).

1.2.3 **O granuloma**

A tuberculose é a causa mais frequente de granulomas, que são agregados organizados, compactos e localizado de células imunes como macrófagos, monócitos, células dendríticas, neutrófilos, células epitelioides e células gigantes multinucleadas que se formam em resposta a estímulos persistentes de natureza infecciosa ou não infecciosa (CADENA; FORTUNE *et al.*, 2017) (**figura 2**). Essa estrutura inicial é circundada por uma camada de linfócitos conferindo-lhe uma estrutura sólida organizada (NDLOVU; MARAKALALA, 2016).

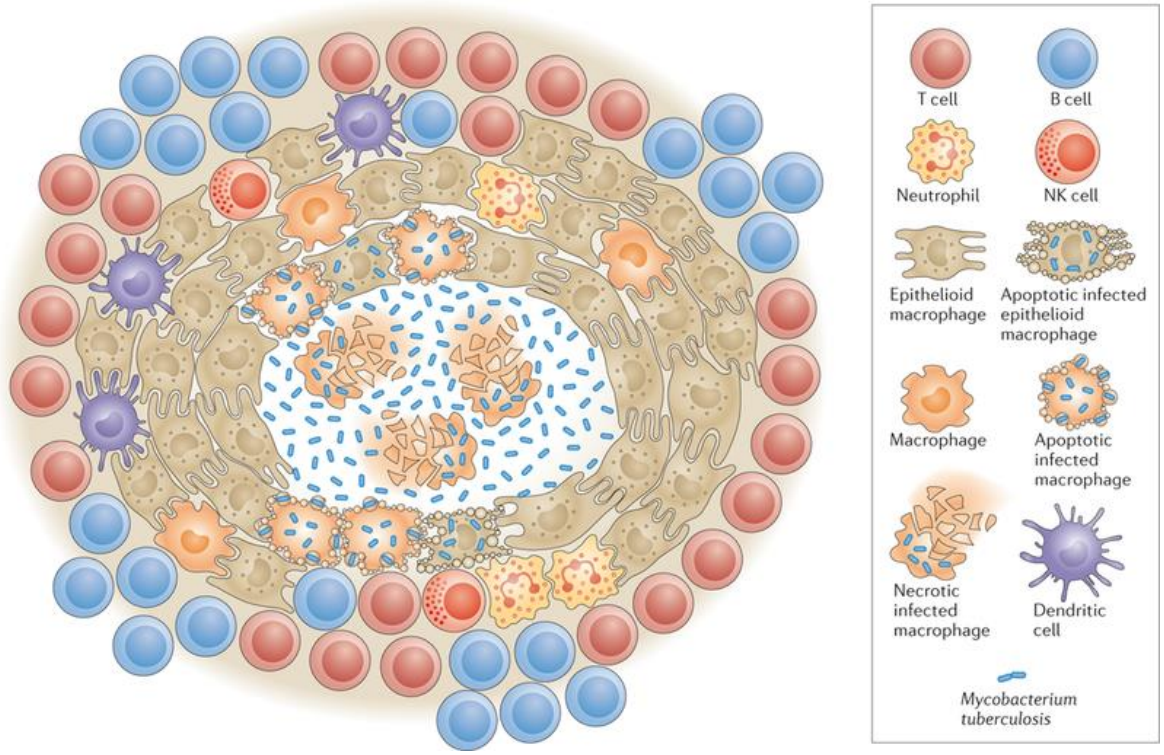

Figura 2 - Composição clássica de um granuloma produzido pela infecção do *Mycobacterium tuberculosis*
Fonte: (Cadena; Fortune; Flynn, 2018).

Após a infecção bem-sucedida do *Mtb* inicia-se um choque de sinais pró-inflamatórios e anti-inflamatórios nos pulmões que levam ao desenvolvimento do granuloma que pode impedir a disseminação bacteriana para os locais extrapulmonares (SIA; RENGARAJAN, 2019). Os mediadores da inflamação podem tanto promover quanto limitar a disseminação bacteriana, um estado pro-inflamatório pode levar à remodelação dentro do granuloma, este processo está ligado ao início da doença ativa, e são necessários para cavitação nas vias aéreas vizinhas e transmissão bem-sucedida de *Mtb* (CADENA; FORTUNE *et al.*, 2017). Ao contrário, a resolução da inflamação no granuloma e nos pulmões está associada a um melhor resultado do hospedeiro, um risco reduzido de reativação e um melhor prognóstico a longo

prazo após o tratamento (CADENA; FORTUNE *et al.*, 2017).

Os primeiros eventos que levam a formação do granuloma foram estudados em um modelo de peixe-zebra (DAVIS; RAMAKRISHNAN, 2009). O estudo mostrou que as bactérias se replicam livremente nos macrófagos e que as *Mtb* intracelulares usam o lócus ESX-1 / RD1 para induzir o recrutamento e a motilidade de novos macrófagos não infectados para as áreas circundantes das células (DAVIS; RAMAKRISHNAN, 2009). Além disso, a proteína de virulência micobacteriana ESAT-6 induz a secreção de metaloproteinase-9 da matriz do hospedeiro por células epiteliais para conduzir o recrutamento de novos macrófagos para o granuloma (NDLOVU; MARAKALALA, 2016). Assim o granuloma está composto por um agregado de macrófagos infectados e não infectados com *Mtb* em vários estágios de maturação e diferenciação (SIA; RENGARAJAN, 2019). Os macrófagos no granuloma podem sofrer uma transformação epitelioide, tornar-se macrófagos espumosos cheios de lipídios ou fundir-se em células gigantes multinucleadas. Este granuloma se desenvolve progressivamente em um ambiente com vários tipos de estresse ambiental, mais notavelmente hipóxia onde muitas células sofrem morte necrótica (macrófagos, células *natural killer* (NK), neutrófilos, células dendríticas e fibroblastos circunscritos por linfócitos T e B) para formar um núcleo acelular denominado caseum (RAMAKRISHNAN, 2012). O *Mtb* entra em estado de 'persistência não replicante' para sobreviver no ambiente hostil caracterizado por crescimento lento e mudança metabólica que reduz sua suscetibilidade à pressão ambiental, aliás o *Mtb* por meio da indução de redes transcricionais complexas como o regulon DosR provoca a angiogênese para melhorar o transporte de oxigênio (CADENA; FORTUNE *et al.*, 2017).

O granuloma pode sofrer eventos de remodelação complexos impulsionados por fatores bacterianos e do hospedeiro, resultando em algumas mudanças estruturais que coincidem com a progressão da tuberculose. Citocinas inflamatórias como TNF-α e IFN-γ são críticas para o desenvolvimento de granuloma e são secretadas por macrófagos infectados com *Mtb* no início do processo de infecção, acelerando o recrutamento de células no granuloma (JAGATIA; TSOLAKI, 2021).

Os medicamentos anti-tuberculose atualmente disponíveis para tratamento, continuam sendo o regime padrão de seis meses de isoniazida, rifampicina, pirazinamida e etambutol (QUIST-HANSSEN; THORUD *et al.*, 1979). Estes não apenas tornam os bacilos defeituosos por meio de mecanismos únicos, mas também têm algum efeito sobre a resposta imunológica e o granuloma (JAGATIA; TSOLAKI, 2021). Por exemplo a rifampicina mostrou aumentar a expressão de cluster de diferenciação 1b (CD1b), que é encontrada em macrófagos ativados por citocinas, aumentando assim a resposta das células T ao *Mtb* (TENTORI; GRAZIANI *et al.*,

1998). A isoniazida induz a apoptose de CD4 + Células T, autofagia e maturação fagossômica em resposta a *Mtb* (TOUSIF; SINGH *et al.*, 2014). A pirazinamida modula a resposta imune do hospedeiro à infecção por *Mtb*, reduzindo a produção e liberação de citocinas pró-inflamatórias como, IL-1β, IL-6 e TNF-α em monócitos infectados com *Mtb* (MANCA; KOO *et al.*, 2013). Também existem novas drogas para o tratamento de TB, como a Bedaquiline que inativa a *ATP synthase* bacteriana e, portanto, esgota o ATP no patógeno (CHOLO; MOTHIBA *et al.*, 2017).

1.3 ASPECTOS GERAIS DA TRANSMISSÃO DE *MTB* EM CONTACTANTES

O contato na tuberculose se refere a alguém que foi exposto à infecção por Mtb compartilhando ambiente aéreo com uma pessoa com tuberculose infecciosa. Um contato próximo é definido como viver na mesma casa ou estar em contato frequente com um caso de origem/índice (por exemplo, o cuidador) com TB e baciloscopia positiva(CDC, 2005). Em 1976, a *American Thoracic Society* (ATS) publicou breves diretrizes para a investigação, avaliação diagnóstica e tratamento médico de contatos com TB. Recentemente, em 2005, essas diretrizes foram atualizadas para a investigação de contatos e tratamento de contatos infectados pois são componentes importantes da estratégia para a eliminação da TB (CDC, 2005).

Uma definição de "contato doméstico" é baseada na localização, como uma área comum para comer ou dormir, ou uma duração mínima de exposição ou grau de proximidade. As definições de contato próximo também variaram, incluindo qualquer exposição conhecida, outras como o íntimo, o compartilhar, o ar por um período prolongado ou especificar uma duração mínima de exposição em outros espaços fechados, como o local de trabalho (FOX; BARRY *et al.*, 2013).

As prioridades para investigação de contato são determinadas com base nas características do paciente índice, suscetibilidade e vulnerabilidade dos contatos e circunstâncias das exposições (PANG, 2014).Certos fatores estão contribuindo para a transmissão em contatos (REICHLER; KHAN *et al.*, 2018), a extensão da doença no paciente índice, a duração que a fonte e o contato estão juntos e sua proximidade, circulação de ar local, condições médicas que prejudicam a competência imunológica (PARK; HAN *et al.*, 2020), a carga infecciosa de *Mtb* (WARRIA; NYAMTHIMBA *et al.*, 2020), virulência da cepa particular de *Mtb* (REILING; HOMOLKA *et al.*, 2013) e a predisposição intrínseca de um contato para infecção ou doença (NARASIMHAN; WOOD *et al.*, 2013). As condições intrínsecas e adquiridas do contato afetam a probabilidade de progressão da doença TB após a infecção, embora o valor preditivo de certas condições possa ser impreciso como a única base

para a atribuição de prioridades.

A idade dos contatos: Após a infecção, a tuberculose é mais provável de ocorrer em crianças mais novas (MARAIS; GIE *et al.*, 2004); o período de incubação ou latência é mais breve; e as formas invasivas letais da doença são mais comuns (GOLLA; SNOW *et al.*, 2017). A incidência da doença específica por idade para crianças com resultados de TST positivos diminui até os 4 anos de idade. Crianças com menos de 5 anos que são contatos têm alta prioridade para investigação, porque quando as crianças ficam mais velhas, há uma probabilidade crescente de positividade do TST devido à exposição acumulada à TB relacionada à idade.

Status imunológico dos contatos: A infecção por HIV resulta na progressão da infecção por *Mtb* para TB ativa como doença mais frequente e rapidamente do que qualquer outro fator conhecido e uma maior disseminação da doença extrapulmonar (GOLDEN; VIKRAM, 2005). No caso contrário é também uma coinfecção de interesse na saúde pública (BASTOS; TAMINATO *et al.*, 2019). A TB acelera a progressão da doença HIV, por isso os contatos infectados pelo HIV e / ou adultos com abuso de substâncias (tabagismo, mastigar o khat e abuso de álcool) devem ser priorizados, assim como uma vigilância extra para a doença TB é recomendada (ALEMU; AWOKE *et al.*, 2016).

Contatos com tratamento de >15mg de prednisona ou seu equivalente por um tempo >4 semanas também devem ter alta prioridade (VELAYUTHAM; JAYABAL *et al.*, 2020). Outros agentes imunossupressores, incluindo múltiplos agentes de quimioterapia para câncer, drogas anti-rejeição para transplante de órgãos e antagonistas do TNF-a e pacientes com lúpus eritematosos sistêmico (LES) contribuem para a doença TB após a infecção; esses contatos também precisam de seguimento com alta prioridade (GHOSH; PATWARDHAN *et al.*, 2009).

Outras condições médicas que são preditores importantes para o risco de TB entre os contatos são: o IMC (peso em quilogramas dividido pelo quadrado da altura em metros), especificamente o estado de baixo peso - IMC <18,5 kg / m2 levou a uma previsão de maior risco de TB (AIBANA; ACHARYA *et al.*, 2016), sendo, portanto, peso abaixo do recomendado para determinada altura relatado como um fator fracamente preditivo que promove a progressão para TB (KITONSA; NALUTAAYA *et al.*, 2020); silicose e diabetes mellitus (CHAWLA; GUPTA *et al.*, 2020); período pós-operatório após o bypass gástrico, a deficiência de zinco e proteínas são condições que afetam adversamente as respostas imunológicas mediadas por células e podem ser risco da incidência de TB em contatos (ISRAR UL HAQ; TALIB *et al.*, 2018).

Exposição dos contatos ao caso índice: A ventilação domiciliar está relacionada à

tuberculose pulmonar. O volume de ar, a taxa de exaustão e a circulação predizem a probabilidade de transmissão em um espaço fechado. Houve correlação entre ventilação (difusão e circulação local) e incidência de TB pulmonar. Em grandes ambientes internos, por causa dos padrões de difusão e circulação local, o grau de proximidade entre os contatos e o paciente índice pode influenciar a transmissão (SAUNDERS; WINGFIELD *et al.*, 2017). A circulação local e a ventilação geral do cômodo também diluem as partículas infecciosas. A CDC sugere um sistema para categorizar os contatos por tamanho do cômodo (por exemplo, "1" sendo o tamanho de um veículo ou carro, "2" o tamanho de um quarto ", 3 "o tamanho de uma casa e" 4 "um tamanho maior que uma casa) (CDC, 2005).

As associações existentes de fatores meteorológicos com casos de TB (por exemplo, umidade e luz) são impraticáveis para incorporar na tomada de decisão, porém existe uma correlação entre fatores ambientais domésticos e a incidência de transmissão de TB pulmonar em domicílios (XU; LI *et al.*, 2020). A condição de iluminação foi um fator de risco significativo devido a que com pouca iluminação, o desenvolvimento de germes da TB pulmonar aumenta, pois, a luz solar é um dos fatores que podem matar os germes da TB pulmonar. Assim, se a iluminação for boa, a transmissão e proliferação de germes podem ser reduzidas (FATHMAWATI; RAUF *et al.*, 2021).

O TST não pode discriminar entre infecções recentes e antigas, ou seja, uma exposição mínima aumentada não seria relevante, de maneira que diminui a importância para a saúde pública encontrar resultados positivos de TST. É mais provável que um resultado positivo em contatos com exposição mínima seja o resultado de uma infecção antiga ou de sensibilidade inespecífica à TST (COOK; SHAH *et al.*, 2012). Sempre que a exposição do contato ao paciente com TB ocorreu <8-10 semanas necessárias para a detecção de testes cutâneos positivos, repetir o teste 8-10 semanas após a exposição mais recente ajudará a identificar conversões de TST recentes, que são provavelmente indicativas de infecção recente (COOK; SHAH *et al.*, 2012).

A probabilidade de infecção depende da intensidade, frequência e duração da exposição. Para estimar o risco após a exposição a uma pessoa com TB pulmonar sem cavidades pulmonares inclui um mínimo de 120 horas de exposição por mês ou como importante preditor de LTBI um há limite de risco de 250 horas de exposição (REICHLER; KHAN *et al.*, 2020).

Investigar um contato de maneira eficaz envolve a avaliação sistemática dos contatos de pacientes conhecidos com TB para identificar doença ativa ou LTBI. A infecção por LTBI é definida como quando os bacilos da TB estão em estado latente e os indivíduos que abrigam esses organismos são considerados portadores de infecção latente (PANG, 2014). Portanto, a LTBI pode ser definida como infecção pelo complexo *Mycobacterium tuberculosis*, em que a

bactéria pode estar viva, mas em estado de dormência e não causar atualmente nenhuma doença ou sintomas ativos (PANG, 2014).

A investigação de contatos de pacientes com TB é uma prioridade para o controle da TB em países de alta renda e está cada vez mais sendo considerada em países com recursos limitados (FOX; BARRY *et al.*, 2013). As informações iniciais dos contatos devem ser coletadas, as atribuições prioritárias devem ser reavaliadas para cada contato e um plano médico para testes de diagnóstico e possível tratamento deve ser formulado para contatos de alta e média prioridade. A identificação precoce de TB ativa entre os contatos significa uma melhor chance de cura e uma redução na transmissão posterior (PANG, 2014).

Além disso, a investigação de contato também permite a identificação de pessoas que estão infectadas de forma latente e com alto risco de TB ativa, as informações de saúde podem incluir (SCHWOEBEL; KOURA *et al.*, 2020):

1 Infecção ou doença por *Mtb* anterior e tratamento relacionado;

2 Relatório verbal do contato e documentação dos resultados anteriores do TST;

3 Sintomas atuais de tuberculose (por exemplo, tosse, dor no peito, hemoptise, febre, calafrios, suores noturnos, perda de apetite, perda de peso, mal-estar ou fadiga fácil);

4 Condições médicas ou fatores de risco que tornam a tuberculose mais provável (por exemplo, infecção por HIV, uso de drogas intravenosas, diabetes mellitus, silicose, corticoterapia prolongada, outra terapia imunossupressora, câncer de cabeça ou pescoço, doenças hematológicas e reticuloendoteliais, doença renal em estágio terminal, bypass intestinal ou gastrectomia, síndrome de má absorção crônica ou baixo peso corporal);

5 Distúrbios de saúde mental (por exemplo, doenças psiquiátricas e distúrbios de abuso de substâncias);

6 Tipo, duração e intensidade da exposição à TB; e

7 Fatores sociodemográficos (por exemplo, idade, raça ou etnia, residência e país de nascimento).

O uso dessas sete etapas de informações de saúde pode permitir uma pontuação de risco para uma investigação de contato em nível individual e pode facilitar a triagem direcionada, vigilância e terapia preventiva para contatos adultos/crianças que têm maior probabilidade de se beneficiar.

Para crianças e outras pessoas de alto risco, será necessária profilaxia (tratamento preventivo de infecção presumida por TB durante o tempo que normalmente levaria para um TST ou IGRA para se tornarem positivos após exposição (COLE; NILSEN *et al.*, 2020).

Um ponto notável é que o único fator de risco independente para desenvolver TB ativa ou infecção latente foi o contato de um caso índice com esfregaço positivo (MANDAL; CRAXTON *et al.*, 2012). A triagem de contatos casuais identificou TB em pacientes com TB pulmonar com baciloscopia negativa e TB não pulmonar. As diretrizes atuais do NICE recomendam o rastreamento de contatos casuais apenas se o índice caso seja esfregaço positivo ou se o hospedeiro for imunocomprometidos (PAI; KALANTRI *et al.*, 2006). A triagem de contatos casuais deve ser limitada a casos em que a doença ativa tenha sido identificada em contatos próximos ou onde contatos casuais suscetíveis já tenham sido identificados devido ao custo-benefício do rastreamento de contato (MANDAL; CRAXTON *et al.*, 2012).

1.4 FATORES GENÉTICOS SUBJACENTES À PATOGÊNESE DA TB

Uma das grandes perguntas sobre a infecção do *Mtb* é, por que todos os indivíduos expostos à bactéria não são infectados? pois a maioria dos indivíduos permanecerá assintomática e conterá a bactéria (MOLLER; KINNEAR *et al.*, 2018). A intensidade da exposição é um fator importante para permanecer ou não livre do bacilo e daqueles que se infectam com *Mtb*, só uma pequena proporção, desenvolverá formas clínicas da doença, enquanto a maioria controlará a infecção e perceberá um estado de LTBI (ORLOVA; SCHURR, 2017). Mas não só a intensidade da exposição pode afeitar o resultado da infecção acredita-se que os fatores genéticos humanos também influenciam o resultado da infecção pelo *Mtb* e assim como os fenótipos clínicos da TB (DUBE; FAVA *et al.*, 2021). Em uma revisão do ano 2014 (ABEL; EL-BAGHDADI *et al.*, 2014), acharam que os estudos de agregação familiar forneceram as evidências de que cada etapa subjacente à infecção ou doença é controlada por fatores genéticos do hospedeiro, por exemplo em famílias com um paciente índice de TB com escarro positivo, cônjuges com história familiar de TB desenvolveram TB manifesta com mais frequência do que aqueles sem tal história (ABEL; EL-BAGHDADI *et al.*, 2014). Outro aspecto interessante é como em ambiente de TB endêmicos alguns adultos que são intensa e repetidamente expostos ao *Mtb* permanecem negativos para reatividade no teste de IGRA, esses indivíduos podem ser definidos clinicamente como resistentes à infecção (SIMMONS; STEIN *et al.*, 2018). Alguns estudos genéticos indicam que fatores genéticos do hospedeiro modulam a resistência ao *Mtb* (SIMMONS; STEIN *et al.*, 2018). A heterogeneidade das respostas do hospedeiro ao *Mtb* mostra que o complexo Mycobacterium tuberculosis tem evoluído para parasitar humanos há milênios e, dentro desse tempo, é possível que a relação nos mudou geneticamente também, quando e onde o *Mtb* era endêmico (DUBE; FAVA *et al.*,

2021).

A contribuição dos fatores hereditários para a susceptibilidade ou a resistência clínica a infecção por *Mtb* foi reconhecida em alguns estudos epidemiológicos (MOLLER; KINNEAR *et al.*, 2018). Em 1943, um estudo investigou a concordância de TB e descobriu que gêmeos monozigotos eram significativamente mais propensos a ficarem doentes, quando existisse um grau de parentesco próximo com algum caso índice com TB (KALLMANN; REISNER, 1943). Em outro estudo com famílias sul-africanas (área de tuberculose hiperendêmica), a herdabilidade das respostas quantitativas de liberação de IFN-γ foi estimada entre 43 e 58%, e a herdabilidade da frequência de IFN-γ^{+} $CD4^{+}$ e IFN-γ^{+} $CD8^{+}$ específicos do antígeno foi estimada em 53-74% (COBAT; GALLANT *et al.*, 2010). Existem respostas transcriptômicas especificas para *Mtb*, por isso é possível que o transcriptôma possa ser útil como um biomarcador para identificar estágio distintos da patogênese da TB (ORLOVA; SCHURR, 2017). Estudos em populações isoladas como os indígenas das américas com pouca ou nenhuma exposição ao *Mtb* mostraram uma mortalidade por TB significativamente maior do que populações não indígenas cujos ancestrais tiveram exposição ao *Mtb* (SOUSA; SALEM *et al.*, 1997).

A regulação epigenética surge aqui como uma estratégia empregada para modular as respostas imunes inflamatórias do hospedeiro no início da infecção da TB (MOLLER; KINNEAR *et al.*, 2018). As alterações epigenéticas são alterações moleculares que podem manipular de forma independente a função do gene e o fenótipo subsequente, sem alterar a sequência de nucleotídeos (GAUBA; GUPTA *et al.*, 2021). Metilação do DNA é a adição de grupos metil (-CH3) à 5ª posição de resíduos de citosina e são responsáveis pela repressão transcricional ou silenciamento de genes (GAUBA; GUPTA *et al.*, 2021). A capacidade do *Mtb* de modular o epigenoma do hospedeiro reside na proteína Rv1988, uma DNA metiltransferase que promove a metilação do DNA do hospedeiro e reprime os genes envolvidos na primeira linha de defesa contra as bactérias (KHOSLA; SHARMA *et al.*, 2016). Outra modificação que pode existir ocorre nas histonas, estas são proteínas que ajudam a enrolar o DNA para criar unidades estruturais chamadas nucleossomas (JENUWEIN; ALLIS, 2001). A partir da estrutura o nucleossoma, uma cauda N-terminal se estende para fora e se converte no principal alvo para modificações epigenéticas como fosforilação, metilação, ubiquitinação, acetilação entre outras (JENUWEIN; ALLIS, 2001). *Bacillus Calmette – Guérin* (BCG) causa modificações epigenéticas nas histonas que remodelam a cromatina, resultando na expressão gênica para uma resposta mais robusta como, por exemplo, *in vitro*, onde o BCG induziu o aumento da trimetilação na histona H3 lisina 4 (H3K4), um marcador ativador, de várias citocinas pró-

inflamatórias (GAUBA; GUPTA *et al.*, 2021). Contudo, O *Mtb* pode causar hipoacetilação da histona H3K4 que já foi associada à TB pulmonar ativa (CHEN; CHAO *et al.*, 2017). A modificação epigenética da acetilação de histonas tem implicações para resistência a TB e desempenha um papel regulador significativo na expressão de genes e na secreção de enzimas metaloproteinase de matriz que conduzem a imunopatologia de TB (MOLLER; KINNEAR *et al.*, 2018).

Os micros RNAs (miRNAs) são moléculas pequenas de RNA não codificantes que atuam na regulação pós-transcricional da expressão gênica e afetam a função de muitos tipos de células imunológicas (MEHTA; LIU, 2014). Os miRNAs são reguladores poderosos de várias atividades celulares, incluindo crescimento celular, diferenciação, desenvolvimento e apoptose (GHANAVI; FARNIA *et al.*, 2020). Atualmente o papel dos miRNAs e sua relação com muitas doenças continua a ser desvendado, sendo umas das mais estudadas a interação entre os miRNAs e as infecções por micobactérias (SALIMINEJAD; KHORRAM KHORSHID *et al.*, 2019). Os primeiros estudos dos miRNAs mostraram que estes podiam ser usados como marcadores para distinguir entre TB ativa, LTBI ou outras infecções microbianas (MOLLER; KINNEAR *et al.*, 2018). Foi revelado que os perfis de expressão gênica nos macrófagos e nas NK são alterados entre a TB ativa e a LTBI, entre a TB e os controles saudáveis, e está regulada provavelmente por miRNAs (HARAPAN; FITRA *et al.*, 2013). O miRNA-29 é um dos mais estudados na patologia da TB, tendo sido documentado em experimentos clínicos e *in vitro* que este miRNA tem uma superexpressão em vários tipos de células após a infecção por *Mtb* e pode ser devido à relação com a supressão da resposta imune contra o *Mtb* pela regulação negativa que causa do IFN-γ (HARAPAN; FITRA *et al.*, 2013).

1.5 INFLUÊNCIA DOS POLIMORFISMOS GENÉTICOS NA SUSCETIBILIDADE À TUBERCULOSE

A progressão e o resultado patogênico da TB estão predeterminados por fatores como a complexidade do sistema imunológico, a genética intrínseca, polimorfismos e interações com o ambiente extrínseco (SINGH; BAGAM *et al.*, 2017). As variações genéticas podem gerar resultados variados na resposta imunológica em diferentes hospedeiros, isto significa que ainda existem muitas perguntas sobre como os fatores genéticos do hospedeiro determinam o resultado das interações com o *Mtb* (FOL; DRUSZCZYNSKA *et al.*, 2015). Avanços recentes no sequenciamento de DNA levaram à descoberta de polimorfismos de nucleotídeo único (SNPs), está variação genética mudou nossa compreensão de como os SNPs estão relacionados com o desenvolvimento da TB (STUCKI; GAGNEUX, 2013).

SNPs são a forma mais comum de variação genética que existem em todos os organismos vivos. Todos os humanos têm quase a mesma sequência de mais ou menos 3 bilhões de bases de DNA, mas em certos locais há diferenças essas variações são chamadas de SNPs (STUCKI; GAGNEUX, 2013). O termo "SNP" é, com frequência, usado alternadamente com "mutação", "polimorfismo" ou "substituição, mas para poder definir uma mutação como um SNP é preciso estar presente a uma frequência de pelo menos 1% em uma determinada população humana (CASILLAS; BARBADILLA, 2017). Se uma nova variante se torna fixa em uma população (100% da população tem a nova variante) se denomina como "substituição" (CASILLAS; BARBADILLA, 2017). As estimativas atuais indicam que até 0,1% do DNA humano pode variar um pouco, o que significa que dois indivíduos não relacionados podem diferir em menos de 3 milhões de posições de DNA (STUCKI; GAGNEUX, 2013). A importância dos SNPs vem de sua capacidade de influenciar o risco de doenças, a eficácia dos medicamentos e os efeitos colaterais, de falar sobre sua ancestralidade e prever aspectos de sua aparência e até mesmo de sua ação.

