UNIVERSIDADE FEDERAL DA BAHIA

INSTITUTO DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA

TRABALHO DE TESE

RYAN DOS SANTOS COSTA

ASSOCIAÇÃO DE POLIMORFISMOS NO GENE DO TGF-β1 COM ASMA, MARCADORES DE ALERGIA E INFECÇÕES HELMÍNTICAS

Orientadora: Profª. Drª. Camila Alexandrina Viana Figueiredo

Co-orientadora: Profª. Drª. Neuza Maria Alcântara Neves

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Tese apresentada ao Programa de Pós-Graduação em Imunologia, da Universidade Federal da Bahia, como requisito parcial para obtenção do título de Doutor em Imunologia.

Orientadora: Profª. Drª. Camila A. Viana Figueiredo

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UNIVERSIDADE FEDERAL DA BAHIA
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ATA DA SESSÃO PÚBLICA DO COLEGIADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM IMUNOLOGIA PARA JULGAMENTO DO TRABALHO DE TESE DO DOUTORANDO **RYAN DOS SANTOS COSTA**

Ao primeiro dia do mês de abril do ano de dois mil e quatorze às 14 horas, se reúne em sessão pública no Auditório III, 2º andar do Instituto de Ciências da Saúde, a Banca Examinadora composta pelos Professores: Dra.Camila Alexandrina Viana de Figueiredo orientadora, Dra. Soraya Castro Trindade, Dr. Fabrício Rios Santos, Dra. Marilda de Souza Gonçalves, Dra. Valdirene Leão Carneiro com a finalidade de discutir, avaliar e julgar o trabalho de Tese intitulado: "**Associação de polimorfismos no gene do TGF-β1 com asma, marcadores de alergia e infecções helmínticas**" do Doutorando Ryan dos Santos Costa. Após a apresentação, foram feitos os comentários pelos examinadores. Havendo cumprido as exigências para a defesa, a Banca Examinadora conclui que o pós-graduando teve a sua defesa de Tese APROVADA, emitindo pareceres individuais que serão anexados à ata. Nada mais havendo a tratar, encerra-se a sessão, da qual é lavrado a presente ata que após lida e aprovada vai assinada pelos componentes da Banca examinadora, pelo Doutorando e pela Coordenadora do Programa de Pós-Graduação. Salvador, 1º de abril de 2014.

Dra.Camila Alexandrina Viana de Figueiredo
Orientadora

Dr. Fabrício Rios Santos
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Dra. Marilda de Souza Gonçalves,
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Dra. Soraya Castro Trindade
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Ryan dos Santos Costa
Doutorando

Dra. Silvia Lima Costa
Coordenadora do PPGIm

“Quanto mais aumenta nosso conhecimento, mais evidente fica nossa ignorância”.

John F. Kennedy

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CONSIDERAÇÕES

A realização desse trabalho foi possível pela colaboração com o grupo de pesquisa em genética da *Johns Hopkins University* coordenado pelo Drª Kathleen Barnes, através de estágio de doutorado sanduíche, que viabilizou a genotipagem das amostras. Com igual importância para o desenvolvimento desse trabalho, destaca-se o acesso aos dados e às amostras de DNA do programa *Social Change Asthma and Allergy in Latin America* (SCAALA), coordenado pelo Profº Drº Maurício Barreto.

RESUMO

A prevalência das doenças alérgicas vem crescendo em ritmo acelerado em todo o mundo estando inversamente associado à redução dos níveis de infecções. Estudos sugerem que no caso de infecções por helmintos existe a ativação de uma rede regulatória caracterizada pela mobilização de linfócitos T regulatórios produtores de IL-10 e TGF-β1 que permitem a sobrevivência do parasito no hospedeiro e parece contribuir para a proteção contra doenças imunomediadas, tais como a asma. Entretanto, as alergias são doenças complexas determinadas não somente por fatores ambientais, mas também pela variabilidade genética interindividual. Neste contexto, o objetivo do presente estudo foi verificar como polimorfismos no gene da citocina imunoregulatória TGF-β1 se associam com infecções por helmintos e com sintomas e marcadores de asma alérgica. As variantes genéticas rs4803455, rs1800470, rs1800469 e rs2241712 foram selecionadas de acordo com estudos prévios na literatura. Foram analisadas as associações de tais SNPs com marcadores de infecção por Ascaris lumbricoides, Trichuris trichiura e Toxocara spp., tais como carga parasitária, IgE total, IgE e IgG4 anti-A. lumbricoides. Além disso, foi avaliada a associação entre variações no gene do TGF-β1 com marcadores de doenças alérgicas, tais como, sintomas de asma, teste cutâneo e IgE específica contra alérgenos. Adicionalmente, foi realizado um estudo de associação deste gene candidato em quatro populações de ancestralidade africana, avaliando a relação de polimorfismo no gene do TGF-β1 com asma. Foi observada associação negativa entre o rs1800470 com asma atópica, IgE e teste cutâneo para alérgenos. Em contra partida, foi observada associação positiva entre os haplótipos das variantes no TGF-β1 e infecções helmínticas. Tais haplótipos também foram associados com maior produção de IL-10. O estudo de associação de gene candidato revelou novos SNPs no TGF-β1 associados com asma. Essas associações foram divergentes entres as populações analisadas. Dessa forma, a realização deste estudo demonstrou que indivíduos carregando determinados polimorfismos no gene do TGF-β1 apresentam menor risco para alergia e maior susceptibilidade para infecções helmínticas, especialmente devido a um aumento da produção de IL-10, destacando a importância da variabilidade genética sobre a modulação das alergias atribuída aos helmintos. Também foram reveladas novas variantes genéticas no TGF-β1 associadas com maior risco de asma.. Dessa forma, o polimorfismo no TGF-β1 parece contribuir para a asma na dependência de outros fatores genéticos e também fatores ambientais.

Palavras-chave: Alergia. Helmintos. Imunorregulação. Polimorfismos genéticos. TGF- β.

ABSTRACT

The prevalence of allergic diseases has been growing rapidly throughout the world, being inversely associated to reduction of the infection level. Studies suggest that in the case of helminth infections there is an activation of regulatory network characterized by the mobilization of regulatory T cells producing IL-10 and TGF- β1 that allow the parasite survival in the host and seems to contribute to protection against immune-mediated diseases such as asthma. However, the allergies are complex diseases determined by not only by environmental factors, but also to genetic variability. In this context, the aim of this study was to investigate how polymorphisms in the gene of immune regulatory cytokine TGF-β1 and its promoter region are associated with helminth infections and allergic asthma symptoms and other markers. The genetic variants rs4803455, rs1800470, rs2241712 and rs1800469 were selected according to previously studies in the literature. Associations of these SNPs with markers of parasitic infection such as parasite burden, the total IgE and specific IgE and IgG4 anti- *A. lumbricoide* were analyzed. Also, it was assessed the association between genetic variation in TGF-β1 with markers of allergic diseases such as asthma symptoms and specific IgE to allergens. Additionally, a candidate gene association study in four populations of African ancestry was conducted by evaluating the ratio of polymorphism in the TGF-β1 gene with asthma. Negative association was observed between rs1800470 with atopic wheezing and markers of allergy. In contrast, a positive association was observed between the haplotypes of the variants in TGF-β1 and helminth infections. These haplotypes were also associated with increased production of IL-10. The candidate gene association study revealed new SNPs in TGF-β1 associated with asthma. These associations were divergent among the analyzed populations. Thus , this study demonstrated that individuals carrying certain polymorphisms in the TGF-β1 gene have a lower risk for allergy and increased susceptibility to helminth infections, especially due to an increased production of IL-10, highlighting the importance of genetic variability on the allergies modulation attributed to helminths. Were also revealed new genetic variants in TGF-β1 associated with increased asthma risk. However, different results were found in each population. Thus, the polymorphisms in *TGFB1* seem to contribute to asthma but also depends on other genetic variants and/or exposures where the target population is sited.

Keywords: Allergy. Genetic polymorphisms. Helminths. Immunoregulation. TGF-β.

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LISTA DE ABREVIATURAS

DNA - Ácido desoxirribonucléico

EMT - Transição Epitelial-Mesenquimal

GWAS - Estudos de associação de genoma completo (*Genome-Wide Association Studies*)

IFN-γ - Interferon Gama

IgE – Imunoglobulina E

IL – Interleucina

mRNA - RNA mensageiro

SCAALA - Social Changes, Asthma and Allergy in Latin America

SNP - Polimorfismo em único nucleotídeo (Single Nucleotide Polymorphism)

TGF-β - Fator de transformação do crescimento Beta

Th2 – Células T *helper* tipo 2

Treg – Células T regulatórias

SUMÁRIO

INTRODUÇÃO

1.1 IMPACTO DAS DOENÇAS ALÉRGICAS E SUA PATOGENIA

As doenças alérgicas que afetam o trato respiratório superior e inferior, tais como rinite alérgica e asma alérgica, são as doenças inflamatórias crônicas mais prevalentes, sendo estimado afetar 300 milhões de pessoas no mundo todo (Pearce et al., 2007; Aït-Khaled et al., 2009). Essa prevalência vem aumentando progressivamente nas últimas décadas entre indivíduos vivendo em países industrializados e, mais recentemente, em países em desenvolvimento, constituindo-se em uma verdadeira epidemia e representando um problema global de saúde pública (Yazdanbakhsh, Kremsner e Van Ree, 2002; Pearce et al., 2007; To et al., 2012). Estudos realizados em capitais de estados do Brasil e de países da América do Sul demonstraram que as taxas de prevalência de alergia são também altas nessas cidades, apesar de serem cidades de países em desenvolvimento, sendo Salvador um das cidades com maior prevalência de alergias no mundo (Solé et al., 2007; Solé et al., 2010). Em um trabalho desenvolvido por nosso grupo, foram encontradas prevalências de alergias respiratórias de 32 % em adultos de classe baixa e 45% em adultos de classe média alta nesta cidade (Baqueiro et al., 2007). Em crianças de classes baixas, foi encontrada prevalência de 22 % de sintomas de asma (Rodrigues et al., 2008).

As alergias representam uma grande carga financeira para a sociedade, seja diretamente, pelos custos com o diagnóstico e tratamento; seja indiretamente, pela perda de dias de trabalho e escola (Bahadori et al., 2009). Além disso, têm um impacto muito significativo na qualidade de vida dos pacientes.

A asma é uma desordem comum das vias aéreas inferiores caracterizada por uma interação complexa da obstrução do fluxo de ar, hiperresponsividade brônquica (BHR), e inflamação das vias aéreas que levam a recorrentes episódios de sibilo, falta de ar, aperto no peito e tosse. A asma é marcada pela heterogeneidade relacionada aos fenótipos inflamatórios e à resposta ao tratamento (Pascual e Bleecker, 2010). As diversas expressões fenotípicas da asma são determinadas por fatores ambientais e,

principalmente, pela variabilidade genética (Williams, 1992; Woolcock e Peat, 1997; Upton et al., 2000).

Classicamente, a inflamação das vias aéreas relacionada ao processo alérgico é tipicamente eosinofílica e acompanhada do aumento de citocinas Th2. A ativação de respostas aberrantes envolvendo citocinas tipo Th2 em resposta a antígenos inócuos desencadeia uma inflamação crônica e dano tecidual que caracterizam estes distúrbios imunopatológicos (Brandtzaeg, 2010). As células Th2 são importantes tanto para a resposta IgE-específica quanto para a resposta eosinofílica que caracterizam as doenças alérgicas (Fallon e Mangan, 2007). Interleucina (IL)-4 é a citocina que promove um *feedback* positivo para a diferenciação das células Th2 e é o principal mediador para a mudança de classe dos anticorpos produzidos pelos plasmócitos para IgE. Por outro lado, a IL-5 é a principal citocina ativadora de eosinófilos *in vivo* (Stone, Prussin e Metcalfe, 2010). A coordenação desses fatores solúveis e de células, especialmente orquestradas pelos linfócitos Th2, é que determina a patogenia das doenças alérgicas, dentre elas a asma.

Entretanto, apenas a resposta Th2 não explica todos os aspectos da asma. Por exemplo, a hiperresponsividade das vias aéreas e o remodelamento tecidual não são completamente relacionados ao processo inflamatório, sendo considerados distintos aspectos do processo alérgico (Bai e Knight, 2005).

O remodelamento crônico das vias aéreas é caracterizado por mudanças estruturais na parede das vias aéreas incluindo lesão epitelial, deposição de proteínas da matriz extracelular, espessamento da membrana basal, metaplasia de células caliciformes, hipertrofia e hiperplasia da musculatura lisa, angiogênese, entre outras modificações (Holgate, 2012). Nesse sentido, a linhagem Th17 foi descrita pela primeira vez em 2005, contribuindo para uma melhor compreensão do processo inflamatório asmático, especialmente sobre o fenótipo não atópico (Harrington et al., 2005; Park et al., 2005). Foi descrito recentemente que o Fator de transformação do crescimento-beta (TGF-β), associado com a IL-6, induz a diferenciação de células T *naive* para linfócitos Th17

levando a expressão do fator de transcrição RORγt e consequente produção de citocinas da família da IL-17 (IL-17A, IL-17F, IL-22) por meio do fator de transcrição Smad2/3 e do STAT3 e do NF-κB (Kudo, Ishigatsubo e Aoki, 2013). Estudo em modelo murino de alergia respiratória demonstrou a participação de resposta Th17 caracterizada por elevação dos níveis de IL-17 e remodelamento das vias aéreas, através da ação direta sobre o músculo liso brônquico (Wang et al., 2010; Bellini et al., 2012; Kudo et al., 2012).

Diante do exposto, fica evidente a complexidade e heterogeneidade da asma, podendo, portanto, ser classificada em diferentes fenótipos especialmente a asma atópica (alérgica) e a asma não atópica.

1.2 INFECÇÕES HELMÍNTICAS E A MODULAÇÃO IMUNOLÓGICA

As infecções parasitárias mais comuns são causadas por geohelmintos, e dentre eles, destacam-se o *Ascaris lumbricoides*, *Trichuris trichiura* e ancilostomídeos (*Ancylostoma duodenale* e *Necator americanus*) que possuem uma distribuição mundial e que estima-se infectar um quarto da população mundial (Bethony et al., 2006), sendo mais prevalentes entre as crianças que vivem em áreas rurais dos trópicos de acesso limitado à água tratada e saneamento e com condições de vida precárias (Bethony et al., 2006; Cooper, 2009; Figueiredo, C. et al., 2009). Outros importantes helmintos são o *Toxocara canis* e *T. cati* que apesar de serem parasitos de cães e gatos respectivamente, também infectam o ser humano em todo o mundo. Embora estes parasitos não atinja a fase adulta no hospedeiro humano, as larvas migram para diferentes órgãos e podem persistir por muitos anos causando a larva migrans visceral (Pinelli e Aranzamendi, 2012).

Infecções crônicas por helmintos são imunologicamente caracterizadas por desvio para a resposta T helper tipo 2, na qual células T CD4 liberam interleucina (IL) -4, IL-5, IL-13 atuando concomitantemente com eosinófilos, basófilos, mastócitos e células

caliciformes contribuindo para o controle da infecção. No entanto, como mecanismo de resistência destes parasitas, infecções crônicas por helmintos podem induzir respostas imunorreguladoras no hospedeiro, com o aumento da produção de IL-10 e TGF-β por linfócitos Treg, para evitar constante ativação imunológica contra os parasitos, permitindo a sua sobrevivência a longo prazo e restringindo a patologia causada pela inflamação gerada (Van Riet, Hartgers e Yazdanbakhsh, 2007).

Crianças que vivem em um ambiente de higiene escassa são mais propensas a se infectar com potentes agentes imunomoduladores, como helmintos, e têm elevada produção espontânea de IL-10 (Figueiredo, C. A. et al., 2009) sendo relacionados à reduzida produção de citocinas Th1 e Th2 por leucócitos de sangue periférico (PBL) (Figueiredo et al., 2011). De fato, as crianças sob condições de exposição hiperendêmica aos nematóides intestinais *Ascaris lumbricoides* e/ou *Trichuris trichiura* mostram evidências de produção espontâneas de citocinas imunorreguladoras (IL-10 e TGF-β1) (Turner et al., 2008; Figueiredo et al., 2011). Também é descrito que a infecção por *S. mansoni*, induz-se a modulação de respostas imunes em indivíduos infectados mediadas pela produção IL-10 (Cardoso et al., 2006)

Vários estudos epidemiológicos têm fornecido evidências de que a imunomodulação induzida por infecções helmínticas pode ter efeitos na alteração de resposta inflamatória aos antígenos não relacionados com o parasito, como alérgenos ou auto-antígenos, possivelmente levando a controlar desordens imunológicas (Van Riet, Hartgers e Yazdanbakhsh, 2007). Estes efeitos podem ser dependentes do tempo (idade no momento da infecção e a duração da infecção) e da dose (isto é, quanto maior a carga de infecção, maior o efeito modulatório) e varia entre diferentes helmintos intestinais (Mutapi et al., 2011; Alcantara-Neves et al., 2012; Rujeni et al., 2012).

Estudos epidemiológicos apoiam o potencial papel imunomodulatório da exposição a parasitos intestinais sobre as doenças alérgicas. A ocorrência da infecção por *T. trichiura* na primeira infância (em média dois anos de idade) pode reduzir o risco de reatividade para teste cutâneo a alérgenos na segunda infância, mesmo na ausência de

infecção (Rodrigues et al., 2008). Também, indivíduos vivendo em áreas endêmicas de *Schistosoma* spp. não respondem para testes cutâneos á aeroalérgenos e esta infecção também tem sido relacionada á redução da gravidade da asma (Araújo *et al.*, 2004; Cardoso et al., 2012; Rujeni et al., 2012). Estudos têm demonstrado que a infecção por *A*. *lumbricoides* é capaz de reduzir a prevalência de resposta positiva de testes cutâneos e o risco de asma (Alcantara-Neves et al., 2012; Cardoso et al., 2012) embora esses dados sejam controversos (Ponte et al., 2006).

Uma estratégia para demonstrar a direta relação entre infecções por helmintos e a modulação de doenças imunomediadas é através da avaliação do impacto da terapia anti-helmíntica sobre marcadores e sintomas da doença. A redução da infecção de parasitos intestinais por tratamento anti-helmintico resulta em um significante aumento da reatividade para testes cutâneos e dos níveis séricos de IgE especifica para alérgenos ambientais (Lynch et al., 1993; Van Den Biggelaar et al., 2004; Cardoso et al., 2006). Similarmente, tratamento anti-helmíntico tem sido associado a aumento de sinais clínicos em pacientes com doença autoimune, bem como, com a diminuição de células Treg CD4+CD25+FoxP3+ secretoras de TGF-β e IL-10 nesses pacientes (Correale e Farez, 2011).

Neste contexto, a hipótese da higiene originalmente descrita por Strachan (1989) tenta explicar o aumento na prevalência de doenças alérgicas nas últimas décadas em países industrializados, por alterações na resposta do hospedeiro aos alérgenos ambientais causados pela exposição diminuída a infecções durante a primeira infância especialmente decorrentes de melhorias na higiene e ao maior acesso a antibióticos e vacinas (Strachan, 1989), contribuindo para uma falta de regulação imunológica, especialmente mediada pela IL-10 e TGF- β, resultando em doenças imunomediadas (Okada et al., 2010).

1.3 TGF-β1 COMO FATOR MODULATÓRIO DE INFECÇÕES HELMÍNTICAS E DOENÇAS ALÉRGICAS

Com o objetivo de evitar a ativação crônica de células e processo inflamatório gerados por antígenos não patogênicos expostos ao organimo via ingestão e inalação, o sistema imunológico desenvolveu ao longo dos anos mecanismos periféricos de tolerância eficientes. Tem sido demonstrado que diferentes subtipos de células regulatórias e supressoras podem desempenhar um papel na tolerância periférica, e sua biologia tem sido objeto de intensa investigação (Shevach, 2002). Um dos principais mecanismos relacionados a esta modulação se dá através das células T regulatórias (Treg) que suprimem respostas imunológicas especialmente através de interações célula-célula e/ou da produção de TGF-β e IL-10 (Read e Powrie, 2001).

O TGF-β é um fator de crescimento pleiotrópico produzido por diversas células do sistema imunológico (células epiteliais, eosinófilos, linfócitos Th2, macrófagos,fibroblastos e plaquetas) que desempenha papel fundamental na regulação da resposta imune durante infecções e doenças inflamatórias através da sua atividade imunomodulatória e fibrogênica, dependendo do ambiente tecidual (Yang et al. 2012). A superfamília da TGF-β possui mais de 33 membros incluindo principalmente os TGF-βs, Fator de Crescimento e Diferenciação (GDFs) e as Inibinas, sendo o TGF-β1 a isoforma mais prevalente (Klass, Grobbelaar e Rolfe, 2009).

TGF-β1 é um dos principais reguladores da resposta imunológica e exerce potente atividade anti-inflamatória através da inibição da diferenciação de células imunes (Linfócitos Th1, Th2, T citotóxicos e células B), bem como inibição da produção de citocinas (IFN-γ e IL-2) (Li et al., 2006; Yoshimura, Wakabayashi e Mori, 2010). A produção e atividade do TGF-β1 e da IL-10 tem sido inter-relacionada e, provavelmente envolve um feedback positivo cíclico, no qual IL-10 aumenta a expressão de TGF-β1, e vice-versa (Travis e Sheppard, 2013). Além disso, atua como um importante fator de diferenciação para algumas células que exercem poderosos e variados efeitos imunossupressores, como, por exemplo, as células T regulatórias $FOXP3^{+}$ (Dardalhon

et al., 2008; Shull et al., 1992; Tran, 2012).

Devido a sua capacidade regulatória sobre o sistema imunológico, o TGF-B1 tem sido descrito associado a infecções por helmintos. Estudo realizado por Turner e colaboradores (2008) com crianças infectadas por helmintos apresenta evidência para a elevada produção de TGF- β1 por leucócitos do sangue periférico não estimulados, associada positivamente com a carga parasitária e negativamente com a reatividade imune, determinada através da capacidade de produção de IL-4 e IFN-γ e da proliferação celular em resposta a estímulos antigênicos. Esta intensa imunorregulação desempenhada pelo TGF- β1 e induzida nos indivíduos infectados pode explicar a proteção descrita na literatura contra o desenvolvimento de doenças imunomediadas (Moreau e Chauvin, 2010) (Turner et al., 2008).

