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O polimorfismo do antagonista do receptor da interleucina-1 (IL1RN) como fator contribuinte para gravidade da cardite reumática em brasileiros

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

Área de concentração: Processos imunes e Infecciosos
Orientadora: Profa. Dra. Rosa Maria Rodrigues Pereira

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Dedico este trabalho às crianças e adolescentes com febre reumática e suas famílias

À minha esposa Caroline e ao meu filho Caio, sem os quais nada disso, ou qualquer outra coisa, teria a menor importância.

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Abreviaturas dos títulos dos periódicos de acordo com *List of Journals Indexed in Index Medicus*.

Sumário

Lista de Siglas

A1	Alelo 1 para o gene codificador do antagonista do receptor da interleucina-1
A2	Alelo 2 para o gene codificador do antagonista do receptor da interleucina-1
A1/A1	Genótipo homozigoto para o alelo 1 do gene codificador do antagonista do receptor da interleucina-1
A2/A2	Genótipo homozigoto para o alelo 2 do gene codificador do antagonista do receptor da interleucina-1
FRA	Febre reumática aguda
CRA	Cardite reumática aguda
ICC	Insuficiência cardíaca congestiva
IC	Intervalo de confiança
CRC	Cardite reumática crônica
DNA	*DesoxiriboNucleic Acid* (ácido desoxiribonucleico)
HLA	*Human Leukocyte Antigen* (antígeno leucocitário humano)
IL	*Interleukin* (interleucina)
IL1-RA	*Interleukin-1 Receptor Antagonist* (antagonista do receptor da interleucina-1)
IL1RN	*Gene codifing the Interleukin-1 Receptor Antagonist* (gene codificador do antagonista do receptor da interleucina-1)

IL-2r	*Interleukin-2 Receptor* (receptor da interleucina-2)
IFN-γ	*Gama Interferon* (interferon gama)
MHC	*Major Histocompatibility Complex* (complexo de histocompatibilidade principal)
MVL	*Multi Valvar Lesion* (lesão multi-valvar)
OR	*Odds Ratio* (razão de probabilidade)
PBMC	*Peripheral Blood Mononuclear Cells* (células mononucleares derivadas de sangue periférico)
FR	Febre reumática
CG	Cardite Grave
FE	Faringite streptococica
Th1	T helper lynphocyte Type 1 (linfócitos T auxiliadores tipo 1)
Th2	T helper lynphocyte Type 2 (linfócitos T auxiliadores tipo 2)
TNF-α	*Tumor Necrosis Factor alpha* (fator de necrose tumoral alfa)

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Resumo

Azevedo PM. *O polimorfismo do antagonista do receptor da interleucina-1 (IL1RN) como fator contribuinte para gravidade da cardite reumática em brasileiros* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2009. 34p.

A febre reumática (FR) é uma doença imuno-mediada, na qual citoquinas pró-inflamatórias têm um importante papel. Uma produção exacerbada de interleucina-1 (IL-1) parece ser um evento precoce entre as anormalidades imunológicas observadas na FR. O antagonista do receptor de IL-1 (IL1-RA) é um inibidor competitivo endógeno do receptor da IL-1. A razão IL-1RA/IL-1 é importante na determinação da intensidade e duração da resposta inflamatória. O alelo 2 (A2) do gene codificador do IL1-RA (IL1RN) tem sido relacionado a um número de doenças inflamatórias e autoimunes, bem como a uma maior resistência a infecções. Considerando que a FR é uma doença inflamatória autoimune desencadeada por uma infecção bacteriana, nós avaliamos o polimorfismo do IL1RN com o intuito de determinar possível relevância na susceptibilidade à FR e suas manifestações clínicas. O genótipo de 84 pacientes com FR e 84 controles pareados por raça foram determinados através da análise do número de repetições em tandem de 86pb no segundo íntron do IL1RN. O DNA foi extraído de leucócitos de sangue periférico e amplificado com sondas específicas. Dados sobre as manifestações clínicas da FR foram obtidos através de questionários padronizados e extensa revisão de prontuários. Cardite foi definida como sopro cardíaco novo auscultado por médico treinado com a correspondente regurgitação ou estenose valvar ao ecocardiograma. Cardite foi definida como grave na presença de insuficiência cardíaca congestiva ou da indicação de cirurgia cardíaca. A associação estatística entre genótipos, FR e suas variações clínicas foram determinadas. A presença do alelo 1 (A1) e do genótipo A1/A1 foram menos freqüentemente encontradas entre pacientes com cardite severa quando comparado a pacientes sem esta manifestação (OR= 0.11, p=0.031; OR= 0.092, p=0.017). Nenhum dos dois alelos, A1 e A2, foram associados à presença de FR (p=0.188; p=0.106), cardite *sensu latu*, (p=0.578 e p=0.767), poliartrite (p=0.343 e p=0.313) ou coréia (p=0.654 e p=0.633).
Em conclusão, na população brasileira estudada o polimorfismo do IL1RN parece ser um fator relevante para a gravidade da cardite reumática.