Uma das novas técnicas para estudo da herdabilidade das respostas imunes no *Mtb* são os estudos de ampla associação do genoma (GWAS) que descobriram que os SNPs influenciam as respostas imunes múltiplas (MESSINA; NETEA *et al.*, 2020). Estes estudos, ao invés, focarem em alguns genes candidatos biológicos, optaram por focar em um método livre de hipótese, para identificar novos biomarcadores genéticos e para avaliar sua contribuição para fenótipos distintos (VAN TONG; VELAVAN *et al.*, 2017). Um estudo longitudinal no Peru realizou GWAS de 2.157 casos de TB de início precoce e 1827 controles domiciliares saudáveis com TST positivo (LUO; SULIMAN *et al.*, 2019). Este estudo indicou que a rápida progressão para a doença tem uma base genética diferente daquela da reativação da TB. Um exemplo foi a variante rs73226617 associada à progressão precoce, mas sem relação nos pacientes com reativação da TB (LUO; SULIMAN *et al.*, 2019). Outro estudo usando GWAS também encontrou associação com o SNP rs9272785 em *HLA-DQA1*, que foi associado ao início precoce da doença em indivíduos (definido como 20–40 anos de idade) (TANG; WANG *et al.*, 2019). A lesão hepática induzida por medicamentos é um efeito colateral durante o tratamento da TB possivelmente associados à genética. Mais um estudo utilizando GWAS, com uma coorte de 79 casos de TB com lesão hepática induzida por drogas anti-TB e 239 controles, recebendo tratamento para TB confirmou uma associação com o SNP rs1495741 na região do gene N-acetiltransferase 2, na Tailândia (SUVICHAPANICH; WATTANAPOKAYAKIT *et al.*, 2019). Em outro estudo GWAS, o SNP rs10946737 no gene *RIPOR2* foi associado à toxicidade hepática induzida por drogas anti-TB em pacientes (PETROS; LEE *et al.*, 2017).

A sequência do genoma do *Mtb* em 1998 abriu portas para novos estudos, que não se limitam a estudar um único gene ou proteína, estes novos estudos são sobre os SNPs no *Mtb* e mostraram que o *Mtb* presenta uma maior diversidade genética que os outros membros do complexo Mycobacterium tuberculosis (STUCKI; GAGNEUX, 2013). Atualmente os SNPs representam marcadores robustos para inferir filogenias e para classificação de cepas e podem ser usados para estimar as distâncias evolutivas entre cepas (FORD; LIN *et al.*, 2011). A primeira vez que se aplicou isto foi em 1997, quando em um sequenciamento de 6 genes associados à resistência aos medicamentos se identificaram dois SNPs que não estavam relacionados à resistência aos medicamentos, e para dois SNPs, foi desenvolvido um esquema de classificação (SREEVATSAN; PAN *et al.*, 1997). Os SNPs no *Mtb* carregam informações funcionais como a resistência a medicamentos (RISKA; JACOBS *et al.*, 2000). Esse tipo de informação molecular é crucial para o desenvolvimento de métodos diagnósticos para detectar a resistência aos medicamentos (STUCKI; GAGNEUX, 2013). O teste diagnostico Xpert MTB/RIF, é um teste molecular automatizado para *Mtb* e resistência à rifampicina, baseado em SNP que foram identificados e incorporados nesta nova ferramenta de diagnóstico (BOEHME; NABETA *et al.*, 2010), mas muitas mutações permanecem desconhecidas, incluindo muitas daquelas que causam resistência aos medicamentos de 2ª linha.

Os fatores genéticos humanos que contribuem para a suscetibilidade ou resistência à patogênese da TB foram investigados por estudos de genes candidatos. Os estudos de genes são estudos de associação genética, que identificam variantes de risco associadas a uma doença específica (PATNALA; CLEMENTS *et al.*, 2013). Os estudos de genes candidatos de menor custo são mais rápidos de realizar (PATNALA; CLEMENTS *et al.*, 2013). Estes estudos têm como foco a seleção de genes que foram de alguma forma relacionados anteriormente à doença e que vem precedida de um conhecimento prévio sobre a função do gene (CORREA-MACEDO; CAMBRI *et al.*, 2019). Neste tipo de estudo, foram relatados SNPs em mais de 111 genes que influenciam o risco de TB e as respostas imunológicas ao *Mtb* (MESSINA; NETEA *et al.*, 2020).

Os Receptores Toll-like (TLRs) constituem uma família de proteínas transmembrana do tipo receptores de reconhecimento de padrões (PRRs) (DUBE; FAVA *et al.*, 2021). No total, existem 10 TLRs em humanos, que eles são expressos em várias células imunes e não imunes, com diferentes ligantes microbianos e efeitos ligeiramente variáveis. Eles desempenham um papel fundamental no sistema imunológico inato (FOL; DRUSZCZYNSKA *et al.*, 2015). TLR2, TLR1 e TLR6, junto como outros PRRs como *CD14*, detectam lipoproteínas micobacterianas (MUKHERJEE; HUDA *et al.*, 2019). Nos genes que codificam TLRs, foram

encontrados SNPs que podem gerar suscetibilidade ou resistência em TB (BRITES; GAGNEUX, 2015). Nos Estados Unidos (SMITH, 1991) encontrou-se que SNPs em TLR6-TLR1-TLR10 foram significativamente representados entre afro-americanos com TB e tiveram um risco aumentado de infecção por *Mtb*. Recentemente na Índia (WANI; SHEHJAR *et al.*, 2021) descobriu-se que SNPs em TLR4 e TLR2 atuam como fatores de risco significativos para a predisposição à tuberculose extrapulmonar.

A vitamina D é relevante na modulação das respostas imunes inatas e adaptativas, esta tem sido amplamente estudada devido a associação do risco de TB e os polimorfismos do gene do receptor da vitamina D (*VDR*) em várias populações (VAN TONG; VELAVAN *et al.*, 2017). Uma meta-analises recente achou que o SNP rs2228570 presentava uma associação significativa com a suscetibilidade a TB na população asiática (YADAV; KUMAR *et al.*, 2021). Numa população Han taiwanesa composta por 198 pacientes com TB e 170 controles saudáveis os SNPs rs1544410 e rs731236 foram significativamente associados à susceptibilidade à TB (LEE; CHUANG *et al.*, 2016). Esta mesma variante rs731236 foi igualmente associada à meningite tuberculosa em uma população indiana, que mostrou uma maior deficiência de vitamina D entre pacientes com meningite tuberculosa quando comparada com controles (RIZVI; GARG *et al.*, 2016).

Outros genes foram estudados como o gene *CD53*, onde o SNP rs4839583 foi associado aos casos de TB na população coreana. Em um estudo de caso controle o SNP rs763780 do gene *IL-17* foi associado a um risco aumentado de TB em 428 casos de TB e 428 controles de uma população chinesa (DU; HAN *et al.*, 2015). Foi realizada uma meta-análise para avaliar possíveis associações de quatro SNPS no gene do *TNF-α*, encontrou-se que as variantes rs1800629 e rs361525 estavam associadas à TB pulmonar independentemente da etnia e do status de HIV, mas a variante rs1800629 foi associada majoritariamente à TB pulmonar em asiáticos, enquanto a variante rs361525 foi associada à TB pulmonar em indivíduos africanos(YI; HAN *et al.*, 2015).

Finalmente, várias linhas de evidência, incluindo genética clínica, epidemiologia genética e genética populacional e funcional, apoiam o papel do componente genético do hospedeiro e entre estes os SNPs, na suscetibilidade à TB (COLL; PHELAN *et al.*, 2018). Todos os esforços feitos em estes estudos para explorar o uso de SNPs como biomarcadores para predição do risco de desenvolvimento futuro de tuberculose tem que ser traduzidos em aplicações na saúde pública (WALZL; MCNERNEY *et al.*, 2018). Por exemplo, é necessário um esforço em realizar estudos GWAS que permitam o cálculo de um escore de risco poligênico que ajudara na estimativa de diversas variantes genéticas para a predição de um fenótipo de

alguns dos espectros da TB (MOLLER; KINNEAR, 2020). Embora não existam testes de diagnóstico validados com base em marcadores como SNPS do hospedeiro, alguns SNPs podem ser vistos como marcadores promissórios no hospedeiro e estão sob investigação clínica já que é necessário que o contexto clínico dos pacientes este bem definido (WALZL; MCNERNEY *et al.*, 2018).

2 **JUSTIFICATIVA**

A TB é um importante problema de saúde pública, pois juntamente com Covid-19 causa mais mortes do que qualquer outro patógeno (WHO, 2020). Atualmente, não é possível diagnosticar diretamente a infecção por *Mtb* em humanos; portanto, a infecção latente de TB (LTBI) é diagnosticada pela resposta à estimulação *in vivo* ou *in vitro* por antígenos de *Mtb* com o uso do TST ou ensaios de liberação de interferon-γ (IGRAs) (PAI; BEHR, 2016). Vários fatores de risco para o desenvolvimento de TB ativa foram descritos como a pandemia de HIV, tabagismo, Diabetes mellitus, transtornos de saúde mental, e circunstâncias socioeconômicas baixas, mas intrigantemente alguns pacientes com TB não apresentam quaisquer fatores de risco conhecidos (ANKRAH; GLAUDEMANS *et al.*, 2018). Neste contexto, os fatores genéticos do hospedeiro desempenham um papel importante na determinação da diferença interindividual na suscetibilidade ou resistência da TB, mas a natureza dos fatores genéticos envolvidos permanece amplamente desconhecida. Foram relatados estudos onde se observaram interações entre polimorfismos genéticos humanos e *Mtb*, por exemplo estudos com gêmeos revelaram uma base genética clara para a suscetibilidade à infecção e doença por TB (ABEL; EL-BAGHDADI *et al.*, 2014). Os polimorfismos em genes relacionados na resposta imune à infecção por *Mtb* podem ter uma influência diversa na suscetibilidade ou proteção contra a TB entre famílias, etnias e raças particulares (HARISHANKAR; SELVARAJ *et al.*, 2018).

No presente estudo, buscamos identificar potenciais biomarcadores genéticos de suscetibilidade à infecção por *Mtb*, em uma coorte de contatos próximos de pacientes com TB pulmonar confirmados microbiologicamente para estimar o risco de infecção por *Mtb* (conversão de TST) e desenvolvimento de TB ativa de forma prospectiva e retrospectiva em uma coorte de Rio de Janeiro, e finalmente realizamos uma revisão sistemática onde avaliamos os trabalhos publicados até o momento sobre a influência dos polimorfismos dos genes *CD14* e *NOD2* no risco de infecção por *Mtb*.

Tendo em vista que estes trabalhos possuem três abordagens, duas em relação a identificação de polimorfismos, como biomarcadores de adoecimento para saber quem tem mais risco na coorte de contatos próximos de pacientes de TB, e casos de TB na revisão sistemática, assim achamos conveniente dividi-lo em três partes.

3 **PARTE I**

3.1 HIPÓTESE

Os polimorfismos de nucleotídeo único rs5743708 (*TLR2*), rs4986791 (*TLR4*), rs361525 (*TNFA*), rs2430561 (*IFN*G), e rs1143627 *(IL1B*) são fatores de risco ou proteção para conversão do TST ou desenvolvimento de TB ativa em contatos de casos de TB ativa.

3.2 OBJETIVOS

3.3 **Objetivo geral**

Avaliar potenciais biomarcadores genéticos de suscetibilidade à infecção por *Mtb* e adoecimento por TB em contatos próximos de pacientes com TB pulmonar

3.4 **Objetivos específicos**

- Descrever a população do estudo em suas características clínicas e demográficas.
- Investigar as frequências dos 5 polimorfismos na população de contatos.
- Analisar quais dos 5 polimorfismos estão associados com à conversão do Teste Tuberculínico e com resultado TST negativo na população de contatos.
- Avaliar os 5 polimorfismos gênicos de contatos de casos de TB pulmonar que estão associados no desenvolvimento de TB ativa.

4 **MANUSCRITO I**

Clinical Infectious Diseases

MAJOR ARTICLE

Polymorphisms in *TLR4* and *TNFA* and Risk of *Mycobacterium tuberculosis* Infection and Development of Active Disease in Contacts of Tuberculosis Cases in Brazil: A Prospective Cohort Study

Juan Manuel Cubillos-Angulo,[1,2,3,a] María B. Arriaga,[1,2,3,a] Elisângela C. Silva,[4,5,a] Beatriz L. A. Müller,[4,6] Daniela M. P. Ramalho,[4] Kiyoshi F. Fukutani,[1,3] Pryscila F. C. Miranda,[4] Adriana S. R. Moreira,[4] Antonio Ruffino-Netto,[7] Jose R. Lapa e Silva,[4] Timothy R. Sterling,[8] Afrânio L. Kritski,[4] Martha M. Oliveira,[9] and Bruno B. Andrade[1,3,8,10,11,12]

[1]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, [2]Faculdade de Medicina, Universidade Federal da Bahia, and [3]Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Fundação José Silveira, Salvador, Bahia, [4]Programa Acadêmico de Tuberculose, Faculdade de Medicina e Complexo Hospitalar HUCFF-IDT, Universidade Federal do Rio de Janeiro, [5]Recognize the Biology Laboratory, Center of Bioscience and Biotechnology, State University of North Fluminense Darcy Ribeiro, and [6]Laboratório de Genômica Funcional e Bioinformática, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, and [7]Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brazil; [8]Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee; [9]Centro de Desenvolvimento Tecnológico em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [10]Wellcome Centre for Infectious Disease Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, South Africa; [11]Universidade Salvador (UNIFACS), Laureate University, and [12]Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil

Background. The role of genetic polymorphisms in latent tuberculosis (TB) infection and progression to active TB is not fully understood.

Methods. We tested the single-nucleotide polymorphisms (SNPs) rs5743708 (*TLR2*), rs4986791 (*TLR4*), rs361525 (*TNFA*), rs2430561 (*IFNG*) rs1143627 (*IL1B*) as risk factors for tuberculin skin test (TST) conversion or development of active TB in contacts of active TB cases. Contacts of microbiologically confirmed pulmonary TB cases were initially screened for longitudinal evaluation up to 24 months, with clinical examination and serial TST, between 1998 and 2004 at a referral center in Brazil. Data and biospecimens were collected from 526 individuals who were contacts of 177 active TB index cases. TST conversion was defined as induration ≥5 mm after a negative TST result (0 mm) at baseline or month 4 visit. Independent associations were tested using logistic regression models.

Results. Among the 526 contacts, 60 had TST conversion and 44 developed active TB during follow-up. Multivariable regression analysis demonstrated that male sex (odds ratio [OR]: 2.3, 95% confidence interval [CI]: 1.1–4.6), as well as SNPs in *TLR4* genes (OR: 62.8, 95% CI: 7.5–525.3) and *TNFA* (OR: 4.2, 95% CI: 1.9–9.5) were independently associated with TST conversion. Moreover, a positive TST at baseline (OR: 4.7, 95% CI: 2.3–9.7) and SNPs in *TLR4* (OR: 6.5, 95% CI: 1.1–36.7) and *TNFA* (OR: 12.4, 95% CI:5.1–30.1) were independently associated with incident TB.

Conclusions. SNPs in *TLR4* and *TNFA* predicted both TST conversion and active TB among contacts of TB cases in Brazil.

Keywords. single-nucleotide polymorphism; tuberculin skin test; *Mycobacterium tuberculosis*; tumor necrosis factor; Toll-like receptor.

Approximately 1.7 billion individuals are infected with *Mycobacterium tuberculosis* (Mtb), representing one-quarter of the global population [1]. Because bacille Calmette-Guerin (BCG) vaccine does not protect either against infection or tuberculosis (TB) disease in adults, the only currently effective strategy to prevent active TB in adults is treatment of latent TB infection (LTBI). Treatment is efficacious in decreasing TB risk; however, compliance is low, and effectiveness therefore decreased, particularly with longer-course regimens [2]. Although the World Health Organization has recently emphasized the need to treat LTBI, high burden countries are unable to implement full-scale contact investigations and LTBI treatment. Of note, if left untreated, only a small proportion (5–10%) of infected persons will develop active disease [3]. Although some risk factors for developing TB disease have been recognized, such as human immunodeficiency virus (HIV) coinfection, diabetes, young age, and recently acquired Mtb infection [4], many TB patients do not have any known risk factors. To identify those who would most benefit from LTBI treatment, biomarkers for susceptibility have been investigated. Interferon-gamma

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[a]J.M. C.-A., M. B. A., and E. C. S. equally contributed to the work.

Correspondence: B. B. Andrade, Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, No. 121, Candeal, Salvador, Bahia 40269-710, Brazil (bruno.andrade@bahia.fiocruz.br).

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release assays have been widely tested as a marker of LTBI and, to a lesser extent, susceptibility to TB disease [5]. However, these tests do not discriminate between active disease and LTBI and, more importantly, have a low predictive value for progression to TB [6].

In addition, not all contacts of pulmonary TB patients acquire Mtb infection. A meta-analysis reported great variability in the proportion of infected household contacts with a positive tuberculin skin test (TST) [7]. Transmission of Mtb depends on index case-related factors, such as bacillary burden and duration of cough [7] and on contact-related factors, such as degree of exposure and individual genetic susceptibility [8]. Mtb infection and progression to TB disease may have distinct genetic influences that underlie the biological mechanisms involved in individual susceptibility [9]. Robust activation of the innate immune response is considered an essential prerequisite for protective immunity and vaccine efficacy. However, data published to date provide an incomplete view of the functional importance of innate immunity in TB [10].

Some key genetic components of protective immunity in human TB include Toll-like receptor (TLR)2, TLR4, tumor necrosis factor (TNF)A, interferon (IFN)G and interleukin (IL)1B [11–14]. Indeed, immune-related single-nucleotide polymorphisms (SNPs) such as *TLR2* rs5743708 [15], *TLR4* rs4986791 [13],*TNFA* rs361525 [16], IFNG rs2430561 [17], IL1B rs1143627 [18], and many others, have all been suggested to influence susceptibility to TB, but the functional immunologic correlates are still unclear. The objective of this study was to evaluate potential genetic biomarkers of susceptibility to Mtb infection and TB disease. We studied close contacts of microbiologically confirmed pulmonary TB patients to estimate the risk of Mtb infection (TST conversion) and development of active TB according to the presence of 5 immune-related SNPs, while also accounting for clinical and epidemiological factors.

MATERIALS AND METHODS

Ethics Statement

Written informed consent was obtained from all participants or their legally responsible guardians, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. The study was approved by the Clementino Fraga Filho University Hospital (HUCFF), Federal University of Rio de Janeiro Ethics Review Board. The anonymity of study subjects was preserved, and all study specimens were de-identified.

Study Design

We performed a longitudinal study of contacts of pulmonary TB patients at the time of diagnosis from November 1998 through March 2004. TB was diagnosed by acid-fast bacilli (AFB) smear and/or culture, according to Brazilian Ministry of Health Guidelines [19]. All TB index cases diagnosed at HUCFF >18 years old. Investigation of TB cases included data on cough, AFB sputum grade, and chest radiographs. After identifying TB cases, we searched for their close contacts. TB contacts were defined as living in the same household or reporting contact with the TB index case for ≥20 hours weekly for 2 months. All individuals identified who were ≥9 years old were invited to participate in the study and were evaluated and screened for active TB following Brazilian guidelines [19]. Prevalent TB cases among close contacts were excluded from analyses.

Procedures

Close contacts were evaluated at baseline and also 4 and 12 months after identification of the TB index case. At first visit, a standardized questionnaire was administered to obtain demographic and clinical data, including type and duration of contact with the index case, and history of risk factors for TB (eg, HIV, diabetes, hematologic malignancies, and use of immunosuppressant drugs). If a contact was a grandparent, parent or sibling of the index case, they were considered to have consanguinity (this definition extended to children with the index case), whereas spouses or other relationships did not. At study baseline, a medical visit and chest radiograph were scheduled. BCG scar was assessed, and TST performed by a trained nurse using the Mantoux technique [19], with 2 tuberculin units of the purified protein derivative RT 23 (Statens Serum Institute, Copenhagen, Denmark). TST reading was performed 48–72 hours after administration. Additional TST screening was performed at months 4 and 12 to evaluate for possible TST conversion.

TST Interpretation and TB Diagnosis

A positive TST was defined as ≥5 mm induration, according to the Brazilian Ministry of Health [19]. A positive TST at the first visit was considered to represent LTBI. Contacts with any TST ≥5 mm were not retested with TST. During the study period, the Brazilian National TB Guidelines indicated that treatment of TST-positive individuals was not mandatory, and assessment of cost-benefit of therapy with isoniazid for 6 months was performed by healthcare workers prior to a decision to treat [19]. If treatment was not initiated, individuals were followed up with periodic examinations to identify development of active TB disease. Twenty-nine participants received isoniazid.

Contacts with signs or symptoms suggestive of active TB underwent medical visits and investigation for TB disease by acid-fast bacilli (AFB) smear and culture in Lowenstein Jensen (LJ) medium. Active TB was diagnosed when ≥1 specimen yielded a positive microbiologic (AFB smear or culture) result. An incident active TB case was defined as TB diagnosed after baseline study assessment. All patients (n = 526) were contacted after 12 additional months (24 months after study enrollment) to assess for incident TB disease. Data on TB incidence from all individuals who could not be contacted at month 24 (n = 168)

were collected by searching the Brazil's Information System for Notifiable Diseases (SINAN). Of 44 incident TB cases, 8 (18%) had TB diagnosis extracted from SINAN rather than at month 24 interview.

Genotyping

Genomic DNA was extracted from peripheral blood collected from TB contacts at study enrollment. DNA extraction and genotyping were performed using the FlexiGene kit (Qiagen, Germany). Genotypes of 5 gene polymorphisms *TLR2* (rs5743708), *TLR4* (rs4986791), *TNFA* (rs361525), *IFNG* (rs2430561), and *IL1B* (rs1143627) were detected using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) method [20, 21]. The primer sequences are in Supplementary Table 1. The PCR products were digested by the enzymes *Msp I* for *TLR2*, *Hinf I* for *TLR4*, *BamHI* for *TNFA*, *AvaII* for *IFNG,* and *AluI* for *IL1B*.

Data Analysis

Categorical data were presented as proportions and continuous data as medians and interquartile ranges (IQR). The frequency distributions of alleles (wild type vs variant) for each polymorphism were compared. The Fisher and χ^2 tests were used to compare categorical variables between study groups. Continuous variables were compared using the Mann-Whitney *U* test. A multivariable regression model using variables with univariate *P*-value ≤ .2 was performed to assess the odds ratios (OR) and 95% confidence intervals (CIs) of the associations with TST conversion and incident active TB. For analysis of *TLR4* in the multivariable model, there was no event among participants who remained TST negative; thus for OR calculation, we added "1" to the group without detected events. In addition, we employed Bayesian Network modeling [22] to infer causal relationships between TST conversion and active TB disease and sociodemographic, clinical, laboratory, and genetic parameters, with 100× bootstrapping. Only associations which remained statistically significant in >20 of 100× bootstraps were considered significant. A *P*-value < .05 was considered statistically significant.

RESULTS

Characteristics of the Study Participants

We approached 1458 contacts of 1191 microbiologically confirmed TB index cases who attended HUCFF between 1998 and 2004. Of those, 932 persons were excluded for the reasons listed in Figure 1. The final study population, from which we collected data and samples, included 526 contacts of 177 TB index

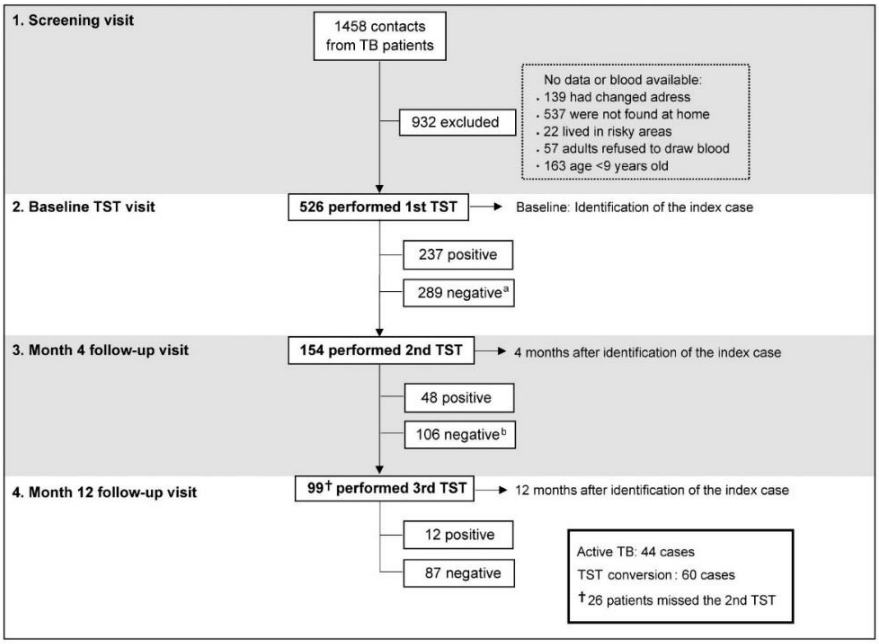

Figure 1. Study flow chart. Index case: first tuberculosis case identified in the household. [a]Missing 2nd TST: 135 cases; [b]Missing 3rd TST: 33 cases and 26 people who missed the 2nd TST showed up. Abbreviation: TST, tuberculin skin test.

cases. The description of the study population is in Table 1. The study population was mostly female, household contacts, and consanguineous with the index case. Indeed, 474 persons (90.5%) were household contacts, with a high rate of consanguinity with the index case (62.5%). There were low frequencies of HIV infection, alcohol use, illicit drug use, and use of immunosuppressant drugs. Only 8 persons (1.8%) had a history of TB. At baseline, few reported cough for more than 4 weeks, and of these, only 3 had a positive AFB smear and were then treated for TB. During the evaluation of the index cases associated with the contacts, almost all had TB diagnosis confirmed by culture and cough for more than 4 weeks. TB index cases frequently exhibited high bacterial loads in sputum (41.1% had AFB grade ≥ +2). In addition, 84 index TB patients had cavitary lesions on chest radiograph.

Variant alleles of *IFNG* were the most common polymorphism in the study population, present in 82.9% of the participants (Table 2). Variations in the *IL1B* gene were also common (47%), whereas polymorphisms in *TLR2*, *TLR4*, and *TNFA* genes were less common (Table 2).

Table 2. Gene Polymorphisms of the Study Participants

SNP	n	(%)
rs5743708 (*TLR2*)		
GG	365	(83.9)
GA+AA[a]	70	(16.1)
rs4986791 (*TLR4*)		
CC	410	(96.7)
CT+TT[a]	14	(3.3)
rs361525 (*TNFA*)		
GG	447	(85.8)
GA+AA[a]	74	(14.2)
rs2430561 (*IFNG*)		
TT	69	(17.1)
TA+AA[a]	335	(82.9)
rs1143627 (*IL1B*)		
TT	254	(53.0)
TC+CC[a]	225	(47.0)

Data on 526 individuals are shown.

Abbreviations: *IFNG*, interferon gamma; *IL1B*, interleukin-1 beta; SNP, single-nucleotide polymorphism, TLR, Toll-like receptor, *TNFA*, tumor necrosis factor α.

[a]Variant alleles of SNP.