O TGF-β1 tem sido descrito como um mediador central no processo do remodelamento brônquico, atuando através da indução de genes relacionados, incluindo o Fator de Crescimento de Tecido Conjuntivo (CTGF), αlfa-actina do músculo liso (α-SMA) e colágeno, além de induzir proliferação e quimioatração de fibroblastos, bem como, diferenciação destas células em miofibroblastos que irão induzir fibrose e contração da matriz extracelular e indução da Transição Epitelial-Mesenquimal (TEM) (Crosby e Waters, 2010); (Willis e Borok, 2007). Diversos estudos têm demonstrado evidências de que o TGF-β1 induz TEM em modelo de fibrose experimental e no epitélio alveolar humano através da fosforilação da SMAD 2/3 ou da ativação de vias dependentes da MAPK (Saika et al., 2004); (Pechkovsky et al., 2008).

1.4 POLIMORFISMOS NO GENE DO TGF-B1 E IMUNORREGULAÇÃO

Dado o importante papel regulador do TGF-β1 na infecção por helmintos e nas doenças inflamatórias, é de interesse saber se a variação genética no gene dessa citocina ou na sua região promotora influencia na imunidade do hospedeiro contra infecção hemíntica, bem como na modulação da alergia e seus sintomas.

O gene do TGF-β1 está localizado no braço longo do cromossomo 19, na posição 13.1 (19q13.1) localizado entre a posição 41.330.530 e a posição 41.353.932.

Polimorfismo em único nucleotídeo (*Single Nucleotide Polymorphism*, SNP) é a forma mais simples de variação genética entre os indivíduos, ocorrendo com uma frequência de aproximadamente 1 a cada 1000 pb, contribuindo para a diversidade entre os indivíduos; evolução genômica; diferença inter-individual na resposta de drogas; e o desenvolvimento de doenças complexas, tais como a diabetes, a hipertensão e a asma (Shastry, 2009). SNPs podem mudar o aminoácido codificado (não-sinônimo) ou pode ser silencioso (sinônimo); ou pode ainda ocorrer em uma região não codificante e, dessa forma, podem influenciar a atividade promotora (expressão gênica), a conformação do RNA mensageiro (mRNA) (estabilidade); bem como o splicing, contribuindo para o desenvolvimento de certas doenças (Shastry, 2009). Polimorfismos em genes relacionados com a resposta imunológica podem contribuir para uma maior susceptibilidade ou maior proteção para o desenvolvimento de doenças imunomediadas. Sendo assim, a identificação de polimorfismos genéticos e os estudos de associação com os seus efeitos biológicos podem contribuir para uma melhor compreensão do seu impacto na relação saúde-doença. Adicionalmente, o estudo dos SNPs deverá permitir o desenvolvimento de marcadores genéticos para o diagnóstico clínico e individualização terapêutica.

Estudos de associação avaliando SNPs representam atualmente uma importante estratégia utilizada para desvendar os determinantes genéticos de doenças complexas, podendo, basicamente, ser realizado de duas maneiras, a saber: (1) Estudos de associação de genoma completo (*Genome-Wide Association Studies*, GWAS), em que são realizados estudos de associação usando uma ampla gama de SNPs distribuídos ao longo de todo o genoma humano e assim testar a associação com a característica de interesse; (2) Estudo de gene candidato, em que são avaliados SNPs presentes em genes plausivelmente envolvidos na patogênese ou em sua região promotora, objetivando detectar o seu papel na susceptibilidade à doença (Cousin et al., 2003).

GWAS têm revelado uma variedade de genes relacionados com susceptibilidade à asma em diferentes populações (Mathias et al., 2010; Galanter et al., 2014). Adicionalmente, diversos SNPs têm sido identificados por estudos de associação em genes candidatos relacionadas com a asma. (Vercelli, 2008). Entretanto, não existe estudo avaliando a variabilidade no gene do TGF-β1 com a susceptibilidade para infecções helmínticas.

Em estudo de associação genética, variações nos genes que codificam proteínas envolvidas no desenvolvimento e função das células Treg tais como Toll-like receptor (TLR-2), TGF-β1, IL10, e hemeoxygenase 1 (HMOX1), foram previamente associados a atopia e fenótipos de asma (Bottema et al., 2010).

Dentre os SNPs no gene do TGF-β1 relatados na literatura como estando associados à doença alérgica, os mais estudados e replicados são os seguintes:

rs1800470 (869 T>C)

O rs1800470 está localizado no códon 10 do exon 1 do gene do TGF-β1 sendo responsável pela mudança de aminoácido leucina para prolina (Awad et al., 1998), estando associado com os níveis séricos da citocina, sendo o genótipo CC associado com maiores concentrações de TGF-β1 quando comparado aos demais genótipos (Yamada et al., 1998; Salam et al., 2007). Estudos prévios têm mostrado que o alelo C do rs1800470 é positivamente associado com asma numa população chinesa e mexicana (Mak et al., 2006; Li et al., 2007) e que o alelo T está associado a um risco reduzido de hospitalização por asma em crianças brancas não hispânicas (Sharma et al., 2009).

rs1800469 (-509 C>T)

O rs1800469 está localizado na região promotora do gene do TGF-β1 e pode modular a função e os níveis séricos da citocina (Li et al., 2007), sendo o alelo T relacionado ao aumento da produção de TGF-β1 (Grainger et al., 1999). A associação desse polimorfismo com asma foi descrito em um GWAS realizado na população do México (Wu et al., 2010) e replicado em outros estudos (Li et al., 2007; Yang et al., 2011). Estudo com 3.023 crianças da Califórnia revelou que indivíduos com o genótipo -509TT no gene do TGF-β1 apresentou risco aumentado para asma persistente (Salam et al. 2007). Adicionalmente, Zhu e cols. (2010) demonstraram que o polimorfismo 509 C/T no gene do TGF-β1 parece ter relação com a gravidade de rinite alérgica persistente em crianças (Zhu et al., 2010).

rs2241712 (-10807 G>A)

O rs2241712 está localizado na região promotora do gene do TGF-β1 e tem sido associado com doenças respiratórias como asma e doença pulmonar obstrutiva crônica (*Chronic Obstructive Pulmonary Disease,* COPD) (Celedón et al., 2004). Estudo realizado na Costa Rica revelou a associação do alelo A desse polimorfismo com aumento na hiperesponsividade das vias aéreas (Sharma et al., 2009). Embora a relação entre o rs2241712 e os níveis séricos de TGF-β1 não tenha sido avaliada, tal polimorfismo apresenta escore 2b no banco de dados do *Regulome DB* que significa que o rs2241712 está relacionado com a ligação de fatores transcripcionais ao DNA e, potencialmente, alteração dos níveis de produção da citocina (Boyle et al., 2012).

Embora não existam estudos avaliando o efeito da variabilidade genética no TGF-β1 sobre a imunomodulação realizada por helmintos, estudos prévios têm destacado polimorfismos no gene da IL-10 como importante fator sobre tal processo. Grant e colaboradores (2010) descreveram que polimorfismos no gene da IL10 estão associados com altos níveis de imunoglobulina E total e com a intensidade de infecção pelo *Schistosoma mansoni* em uma população brasileira (Grant *et al.*, 2010). Em estudo envolvendo o programa SCAALA, nosso grupo de pesquisa verificou que polimorfismos

no gene da IL-10 estão negativamente associados à produção de IL-10 (rs3024496) e infecções por *A. lumbricoides* e *T. trichiura* (rs3024498 e rs3024492) e positivamente associados com asma atópica (rs3024492) e teste de puntura cutâneo (rs3024492) para alérgenos comuns a esta população (Figueiredo et al., 2013). A interação gene-gene entre os genes da IL-10 e do TGF-β1 também vem sendo implicada em alergias e asma. Polimorfismo no gene do TGF-β1 localizado na região 509C/T foi apontado como tendo um efeito sinérgico com a IL10 em pacientes com rinosinusite (Kim et al., 2009).

O entendimento de como esses fatores regulatórios conjuntamente podem estar relacionados à asma e outras alergias na população de estudo e como fatores ambientais estão contribuindo para isto, é essencial para o entendimento da patogênese da asma tendo em vista a adoção de medidas preventivas e de melhor intervenção terapêutica para o tratamento desta patologia.

Neste contexto, no presente trabalho pretendemos explorar como a variação no gene do TGF-β1 ou na sua região promotora se associam com marcadores de doenças alérgicas e asma e com infecções helmínticas.

Os resultados desse trabalho, bem como suas respectivas metodologias e discussões, serão apresentados na estrutura de artigos conforme recomendação do Programa de Pós-Graduação em Imunologia. O primeiro manuscrito foi uma revisão abordando a associação de polimorfismos genéticos no TGF-β1 com alergia, publicado no *OA Immunology*. O segundo manuscrito se encontra formatado de acordo com o *Journal of Allergy and Clinical Immunology*, ao qual será submetido. O terceiro manuscrito se encontra no formato da revista *Gene & Immunity*, ao qual o mesmo será submetido.

OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar a associação entre os polimorfismos no gene do TGF-β1 e atopia, asma e infecções helmínticas.

2.2 OBJETIVOS ESPECÍFICOS

1. Determinar as frequências dos polimorfismos do TGF-β1 (rs4803455, rs1800470, rs1800469, rs2241712) na população do SCAALA;

2. Investigar se existe associação entre os polimorfismos no gene do TGF-β1 e sintomas de asma alérgica, produção de IgE específica e teste cutâneo para alérgenos;

3. Investigar se existe associação entre os polimorfismos no gene do TGF- β1 e níveis séricos de IgE total;

4. Investigar se existe associação entre os polimorfismos no gene do TGF- β1 e infecções por *Ascaris lumbricoides*, *Trichuris trichiura e Toxocara spp.* em crianças residentes em Salvador - Brasil;

5. Determinar se existe associação entre os polimorfismos no gene do TGF- β1 e a produção de IL-10 em cultura de sangue total estimulada com mitógeno;

6. Investigar se existe associação entre os polimorfismo no gene do TGF- β1 com a asma através de estudo de associação de gene candidato em quatro populações de ancestralidade africana.

RESULTADOS E DISCUSSÃO

Manuscrito 1. Are TGF-β gene polymorphisms associated with asthma risk?

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Are TGF-β gene polymorphisms associated with asthma risk?

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Abstract

Introduction: Asthma is an inflammatory disease, leading to airway obstruction, hyper-response, heightened mucus production, and it can affect remodelling of the airway wall. Almost 300 million people in the world are estimated to be affected by asthma. Several studies have shown that regulatory T cells (Treg cells) have a key role in controlling allergic diseases and chronic inflammation on asthma. Thus, recently, attention has been given to Treg cells producing interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). *TGF-β1* is an important replicated asthma candidate gene, and few studies have evaluated the direct association of *TGF*-β polymorphisms and risk to allergic diseases. Thus, the aim of this article was to critically review the main polymorphisms on TGF-β gene described so far and depict the role of such polymorphisms on asthma development.

Conclusion: Although these data seem controversial, polymorphisms on TGF-β1 may be an interesting marker for asthma since it is related to an increase on TGF-β1 levels, and it may be related to tissue remodelling. However, more studies are required to better understand the results observed so far.

Introduction

Asthma and its pathophysiology

Asthma is the most common chronic diseases that affect childhood and is the main cause of morbidity in adults. Epidemiological data show that this illness affects 4%–17% children and 7.7% adults in the United States. Furthermore, almost 300 million people worldwide could be affected by asthma[1]. In Brazil, Salvador has extremely high rates of up to 24.6% among school-age children (13–14 years)[2].

Studies have shown that asthma develops during the intrauterine foetal programming[3]. Atopic asthma is most frequent in children because there is a predominant immunological Th2 response at this stage of life[4]. This condition is an inflammatory disease, leading to airway obstruction, hyper-response, heightened mucus production, and it can affect remodelling of the airway wall. The increasing number of studies about this pathology allowed a paradigm shift in immunology and molecular biology that led to an extensive analysis of inflammatory cells and mediators involved in the pathophysiology of asthma. The pathophysiological characteristics of asthma are not limited to the Th2 response. Studies have shown that the immune response also involves T helper cells (Th)-17, Th9, as well as the Th1 and Th33 profile[5].

The Th2 response is characterised by the production of Th2 cytokines; interleukin-4 (IL-4) and IL-13 are main cytokines responsible for the stimulation of plasma cells to secrete IgE and continue the inflammatory process. IL-4 also stimulates increased expression of IgE receptors on mast cells, eosinophils and basophils[6]. Other mediators are also involved in the inflammatory process, such as Tumour Necrosis Factor (TNF) and nitric oxide released by macrophages which produce neutrophil elastase. Rather than Th2-type cytokines, it has been well documented that IL-2, IL-12, interferon-γ (IFN-γ), TNF-α and transforming growth factor-β (TGF-β) produced by CD4+ T helper 1 cells (Th1) are implicated in the pathogenesis of asthma[5–8]. Epithelial cells in repair phase produce TGF-β, fibroblast growth factor and endothelin, which regulate fibroblasts and myofibroblasts to release collagen, elastic fibre, proteoglycan and glycoprotein, and

these substances induce airway wall thickening[9]. Antigen-presenting cells, especially dendritic cells (DCs), play a critical role in initiating and regulating early inflammatory events at epithelial surfaces and control the recruitment and activation of Th2 cells[6,10]. A cooperation of airway epithelium and DC controls asthma development, and Th2 activation requires DC-mediated antigen presentation. Thus, cytokines such as IL-33 are induced[5]. IL-33 is a member of the IL-1 family that can induces activation of DCs, mast cells, eosinophils and basophils, ultimately leading to increased expression of Th2-associated cytokines and IgE. In addition, IL-33 administration provokes hypertrophy of bronchial epithelial cells, as well as mucus secretion [11,12]. Studies have shown that IL-33 is an attractive candidate for therapeutic intervention, either by its soluble receptor, ST2, targeting the lung or by its small molecule inhibitors that could act systemically as a central regulator of the inflammatory response characteristic of asthma[11].

Based on the central role of TGF-β, especially on its ability to modulate various immunological profiles the aim of this review was to discuss whether TGF-β gene polymorphisms are associated with asthma risk.

Discussion

The authors referenced studies that have been conducted in accordance with the Declaration of Helsinki (1964), and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies.

TGF-β

TGF-β is a pleiotropic and multifunctional growth factor released by several immunologic cells (epithelial cells, eosinophils, Th2 lymphocytes, macrophages and fibroblast)

showing key roles in immune response on homeostasis, infections and inflammatory diseases through its immune modulatory and fibrogenic activity[13]. The TGF-β superfamily consists of more than 33 members, all holding a similar prodomain fold, including bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), activins and inhibins[14]. The TGF-β family has three different TGF-β isoforms in mammals: TGF-β1, the most prevalent isoform, TGF-β2 and TGF-β3 that have similar properties *in vitro*[14,15].

TGF-β is the main regulator of the immune response and exerts potent anti-inflammatory activity by inhibiting the differentiation of immune cells (Th1, Th2, cytotoxic T cells and B cells) as well as inhibition of cytokine production (IFN-γ and IL-2)[15,16]. Furthermore, it acts as an important differentiating factor for some cells that exert powerful immunosuppressive effects, such as regulatory T cells (Treg cells) FOXP3+ [17–19].

Additionally, TGF-β1 plays an important role in extracellular matrix remodelling and fibrosis through the induction of target genes such as connective tissue growth factor (CTGF), α-smooth muscle actin (α-SMA), collagen 1a2 (col1a2)[20]inducing proliferation and chemo attraction of fibroblasts and their differentiation into myofibroblasts, which finally will induce fibrosis and contraction of the extracellular matrix[13].

TGF-β and asthma immune response

Allergic diseases are caused by inappropriate immunological responses to allergens[5]. Several studies have shown that regulatory T cells (Treg cells) have a key role in controlling allergic diseases and chronic inflammation in asthma. A defect on immune regulation leads to an exacerbation of Th2 response[21,22].

Treg cells are essential in the maintenance of immunological tolerance to self-antigens and in the regulation of the immune response to infectious organisms and represent a

major pathway proposed to keep immune homeostasis in the airways. Thus, recently, attention has been given to Treg cells producing IL-10 and TGF-β. These immune modulatory cytokines can down regulate the production of both Th1 and Th2 cytokines and suppress the inflammatory response on asthma (Figure 1)[23–25].

Accordingly, TGF-β is crucial in the development and function of $CD4^+$ $CD25^+$ Treg and induces expression of the master regulatory transcription factor Foxp3, a critical gene regulator for Treg cells development. TGF-β inhibits IgE, the main antibody associated with allergic diseases and asthma[26] and has been shown to induce IL-10 expression in T cells (Figure 1)[27].

Hansen et al. have demonstrated that T cells producing TGF-β are able to reduce inflammation and airway hyperreactivity in a mouse model[28]. It was also demonstrated that blocking the TGF-β signalling pathway in T lymphocytes, an increasing inflammation and airway hyperreactivity is observed, suggesting that TGF-β-induced immune regulation reduces the pulmonary inflammatory response *in vivo* (Figure 1)[23].

In contrast, several studies have shown that TGF-β is associated to an increased airway remodelling by inducing apoptosis of airway epithelial cells and is potentially involved in the regulation of epithelial cells adhesion leading to tissue damage (Figure 1)[29]. The neutralisation of TGF-β in two different models of chronic allergen challenge reduced airway remodelling[30]. Asthmatic patients showed increased TGF-β expression in both bronchial biopsy sections and bronchoalveolar lavage in comparison with normal subjects and expression correlated with the lung fibrosis degree[31].

These data reinforce the idea that TGF-β acts to regulate immune response in the lungs and that perturbations in the level of expression of that cytokine or even in one of the TGF-β signalling pathway molecules may have severe consequences for maintenance of pulmonary homeostasis. However, many studies have investigated the rationale that increased TGF-β expression may be associated with asthma severity by increasing airway remodelling.

TGF-β gene polymorphisms and allergic disease risk

Polymorphisms in gene sequences can affect the expression of proteins in various ways: levels of gene transcription, splicing, stability and levels of mRNA translation[32]. Polymorphisms in genes that participate on immunity may influence the development of several diseases. Susceptibility to many diseases is associated with a particular 'pro-inflammatory' profile, which can be explained by individual genetic determinants. TGF-β1 is an important replicated asthma candidate gene, and few studies have evaluated the direct association of *TGF*-β polymorphisms and risk to allergic diseases, in particular, asthma[33,34]. The TGF-β gene is located on chromosome 19q13.1–13.3[35], and some polymorphisms were shown in this gene and can be found in exons, introns and promoter gene sequences. The most studied polymorphisms on TGF-β gene are described in Table 1. Below we present some of them and discuss their role on cytokine levels as well as on asthma development:

rs1800469 (-509 C>T)

The -509 C>T polymorphism is located in the promoter region of *TGFB1* and can modulate TGF-β1 function and circulating TGF-β1 levels[34](Table 1). The T allele has been associated with higher TGF-β1 plasma levels[36]. An interesting Genome-wide association study (GWAS) previously reported that -509 C>T was associated with asthma in a Mexican population[37]. These results were consistent with several authors[34,38]. In contrast, other authors found no association between -509 polymorphisms and risk to clinically manifested atopic asthma or other allergies[33,39]. Although these data seem controversial, this single-nucleotide polymorphism (SNP) can be an interesting marker for allergic diseases. However, more studies are needed to better understand the results obtained so far. Lack of associations can be achieved when sample size is small and therefore may have lacked power to detect statistically significant associations. Moreover this polymorphism was associated with IgE levels. One study described phenotypic association between total IgE levels in serum and -509

C>T(39) and also association with persistent IgE-mediated cow's milk allergy in children[40].

rs2241712 (-10807 G>A)

The rs2241712 (-10807) is located in the promoter region of the TGF-β1 gene and has been associated with some respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD)[41] (Table 1) Studies have analysed associations between rs2241712 and asthma. In one of these studies, the authors have shown an association between rs2241712 and asthma in atopic subjects. The G/A genotype was associated with decreasing asthma risk; however, this association was not statistically significant[38]. Furthermore in a study conducted in Costa Rica revealed an association of the A allele with increased airway responsiveness[42]. However, another study showed an association between the G allele with asthma risk, but this difference was not statistically significant[38]. It seems that this SNP may have an important role in the respiratory disease development. Therefore more studies are needed to elicit this association. Although the relationship between rs2241712 and TGF-β1 serum levels has not been evaluated, this polymorphism presents score 2b in the Regulome DB database being likely to affect binding to transcription factor and, potentially, change the TGF-β1 production (Table 1).

rs1800468 (-800 G>A)

We found only one study that analysed the SNP-800 in association with asthma and serum IgE levels[33]. Moreover, the authors found no association between the SNP-800 and clinical manifestation of atopic diseases and serum IgE levels (Table 1).

rs1800470 (869 T>C)

The rs1800470 is located in codon 10 of exon 1 and is related to change the amino acid sequence, leucine to proline in the signal peptide[43]. Codon 10 polymorphism has been studied in several diseases as nephropathy in Type 1 diabetes[44], allergic rhinitis[45] and asthma[34]. The 869 polymorphism was found to be associated with an increased circulating TGF-β1 concentration[46], increased production of TGF-β1 *in vitro*[47] and increased risk of asthma[34] (Table 1)

rs1800471 (codon 25)

The 915 G>C polymorphism results in an amino acid change (arginine to proline) at codon 25; however, the functional impact of this polymorphism is unknown (Table 1). In allergic diseases, no association was found for this SNP[45]. Although, the haplotype analysis including rs1800471 (codon 10) was associated with a history of parental asthma[45].

Conclusion

Several SNPs on TGF-β1 have been studied in asthma diseases. Some SNPs were positively associated with asthma but not always replicated by all authors. Although these data seem controversial, polymorphisms on TGF-β1 may be an interesting marker for asthma since it is related to an increase on TGF-β1 levels, and it may be related to tissue remodelling. However, more studies are required to better understand the results observed so far. It is important to point out that in some studies, lack of associations can be related to small sample size and consequently no power to detect statistically significant associations. Moreover, the prevalence of these polymorphisms may vary from population to population, which can also interfere with these controversial results. Greater sample size and studies using a considerable number of informative ancestry

markers are required to confirm these findings and also to identify populations, where TGF-β1 variants are mediating the casual pathway of allergic diseases, in special, asthma.

References

1. Bang DW, Yang HJ, Ryoo E, Al-Hasan MN, Lahr B, Baddour LM, et al. Asthma and risk of non-respiratory tract infection: a population-based case-control study. BMJ Open. 2013;3(10):e003857.
2. Toledo MF, Rozov T, Leone C. Prevalence of asthma and allergies in 13- to 14-year-old adolescents and the frequency of risk factors in carriers of current asthma in Taubaté, São Paulo, Brazil. Allergol Immunopathol (Madr). 2011;39(5):284–90.
3. Warner JO, Marguet C, Rao R, Roche WR, Pohunek P. Inflammatory mechanisms in childhood asthma. Clin Exp Allergy. 1998;28Suppl 5:71–5; discussion 90–1.
4. Ocampo Martínez DM, Gómez Vera J, Flores Sandoval G, OreaSolano M. [Asthma phenotype during the first six years of life]. Rev Alerg Mex. 1999;46(5):124–9.
5. Kudo M, Ishigatsubo Y, Aoki I. Pathology of asthma. Front Microbiol. 2013;4:263.
6. Holgate ST. Innate and adaptive immune responses in asthma. Nat Med. 2012;18(5):673–83.
7. Kim YM, Kim YS, Jeon SG, Kim YK. Immunopathogenesis of allergic asthma: more than the th2 hypothesis. Allergy Asthma Immunol Res. 2013;5(4):189–96.
8. Figueiredo CA, Rodrigues LC, Alcantara-Neves NM, Cooper PJ, Amorim LD, Silva NB, et al. Does IFN-γ play a role on the pathogenesis of non-atopic asthma in Latin America children? Allergy Asthma Clin Immunol. 2012;8(1):18.
9. Holgate ST, Davies DE, Lackie PM, Wilson SJ, Puddicombe SM, Lordan JL. Epithelial-mesenchymal interactions in the pathogenesis of asthma. J Allergy ClinImmunol. 2000;105(2 Pt 1):193–204.
10. Schuijs MJ, Willart MA, Hammad H, Lambrecht BN. Cytokine targets in airway inflammation. Curr Opin Pharmacol. 2013;13(3):351–61.
11. Borish L, Steinke JW. Interleukin-33 in asthma: how big of a role does it play? Curr Allergy Asthma Rep. 2011;11(1):7–11.
12. Mato N, Bando M, Yamasawa H, Hosono T, Mizushina Y, Sata M, et al. Role of IL-33 in bronchial asthma. Nihon Kokyuki Gakkai Zasshi. 2010;48(6):419–25.
13. Yang YC, Zhang N, Van Crombruggen K, Hu GH, Hong SL, Bachert C. Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. Allergy. 2012;67(10):1193–202.
14. Klass BR, Grobbelaar AO, Rolfe KJ. Transforming growth factor beta1 signalling, wound healing and repair: a multifunctional cytokine with clinical implications for wound repair, a delicate balance. Postgrad Med J. 2009;85(999):9–14.
15. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol. 2006;24:99–146.

16. Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by TGF-beta. J Biochem. 2010;147(6):781–92.
17. Dardalhon V, Awasthi A, Kwon H, Galileos G, Gao W, Sobel RA, et al. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. Nat Immunol. 2008;9(12):1347–55.
18. Tran DQ. TGF-β: the sword, the wand, and the shield of FOXP3(+) regulatory T cells. J Mol Cell Biol. 2012;4(1):29–37.
19. Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, Yin M, et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature. 1992;359(6397):693–9.
20. Crosby LM, Waters CM. Epithelial repair mechanisms in the lung. Am J Physiol Lung Cell Mol Physiol. 2010;298(6):L715–31.
21. Umetsu DT, Akbari O, Dekruyff RH. Regulatory T cells control the development of allergic disease and asthma. J Allergy Clin Immunol. 2003;112(3):480–7; quiz 8.
22. Jaffar Z, Sivakuru T, Roberts K. CD4+CD25+ T cells regulate airway eosinophilic inflammation by modulating the Th2 cell phenotype. J Immunol. 2004;172(6):3842–9.
23. Nakao A, Miike S, Hatano M, Okumura K, Tokuhisa T, Ra C, et al. Blockade of transforming growth factor beta/Smad signaling in T cells by overexpression of Smad7 enhances antigen-induced airway inflammation and airway reactivity. J Exp Med. 2000;192(2):151–8.
24. Leech MD, Benson RA, De Vries A, Fitch PM, Howie SE. Resolution of Der p1-induced allergic airway inflammation is dependent on CD4+CD25+Foxp3+ regulatory cells. J Immunol. 2007;179(10):7050–8.
25. Lloyd CM, Saglani S. T cells in asthma: influences of genetics, environment, and T-cell plasticity. J Allergy Clin Immunol. 2013;131(5):1267–74; quiz 75.
26. Larché M. Regulatory T cells in allergy and asthma. Chest. 2007;132(3):1007–14.
27. Kitani A, Fuss I, Nakamura K, Kumaki F, Usui T, Strober W. Transforming growth factor (TGF)-beta1-producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-beta1-mediated fibrosis. J Exp Med. 2003;198(8):1179–88.
28. Hansen G, McIntire JJ, Yeung VP, Berry G, Thorbecke GJ, Chen L, et al. CD4(+) T helper cells engineered to produce latent TGF-beta1 reverse allergen-induced airway hyperreactivity and inflammation. J Clin Invest. 2000;105(1):61–70.
29. Szefler SJ. Airway remodeling: therapeutic target or not? Am J Respir Crit Care Med. 2005;171(7):672–3.
30. McMillan SJ, Xanthou G, Lloyd CM. Manipulation of allergen-induced airway remodeling by treatment with anti-TGF-beta antibody: effect on the Smad signaling pathway. J Immunol. 2005;174(9):5774–80.
31. Doherty TA, Soroosh P, Khorram N, Fukuyama S, Rosenthal P, Cho JY, et al. The tumor necrosis factor family member LIGHT is a target for asthmatic airway remodeling. Nat Med. 2011;17(5):596–603.
32. Bidwell JL, Wood NA, Morse HR, Olomolaiye OO, Laundy GJ. Human cytokine gene nucleotide sequence alignments, 1998. Eur J Immunogenet. 1998;25(2–3):83–265.

33. Buckova D, Izakovicová Hollá L, Benes P, Znojil V, Vácha J. TGF-beta1 gene polymorphisms. Allergy. 2001;56(12):1236–7.
34. Li H, Romieu I, Wu H, Sienra-Monge JJ, Ramírez-Aguilar M, del Río-Navarro BE, et al. Genetic polymorphisms in transforming growth factor beta-1 (TGFB1) and childhood asthma and atopy. Hum Genet. 2007;121(5):529–38.
35. Fujii D, Brissenden JE, Derynck R, Francke U. Transforming growth factor beta gene maps to human chromosome 19 long arm and to mouse chromosome 7. Somat Cell Mol Genet. 1986;12(3):281–8.
36. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, et al. Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet. 1999;8(1):93–7.
37. Wu H, Romieu I, Shi M, Hancock DB, Li H, Sienra-Monge JJ, et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. J Allergy Clin Immunol. 2010;125(2):321–7.e13.
38. Yang XX, Li FX, Wu YS, Wu D, Tan JY, Li M. Association of TGF-beta1, IL-4 and IL-13 gene polymerphisms with asthma in a Chinese population. Asian Pac J Allergy Immunol. 2011;29(3):273–7.
39. Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. Am J Respir Crit Care Med. 1998;158(6):1958–62.
40. Jacob CM, Pastorino AC, Okay TS, Castro AP, Gushken AK, Watanabe LA, et al. Interleukin 10 (IL10) and transforming growth factor β1 (TGF-β1) gene polymorphisms in persistent IgE-mediated cow's milk allergy. Clinics (Sao Paulo). 2013;68(7):1004–9.
41. Celedón JC, Lange C, Raby BA, Litonjua AA, Palmer LJ, DeMeo DL, et al. The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). Hum Mol Genet. 2004;13(15):1649–56.
42. Sharma S, Raby BA, Hunninghake GM, Soto-Quirós M, Avila L, Murphy AJ, et al. Variants in TGFB1, dust mite exposure, and disease severity in children with asthma. Am J Respir Crit Care Med. 2009;179(5):356–62.
43. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation. 1998;66(8):1014–20.
44. Patel A, Scott WR, Lympany PA, Rippin JD, Gill GV, Barnett AH, et al. The TGF-beta 1 gene codon 10 polymorphism contributes to the genetic predisposition to nephropathy in Type 1 diabetes. Diabet Med. 2005;22(1):69–73.
45. Gentile DA, Doyle WJ, Zeevi A, Howe-Adams J, Trecki J, Skoner DP. Association between TNF-alpha and TGF-beta genotypes in infants and parental history of allergic rhinitis and asthma. Hum Immunol. 2004;65(4):347–51.
46. Yamada Y. Association of a Leu(10)-->Pro polymorphism of the transforming growth factor-beta1 with genetic susceptibility to osteoporosis and spinal osteoarthritis. Mech Ageing Dev. 2000;116(2–3):113–23.
47. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, et al. A transforming growth factorbeta1 signal peptide variant increases secretion in vitro and

is associated with increased incidence of invasive breast cancer. Cancer Res. 2003;63(10):2610–5.

Table 1. Description of most studied *TGF-β1* gene polymorphisms, their association with asthma and *TGF-β1* protein levels.

Figure 1. TGF-β on asthma immune response. TGF-β is crucial to induce expression of the main regulatory transcription factor Foxp3 and to regulate the development, function and IL-10 production from of $CD4^+CD25^+$ Treg cells. Moreover, TGF-β can down-regulates the activation of Th2 lymphocytes, suppress the inflammatory response and airway hyperreactivity and inhibits IgE release. TGF-β also seems to be involved in the airway remodelling and, consequently, severity of asthma.

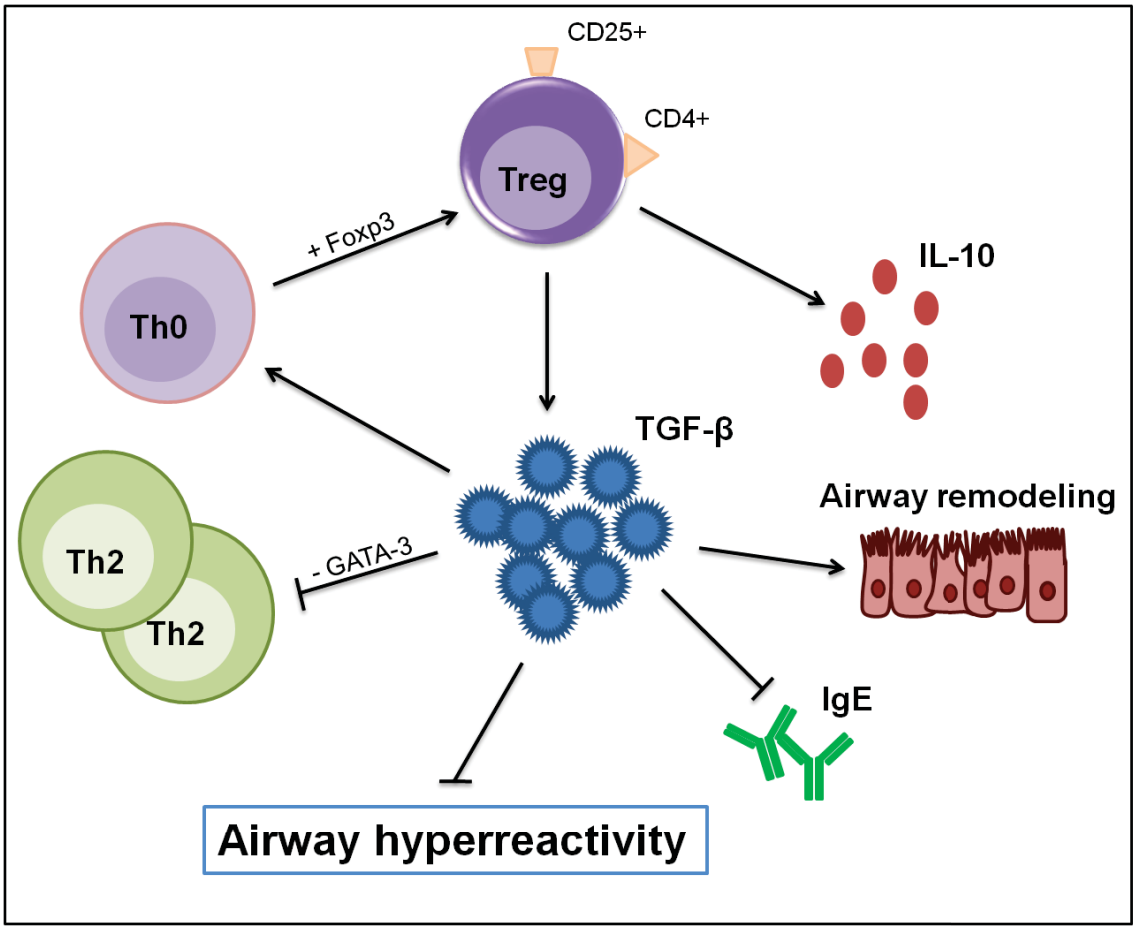
CD25+
CD4+
Treg
+ Foxp3
Th0
IL-10
TGF-β
Airway remodeling
Th2
Th2
- GATA-3
IgE
Airway hyperreactivity

Figure 1

Table 1. Description of *TGF-β1* polymorphisms

SNP	Location	Location allele	DB Score*	Asthma risk	References[1]	TGF-β levels	References[2]
rs1800469	41860296	promoter	1b	+	Wu, 2010 Hulling, 2007 Xue, 2011	⬆	Grainger, 1999 .
				No association	Buckova, 2001 Hobbs 1998		
rs2241712	41869756	promoter	2b	**No association**	Yang, 2011	Unknown	
rs1800470	41858921	exon	4	+	Hulling, 2007	⬆	Yamada, 2001
rs1800471	41858876	exon	4	**No association**	Gentili, 2007	⬆	Awad, 1988
rs1800468	41860587	promoter	5	**No association**	Buckova, 2001	Unknown	

*RegulomeDB Score is a computer score based on the integration of multiple high-throughput datasets that represent the polymorphism impact in the regulation of gene transcription.

[1]References to asthma risk.

[2]References to TGF-β levels.

Manuscrito 2. Transforming Growth Factor-β1 (TGF-β1) gene polymorphisms are associated with atopic asthma and helminth infections in an admixture population

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Transforming Growth Factor-β1 (TGF-β1) gene polymorphisms are associated with atopic asthma and helminth infections in an admixture population

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Abstract

Background: Allergic asthma is a complex disorder resulting from a combination of genetic and environmental factors. Studies suggest that helminth infections can active a regulatory network characterized by Transforming growth factor-beta 1 (TGF-β1) production and then to protect against immune-mediated diseases such as asthma. TGF-β1 is increased in the lungs of individuals with asthma and may modulate airway inflammation. The role of single nucleotide polymorphisms of TGF-β1 in allergy remains inconclusive.

Objective: To evaluate the effects of genetic variations in the TGF-β1 on allergy and helminth infections in children.

Methods: We tested for association between four SNPs in TGF-β1 and atopic asthma, specific IgE, skin prick test and IL-10 production in 1,335 Brazilian children. In addition, we analyzed the association with markers of helminth infections such as parasite burden, anti-*A. lumbricoides* IgE and IgG4. The polymorphisms were genotyped using Taq Man probes.

Results: Negative association was observed between rs1800470 and atopic asthma, specific IgE to allergens and skin test reactivity to allergens, including house dust mite *B. tropicalis*. Also, a positive association was observed between the haplotypes of the variants in the TGF-β1 and infections with *Ascaris lumbricoides*, *Trichuris trichiura* and *Toxocara spp*. The same haplotypes were positively associated with IL-10 production.

Conclusion: Individual with TGF-β1 polymorphisms have a lower risk of developing allergy and increased susceptibility to helminth infections. Additionally, we have shown that immune modulation of allergy result not only of the environmental factors but also of the genetic polymorphisms, especially by modulating IL-10 production.

Key Messages

TGF-β1 genetic variants are negatively associated with atopy and positively associated with helminth infections through of up-regulation of IL-10 production.

TGF-β1 genetic variants seem to contribute to immune regulation in the atopy assigned to helminth infections.

Capsule summary

TGF-β1 gene polymorphisms are related with susceptibility to helminth infection as well as protective effect of atopy appearing to have a key role on immune modulation of allergy previously attributed to helminth products only.

Key words: TGF-β1, polymorphisms, helminth infections, allergy, immune regulation.

INTRODUCTION

Asthma is a chronic heterogeneous inflammation characterized by reversible airway obstruction, and represents the final setting of different etiologies and pathways that are associated with complex genetic backgrounds and environment exposure[1]. It is estimated that 300 million people worldwide have asthma, and the prevalence of this disease has increased in recent decades among individuals living in industrialized countries and more recently in developing countries[2, 3, 4].

The temporal trend in the prevalence of allergic disease is explained by the "hygiene hypothesis", originally proposed by Strachan (1989), as consequence of decreased exposures to pathogens (e.g. helminth and bacteria) in the environment during childhood[5]. It may lead to a failure to develop robust immune regulatory mechanisms mediated by regulatory T (Treg) cells that act through the production of Transforming Growth Factor-Beta (TGF-β) and IL-10[6-8], resulting in immune-mediated diseases.

TGF-β is a pleiotropic growth factor produced by various immune cells (epithelial cells, eosinophils, Th2 lymphocytes, macrophages, fibroblasts and platelets) that plays a key role in regulation of the immune response during infections and inflammatory diseases inhibiting the differentiation of immune cells (Th1 lymphocytes, Th2 cells, cytotoxic T cells and B cells) as well as cytokine production (IFN-γ and IL-2)[9 10 11]. Furthermore, TGF-β1 is shown as an important differentiation factor for regulatory T cells exerting powerful and diverse immunosuppressive effects.[12]

In genetic association studies, variations in genes encoding proteins involved in the development and function of Treg cells, such as IL-10 and TGF-β1, were previously associated with atopy and asthma phenotypes[13, 14]. Several studies have associated the single nucleotide polymorphisms (SNPs) in TGF-β1 gene with asthma and atopy, however, this association remains uncertain[15-17] especially in admixture populations such as in Brazil. Additionally, there is no data demonstrating the association between helminth infections and TGF-β1 polymorphisms and considering that exposure to helminth infections in some population may constitute a natural selection for regulatory

genetic background, helminth parasites have been extensively associated to allergic diseases and autoimmune protection[18, 19].

Due the important regulatory role of TGF-β1 on inflammatory diseases and infection to helminths the aim of this study was to assess whether the variation in TGF-β1 gene or in its promoter region are associated with atopy and asthma as well as its role on immunity of host against helminth infections.

METHODS

Study population and design

This study was conducted in the city of Salvador in northeastern Brazil. The general study design has been reported elsewhere[6, 7, 20]. Briefly, the study population included 1335 unrelated children between 4 and 11 years old originally recruited in infancy for a prospective study that analyzed the effect of a citywide sanitation program on childhood morbidity[21].

Data were collected from children born between 1994 and 2001 who lived in sentinel neighborhoods in the city. In 2000, stool samples were collected to characterize intestinal helminth infections. Children were resurveyed in 2005 to collect data on asthma status and to obtain stool and blood samples. Written informed consent was obtained from parents or legal guardian of the children and ethical approval was provided by the Ethical Committee of the Instituto de Saúde Coletiva, Universidade Federal da Bahia and by the Brazilian National Ethical Committee.

Wheezing definition

As previously described [22], children were classified as having current wheeze by using a Portuguese-adapted phase II International Study of Asthma and Allergies in Childhood questionnaire (wheezing in the last 12 months) and were considered to have current wheeze plus symptoms if parents reported wheezing in the previous 12 months and at least one of the following: (1) asthma diagnosis by doctor; (2) wheezing with exercise in the last 12 months; (3) 4 or more episodes of wheezing in the last 12 months; and (4) waking up at night because of wheezing in the last 12 months. We defined atopic wheeze phenotype according to having a positive result (>0.70 kU/L) for at least one sIgE against aeroallergens.

Skin prick tests

Skin prick tests (SPTs) were performed on the right forearms of the children by using standardized extracts (ALK-Abelló, São Paulo, Brazil) of *Dermatophagoides pteronyssinus, Blomia tropicalis, Blatella germanica, Periplaneta americana*, cat and dog epithelia and a fungi mix (*Aspergillus amstelodami, Aspergillus fumigatus, Aspergillus niger, Aspergillus terrus, Penicillium brevicompactum, Penicillium expansum, Penicillium notatum, Penicillium roqueforti, Cladosporium fulvum,* and *Cladosporium herbarum)*. Saline and 10mg/mL histamine solution were used as negative and positive controls, respectively. The reaction was read after 15 minutes. It was considered positive a reaction whose wheal size was at least 3 mm greater than that elicited by the negative control.

Allergens specific IgE

Determination of sIgE serum level was performed for *D. pteronyssinus, B. tropicalis, B. germanica, and P. americana* by using the ImmunoCAP assay (Phadia Diagnostics AB, Uppsala Sweden). Result equal or greater than 0.70 kU/L was considered positive.

Parasitological analysis for intestinal helminth

Stool samples were collected twice and analyzed for *A. lumbricoides* and *T. trichiura* infection at each of the 2 sampling times 2 weeks apart. Stool samples were analyzed by using the Hoffman technique[6] and the Kato-Katz technique [23] to determine the presence and numbers of helminth eggs. For each sample were analyzed two slides. All children with positive results were appropriately treated [20].

Occurrence and chronicity of infections were defined as follows: (1) current infections: infections with *A. lumbricoides*, or *T. trichiura* detected only later in childhood (ie, survey conducted in 2005); (2) chronic infections: children infected with either *A. lumbricoides* or *T. trichiura* in both early (ie, survey conducted in 2000) and later (2005); and (3) coinfection: children infected with both *A. lumbricoides* and *T. trichiura* in 2005.

Total IgE level and markers of infection: anti- *A. lumbricoides* IgE and IgG4 antibodies and anti- *T. canis* IgG

Total IgE level were measured as previously described [6]. Briefly, plate wells were coated with 4 mg/mL of an anti-human IgE antibody (BD PharMingen) overnight at 4ºC, followed to blocking overnight at 4ºC. Samples were diluted 1:10 in diluent solution and incubated overnight at 4ºC. Plates were incubated with biotinylated anti-human IgE (Sigma), followed by streptavidin/peroxidase (BD PharMingen) and H_2O_2/orthophenylenediamine substrate (Merck) and read with a 480-nm filter.