Association Between Polymorphisms and TST Conversion

Exposure to Mtb at the time of study enrollment was examined by TST screening of the 526 individuals; 237 (45.1%) had a positive TST (Figure 1). There were 135 individuals who missed the month 4 visit, and 154 (53.3% of those TST negative at baseline) were retested. A positive TST was detected in 48 individuals, representing 16.6% of the participants with an initially negative TST. At month 12, a third TST was performed in TB contacts who remained TST negative at month 4. In addition, 26 participants who missed TST testing at month 4 were tested at month 12. A total of 99 individuals were tested. Twelve individuals had a positive TST at this time point. Thus, during the study period, 60 persons converted to a positive TST, suggesting recent Mtb infection.

Table 1. Clinical and Demographic Characteristics of the Study Population

Characteristics of Contact	n/N	n	(%)
Age, median (IQR)	526/526	35	(33–38)
Male	526/526	181	(34.4)
Consanguinity with index case	526/526	329	(62.5)
BCG vaccination	521/526	177	(33.9)
HIV infection	31/526	4	(12.9)
IDU	439/526	7	(1.6)
Smoking	524/526	131	(25.0)
Alcohol use	444/526	1	(0.2)
Prior tuberculosis	440/526	8	(1.8)
Household contact[a]	523/526	474	(90.5)
Frequency of contact (>20 hours)	526/526	489	(93.0)
Comorbid conditions[b]	500/526	127	(25.4)
Immunosuppressant drugs	444/526	3	(0.7)
Cough (> 4 weeks)	518/526	19	(3.6)
Positive AFB screening	429/526	3	(0.7)
Characteristics of TB index case			
Cavities on chest x-ray	517/526	84	(16.2)
Cough (> 4 weeks)	518/526	470	(90.7)
≥2+ AFB	444/526	200	(41.1)
Positive culture	367/526	352	(95.9)

Abbreviations: AFB, acid fast bacilli; BCG, bacille Calmette-Guerin; HIV, human immunodeficiency virus; IDU, illicit drug use; IQR, interquartile range; n, number of persons for whom such data were available; N, number total that participants from the study available; TB, tuberculosis.

[a]Household contact is defined as living in the same household or reporting contact with the TB index case for >20 hours weekly for 2 months.

[b]Comorbidities: renal failure, diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous, and hepatitis.

TST converters were more commonly male and more frequently household contacts than nonconverters (Table 3). Other characteristics were similar between converters and nonconverters. Univariate analyses indicated that variant alleles in *TLR2* ($P = .03$), *TLR4* ($P < .01$), and *TNFA* ($P = .001$) were associated with TST conversion, whereas mutant *IL1B* ($P = .006$) alleles were more common in those who did not convert (Table 4). Multivariable regression analysis confirmed that male sex and genetic variants in *TLR4 and TNFA* were all independently associated with increased odds of TST conversion (Figure 2A), whereas *IL1B* SNP was not significant (adjusted OR: 0.6, 95% CI: 0.28–1.29, $P = .191$).

Furthermore, we applied Bayesian network modeling to infer causal relationships between the presence of polymorphisms and TST conversion, and all recorded statistically relevant demographic, epidemiologic, and behavioral information from univariate analyses were cited above. This approach confirmed the strong direct associations between male sex, polymorphisms in *TLR4* and *TNFA*, in addition to *IL1B*, with TST conversion (Figure 2B). The *TLR2* polymorphism was not directly

Table 3. Characteristics of the Study Participants Evaluated for Conversion From TST Negative to TST Positive

Characteristic	n/N	Conversion n = 60	TST Negative n = 224	OR (95% CI)	*P*-Value
Age, median (IQR)	284/284	37 (15.59)	34 (21–53)		.85
Male	284/284	28 (46.7)	76 (33.9)	1.7 (1.0–3.0)	.072
Consanguinity with index case	284/284	36 (60.0)	142 (63.4)	0.9 (0.5–1.6)	.65
BCG vaccination	281/284	17 (28.8)	74 (33.3)	0.8 (0.4–1.5)	.54
HIV infection	20/284	1 (5.9)	2 (66.7)	0.03 (0.0–0.7)	.05
Nonwhite race	275/284	28 (48.3)	117 (53.9)	0.8 (0.4–1.4)	.46
IDU	230/284	0 (0)	3 (1.5)	…	1
Smoking	283/284	15 (25.0)	56 (25.1)	1 (0.5–1.9)	1
Alcohol consumption	231/284	0 (0)	0 (0)	…	…
Prior tuberculosis	229/284	1 (2.9)	0 (0)	…	…
Household contact	282/284	50 (83.3)	195 (87.8)	0.7 (0.3–1.5)	.39
Frequency of contact (>20 hours)	284/284	56 (93.3)	206 (92.0)	1.2 (0.4–3.8)	1
Comorbid conditions	266/284	9 (16.1)	54 (25.7)	0.5 (0.3–1.2)	.16
Immunosuppressant drugs	231/284	0 (0)	0 (0)	…	
Cough (> 4 weeks)	283/284	2 (3.3)	5 (2.2)	1.5 (0.3–7.9)	.64
Positive AFB	231/284	0	1 (0.2)	…	.92
Characteristics of TB index case					
Cavities on chest x-ray	276/284	4 (7.1)	24 (10.9)	0.6 (0.2–1.9)	.62
Cough (> 4 weeks)	283/284	30 (50.0)	88 (39.5)	1.5 (0.9–2.7)	.18
≥2 AFB	256/284	16 (30.8)	79 (38.7)	0.7 (0.4–1.4)	.37
Positive culture	201/284	39 (95.1)	151 (94.4)	1.2 (0.2–5.6)	1

Data represent no. (%). Comorbidities: diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous, and hepatitis.

Abbreviations: AFB, acid fast bacilli; CI, confidence interval; IDU, illicit drug use; n, number of persons for whom such data were available; N, number total that participants from the study available; OR, odds ratio; TB, tuberculosis; TST, tuberculin skin test.

connected to TST conversion but was associated with *TLR4* SNP using the Bayesian network approach. In fact, 10 out of 11 individuals with TST conversion and the *TLR4* SNP also had the *TLR2* polymorphism.

Individuals who were TST positive at study baseline (n = 203) were similar to those who were TST negative and did not convert nor develop active TB during study follow-up (n = 224) with regard to most of the characteristics evaluated, including the SNPs (Supplementary Table 2). Cavitary lesions as well as cough in the index TB cases were more frequent in participants who were TST positive at the first visit compared to those who remained TST negative ($P = .005$ and $P = .009$, respectively).

Association Between Polymorphisms and Incident TB

Incident TB was higher in those who were TST positive at baseline (Table 5). Only 2 of the 29 individuals who received isoniazid therapy developed incident TB. In addition, index cases from participants who developed active TB more frequently had cavitary lung lesions identified on chest x-ray compared to index cases of contacts who did not develop TB (Table 5). Lastly, incident TB was more frequent in participants who had allelic variants in both *TLR4* and *TNFA* genes (Table 6).

Multivariable regression analysis revealed that contacts who were TST positive at baseline had 7 times greater odds of developing active TB than those who remained TST negative

Table 4. Gene Polymorphisms of the Study Participants Evaluated for Conversion From TST Negative to TST Positive

SNP	Conversion n = 60	TST Negative n = 224	OR	95% CI	*P*-Value
rs5743708–*TLR2*	15 (29.4)	27 (15.1)	2.3	(1.1–4.9)	.03
rs4986791–*TLR4*	11 (21.6)	0 (0)	…	…	<.01
rs361525–*TNFA*	18 (30.0)	24 (10.9)	3.5	(1.7–7.0)	.001
rs2430561–*IFNG*	36 (78.3)	140 (83.8)	0.6	(0.3–1.6)	.38
rs1143627–*IL1B*	14 (25.5)	94 (46.3)	0.4	(0.2–0.8)	.006

Data represent no. (%).

Abbreviations: CI, confidence interval; *IFNG*, interferon gamma; *IL1B*, interleukin-1beta; OR, odds ratio; SNP: single-nucleotide polymorphism; TLR, Toll-like receptor; *TNFA*, tumor necrosis factor α; TST, tuberculin skin test.

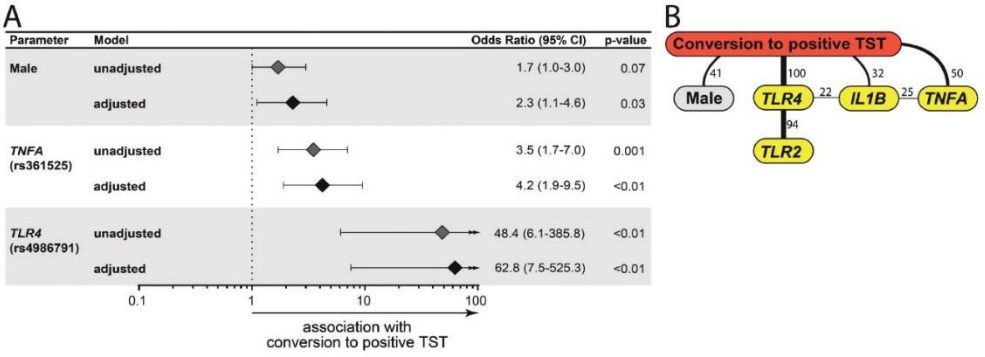

Figure 2. Factors associated with TST conversion. *A*, Multivariable regression model of variables shown in Tables 3 and 4, which displayed univariate *P*-value ≤ .2. *B*, Bayesian network with bootstrap (100×) was used to illustrate the statistically significant associations between the parameters and the presence of TST conversion in the study population. Lines represent direct associations. Associations that remained statistically significant on ≥20 of 100 bootstraps are plotted. Numbers of times each association persisted during bootstrap are shown. Bold lines highlight the strongest associations. All parameters from Table 3 were included. Only those displaying significant associations are shown. Abbreviation: TST, tuberculin skin test.

(Figure 3A). Occurrence of allelic variants in either *TLR4* or *TNFA* genes was independently associated with odds of incident TB. Bayesian networks confirmed the associations between *TNFA* and *TLR4* polymorphisms and incident TB (Figure 3B). Three participants had both SNPs: all 3 were TST converters, of whom 2 also developed active TB. A total of 5 TST converters developed TB disease. Of these, 2 had 2 SNPs, *TLR4* and *TNFA*, 1 had only the TLR4 variants and 1 had only the *TNFA* polymorphism. In addition, prior TB and being TST positive at baseline were robustly associated with development of active TB (Figure 3B). Interestingly, this model indicated that *TLR2* SNPs were again indirectly associated with incident TB through

Table 5. Characteristics of Contacts of Pulmonary TB Cases Evaluated for Development of Active TB Disease

Characteristic	n/N	Active TB n = 44	No Active TB n = 482	OR (95% CI)	*P*-Value
Age – median (IQR)	526/526	32 (29–39)	39 (34–40)	…	.04
Male	526/526	18 (40.9)	163 (33.8)	1.4 (0.7–2.5)	.4
Consanguinity with index case	526/526	31 (70.5)	299 (62.0)	1.5 (0.8–2.6)	.3
BCG vaccination	521/526	14 (31.8)	163 (34.1)	0.9 (0.5–1.7)	.8
HIV infection	31/526	2 (22.2)	2 (9.1)	2.9 (0.3–24.3)	.6
Nonwhite race	505/526	21 (46.9)	258 (55.8)	0.8 (0.4–1.4)	.4
IDU	439/526	1 (3.4)	6 (1.5)	2.4 (0.3–20.7)	.4
Smoking	524/526	12 (27.3)	119 (24.8)	1.1 (0.6–2.3)	.7
Alcohol use	444/526	1 (3.1)	0	…	.07
Prior TB	440/526	6 (19.4)	2 (0.5)	48.8 (9.4–254.4)	<.01
Household contact	523/526	41 (93.2)	433 (92.9)	1.5 (0.4–5.0)	.8
Frequency of contact (>20 hours)	526/526	42 (95.5)	448 (92.9)	1.6 (0.4–6.9)	.4
Comorbid conditions	500/526	10 (25.0)	117 (25.4)	1.0 (0.4–2.1)	1.0
Immunosuppressant drugs	444/526	1 (3.1)	2 (0.5)	6.6 (0.6–75.0)	.2
Cough (> 4 weeks)	518/526	8 (18.2)	7 (1.5)	15.0 (5.2–43.8)	<.01
Conversion	526/526	5 (11.4)	55 (11.4)	1.0 (0.4–1.1)	1.0
Positive TST at baseline	526/526	34 (77.3)	203 (42.1)	4.7 (2.3–9.7)	<.01
Characteristics of TB index case					
Cavities on chest x-ray	517/526	13 (29.5)	71 (15.0)	2.4 (1.2–4.8)	.04
Cough (> 4 weeks)	518/526	43 (97.7)	427 (90.1)	4.7 (0.6–35.2)	.1
≥2+ AFB	444/526	21 (47.7)	179 (40.4)	1.3 (0.7–2.5)	.4
Positive culture	367/526	29 (96.7)	323 (95.8)	1.3 (0.2–9.9)	1.0

Data represent no. (%). Comorbidities: diabetes, heart failure, and/or hypertension, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous, and hepatitis.

Abbreviations: AFB, acid fast bacilli, CI, confidence interval; HIV, human immunodeficiency virus; IDU, illicit drug use; n, number of persons for whom such data were available; N, number total that participants from the study available; OR, odds ratio; TB, tuberculosis.

Table 6. Gene Polymorphisms of Contacts of Pulmonary TB Cases Evaluated for Development of Active TB Infection

SNP	Active TB n = 44		No Active TB n = 482		OR	95% CI	*P*-Value
rs5743708–*TLR2*	8	(23.5)	62	(15.5)	1.7	(0.7–3.9)	.2
rs4986791–*TLR4*	5	(14.7)	9	(2.3)	7.3	(2.3–23.2)	<.01
rs361525–*TNFA*	23	(52.3)	51	(10.7)	9.0	(4.7–17.7)	<.01
rs2430561–*IFNG*	25	(78.1)	310	(83.3)	0.7	(0.3–1.7)	.5
rs1143627–*IL1B*	17	(44.7)	208	(47.2)	0.9	(0.5–1.8)	.8

Data represent no. (%).

Abbreviations: CI, confidence interval; *IFNG*, interferon gamma; *IL1B*, interleukin-1β; OR, odds ratio; SNP, single-nucleotide polymorphism; TLR, Toll-like receptor; *TNFA*, tumor necrosis factor α.

TLR4 polymorphisms, suggesting that the combination of allelic variants in these genes may be associated with increased risk of Mtb infection and development of active TB.

DISCUSSION

In this study we tested associations between SNPs from immune related genes in a large cohort of TB contacts from a highly endemic region in Brazil. The most important finding was that *TLR4* Thr399Ile (rs4986791) and *TNFA*-238 (rs361525) were independently associated with both TST conversion and subsequently developing TB disease. These findings highlight the importance of innate immunity, particularly of these molecules, in the pathogenesis of human Mtb infection and TB disease.

Our results are consistent with our current understanding of TB pathogenesis, in which TLRs are considered critical for host immunity against Mtb in both experimental and clinical settings. Indeed, several groups have shown that polymorphisms in TLR genes are associated with increased susceptibility to TB disease [13]. The *TLR4* ectodomain plays a key role in recognition of pathogen-associated molecular patterns. Interestingly, *TLR4* Thr399Ile has been associated with hypo-responsiveness to ligand interaction due its location near the central ectodomain region [23]. This polymorphism has been associated with more severe forms of pulmonary TB as quantified by sputum bacillary loads and chest radiographs [24]. Our findings on TB contacts provide additional evidence for the critical role of TLR4 in susceptibility to TB. Upon activation through interaction between Mtb ligands and TLR4, myeloid cells produce IL-12 among other proinflammatory mediators [25], which are important to drive T helper 1 (Th1) responses. Exposure to mycobacteria also triggers production of TNF-α and IL-1β [26]. Thus, TLR4 may be critical to drive the protective Th1 responses in the context of Mtb infection and hypo-responsiveness may drive increased susceptibility to TB.

TNF-α has a central role both in the host immune response to Mtb infection and in the immunopathology of TB. TNF-α

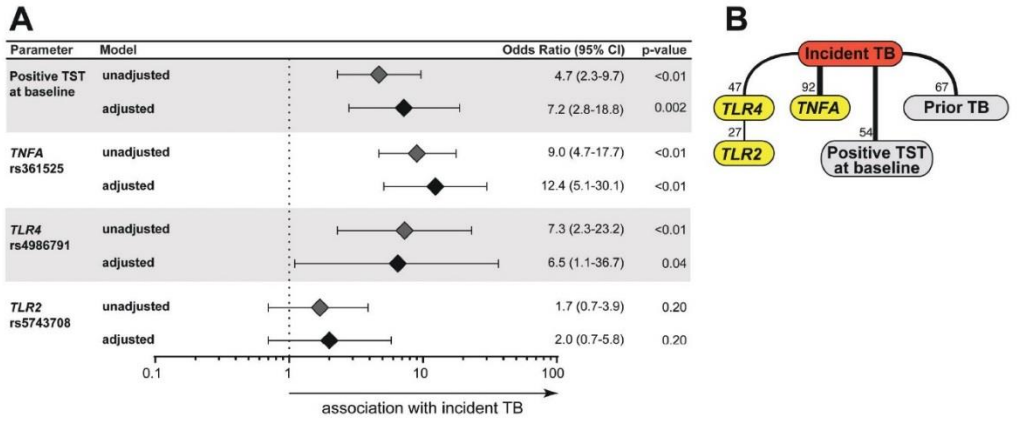

Figure 3. Variables associated with development of active TB among contacts of pulmonary TB. *A*, Multivariable regression model of variables shown in Tables 5 and 6 which displayed univariate *P*-value ≤ .2. *B*, Bayesian network with bootstrap (100×) was used to illustrate the statistically significant associations between the parameters and the occurrence of incident TB in the study population. Lines represent direct associations. Associations that remained statistically significant on ≥20 of 100 bootstraps are plotted. Numbers of times each association persisted during bootstrap are shown. Bold lines highlight the strongest associations. All parameters from Table 5 were included. Only those displaying significant associations are shown. Abbreviation: TB, tuberculosis.

is produced by many cell types and has cytotoxic synergy with human interferon [27]. Experimental studies have shown that TNF-α is required for the formation and maintenance of granulomas [28]. In humans, anti-TNF drugs are associated with heightened risk of a number of severe respiratory infections including TB [29] In a Chinese population, the *TNFA*-308 allele was associated with elevated odds of pulmonary TB [21]. To our knowledge, no previous study has tested the *TNFA* SNP in the context of TB in Brazil. While examining a Brazilian population, Rocha et al. reported that *TNFA*-238 (rs361525) was associated with spondylarthritis [30]. Our results argue that screening for *TNFA* SNPs could serve as a tool to guide implementation of preventive therapy in TB contacts.

In the present study, the LTBI cases identified at baseline may reflect a cumulative risk for infection before the programmatic contact tracing. Initial LTBI was associated with nonwhite ethnicity and with the presence of cavity on chest radiograph of the index case. Nonwhite ethnicity has been found as a risk factor for extrapulmonary TB [31], but in our study, this characteristic may be a proxy variable for socioeconomic conditions in Brazil, reflecting crowding and higher community exposure.

Both logistic regression and Bayesian network analyses demonstrated that male sex was associated with TST conversion. This relationship has been reported previously [25, 32]. Other direct associations with TST conversion found here included *TLR4* and *TNFA* SNPs. The Bayesian network analyses refined these relationships while suggesting that *TLR2* and *TLR4* SNPs may sometimes act combined to increase odds of TST conversion. Both *TLR2* and *TLR4* are expressed on cell surface and share common intracellular signaling adaptors [33]. Our findings are intriguing and deserve additional investigations to validate the results and narrow down potential interdependency between TLR2 and TLR4 in the immune response against Mtb.

We examined the characteristics associated with development of active TB in our study population and found that polymorphisms in *TLR4* and *TNFA* were independent risk factors. Importantly, such SNPs were also associated with TST conversion, reinforcing the idea that TLR4 signaling and TNF-α production are critically involved in TB pathogenesis. As TNF-α is important for maintenance of granulomas [34], it is possible that the SNP reported here could affect this process and favor development of active TB. The *TLR4* polymorphism was also directly associated with development of active TB as well as with the *TLR2* polymorphism, which although not significantly linked to this clinical outcome in logistic regression, was identified by the Bayesian network and indirectly linked through *TLR4*, reinforcing the idea of interdependency between these TLRs. The same analyses revealed that a prior history of TB was also a risk factor, which has already been demonstrated previously [35].

Our study has several strengths such as serial TST testing (currently recommended as the diagnostic test for LTBI in most resource-restrained countries), microbiologically confirmed TB, and SNPs closely related to immune responses against TB. This study had some limitations. Approximately 20% (n = 109) of the study population were lost to follow-up, but this proportion was lower than the average reported by studies of TB contacts [36]. In addition, most contacts were consanguineous with the index TB case, but there was no impact on the outcomes evaluated. Furthermore, we assumed that within a household all were infected by a common Mtb strain, which may not have always been true and might influence the host immune response.

In conclusion, our study provides strong evidence for associations between polymorphisms in innate immune genes and the risk of Mtb infection and development of active TB in Brazil. Further translational studies are warranted to delineate the molecular events behind these associations.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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5 **PARTE II**

5.1 HIPÓTESE

Sete polimorfismos dos genes da via do IFN Tipo I envolvidos na detecção de DNA e RNA na via endocitose mediada por receptor, estão associados à positividade do TST em contatos próximos de pacientes com TB pulmonar confirmados microbiologicamente no Brasil.

5.2 OBJETIVOS

5.2.1 O**bjetivo geral**

Identificar potenciais biomarcadores genéticos associados à suscetibilidade na positividade do TST em contatos próximos de pacientes com TB.

5.2.3 **Objetivos específicos**

- Caracterizar clinicamente uma coorte de contatos de casos de índice de TB pulmonar;
- Avaliar associação entre os sete polimorfismos de genes candidatos e TST;
- Analisar quais dos sete polimorfismos de genes candidatos presenta uma associação com o risco de positividade do TST em um modelo multivariável que incluiu ajuste para raça / etnia, parentesco familiar, gênero e idade;
- Distinguir quais dos sete polimorfismos de genes candidatos presenta uma associação do com o risco de positividade do TST em um modelo multivariável que incluiu ajuste para idade, sexo, raça/etnia, parentesco familiar, contato domiciliar e características do caso índice de TB: Cavidades no raio-X do tórax, ≥ 2 BAAR esfregaço de escarro e cultura *Mtb* positiva.

6 **MANUSCRITO II**

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Polymorphisms in interferon pathway genes and risk of *Mycobacterium tuberculosis* infection in contacts of tuberculosis cases in Brazil

Juan Manuel Cubillos-Angulo[a,b,c,1], María B. Arriaga[a,b,c,1], Mayla G.M. Melo[d,e], Elisangela C. Silva[d,f], Lucia Elena Alvarado-Arnez[g,h], Alexandre S. de Almeida[e], Milton O. Moraes[g], Adriana S.R. Moreira[e], Jose R. Lapa e Silva[e], Kiyoshi F. Fukutani[a,c], Timothy R. Sterling[i], Thomas R. Hawn[j,2], Afrânio L. Kritski[e,2], Martha M. Oliveira[k,2], Bruno B. Andrade[a,b,c,i,l,m,n,*,2]

[a] *Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil*
[b] *Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Bahia, Brazil*
[c] *Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Fundação José Silveira, Salvador, Bahia, Brazil*
[d] *Laboratório de Micobacteriologia Molecular, Centro de Pesquisas em Doenças Infecciosas e Parasitárias- CEPEDIP – Universidade Federal do Rio de Janeiro, Rio de janeiro, Brazil*
[e] *Programa Acadêmico de Tuberculose, Faculdade de Medicina e Complexo Hospitalar HUCFF-IDT, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[f] *Laboratório de Biologia do Reconhecer, Centro de Biociências e Biotecnologia, Universidade Federal do Norte Fluminense Darcy Ribeiro, Rio de Janeiro, Brazil*
[g] *Laboratório de Hanseníase, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil*
[h] *Coordinación Nacional de Investigación, Universidad Franz Tamayo (UNIFRANZ), La Paz, Bolivia*
[i] *Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA*
[j] *Department of Medicine, University of Washington, Seattle, WA, USA*
[k] *Centro de Desenvolvimento Tecnológico em Saúde, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil*
[l] *Wellcome Centre for Infectious Disease Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa*
[m] *Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Bahia, Brazil*
[n] *Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil*

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ABSTRACT

Background: Host genetic polymorphisms may be important in determining susceptibility to *Mycobacterium tuberculosis* (Mtb) infection, but their role is not fully understood. Detection of microbial DNA and activation of type I interferon (IFN) pathways regulate macrophage responses to Mtb infection.
Methods: We examined whether seven candidate gene SNPs were associated with tuberculin skin test (TST) positivity in close contacts of microbiologically confirmed pulmonary TB patients in Brazil. Independent associations with TST positivity were tested using multivariable logistic regression (using genotypes and clinical variables) and genetic models.
Results: Among 482 contacts of 145 TB index cases, 296 contacts were TST positive. Multivariable regression analysis adjusted for population admixture, age, family relatedness, sex and clinical variables related to increased TB risk demonstrated that SNPs in *PYHIN1-IFI16-AIM2* rs1101998 (adjusted OR [aOR]: 3.72; 95% CI = 1.15–12.0; p = 0.028) and in *PYHIN1-IFI16-AIM2* rs1633256 (aOR = 24.84; 95%CI = 2.26–272.95; p = 0.009) were associated with TST positivity in a recessive model. Furthermore, an *IRF7* polymorphism (rs11246213) was associated with reduced odds of TST positivity in a dominant model (aOR: 0.50, 95%CI:

* Corresponding author at: Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, no. 121, Candeal, Salvador, Bahia 40269-710, Brazil.
E-mail address: bruno.andrade@fiocruz.br (B.B. Andrade).
[1] JMC-A and MBA equally contributed to the work.
[2] TRH, ALK, MMO and BBA equally contributed to the work.

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0.26-0.93; p = 0.029).
Conclusions: Polymorphisms in PYHIN1-*IFI16*-AIM2 rs1633256, rs1101998 and in *IRF7* rs11246213 were associated with altered susceptibility to Mtb infection in this Brazilian cohort.

Introduction

Tuberculosis (TB) is the leading cause of death from a single infectious agent (WHO, 2018). Approximately one-quarter of the global population is infected with *Mycobacterium tuberculosis* (Mtb) (Houben and Dodd, 2016). Latent tuberculosis infection (LTBI) is defined by immunological sensitization to Mtb antigens in the absence of clinical symptoms of disease and the diagnosis is based on the tuberculin skin test (TST) and/or Interferon-γ (IFN-γ) release assay (IGRA) (Robertson et al., 2012). Nevertheless, these tests do not discriminate between active disease and LTBI and, more importantly, have a low predictive value for progression to active TB (Rangaka et al., 2012). Many risk factors for developing active TB have been described, including HIV co-infection, diabetes, young age and recently acquired Mtb infection (Reid et al., 2019), but intriguingly some TB patients do not exhibit any known risk factors (Yan et al., 2015). TB occurs as the result of an intricate and dynamic relationship involving host genetics (van Tong et al., 2017) as well as immunological (Mahan et al., 2012; Tameris et al., 2013), and epidemiological (Shin et al., 2016) factors, in addition to characteristics of the Mtb strain itself (Koch and Mizrahi, 2018), that contribute to disease susceptibility (Pai et al., 2016).