Determination of sIgE serum level was performed for *A. lumbricoides* by using the ImmunoCAP assay (Phadia Diagnostics AB, Uppsala, Sweden). Anti- *A lumbricoides*-sIgE level equal or greater than 0.35 kU/L was considered positive results.

Anti- *A. lumbricoides* IgG4 was detected by using indirect ELISA as previously described [6]. Briefly, plate wells were sensitized with 20 mg/mL of *A. lumbricoides* antigen. Sera were diluted 1:50 in diluent solution. Plates were incubated with biotinylated anti-human IgG4 (Sigma Chemical Co), followed by streptavidin/peroxidase (BD PharMingen) and H_2O_2/orthophenylenediamine substrate (Merck, White House Station, NJ) and read with a 480-nm filter. The assay cutoff for IgG4 for *A. lumbricoides* was determined as the mean plus an standard deviations (SD) of negative controls (sera from children with 3 negative stool samples collected serially). Antibody levels of anti–A lumbricoides IgG4 more than the cutoff were defined as positive.

Anti- *T. canis* IgG antibodies were detected in sera by indirect ELISA assay using excretory-secretory *T. canis* larval antigens as previously described [24]. The cut-off obtained (0.23) was calculated by the OD from the mean of the 14 negative controls (children without history of contact with dogs and cats) plus three SD of this mean. Five previously assayed sera samples were used as positive controls.

Cell culture

Venous blood was collected into heparinized tubes and cultured at a dilution of 1:4 in RPMI (Gibco, Auckland, New Zealand) containing 10 mmol/L glutamine (Sigma-Aldrich, St Louis, Mo) and 100 mg/mL gentamicin (Sigma-Aldrich). The cell cultures were started within 6 hours after the blood collection and were maintained in a humidified environment of 5% CO_2 at 37°C for 24 hours for IL-10 detection in the presence of pokeweed mitogen (PWM; Sigma-Aldrich, St. Louis, MO, USA) (2.5 µg/mL).

IL-10 measurement using ELISA

The IL-10 concentrations were measured in whole-blood culture supernatant stimulated with pokeweed mitogen by sandwich ELISA, according to the manufacturer's instructions (BD PharMingen, San Diego, Calif). Cytokine concentrations were determined by means of interpolation of standard curves. The detection limits (low/high) were 31.25/500 pg/ml.

Genotyping

Four TGF-β1 single nucleotide polymorphisms (SNPs) with prior associations with related phenotypes (rs4803455, rs1800470, rs1800469, rs2241712) were selected for genotyping[15, 16].

DNA was extracted from peripheral blood samples by using commercial standard protocols (Gentra Puregene Blood Kit; Qiagen, Hilden, Germany). SNPs were typed by using the TaqMan probe–based, 59 nuclease assay minor groove binder chemistry [25] on the 7900HT Sequence Detection System (Applied Biosystems, Foster City, Calif). TaqMan-validated assays and master mix were manufactured by Applied Biosystems.

PCR was conducted in a 5μL volume by using a universal master mix and 4 predesigned and validated TaqMan assays for the SNPs (list of SNPs is shown in Table I). The thermal cycling conditions were as follows: 95ºC for 10 minutes, followed by 40 cycles of 95ºC for 15 seconds/60ºC for 1 minute and an extension step of 60ºC for 5 minutes. Nontemplate negative and genotyping-positive controls were included in each genotyping plate. Automatic calling was performed with a quality value of greater than 99%.

Ten percent of the samples were genotyped in duplicate with 100% reproducibility. All 4 SNPs were in Hardy-Weinberg equilibrium. Allele frequencies of the SNPs and SNP localization in the TGF-β1 gene (chromosome 19, approximately 23 Kbp) are

summarized in Table I. The rs1800469 and the rs2241712 are in strong linkage disequilibrium (Figure E1 in the Online Repository).

Statistical analyses

Genotype and haplotype analyses were conducted for genetic associations by using logistic regression (including sex, age, helminth infection as covariates, when appropriated). For genotype analysis was used the additive, dominant and recessive models. In addition, the first 2 principal components delineated through Eigenstrat on 269 ancestry informative markers were included in the model to address the potential effects of population stratification. For continuous data, such as total IgE and IL-10 levels analyses were conducted by using linear regression adjusted by sex, age, helminth infection and principal components 1 and 2 where appropriate. All genetic analyses were performed with PLINK 1.07[26] and the Pairwise LD was created with Haploview[27].

RESULTS

4.1 Description of the study population

Table 2 summarizes the clinical characteristics of the study population. We observed greater proportions of children with atopic and nonatopic wheeze in the younger group (<5 years old). Markers of allergy, such as SPT reactivity (65.73%) and total IgE levels (1.71 kU/mL), were greater in the group with atopic wheeze. IgE anti-*A. lumbricoides* (77.53%) was great in atopic asthmatics subjects.

4.2 Association of TGF-β1 SNPs with asthma, atopy and markers of allergy

The SNP rs1800470 was negatively associated (OR 0.60, p <0.05) with atopic asthma in the recessive model (**Table 3**). The rs1800470 was negatively associated with specific IgE to at least one allergen tested (OR 0.52, p <0.05), with skin test to at least one allergen (OR 0.41, p <0.01) and skin test reactivity to *Blomia tropicalis* (OR 0.39, p <0.05), in the recessive model (**Table 4**).

4.3 Association of TGF-β1 SNPs and total IgE

The analysis between the SNPs and serum total IgE levels showed no genotypic association in any of the models evaluated (data not shown). However, the haplotypes AT (p<0.05), CTT (p< 0.05), TTG (p<0.01) and CTTG (p<0.01) were negatively associated with total IgE serum levels (**Table 5**).

4.4 Association of TGF-β1 SNPs and helminth infections

No association was found considering analysis between single genotype with helminth infections and markers of infection (data no shown). However, evaluating the association of possible haplotypes with helminth infections, significant association was observed (**Table 6**). Specially, the haplotypes AC, ACC and ACCA showed a positive association with *T. canis* infection (OR 1.73, 2.09 and 2.07, respectively) (p <0.001*), T. trichiura* acute infection (OR 1.80, 1.80 and 1.85, respectively) (p <0.01) and *T. trichiura* chronic (OR 2.00, 2.00 and 2.07, respectively) (p <0.05), *A. lumbricoides* chronic infection (OR 1.69, 1.77 and 1.85, respectively) (p <0.05) and co-infection with *T. trichiuras* and *A. lumbricoides* (OR 1.61, 1.63, 1.67, respectively) (p <0.01).

Additionally, the haplotypes TT (OR 2.23, p <0.05), CTT (OR 2.61, p <0.05), TTG (OR 2.5, p <0.05) and CTTG (OR 2.62, p <0.05) were positively associated with *T. trichiura* chronic infection by logistic regression (p <0.05) (Table E1 in the Online Repository).

By logistic regression, CC (OR 1.67, p<0.001), CCC (OR 1.48, p<0.05), CCA (OR 1.57, p<0.001) and CCCA (OR 1.4, p<0.05) haplotypes were positively associated with IgG4 anti-*A. lumbricoides* serum levels (Table E2 in the Online Repository).

4.5. Association of TGF-β1 SNPs with IL-10 levels

No association was found considering analysis between single genotype with IL-10 levels (data no shown). However, evaluating the possible haplotypes with levels of IL-10 production under mitogen stimulation, we found a positive association with the haplotypes AC (Beta 46.8, p<0.001), ACC (Beta 51.1, p< 0.001) and ACCA (Beta 50.7, p<0,001) (**Table 7**).

4.6. Association of TGF-β1 SNPs with atopic asthma in helminth-infected individuals

The rs1800470, in the recessive model, was negatively associated (OR 0.31, p <0.001) with atopic asthma in the *Toxocara canis* infected subjects (**Table 8**). There was no association with atopic asthma in *A. lumbricoides* or *T. trichiura*- infected individuals.

DISCUSSION

Allergy is a complex disease in which there are multiple genetic effects interacting with the environment able to modify its susceptibility and severity. In order to elucidate the impact of the immune regulatory network on allergies and parasitic diseases, we investigated the genetic polymorphism on TGF-β1, an important immune regulatory cytokine. We found that genetic polymorphisms in TGF-β1 is negatively associated with

allergy and its markers and positively associated with helminth infections in a population of children living in Salvador/BA. This observation may contribute to the better understanding of the importance of genetic variability on the modulation of allergic processes by helminth infections.

Of the four SNPs evaluated in this study, the C allele of rs1800470 (T869C) showed a negative association in the recessive model with atopic asthma, serum sIgE to allergens and skin test reactivity to allergens, including house dust mite *B. tropicalis*. The biological consequence of such polymorphism is the change of nucleotide triplets and subsequent exchange of amino acid from leucine to proline in the cytokine molecule, being potentially related to structural alteration of TGF-β1. The rs1800470 also has been reported to be associated with the serum level of the gene product, being the CC genotype associated with higher TGF-β1 concentration than other genotypes [28].

In our study, the rs1800470 seems to correlate with increased immune modulatory activity of TGF-β1 on the allergic disease. The association of TGF-β1 with allergy has been explored by several experimental studies. Intratracheal delivery of TGF-β1 suppressed allergen induced inflammation [8]. In contrast, blocking transforming growth factor beta/Smad signaling in T cells enhances antigen-induced airway inflammation, airway reactivity and increased Th2 cytokine production [29]. Moreover, reduced expression of TGF-β1 exacerbates pathology in an experimental asthma model related with increased eosinophilic inflammation and increased levels of specific IgE in serum [30].

We found no association with wheeze in the analysis using helminth exposed and non-exposed individuals. However, previous studies have shown that the C allele of rs1800470 is positively associated with increased risk of asthma in the Chinese and Mexican populations [17, 31] and the T allele is associated with a reduced risk of asthma hospitalizations in non-Hispanic white children [32]. The patients with severe asthma have higher TGF-β1 levels than patients with intermittent, mild or moderate asthma which may be correlated with its fibrogenic activity [33 34].

The IgE antiboby is an important mediator involved in the allergic process as well as in the immune response against helminth. We found four haplotypes in TGF-β1 gene negatively associated with total IgE serum levels. Previous study has found association between TGF-β1 SNPs and total IgE levels [35], however it remain controvertial.[34] .

Although no association was found between single genotypes in TGF-β1 gene and helminth infections, analysis of possible haplotypes as a mean of simultaneous SNPs occurring together, especially the haplotypes formed by the C allele of rs1800470 with the others SNPs, were positively associated with helminth infections, indicating that the presence of such polymorphisms may contribute to susceptibility to parasite infections. This study was the first to describe the association of polymorphism in the TGF-β1 gene and infection by *Toxocara* spp.*, T. trichiura* and *A. lumbricoides.* A study with children infected with helminth presented evidences for the increased production of TGF-β1 by unstimulated peripheral blood leukocytes, being positively associated with burden of infection and negatively associated with immune reactivity, determined by IL- 4 and IFN- γ production and cell proliferation in response to antigenic stimuli[36].

Exposure to pathogens and their products, in particular helminth, appear to protect against the development of allergic diseases[37, 38, 39]. The intense immune regulatory role played by TGF-β1 induced in infected individuals may explain the protection against the development of immune-mediated diseases such as was discussed by the “hygiene hypothesis” [5, 36, 40]. However, the genetic variability in immune regulatory genes can represent important role on this relationship. In fact, we found that in individuals infected with *Toxocara* spp. the CC genotype of rs1800470 is associated with lower risk of atopic asthma. Thus, we demonstrated that not only the infection can modulate the immunologic response on allergic response, but also the genetic variability contributes to the immune regulation and protection from atopy.

Our group previously demonstrated in the same population that children chronically infected with helminth produce higher levels of immune regulatory cytokine IL-10[6]. Also in the SCAALA population, our group demonstrated that the relationship between allergies and IL-10 levels are determined not only by environmental factors, but also as

a result of polymorphisms in the IL-10 gene that are positively associated with allergy and negatively associated with helminth infections[14]. In this study, we found that the same haplotypes associated with helminth infections, were negatively associated with atopy and positively associated with IL-10 levels. Thus, not only polymorphisms in the IL-10 gene but also in TGF-β1 are involved in susceptibility to infection and potential modulation of allergy. The TGF-β1 is the main regulator of the immune response acting by inhibiting the proliferation of Th cells and cytokine production as well as by inducing the differentiation and development of Foxp3+ regulatory T cells that are responsible for the IL-10 production [41 42]. Previous study has shown that rs1800470 is associated with higher TGF-β1 levels [28], although we were unable to reproduce this finding due to technical limitations. Therefore, TGF-β1 polymorphisms seem to contribute indirectly to the regulation of IL-10 levels through the increase of TGF-β1 production.

In conclusion, individuals with genetic polymorphisms in TGF-β1 gene have a lower risk of developing allergy and increased susceptibility to helminth infections. Additionally, we have shown that immune modulation of allergy is a complex response resulting not only from the environmental factors but also by genetic polymorphisms, especially upon IL-10 production. Future works are needed to further elucidate the potential role of TGF-β1 on asthma and whether it could be a strategy to control this disease and other allergies.

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REFERENCES

1. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. Immunol Rev 2011; 242:10-30.
2. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health 2012; 12:204.

3. Pearce N, Aït-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax 2007; 62:758-66.
4. Cooper PJ, Rodrigues LC, Cruz AA, Barreto ML. Asthma in Latin America: a public heath challenge and research opportunity. Allergy 2009; 64:5-17.
5. Strachan DP. Hay fever, hygiene, and household size. BMJ 1989; 299: 1259-60
6. Figueiredo CA, Barreto ML, Rodrigues LC, Cooper PJ, Silva NB, Amorim LD, et al. Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. Infect Immun 2010; 78:3160-7.
7. Figueiredo CA, Alcantara-Neves NM, Amorim LD, Silva NB, Carvalho LC, Cooper PJ, et al. Evidence for a modulatory effect of IL-10 on both Th1 and Th2 cytokine production: the role of the environment. Clin Immunol 2011; 139:57-64.
8. Joetham A, Takeda K, Takada K, Taube C, Miyahara N, Matsubara S, et al. Naturally occurring lung CD4(+)CD25(+) T cell regulation of airway allergic responses depends on IL-10 induction of TGF-beta. J Immunol 2007; 178:1433-42.
9. Yang YC, Zhang N, Van Crombruggen K, Hu GH, Hong SL, Bachert C. Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. Allergy 2012; 67:1193-202.
10. Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by TGF-beta. J Biochem 2010; 147:781-92.
11. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 2006; 24:99-146.
12. Li MO, Sanjabi S, Flavell RA. Transforming growth factor-beta controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and -independent mechanisms. Immunity 2006; 25:455-71.
13. Bottema RW, Kerkhof M, Reijmerink NE, Thijs C, Smit HA, van Schayck CP, et al. Gene-gene interaction in regulatory T-cell function in atopy and asthma development in childhood. J Allergy Clin Immunol 2010; 126:338-46, 46.e1-10.
14. Figueiredo CA, Barreto ML, Alcantara-Neves NM, Rodrigues LC, Cooper PJ, Cruz AA, et al. Coassociations between IL10 polymorphisms, IL-10 production, helminth infection, and asthma/wheeze in an urban tropical population in Brazil. J Allergy Clin Immunol 2013; 131:1683-90.
15. Wu H, Romieu I, Shi M, Hancock DB, Li H, Sienra-Monge JJ, et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. J Allergy Clin Immunol 2010; 125:321-7.e13.
16. Yang XX, Li FX, Wu YS, Wu D, Tan JY, Li M. Association of TGF-beta1, IL-4 and IL-13 gene polymerphisms with asthma in a Chinese population. Asian Pac J Allergy Immunol 2011; 29:273-7.
17. Li H, Romieu I, Wu H, Sienra-Monge JJ, Ramírez-Aguilar M, del Río-Navarro BE, et al. Genetic polymorphisms in transforming growth factor beta-1 (TGFB1) and childhood asthma and atopy. Hum Genet 2007; 121:529-38.
18. Amoah AS, Boakye DA, van Ree R, Yazdanbakhsh M. Parasitic worms and allergies in childhood: Insights from population studies 2008-2013. Pediatr Allergy Immunol 2013.
19. El-Malky M, Nabih N, Heder M, Saudy N, El-Mahdy M. Helminth infections: therapeutic potential in autoimmune disorders. Parasite Immunol 2011; 33:589-93.
20. Barreto ML, Cunha SS, Alcântara-Neves N, Carvalho LP, Cruz AA, Stein RT, et al. Risk factors and immunological pathways for asthma and other allergic diseases in children: background and methodology of a longitudinal study in a large urban center in Northeastern Brazil (Salvador-SCAALA study). BMC Pulm Med 2006; 6:15.

21. Barreto ML, Genser B, Strina A, Teixeira MG, Assis AM, Rego RF, et al. Impact of a citywide sanitation program in Northeast Brazil on intestinal parasites infection in young children. Environ Health Perspect 2010; 118:1637-42.
22. Alcantara-Neves NM, Veiga RV, Dattoli VC, Fiaccone RL, Esquivel R, Cruz Á, et al. The effect of single and multiple infections on atopy and wheezing in children. J Allergy Clin Immunol 2012; 129:359-67, 67.e1-3.
23. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo 1972; 14:397-400.
24. Mendonça LR, Figueiredo CA, Esquivel R, Fiaccone RL, Pontes-de-Carvalho L, Cooper P, et al. Seroprevalence and risk factors for Toxocara infection in children from an urban large setting in Northeast Brazil. Acta Trop 2013; 128:90-5.
25. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 1999; 14:143-9.
26. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559-75.
27. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263-5.
28. Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, et al. Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. J Bone Miner Res 1998; 13:1569-76.
29. Nakao A, Miike S, Hatano M, Okumura K, Tokuhisa T, Ra C, et al. Blockade of transforming growth factor beta/Smad signaling in T cells by overexpression of Smad7 enhances antigen-induced airway inflammation and airway reactivity. J Exp Med 2000; 192:151-8.
30. Scherf W, Burdach S, Hansen G. Reduced expression of transforming growth factor beta 1 exacerbates pathology in an experimental asthma model. Eur J Immunol 2005; 35:198-206.
31. Mak JC, Leung HC, Ho SP, Law BK, Ho AS, Lam WK, et al. Analysis of TGF-beta(1) gene polymorphisms in Hong Kong Chinese patients with asthma. J Allergy Clin Immunol 2006; 117:92-6.
32. Sharma S, Raby BA, Hunninghake GM, Soto-Quirós M, Avila L, Murphy AJ, et al. Variants in TGFB1, dust mite exposure, and disease severity in children with asthma. Am J Respir Crit Care Med 2009; 179:356-62.
33. Yang ZC, Yi MJ, Ran N, Wang C, Fu P, Feng XY, et al. Transforming growth factor-β1 induces bronchial epithelial cells to mesenchymal transition by activating the Snail pathway and promotes airway remodeling in asthma. Mol Med Rep 2013; 8:1663-8.
34. Acevedo N, Vergara C, Gusmão L, Jiménez S, Martínez B, Mercado D, et al. The C-509T promoter polymorphism of the transforming growth factor beta-1 gene is associated with levels of total and specific IgE in a Colombian population. Int Arch Allergy Immunol 2010; 151:237-46.
35. Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L. Interleukin-10 and Transforming Growth Factor-b. Am J Respir Crit Care Med 1998; 158: 1958-1962.
36. Turner JD, Jackson JA, Faulkner H, Behnke J, Else KJ, Kamgno J, et al. Intensity of intestinal infection with multiple worm species is related to regulatory cytokine output and immune hyporesponsiveness. J Infect Dis 2008; 197:1204-12.
37. Cooper PJ. Interactions between helminth parasites and allergy. Curr Opin Allergy Clin Immunol 2009; 9:29-37.

38. Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections protect against allergic diseases by active regulatory processes. Curr Allergy Asthma Rep 2010; 10:3-12.
39. Rodrigues LC, Newcombe PJ, Cunha SS, Alcantara-Neves NM, Genser B, Cruz AA, et al. Early infection with Trichuris trichiura and allergen skin test reactivity in later childhood. Clin Exp Allergy 2008; 38:1769-77.
40. Moreau E, Chauvin A. Immunity against helminths: interactions with the host and the intercurrent infections. J Biomed Biotechnol 2010; 2010:428593.
41. Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. J Immunol 2001; 166:7282-9.
42. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity 2008; 28:546-58.

Table 1. Description of SNPs analyzed in this study, including allelic frequency and Hardy-Weinberg equilibrium data.

SNP	Base pairs	Allele	MAF	HWE	Function	Regulome DB Score
rs4803455	41855515	A/C	0.49	0.53	intron	7
rs1800470	41858921	C/T	0.47	0.57	missense	4
rs1800469	41860296	T/C	0.33	0.79	near-gene-5	2b
rs2241712	41869756	G/A	0.28	1	intron	2b

Table 2. Characteristics of the Social Changes in Asthma and Allergy in Latin American population according to asthma status and variables included in this study

	Nonasthmatic (n=962)	Nonatopic Asthmatic (n=212)	Atopic Asthmatic (n=178)	P value
Age				
≤5 y	290 (30.40%)	108 (50.94%)	75 (42.13%)	
6-7 y	351 (36.79%)	67 (31.60%)	59 (33.15%)	
≥8 y	313 (32.81%)	37 (17.45%)	44 (24.72%)	**<0.0001**
Sex				
Male	517 (53.74%)	101 (47.64%)	107 (60.11%)	
Female	445 (46.26%)	111 (52.36%)	71 (39.89%)	**0.048**
Skin prink test response ≥ 1 allergen (>3mm)	268 (27.86%)	21 (9.91%)	117 (65.73%)	**<0.0001**
Skin prink test to *B. Tropicalis* (>3mm)	192 (19.96%)	10 (4.72%)	90 (50.56%)	**<0.0001**
Specific IgE for ≥ 1 allergen (> 0.70KU/L)	331 (34.41%)	0 (0.00%)	178 (100.00%)	**<0.0001**
Total IgE (KU/L) mean ± SD	0.80 ± 5.46	0.28 ± 0.60	1.71 ± 4.59	**0.0155**
***Toxocara spp.* current infection**	443 (46.05%)	101 (47.64%)	88 (49.44%)	0.888
***T. trichiura* current infection**	124 (12.89%)	39 (18.40%)	21 (11.80%)	0.193
***T. trichiura* chronic infection**	48 (4.99%)	15 (7.08%)	8 (4.49%)	0.095
***A. lumbricoides* chronic infection**	50 (5.20%)	17 (8.02%)	12 (6.74%)	0.203
IgG4 anti-Asc	145 (15.07%)	36 (16.98%)	38 (21.35%)	0.233
IgE anti-Asc	462 (48.02%)	80 (37.74%)	138 (77.53%)	**<0.0001**
Coinfection (*A. lumbricoides* + *T. trichiura*)	210 (21.83%)	62 (29.25%)	39 (21.91%)	0.167

Table 3. Association between the TGF-β1 SNPs and atopic wheezing by logistic regression adjusted for age, sex, helminth infections and principal components 1 and 2**.**

Marker	Model	OR	95% CI	P Value
Atopic wheezing				
rs1800470	Recessive	0.60	0.37-0.95	**0.030**

Table 4. Association between TGF-β1 SNPs and specific IgE and skin tests in the group of asthmatic subjects by logistic regression adjusted for age, sex, helminth infections and major components 1 and 2.