Genetic factors are important for TB susceptibility, but the major genes involved remain unknown (van Tong et al., 2017). Candidate gene/pathway studies interrogate selected pathways that are important in the human host response to mycobacterial infection (Kinnear et al., 2017). Type I IFN pathways mediate an important role in TB pathogenesis. Whole blood RNA signatures dominated by Type I IFN-signaling identify individuals who will develop active disease (Berry et al., 2010). In Mtb-infected mice, increased expression of type I IFNs is deleterious for survival in association with reduced Th1 immunity (Manca et al., 2005). The Type I IFN pathway is activated by DNA (e.g. *IFI16-PYHIN1-AIM2*, cGAS, STING) and RNA sensors (e.g. *IFIT1* and 5), and contains several important signaling molecules and transcription factors (e.g. *IRF* family). For example, the cytosolic DNA sensor cGAS regulates IFN production during Mtb infection of macrophages (Watson et al., 2015). Although these murine and cellular studies suggest an important role for Type I IFNs in TB pathogenesis, the human genetics of this pathway in the context of Mtb infection are poorly understood (Donovan et al., 2017).

In a longitudinal investigation examining TB contacts from Brazil, we recently found that polymorphisms in toll-like receptor 4 (*TLR4*) and tumor necrosis factor (*TNFA*) are associated with increased risk of TST conversion and development of active TB (Cubillos-Angulo et al., 2019). Here we investigated in this same cohort whether genetic variation of Type I IFN pathway genes were associated with susceptibility to Mtb infection by examining single nucleotide polymorphisms (SNPs) involved in DNA and RNA sensing: (rs1101998, rs1633256, rs866484 in *IFI16-PYHIN1-AIM2 region*, rs59633641 and rs10887959 in *IFIT5*), rs304478 and rs304498 in *IFIT1* and the IFN signaling pathway (rs11246213 [*IRF7*]). The objective of this study was to identify potential genetic biomarkers of susceptibility to Mtb infection. We studied close contacts of microbiologically confirmed pulmonary TB patients to estimate factors associated with a positive versus negative TST.

Methods

Study design

The present study was based on analyses performed retrospectively on a cohort of contacts of pulmonary TB patients, recruited between November 1998 through March 2004. The parent study was reported previously (Cubillos-Angulo et al., 2019). The cases and controls were enrolled in the state of Rio de Janeiro, Brazil where the population is mostly white and brown ('*parda,* mixed ethnic ancestries) (IBGE, 2012). Racial/ethnic background was self-reported and used the definitions/approaches employed by the Brazilian government for race documentation. TB index cases were diagnosed by acid-fast bacilli (AFB) smear and/or culture, according to Brazilian Ministry of Health Guidelines (Ministério da Saúde, Brasil, 2019). TB index case variables included cough, AFB sputum grade, and chest radiographs. TB contacts were defined as living in the same household or reporting contact with the TB index case for >20 h weekly for 2 months (Cubillos-Angulo et al., 2019). In the analyses presented here, we used data from a subgroup of 482 individuals, which were selected by such criteria and included contacts with TST-positive or TST-negative results. Patients who developed active TB were excluded from the analysis. Additional details on inclusion and exclusion criteria as well as patient characteristics have been described previously (Cubillos-Angulo et al., 2019).

A standardized questionnaire was administered to obtain demographic and clinical data, including a history of risk factors for TB (e.g., HIV, diabetes, hematologic malignancies, and use of immunosuppressant drugs) and duration of contact with the index case. Consanguinity was considered if a contact was a grandparent, parent or sibling of the index case, whereas spouses or other relationships were not. At study baseline, a medical visit and chest radiograph were performed. BCG scar was assessed and TST reading was performed 48–72 h after administration at baseline, using 2 tuberculin units of the purified protein derivative RT 23 (Statens Serum Institute, Copenhagen, Denmark).

TST interpretation and TB diagnosis

A positive TST was defined as an induration larger than ≥5 mm induration, according to the Brazilian Ministry of Health (Ministério da Saúde, Brasil, 2019). Contacts with any TST ≥ 5 mm were not re-tested with TST. The Brazilian National TB Guidelines indicated that treatment of TST-positive individuals was systematically offered but implementation was not mandatory during the study period (Ministério da Saúde, Brasil, 2019). For the index case, active TB was diagnosed when ≥1 specimen yielded a positive microbiologic (AFB smear or culture) result by AFB smear and/or culture in Lowenstein Jensen (LJ) medium (Cubillos-Angulo et al., 2019).

Genotyping

Genomic DNA was extracted from peripheral blood collected from TB contacts at study enrollment. DNA extraction and genotyping were performed using the FlexiGene kit (Qiagen,

Germany). Genotypes of 8 gene polymorphisms were chosen for convenience since an RFLP assay was available: rs1101998 (*IFI16-PYHIN1-AIM2*), rs1633256 (*IFI16-PYHIN1-AIM2*), rs866484 (*IFI16-PYHIN1-AIM2*), rs304478 (*IFIT1*), rs304498 (*IFIT1*), rs11246213 (*IRF7*), rs59633641 (*IFIT5*) and rs10887959 (*IFIT5*) were detected using polymerase chain reaction restriction fragment length polymorphism (RFLP) method (Cubillos-Angulo et al., 2019). The primer sequences are in Supplementary Table S1. The PCR products were digested by the enzymes *EcoRII* for rs1101998 (*IFI16*), *AgsI* for rs1633256 (*IFI16*), *AgsI* for rs866484 (*IFI16*), *AarI* for rs304478 (*IFIT1*), *TfiI* for rs304498 (*IFIT1*), *BsaAI* for rs11246213 (*IRF7*), *ApoI* for rs59633641 (*IFIT5*) and *AgsI* for rs10887959 (*IFIT5*). Hardy-Weinberg equilibrium was tested for each SNP. We did not find significant deviation from Hardy Weinberg equilibrium except in rs304498 (*IFIT1*), and thus this SNP was excluded from further analysis. Linkage disequilibrium coefficients were calculated using Package "LDheatmap" (Shin et al., 2006) in the stats package in R 3.5.2 and using an R^2 and D' cutoff of 0.8. Haplotypes analysis were constructed in the stats package R 3.5.2 using the haplo.stats (version 1.6.0) R package (Sinnwell and Schaid, 2018).

Data analysis

Categorical data were presented as proportions and continuous data as medians and interquartile ranges (IQR). For clinical characteristics, a Fisher's exact test was used to perform 2×2 comparisons. Continuous variables were compared using the Mann–Whitney *U* test. For genetic analysis, a Cochrane-Armitage test for trend was used initially to examine the association of genotypes with TST positivity. SNPs were then evaluated with a Fisher's exact test using dominant (00 vs 01/11) and recessive (00/01 vs 11) models. We also estimated significant associations between indicated SNPs and TST positivity using multivariable logistic regression adjusted for race/ethnicity, family relatedness, gender and age in both dominant and recessive models. Finally, we also performed additional investigations with dominant and recessive models in a multivariable analysis with adjustment for age, gender, race/ethnicity, family relatedness, household contact status and characteristics of TB index case, such as cavities on chest X-ray, ≥2+ AFB sputum smear grade and positive sputum culture for Mtb. We also used the GTEx portal (https://gtexportal.org/home/) to evaluate the expression quantitative trait loci (eQTL) of the SNPs (Consortium, 2013). Furthermore, the likelihood of being a regulatory SNP was examined using the RegulomeDB dataset (http://www.regulomedb.org/snp/chr10/91150921) (Boyle et al., 2012).

Results

Characteristics of the study participants

We used a retrospective cohort study of contacts (N = 482) of pulmonary TB index cases (N = 145) to examine whether genetic variants of candidate genes were associated with TST positivity. Household contacts were more frequently observed in the group of individuals presenting with a positive TST result than in those with a negative TST (Table 1). Cavitary lesions as well as cough in the index TB cases were more frequent in participants who were TST positive compared to those who had negative results (p = 0.04 and p = 0.008, respectively). Other characteristics were similar between TST positive and TST negative individuals.

The study population was mostly female (n = 321, 67%), with a high frequency of first degree relatives with the index case (n = 229, 62%) (Table 1). In addition, the vast majority of participants were household contacts (n = 434, 90%). There were low frequencies of HIV infection, illicit drug use, prior TB and use of immunosuppressive drugs. Approximately 97% (n = 141) of the index cases had TB confirmed by culture. TB index cases frequently reported cough for more than 4 weeks (80%) and had high bacterial loads in sputum (60% had AFB grade ≥ +2;). In addition, 100 index TB patients had cavitary lesions on chest radiograph.

Association between polymorphisms and TST positivity

Two of seven polymorphisms were associated with TST positivity (rs1633256 and rs59633641 with an unadjusted genotypic trend test, Table 2). *PYHIN1-IFI16-AIM2* SNPs rs1101998 allele C (p = 0.01) and rs1633256 allele A (p = <0.01) were more common in TST positive participants and fit a recessive model (Table 2). *IFIT5* rs59633641 allele G (p = 0.04) was more

Table 1
Clinical characteristics of the study participants and association with tuberculin skin test (TST) positivity.

Characteristic	n	TST negative n = 219	TST positive n = 263	P-value
Age -median (IQR)	482	34 (23–50)	37 (24–49)	0.40
Male sex	482	74 (34)	87 (33)	0.92
First-degree relative of the index case	482	138 (63)	161 (61)	0.71
HIV infection	21	2 (67)	1 (6)	0.04
Race (% white)	462	113 (53)	144 (58)	0.40
Illicit drug use[a]	412	3 (2)	3 (1)	1.00
Prior tuberculosis	411	0 (0)	3 (1.4)	0.25
Household contact	480	190 (88)	244 (93)	0.06
Duration of contact (>20 h)	482	202 (92)	248 (94)	0.46
Comorbid conditions[b]	459	53 (26)	65 (26)	1.00
Immunosuppressant drugs[c]	414	0 (0)	2 (1)	0.50
Cough (>4 weeks)	481	5 (2)	5 (2)	0.76
Characteristics of TB index case				
Cavities on chest X-ray	473	24 (11)	47 (18)	0.04
Cough (>4 weeks)	481	86 (39)	136 (52)	0.008
≥2 AFB sputum smear	443	77 (39)	103 (42)	0.44
Mtb positive culture	339	147 (94)	178 (97)	0.18

"n" is the number of TB contacts for whom such data were available, out of a total of 482 included in the study. Data represents no. (%) or median and interquartile range (IQR) and were compared using the Fisher's exact test (categorical variables) or the Mann–Whitney *U* test (for age). TST: tuberculin skin test; AFB: acid-fast bacilli on sputum smear, CI: confidence interval; OR: odds ratio.

[a] Illicit drugs: cannabis, cocaine, or crack.

[b] Co-morbid conditions: diabetes mellitus, heart failure, chronic obstructive pulmonary disease, neoplasia, systemic lupus erythematous and hepatitis.

[c] Immunosuppressant drugs: corticosteroids, tumor necrosis factor blockers, calcineurin inhibitors, or interleukin inhibitors.

Table 2
Association between candidate gene polymorphisms and TST.

SNP	Genotype Frequency in TST negative				Genotype Frequency in TST positive				P-value			
	00	01	11	Total	00	01	11	Total	HWE	Genotypic Trend[b]	Dominant 00 vs 01/11	Recessive 00/01 vs 11
rs1101998 - PYHIN1- IFI16-AIM2 (T/C)	0.43	0.49	0.09	105	0.43	0.35	0.22	102	0.21	0.20	1.00	0.01
rs1633256 - *PYHIN1- IFI16-AIM2* (G/A)	0.56	0.41	0.03	80	0.53	0.26	0.22	78	0.02	0.04	0.75	<0.01
rs866484 - *PYHIN1- IFI16-AIM2* (C/G)	0.55	0.36	0.09	120	0.49	0.39	0.13	127	0.1	0.27	0.37	0.42
rs304478 - *IFIT1* (T/G)	0.37	0.47	0.17	131	0.44	0.48	0.08	135	0.66	0.06	0.26	0.04
rs59633641 - *IFIT5*[a] (C/G)	0.97	0.03	0	121	0.9	0.1	0	117	0.59	0.04	–	–
rs10887959 - *IFIT5* (C/T)	0.64	0.3	0.06	115	0.46	0.46	0.08	119	1.00	0.06	0.009	0.80
rs11246213 - *IRF7* (A/G)	0.48	0.4	0.12	126	0.37	0.46	0.17	133	0.25	0.07	0.08	0.29

Data represent genotype frequency of SNP TST: tuberculin skin test; SNP: single-nucleotide polymorphism; 00, homozygous common allele; 01, heterozygous allele; 11, homozygous rare allele. HWE: Hardy Weinberg equilibrium. Data were analyzed using the Fisher's exact test (2 × 2 comparisons) or the chi-square trend test (3 × 2 comparisons).

[a] No uncommon homozygous mutation; in this particular case, the test employed for the genotypic analysis was based on 2 × 2 comparison. P-value represents comparison of genotype frequencies without adjustment for any covariates.

[b] Cochrane-Armitage trend test.

common in TST positive individuals (trend test p = 0.04, Table 2). *IFIT1* rs304478 and *IFIT5* rs10887959 were also significantly associated with outcomes in recessive and dominant models, respectively.

In a multivariable model that included adjustment for race/ethnicity, family relatedness, gender, and age (Figure 1), we observed in the recessive model that *PYHIN1-IFI16-AIM2* rs1101998 (adjusted OR [aOR] = 2.90; 95%CI = 1.24–6.78; p = 0.014) and rs1633256 (aOR = 10.1; 95%CI = 2.20–46.28; p = 0.003) were associated with an increased risk of TST positivity. Moreover, in the dominant model, *IFIT5* rs10887959 (aOR = 0.49; 95%CI = 0.28–0.84; p = 0.01) and *IRF7* rs11246213 (aOR = 0.60; 95% CI = 0.36–1.00; p = 0.049) were also linked to a lower likelihood of positive TST.

We next used a multivariable regression analysis to adjust for household contact and characteristics of TB index case (cavities on chest X-ray, ≥2 AFB sputum smear and positive Mtb culture) as well as race/ethnicity, family relatedness, gender, and age (Figure 2). We confirmed in the recessive model that *PYHIN1-IFI16-AIM2* rs1101998 (aOR = 3.72; 95%CI = 1.15–12.0; p = 0.028) and rs1633256 (aOR = 24.84; 95%CI = 2.26–272.95; p = <0.009) were independently associated with increased odds of a positive TST. In addition, in the dominant model, *IRF7* rs11246213 was also independently associated with a lower likelihood of a positive TST (aOR: 0.50, 95%CI: 0.26–0.93; p = 0.029).

We next examined effects of linkage disequilibrium and SNP-SNP interactions in the *PYHIN1-IFI16-AIM2* region on chromosome 1. *PYHIN1-IFI16-AIM2 SNPs* rs8666484, rs1101998 and rs1633256 were all in moderate to high linkage disequilibrium (Supplemental Figure S1). In a haplotype analysis of chromosome 1 adjusted for age, gender, race/ethnicity, family relatedness and household contact, the haplotypes containing allele C from rs1101998 and allele A from rs1633256 did not have a higher risk of TST positivity compared to single SNP analyses (Figure 3 compared to Figure 2).

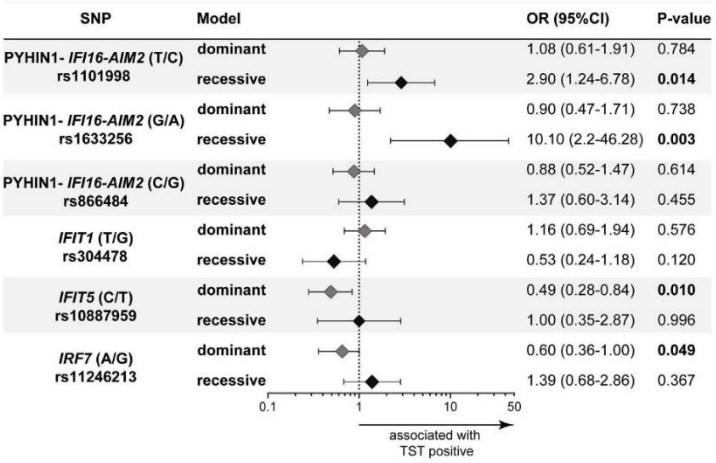

Figure 1. Multivariable model of association between genetic variants and TST positivity.
Analysis in all study participants Data represent no. SNP: single-nucleotide polymorphism; OR: odds ratio, 95% CI: confidence interval; P-value represents comparison of genotype frequencies in a dominant and recessive model with adjustment for race/ethnicity, family relatedness, gender, and age. OR (Odds ratio) represents association of minor allele with risk of TST positivity.

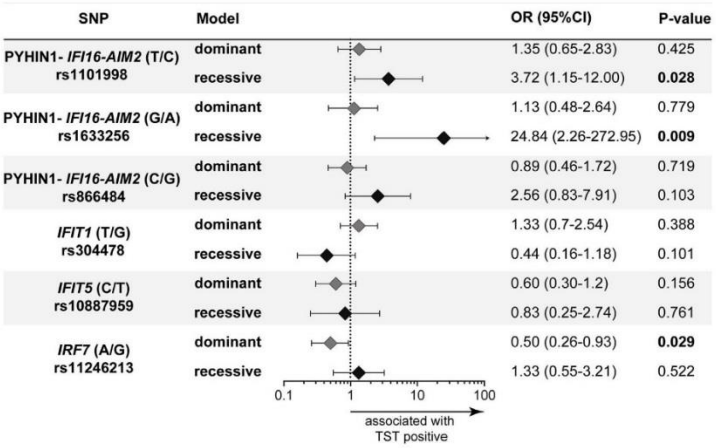

Figure 2. Multivariable model of association between genetic variants and TST positivity including clinical variables.
Analysis in all study participants Data represent no. SNP: single-nucleotide polymorphism; OR: odds ratio, 95% CI: confidence interval; P-value represents comparison of genotype frequencies in a dominant and recessive model with adjustment for age, gender, race/ethnicity, family relatedness, household contact and characteristics of TB index case: Cavities on chest X-ray, ≥2 AFB sputum smear and positive Mtb culture.

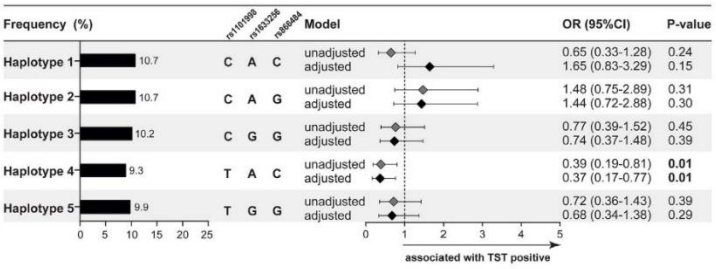

Figure 3. Haplotype analysis chromosome 1.
Haplotype analysis chromosome 1 of SNPs rs1101998, rs1633256, rs866484- *PYHIN1- IFI16-AIM2*. P-value represents comparison of haplotype frequencies with TST conversion in an unadjusted and adjusted model for age, gender, race/ethnicity, family relatedness and household contact.

Using an in silico approach with data from the GTEx portal tool (see Methods for details and also in (Consortium, 2013), we found that six polymorphisms (rs1101998, rs1633256, rs866484, rs304478, rs10887959 and rs11246213) were eQTLs in different tissues (Supplementary Table S2). Interestingly, three different SNPs were reported to be expressed in the spleen and/or lung, which are organs commonly affected by TB (Figure 4). The findings indicated that the *PYHIN1-IFI16-AIM2* rs1101998 genotype CC was linked to decreased expression of *AIM2* in spleen (Figure 4). The *PYHIN1-IFI16-AIM2* rs1633256 genotype AA was also associated with dampened expression of *AIM2* in spleen tissue (Figure 4). The *IFIT5* rs10887959 genotype CC was associated with lower expression of *IFIT5* in spleen and lung tissues (Figure 4). Finally, using a different online tool, the RegulomeDB dataset, we observed that *PYHIN1-IFI16-AIM2* rs1101998 exhibited high likelihood of being a regulatory SNP for a DNAase I hypersensitivity peak or transcription factor binding. Moreover, *IFIT5* rs10887959 displayed a high likelihood of being a regulatory SNP for transcription factor binding and a DNAase I hypersensitivity peak. Together, these data suggest that rs1101998, rs1633256, and rs10887959 are eQTLs.

Discussion

In the present study, we tested associations between SNPs from related genes in different pathways of DNA and RNA sensing and the type I IFN pathway in a large number of TB contacts. The notable finding was that *PYHIN1- IFI16-AIM2* rs1633256 and rs1101998 were associated with an increased risk of TST positivity whereas *IRF7* rs11246213 was associated with a lower probability of TST positivity. To our knowledge, SNPs in these genes have not previously been reported to be associated with the pathogenesis of Mtb infection in contacts.

Our results suggest that the *PYHIN1- IFI16-AIM2* rs1633256 and rs1101998 polymorphisms are associated with increased susceptibility to Mtb infection (i.e., a positive TST). The two polymorphisms are in a 3-gene locus on chromosome 1q23.1; thus, it is not possible

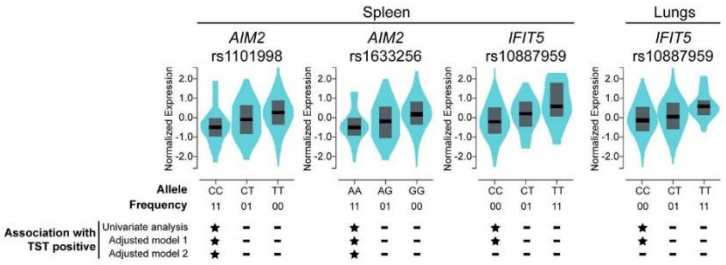

Figure 4. *In silico* expression of SNPs rs1101998, rs1633256 and rs10887959 adapted from GTEx eQTL database.
Normalized expression values were obtained from the GTEx eQTL database and violin plots (with median and interquartile range values) were used to represent the trends in data variation between the different SNPs. The full list of the SNPs and tissues evaluated is described in the Supplementary Table S2. The Figures describe the SNPs that had publicly available data on expression in spleen and/or lungs, due to its importance in TB pathogenesis. Thus, data on the SNPs rs1101998, rs1633256 and rs10887959 are shown. Allele frequency was determined as the following: 00, homozygous common allele; 01, heterozygous allele; 11, homozygous rare allele. A summary of the results of the analysis from the present study testing the association between each indicated allele and a positive TST result is shown at the bottom of the graphs. A star denotes statistically significant associations with TST positivity in the following conditions: (i) Univariate analysis: a comparison of genotype frequencies without adjustment for any covariates; (ii) Adjusted model 1: analysis in a dominant and recessive model with adjustment for race/ethnicity, family relatedness, sex, and age; and (iii) Adjusted model 2: analysis in a dominant and recessive model with adjustment for age, sex, race/ethnicity, family relatedness, household contact and characteristics of TB index case (cavity on chest X-ray, ≥2 AFB sputum smear and positive Mtb culture). SNP: single-nucleotide polymorphism.

to know which specific gene is most likely to exert a functional effect related to these genetic variants. The gene encoding Interferon-γ-inducible protein 16 (*IFI16*) (Trapani et al., 1994) is a multifunctional and ubiquitous host protein (Trapani et al., 1992), and a member of the PyHIN (pyrin and HIN200 domain-containing) protein family that consists of four family members: PYHIN1 (alias *IFIX*), *IFI16* (alias PYHIN2), *MNDA* (alias PYHIN3) and *AIM2* (alias PYHIN4) (Thompson et al., 2011). During Mtb infection of macrophages, *IFI16* is reported to be localized into the cytosolic compartment (Thompson et al., 2011) and Mtb DNA activates the cytosolic surveillance pathway. Mice genetically lacking IFI204 (a homolog gene of human *IFI16*) show reduced *IFIT1* and *IFN-β* induction against Mtb infection (Manzanillo et al., 2012).Furthermore, mycobacterial infection of $AIM2^{-/-}$ (absent in melanoma 2) mice induces elevated IFN-γ and reduced IFN-γ responses, leading to higher infection burdens and more severe pathology (Yan et al., 2018). In addition, in vitro studies demonstrated that AIM2-deficient macrophages display impaired activation of the inflammasome and defective production of IL-1b and IL-18 upon Mtb infection, making such cells highly susceptible to bacterial proliferation and cell death (Saiga et al., 2012). To the best of our knowledge, there are no previously reported studies on the relationship of PYHIN1 and TB. PYHIN1 detects Herpes Simplex (HSV-1) DNA and contributes to the induction of interferon response in human fibroblasts (Diner et al., 2015). In the present study, the SNPs associated with TST positivity (rs1633256 and rs1101998) are part of a large locus; thus it is possible that at least these two SNPs could be associated with any one of the 3 genes described above (*PYHIN1- IFI16-AIM2*) and influence the detection of Mtb DNA during infection. Future studies are warranted to directly elucidate the molecular mechanisms underlying these associations.

The human *IRF7* gene is located on chromosome 11p15.5 and is a member of the interferon regulatory factor family of transcription factors, comprised of nine members (IRF1 to 9) (Ning et al., 2011). This family is recognized by the regulation of many facets of innate and adaptive immune responses (Tamura et al., 2008). *IRF7* is the central transcription factor that induces *IFNA/B* gene transcription in response to cytosolic viral DNA and RNA in host cells (McNab et al., 2015). In addition, *IRF7* is produced by murine bone marrow–derived macrophage infected with Mtb (Cheng and Schorey, 2018; Leisching et al., 2017). In a recent meta-analysis, Mtb infection of THP-1 macrophages induced differential expression of *IRF7* (Zhang et al., 2019). Excessive type I IFN expression has been linked to increased TB-associated immunopathology and susceptibility to severe TB (Mayer-Barber et al., 2011; Mayer-Barber et al., 2014). *IRF7* SNPs have been reported to significant reduce IFNα production by plasmacytoid dendritic cells following stimulation with HIV-1 (Chang et al., 2011). The effect of IRF7 SNPs on reduced IFNα production, if present also in exposure to Mtb, could be a factor explaining the decreased susceptibility to Mtb infection reported here.

We also found that *IFIT5* rs59633641 was less frequently observed in individuals with a positive TST whereas *IFIT5* rs10887959 was more commonly detected in individuals with positive TST. *IFIT5* (IFN-induced protein with tetratricopeptide repeats-5) is a member of an interferon-induced protein with tetratricopeptide repeats (IFIT) family with five members (*IFIT1*, *IFIT2*, *IFIT3*, *IFIT1B* and *IFIT5*) localized in chromosome 10q23 (Diamond, 2014). The multivariable model with adjustment for race/ethnicity, family relatedness, gender and age demonstrated associations between the *IFIT5* rs10887959 and increased chance of negative TST. It has been recently demonstrated that *IFIT5* physically interacts with MAP3K7/TAK1 and IκB kinase (IKK) to activate the transcription factor NF-κB, which is a key regulator of the expression of genes involved in immune responses, inflammation, cell survival and cancers (Zheng et al., 2015). *IFIT5* is one of the main genes upregulated in active TB patients (Ahmed et al., 2016). The IFN-induced proteins regulate immune response against viruses. For example, it has been recently shown that *IFIT3* has a protective role in response to dengue virus infection of human lung epithelial cells (Hsu et al., 2013).

Our study has several strengths such as systematic TST testing (currently recommended as the diagnostic test for LTBI in most resource-restrained countries) and inclusion criteria that ensure microbiological confirmation of TB index cases. However, it is also important to highlight potential limitations of our investigation, such as the cross-sectional nature of the analyses, which are not able to establish causal relationships. We have not performed functional validation of the findings; however, we showed an analysis of gene expression data in silico. In addition, we considered a common Mtb strain to be responsible for infections within a household, but it is possible that that may not have always been true. In addition, LTBI was only measured by TST with no IGRA

assessments. These two tests are not perfectly concordant, so the TST negative group could probably include some individuals with positive IGRA results. Of note, IGRA was not available in Brazil at the time of the patient enrollment. The Food and Drug Administration (FDA) approved IGRA in 2001, and this test was introduced in Brazil in 2014, 10 years after the data collection of the present study was finalized. Regardless, our results clearly indicate associations between polymorphisms in innate immune genes linked to interferon responses and odds of Mtb infection assessed by TST positivity. Further translational studies are required to delineate the molecular events behind these associations.

Ethics statement

The study was approved by the Clementino Fraga Filho University Hospital (HUCFF), Federal University of Rio de Janeiro Ethics Review Board. Written informed consent was obtained from all participants or their legally responsible guardians, and all clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. The anonymity of study subjects was preserved with a code created with a link to personal identifiers.

Contributions

Study design: BBA, ALK, MMO, JRLS. Data collection: ECS, LEAA, ASdA, MOM, ASRM. Data analysis: MGMM, ECS, JMCA, MBA, KFF, TRS, TRH, MMO, BBA. Writing: JMCA, MBA, TRS, TRH, BBA.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.12.013.

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7 **PARTE III**

7. 1 HIPÓTESE

Os polimorfismos dos genes *CD14* e *NOD2* presentam uma associação com o risco de doenças causadas pela infecção por *Mtb* em diferentes estudos feitos em várias populações.

7.2 OBJETIVOS

7.2.1 **Objetivo geral**

Avaliar os trabalhos publicados até o momento sobre a influência dos polimorfismos dos PRR *CD14* e *NOD2* no risco de infecção por *Mtb*.

7.2.2 **Objetivos específicos**

- Selecionar todos os artigos elegíveis para a revisão sistemática sobre a influência dos polimorfismos nos genes *CD14* e *NOD2* no risco de infecção por *Mtb*;
- Caracterizar todos os estudos selecionados para obter a informação sobre os polimorfismos dos genes *CD14* e *NOD2* e sua associação com o risco de infecção por *Mtb*;
- Identificar a distribuição geográfica dos polimorfismos dos genes *CD14* e *NOD2* associados ao risco de infecção por *Mtb*;
- Avaliar as associações dos polimorfismos dos genes *CD14* e *NOD2* com o risco de infecção por *Mtb*.

8 **MANUSCRITO III**

Cubillos-Angulo *et al. Systematic Reviews* (2021) 10:174
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Systematic Reviews

RESEARCH Open Access

The influence of single nucleotide polymorphisms of *NOD2* or *CD14* on the risk of *Mycobacterium tuberculosis* diseases: a systematic review

Juan M. Cubillos-Angulo[1,2,3†], Catarina D. Fernandes[1,3,4†], Davi N. Araújo[1,2,3†], Cristinna A. Carmo[4], María B. Arriaga[1,2,3] and Bruno B. Andrade[1,2,3,4,5,6,7,8*]

Abstract

Background: Tuberculosis (TB) is still one of the leading causes of death worldwide. Genetic studies have pointed to the relevance of the *NOD2* and *CD14* polymorphic alleles in association with the risk of diseases caused by *Mycobacterium tuberculosis* (*Mtb*) infection.

Methods: A systematic review was performed on PubMed, EMBASE, Scientific Electronic Library Online (SciELO), and Literatura Latino-Americana e do Caribe em Ciências da Saúde (Lilacs) to examine the association between single nucleotide polymorphisms (SNP) and risk of *Mtb* diseases. Study quality was evaluated using the Newcastle-Ottawa Quality Scale (NOQS), and the linkage disequilibrium was calculated for all SNPs using a webtool (Package LDpop).

Results: Thirteen studies matched the selection criteria. Of those, 9 investigated *CD14* SNPs, and 6 reported a significant association between the T allele and TT genotypes of the rs2569190 SNP and increased risk of *Mtb* diseases. The genotype CC was found to be protective against TB disease. Furthermore, in two studies, the *CD14* rs2569191 SNP with the G allele was significantly associated with increased risk of *Mtb diseases*. Four studies reported data uncovering the relationship between *NOD2* SNPs and risk of *Mtb* diseases, with two reporting significant associations of rs1861759 and rs7194886 and higher risk *of Mtb* diseases in a Chinese Han population. Paradoxically, minor allele carriers (CG or GG) of rs2066842 and rs2066844 *NOD2* SNPs were associated with lower risk of *Mtb* diseases in African Americans.

Conclusions: The *CD14* rs2569190 and rs2569191 polymorphisms may influence risk *of Mtb* diseases depending on the allele. Furthermore, there is significant association between *NOD2* SNPs rs1861759 and rs7194886 and augmented risk *of Mtb* diseases, especially in persons of Chinese ethnicity. The referred polymorphisms of *CD14* and *NOD2* genes likely play an important role in risk *of Mtb* diseases and pathology and may be affected by ethnicity.

* Correspondence: bruno.andrade@fiocruz.br
†Juan M. Cubillos-Angulo, Catarina D. Fernandes and Davi N. Araújo contributed equally to this work.
[1]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil
[2]Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Bahia, Brazil
Full list of author information is available at the end of the article

Systematic review registration: CRD42020186523

Keywords: NOD2, CD14, Single nucleotide polymorphism, Tuberculosis

Background

Tuberculosis (TB) is one of the 10 leading causes of death around the world [1]. Approximately 1.7 billion people are infected by *Mycobacterium tuberculosis* (*Mtb*) worldwide [1]. The occurrence of this infection at different rates across countries and ethnicities indicates that genetic determinants may underlay the risk of developing diseases caused by *Mtb* infection such as pulmonary or extrapulmonary TB (referred hereafter as *Mtb* diseases) [2]. Work to date has highlighted notable gaps in factors that influence the risk *of Mtb* diseases [3]. For example, the associations of host genetic factors with *Mtb* infection have not been validated in multiple populations, and some study findings are inconsistent [3].

The immune system has a fundamental role in response to *Mtb* [4]. Thus, it is expected that polymorphisms in immune-related genes may directly affect the capacity of a host exposed to Mtb to control infection. Indeed, many studies have reported relationships between SNPs of immune-related genes and risk *of Mtb* diseases, such as the association between SNPs in *TLR4* [5], *TNFA* [6], and increased risk of active TB among highly exposed individuals. In addition to these genes, the nucleotide-binding oligomerization Domain-Containing protein 2 (*NOD2*) and Cluster Differentiation antigen 14 (*CD14*) genes are frequently studied in this setting, as these genes account for proteins that act in the recognition of mycobacterial molecular patterns and lead to immune activation against Mtb [7, 8]. While prior studies reported on the role of *NOD2* and *CD14*, many have disparate results, and often are restricted to certain populations [9].

The *CD14* gene codifies a glycosylphosphatidylinositol-anchored surface molecule present on the surface of monocytes, macrophages, and polymorphonuclear leucocytes, which functions as a key pattern recognition receptor (PRR) protein in innate immunity. CD14 plays a role in mediating signals from Toll-like receptors (TLRs) that recognize Mtb [10]. Additionally, *CD14* is critical to mounting an adequate innate response to aerogenic infection with Mtb [11]. Several studies have investigated whether risk *of Mtb* diseases is influenced by polymorphisms of this gene, though results have been inconsistent and inconclusive [4, 12]. For that reason, it remains difficult to determine the role of CD14 on risk *of Mtb* diseases in different populations, as studies with distinct ethnicities have conflicting results.

NOD2 is expressed in numerous cell types of the immune system, including macrophages, neutrophils, and eosinophils [13, 14]. It encodes a specialized protein that functions as an intracellular PRR of peptidoglycan through the recognition of muramyl dipeptide (MDP), a motif common to all bacteria [15], with a stimulating signal towards activation of immune responses [16]. When *NOD2* is activated by specific substances produced by bacteria, it turns on a protein complex named nuclear factor kappa-B (NFkB), resulting in transcription of pro-inflammatory mediators [17]. As such, there is mounting evidence that deregulation of *NOD2* signaling causes or contributes to a variety of human diseases, including asthma [18], cancer [19], inflammatory bowel disease [20], and TB [21]. Of note, studies have reported conflicting results on the relationship between *NOD2* SNPs and TB infection, finding mutations in the *NOD2* gene that may lead to both the increased and decreased risk *of Mtb* diseases [22]. Notwithstanding, like studies of *CD14* SNPs, most results diverge depending on the investigated population, leaving several knowledge gaps for a complete understanding of these relationships.

The present study aimed to evaluate work published to date on the influence of polymorphisms of the abovementioned PRRs on risk *of Mtb* diseases. We performed a systematic review to evaluate the association between all reported polymorphisms of *CD14* and *NOD2* and occurrence of *Mtb* diseases, and how such association may differ in distinct ethnic populations.

Methods

Study aim

We performed a systematic review on the influence of *CD14* and *NOD2* SNPs on the risk *of Mtb* diseases following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations.

Literature search

A systematic search was conducted between June 01, 2019, and June 25, 2020, by two independent researchers (the authors JMC-A and DNA) in the following databases: PubMed, EMBASE, Scientific Electronic Library Online (SciELO), and Literatura Latino-Americana e do Caribe em Ciências da Saúde (Lilacs). The keywords used in the search were '*Mycobacterium tuberculosis*', 'tuberculosis'; '*CD14*' or '*NOD2*'; and 'polymorphism', 'SNPs' or 'genetic polymorphism' with various combinations. The exact search strategy per database and the number of hits per database are illustrated in the Additional File 1.

Every original research article found in the search that was in English, Spanish, or Portuguese was considered, with no restriction on the publication date. Reviews, letters to the editor, and comments were not included but were sources of additional references that did not appear in the first search.

Selection of studies

Initially, titles and abstracts were reviewed and analyzed for eligibility (Fig. 1). Thereafter, all the eligible articles were fully read. These two steps were performed by two independent reviewers (JMC-A and DNA). The inclusion criteria were (1) the main subject of the article must have been the genetic influence on TB and (2) the study must have been related to a SNP in *CD14* and/or *NOD2* genes. Articles that did not mention TB susceptibility or risk *of Mtb* diseases, polymorphism in the genes indicated above, had non-sufficient data, reviews, meta-analyses, animal model studies, letters to the editor, or which were clearly not related to the theme were excluded.

Data extraction

Data extraction was performed individually by two researchers (DNA and CDF), and discrepancies between them were resolved by a third reviewer (JMC-A). All the information on important variables, publication date, methods, results, and conclusions of the included articles were registered in tables built in Microsoft Excel, made by two different researchers. Lastly, the content of those Excel tables was checked by a third reviewer (JMC-A), attesting the registry compliance.

Quality assessment

The quality assessment of each individual study was further performed according to the Newcastle-Ottawa Quality Scale (NOQS) [34] (Table 2), which measures the quality of a study based on three aspects: selection (maximum, 4 stars), comparability (maximum, 2 stars), and exposure (maximum, 3 stars). Thus, in the processing of the article quality analysis, a maximum of 9 stars could be obtained. Publication with a total score of 0–3

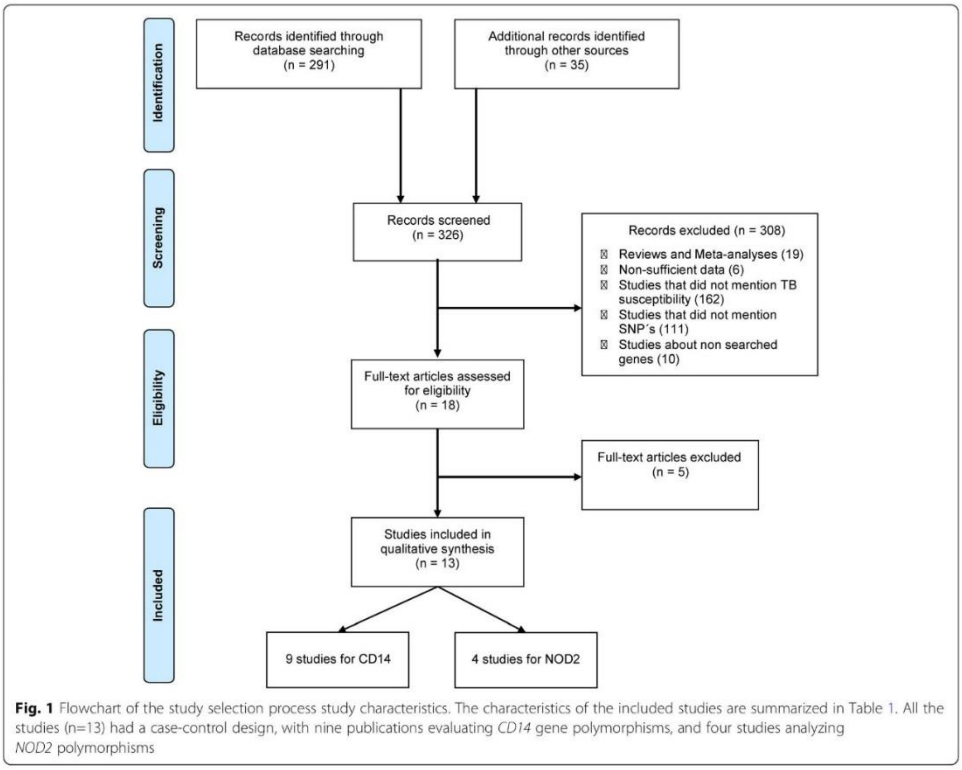

Fig. 1 Flowchart of the study selection process study characteristics. The characteristics of the included studies are summarized in Table 1. All the studies (n=13) had a case-control design, with nine publications evaluating *CD14* gene polymorphisms, and four studies analyzing *NOD2* polymorphisms

was classified as low quality, 4–6 as moderate quality, and ≥7 as high quality.

Linkage disequilibrium

Linkage disequilibrium coefficients were calculated and reported in only three studies [24, 30, 32]. In order to examine the overall profile of the linkage disequilibrium of the SNPs reported in our systematic review, we calculated the linkage disequilibrium for all SNPs of *CD14* and *NOD2* using the Package LDpop [35], establishing an R^2 cutoff of ≥ 0.8. LDpop takes as input two dbSNP reference SNP numbers and a selection of desired populations from the 1000 Genomes Project which includes sequencing data for 2504 individuals in 26 ancestral populations which are divided into 5 "super populations" [35]. In this approach, we used for the linkage disequilibrium the R^2 values for all individuals that had reported information for the SNPs.

Results

Selection of articles

Our primary search identified a total of 326 articles (Fig. 1). Through the study selection process, 13 articles met the inclusion criteria and were included in the systematic review [4, 12, 23–33]. The majority of the studies evaluated *CD14* gene polymorphisms (n= 9), whereas four studies analyzed *NOD2* polymorphisms. All of the selected studies adopted the case-control design, in which the case was defined as patients with tuberculosis, whether pulmonary, extrapulmonary, or both, and controls were defined as individuals not infected with Mtb. The majority of articles investigated the relationship between presence of polymorphisms and the risk *of Mtb* diseases (n= 7), whereas five studies tested association of SNPs with pulmonary and other forms of TB, and one assessed the relationship with spinal TB (Table 1).

In this systematic review, data on 4054 TB patients were examined, whereas 3993 individuals were identified as controls. The median sample size (IQR) per study was 267 (123–401) and 187 (127–413) for TB patients and healthy controls, respectively. The detailed characteristics of each study are shown in Table 1.

As observed in Fig. 2, most studies originated from Asia (n= 8) [23–28, 31, 32], with China leading as the most frequent study site (n= 5) [23–25, 31, 32], followed by Turkey (n= 1) [27], Iran (n= 1) [26], and South Korea (n= 1) [28]. The American continent also contributed to studies (n= 3): 1 in the USA [33], 1 in Mexico [4], and 1 in Colombia [12]. Only one study was set in Africa, specifically in Uganda [30], and Europe was represented by one study from Poland [29]. It is also possible to visualize in Fig. 2 that the *CD14* polymorphisms were studied in diverse populations from various ethnicities, including Mexican, Colombian, Polish, Turkish, Iranian, South Korean, and Chinese. In contrast, the *NOD2* polymorphisms were studied in 3 restricted populations: North Americans, Chinese, and Ugandan.

Quality assessment and sensitivity analysis

The quality scores of the studies, assessing the risk of bias, are displayed in Table 2. All the studies were of moderate quality (Table 2). Of note, 5 studies [12, 27, 28, 30, 33] were clear about the procedures used to test the control populations (persons without TB infection/exposure), affecting the quality score with regard to comparability between the experimental groups (Table 2).

We performed sensitivity analysis with five studies [12, 27, 28, 30, 33] that specifically mentioned use of tuberculin skin test to exclude TB in the control groups. The five studies investigated different SNPs, and only 3 studies [12, 27, 28] reported results from the same SNP (rs2569190). A summary of the observations is illustrated in Additional File 2. Hence, a sensitivity analysis could not be performed for the other SNPs.

CD14 polymorphisms and risk *of Mtb* diseases

In the present review, *CD14* polymorphisms were the most frequently studied in the context of the risk of *Mtb* diseases. In total, 9 studies reported potential associations, presenting data for different populations. The sample sizes in total were 1976 TB cases and 2011 controls. The studies reported data on 7 *CD14* SNPs: rs2569190 [4, 12, 23, 24, 26–29, 31], rs2569191 [24, 31], rs3138078 [24], rs2915863 [24], rs3138076 [24], rs5744455 [24], and rs5744454 [24].

Linkage disequilibrium

The study of Xue et al. [24] was the only one that evaluated the magnitude of linkage disequilibrium and found that rs2569191 and rs2569190 were in high linkage disequilibrium ($R^2 = 0.90$). We next examined effects of linkage disequilibrium and SNP-SNP interactions in the CD14 gene with the Package LDpop as described in the "Methods" section [35]. The SNPs rs2569190-rs2569191 ($R^2 = 0.9715$), rs3138078-rs3138076 ($R^2 = 0.9924$), rs3138078-5744454 ($R^2 = 0.9924$), and rs3138076-rs5744454 ($R^2 = 1$) were in high linkage disequilibrium and represented a haplotype block. The tables with all values of linkage disequilibrium for all SNPs can be found in supplementary information Additional File 3.

The findings on linkage disequilibrium are described below narratively for each SNP of CD14 identified in the search from here onwards as subsections. Likewise, given the heterogeneity in populations and SNPs, only a narrative description was feasible.

Table 1 Characteristics of included studies

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
Zheng et al., 2018 [23]	China	Case control	Chinese Han	**CD14** rs2569190 C>T	Spinal	240 TB and 150 controls; N= 390	Risk of *Mtb* diseases	Healthy controls	Not reported	Patients diagnosed with extra-pulmonary TB, who tested negative for PTB. Every included patient is free of co-morbidities. The spinal TB patients with comorbid disorders or other complications, such as rheumatoid arthritis, congenital cervical anomalies, trauma, prior spinal cervical surgery, HIV-positive, or ankylosing spondylitis were excluded from the present study.	Age- and sex-matched healthy subjects were enrolled as controls.	The frequency of the rs2569190 T allele was significantly higher in spinal TB patients than in controls (OR= 1.97, 95% CI= 1.24–3.42) ($p< 0.01$), and the frequency of the CT+TT genotypes (OR=2.10, 95% CI= 1.09–3.85) was also significantly higher in spinal TB patients than in controls ($p< 0.05$).
Xue et al., 2012 [24]	China	Case control	Chinese Han	**CD14** rs2915863 G>A; rs3138078 T>G; rs2569190 C>T; rs2569191 A>G; rs3138076 T>C; rs5744454 T>G, rs5744455 G>T	Pulmonary	318 TB and 380 controls; N= 698	Risk of *Mtb* diseases	The control group was unrelated blood donors with no history of TB or other immune diseases.	Not reported	Patients with PTB confirmed by clinical, radiological, and bacteriological investigation. Patients were excluded if they tested positive for HIV or if they were undergoing immunosuppressive agents.	Healthy individuals, with no history of TB or immune diseases.	The G allele of rs2915863 (OR=1.41, 95% CI=1.12–1.76), G allele of rs3138078 (OR=1.77, 95% CI=1.39–2.24), G allele of rs2569191 (OR=1.78, 95% CI=1.43–2.22), and T allele of rs2569190 (OR=1.73, 95% CI=1.40–2.15,

Table 1 Characteristics of included studies *(Continued)*

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
												respectively) were significantly associated with TB. rs3138076, rs5744454, and rs5744455, there were no statistically relevant associations
Zhao et al., 2012 [25]	China	Case control	Chinese Han	**CD14** rs2569190 C>T; rs2569191 G>A	Pulmonary and extrapulmonary TB	432 TB and 404 controls; N= 836	Risk of *Mtb* diseases	The control group comprised unrelated blood donors with no history of TB or other immune diseases.	Not reported	Patients were undergoing standard TB treatment at the TB clinic of the Sixth Hospital of Shaoxing and Hangzhou Red Cross Hospital between October 2005 and October 2009. They were excluded if HIV+ or were taking immunosuppressive agents.	Healthy, unrelated blood donors with no history of TB or other immune diseases. All control subjects were from the same ethnic population and geographical origin and were living in the same region as the patients with TB.	Both the frequency of allele T in the rs2569190 (OR= 1.4, 95% CI = 1.148–1.708) and allele G in the rs2569191 (OR = 1.512, 95% CI = 1.236–1.849) were significantly more frequent in cases than in controls and were also significantly associated with TB. The frequencies of genotypes CT and CC in the rs2569190 (OR = 0.46 and 0.63, respectively; 95% CI = 0.34–0.63 and 0.42–

Table 1 Characteristics of included studies *(Continued)*

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
												0.93), as well as the frequencies of genotypes AG and AA in the rs2569191 (OR = 0.60 and 0.44, respectively; 95% CI = 0.44–0.83 and 0.29–0.65) were lower in cases than in controls and were also protective against the disease.
Alavi-Naini et al., 2012 [26]	Iran	Case control	Persian, Balouch, and Afghan Iranians	**CD14** rs2569190 C>T	Pulmonary	120 TB and 131 controls; N= 251	Risk of *Mtb* diseases	The control group was healthy subjects with absence of clinical symptoms and signs suggestive of active pulmonary TB and normal chest X-ray.	Not reported	Culture-positive PTB patients were included. They had no other comorbidities such as myocardial infarction, septic shock, liver cirrhosis, or pancreatitis.	Healthy subjects matched for age, sex, and ethnicity. The inclusion criteria were absence of clinical symptoms or signs for active TB and normal chest X-ray, no medical history of TB or other infectious or autoimmune diseases.	The frequency of the rs2569190 T allele was 57% in TB patients and 44% in controls and was significantly different ($p < 0.002$). The risk of *Mtb* diseases was 2.3-fold greater in individuals with the T-allele (CT + TT) than in those without (OR= 2.3, 95% CI= 1.2–4.3, p = 0.006).

Table 1 Characteristics of included studies *(Continued)*

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
Rosas-Taraco et al., 2007 [4]	Mexico	Case control	White and Mestizo Mexican	**CD14** rs2569190 C>T	Pulmonary	111 TB and 174 controls; N = 285	Risk of *Mtb* diseases	Healthy individuals as control subjects	Not reported	All patients had active pulmonary TB diagnosed on the basis of clinical findings and smear or culture positive for PTB. Also, 67 were household contacts who were or were not genetically related to the patients. All participants were negative for HIV and diabetes and not treated with steroids or immunosuppressive agents.	114 healthy individuals. All of them were Mexican older than 18 years.	The frequency of the rs2569190 homozygous TT genotype was highest in patients with pulmonary TB (OR= 3.37, 95% CI= 1.58–7.19 p=<0.002). The frequency of the rs2569190 allele T had a significantly higher risk for the development of pulmonary TB (OR= 2.267; 95% CI= 1.5–3.3).
Ayaslioglu et al., 2012 [27]	Turkey	Case control	Turkish	**CD14** rs2569190 C>T	Pulmonary and extrapulmonary TB	88 TB and 116 controls; N = 204	Risk of *Mtb* diseases	Control group was selected from the adult population who had no underlying comorbidity and no diagnosis of tuberculosis.	Tuberculin skin test (TST)	Subjects who had a diagnosis of tuberculosis; age ≥16 years; and consented to be included into the study. Patients who had infectious diseases in the last 6 weeks, had significant chronic immunosuppressive systemic diseases, was pregnant, or HIV+ were excluded.	Subjects with no known diseases. Patients who had infectious diseases in the last 6 weeks, had significant chronic immunosuppressive systemic diseases, was pregnant, or HIV+ were excluded.	There was no significant difference in terms of genotype distribution between patients with tuberculosis and controls.
Kang et al., 2009 [28]	South Korea	Case control	Korean	**CD14** rs2569190	Pulmonary and extrapulmonary	274 TB and 422	Risk of *Mtb* diseases	A control group	Tuberculin skin test	Patients with confirmed	A group of healthy blood donors with	The frequency of the

Table 1 Characteristics of included studies *(Continued)*

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
				C>T	TB	controls; N = 696		consisting of 422 healthy blood donors with normal chest X-ray and without respiratory symptoms and signs	(TST)	tuberculosis were enrolled from Seoul National University Hospital in Korea. Patients with a positive HIV test were excluded.	normal chest X-ray and without respiratory symptoms and signs were recruited from medical students and employees of Seoul National University College of Medicine/Seoul National University Hospital.	rs2569190 T allele was higher in tuberculosis patients than in healthy controls (64% vs. 57%; p = 0.01), and rs2569190 TT genotypes (OR= 1.60; 95% CI, 1.01–2.54) were over-represented among tuberculosis patients (43% vs. 32%; p = 0.016).
Druszczyńska et al., 2006 [29]	Poland	Case control	Caucasian Polish	**CD14** rs2569190 C>T	Pulmonary TB	126 TB and 122 controls; N = 248	Risk of *Mtb* diseases	Healthy volunteers who had no past history of TB	Not reported	Not reported	Not reported	No association was found between the rs2569190 and the presence of TB.
Pacheco et al., 2004 [12]	Colombia	Case control	Caucasian and Mestizo Colombian	**CD14** rs2569190 C>T	Pulmonary and extrapulmonary TB	267 TB and 112 controls; N = 379	Risk of *Mtb* diseases	Healthy control individuals were recruited	Tuberculin skin test (TST)	Patients were recruited from different health units in the metropolitan area of Medellin, Colombia. Individuals, who were HIV+, or with a history of cancer, autoimmune, metabolic, or endocrine diseases, as well as pregnant women, were	Tuberculin-positive healthy control individuals were recruited from the Facultad de Medicina at the Universidad de Antioquia, and the institutions from where the patients were recruited.	No association was found between the allele and genotype frequencies and the presence of TB or between the different forms of the disease.