Marker	Model	OR	95% CI	p Value
Specific IgE for at least one aeroallergen (>0.70kU/L)				
rs1800470	Recessive	0.52	(0.29-0.91)	**0.02171**
SPT response for at least one specific aeroallergen (≥3mm)				
rs1800470	Recessive	0.41	(0.22 - 0.79)	**0.006929**
SPT response to *B. tropicalis* (≥3mm)				
rs1800470	Recessive	0.39	(0.19-0.81)	**0.01183**
Specific IgE to *B tropicalis*				
rs1800470	Recessive	0.57	(0.32-1)	0.05

Table 5. Association between the TGF-β1 SNPs and total IgE in total case-control subjects by linear regression adjusted by age, sex, helminth infections and principal components 1 and 2.

# SNP	SNP1	SNP2	Haplotype	Freq.	Beta	p Value
2	rs1800470	rs1800469	TT	0.0343	-0.156	**0.0137**
3	rs4803455	rs1800469	CTT	0.0281	-0.162	**0.0243**
3	rs1800470	rs2241712	TTG	0.029	-0.211	**0.00284**
4	rs4803455	rs2241712	CTTG	0.0271	-0.197	**0.00614**

Table 6. Association between haplotypes of TGF-β1 SNPs and Infections with helminth in total case-control subjects by additive logistic regression model adjusted for age, sex and principal components 1 and 2.

Trait	AC			ACC			ACCA		
	Freq	**OR**	**p Value**	**Freq**	**OR**	**p Value**	**Freq**	**OR**	**p Value**
***Toxocara* spp present or past infection**	0.8	1.73	**0.00078**	0.08	2.09	**0.00014**	0.08	2.07	**0.00017**
***Tricuris trichiura* current infection**	0.12	1.80	**0.00172**	0.09	1.80	**0.0052**	0.09	1.85	**0.00349**
***Tricuris trichiura* chronic infection**	0.13	2.00	**0.0124**	0.11	2.00	**0.0185**	0.11	2.03	**0.0159**
***A. lumbricoides* chronic infection**	0.13	1.69	**0.0471**	0.10	1.77	**0.0435**	0.11	1.85	**0.0273**
Anti-*A. lumbricoides* IgE	0.09	1.49	**0.013**	0.07	1.58	**0.0134**	0.07	1.61	**0.0109**
Anti-*A. lumbricoides* IgG4	0.12	1.80	**0.00081**	0.09	1.92	**0.00087**	0.10	2.01	**0.00033**
Coinfection (*A. lumbricoides* + *T. trichiura*)	0.11	1.61	**0.00434**	0.08	1.63	**0.0104**	0.08	1.67	**0.00679**

Table 7. Association between haplotypes of TGF-β1 SNPs and levels of IL-10 production under mitogen stimulation in total of cases-controls subjects by linear regression adjusted for age, sex, principal components 1 and 2 and helminth infections.

# SNP	SNP1	SNP2	Haplotype	Freq.	Beta	P
2	rs4803455	rs1800470	AC	0.0812	46.8	**0.000413**
3	rs4803455	rs1800469	ACC	0.0594	51.1	**0.000575**
4	rs4803455	rs2241712	ACCA	0.0592	50.7	**0.000632**

Table 8. Association between the TGF-β1 SNPs and atopic asthma in *Toxocara spp.* infected subjects by logistic regression adjusted for age, sex, helminth infections and principal components 1 and 2**.**

Marker	Model	OR	95% CI	P value
Wheezing atopic				
rs1800470	Recessive	0.31	(0.14-0.72)	**0.005894**

Online Repository

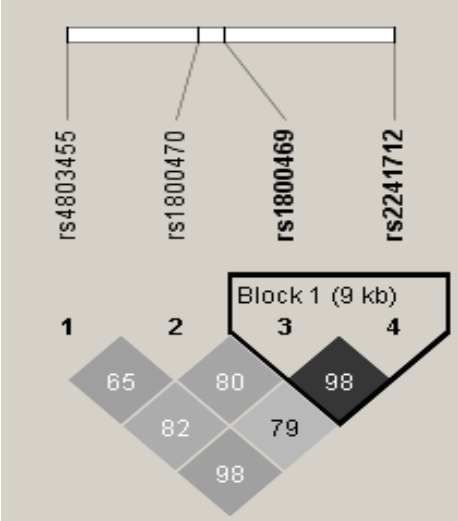

Figure E1. Pairwise LD within Haploview by using the R` squared statistic for the TGF-β1 gene. Intensity of shading indicates the degree of confidence in the R` value.

Table E1. Association between haplotypes of TGF-β1 SNPs and chronic infection *Trichuris trichiura i*n total cases and controls subjects by logistic regression adjusted for age, sex and principal components 1 and 2.

2	rs1800470	rs1800469	TT	0.0343	2.23	**0.0138**
3	rs4803455	rs1800469	CTT	0.0281	2.61	**0.0111**
3	rs1800470	rs2241712	TTG	0.029	2.5	**0.0209**
4	rs4803455	rs2241712	CTTG	0.0271	2.62	**0.016**

Table E2. Association between haplotypes of TGF-β1 SNPs and levels of IgG4 anti-*Ascaris lumbricoide* in total of cases-controls subjects by logistic regression adjusted for age, sex and principal components 1 and 2

# SNP	SNP1	SNP2	Haplotype	Freq.	OR	p Value
Anti Asc- IgG4						
2	rs1800470	rs1800469	CC	0.178	1.67	**$9.3x10^{-5}$**
3	rs4803455	rs1800469	CCC	0.116	1.48	**0.0147**
3	rs1800470	rs2241712	CCA	0.18	1.57	**0.000405**
4	rs4803455	rs2241712	CCCA	0.12	1.4	**0.0326**

Manuscrito 3. Evaluation of TGF-β1 polymorphisms in a candidate gene association study for asthma in four African-ancestry populations

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Evaluation of TGF-β1 polymorphisms in a candidate gene association study for asthma in four African-ancestry populations

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Abstract

Background: Asthma is a complex disease characterized by ethnic disparities not explained completely by environmental factors. TGF-β1 is a multifunctional cytokine that plays a controversial key role in asthma. Genetic association studies have shown the relationship of TGF-β1 polymorphisms with asthma, however these associations are controversial.

Objective: To evaluate the association of TGF-β1 gene polymorphisms with asthma in four independent populations of African-ancestry.

Methods: We performed a candidate gene association study in four independent populations of African ancestry (Honduras, African Caribbean, African American and South American) to indent single nucleotide polymorphisms (SNPs) associated with asthma. Were analyzed 501 SNPs in TGF-β1 gene and its promoter region.

Results: There were seven TGF-β1 SNPs positively associated with asthma in Honduras population (rs11083616, rs11083617, rs1982072, rs11668109, rs10416269, rs4803455, rs10406816.) In the African Caribbean population we found three TGF-β1 SNPs with statistically significant differences between the asthma group and the control group (rs11466345, rs11466340, rs11466339). There is no polymorphism significantly associated with asthma in African American neither South American populations.

Conclusion: This candidate gene association study suggests that polymorphisms in TGF-β1 may contribute to asthma susceptibility in populations of African descendants. However, it was found different results in each population. Thus, the TGF-β1 polymorphisms seem to contribute to asthma process depending on the genetic background of the population and environmental factors.

Keywords: Asthma, TGF-β1, polymorphisms, African ancestry, genetic association

INTRODUCTION

Asthma is a disease characterized by inflammation and remodeling of the airways, leading to recurrent episodes of wheezing, breathlessness, chest tightness and cough. The prevalence of asthma has increased and is disproportional among ethnic groups (1). However it cannot be explained only by environmental factor only (2).

Like other complex diseases, asthma is influenced by both genetic and environmental factors, being suggested that approximately 60% of asthma susceptibility is attributed to genetic variants (3). Genome-wide association studies (GWAS) have revealed a large number of asthma- related genes with susceptibility in different populations (2, 4). Additionally, several polymorphisms have been identified by candidate gene association studies that focus on genes plausibly involved in disease pathogenesis or located in a region of linkage for the disease (5).

TGF-β1 is a multifunctional cytokine that plays a controversial key role in asthma, been important in the immune modulation of inflammation, inhibiting Th1 and Th2 cell responses, and promoting the differentiation of Treg cells (6, 7). In contrast, increased levels of TGF-β1 in bronchoalveolar lavage (BAL) fluid from asthmatic patients have been observed (8) which may be correlated with fibrosis induction and bronchial remodeling (9, 10). TGF-β1 is widely replicated as asthma candidate gene, and SNPs in this gene have been associated with asthma and allergy markers in some previous studies (11, 5).

In this candidate gene study, we evaluated the association of TGF-β1 gene polymorphisms with asthma in four independent populations of African-ancestry: Honduras, African Caribbean, African American and South American.

MATERIAL AND METHODS

Subjects

The data for this study were obtained from Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA) that include high coverage whole genome sequence (WGS) data on 1,075 subjects of African ancestry. Here, these individuals were classified as African Caribbean, African American, South American and Honduras.

African Caribbean

Two groups of African Caribbean were included in this study (**Table 1**). We included a group of 39 Barbadian nonrelated individuals (22 cases and 17 nonasthmatic controls) from a study on the genetics of asthma as previously described (12, 2, 13) and a second group, comprising 45 unrelated individuals (23 asthmatics, 22 nonasthmatic controls) was recruited from Kingston, St. Andrew, and St. Catherine, Jamaica as part of the Jamaican Adolescent Asthma Study as previously described (14, 15, 13). All subjects provided informed written consent to participate as approved by the University of the West Indies, Mona campus.

African Americans

Two groups of self-reported African Americans were included in this study (**Table 1**). A group of 47 individuals (24 asthma and 23 nonasthmatic controls) ascertained participating in *Genomic Research on Asthma in the African Diaspora* (GRAAD) recruited in the Baltimore-Washington D.C. metropolitan area and participants in the *Baltimore Asthma Severity Study* (BASS) residents of Baltimore City and a second group comprising 39 individuals (18 asthmatic cases and 21 nonasthmatic controls) were recruited in New York and participated in *The Reducing Emergency Asthma Care in Harlem* (REACH) study as previously described elsewhere (16, 17, 18, 13). The

study protocols, recruitment procedures, and consent forms were approved by the Institutional Review Board of Columbia University, Johns Hopkins University, and Howard University.

South American

Three groups of South American were included in this study (**Table 1**). These groups were put together based in its geographical proximity and genetic similarity (13). A group of 48 unrelated individuals (23 asthmatics, 25 nonasthmatic controls) was included from Salvador in northeastern Brazil who were recruited in infancy for the Social Changes, Asthma and Allergy in Latin America (SCAALA) study as previously described (19, 20). Ethical approval for the study was obtained from the Brazilian National Ethical Committee, and written informed consent was obtained from the legal guardian of each child. A second group comprising 33 unrelated individuals (6 asthmatic and 27 nonasthmatic controls) were recruited in five rural communities (Buri, Camarao, Genipapo, Sempre Viva, and Cobo) in the district of Conde, Bahia, located in the North East Coast of Brazil, as described in detail elsewhere (21, 13, 22). Written consent was obtained from adult individuals or parent or guardian of children as approved by the Johns Hopkins Bayview Medical Center and Universidade Federal da Bahia. And a sample of 31 unrelated individuals (13 asthmatics and 18 nonasthmatic controls) was recruited from the Caribbean coastal city of Cartagena as previously described in detail elsewhere (13, 22, 23). All subjects or their guardian/responsible adult gave written consent for their inclusion in the present study as approved by the Bioethics Committee of the School of Medicine of The University of Cartagena.

Honduras

A group of 41 unrelated individuals (23 asthmatics, 18 nonasthmatic controls) was included from Honduras (**Table 1**). The study protocols, recruitment procedures, and consent forms were previously approved by appropriated Ethical Committee.

Genotyping

Blood samples were collected from all subjects and genomic DNA was extracted using standard protocols. The SNPs were identified by Whole Genome Sequecing (WGS) using Illumina platform. The polymorphisms are located between 41,836,811 and 41,869,756 bp on chromosome 19, relative to TGF-β1 gene and its promoter region, totaling 501 SNP's involved in distinct genetic functions (**Table 2**). The table with all SNPs studied is available in supplementary materials and methods.

Statistical Methods

Quality Control

Hardy–Weinberg equilibrium (HWE) was tested for all the SNPs using PLINK ver. 1.07 (24). For the candidate gene association analysis, more stringent SNP exclusion thresholds were used: Hardy Weinberg equilibrium p-value less than 1×10^{-4}, and missing locus more than 1%. In the African American population, of the 501 SNPs 498 passed in quality control and were analyzed in complete case-control study. For the other populations (African Caribbean, Honduras, South American), only 2 SNP's were missed.

Tests for association

Association analyses were conducted using PLINK. 1.07 (24). Each population was analyzed separately with adjustment for principal component (PC) 1 and 2 under an additive model using multivariate logistic regression for SNP with minor allele frequency (MAF) more than 10%. For SNP with MAF less than 10% the association analysis was made by Fisher's Test. The asymptotic P values for this test were observed while the odds ratio (OR) were estimated. The plot with P value and MAF to all SNPs was made by using R program. Linkage disequilibrium (LD) structure was constructed using Haploview 4.2 (25).

RESULTS

Association between TGF-β1 polymorphism and asthma in Honduras population

All genotype frequencies were consistent with Hardy-Weinberg equilibrium (P>0.05). The **figure 2** show the plot with MAF and p Value of all SNPs analyzed. There were seven TGF-β1 SNPs positively associated with asthma in genotype distribution (**Table 3**). These are rs11083616 (OR 3.27, p=0.030), rs11083617 (OR 3.27, p=0.030), rs1982072 (OR 4.21, p=0.031), rs11668109 (OR3.88, p=0.032), rs10416269 (OR 4.74, p=0.042), rs4803455 (OR 3.00, p=0.045), rs10406816 (OR 3.00, p=0.045). There are two blocks in LD (**Figure 1A**). The first one is formed by rs4803455 and rs10406816. The second one is formed by rs1982072, rs11083616 and rs11083617.

Association between TGF-β1 polymorphism and asthma in African Caribbean population

All genotype frequencies were consistent with Hardy-Weinberg equilibrium (P>0.05). The figure 2 shows the plot with MAF and p Value of all SNPs analyzed. As shown

in **Table 4**, there were three TGF-β1 SNPs identified with statistically significant differences between the asthma group and the control group in genotype distribution. These are rs11466345 (OR 0.22, p=0.003), rs11466340 (p=0.015), rs11466339 (OR 7.51, p=0.038). The rs11466340 and rs11466339 are in LD (**Figure 1B**).

Association between TGF-β1 polymorphism and asthma in African American and South American populations

All genotype frequencies were consistent with Hardy-Weinberg equilibrium (P>0.05). The figure 4 and 5 show the plot with MAF and p Value of all SNPs analyzed. No SNP was significantly associated with asthma in these populations.

DISCUSSION

TGF-β1 is a cytokine involved in very early events of respiratory diseases as well as late persistent diseases being implicated in a variety of biological processes that may influence asthma by acting as an anti-inflammatory cytokine suppressing allergic inflammation and hyper-reactivity (26). However, during ongoing inflammation of the lungs, TGF-β1 can induce fibrosis and airway remodeling as seen in chronic disease (27). Therefore, TGF-β1 is a promising candidate gene to contribute to asthma.

In this study, the TGF-β1 gene segment was genotyped in asthmatics and healthy controls covering 501 SNPs in four populations of African descendents. Our results show the rs11083616, rs11083617, rs1982072, rs11668109, rs10416269, rs4803455 and rs10406816 to be positively associated with asthma in Honduras population. Three different SNPs (rs11466345, rs11466340 and rs11466339) were associated with asthma in African Caribbean population. It was found no association of polymorphism in TGF-β1gene with asthma in African American and South American populations.

The C allele of the rs4803455 was the minor allele and is positively associated with asthma. In a genome-wide association study on Mexican children the A allele had minor frequency and was negatively associated with asthma (11). The rs4803455 is in LD with the rs1982072 in Honduras population. The other block in this population is formed by rs11083616, rs11083617 and rs1982072. There is no previous study showing the association of these polymorphisms with asthma. According with Regulome BD database (28) the rs1982072 is likely to affect binding of transcription factor and linked to expression of TGF-β1 (1f score) being potentially associated with TGF-β1 level and therefore an important SNP related with this disease.

The seven SNPs associated with asthma in Honduras population were not associated in the African Caribbean population also analyzed in this study. However the rs11466339, other polymorphism present on intron region of TGF-β1 was positively associated with asthma in this population and is in LD with rs11466340. In contrast, the rs11466345 was negatively associated with asthma in the same population representing a protective factor. It is the first report of the association of these SNPs with asthma in the literature.

Candidate gene association studies are strategies that use SNPs in/or around genes potentially related with the trait of interest to detect their role in disease susceptibility, contributing to reveal anonymous genetic determinants of complex diseases, such as asthma (29). TGF-β1 has been associated with asthma in candidate gene and genome-wide association studies and SNPs in TGF-β1 have been replicated in several asthma studies (11, 30, 5). Here we reported six new SNPs associated with asthma in African Caribbean or Honduras populations.

The analysis of the TGF-β1 SNPs in African American and South American populations revealed no association with asthma. Thus, we found different results in each population in this study. The allelic frequency and the sample size were varied in the different populations which may have contributed to such discrepancies in the associations. Also the sample size may to represent a limitation of this study. Besides, as already noted earlier, not only the genetic factor is responsible for the asthma. Also environmental

exposure is involved in the asthmatic response contributing to evidence the genetic predisposition.

In conclusion, the TGF-β1 gene polymorphisms are associated with asthma in African-ancestry populations. However, the association profile is distinct among the studied populations highlighting the contribution of other genes involved in the asthmatic response as well as environmental factors that will raise its modulation. These findings may serve as a resource for replication in other populations contributing to a wide description of the genetic background of asthmatic patients. Future functional study on TGF-β1 gene may help to better elucidate the potential role of these genetic variants on the asthma.

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REFERENCES

1. Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, et al. National surveillance for asthma--United States, 1980-2004. MMWR Surveill Summ. 2007;56(8):1-54.
2. Mathias RA, Grant AV, Rafaels N, Hand T, Gao L, Vergara C, et al. A genome-wide association study on African-ancestry populations for asthma. J Allergy Clin Immunol. 2010;125(2):336-46.e4.
3. Los H, Postmus PE, Boomsma DI. Asthma genetics and intermediate phenotypes: a review from twin studies. Twin Res. 2001;4(2):81-93.
4. Galanter JM, Gignoux CR, Torgerson DG, Roth LA, Eng C, Oh SS, et al. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: The Genes-environments & Admixture in Latino Americans study. J Allergy Clin Immunol. 2014.
5. Vercelli D. Discovering susceptibility genes for asthma and allergy. Nat Rev Immunol. 2008;8(3):169-82.
6. Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and its role in asthma. Pulm Pharmacol Ther. 2003;16(4):181-96.

7. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. Immunity. 2009;31(3):438-49.
8. Howell JE, McAnulty RJ. TGF-beta: its role in asthma and therapeutic potential. Curr Drug Targets. 2006;7(5):547-65.
9. Lee CG, Cho SJ, Kang MJ, Chapoval SP, Lee PJ, Noble PW, et al. Early growth response gene 1-mediated apoptosis is essential for transforming growth factor beta1-induced pulmonary fibrosis. J Exp Med. 2004;200(3):377-89.
10. McMillan SJ, Xanthou G, Lloyd CM. Manipulation of allergen-induced airway remodeling by treatment with anti-TGF-beta antibody: effect on the Smad signaling pathway. J Immunol. 2005;174(9):5774-80.
11. Wu H, Romieu I, Shi M, Hancock DB, Li H, Sienra-Monge JJ, et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. J Allergy Clin Immunol. 2010;125(2):321-7.e13.
12. Barnes KC, Neely JD, Duffy DL, Freidhoff LR, Breazeale DR, Schou C, et al. Linkage of asthma and total serum IgE concentration to markers on chromosome 12q: evidence from Afro-Caribbean and Caucasian populations. Genomics. 1996;37(1):41-50.
13. Vergara C, Murray T, Rafaels N, Lewis R, Campbell M, Foster C, et al. African ancestry is a risk factor for asthma and high total IgE levels in African admixed populations. Genet Epidemiol. 2013;37(4):393-401.
14. Ashley D, McCaw-Binns A. Informing Maternal and Child Health (MCH) policy through research. West Indian Med J. 1998;47 Suppl 4:16-9.
15. Tulloch-Reid MK, Ferguson TS, Younger NO, Van den Broeck J, Boyne MS, Knight-Madden JM, et al. Appropriate waist circumference cut points for identifying insulin resistance in black youth: a cross sectional analysis of the 1986 Jamaica birth cohort. Diabetol Metab Syndr. 2010;2:68.
16. Ford JG, Meyer IH, Sternfels P, Findley SE, McLean DE, Fagan JK, et al. Patterns and predictors of asthma-related emergency department use in Harlem. Chest. 2001;120(4):1129-35.
17. Murray T, Beaty TH, Mathias RA, Rafaels N, Grant AV, Faruque MU, et al. African and non-African admixture components in African Americans and an African Caribbean population. Genet Epidemiol. 2010;34(6):561-8.
18. Pesola GR, Xu F, Ahsan H, Sternfels P, Meyer IH, Ford JG. Predicting asthma morbidity in Harlem emergency department patients. Acad Emerg Med. 2004;11(9):944-50.
19. Figueiredo CA, Barreto ML, Rodrigues LC, Cooper PJ, Silva NB, Amorim LD, et al. Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. Infect Immun. 2010;78(7):3160-7.
20. Figueiredo CA, Alcantara-Neves NM, Amorim LD, Silva NB, Carvalho LC, Cooper PJ, et al. Evidence for a modulatory effect of IL-10 on both Th1 and Th2 cytokine production: the role of the environment. Clin Immunol. 2011;139(1):57-64.
21. Grant AV, Araujo MI, Ponte EV, Oliveira RR, Cruz AA, Barnes KC, et al. Polymorphisms in IL10 are associated with total Immunoglobulin E levels and Schistosoma mansoni infection intensity in a Brazilian population. Genes Immun. 2011;12(1):46-50.

22. Vergara C, Tsai YJ, Grant AV, Rafaels N, Gao L, Hand T, et al. Gene encoding Duffy antigen/receptor for chemokines is associated with asthma and IgE in three populations. Am J Respir Crit Care Med. 2008;178(10):1017-22.
23. Vergara C, Jiménez S, Acevedo N, Martínez B, Mercado D, Gusmão L, et al. Association of G-protein-coupled receptor 154 with asthma and total IgE in a population of the Caribbean coast of Colombia. Clin Exp Allergy. 2009;39(10):1558-68.
24. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81(3):559-75.
25. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21(2):263-5.
26. Yang YC, Zhang N, Van Crombruggen K, Hu GH, Hong SL, Bachert C. Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. Allergy. 2012;67(10):1193-202.
27. Van Bruaene N, C PN, Van Crombruggen K, De Ruyck N, Holtappels G, Van Cauwenberge P, et al. Inflammation and remodelling patterns in early stage chronic rhinosinusitis. Clin Exp Allergy. 2012;42(6):883-90.
28. Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 2012;22(9):1790-7.
29. Cousin E, Genin E, Mace S, Ricard S, Chansac C, del Zompo M, et al. Association studies in candidate genes: strategies to select SNPs to be tested. Hum Hered. 2003;56(4):151-9.
30. Genuneit J, Cantelmo JL, Weinmayr G, Wong GW, Cooper PJ, Riikjärv MA, et al. A multi-centre study of candidate genes for wheeze and allergy: the International Study of Asthma and Allergies in Childhood Phase 2. Clin Exp Allergy. 2009;39(12):1875-88.

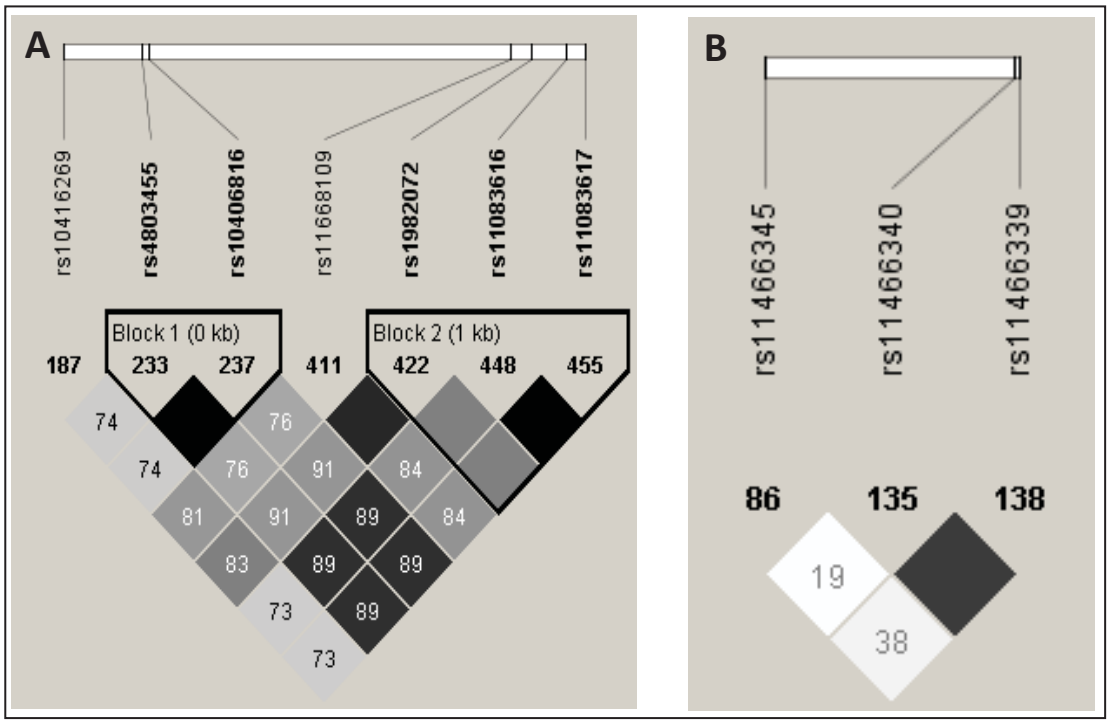

Figure 1. Linkage Disequilibrium in A) Honduras population; B) African Caribbean population.

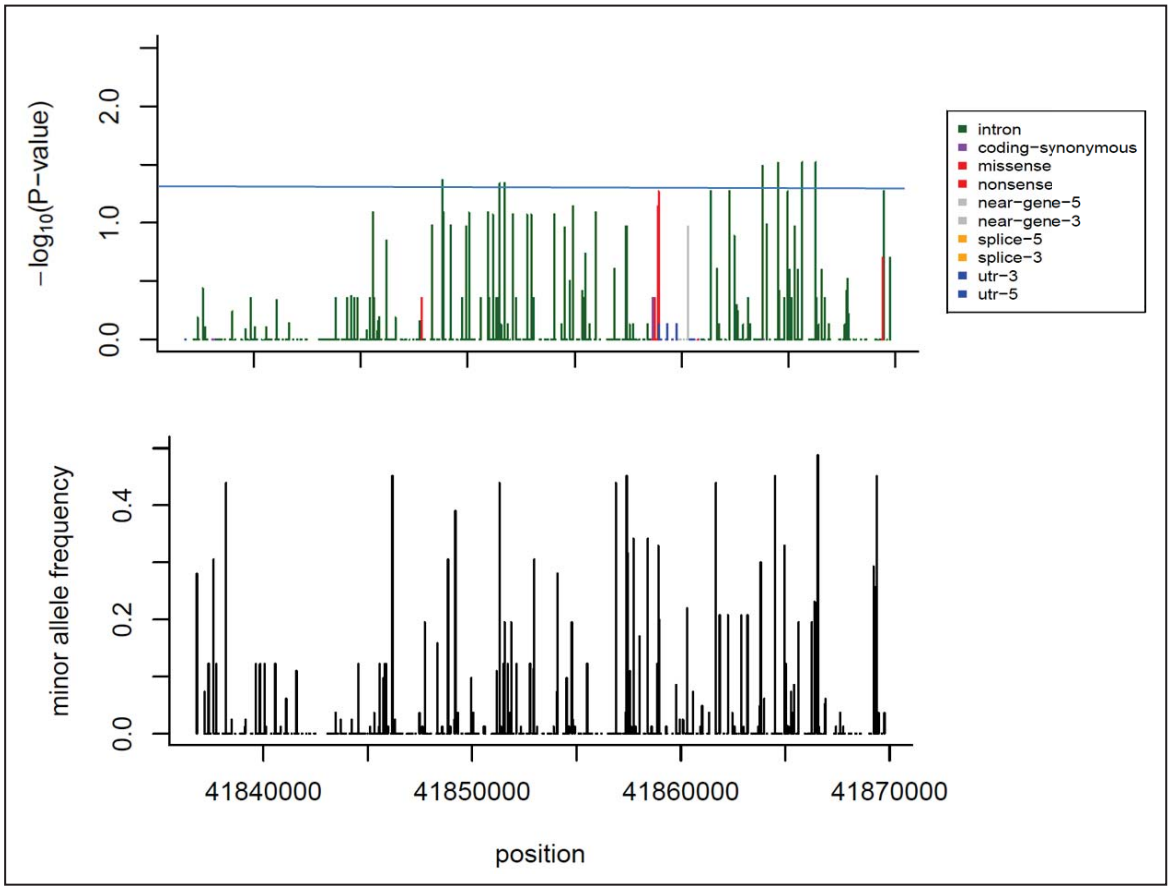

Figure 2. Association between *TGFB1* SNPs and asthma in Honduras population by using logistic regression adjusted by Principal Components 1 and 2, if MAF >0.1 or Fisher's Test, if MAF<0.1.

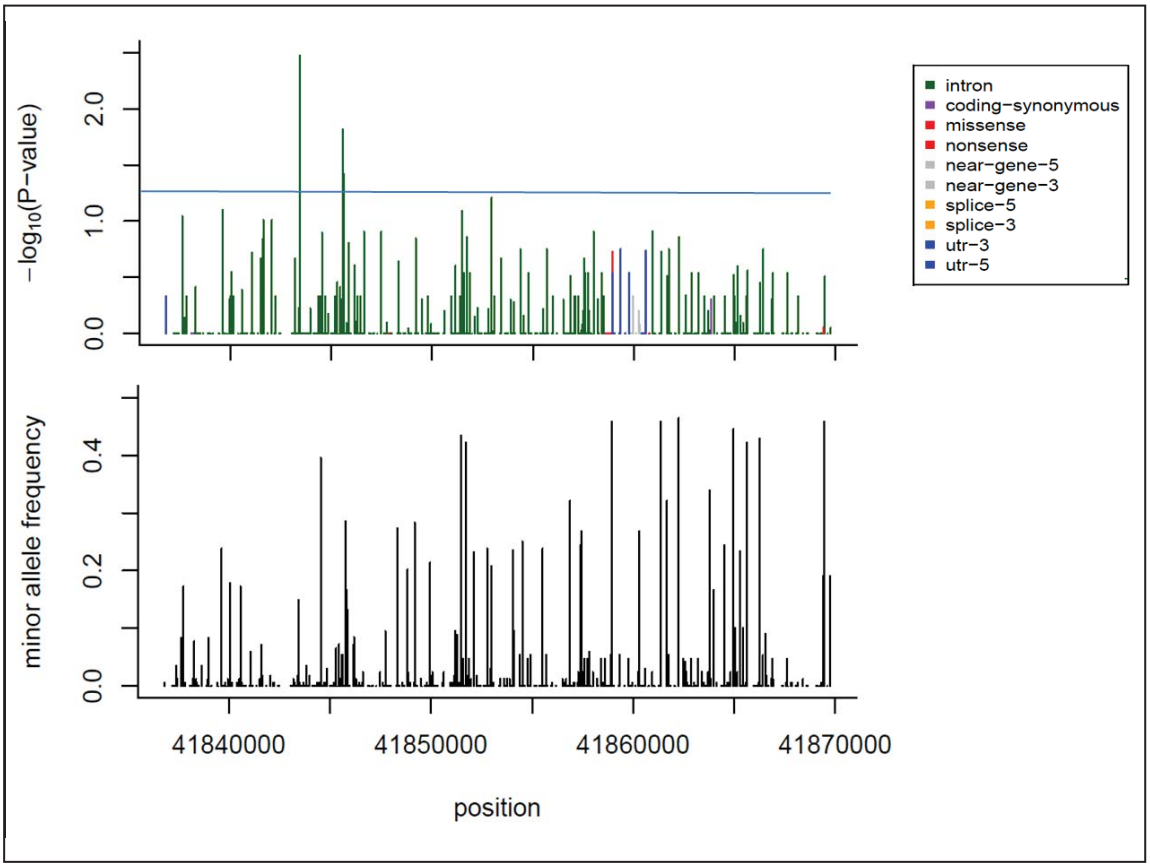

Figure 3. Association between *TGFB1* SNPs and asthma in African Caribbean population by using logistic regression adjusted by Principal Components 1 and 2, if MAF >0.1 or Fisher's Test, if MAF<0.1.

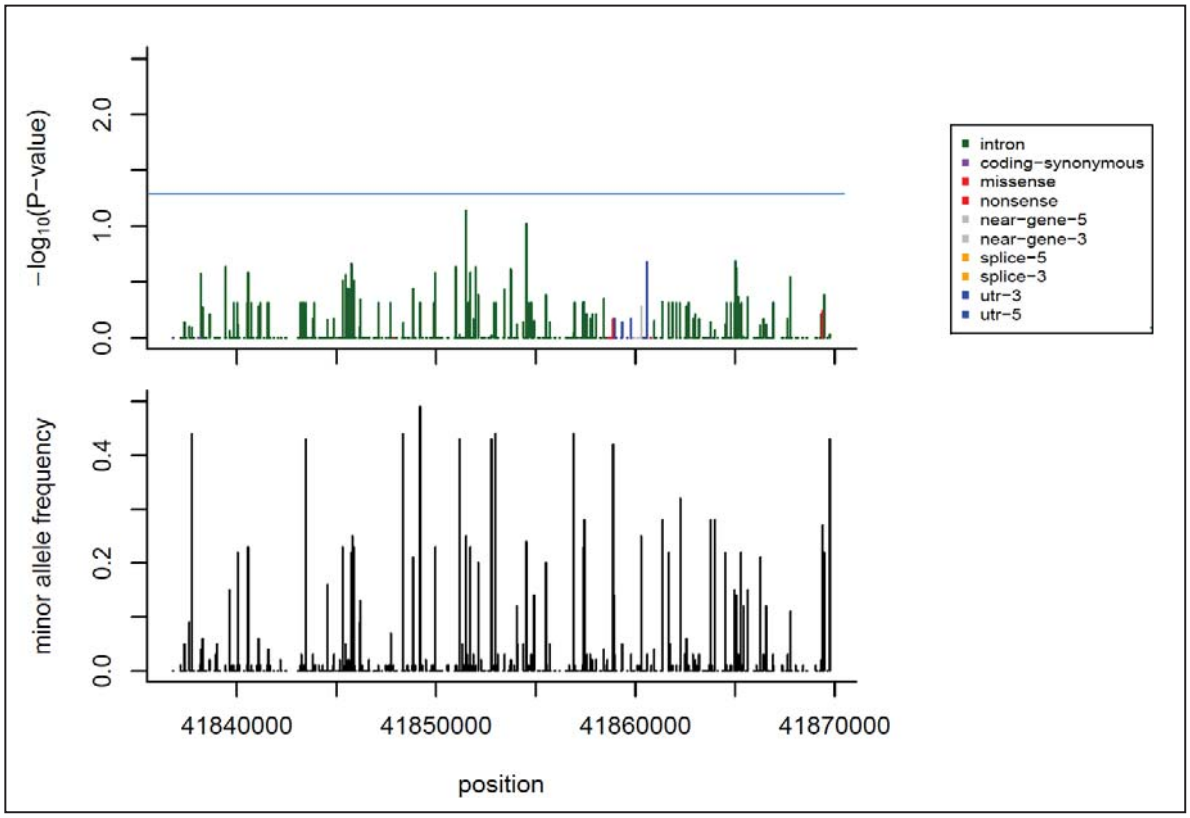

Figure 4. Association between *TGFB1* SNPs and asthma in African American population by using logistic regression adjusted by Principal Components 1 and 2, if MAF >0.1 or Fisher's Test, if MAF<0.1.

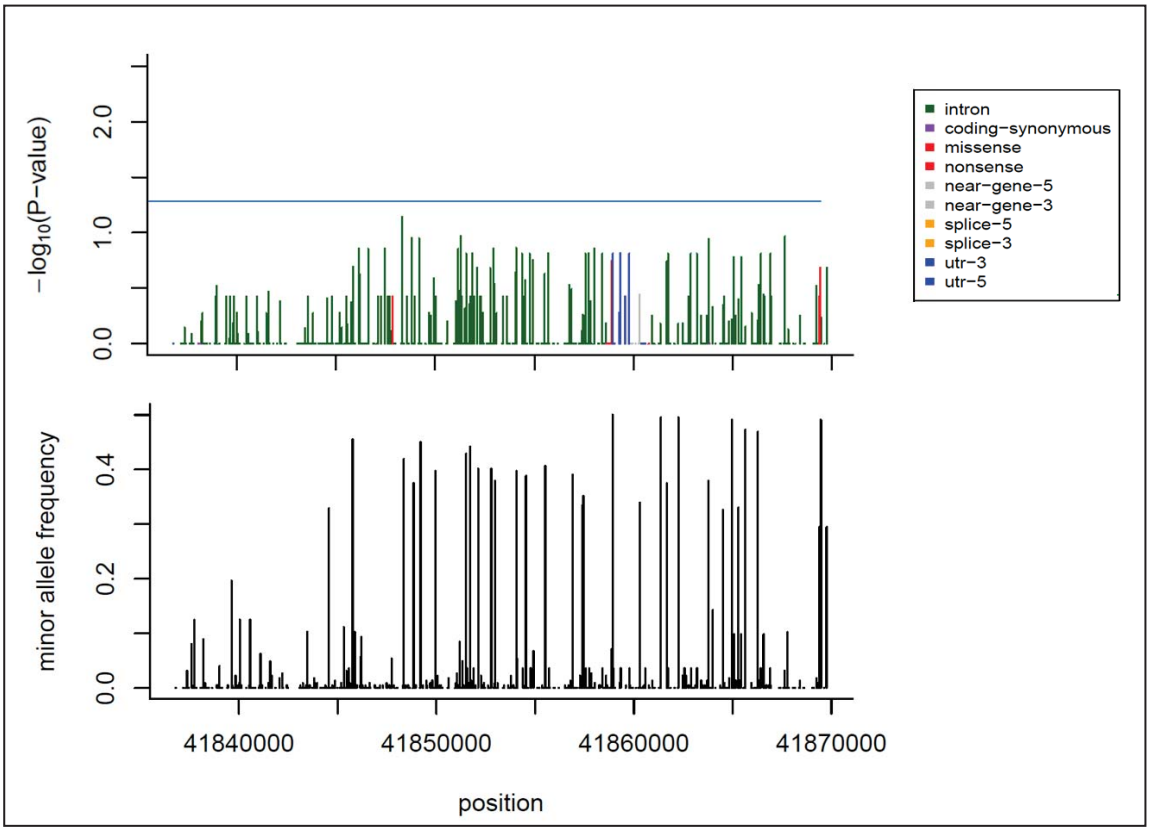

Figure 5. Association between *TGFB1* SNPs and asthma in South American population by using logistic regression adjusted by Principal Components 1 and 2, if MAF >0.1 or Fisher's Test, if MAF<0.1

Table 1. Characteristics of four independent populations of African descent according to asthma status, sex and site.

Population	Total	Case	Control
African American			
N	86	42 (48.84%)	44 (0.57%)
Sex			
Female	39	18 (42.86%)	21 (47.73%)
Male	47	24 (57.14%)	23 (52.27%)
Site			
Baltimore DC	47	24 (57.14%)	23 (52.27%)
New York	39	18 (42.86%)	21 (47.73%)
African Caribbean			
N	84	45 (53,57%)	39 (46,43%)
Sex			
Female	39	22 (48.89%)	17 (37.78%)
Male	45	23 (51.11%)	22 (48.89%)
Site			
Barbados	39	22 (48.89%)	17 (37.78%)
Jamaica	45	23 (51.11%)	22 (48.89%)
Honduras			
N	41	23 (56.19%)	18 (43.90%)
Sex			
Female	21	10 (43.48%)	11 (61.11%)
Male	20	13 (56.52%)	7 (38.89%)
South American			
N	112	42 (37.50%)	70 (62.50%)
Sex			
Female	58	15 (35.71%)	43 (61.43%)
Male	54	27 (64.29%)	27 (38.57%)
Site			
Conde, BR	33	6 (14.29%)	27 (38.57%)
Salvador, BR	48	23 (54.76%)	25 (35.71%)
Cartagena	31	13 (30.95%)	18 (25.71%)

Table 2. Function of *TGFB1* SNPs included in this study.

Function GVS	# SNPs
Intron	462
Missense	9
Utr-3	9
Coding-synonymous	7
Near-gene-3	7
Utr-5	7

Table 3. Significant association between *TGFB1* SNPs and asthma in Honduras population by using logistic regression adjusted by Principal Components 1 and 2, if MAF >0.1 or Fisher's Test, if MAF<0.1.

Marker	Distance	MAF	OR	95% CI	p value	Function	Regulome DB Score
rs11083616	41865643	0.44	3.27	1.12-9.54	0.030	intron	6
rs11083617	41866273	0.44	3.27	1.12-9.54	0.030	intron	7
rs1982072	41864509	0.28	4.21	1.14 – 15.50	0.031	intron	1f
rs11668109	41863777	0.32	3.88	1.12 – 13.38	0.032	intron	5
rs10416269	41848848	0.22	4.74	1.06 – 21.30	0.042	intron	7
rs4803455	41851509	0.44	3.00	1.02 – 8.82	0.045	intron	5
rs10406816	41851716	0.44	3.00	1.02 – 8.82	0.045	intron	7

Table 4. Significant association between *TGFB1* SNPs and asthma in African Caribbean population by using logistic regression adjusted by Principal Components 1 and 2, if MAF >0.1 or Fisher's Test, if MAF<0.1.

Marker	Distance	MAF	OR	95% CI	p Value	Function	Regulome DB Score
rs11466345	41843461	0.15	0.22	0.08 – 0.60	0.003	intron	7
rs11466340	41845579	0.04	NA	-	0.015	intron	7
rs11466339	41845622	0.05	7.51	-	0.038	Intron	6

Para S*upplementary Materials and Methods* ver ANEXO III.

CONCLUSÃO GERAL

Indivíduos que apresentam polimorfismo genético no TGF-β1 possuem menor risco de desenvolver alergia e aumentada susceptibilidade para infecções por helmintos. Dessa forma, a modulação sobre a resposta alérgica classicamente atribuída aos helmintos é resultante não apenas de fatores ambientais, mas também da predisposição genética, especialmente sobre a regulação da produção de IL-10.

Adicionalmente o estudo de associação de gene candidato revelou que polimorfismos no gene do TGF-β1 estão relacionados com asma em algumas populações de ancestralidade africana. Dentre estes, seis polimorfismos foram associados com asma pela primeira vez nesse trabalho. Entretanto, os perfis de associação são distintos entre as quatro populações estudadas, evidenciando a contribuição de outros genes envolvidos na resposta asmática, bem como de fatores ambientais diversos.

Futuros estudos são necessários para um melhor estabelecimento da contribuição potencial de polimorfismos no gene do TGF-β1 sobre a asma e se estes polimorfismos podem ser utilizados como ferramenta terapêutica da asma ou de outras doenças alérgicas. Além disso, esses achados podem servir como fonte para a replicação de tais polimorfismos genéticos em outras populações colaborando para ampliar a compreensão da importância dos fatores genéticos sobre a asma.