Table 1 Characteristics of included studies *(Continued)*

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
										excluded from the study.		
Hall et al., 2015 [30]	Uganda	Case control	USA	**NOD2** rs6500328 A>G, rs2111234 G>A and rs17313265 C>T	TB[a]	240 TB 595 controls; N= 835	Risk of *Mtb* diseases	Healthy household contacts without active disease were included in the control group	Tuberculin skin test (TST)	Analysis was gathered from two phases of a household contact study conducted in Kampala, Uganda. Subjects from the Household Contact Study were enrolled from 1995 to 1999. Individuals who presented at the study clinic with active culture-positive pulmonary TB were enrolled as index cases.	Analysis was gathered from two phases of a household contact study conducted in Kampala, Uganda. Subjects from the Household Contact Study were enrolled from 1995 to 1999. Healthy household contacts underwent a follow-up evaluation every 3 months for the first 6 months and were enrolled as control	rs17313265 association with TB in adults (examination of age-specific effects with TB) (OR= 2.82, 95% CI= 1.05–7.53). rs6500328 (OR= 2.44, 95% CI=1.01–5.88) and rs2111234 (OR= 1.56 95% CI= 1.07–2.28) showed a nominal association with resistance to *Mycobacterium tuberculosis* (Mtb) infection.
Zhao et al., 2012 [31]	China	Case control	Chinese Han, Uygur and Kazak	**NOD2** rs1861759 T>G	Pulmonary	425 TB and 380 controls; N=805	Risk of *Mtb* diseases	Healthy controls were HIV negative and none was known to present any autoimmune, chronic inflammatory or any other disease conditions.	Not reported	Han population 219 PTB and 215 healthy controls; For the Uygur population 86 PTB patients and 72 controls; for the Kazak 120 PTB patients and 93 healthy controls. The patients were diagnosed based on the TST test, chest X-ray, or sputum smear culture results.	They were HIV-negative patients and controls without any auto immune, chronic inflammatory, or any other disease condition. All selected patients had no mixed descendants within 3 generations.	By comparing the TG genotype frequencies of rs1861759 in the Han population, a significant difference was observed between the patients with TB and the healthy controls (OR=

Table 1 Characteristics of included studies *(Continued)*

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
												2.16; 95% CI= 1.31–3.58; p= 0.0023).
Pan et al., 2012 [32]	China	Case control	Chinese Han	**NOD2** rs7194886 C>T and rs9302752 T>C	Pulmonary	1043 TB and 808 controls; N= 1851	Risk of *Mtb* diseases	The controls were selected from a pool of individuals who participated in the local community-based health examination programs. None of controls had a history of active tuberculosis and/or a malignancy	Not reported	Patients older than 15 years, Han Chinese. They were divided in 3 groups: clinical symptoms of TB, sputum smear positive, and bacteriologically confirmed TB. 234 of 1043 TB patients were sputum smear negative.	Every control was older than 15 years, without history of TB and/or a malignancy.	The individuals carrying the CT/TT genotype of rs7194886 had an increased risk of pulmonary tuberculosis (OR= 1.35, 95% CI= 1.05–1.72). Allele frequency analysis found that variant allele T of rs7194886 (OR= 1.25, 95% CI= 1.00–1.57) was associated with an increased risk of tuberculosis. Haplotype rs9302752 C–rs7194886 T was associated with an increased risk of being sputum culture-positive tuberculosis (p = 0.039).
Austin et al., 2008 [33]	USA	Case control	African Americans	**NOD2** rs2066842	Pulmonary and extrapulmonary	377 TB and 187	Risk of *Mtb* diseases	Control subjects for	Tuberculin skin test	All cases patients were HIV negative	African Americans without history of	Minor allele carriers

Table 1 Characteristics of included studies *(Continued)*

Author/year	Country	Study design	Ethnicity	Gene/ studied SNP	TB diagnosis	Sample size	Type of study (Susceptibility/ resistance to *Mtb* diseases or risk of *Mtb* diseases)	Type of control	TB screening controls (Tuberculin skin test (TST) or Interferon -gamma Release Assay (IGRA))	Inclusion and exclusion criteria cases	Inclusion and exclusion criteria controls	Outcomes
				C>T rs2066844 C>T, and rs5743278 C>G	TB	controls; N = 564		this study were recruited without a history of TB, autoimmune disease, or other infectious diseases	(TST)	and had their ethnicities determined by self-indication. TB diagnosis was given based on bacille culture (286/312 PTB cases and 33/43 of EPTB); in these negative patients, diagnosis was based on clinical manifestations, chest X-ray, and clinical improvement to anti-mycobacterial treatment.	TB, autoimmune disease, or other diseases.	(heterozygous and homozygous) of rs2066842 (OR= 0.55, 95% CI=0.32–0.94, p= 0.02) and rs2066844 (OR= 0.27, 95% CI= 0.08–0.88; p= 0.01) presented decreased risks for TB disease. Conversely, the minor allele carrier (heterozygous) of rs2066844 (OR= 2.16, 95% CI= 1.10–4.72; p= 0.03) showed an increased risk for TB disease.

Abbreviations: *NOD2* nucleotide-binding oligomerization domain-containing protein 2, *CD14* Cluster Differentiation antigen 14, *TB* tuberculosis, *PTB* pulmonary tuberculosis, *EPTB* extrapulmonary tuberculosis, *OR* odds ratio, *CI* confidence intervals, *USA* United States of America

[a]Did not specify the TB type

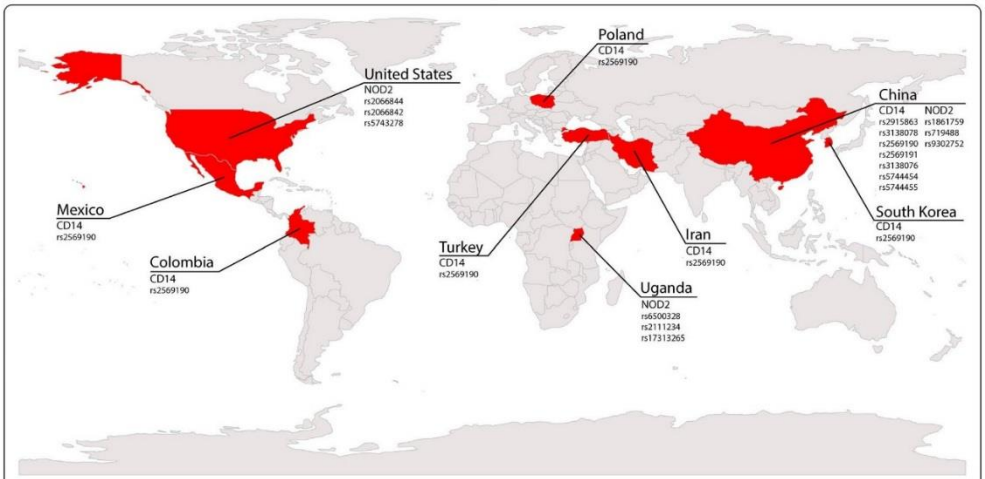

Fig. 2 Geographical distribution of CD14 and NOD2 SNPs associated with risk of *Mtb* diseases. Studies originated from nine different countries. Most studies were from Asia (n=8), with China leading as study site (n=5) followed by Iran (n= 1), Turkey (n= 1), and South Korea (n= 1). Three studies were performed in the American continent: one in the USA, one in Mexico, and one in Colombia. Only one study was set in Europe (Poland), and one was performed in Africa (Uganda)

SNP rs2569190

Of the 9 publications which investigated this gene locus, six studies reported a significant association between the T allele of SNP rs2569190 and higher odds of TB [4, 23, 24, 26, 28, 31].

Zhao et al. identified the T allele as the major allele of the cases that was higher in TB cases compared to healthy controls (63.53% vs 55.44%, respectively) [31]. Hence, the frequencies of the allele C in the rs2569190 polymorphism were lower in TB cases than in controls suggesting that CT and CC genotypes are likely protective against TB (OR = 0.46 and 0.63, respectively) [31]. Similarly, Alavi-Naini et al. observed that the risk *of Mtb* diseases was greater in individuals with the T-allele (CT

Table 2 Quality assessment of studies included in the systematic review by Newcastle-Ottawa Scale

N°	Source	Selection				Comparability		Exposure			Overall score[a]
		1	2	3	4	5A	5B	6	7	8	
1	Zhao et al. [25]	*	*	0	0	*	NA	*	*	NA	5
2	Kang et al. [28]	*	*	*	0	*	NA	*	*	NA	6
3	Alavi-Naini et al. [26]	*	*	0	0	*	NA	*	*	NA	5
4	Zhao et al. [31]	*	*	*	0	*	NA	*	*	NA	6
5	Rosas-Taraco et al. [4]	*	*	*	0	*	NA	*	*	NA	6
6	Pacheco et al. [12]	*	*	*	0	*	NA	*	*	NA	6
7	Austin et al. [33]	*	*	0	0	*	NA	*	*	NA	5
8	Zheng et al. [23]	*	*	0	0	*	NA	*	*	NA	5
9	Xue et al. [24]	*	*	*	0	*	NA	*	*	NA	6
10	Hall et al. [30]	*	*	*	0	*	NA	*	0	NA	5
11	Pan et al. [32]	*	0	*	0	*	NA	*	*	NA	5
12	Ayaslioglu et al. [27]	*	*	*	0	*	NA	*	*	NA	6
13	Druszczyñska et al. [29]	*	0	0	0	*	NA	*	*	NA	4

Abbreviations: *NA* not applicable
Star (*) indicates the score given to the study according to the NOS quality assessment scale
[a]Determined by the total number of stars assigned to study: 0–3 stars = poor; 4–6 stars = moderate; ≥7 stars = good quality

and TT) than in those without, finding that the T allele was more common in TB patients (57%) than in controls (44%) [26]. Moreover, in this same study, the C allele in homozygosis was a protective factor in a sub-analysis of Iranian subjects, with an OR of 0.44 (95% CI 0.23–0.83; p= 0.006) [26].

Zheng et al. [23] found that the frequency of the rs2569190 T allele was significantly higher in spinal TB patients compared to healthy controls (57.5% v 44%; p< 0.01), demonstrating that those with TT and CT genotypes was more frequent in spinal TB patients than in healthy controls (85% vs 44.17%; p<0.05) [23]. In contrast, Rosas-Taraco et al. [4] found that the most frequent allele of the rs2569190 was allele C in all study population, but the highest frequency of the rs2569190 T allele was 71% in household contacts of TB index cases who developed active TB, and 60% in those with pulmonary TB. In contrast, this allele was present in only 40% the healthy controls and 39.2% in the household contacts without TB (p < .0001) [4].

Xue et al. [24] observed allele T as the common allele of rs2569190 in the study population [24]. The TT genotype of rs2569190 was significantly associated with increased risk of *Mtb* diseases, present in 46% in patients diagnosed with pulmonary TB and 30% in healthy controls (p<0.001) [24]. Finally, Kang et al. [28] identified that the TT genotypes increased the risk *of Mtb* diseases and was significantly more frequent in TB patients than in healthy controls (43% vs 32%; p = 0.016) [28].

Notably, two articles, by Ayaslioglu et al. [27] and Pacheco et al. [12], described more reliably "control" groups; in such studies, the authors found no statistically significant difference between the presence of SNP rs2569190 and increased TB risk. Moreover, another study [29] also did not find any evidence of a significant association between SNP rs2569190 and TB development. Furthermore, no association was found between the *CD14*-159C/T polymorphism and TB clinical severity in studies that evaluated Turkish [27], Caucasian Polish [29], or White and Mestizo Colombian patients [12]. In the Turkish study performed by Ayaslioglu et al. [27], one hypothesis for the lack of association was the small sample size (88 TB cases and 116 controls). While investigating Caucasian Polish individuals, Druszczyñska et al. [29] did not specify whether TB patients and controls were from the same region, which could possibly account for the lack of association.

SNP rs2569191

Additional investigations evaluating the CD14 SNP rs2569191 revealed significant associations with odds of TB in 2 distinct publications from China [24, 31]. In one of these studies [24], individuals with the GG genotype of A-1145G were more likely to present with TB (p< 0.001), and this genotype was more common in the patients diagnosed with pulmonary TB (46%) than the healthy controls (28%). The second investigation [31] suggested that the frequencies of genotypes AG and AA were lower in TB cases compared to healthy controls [AG=181 (41.90%) vs 174 (47.41%) and AA= 65 (15.05%) vs 85 (23.16%) respectively], arguing for a protective role against TB and not found significance with the GG genotype. Both studies reported that the G allele of A-1145G was more prevalent in TB cases than in controls, indicating an increased risk *of Mtb* diseases.

Other SNPs

CD14 SNPs (rs3138078, rs2915863, rs2569192, rs3138076, rs5744455, and rs5744454) were evaluated by only one study [24], finding the following alleles to be significantly associated with TB: G allele of rs2915863 and the G allele of rs3138078. For the SNPs rs2569192, rs3138076, rs5744455, and rs5744454, there were no statistically relevant associations

NOD2 polymorphisms and risk *of Mtb* diseases

In regard to the *NOD2* gene, the results from the studies were diverse, with SNPs associated with either an increased or decreased risk *of Mtb* diseases in each of the study populations based on ethnicity, age group, or biological sex. In such studies, a total of 2085 TB cases and 2347 controls were investigated. These studies were performed in different countries including China, Uganda, and North America, with the last being focused on African Americans. Two publications reported data on *NOD2* SNPs in China [25, 32], with a total of 2651 individuals.

Linkage disequilibrium

The study of Hall et al. [30] used the linkage disequilibrium for selected SNPs with linkage disequilibrium R2 ≥ 0.8. The other study that used linkage disequilibrium was Pan et al. [32]. In such investigation, the selected haplotype blocks where haplotype rs9302752C–rs7194886T (block 1) which was associated with an increased risk of being a case of sputum culture-positive tuberculosis. We next examined the effects of linkage disequilibrium and SNP-SNP interactions in the NOD2 gene with the Package LDpop [35]. Here, we found that no reported SNP was in high linkage disequilibrium. The tables with all values of linkage disequilibrium for all SNPs can be found in supplementary information Additional File 4.

Hereafter, the findings on linkage disequilibrium will be reported narratively for each NOD2 SNP identified in the search as subsections. Once again, given the heterogeneity in populations and SNPs, only a narrative description was feasible.

SNP rs1861759

Zhao et al. [25] showed that in the Han population, the T allele frequency of the SNP rs1861759 in the patient group and in the control group was the most common. The TG genotype in rs1861759 SNP was substantially associated with TB (p = 0.0023) in the Han population with a frequency of 53 (24.2%) in pulmonary TB patients and 28 (13%) in healthy controls. Interestingly, the same study did not identify this relationship between the TG genotype and TB in Chinese participants of Kazak or Uygur ancestry.

SNP rs7194886

A different study by Xue et al. investigating Han Chinese individuals [24] found that the frequency of the rs7194886 T allele was associated with risk *of Mtb* diseases (p=0.042), but allele C was the most common in all study subjects. Patients who presented CT or TT genotypes of rs7194886 were more likely to present with TB when compared to individuals with the CC genotype (p=0.018). The genotype CT was more frequent in the pulmonary TB case compared with uninfected controls (CT= 271 [26.03%] vs 179 [22.43%]). Furthermore, the study found a higher frequency of rs7194886 polymorphism in either smokers (p=0.019) or men (p=0.014) [28].

SNP rs9302752

In the same study [32], the haplotype rs9302752 C–rs7194886 T was linked with a higher risk of presenting with sputum culture-positive TB (p = 0.039).

SNPs rs2066842, rs2066844, and rs5743278

The study by Austin et al., which predominantly recruited African Americans in the USA [33], found C allele of the SNPs rs2066842, rs2066844, and rs5743278 in all case patients and control subjects. This study reported an association between NOD2 SNPs rs2066842, rs2066844, and rs5743278 and odds of TB. The study participants who were carriers of the CC genotype in SNPs rs2066842 (p=0.02) and rs2066844 (p=0.01) were less likely to have TB. The frequencies of the CC genotype (340 vs 156) in rs2066842 and in rs2066844 (372 vs 178) were higher in patients with TB compared with control subjects. Moreover, individuals who were heterozygous (CG) in rs5743278 were more frequent in those with TB compared to the control group (39 vs 10) and exhibited an increased chance of having TB (p=0.03).

SNPs rs17313265, rs6500328, and rs2111234

The study by Hall et al. in the adult African population from Uganda [30] reported that presence of the SNP rs17313265 was associated with increased risk *of Mtb* diseases (OR= 2.82, 95% CI= 1.05, 7.53; p= 0.0052). Notably, increased frequency of the rs6500328 and rs2111234 SNPs was found in those without TB, suggesting that such SNPs may protect against Mtb infection, with OR of 2.44 (95% CI= 1.01. 5.88; p= 0.047) and 1.56 (95% CI= 1.07, 2.28, p=0.020), respectively.

Discussion

The molecular basis of immune response to infectious diseases is an indispensable approach to understand how gene regulation ultimately may impact clinical outcomes. In recent decades, a great deal of research has sought to identify associations between genetic polymorphisms and risk *of Mtb* diseases [36, 37]. Most of the target loci are PRRs contributing to control of mycobacterial diseases, especially TB [38]. *CD14* and *NOD2* are considered to be key PRRs in the innate immune system [9]. In this systematic review, a variety of studies were identified evaluating seven *CD14* SNPs and four evaluating nine *NOD2* SNPs, finding several genetic variants associated with Mtb infection.

We identified studies that found significant association between the T allele of *CD14* SNP rs2569190 and increased risk *of Mtb* diseases in different ethnic groups. This polymorphism is located in the promoter region of the *CD14* gene [9], and the T allele seems to act as a negative regulator of in vitro T-cell proliferation and decreased production of cytokines, including interferon-γ (IFN-γ) [28]. IFN-γ is an essential cytokine for the control of mycobacterial infection [39], and therefore, rs2569190 may produce an environment that favors development of TB. Interestingly, other studies found this SNP associated with ischemic stroke [40], cardiovascular disease [41], and asthma [42], indicating that these polymorphisms could actually lead to a more profound alteration in immune responses that may affect a large number of clinical conditions.

Another important association of *CD14* polymorphism with TB was described in this review, involving the SNP rs2569191. In this setting, the G allele of A-1145G was more prevalent in TB cases than in controls in two Chinese studies. An interesting link between such SNP and circulating concentrations of IgE has been proposed. In a study performed in patients with asthma, the authors identified heightened IgE concentrations in those who had the G allele of A-1145G [43]. Moreover, other studies identified IgE as a marker of Mtb infection, in which pre-treatment levels of serum total IgE concentrations in TB patients were significantly higher than in healthy individuals; such levels decreased after successful antitubercular treatment [44, 45]. It is possible that the polymorphism rs2569191 plays an important role in TB infection which may be related to IgE concentrations. Future studies in other ethnic groups are warranted to directly test this hypothesis. Interestingly, the two SNPs rs2569191 and rs2569190 are found to be in linkage disequilibrium [46]. For this reason, future studies will need

to determine whether these SNPs influence risk *of Mtb* diseases individually when solely associated.

NOD2 is one of the most well-studied genes in the context of the innate immune response against microbial pathogens [47]. This gene accounts for a cytoplasmic receptor belonging to the NOD-like receptor family [48] and is known to participate in the induction of inflammation during Mtb infection [49]. In experimental conditions, Mtb recognition is *NOD2*-dependent [50]. Mice genetically deficient in *NOD2* are shown to be more susceptible to TB [51]. Here, we found two studies [25, 32] in the Chinese population that reported three different polymorphisms (rs1861759, rs7194886, and rs9302752) associated with increased risk *of Mtb* diseases. The rs1861759 (synonymous variant) TG genotype is associated with increased risk *of Mtb* diseases [25]. Individuals with the rs7194886 CT or TT genotype are more likely to develop TB [32]. The rs9302752 C allele has been linked to a higher risk of being sputum culture-positive TB. To our knowledge, these three polymorphisms do not appear in other TB studies. Curiously, such SNPs have been also related to increased risk of leprosy [52].

In an African-American study [33], it was observed that the three *NOD2* polymorphisms exhibit impact on risk *of Mtb* diseases. The polymorphism rs2066844 represents missense mutations whose variants are located in the C-terminal region and cause defective production of proinflammatory mediators [53]. The rs2066842 is a missense variant but, when presented alone, does not alter gene function [54]. Importantly, the two polymorphisms in our systematic review appear to protect against TB when in the presence of allele C [33]. In contrast, the presence of the T allele in such mutations was associated with increased risk *of Mtb* diseases. The association between the presence of the T allele and augmented pathology has been described for other diseases such as Crohn's disease [55] and gastric cancer [56]. The genotyping heterozygous (CG) of rs5743278 has been linked to higher risk of *Mtb* diseases [55]. It is possible that the rs5743278 SNP causes an amino acid change from a low hydrophobic arginine to a highly hydrophobic tryptophan, modifying the stability of the *NOD2* structure or its ability to properly interact with Mtb ligands [55]. Finally, one study in a population from Uganda described the *NOD2* rs17313265 polymorphism associated with increased risk *of Mtb* diseases in adults whereas the SNPs rs6500328 and rs2111234 exhibited decreased risk *of Mtb* diseases [30]. Furthermore, these three polymorphisms have not been reported in other studies with TB patients. These findings indicate that NOD2 polymorphisms may be dramatically affected by ethnicity and/or ancestry. This scenario reinforces the need of additional investigations performed in a variety of ethnic populations, particularly when study design uses family-based control subjects to eliminate the bias in stratification of the populations.

To our knowledge, the present study is the first systematic review to explore the relation of all *CD14* and *NOD2* SNP polymorphisms with the risk *of Mtb* diseases in different ethnicities. A strength of our study was the comprehensive search strategy, which used detailed inclusion and exclusion criteria. Moreover, methodological quality was assessed in duplicate using the Newcastle-Ottawa scale [34], which reduced the subjectivity of the selection of studies and allowed for precise evaluation of the risk of bias in several domains. There was no report categorized as low quality, resulting in a review with reduced risk of bias. Another important strength is that the inclusion of the SNPs was not limited to one specific locus or ethnicity, allowing the study to observe different loci associated with risk *of Mtb* diseases or not in various populations.

Our study has some limitations. It was not possible to perform a meta-analysis and, consequently, a sensitivity analysis, mainly because a considerable part of the SNP polymorphisms appeared in only one study at a time. Only two SNPs, rs2569191 which was seen in two studies and rs2569190 which was reported in nine publications were consistently investigated. However, the need for a study to compile and critically revise these results in different ethnicities reinforces the importance of our work, regardless of quantitative analyses. Another limitation was the moderate quality of those studies included in the systematic review. One reason for this quality is the ambiguous concept of the control groups reported in most of the studies where the precise screening method to categorize these groups was not specifically described. To circumvent such concern, we have contacted the authors individually but obtained replies from just two articles. Only 5 of the 13 included studies reported active TB screening of asymptomatic individuals with either tuberculin skin test (TST) or interferon-gamma releasing assay (IGRA). This imprecision in the definition of control groups may limit the full interpretation of the study results. Our study highlights the need of studies standardizing description of the control groups to more accurately delineate the associations between SNPs and risk *of Mtb* diseases. Other determinants for the moderate quality were the small sample size of some studies and lack of clarity regarding description of the tools/approaches used to choose the SNPs, such as the examination of the linkage disequilibrium.

This review identified multiple studies that determined an association of the minor allele T at position chr5: 140633331 of rs2569190 CD14 polymorphism with increased risk *of Mtb* diseases in persons from different ethnicities. In addition, the *CD14* SNP rs2569191 and

the *NOD2* SNPs rs1861759 and rs7194886 are shown here to be associated with a high risk *of Mtb* diseases in the Chinese population. In contrast, genotypes CG or GG of rs2066842 and rs2066844 were at low risk *of Mtb* diseases in African Americans. Since such genes account for key molecules of the immune system, the referred polymorphisms of *CD14* and *NOD2* genes likely play an important role in TB physiopathology. These results add knowledge to the field by reinforcing the genetic influence on the risk *of Mtb* diseases. Such knowledge, if validated by larger studies, may help development of tools for assessment of the risk *of Mtb* diseases and hopefully predict clinical outcomes in precision medicine approaches.

Abbreviations

MONSTER: Multinational Organization Network Sponsoring Translational and Epidemiological Research; UNIFACS: Universidade Salvador; FTC: Faculdade de Tecnologia e Ciências; TB: Tuberculosis; SNP: Single nucleotide polymorphisms; NOQS: Newcastle-Ottawa Quality Scale; Mtb: *Mycobacterium tuberculosis*; *NOD2*: Domain containing protein 2; CD14: Cluster differentiation antigen 14; PRR: Pattern recognition receptor; TLRs: Toll-like receptors; MDP: Muramyl dipeptide; NFkB: Nuclear factor kappa-B; PRIS MA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SciELO: Scientific Electronic Library Online; Lilacs: Literatura Latino-Americana e do Caribe em Ciências da Saúde; IQR: Median sample size; OR: Odds ratio; IFN-γ: Interferon-γ; CNPq: *Conselho Nacional de Desenvolvimento Científico e Tecnológico*; OAS-PAEC : Organization of American States - Partnerships Program for Education and Training; CAPES: *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*—Brazil; FAPESB: *Fundação de Amparo à Pesquisa da Bahia*; PTB: Pulmonary tuberculosis; EPTB: Extrapulmonary tuberculosis; CI: Confidence intervals; TST: Tuberculin skin test; IGRA : Interferon-gamma releasing assay; USA: United States of America; NA: Not applicable

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13643-021-01729-y.

Additional file 1. Table with the exact search strategy per database and the number of hits per database.

Additional file 2. Table of the five studies that evaluated the control groups with the tuberculin skin test.

Additional file 3. Tables describing the frequencies and the linkage disequilibrium of the seven CD14 SNPs.

Additional file 4. Tables depicting the frequencies and the linkage disequilibrium of the nine NOD2 SNPs.

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Authors' contributions

JMC-A, CDF, and DNA conceived the study. JMC-A, CDF, DNA, and MBA were responsible for the data collection. JMC-A, CDF, DNA, MBA, and BBA analyzed the data and built the figures and tables. JMC-A, CDF, and DNA performed the scientific literature search. JMC-A, CDF, and DNA participated in the writing of the first draft of the manuscript. BBA critically revised the article. The authors read and approved the final version of the manuscript.

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Availability of data and materials

All data used in the present study were retrieved from the publications used in the systematic review and are publicly available.

Declarations

Ethics approval and consent to participate

There were no patients directly involved in the research. The present study used public data from previously published studies to perform a systematic review. All information given to the research team was de-identified. Thus, the study was exempted from revision by the Institutional Review Board of the Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil, and did not require signed consent forms.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil. [2]Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Bahia, Brazil. [3]Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador, Bahia, Brazil. [4]Curso de Medicina, Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Bahia, Brazil. [5]Curso de Medicina, Faculdade de Tecnologia e Ciências (FTC), Salvador, Bahia, Brazil. [6]Curso de Medicina, Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil. [7]Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, USA. [8]Wellcome Centre for Infectious Disease Research in Africa, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa.

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9 **DISCUSSÃO**

Um número crescente de estudos e investigadores tem focado suas atenções para novos biomarcadores buscando superar as limitações dos testes de imunodiagnóstico existentes para infecção por TB, incluindo sua incapacidade de diferenciar entre TB ativa e LTBI e, mais importante, pelo fato de tais testes ainda apresentarem baixo valor preditivo de progressão para TB (SUDBURY; CLIFFORD *et al.*, 2020).

Esta incapacidade é dada pelo processo complexo e de vários estágios desde o primeiro encontro com a bactéria na infecção pelo *Mtb* (MATHEMA; KUREPINA *et al.*, 2006). Não todos os indivíduos expostos à *Mtb* são infectados e, dependendo da pressão da infecção, muitos permanecerão livres da infecção (MOLLER; KINNEAR *et al.*, 2018). Alguns dos indivíduos permanecerão assintomáticos e entraram em um estágio denominado LTBI, isto graças aos granulomas que limitam a propagação e a replicação das bactérias. Nesse estágio, o sistema imunológico pode conter a infecção, mas, se falhar, a infecção pode progredir para doença ativa (FRIEDEN; STERLING *et al.*, 2003). Esta progressão para TB ativa é dada pela disseminação a partir de granulomas (reativação), aliás pode ocorrer reinfecção com outra cepa micobacteriana, ou pode se dar uma doença subclínica caracterizada por sintomas intermitentes e infecciosidade periódica (HOUBEN; DODD, 2016).

Todos estes estágios da infecção de *Mtb* e o processo complexo da progressão para TB ativa pode estar sendo influenciada por fatores ambientais e genético envolvidos na suscetibilidade individual (COMMANDEUR; VAN MEIJGAARDEN *et al.*, 2013). O surgimento dos estudos de SNPs geram uma possibilidade de entender melhor sua relevância durante a infecção humana causada por *Mtb*.

No nosso primeiro trabalho buscamos examinar associações entre 5 SNPs de genes relacionados ao sistema imunológico em uma grande coorte de contatos próximos de pacientes com TB pulmonar confirmados microbiologicamente, provenientes de uma região altamente endêmica no Brasil, como é o caso do Rio de janeiro (manuscrito 1). Os polimorfismos como o *TLR2* e *TLR4*, já foram analisados na população marroquinas, mas o papel de estes polimorfismos na infecção por LTBI e na progressão para TB ativa não estava totalmente compreendido.