REFERÊNCIAS BIBLIOGRÁFICAS

ALCANTARA-NEVES, N. M. et al. The effect of single and multiple infections on atopy and wheezing in children. **J Allergy Clin Immunol,** v. 129, n. 2, p. 359-67, 367.e1-3, Feb 2012. ISSN 1097-6825. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22035877 >.

ARAÚJO, M. I. et al. Schistosoma mansoni infection modulates the immune response against allergic and auto-immune diseases. **Mem Inst Oswaldo Cruz,** v. 99, n. 5 Suppl 1, p. 27-32, 2004. ISSN 0074-0276. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15486631 >.

AWAD, M. R. et al. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. **Transplantation,** v. 66, n. 8, p. 1014-20, Oct 1998. ISSN 0041-1337. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9808485 >.

AÏT-KHALED, N. et al. Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. **Allergy,** v. 64, n. 1, p. 123-48, Jan 2009. ISSN 1398-9995. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19132975 >.

BAHADORI, K. et al. Economic burden of asthma: a systematic review. **BMC Pulm Med,** v. 9, p. 24, 2009. ISSN 1471-2466. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19454036 >.

BAQUEIRO, T. et al. Asthma and rhinitis symptoms in individuals from different socioeconomic levels in a Brazilian city. **Allergy Asthma Proc,** v. 28, n. 3, p. 362-7, 2007 May-Jun 2007. ISSN 1088-5412. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17619568 >.

BELLINI, A. et al. Interleukin (IL)-4, IL-13, and IL-17A differentially affect the profibrotic and proinflammatory functions of fibrocytes from asthmatic patients. **Mucosal Immunol,** v. 5, n. 2, p. 140-9, Mar 2012. ISSN 1935-3456. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22189956 >.

BETHONY, J. et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. **Lancet,** v. 367, n. 9521, p. 1521-32, May 2006. ISSN 1474-547X. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16679166 >.

BOTTEMA, R. W. et al. Gene-gene interaction in regulatory T-cell function in atopy and asthma development in childhood. **J Allergy Clin Immunol,** v. 126, n. 2, p. 338-46,

346.e1-10, Aug 2010. ISSN 1097-6825. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20599261 >.

BOYLE, A. P. et al. Annotation of functional variation in personal genomes using RegulomeDB. **Genome Res,** v. 22, n. 9, p. 1790-7, Sep 2012. ISSN 1549-5469. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22955989 >.

BRANDTZAEG, P. Food allergy: separating the science from the mythology. **Nat Rev Gastroenterol Hepatol,** v. 7, n. 7, p. 380-400, Jul 2010. ISSN 1759-5053. Disponível em: < http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20606633 >.

CARDOSO, L. S. et al. Risk factors for asthma in a helminth endemic area in bahia, Brazil. **J Parasitol Res,** v. 2012, p. 796820, 2012. ISSN 2090-0031. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22970348 >.

_____. Schistosoma mansoni antigen-driven interleukin-10 production in infected asthmatic individuals. **Mem Inst Oswaldo Cruz,** v. 101 Suppl 1, p. 339-43, Sep 2006. ISSN 0074-0276. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17308794 >.

CELEDÓN, J. C. et al. The transforming growth factor-beta1 (TGF-β1) gene is associated with chronic obstructive pulmonary disease (COPD). **Hum Mol Genet,** v. 13, n. 15, p. 1649-56, Aug 2004. ISSN 0964-6906. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15175276 >.

COOPER, P. J. Interactions between helminth parasites and allergy. **Curr Opin Allergy Clin Immunol,** v. 9, n. 1, p. 29-37, Feb 2009. ISSN 1473-6322. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19106698 >.

CORREALE, J.; FAREZ, M. F. The impact of parasite infections on the course of multiple sclerosis. **J Neuroimmunol,** v. 233, n. 1-2, p. 6-11, Apr 2011. ISSN 1872-8421. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21277637 >.

COUSIN, E. et al. Association studies in candidate genes: strategies to select SNPs to be tested. **Hum Hered,** v. 56, n. 4, p. 151-9, 2003. ISSN 0001-5652. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15031617 >.

CROSBY, L. M.; WATERS, C. M. Epithelial repair mechanisms in the lung. **Am J Physiol Lung Cell Mol Physiol,** v. 298, n. 6, p. L715-31, Jun 2010. ISSN 1522-1504. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20363851 >.

DARDALHON, V. et al. IL-4 inhibits TGF-beta-induced Foxp3+ T cells and, together with TGF-beta, generates IL-9+ IL-10+ Foxp3(-) effector T cells. **Nat Immunol,** v. 9, n. 12, p. 1347-55, Dec 2008. ISSN 1529-2916. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18997793 >.

FALLON, P.; MANGAN, N. Suppression of TH2-type allergic reactions by helminth infection. **Nat Rev Immunol,** v. 7, n. 3, p. 220-30, Mar 2007. ISSN 1474-1733. Disponível em: < http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17318233 >.

FIGUEIREDO, C. et al. Spontaneous cytokine production in children according to biological characteristics and environmental exposures. **Environ Health Perspect,** v. 117, n. 5, p. 845-9, May 2009. ISSN 1552-9924. Disponível em: < http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19478971 >.

_____. Evidence for a modulatory effect of IL-10 on both Th1 and Th2 cytokine production: the role of the environment. **Clin Immunol,** v. 139, n. 1, p. 57-64, Apr 2011. ISSN 1521-7035. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21285005 >.

_____. Coassociations between IL10 polymorphisms, IL-10 production, helminth infection, and asthma/wheeze in an urban tropical population in Brazil. J Allergy Clin Immunol, v. 131, p.1683-90, 2013.

GALANTER, J. M. et al. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: The Genes-environments & Admixture in Latino Americans study. **J Allergy Clin Immunol**, Jan 2014. ISSN 1097-6825. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24406073 >.

GRAINGER, D. J. et al. Genetic control of the circulating concentration of transforming growth factor type beta1. **Hum Mol Genet,** v. 8, n. 1, p. 93-7, Jan 1999. ISSN 0964-6906. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9887336 >.

GRANT, A. V. et al. Polymorphisms in IL10 are associated with total Immunoglobulin E levels and Schistosoma mansoni infection intensity in a Brazilian population. **Genes Immun**, Oct 2010. ISSN 1476-5470. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20927126 >.

HARRINGTON, L. E. et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. **Nat Immunol,** v. 6, n. 11, p. 1123-32, Nov 2005. ISSN 1529-2908. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16200070 >.

HOLGATE, S. T. Innate and adaptive immune responses in asthma. **Nat Med,** v. 18, n. 5, p. 673-83, May 2012. ISSN 1546-170X. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22561831 >.

KIM, S. H. et al. Combined effect of IL-10 and TGF-beta1 promoter polymorphisms as a risk factor for aspirin-intolerant asthma and rhinosinusitis. **Allergy,** v. 64, n. 8, p. 1221-5,

Aug 2009. ISSN 1398-9995. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19222424 >.

KLASS, B. R.; GROBBELAAR, A. O.; ROLFE, K. J. Transforming growth factor beta1 signalling, wound healing and repair: a multifunctional cytokine with clinical implications for wound repair, a delicate balance. **Postgrad Med J,** v. 85, n. 999, p. 9-14, Jan 2009. ISSN 1469-0756. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19240282 >.

KUDO, M.; ISHIGATSUBO, Y.; AOKI, I. Pathology of asthma. **Front Microbiol,** v. 4, p. 263, 2013. ISSN 1664-302X. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24032029 >.

KUDO, M. et al. IL-17A produced by αβ T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction. **Nat Med,** v. 18, n. 4, p. 547-54, Apr 2012. ISSN 1546-170X. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22388091 >.

LI, H. et al. Genetic polymorphisms in transforming growth factor beta-1 (TGF-β1) and childhood asthma and atopy. **Hum Genet,** v. 121, n. 5, p. 529-38, Jun 2007. ISSN 0340-6717. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17333284 >.

LI, M. O. et al. Transforming growth factor-beta regulation of immune responses. **Annu Rev Immunol,** v. 24, p. 99-146, 2006. ISSN 0732-0582. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16551245 >.

LYNCH, N. R. et al. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. **J Allergy Clin Immunol,** v. 92, n. 3, p. 404-11, Sep 1993. ISSN 0091-6749. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8360391 >.

MAK, J. C. et al. Analysis of TGF-beta(1) gene polymorphisms in Hong Kong Chinese patients with asthma. **J Allergy Clin Immunol,** v. 117, n. 1, p. 92-6, Jan 2006. ISSN 0091-6749. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16387590 >.

MATHIAS, R. A. et al. A genome-wide association study on African-ancestry populations for asthma. **J Allergy Clin Immunol,** v. 125, n. 2, p. 336-346.e4, Feb 2010. ISSN 1097-6825. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19910028 >.

MOREAU, E.; CHAUVIN, A. Immunity against helminths: interactions with the host and the intercurrent infections. **J Biomed Biotechnol,** v. 2010, p. 428593, 2010. ISSN 1110-7251. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20150967 >.

MUTAPI, F. et al. Schistosome infection intensity is inversely related to auto-reactive antibody levels. **PLoS One,** v. 6, n. 5, p. e19149, 2011. ISSN 1932-6203. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21573157 >.

OKADA, H. et al. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. **Clin Exp Immunol,** v. 160, n. 1, p. 1-9, Apr 2010. ISSN 1365-2249. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20415844 >.

PARK, H. et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. **Nat Immunol,** v. 6, n. 11, p. 1133-41, Nov 2005. ISSN 1529-2908. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16200068 >.

PASCUAL, R. M.; BLEECKER, E. R. Pharmacogenetics of asthma. **Curr Opin Pharmacol,** v. 10, n. 3, p. 226-35, Jun 2010. ISSN 1471-4973. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20646961 >.

PEARCE, N. et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). **Thorax,** v. 62, n. 9, p. 758-66, Sep 2007. ISSN 0040-6376. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17504817 >.

PECHKOVSKY, D. V. et al. Transforming growth factor beta1 induces alphavbeta3 integrin expression in human lung fibroblasts via a beta3 integrin-, c-Src-, and p38 MAPK-dependent pathway. **J Biol Chem,** v. 283, n. 19, p. 12898-908, May 2008. ISSN 0021-9258. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18353785 >.

PINELLI, E.; ARANZAMENDI, C. Toxocara infection and its association with allergic manifestations. **Endocr Metab Immune Disord Drug Targets,** v. 12, n. 1, p. 33-44, Mar 2012. ISSN 2212-3873. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22214330 >.

PONTE, E. V. et al. Skin test reactivity and Der p-induced interleukin 10 production in patients with asthma or rhinitis infected with Ascaris. **Ann Allergy Asthma Immunol,** v. 96, n. 5, p. 713-8, May 2006. ISSN 1081-1206. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16729785 >.

READ, S.; POWRIE, F. CD4(+) regulatory T cells. **Curr Opin Immunol,** v. 13, n. 6, p. 644-9, Dec 2001. ISSN 0952-7915. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11677084 >.

RODRIGUES, L. C. et al. Early infection with Trichuris trichiura and allergen skin test reactivity in later childhood. **Clin Exp Allergy,** v. 38, n. 11, p. 1769-77, Nov 2008. ISSN 1365-2222. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18547322 >.

RUJENI, N. et al. Atopy is inversely related to schistosome infection intensity: a comparative study in Zimbabwean villages with distinct levels of Schistosoma haematobium infection. **Int Arch Allergy Immunol,** v. 158, n. 3, p. 288-98, 2012. ISSN 1423-0097. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22398631 >.

SAIKA, S. et al. Smad3 signaling is required for epithelial-mesenchymal transition of lens epithelium after injury. **Am J Pathol,** v. 164, n. 2, p. 651-63, Feb 2004. ISSN 0002-9440. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14742269 >.

SALAM, M. T. et al. Transforming growth factor- 1 C-509T polymorphism, oxidant stress, and early-onset childhood asthma. **Am J Respir Crit Care Med,** v. 176, n. 12, p. 1192-9, Dec 2007. ISSN 1535-4970. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17673695 >.

SHARMA, S. et al. Variants in TGF-β1, dust mite exposure, and disease severity in children with asthma. **Am J Respir Crit Care Med,** v. 179, n. 5, p. 356-62, Mar 2009. ISSN 1535-4970. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19096005 >.

SHASTRY, B. S. SNPs: impact on gene function and phenotype. **Methods Mol Biol,** v. 578, p. 3-22, 2009. ISSN 1940-6029. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19768584 >.

SHEVACH, E. M. CD4+ CD25+ suppressor T cells: more questions than answers. **Nat Rev Immunol,** v. 2, n. 6, p. 389-400, Jun 2002. ISSN 1474-1733. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12093005 >.

SHULL, M. M. et al. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. **Nature,** v. 359, n. 6397, p. 693-9, Oct 1992. ISSN 0028-0836. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/1436033 >.

SOLÉ, D. et al. Prevalence of rhinitis among Brazilian schoolchildren: ISAAC phase 3 results. **Rhinology,** v. 45, n. 2, p. 122-8, Jun 2007. ISSN 0300-0729. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17708458 >.

_____. Prevalence of rhinitis-related symptoms in Latin American children - results of the International Study of Asthma and Allergies in Childhood (ISAAC) phase three. **Pediatr Allergy Immunol,** v. 21, n. 1 Pt 2, p. e127-36, Feb 2010. ISSN 1399-3038. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19788538 >.

STONE, K.; PRUSSIN, C.; METCALFE, D. IgE, mast cells, basophils, and eosinophils. **J Allergy Clin Immunol,** v. 125, n. 2 Suppl 2, p. S73-80, Feb 2010. ISSN 1097-6825. Disponível em: < http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20176269 >.

STRACHAN, D. P. Hay fever, hygiene, and household size. **BMJ,** v. 299, n. 6710, p. 1259-60, Nov 1989. ISSN 0959-8138. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/2513902 >.

TO, T. et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. **BMC Public Health,** v. 12, p. 204, 2012. ISSN 1471-2458. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22429515 >.

TRAN, D. Q. TGF-β: the sword, the wand, and the shield of FOXP3(+) regulatory T cells. **J Mol Cell Biol,** v. 4, n. 1, p. 29-37, Feb 2012. ISSN 1759-4685. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22158907 >.

TRAVIS, M. A.; SHEPPARD, D. TGF-β Activation and Function in Immunity. **Annu Rev Immunol**, Dec 2013. ISSN 1545-3278. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24313777 >.

TURNER, J. D. et al. Intensity of intestinal infection with multiple worm species is related to regulatory cytokine output and immune hyporesponsiveness. **J Infect Dis,** v. 197, n. 8, p. 1204-12, Apr 2008. ISSN 0022-1899. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18462167 >.

VAN DEN BIGGELAAR, A. H. et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. **J Infect Dis,** v. 189, n. 5, p. 892-900, Mar 2004. ISSN 0022-1899. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/14976607 >.

VAN RIET, E.; HARTGERS, F. C.; YAZDANBAKHSH, M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. **Immunobiology,** v. 212, n. 6, p. 475-90, 2007. ISSN 0171-2985. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17544832 >.

VERCELLI, D. Discovering susceptibility genes for asthma and allergy. **Nat Rev Immunol,** v. 8, n. 3, p. 169-82, Mar 2008. ISSN 1474-1741. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18301422 >.

WANG, Y. H. et al. A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. **J Exp Med,** v. 207, n. 11, p. 2479-91, Oct 2010. ISSN 1540-9538. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20921287 >.

WILLIS, B. C.; BOROK, Z. TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. **Am J Physiol Lung Cell Mol Physiol,** v. 293, n. 3, p. L525-34, Sep 2007. ISSN 1040-0605. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17631612 >.

WU, H. et al. Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. **J Allergy Clin Immunol,** v. 125, n. 2, p. 321-327.e13, Feb 2010. ISSN 1097-6825. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19910030 >.

YAMADA, Y. et al. Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. **J Bone Miner Res,** v. 13, n. 10, p. 1569-76, Oct 1998. ISSN 0884-0431. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9783545 >.

YANG, X. X. et al. Association of TGF-beta1, IL-4 and IL-13 gene polymerphisms with asthma in a Chinese population. **Asian Pac J Allergy Immunol,** v. 29, n. 3, p. 273-7, Sep 2011. ISSN 0125-877X. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22053598 >.

YAZDANBAKHSH, M.; KREMSNER, P.; VAN REE, R. Allergy, parasites, and the hygiene hypothesis. **Science,** v. 296, n. 5567, p. 490-4, Apr 2002. ISSN 1095-9203. Disponível em: < http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11964470 >.

YOSHIMURA, A.; WAKABAYASHI, Y.; MORI, T. Cellular and molecular basis for the regulation of inflammation by TGF-beta. **J Biochem,** v. 147, n. 6, p. 781-92, Jun 2010. ISSN 1756-2651. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20410014 >.

ZHU, X. J. et al. [Association of TGF-β1 gene polymorphism -509C/T with disease severity in childhood allergic rhinitis]. **Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi,** v. 45, n. 6, p. 459-64, Jun 2010. ISSN 1673-0860. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21055322 >.

Anexo I _ Aprovação do Comitê de Ética

Universidade Federal da Bahia
Instituto de Saúde Coletiva
COMITÊ DE ÉTICA EM
PESQUISA

Formulário de Aprovação do Comitê de Ética em Pesquisa

Registro CEP: 003-05/CEP-ISC
Projeto de Pesquisa: "Fatores de risco para a asma e alergia e perfil imunológico em crianças na cidade de Salvador"
Pesquisador Responsável: Maurício Lima Barreto
Área Temática: Grupo I

Os Membros do Comitê de Ética em Pesquisa, do Instituto de Saúde Coletiva/Universidade Federal da Bahia, reunidos em sessão ordinária no dia 25 de fevereiro de 2005, e com base em Parecer Consubstanciado, resolveu pela situação do projeto abaixo descrito.

Situação: APROVADO

Salvador, 28 de fevereiro de 2005

VILMA SOUSA SANTANA
Presidente do Comitê de Ética em Pesquisa
Instituto de Saúde Coletiva
Universidade Federal da Bahia

Anexo II – Termo de Consentimento Livre e Esclarecido

INSTITUTO DE SAÚDE COLETIVA e FACULDADE DE MEDICINA
UNIVERSIDADE FEDERAL DA BAHIA

PROJETO: Fatores de risco para asma e doenças alérgicas, e perfil imunológico em crianças na cidade de Salvador

Nome da criança: _____
REGISTRO:_____

Consentimento Informado

Pesquisadores da Universidade Federal da Bahia estão realizando um estudo sobre ASMA E ALERGIA na cidade de Salvador. O objetivo do estudo é saber a proporção de crianças com asma e alergia e estudar a causa dessas doenças. Seu (sua) filho (a) acima mencionado foi selecionado para participar do estudo, porém para isto é necessário que o (a) Senhor (a), como responsável pela criança acima, dê o seu consentimento para que as seguintes atividades sejam realizadas:

1. Que o senhor (a) responda um **questionário** sobre asma e alergia na criança.

2. Permita que se faça um exame na criança para saber se é alérgica. Nesse teste (chamado teste cutâneo) pequenas injeções serão dadas no braço da criança e se procurará ver se ela desenvolve um vermelhidão no lugar da injeção. Se o vermelhidão aparecer, isso quer dizer que a criança tem alergia. O teste dura em torno de 30 minutos e será feito por um médico qualificado.

3. Permita que se faça coleta de uma amostra de sangue da criança que será usado também para saber se a criança tem alergia a ácaros e barata, anemia, já teve infecção por vírus da hepatite A, *Toxocara canis*, *Toxoplasma gondii*, *Ascaris lumbricoides* e para saber o seu estado imunológico (interleucinas Il-4, Il-5, Il-10 e IFN-gama).

4. Nos forneça duas amostras de fezes da criança para exame parasitológico para saber se as crianças tem vermes.

5. Permita que seja medido a altura e peso da criança.

6. Permita que se faça coleta de poeira no leito onde a criança dorme, para saber que tipo de poeira existe na casa e que pode causar alergia.

7. Que o senhor (a) responda um questionários sobre características do quarto e leito onde a criança dorme a ser aplicado no momento da coleta de poeira dos leitos das crianças.

8. Que o senhor (a) permita que o soro que será utilizados para realizar os exames deste estudo, caso não sejam todo utilizados, possam ser guardados para ser utilizado no futuro na realização de outros exames que porventura sejam necessários para maior esclarecimento sobre as doenças estudadas

Toda informação obtido através do questionário ou dos exame é estritamente confidencial e o seu nome ou do seu filho não aparecerá em nenhuma parte do relatório ou publicação deste estudo.

Todos os resultados do exame serão entregue. A amostra de sangue será encaminhada para um laboratório e os exames são demorados, os resultados não são liberados no mesmo dia. Se a criança tiver algum verme forneceremos a orientação e os medicamentos para o tratamento gratuitamente. Se a criança estiver desnutrida ou com peso acima do normal receberá orientação sobre a dieta apropriada. Se a criança tiver asma ou alergia, marcaremos um dia para a criança ser vista por médico no ambulatório no Hospital das Clínicas no Canela, e vocês receberão passe de ônibus para duas pessoas ida e volta. Caso necessário toda orientação será dada para que ela tenha acesso ao melhor tratamento possível.

Devemos enfatizar que a participação na pesquisa é voluntária e o Sr.(a) pode retirar o seu filho a qualquer momento.

Qualquer problema contatar: Dr. Sérgio Souza da Cunha, Instituto de Saúde Coletiva, Universidade Federal da Bahia, Rua Padre Feijó 29/ 4º andar, Canela, telefone 3245-0544, email: cunhass@ufba.br .

Declaro estar ciente do que se trata a pesquisa **Fatores de risco para asma e doenças alérgicas, e perfil imunológico em crianças na cidade de Salvador**, confirmando os itens abaixo.