Nossos resultados mais importantes foram que *TLR4* Thr399Ile (rs4986791) e *TNFA*-238 (rs361525) estiveram independentemente associados com a conversão de TST e subsequentemente com o desenvolvimento de tuberculose. Além disso, os SNPs *TLR2* e *TLR4* podem, às vezes, agir combinados para aumentar as chances de conversão de TST e o

desenvolvimento de TB ativa.

O TLR4 foi sugerido como o principal receptor envolvido na imunidade à TB por meio do reconhecimento de antígenos micobacterianos (SEPEHRI; KIANI *et al.*, 2019). TLR4 é mais específico para *Mtb* do que para outras micobactérias, visto que no estudo realizado por Carmona (CARMONA; CRUZ *et al.*, 2013), foi revelado que o TLR4 reconhece o *Mtb* ao reconhecer outras micobactérias. Além disso, TLR4 é regulado positivamente nos macrófagos derivados de pacientes infectados com *Mtb*, em comparação com pacientes infectados com *Mycobacterium Africanum* com perfil distinto de citosinas (CARMONA; CRUZ *et al.*, 2013).

O TLR4 ativa as células imunes, especialmente macrófagos e células dendríticas, para combater a infecção por *Mtb* (DE OLIVEIRA; PERESI *et al.*, 2014). TLR4 interatua nas funções fagossomicas por meio da ativação de macrófagos e maturação de células dendríticas estimulado pelo *Mtb* (MAZUREK; IGNATOWICZ *et al.*, 2012). TLR4 também induz funções das células imunes adaptativas como células B, por meio de um aumento da expressão de citosinas pró-inflamatórias como são TNF- α, IL-17, IL- 1β e IL-21 (DU PLESSIS; KLEYNHANS *et al.*, 2016).

O TLR4 demonstra ter um papel positivo no combate ao *Mtb*, mas também pode presentar uma relação negativa com a TB em algumas situações. Quando o TLR4 foi inibido em células não imunes a contagem de *Mtb* aumento o que sugere que o TLR4 está vinculado como um fator importante na replicação do *Mtb* em este tipo de células (NAZARI; KARAKOUSIS *et al.*, 2014). A interação entre TLR4 e *Mtb* também é capaz de induzir estresse oxidativo mitocondrial (KO; SARASWATHY *et al.*, 2011). Com estes resultados positivos e negativos da relação do TLR4 e *Mtb*, foi sugerido que algumas variações genéticas dentro do gene *TLR4* pode influenciar o resultado da TB como observamos em nosso artigo (SEPEHRI; KIANI *et al.*, 2019). Numa meta-análise que incluiu 16 estudos de caso-controle em SNPs dos genes *TLR4* e *TLR9*, foi demostrado que o mesmo SNP que foi analisado por nós, o rs4986791, estava significativamente associado ao risco de infecção por *Mtb* em africanos (ZHAO; LIU *et al.*, 2015) e em outro estudo, este mesmo SNP está significativamente associado ao aumento do risco de TB no subgrupo asiático (WU; HU *et al.*, 2015).

Por outro lado, o papel conjunto de *TLR2* e *TLR4* na conversão de TST e desenvolvimento de TB ativa pode estar relacionado à estimulação de apoptose nos macrófagos, pois se apresentou significativamente reduzida nos macrófagos deficientes de TLR2 e TLR4 (LIM; CHOI *et al.*, 2015). O TLR2 é um dos mais estudados e associados a doença da TB. Existem evidências descrevendo que o TLR2 pode reconhecer lipoproteínas bacterianas incluindo *Mtb* (HOOK; CAO *et al.*, 2020). Uma revisão sistemática recente mostrou que o SNP

no gene *TLR2* (rs5743708), também estudado por nós, estava significativamente associado a alto risco de TB e o risco se eleva nas populações asiáticas e caucasianas (HU; TAO *et al.*, 2019).

A TNF-α é uma citocina secretada principalmente por diferentes células do sistema imunológico, que atua como mediador central da inflamação: ativa macrófagos, regula a produção de interferon γ e estimula a produção de IL-1 e IL-6 (AYAZ; DEMIRKAYA *et al.*, 2010). Algumas doenças são tratadas com inibidores de TNF-α, mas na tuberculose todos os antagonistas de TNF-α tem o potencial de predispor os pacientes a infecções granulomatosas (LYON; ROSSMAN, 2017), pois, os antagonistas de TNF-α podem gerar a lise de macrófagos envolvidos na formação do granuloma, promovendo instabilidade no granuloma e, resultando na disseminação da infecção (LYON; ROSSMAN, 2017). Há relato de aumento significativo na incidência de TB extrapulmonar entre pacientes recebendo antagonistas do TNF-α (DANTES; TOFOLEAN *et al.*, 2018); esta relação poderia explicar o porquê em nossos resultados o SNP rs361525 esteve associado a um maior risco de conversão de TST e, subsequentemente, o desenvolvimento de tuberculose. Uma evidência importante é que os pacientes com terapia antagonista de TNF-α tem o potencial de desenvolver TB, devido à sua indução de lise celular que predispor os pacientes a infecções granulomatosas (LYON; ROSSMAN, 2017).

O polimorfismo rs1143627–IL1β é fator protetor para a conversão de TST, este polimorfismo foi mais comum naqueles que não converteram. IL1β é um potente mediador de inflamação envolvido na patogênese de doenças inflamatórias e autoimunes graves, como a TB PMID:20177398. Este papel protetor do IL1β pode ter relação com o demostrado em camundongos, onde aqueles que presentam deficiência de IL-1β sofrem uma infecção aguda com uma pneumonia necrotizante causada pela infecção de *Mtb*, enquanto camundongos com presença de IL-1β controlaram a infecção aguda por *Mtb* e a inflamação pulmonar (BOURIGAULT; SEGUENI *et al.*, 2013). A citocina IL1β quando é produzida em alta quantidades no sangue de pacientes HIV^{+} interrompe a ativação da LTBI o que exibe de novo seu fator protetor (DEVALRAJU; NEELA *et al.*, 2019). Este mesmo polimorfismo (rs1143627) em populações asiáticas, caucásicas e arábicas mostrou-se como um fator protetor contra o queratocono em uma metanalise, sendo evidencia como fator protetor para várias doenças, contudo deveria ser considerado para futuros estudos (HARATI-SADEGH; SARGAZI *et al.*, 2021).

Conhecendo a importância destes polimorfismos em outras populações humanas, surgiu um questionamento sobre a relação dos polimorfismos com a detecção de DNA microbiano e a

ativação das vias do IFNs tipo I que regulam as respostas dos macrófagos à infecção por *Mtb*. Por isso, analisamos o impacto de oito polimorfismos em genes de IFNs tipo I relacionados a redes de resposta imune na patologia e controle de patógenos durante a infecção por *Mtb* pouco compreendidos, na mesma coorte de contatos de pacientes com TB pulmonar utilizada para a elaboração do primeiro manuscrito. Nosso achado mais notável foi que o polimorfismo *PYHIN1-IFI16-AIM2* rs1633256 e rs1101998 estiveram associados a um risco aumentado de positividade de TST, enquanto *IRF7* rs11246213 foi associado a uma probabilidade menor de positividade de TST.

Os SNPs *PYHIN1-IFI16-AIM2* rs1633256 e rs1101998 estão em um locus de 3 genes no cromossomo 1, estes três genes estão presentes nos SNPs já que não é possível saber qual gene específico tem maior probabilidade de exercer um efeito funcional relacionado a essas variantes genéticas. Por isso, mostrou-se ser importante analisar esses três genes que tem sido muito pouco ou nada estudados na TB. Estes genes fazem parte do sistema imunológico inato e dos PRRs, cujos TLRs também fazem parte (UNTERHOLZNER; KEATING *et al.*, 2010). Estes receptores são sensores imunes inatos intracelulares responsáveis pela detecção de DNA, que podem provocar inflamação e/ou resposta autoimune no organismo (CONNOLLY; BOWIE, 2014). O gene *PYHIN1* (também é conhecido como IFIX) codifica seis isoformas de proteínas diferentes como resultado de *splicing* alternativo, e todas estas proteínas estão localizadas no núcleo da célula (CONNOLLY; BOWIE, 2014). Funcionalmente PYHIN1 está relacionado com o crescimento celular e supressão de tumores de mama e outras linhas celulares de câncer de mama (DING; WANG *et al.*, 2004). Num meta-análise de estudos GWAS que incluiu 5.416 casos de asma, observou-se uma relação entre SNPs presentes na região do gene *PYHIN1* relacionado à asma brônquica em populações afro-americanas e afro-caribenhas (TORGERSON; AMPLEFORD *et al.*, 2011). Em outro estudo, as variantes gênicas de *PYHIN1* também estiveram associadas à doença inflamatória intestinal pediátrica (ANDREOLETTI; ASHTON *et al.*, 2015). Recentemente, foi descoberto que *PYHIN1* induziu diretamente a transcrição de citocinas pró-inflamatórias como IL-6 e TNFα (MASSA; BARAN *et al.*, 2020).

O gene *IFI16* está associado a produção de três diferentes proteínas (como resultado de *splicing* alternativo de RNA) e auxilia na indução de IFN-β (UNTERHOLZNER; KEATING *et al.*, 2010). Os primeiros estudos sobre o IFI16 enfocaram seu papel no controle do ciclo celular e na regulação da transcrição (CONNOLLY; BOWIE, 2014). Foi observado que *IFI16* estava atuando como um sensor crítico para dsDNA exógeno, por isto foi sugerido um papel de este gene na detecção de DNA bacteriano durante a infecção (UNTERHOLZNER; KEATING *et al.*, 2010). AIM2, predominantemente citosólico, está implicado na defesa imunológica do

hospedeiro como um sensor de DNA, (CONNOLLY; BOWIE, 2014), ele detecta o DNA de *Mtb* que está presente no citosol, provoca a ativação do inflamassoma (SAIGA; KITADA *et al.*, 2012), e facilita a restrição da replicação do *Mtb* tanto in vitro quanto in vivo (MA; ZHAO *et al.*, 2021). Este foi o primero estudo que identificou a associação de polimorfismos dos genes *PYHIN1-IFI16-AIM2* com TB, do nosso conhecimento, o que permite a possibilidade de novos estudos destes genes para entender melhor a base da progressão da TB.

IRF7 é um Fator regulador de interferon (IRFs), composto por 9 membros de uma família de fatores de transcrição que regulam vários aspectos das respostas imunes inatas e adaptativas, respondendo a patógenos para conduzir respostas pró-inflamatórias e regulando a diferenciação de células imunes (TAMURA; YANAI *et al.*, 2008). IRF7 é regulador positivos da indução do gene do IFNs tipo I por meio dos receptores PRRs e, de mesma maneira, a expressão de IRF7 é induzida pelos IFNs do tipo I, resultando em um loop (HONDA; TAKAOKA *et al.*, 2006). O IRF7 é regulado positivamente na resposta à infecção por *Mtb* (LEISCHING; PIETERSEN *et al.*, 2017). O RNA citosolico de *Mtb* induz a produção de IFN-β por meio da via de detecção de RNA por IRF7, atuando assim o hospedeiro na identificação de infecção bacteriana (CHENG; SCHOREY, 2018). Um resultado muito interessante e que pode ajudar a entender nosso resultado é que em um estudo envolvendo perfis de expressão gênica de sangue total em pacientes com TB ativa e latente, o IRF7 esteve significativamente regulado, sugerindo atuação em prol dos pacientes a resistirem à infecção por TB (LIN; DUAN *et al.*, 2017).

Neste segundo estudo, por meio da avaliação de TST, confirmamos que polimorfismos relacionados com a via de sinalização do IFNs do tipo I desempenham um papel significativo na infecção por *Mtb*. Este resultado fornece um novo ângulo de futuros biomarcadores e alvos terapêuticos que tem sido pouco estudado e que podem auxiliar no entendimento de genes relacionados na resposta da imunidade inata por TB.

Estudos em contatos de pacientes com TB ativa é uma atividade importante no controle da TB e pode chegar a reduzir o 44% da transmissão de *Mtb* (MORRISON; PAI *et al.*, 2008). Nossos dois primeiros estudos foram feitos em contatos para poder gerar conhecimento em estas populações que são sometidas a um alto risco de infecção adquirida no domicílio ou na comunidade (FOX; BARRY *et al.*, 2013). Os contatos de crianças são especialmente estudados já que são uma população com alto risco a se infectar com *Mtb.* Uma revisão sistemática e meta-análise com 26 estudos de crianças que foram expostas à TB descobriu que uma criança exposta a uma pessoa com TB no domicílio tinha quase 4 vezes mais chances de ter infecção por TB e mostraram que em países de todos os níveis de renda os domicílios de casos índice de TB são

áreas de intensa transmissão (MARTINEZ; SHEN *et al.*, 2017). Mas não só as crianças como contatos são estudadas, uma revisão sistemática e meta-análise com 244 estudos em contatos de todas as idades, constatou que a investigar os contatos aumenta a notificação de casos de TB, há detecção de casos de TB, diminuiu a mortalidade e a prevalência de TB na população (VELEN; SHINGDE *et al.*, 2021). Estes estudos mostram que a investigação de contatos é uma das atividades de maior prioridade no controle da TB, além de outros tipos de estudo que procuram biomarcadores que possam detectar conversão em está população já que os procedimentos de triagem da TB em contatos limitavam-se ao TST mas agora o IGRA faz parte da vigilância (COOK; SHAH *et al.*, 2012).

Após estes dois estudos pensamos em outros genes relacionados com os PRRs e encontramos a existência de vários estudos genéticos que apontaram a relevância dos alelos polimórficos para os genes *NOD2* (*Nucleotide Binding Oligomerization Domain Containing 2*) e o *CD14* (*Cluster Differentiation antigen 14*). Desta forma, realizamos uma revisão sistemática com o objetivo de avaliar os trabalhos publicados até o momento sobre a influência dos polimorfismos dos genes *NOD2* e o *CD14*.

O *CD14*, cujo gene codificador está localizado no cromossomo 5, é um mediador particularmente importante da resposta inflamatória, é um correceptor para TLR2 e TLR4 que coordena a ligação do ligante (HERMANN; RAVIKUMAR *et al.*, 2018). O *CD14* está principalmente envolvido no reconhecimento da endotoxina bacteriana com TLR4 (CHUN; SEONG, 2010). A estreita associação de *CD14* com TLR2 e TLR4 no reconhecimento sugere que o CD14 provavelmente desempenha um papel na resposta a infecção por *Mtb*.

Em nossa revisão sistemática, observamos que os polimorfismos de *CD14* foram os mais frequentemente estudados no contexto do risco de *Mtb*. Os SNPs *CD14* rs2569190 e rs2569191 estiveram associados com o aumento do risco de TB em diferentes grupos étnicos. Além da TB humana, foi relatado que polimorfismos do gene CD14 estão associados também à suscetibilidade à doença arterial coronariana (LI; WANG *et al.*, 2015), sepses (WANG; WEI *et al.*, 2014) e doenças alérgicas (DEBINSKA; DANIELEWICZ *et al.*, 2019). A variante rs2569190 do gene CD14 na via da patogênese, pode alterar a estrutura, função e os níveis da proteína (WANG; YANG *et al.*, 2016). Estudos sobre estes dois polimorfismos podem ser úteis na formulação de novas estratégias de terapia individual de TB, mas, há uma necessidade de novos estudos para validar ainda mais os resultados desta revisão sistemática.

NOD2 é um sensor intracelular de peptidoglicano bacteriano que desempenha um papel crítico no controle da defesa e inflamação do hospedeiro (MUKHERJEE; HOVINGH *et al.*, 2019). Em estudos *in vivo* de *Mtb* observou-se que o NOD2 ativa a expressão de citocinas pró-

inflamatórias, incluindo TNFα, IL-1α, IL-1β, CCL5, IL-6 e respostas de células T para restringir a *Mtb* (MUKHERJEE; HOVINGH *et al.*, 2019). Os polimorfismos de NOD2 em humanos estiveram associados ao desenvolvimento de *Mycobacterium leprae* (ZHANG; HUANG *et al.*, 2009), linfoma gástrico associado a *Helicobacter pylori* (ROSENSTIEL; HELLMIG *et al.*, 2006) e suscetibilidade à doença tuberculosa (AUSTIN; MA *et al.*, 2008). Em nossa revisão sistemática, observamos que dois SNPs rs1861759 e rs7194886 estiveram associados com o risco aumentado de *Mtb*, especialmente em pessoas de etnia chinesa, mas os mecanismos pelos quais rs1861759 e rs7194886 influenciam a suscetibilidade á infecção por *Mtb* permanecem obscuros. Neste cenário, no intuito de validar tais resultados, torna-se necessário a realização de estudos de NOD2 *in vivo* e *in vitro.*

Um dos pontos mais interessantes em nossos três artigos são aqueles SNPs que parecem dar uma possível proteção contra a TB. Já tem sido estudado que existe uma pequena porção de indivíduos que se chamam de resistentes inatos, estes indivíduos apresentam resultados negativos contínuos de TST ou/e IGRA apesar de uma exposição intensa e contínua ao *Mtb* e usualmente não estarão em risco de TB clínica (MOLLER; KINNEAR *et al.*, 2018). Nos países com uma alta carga de *Mtb*, os indivíduos expostos que são resistentes tem mais mecanismos imunogenéticos de resistência do que aqueles indivíduos que resistem à infecção após uma exposição menor (MOLLER; KINNEAR *et al.*, 2018), eis esta a razão pela qual os estudos de contatos são tão interessantes já que geralmente são pessoas que estão em uma alta carga de *Mtb* e achar alguns mecanismos protetores pode ajudar muito no entendimento da infecção e no processo de tentar chegar a uma solução definitiva.

Apenas alguns estudos moleculares investigaram os fatores genéticos subjacentes à resistência à infecção por *Mtb* usando a reatividade do TST. Coletivamente, esses estudos sugerem um modelo no qual é provável que padrões complexos de herança poligênica sejam importantes para a resistência ou seja que existem multiplex SNPs que juntos podem ser relevantes para o fenótipo resistente (MOLLER; KINNEAR *et al.*, 2018). Assim muitas variantes genéticas individuais, cada uma com pequenos efeitos individuais, contribuem de forma aditiva para este fenótipo.

Finalmente é importante elucidar os mecanismos imunológicos e genéticos do fenótipo resistente já que as investigações ainda estão em estágio inicial. Os grandes avanços nos últimos anos conseguiram melhorar os métodos moleculares e existem plataformas que agora podem incluir a detecção de polimorfismos no genoma. O analises do todo o genoma permitem estudar genes e polimorfismos não identificados em regiões não codificantes que não são anotados em bancos de dados públicos; polimorfismos funcionais (que afetam a expressão ou sinalização

dos genes, por exemplo), e as associações identificadas com fenótipos clínicos requerem replicação em outras populações. Também, discernir entre os mecanismos de eliminação precoce ou prevenção da infecção por *Mtb* ajudara a entender as diferencias entre resistentes e indivíduos com LTBI. Esses estudos dos mecanismos biológicos podem chegar a fornecer alvos para vias metabólicas que servem para desenvolver novos medicamentos e vacinas que possam melhorar funções imunológicas específicas e melhorar as opções terapêuticas para o tratamento da TB.

10 **PRINCIPAIS ACHADOS DA TESE**

- No primeiro artigo, identificamos que o *TLR4* Thr399Ile (rs4986791) e o *TNFA* -238 (rs361525) estiveram associados ao risco de infecção por *Mtb* e desenvolvimento de TB ativa no Rio de Janeiro, Brasil
- No segundo artigo, observamos associações entre polimorfismos em *IFI16* (rs1633256 e rs1101998) e risco aumentado de positividade de TST e o SNP *IRF7* rs11246213 foi associado a uma probabilidade menor de positividade do TST.
- No terceiro artigo, observamos que o alelo T do polimorfismo rs2569190 *CD14* pode ser considerado um fator de risco genético para doenças *Mtb* em pessoas de diferentes etnias. E identificamos que os SNPs *CD14* rs2569191, *NOD2* rs1861759 e rs7194886 tem o potencial de estar associados a um alto risco de TB na população chinesa.

11 **CONCLUSÃO**

Em conjunto nossos estudos fornecem fortes evidências de associações entre polimorfismos em genes do sistema imunológico inato e o risco de infecção recente ou não por *Mtb*, e desenvolvimento de TB ativa.

Esses resultados agregam conhecimento ao campo da imunogenética da TB, reforçando a influência genética no risco de infecção recente e progressão da infecção latente por Mtb para a doença tuberculosa.

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ANEXOS

PLOS ONE

RESEARCH ARTICLE

Association between neurofibromatosis type 1 and cerebrovascular diseases in children: A systematic review

Beatriz Barreto-Duarte[1,2,3,4☯]*, Fabiana H. Andrade-Gomes[3☯], María B. Arriaga[1,2,5], Mariana Araújo-Pereira[1,2,6], Juan Manuel Cubillos-Angulo[1,2,5], Bruno B. Andrade[1,2,3,4,5,6,7]*

1 Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil, **2** Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador, Bahia, Brazil, **3** Curso de Medicina, Universidade Salvador (UNIFACS), Salvador, Bahia, Brazil, **4** Programa de Pós-Graduação em Clínica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, **5** Curso de Medicina, Centro Universitário Faculdade de Tecnologia e Ciências (UniFTC), Salvador, Bahia, Brazil, **6** Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Bahia, Brazil, **7** Curso de Medicina, Escola Bahiana de Medicina e Saúde Pública (EBMSP), Salvador, Bahia, Brazil

☯ These authors contributed equally to this work.
* bruno.andrade@fiocruz.br (BBA); beatriz.duarte@monsterinitiative.com (BBD)

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Abstract

Background

Neurofibromatosis type 1 (NF-1) is an autosomal dominant disease that affects one in every 3000 individuals. This disease can present a wide range of clinical manifestations, ranging from skin abnormalities to severe vascular damage. Although not commonly recognized in the context of NF-1, cerebrovascular disease (CVD), can be often present since childhood and diagnosed just later in life. When present, NF-1-associated CVD clinical manifestations may include headache, cognitive deficits and ultimately aneurysm rupture, causing death. Thus, CVD plays an important role in the clinical manifestations, disease severity and prognosis of patients with NF-1. This systematic review aims to summarize the body of evidence linking NF-1 and CVD in children.

Methods

Two independent investigators performed a systematic review on the PubMed and EMBASE search platforms, using the following key terms: "neurofibromatosis type 1", "Von Recklinghausen's disease", "children", "adolescents", "stroke", "Moyamoya disease", "vascular diseases", "cerebrovascular disorders", "aneurysm" and "congenital abnormalities". Studies focused on assessing the development of CVD in children with NF-1 were included.

Results

Seven studies met the inclusion criteria. Twelve different clinical manifestations have been associated with cerebrovascular changes in children with NF-1; 44,5% of diagnosed patients were asymptomatic.

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Hydroxyurea treatment is associated with reduced degree of oxidative perturbation in children and adolescents with sickle cell anemia

Caian L. Vinhaes[1,2,3,8], Rozana S. Teixeira[4,5,8], Jay A. S. Monteiro-Júnior[4,8], Rafael Tibúrcio[1,2,5,8], Juan M. Cubillos-Angulo[1,2,5], María B. Arriaga[1,2,5], Adrielle G. Sabarin[4], Amâncio J. de Souza[4], Jacqueline J. Silva[1,4], Isa M. Lyra[6], Ana Marice Ladeia[4,7,9] & Bruno B. Andrade[1,2,3,4,5,6,9]✉

Sickle cell anemia (SCA) is the most common inherited hemolytic anemia worldwide. Here, we performed an exploratory study to investigate the systemic oxidative stress in children and adolescents with SCA. Additionally, we evaluated the potential impact of hydroxyurea therapy on the status of oxidative stress in a case–control study from Brazil. To do so, a panel containing 9 oxidative stress markers was measured in plasma samples from a cohort of 47 SCA cases and 40 healthy children and adolescents. Among the SCA patients, 42.5% were undertaking hydroxyurea. Multidimensional analysis was employed to describe disease phenotypes. Our results demonstrated that SCA is associated with increased levels of oxidative stress markers, suggesting the existence of an unbalanced inflammatory response in peripheral blood. Subsequent analyses revealed that hydroxyurea therapy was associated with diminished oxidative imbalance in SCA patients. Our findings reinforce the idea that SCA is associated with a substantial dysregulation of oxidative responses which may be dampened by treatment with hydroxyurea. If validated by larger prospective studies, our observations argue that reduction of oxidative stress may be a main mechanism through which hydroxyurea therapy attenuates the tissue damage and can contribute to improved clinical outcomes in SCA.

Sickle cell anemia (SCA) is the most common monogenic hemoglobinopathy disease in the world[1,2]. This disease is characterized by altered hemoglobin synthesis (sickle hemoglobin [HbS]), which leads to several pathological effects, including hemolysis, vaso-occlusive crises, progressive organic damage, and eventual early death[3]. Notably, such hereditary hemolytic anemia exhibits a high prevalence in Brazil, especially in the state of Bahia[4]. Due to the scarcity of an effective pharmacological treatment, understanding of fundamental mechanisms underlying SCA may lead to development of novel therapies and optimized patient care[1,5,6].

Numerous aspects are long proposed to influence the pathogenesis of SCA[7,8]. Notably, a large number of studies investigating oxidative stress in SCA patients have indicated that several types of reactive oxygen species (ROS) affect red blood cells (RBCs), resulting in metabolic dysfunction of these cells and alteration of their biochemical properties[9,10]. There is also evidence supporting the idea that oxidative damage mediates cytoskeleton

[1]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Salvador 40296-710, Brazil. [2]Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador 41810-710, Brazil. [3]School of Medicine, Faculdade de Tecnologia E Ciências (UniFTC), Salvador 41741-590, Brazil. [4]Bahiana School of Medicine and Public Health, Bahia Foundation for the Development of Sciences, Salvador 40290-000, Brazil. [5]School of Medicine, Federal University of Bahia, Salvador 40110-100, Brazil. [6]University Salvador (UNIFACS), Laureate International Universities, Salvador 41720-200, Brazil. [7]Catholic University of Salvador, Salvador 41740-090, Brazil. [8]These authors contributed equally: Caian L. Vinhaes, Rozana S. Teixeira, Jay A. S. Monteiro-Júnior and Rafael Tibúrcio. [9]These authors jointly supervised this work: Ana Marice Ladeia and Bruno B. Andrade. ✉email: bruno.andrade@fiocruz.br

PLOS ONE

RESEARCH ARTICLE

In silico transcriptional analysis of mRNA and miRNA reveals unique biosignatures that characterizes different types of diabetes

Juan M. Cubillos-Angulo[1,2,3], Caian L. Vinhaes[1,3,4], Eduardo R. Fukutani[1], Victor V. S. Albuquerque[5], Artur T. L. Queiroz[1,4☯]*, Bruno B. Andrade[1,2,3,4,5,6☯]*, Kiyoshi F. Fukutani[1,3,4☯]*

1 Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil, **2** Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil, **3** Curso de Medicina, Faculdade de Tecnologia e Ciências (FTC), Salvador, Brazil, **4** Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador, Brazil, **5** Escola Bahiana de Medicina e Saúde Pública (EBMSP), Salvador, Brazil, **6** Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Brazil

☯ These authors contributed equally to this work.
* bruno.andrade@fiocruz.br (BBA); arturlopo@gmail.com (ATLQ); ferreirafk@gmail.com (KFF)

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Abstract

Diabetes (DM) has a significant impact on public health. We performed an *in silico* study of paired datasets of messenger RNA (mRNA) micro-RNA (miRNA) transcripts to delineate potential biosignatures that could distinguish prediabetes (pre-DM), type-1DM (T1DM) and type-2DM (T2DM). Two publicly available datasets containing expression values of mRNA and miRNA obtained from individuals diagnosed with pre-DM, T1DM or T2DM, and normoglycemic controls (NC), were analyzed using systems biology approaches to define combined signatures to distinguish different clinical groups. The mRNA profile of both pre-DM and T2DM was hallmarked by several differentially expressed genes (DEGs) compared to NC. Nevertheless, T1DM was characterized by an overall low number of DEGs. The miRNA signature profiles were composed of a substantially lower number of differentially expressed targets. Gene enrichment analysis revealed several inflammatory pathways in T2DM and fewer in pre-DM, but with shared findings such as Tuberculosis. The integration of mRNA and miRNA datasets improved the identification and discriminated the group composed by pre-DM and T2DM patients from that constituted by normoglycemic and T1DM individuals. The integrated transcriptomic analysis of mRNA and miRNA expression revealed a unique biosignature able to characterize different types of DM.