Pergunta	Resposta	Assinatura do responsável
Aceita responder o **questionário**?	SIM–()	
	NÃ0– ()	
Aceita que a criança faça o **teste cutâneo**?	SIM–()	
	NÃ0– ()	
Aceita que seja coletado uma **amostra de sangue** da criança para realização dos testes acima especificados?	SIM–()	
	NÃ0– ()	
Aceita que seja coletado amostra de fezes da criança?	SIM–()	
	NÃ0– ()	
Aceita que seja **coletado poeira** na casa?	SIM–()	

	NÃ0– ()	
Aceita que seja medido **peso e altura**?	SIM–()	
	NÃ0– ()	
Aceita que o soro possa ser guardado, sujeito ao seu consentimento para novos exames alem do acima especificado	SIM–()	
	NÃ0– ()	
Responsável **não aceitou** participar da pesquisa	()	

Salvador, ... de .....de 2005

Assinatura do/a Pesquisador/a: _____

Anexo II - *Supplementary Materials and Methods*

Table 1. The table with all SNPs included in this study. The SNPs are located between 41,836,811 and 41,869,756 bp on chromosome 19, relative to TGF-β1 gene and its promoter region, totaling 501 SNP's involved in distinct genetic functions.

Number	Position	Alleles	Function	rs ID	Amino acids
1	41836811	A/G	utr-3	0	none
2	41836816	C/T	utr-3	0	none
3	41837186	C/T	intron	0	none
4	41837307	C/T	intron	0	none
5	41837457	A/G	intron	0	none
6	41837466	C/T	intron	0	none
7	41837701	C/T	intron	0	none
8	41837715	A/C	intron	0	none
9	41837844	C/T	intron	0	none
10	41838075	C/T	coding-synonymous	11466358	none
11	41838155	A/G	coding-synonymous	0	none
12	41838201	A/G	intron	0	none
13	41838257	C/T	intron	0	none
14	41838288	C/G	intron	0	none
15	41838372	A/C	intron	0	none
16	41838394	A/G	intron	0	none
17	41838406	A/T	intron	0	none
18	41838495	A/G	intron	0	none
19	41838645	A/G	intron	0	none
20	41838887	A/G	intron	0	none
21	41838960	A/G	intron	11466357	none
22	41839016	C/T	intron	11466356	none
23	41839114	A/T	intron	8103575	none
24	41839142	C/G	intron	11466354	none
25	41839416	C/T	intron	0	none
26	41839440	A/G	intron	0	none
27	41839443	C/T	intron	0	none
28	41839456	C/T	intron	0	none
29	41839662	A/G	intron	0	none
30	41839747	A/G	intron	0	none
31	41839845	C/T	intron	11466352	none
32	41839846	A/G	intron	11466351	none
33	41839943	A/G	intron	0	none

34	41840017	C/T	intron	0	none
35	41840018	A/G	intron	0	none
36	41840022	A/G	intron	0	none
37	41840105	A/C	intron	0	none
38	41840137	G/T	intron	0	none
39	41840145	C/T	intron	0	none
40	41840375	C/T	intron	0	none
41	41840517	C/T	intron	11466350	none
42	41840597	C/T	intron	0	none
43	41840624	A/G	intron	0	none
44	41840691	C/T	intron	0	none
45	41840736	C/T	intron	0	none
46	41840831	G/T	intron	0	none
47	41841012	C/T	intron	0	none
48	41841014	A/G	intron	11466348	none
49	41841051	A/G	intron	0	none
50	41841186	C/T	intron	0	none
51	41841363	G/T	intron	0	none
52	41841490	A/G	intron	0	none
53	41841529	C/G	intron	0	none
54	41841546	C/T	intron	0	none
55	41841591	C/T	intron	78683362	none
56	41841592	A/G	intron	0	none
57	41841652	C/G	intron	0	none
58	41841673	C/T	intron	0	none
59	41841854	A/G	intron	0	none
60	41841866	C/T	intron	0	none
61	41842060	C/G	intron	0	none
62	41842192	C/T	intron	57441595	none
63	41842240	C/T	intron	0	none
64	41842431	A/C	intron	0	none
65	41842490	G/T	intron	11466346	none
66	41843067	C/T	intron	0	none
67	41843068	A/G	intron	0	none
68	41843115	C/T	intron	0	none
69	41843194	C/G	intron	0	none
70	41843247	C/T	intron	0	none
71	41843248	A/G	intron	0	none
72	41843263	C/T	intron	0	none
73	41843335	A/G	intron	0	none
74	41843372	C/G	intron	0	none
75	41843383	C/T	intron	0	none
76	41843447	A/G	intron	0	none
77	41843468	C/T	intron	0	none

78	41843472	A/G	intron	0	none
79	41843485	C/T	intron	0	none
80	41843515	A/C	intron	0	none
81	41843586	C/T	intron	0	none
82	41843704	C/T	intron	0	none
83	41843744	C/T	intron	0	none
84	41843819	A/G	intron	0	none
85	41843853	C/T	intron	0	none
86	41843890	A/G	intron	0	none
87	41843960	A/G	intron	0	none
88	41843963	G/T	intron	0	none
89	41843966	A/G	intron	0	none
90	41844121	C/T	intron	0	none
91	41844128	A/G	intron	0	none
92	41844228	A/G	intron	0	none
93	41844229	C/T	intron	0	none
94	41844276	A/G	intron	0	none
95	41844309	A/G	intron	0	none
96	41844389	A/G	intron	0	none
97	41844410	A/C	intron	0	none
98	41844414	A/G	intron	76109053	none
99	41844435	A/G	intron	0	none
100	41844465	C/T	intron	0	none
101	41844609	C/T	intron	0	none
102	41844699	A/G	intron	0	none
103	41844723	A/G	intron	0	none
104	41844757	A/G	intron	0	none
105	41844823	C/G	intron	0	none
106	41844846	A/G	intron	0	none
107	41844871	A/G	intron	0	none
108	41844875	G/T	intron	0	none
109	41844991	A/C	intron	28730295	none
110	41845105	C/T	intron	0	none
111	41845161	C/T	intron	0	none
112	41845180	C/T	intron	0	none
113	41845296	C/T	intron	0	none
114	41845313	C/T	intron	11466344	none
115	41845314	A/G	intron	0	none
116	41845430	A/C	intron	0	none
117	41845455	C/T	intron	11466343	none
118	41845456	A/G	intron	0	none
119	41845512	A/G	intron	0	none
120	41845533	G/T	intron	0	none
121	41845561	C/G	intron	11466342	none

122	41845573	A/C	intron	0	none
123	41845574	C/G	intron	11466341	none
124	41845598	A/G	intron	0	none
125	41845612	C/T	intron	0	none
126	41845622	A/C	intron	11466339	none
127	41845691	C/T	intron	0	none
128	41845731	A/G	intron	0	none
129	41845790	A/G	intron	0	none
130	41845872	A/G	intron	8110090	none
131	41845917	A/G	intron	79225164	none
132	41845971	C/T	intron	0	none
133	41846142	C/T	intron	0	none
134	41846160	C/T	intron	0	none
135	41846162	C/T	intron	72300752	none
136	41846271	C/T	intron	0	none
137	41846290	C/G	intron	0	none
138	41846298	A/C	intron	0	none
139	41846413	C/T	intron	0	none
140	41846418	C/G	intron	11466336	none
141	41846465	C/T	intron	0	none
142	41846543	C/T	intron	0	none
143	41846552	A/C	intron	0	none
144	41846588	A/C	intron	0	none
145	41846625	C/T	intron	0	none
146	41846651	A/G	intron	0	none
147	41846881	A/C	intron	0	none
148	41846963	A/G	intron	0	none
149	41847053	A/G	intron	0	none
150	41847061	C/T	intron	0	none
151	41847110	A/G	intron	0	none
152	41847153	C/T	intron	0	none
153	41847296	C/T	intron	0	none
154	41847481	A/G	intron	0	none
155	41847484	A/T	intron	0	none
156	41847622	C/G	intron	0	none
157	41847623	C/G	intron	0	none
158	41847703	C/T	intron	0	none
159	41847860	A/G	missense	1800472	THR,ILE
160	41847970	C/T	intron	0	none
161	41848021	A/G	intron	0	none
162	41848297	C/T	intron	0	none
163	41848412	A/G	intron	0	none
164	41848451	A/G	intron	0	none
165	41848568	C/T	intron	0	none

166	41848663	C/T	intron	0	none
167	41848811	C/T	intron	11466333	none
168	41848859	A/G	intron	0	none
169	41848909	C/T	intron	0	none
170	41848956	A/G	intron	0	none
171	41849164	C/T	intron	0	none
172	41849196	C/T	intron	0	none
173	41849306	C/T	intron	0	none
174	41849319	A/G	intron	0	none
175	41849495	G/T	intron	11466331	none
176	41849528	A/G	intron	0	none
177	41849694	C/T	intron	11466330	none
178	41849706	A/C	intron	0	none
179	41849752	C/G	intron	0	none
180	41849799	A/G	intron	0	none
181	41849879	C/T	intron	0	none
182	41849958	A/G	intron	1549934	none
183	41849981	A/G	intron	0	none
184	41850040	A/G	intron	0	none
185	41850057	C/T	intron	0	none
186	41850060	G/T	intron	0	none
187	41850121	C/T	intron	0	none
188	41850157	C/T	intron	0	none
189	41850237	C/T	intron	0	none
190	41850314	A/T	intron	0	none
191	41850569	A/G	intron	0	none
192	41850624	A/G	intron	28395768	none
193	41850949	C/T	intron	0	none
194	41850984	C/T	intron	10405403	none
195	41851016	A/G	intron	0	none
196	41851035	A/G	intron	0	none
197	41851042	A/G	intron	11466328	none
198	41851124	C/G	intron	0	none
199	41851128	A/G	intron	0	none
200	41851168	A/G	intron	0	none
201	41851275	A/G	intron	0	none
202	41851327	A/G	intron	0	none
203	41851358	C/T	intron	0	none
204	41851368	C/T	intron	0	none
205	41851380	C/T	intron	0	none
206	41851388	C/T	intron	0	none
207	41851397	C/G	intron	0	none
208	41851467	C/T	intron	0	none
209	41851483	A/C	intron	0	none

210	41851565	A/G	intron	8102918	none
211	41851591	C/G	intron	0	none
212	41851644	C/T	intron	0	none
213	41851755	C/T	intron	0	none
214	41851771	C/T	intron	0	none
215	41851798	C/T	intron	0	none
216	41851815	A/T	intron	0	none
217	41851816	A/G	intron	0	none
218	41851873	C/G	intron	0	none
219	41851874	A/C	intron	0	none
220	41851994	A/G	intron	0	none
221	41852259	A/C	intron	2014015	none
222	41852266	A/G	intron	0	none
223	41852267	A/C	intron	0	none
224	41852332	A/C	intron	0	none
225	41852395	C/T	intron	0	none
226	41852404	A/G	intron	0	none
227	41852460	C/T	intron	0	none
228	41852606	C/T	intron	0	none
229	41852680	C/G	intron	0	none
230	41852725	A/G	intron	0	none
231	41852759	C/T	intron	0	none
232	41852909	A/G	intron	0	none
233	41852952	C/T	intron	0	none
234	41852953	A/G	intron	0	none
235	41853012	C/T	intron	0	none
236	41853013	A/G	intron	0	none
237	41853103	A/T	intron	0	none
238	41853375	A/G	intron	0	none
239	41853419	A/C	intron	0	none
240	41853625	C/T	intron	0	none
241	41853712	A/G	intron	0	none
242	41853755	A/G	intron	0	none
243	41853758	A/G	intron	0	none
244	41853759	A/C	intron	0	none
245	41853776	C/T	intron	0	none
246	41853779	C/T	intron	0	none
247	41853898	G/T	intron	0	none
248	41853906	A/G	intron	0	none
249	41853969	A/G	intron	0	none
250	41854480	A/T	intron	0	none
251	41854535	A/T	intron	0	none
252	41854537	A/T	intron	58675726	none
253	41854551	A/G	intron	0	none

254	41854590	A/G	intron	0	none
255	41854640	C/T	intron	0	none
256	41854773	C/T	intron	8108052	none
257	41854775	C/T	intron	0	none
258	41854819	A/G	intron	0	none
259	41854916	A/G	intron	11466321	none
260	41855110	A/T	intron	0	none
261	41855204	C/T	intron	11466320	none
262	41855360	C/T	intron	0	none
263	41855366	C/T	intron	0	none
264	41855453	C/G	intron	0	none
265	41855523	A/G	intron	0	none
266	41855553	C/T	intron	0	none
267	41855576	A/C	intron	0	none
268	41855611	A/G	intron	0	none
269	41855747	C/T	intron	0	none
270	41855932	C/T	intron	62120999	none
271	41855998	A/G	intron	0	none
272	41856186	A/G	intron	0	none
273	41856511	C/G	intron	0	none
274	41856562	A/G	intron	0	none
275	41856592	A/C	intron	0	none
276	41856678	C/T	intron	0	none
277	41856716	A/T	intron	0	none
278	41856784	A/G	intron	0	none
279	41856804	A/C	intron	0	none
280	41856950	A/C	intron	0	none
281	41856988	A/G	intron	0	none
282	41857048	G/T	intron	0	none
283	41857125	A/G	intron	0	none
284	41857245	A/C	intron	0	none
285	41857277	C/T	intron	0	none
286	41857285	A/G	intron	0	none
287	41857314	A/T	intron	0	none
288	41857355	A/G	intron	0	none
289	41857506	C/G	intron	0	none
290	41857544	C/T	intron	0	none
291	41857553	A/G	intron	0	none
292	41857570	C/T	intron	80224671	none
293	41857623	A/C	intron	0	none
294	41857711	C/G	intron	67392886	none
295	41857734	A/T	intron	76161655	none
296	41857767	A/G	intron	0	none
297	41857792	A/C	intron	79811788	none

298	41857844	G/T	intron	10418010	none
299	41857908	C/G	intron	0	none
300	41858021	A/T	intron	77546077	none
301	41858022	C/T	intron	0	none
302	41858219	C/G	intron	0	none
303	41858285	C/T	intron	0	none
304	41858418	G/T	intron	74865332	none
305	41858440	G/T	intron	0	none
306	41858510	G/T	intron	0	none
307	41858535	C/T	intron	0	none
308	41858590	C/T	intron	11466318	none
309	41858602	A/G	coding-synonymous	56281462	none
310	41858648	A/G	missense	0	ALA,VAL
311	41858659	A/G	coding-synonymous	0	none
312	41858759	C/G	missense	0	SER,THR
313	41858864	C/T	missense	0	GLY,GLU
314	41858876	C/G	missense	1800471	ARG,PRO
315	41858921	A/G	missense	1800470	PRO,LEU
316	41858963	C/T	utr-5	9282871	none
317	41859031	A/G	utr-5	0	none
318	41859277	A/G	utr-5	45457101	none
319	41859298	C/G	utr-5	0	none
320	41859589	C/T	utr-5	0	none
321	41859776	C/G	utr-5	11466315	none
322	41859819	G/T	near-gene-3	0	none
323	41859921	A/G	near-gene-3	0	none
324	41859961	A/G	near-gene-3	0	none
325	41860103	A/G	near-gene-3	35318502	none
326	41860236	C/T	near-gene-3	11466314	none
327	41860257	A/G	near-gene-3	0	none
328	41860395	A/G	utr-3	0	none
329	41860429	A/T	utr-3	0	none
330	41860476	G/T	utr-3	0	none
331	41860477	C/T	utr-3	0	none
332	41860497	C/G	utr-3	0	none
333	41860518	A/G	utr-3	4987025	none
334	41860759	C/T	missense	1054797	ARG,GLN
335	41860797	C/T	coding-synonymous	0	none
336	41860934	C/G	intron	3087453	none
337	41861018	C/T	intron	41445948	none
338	41861053	A/G	intron	0	none

339	41861266	C/T	intron	0	none
340	41861359	C/T	intron	4803457	none
341	41861667	G/T	intron	0	none
342	41862057	C/T	intron	0	none
343	41862071	A/G	intron	0	none
344	41862223	A/G	intron	0	none
345	41862232	A/G	intron	0	none
346	41862463	C/T	intron	13345981	none
347	41862474	A/C	intron	0	none
348	41862475	A/G	intron	0	none
349	41862636	A/G	intron	0	none
350	41862645	A/G	intron	0	none
351	41862669	C/T	intron	0	none
352	41862774	A/C	intron	0	none
353	41862891	C/T	intron	79711096	none
354	41862896	C/T	intron	0	none
355	41863003	C/T	intron	0	none
356	41863055	C/T	intron	0	none
357	41863094	C/G	intron	0	none
358	41863193	G/T	intron	78455916	none
359	41863386	A/G	intron	0	none
360	41863401	C/T	intron	74474357	none
361	41863491	A/G	intron	0	none
362	41863512	C/T	intron	0	none
363	41863599	C/T	intron	0	none
364	41863701	C/T	intron	78999030	none
365	41863781	A/G	intron	0	none
366	41863821	A/G	coding-synonymous	34088631	none
367	41863833	A/G	coding-synonymous	0	none
368	41863958	C/G	intron	0	none
369	41864123	G/T	intron	0	none
370	41864359	A/G	intron	77751159	none
371	41864454	C/G	intron	0	none
372	41864463	C/G	intron	0	none
373	41864535	A/G	intron	0	none
374	41864575	A/G	intron	0	none
375	41864582	C/T	intron	0	none
376	41864673	A/G	intron	0	none
377	41864765	A/G	intron	0	none
378	41864774	A/G	intron	0	none
379	41864802	C/T	intron	0	none
380	41864815	A/G	intron	0	none

381	41864817	G/T	intron	58315091	none
382	41864818	A/G	intron	0	none
383	41864980	A/G	intron	0	none
384	41864991	C/T	intron	0	none
385	41865017	C/G	intron	0	none
386	41865156	C/T	intron	61414323	none
387	41865157	A/G	intron	0	none
388	41865269	C/T	intron	0	none
389	41865313	A/C	intron	0	none
390	41865328	A/C	intron	0	none
391	41865540	G/T	intron	0	none
392	41865581	C/T	intron	0	none
393	41865627	A/G	intron	79198540	none
394	41865952	A/G	intron	0	none
395	41865969	A/G	intron	0	none
396	41866075	C/T	intron	0	none
397	41866085	G/T	intron	0	none
398	41866172	C/G	intron	0	none
399	41866216	A/G	intron	0	none
400	41866287	G/T	intron	0	none
401	41866329	C/G	intron	0	none
402	41866396	C/G	intron	0	none
403	41866418	A/T	intron	0	none
404	41866559	C/T	intron	0	none
405	41866577	A/C	intron	0	none
406	41866585	C/T	intron	0	none
407	41866603	C/G	intron	0	none
408	41866671	A/C	intron	0	none
409	41866703	C/T	intron	0	none
410	41866840	G/T	intron	0	none
411	41866900	A/G	intron	76515864	none
412	41866907	C/T	intron	0	none
413	41866923	A/G	intron	0	none
414	41866925	A/G	intron	0	none
415	41867351	C/T	intron	0	none
416	41867370	A/C	intron	0	none
417	41867432	C/T	intron	0	none
418	41867564	A/G	intron	0	none
419	41867637	A/G	intron	0	none
420	41867641	C/T	intron	0	none
421	41867710	C/T	intron	0	none
422	41867797	C/T	intron	0	none
423	41867841	C/T	intron	0	none
424	41867853	C/T	intron	0	none

425	41868045	C/T	intron	0	none
426	41868168	A/G	intron	0	none
427	41868377	A/G	intron	0	none
428	41868410	C/T	intron	0	none
429	41868565	C/T	intron	0	none
430	41868620	A/G	intron	0	none
431	41869046	G/T	intron	0	none
432	41869107	C/T	intron	0	none
433	41869235	C/T	intron	79033882	none
434	41869311	A/G	intron	0	none
435	41869320	C/G	intron	0	none
436	41869331	A/G	intron	0	none
437	41869366	C/G	missense	0	SER,THR
438	41869392	C/T	missense	2241714	ILE,MET
439	41869468	C/G	intron	2241713	none
440	41869621	A/G	intron	0	none
441	41869756	C/T	intron	2241712	none
442	41861858	C/T	intron	11466310	none
443	41863994	A/G	intron	73931466	none
444	41837615	A/G	intron	11466359	none
445	41867772	A/G	intron	0	none
446	41841098	C/T	intron	11466347	none
447	41846164	C/T	intron	0	none
448	41846183	A/T	intron	73045292	none
449	41838206	A/G	intron	8179181	none
450	41853430	A/G	intron	0	none
451	41867783	A/G	intron	11673525	none
452	41839838	G/T	intron	11466353	none
453	41860587	C/T	utr-3	1800468	none
454	41845758	C/G	intron	2278422	none
455	41837378	C/G	intron	0	none
456	41863970	C/T	intron	0	none
457	41848848	A/G	intron	10416269	none
458	41854052	A/C	intron	2241717	none
459	41865067	A/G	intron	73047208	none
460	41865413	C/T	intron	58479032	none
461	41852775	C/T	intron	2288874	none
462	41851315	C/T	intron	7249191	none
463	41854086	C/T	intron	2241716	none
464	41857439	A/G	intron	12983775	none
465	41863777	A/C	intron	11668109	none
466	41860296	A/G	near-gene-3	1800469	none
467	41852115	C/T	intron	1989457	none
468	41866563	A/G	intron	0	none

469	41840050	C/T	intron	0	none
470	41855515	A/G	intron	7258445	none
471	41857404	C/T	intron	12462166	none
472	41865293	A/G	intron	4803458	none
473	41837747	A/G	intron	747857	none
474	41840569	C/G	intron	11466349	none
475	41852979	A/G	intron	2288873	none
476	41854534	A/T	intron	8108632	none
477	41845579	A/G	intron	11466340	none
478	41862562	A/G	intron	0	none
479	41844559	C/T	intron	6508975	none
480	41843461	C/T	intron	11466345	none
481	41849200	C/G	intron	7408955	None
482	41845801	C/T	intron	11466338	None
483	41866273	G/T	intron	11083617	None
484	41866422	A/T	intron	0	None
485	41846200	C/T	intron	76206979	None
486	41859336	A/G	utr-5	11466316	None
487	41854385	A/C	intron	11466324	None
488	41855695	A/G	intron	78430764	None
489	41861748	A/G	intron	11466311	None
490	41848347	A/C	intron	12461895	None
491	41856886	A/C	intron	2241715	None
492	41861674	C/T	intron	2317130	None
493	41847737	A/G	intron	11466334	None
494	41865643	A/G	intron	11083616	None
495	41851716	A/G	intron	10406816	None
496	41839631	C/T	intron	8105161	None
497	41864509	A/T	intron	1982072	None
498	41851186	C/G	intron	8102501	None
499	41864971	A/G	intron	11670143	None
500	41851509	A/C	intron	4803455	None
501	41862253	A/G	intron	11666933	None