Introduction

Diabetes mellitus (DM) is a group of chronic metabolic disorders characterized by the elevation of blood glucose levels (hyperglycemia) due to defects in insulin secretion and/or activity [1]. The most recent report from the American Diabetes Association (ADA) indicated that in 2017, approximately 425 million adults were diagnosed with DM and estimated that by 2045,

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RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response

Adam Penn-Nicholson[1,34], Stanley Kimbung Mbandi[1,34], Ethan Thompson[2,34], Simon C. Mendelsohn[1,34], Sara Suliman[1,3], Novel N. Chegou[4], Stephanus T. Malherbe[4], Fatoumatta Darboe[1], Mzwandile Erasmus[1], Willem A. Hanekom[1], Nicole Bilek[1], Michelle Fisher[1], Stefan H. E. Kaufmann[5,6], Jill Winter[7], Melissa Murphy[1], Robin Wood[8], Carl Morrow[8], Ildiko Van Rhijn[3], Branch Moody[3], Megan Murray[9], Bruno B. Andrade[10], Timothy R. Sterling[11], Jayne Sutherland[12], Kogieleum Naidoo[13,14], Nesri Padayatchi[13,14], Gerhard Walzl[4], Mark Hatherill[1], Daniel Zak[2], Thomas J. Scriba[1]✉, The Adolescent Cohort Study team*, The GC6-74 Consortium*, The SATVI Clinical and Laboratory Team*, The ScreenTB Consortium*, The AE-TBC Consortium*, The RePORT Brazil Team*, Peruvian Household Contacts Cohort Team* & The CAPRISA IMPRESS team*

Improved tuberculosis diagnostics and tools for monitoring treatment response are urgently needed. We developed a robust and simple, PCR-based host-blood transcriptomic signature, RISK6, for multiple applications: identifying individuals at risk of incident disease, as a screening test for subclinical or clinical tuberculosis, and for monitoring tuberculosis treatment. RISK6 utility was validated by blind prediction using quantitative real-time (qRT) PCR in seven independent cohorts. Prognostic performance significantly exceeded that of previous signatures discovered in the same cohort. Performance for diagnosing subclinical and clinical disease in HIV-uninfected and HIV-infected persons, assessed by area under the receiver-operating characteristic curve, exceeded 85%. As a screening test for tuberculosis, the sensitivity at 90% specificity met or approached the benchmarks set out in World Health Organization target product profiles for non-sputum-based tests. RISK6 scores correlated with lung immunopathology activity, measured by positron emission tomography, and tracked treatment response, demonstrating utility as treatment response biomarker, while predicting treatment failure prior to treatment initiation. Performance of the test in capillary blood samples collected by finger-prick was noninferior to venous blood collected in PAXgene tubes. These results support incorporation of RISK6 into rapid, capillary blood-based point-of-care PCR devices for prospective assessment in field studies.

[1]South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine and Division of Immunology, Department of Pathology, University of Cape Town, Cape Town, South Africa. [2]Center for Infectious Disease Research, Seattle, WA, USA. [3]Brigham and Women's Hospital, Division of Rheumatology, Immunity and Inflammation, Harvard Medical School, Boston, USA. [4]DST-NRF Centre of Excellence for Biomedical Tuberculosis Research; South African Medical Research Council Centre for Tuberculosis Research; Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa. [5]Max Planck Institute for Infection Biology, Berlin, Germany. [6]Hagler Institute for Advanced Study at Texas A&M University, College Station, TX, USA. [7]Catalysis Foundation for Health, San Ramon, CA, USA. [8]Desmond Tutu HIV Centre, and Institute of Infectious Disease and Molecular Medicine (IDM), University of Cape Town, Cape Town, South Africa. [9]Department of Global Health and Social Medicine, and Division of Global Health Equity, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. [10]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil. [11]Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, USA. [12]Vaccines and Immunity, Medical Research Council Unit, Fajara, The Gambia. [13]Centre for the AIDS Programme of Research in Africa, Durban, South Africa. [14]South African Medical Research Council-CAPRISA HIV-TB Pathogenesis and Treatment Research Unit, Durban, South Africa. [34]These authors contributed equally: Adam Penn-Nicholson, Stanley Kimbung Mbandi, Ethan Thompson and Simon C. Mendelsohn. *Lists of authors and their affiliations appear at the end of the paper. ✉e-mail: thomas.scriba@uct.ac.za

Inflammatory profile of patients with tuberculosis with or without HIV-1 co-infection: a prospective cohort study and immunological network analysis

Elsa Du Bruyn, Kiyoshi F Fukutani*, Neesha Rockwood*, Charlotte Schutz, Graeme Meintjes, María B Arriaga, Juan M Cubillos-Angulo, Rafael Tibúrcio, Alan Sher, Catherine Riou, Katalin A Wilkinson, Bruno B Andrade†, Robert J Wilkinson†*

Summary

Background HIV-1 mediated dysregulation of the immune response to tuberculosis and its effect on the response to antitubercular therapy (ATT) is incompletely understood. We aimed to analyse the inflammatory profile of patients with tuberculosis with or without HIV-1 co-infection undergoing ATT, with specific focus on the effect of ART and HIV-1 viraemia in those co-infected with HIV-1.

Methods In this prospective cohort study and immunological network analysis, a panel of 38 inflammatory markers were measured in the plasma of a prospective patient cohort undergoing ATT at Khayelitsha Site B clinic, Cape Town, South Africa. We recruited patients with sputum Xpert MTB/RIF-positive rifampicin-susceptible pulmonary tuberculosis. Patients were excluded from the primary discovery cohort if they were younger than 18 years, unable to commence ATT for any reason, pregnant, had unknown HIV-1 status, were unable to consent to study participation, were unable to provide baseline sputum samples, had more than three doses of ATT, or were being re-treated for tuberculosis within 6 months of their previous ATT regimen. Plasma samples were collected at baseline (1–5 days after commencing ATT), week 8, and week 20 of ATT. We applied network and multivariate analysis to investigate the dynamic inflammatory profile of these patients in relation to ATT and by HIV status. In addition to the discovery cohort, a validation cohort of patients with HIV-1 admitted to hospital with CD4 counts less than 350 cells per μL and a high clinical suspicion of new tuberculosis were recruited.

Findings Between March 1, 2013, and July 31, 2014, we assessed a cohort of 129 participants (55 [43%] female and 74 [57%] male, median age 35·1 years [IQR 30·1–43·7]) and 76 were co-infected with HIV-1. HIV-1 status markedly influenced the inflammatory profile regardless of ATT duration. HIV-1 viral load emerged as a major factor driving differential inflammatory marker expression and having a strong effect on correlation profiles observed in the HIV-1 co-infected group. Interleukin (IL)-17A emerged as a key correlate of HIV-1-induced inflammation during HIV–tuberculosis co-infection.

Interpretation Our findings show the effect of HIV-1 co-infection on the complexity of plasma inflammatory profiles in patients with tuberculosis. Through network analysis we identified IL-17A as an important node in HIV–tuberculosis co-infection, thus implicating this cytokine's capacity to correlate with, and regulate, other inflammatory markers. Further mechanistic studies are required to identify specific IL-17A-related inflammatory pathways mediating immunopathology in HIV–tuberculosis co-infection, which could illuminate targets for future host-directed therapies.

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Introduction

Tuberculosis remains one of the most deadly infectious diseases, with 1·2 million deaths in individuals without HIV-1 co-infection and 208 000 deaths in individuals with HIV-1 co-infection reported globally in 2019.[1] HIV-1 infection is a significant risk factor for tuberculosis infection and although antiretroviral therapy (ART) substantially mitigates tuberculosis risk, it remains higher in individuals with HIV-1 on ART than in individuals without HIV-1.[2]

The influence of HIV-1 co-infection on the immune response to *Mycobacterium tuberculosis* remains poorly understood, as each pathogen compounds the immunopathology associated with the other, adding complexity. Furthermore, the intertwined effects of ART-mediated immune reconstitution and immune clearance of *M tuberculosis* during antitubercular therapy (ATT) makes it difficult to dissect the relative contribution of each to a successful treatment response. There is thus paucity in biomarkers predictive of treatment outcome in HIV–tuberculosis co-infection.

We previously addressed this shortfall by investigating expression of the antioxidant enzyme heme oxygenase 1 (HMOX1) in the context of HIV–tuberculosis

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*These authors contributed equally

†These senior authors contributed equally

Wellcome Centre for Infectious Disease Research in Africa, Institute of Infectious Disease and Molecular Medicine (E Du Bruyn MD, N Rockwood PhD, C Schutz MD, G Meintjes PhD, C Riou PhD, K A Wilkinson PhD, B B Andrade MD, Prof R J Wilkinson FMedSci) **and Department of Medicine** (E Du Bruyn, C Schutz, G Meintjes, Prof R J Wilkinson), **University of Cape Town, Observatory, South Africa; Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil** (K F Fukutani PhD, M B Arriaga MSc, J M Cubillos-Angulo MSc, R Tibúrcio MSc, B B Andrade); **Multinational Organization Network Sponsoring Translational and Epidemiological Research Initiative, Salvador, Brazil** (K F Fukutani, M B Arriaga, J M Cubillos-Angulo, R Tibúrcio, B B Andrade); **Curso de Medicina, Faculdade de Tecnologia e Ciências, Salvador, Brazil** (K F Fukutani); **Department of Infectious Diseases, Imperial College London, London, UK** (N Rockwood, Prof R J Wilkinson); **Department of Microbiology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka** (N Rockwood); **Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA** (A Sher PhD);

PLOS ONE

RESEARCH ARTICLE

Systems biology analysis of publicly available transcriptomic data reveals a critical link between *AKR1B10* gene expression, smoking and occurrence of lung cancer

Juan M. Cubillos-Angulo[1,2,3☯], Eduardo R. Fukutani[1☯], Luís A. B. Cruz[1,3,4], María B. Arriaga[1,2,3], João Victor Lima[1], Bruno B. Andrade[1,2,3,4,5,6‡*], Artur T. L. Queiroz[1‡*], Kiyoshi F. Fukutani[1,3,4‡*]

1 Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil, **2** Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Bahia, Brazil, **3** Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador, Bahia, Brazil, **4** Curso de Medicina, Faculdade de Tecnologia e Ciências, Salvador, Bahia, Brazil, **5** Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Bahia, Brazil, **6** Escola Bahiana de Medicina e Saúde Pública (EBMSP), Salvador, Bahia, Brazil

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* bruno.andrade@fiocruz.br (BBA); arturlopo@gmail.com (ATLQ); ferreirafk@gmail.com (KFF)

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Data Availability Statement: We accessed the Gene Expression Omnibus (GEO-NCBI -https://www.ncbi.nlm.nih.gov/geo/) and have looked for datasets with tobacco smoking information in human biopsies or tissues, without diagnosis of other comorbidities. Five datasets have been found in GEO: GSE4498, GSE3320, GSE20257, GSE17905, GSE13931.

Abstract

Background

Cigarette smoking is associated with an increased risk of developing respiratory diseases and various types of cancer. Early identification of such unfavorable outcomes in patients who smoke is critical for optimizing personalized medical care.

Methods

Here, we perform a comprehensive analysis using Systems Biology tools of publicly available data from a total of 6 transcriptomic studies, which examined different specimens of lung tissue and/or cells of smokers and nonsmokers to identify potential markers associated with lung cancer.

Results

Expression level of 22 genes was capable of classifying smokers from non-smokers. A machine learning algorithm revealed that *AKR1B10* was the most informative gene among the 22 differentially expressed genes (DEGs) accounting for the classification of the clinical groups. *AKR1B10* expression was higher in smokers compared to non-smokers in datasets examining small and large airway epithelia, but not in the data from a study of sorted alveolar macrophages. Moreover, *AKR1B10* expression was relatively higher in lung cancer specimens compared to matched healthy tissue obtained from nonsmoking individuals. Although the overall accuracy of *AKR1B10* expression level in distinction between cancer and healthy

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Determinants of losses in the latent tuberculosis cascade of care in Brazil: A retrospective cohort study

Nélia C.N. Araújo[a,b,c,d,1], Constança M.S. Cruz[a,e,1], María B. Arriaga[c,f,1], Juan M. Cubillos-Angulo[c,f,1], Michael S. Rocha[b,c], Paulo S. Silveira-Mattos[c,f,g], Gisela M. Matos[a], Izabella M.B. Marques[a], Isa Carolina P. Espirito Santo[a], Luiza L. Almeida[a], Caroline M. Andrade[a], Leonardo A. Souza[a], Eduardo M. Netto[b,d], Bruno B. Andrade[a,c,f,g,h,*]

[a] Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil
[b] Instituto Brasileiro para Investigação da Tuberculose (IBIT), Fundação José Silveira, Salvador, Bahia, Brazil
[c] Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER), Salvador, Bahia, Brazil
[d] Complexo Hospitalar Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brazil
[e] Obras Sociais Irmã Dulce, Salvador, Bahia, Brazil
[f] Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil
[g] Curso de Medicina, Faculdade de Tecnologia e Ciências, Salvador, Bahia, Brazil
[h] Universidade Salvador (UNIFACS), Laureate Universities, Salvador, Bahia, Brazil

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ABSTRACT

Background: The present study evaluated factors associated with losses in the latent tuberculosis infection (LTBI) cascade of care in contacts of tuberculosis (TB) patients, in a referral center from a highly endemic region in Brazil.
Methods: Contacts of 1672 TB patients were retrospectively studied between 2009 and 2014. Data on TB screening by clinical investigation, radiographic examination and tuberculin skin test (TST) were extracted from medical records. Losses in the cascade of care and TB incidence within 2-year follow-up were calculated.
Results: From a total of 1180 TB contacts initially identified, only 495 were examined (58% loss), and 20 were diagnosed with active TB at this stage. Furthermore, 435 persons returned for TST result interpretation and 351 (~81%) were TST positive. Among those with positive TST, 249 (73%) were treated with isoniazid for 6 months whereas 51 abandoned therapy early. Three individuals who did not receive LTBI treatment, one with incomplete treatment and another who completed treatment developed active TB. A logistic regression analysis revealed that increases in age were associated with losses in the LTBI cascade independent of other clinical and epidemiological characteristics.
Conclusions: Major losses occur at initial stages and older patients are at higher risk of not completing the LTBI cascade of care.

Introduction

The majority of new cases of Tuberculosis (TB) occur in 30 countries with high disease burden such as Brazil, India, China, and South Africa (WHO, 2019). Furthermore, approximately 1.5 million deaths attributable to TB globally were reported in 2018 (WHO, 2019). Factors that may underlie the slow improvement of TB control include inaccurate diagnosis and loss to follow up of patients or household contacts undergoing anti-TB treatment (Zelner et al., 2018). In Brazil, despite significant investment from the government, the reported reduction in TB incidence (−1.34% per year) is considered insufficient to meet targets established by the World Health Organization (WHO) to reduce the incidence of TB by 90% by 2035 and eliminate TB (less than 1 incident case per 1,000,000 per year) by 2050 (Houben and Dodd, 2016). To achieve

* Corresponding author at: Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Rua Waldemar Falcão, No. 121, Candeal, Salvador, Bahia 40269-710, Brazil.
E-mail address: bruno.andrade@fiocruz.br (B.B. Andrade).
[1] NCNA, CMSC, MBA and JMCA equally contributed to the work.

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Molecular degree of perturbation of plasma inflammatory markers associated with tuberculosis reveals distinct disease profiles between Indian and Chinese populations

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Deivide Oliveira-de-Souza[1,2,3], Caian L. Vinhaes[1,2,3], Maria B. Arriaga[1,2], Nathella Pavan Kumar[4], Juan M. Cubillos-Angulo[1,2], Ruiru Shi[5], Wang Wei[5], Xing Yuan[5], Guolong Zhang[6], Ying Cai[7], Clifton E. Barry III[7], Laura E. Via[7], Alan Sher[8], Subash Babu[4], Katrin D. Mayer-Barber[7], Helder I. Nakaya[9], Kiyoshi F. Fukutani[1,2,3] & Bruno B. Andrade[1,2,3,10,11]

Tuberculosis (TB) is a chronic inflammatory disease caused by *Mycobacterium tuberculosis* infection which causes tremendous morbidity and mortality worldwide. Clinical presentation of TB patients is very diverse and disease heterogeneity is associated with changes in biomarker signatures. Here, we compared at the molecular level the extent of individual inflammatory perturbation of plasma protein and lipid mediators associated with TB in patients in China versus India. We performed a cross-sectional study analyzing the overall degree of inflammatory perturbation in treatment-naïve pulmonary TB patients and uninfected individuals from India (TB: n = 97, healthy: n = 20) and China (TB: n = 100, healthy: n = 11). We employed the molecular degree of perturbation (MDP) adapted to plasma biomarkers to examine the overall changes in inflammation between these countries. *M. tuberculosis* infection caused a significant degree of molecular perturbation in patients from both countries, with higher perturbation detected in India. Interestingly, there were differences in biomarker perturbation patterns and the overall degree of inflammation. Patients with severe TB exhibited increased MDP values and Indian patients with this condition exhibited even higher degree of perturbation compared to Chinese patients. Network analyses identified IFN-α, IFN-β, IL-1RI and TNF-α as combined biomarkers that account for the overall molecular perturbation in the entire study population. Our results delineate the magnitude of the systemic inflammatory perturbation in pulmonary TB and reveal qualitative changes in inflammatory profiles between two countries with high disease prevalence.

Tuberculosis (TB) is now the leading cause of mortality worldwide due to a single infectious agent[1]. In addition, *Mycobacterium tuberculosis* (Mtb) is widely disseminated geographically, with up to 23% of the world's population

[1]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, 40296-710, Brazil. [2]Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Fundação José Silveira, Salvador, 40210-320, Brazil. [3]Curso de Medicina, Faculdade de Tecnologia e Ciências (FTC), Salvador, 40290-150, Brazil. [4]National Institutes of Health- National Institute for Research in Tuberculosis, International Center for Excellence in Research, Chennai, 600031, India. [5]Henan Chest Hospital, Zhengzhou, 450000, China. [6]Sino-US International Research Center for Tuberculosis, and Henan Public Health Center, Zhengzhou, 450000, China. [7]Laboratory of Clinical Immunology and Microbiology, NIAID, NIH, Bethesda, 20892, USA. [8]Laboratory of Parasitic Diseases, NIAID, NIH, Bethesda, 20892, USA. [9]Department of Pathophysiology and Toxicology, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, 05508, Brazil. [10]Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, 37232, USA. [11]Universidade Salvador (UNIFACS), Laureate Universities, Salvador, 41720-200, Brazil. Deivide Oliveira-de-Souza, Caian L. Vinhaes, Kiyoshi F. Fukutani and Bruno B. Andrade contributed equally. Correspondence and requests for materials should be addressed to B.B.A. (email: bruno.andrade@fiocruz.br)

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Review

Systemic Inflammation Associated with Immune Reconstitution Inflammatory Syndrome in Persons Living with HIV

Caian L. Vinhaes [1,2,3,†], Mariana Araujo-Pereira [1,2,4,†], Rafael Tibúrcio [1,2,4], Juan M. Cubillos-Angulo [1,2,4], Fernanda O. Demitto [2], Kevan M. Akrami [1,2,4,5] and Bruno B. Andrade [1,2,3,4,6,*]

1 Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador 40296-710, Brazil; caianleal@gmail.com (C.L.V.); araujopereira.mariana@gmail.com (M.A.-P.); rafael.santos@aluno.bahia.fiocruz.br (R.T.); j.cubillosangulo@gmail.com (J.M.C.-A.); Kevan.akrami@gmail.com (K.M.A.)
2 Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador 40210-320, Brazil; fernandademitto@gmail.com
3 Bahiana School of Medicine and Public Health, Bahia Foundation for the Development of Sciences, Salvador 40290-000, Brazil
4 Faculdade de Medicina, Universidade Federal da Bahia, Salvador 40110-100, Brazil
5 Divisions of Infectious Diseases and Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University of California, San Diego, CA 92093, USA
6 Curso de Medicina, Centro Universitário Faculdade de Tecnologia e Ciências (UniFTC), Salvador 41741-590, Brazil
* Correspondence: bruno.andrade@fiocruz.br; Tel.: +55-71-3176-2264
† These authors contributed equally to this work.

Abstract: Antiretroviral therapy (ART) has represented a major advancement in the care of people living with HIV (PLWHH), resulting in significant reductions in morbidity and mortality through immune reconstitution and attenuation of homeostatic disruption. Importantly, restoration of immune function in PLWH with opportunistic infections occasionally leads to an intense and uncontrolled cytokine storm following ART initiation known as immune reconstitution inflammatory syndrome (IRIS). IRIS occurrence is associated with the severe and rapid clinical deterioration that results in significant morbidity and mortality. Here, we detail the determinants underlying IRIS development in PLWH, compiling the available knowledge in the field to highlight details of the inflammatory responses in IRIS associated with the most commonly reported opportunistic pathogens. This review also highlights gaps in the understanding of IRIS pathogenesis and summarizes therapeutic strategies that have been used for IRIS.

Keywords: systemic inflammation; mycobacteria; HIV; immune reconstitution inflammatory syndrome (IRIS)

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1. Introduction

Globally, nearly 38 million people are living with HIV (PLWH) [1]. The most critical advancement in this epidemic was the development and increased access to antiretroviral therapy (ART), which led to significant reductions in morbimortality through immune reconstitution and attenuation of homeostatic disruption [2]. This has reduced the incidence and severity of opportunistic infections (OI) such as *Mycobacterium tuberculosis* (Mtb) and *Avium complex* (MAC), *Cytomegalovirus* (CMV), Kaposi sarcoma-associated herpesvirus (KSHV), hepatitis C (HCV) and B (HBV) virus, *Cryptococcus neoformans*, *Pneumocystis jirovecci* and *Toxoplasma gondii*. However, paradoxically in a subset of PLWH, ART initiation may trigger clinical worsening with pathologic immune activation against these OIs, characterized by uncontrolled cytokine production known as immune reconstitution inflammatory syndrome (IRIS) [2].

IRIS is defined as a condition occurring shortly after ART initiation (up to 3 months) marked by rapid clinical deterioration with uncontrolled inflammatory processes despite

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Prevalence and Clinical Profiling of Dysglycemia and HIV Infection in Persons With Pulmonary Tuberculosis in Brazil

María B. Arriaga[1,2,3†], Mariana Araújo-Pereira[1,2,3†], Beatriz Barreto-Duarte[1,2,4,5†], Caio Sales[1,2,4], João Pedro Miguez-Pinto[1,2,4], Evelyn B. Nogueira[1,2,4], Betânia M. F. Nogueira[1,2,3,6], Michael S. Rocha[1,2,6,7], Alexandra B. Souza[8,9], Aline Benjamin[10], Jamile G. de Oliveira[11], Adriana S. R. Moreira[12], Artur T. L. Queiroz[2,13], Moreno M. S. Rodrigues[14], Renata Spener-Gomes[8,9], Marina C. Figueiredo[15], Betina Durovni[11], Solange Cavalcante[11], José R. Lapa-e-Silva[8,12], Afrânio L. Kristki[8,12], Marcelo Cordeiro-Santos[8,9,16], Timothy R. Sterling[15], Valeria C. Rolla[10], Bruno B. Andrade[1,2,3,4,7,15*] and the RePORT-Brazil consortium

[1]Laboratório de Inflamação e Biomarcadores, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil
[2]Multinational Organization Network Sponsoring Translational and Epidemiological Research (MONSTER) Initiative, Salvador, Brazil
[3]Faculdade de Medicina, Universidade Federal da Bahia, Salvador, Brazil
[4]Escola de Medicina, Universidade Salvador (UNIFACS), Salvador, Brazil
[5]Programa de Pós-Graduação em Clínica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[6]Instituto Brasileiro para Investigação da Tuberculose, Fundação José Silveira, Salvador, Brazil
[7]Escola Bahiana de Medicina e Saúde Pública, Salvador, Brazil
[8]Fundação Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Brazil
[9]Programa de Pós-Graduação em Medicina Tropical, Universidade do Estado do Amazonas, Manaus, Brazil
[10]Laboratório de Pesquisa Clínica em Micobacteriose, Instituto Nacional de Infectologia Evandro Chagas, Fiocruz, Rio de Janeiro, Brazil
[11]Secretaria Municipal de Saúde do Rio de Janeiro, Rio de Janeiro, Brazil
[12]Programa Acadêmico de Tuberculose da Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[13]Center of Data and Knowledge Integration for Health, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Brazil
[14]Laboratório de Análise e Visualização de Dados, Fundação Oswaldo Cruz, Porto Velho, Brazil
[15]Division of Infectious Diseases, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN, United States
[16]Faculdade de Medicina, Universidade Nilton Lins, Manaus, Brazil

Background: There are scarce data on the prevalence and disease presentation of HIV in patients with tuberculosis (TB) and dysglycemia (diabetes [DM] and prediabetes [PDM]), especially in TB-endemic countries.

Methods: We assessed the baseline epidemiological and clinical characteristics of patients with culture-confirmed pulmonary TB, enrolled in a multicenter prospective cohort in Brazil (RePORT-Brazil) during 2015–2019. Dysglycemia was defined by elevated glycated hemoglobin and stratified as PDM or DM. Additionally, we used data from TB cases obtained through the Brazilian National Notifiable Diseases Information System (SINAN), during 2015–2019. In SINAN, diagnosis of diabetes was based on self-report. Logistic regression models were performed to test independent associations between HIV, dysglycemia status, and other baseline characteristics in both cohorts.

Results: In the RePORT-Brazil cohort, the prevalence of DM and of PDM was 23.7 and 37.8%, respectively. Furthermore, the prevalence of HIV was 21.4% in the group of persons with TB-dysglycemia and 20.5% in that of patients with TBDM. In the SINAN cohort, the prevalence of DM was 9.2%, and among the TBDM group the prevalence of HIV was 4.1%. Logistic regressions demonstrated that aging was independently associated with PDM or DM in both the RePORT-Brazil and SINAN cohorts. In RePORT-Brazil, illicit drug use was associated with PDM, whereas a higher body mass index (BMI) was associated with DM occurrence. Of note, HIV was not associated with an increased risk of PDM or DM in patients with pulmonary TB in both cohorts. Moreover, in both cohorts, the TBDM-HIV group presented with a lower proportion of positive sputum smear and a higher frequency of tobacco and alcohol users.

Conclusion: There is a high prevalence of dysglycemia in patients with pulmonary TB in Brazil, regardless of the HIV status. This reinforces the idea that DM should be systematically screened in persons with TB. Presence of HIV does not substantially impact clinical presentation in persons with TBDM, although it is associated with more frequent use of recreational drugs and smear negative sputum samples during TB screening.

Introduction

Approximately one-quarter of the world population is thought to be infected with *Mycobacterium tuberculosis* (Mtb) and about 5–10% of those will develop active disease at some point in their lives, which represents a substantial public health problem (1). Several factors are related to the development of active tuberculosis (TB), such as immunological, genetic, and metabolic factors. Importantly, metabolic disorders associated