Descritores: 1.IL1RN 2.Polimorfismo Genético 3.Febre Reumática 4.Doença reumática cardíaca.

Summary

Azevedo PM. *Interleukin-1 receptor antagonist gene (IL1RN) polymorphism as a contributing factor for the severity of Rheumatic Carditis in a Brazilian cohort* [Thesis]. São Paulo: "Faculdade de Medicina, Universidade de São Paulo"; 2009. 34p.

Rheumatic fever (RF) is an immune-mediated disease in which pro-inflammatory cytokines play an important role. Exacerbated Interleukin-1 (IL-1) production seems to be an early event in the immunological abnormalities that are observed in RF. The Interleukin-1 receptor antagonist (IL-1ra) is an endogenous competitive inhibitor of IL-1. The IL-1ra/IL-1 ratio is important in evaluating the intensity and duration of the inflammatory response. The second allele (A2) for the IL-1ra gene (IL1RN) has been related to a number of inflammatory and autoimmune diseases as well as to a greater resistance to infections. Considering that RF is an inflammatory autoimmune disease that is triggered by a bacterial infection, we have evaluated the IL1RN polymorphism and its possible relevance to the susceptibility to RF and its clinical manifestations. The genotypes of 84 RF patients (Jones criteria) and 84 normal race-matched controls were determined through the analysis of the number of 86-bp tandem repeats in the second intron of IL1RN. The DNA was extracted from peripheral-blood leukocytes and amplified with specific primers. Clinical manifestations of RF were obtained through a standardized questionnaire and an extensive chart review. Carditis was defined as new onset cardiac murmur that was perceived by a trained physician with corresponding valvae regurgitation or stenosis on echocardiogram. Carditis was classified as severe in the presence of congestive heart failure or upon the indication for cardiac surgery. The statistical association among the genotypes, RF and its clinical variations was determined. The presence of allele 1 and the genotype A1/A1 were found less frequently among patients with severe carditis when compared to patients without this manifestation (OR= 0.11, p=0.031; OR= 0.092, p=0.017). Neither allele 1 nor allele 2 were associated with the presence of RF (p=0.188; p=0.106), overall carditis (p=0.578 and p=0.767), polyarthritis (p=0.343 and p=0.313) and chorea (p=0.654 and p=0.633). In conclusion, for this Brazilian cohort, the polymorphism of the IL-1ra gene is a relevant factor for rheumatic heart disease severity.

Descriptors: 1.IL1RN 2.Gene Polymorphism 3.Rheumatic Fever 4.Rheumatic Heart Disease.

1. INTRODUÇÃO

1.1 DEFINIÇÃO

Febre Reumática é uma complicação tardia não supurativa de uma infecção faríngea por cepas de Streptococcus do grupo A de Lancefield. O quadro clínico característico consiste em um quadro febril agudo acompanhado de artrite migratória predominantemente de grandes articulações e/ou sinais clínicos ou laboratoriais de cardite e valvulite duas a três semanas após a faringite desencadeante (Ayoub, 2001). Sintomas secundários ao acometimento de sistema nervoso central (Coréia de Sydenham, alterações de comportamento) são menos freqüentes, mas muito sugestivos, e irão ocorrer após um período de latência mais longo, geralmente de 3 a 6 meses após a infecção (Ayoub, 2001). Os episódios agudos são auto limitados, mas as lesões valvares podem ser crônicas e progressivas, levando a disfunção cardíaca. O diagnóstico tem sido baseado nos critérios de Jones (Tabela 1).

Tabela 1. Critérios de Jones (1992)

Critérios Modificados de Jones para o diagnóstico de Febre Reumática	
Manifestações Menores	**Manifestações Menores**
Cardite	**Achados Clínicos**
Poliartrite	Artralgia
Coréia	Febre
Eritema *Marginatum*	
Nódulos Subcutâneos	**Achados Laboratoriais**
	Elevação de Reagentes de Fase Ativa
	Elevação na Velocidade de Hemossedimentação
	Elevação na Proteína C Reativa
	Prolongamento do intervalo PR no ECG
Evidências de Infecção previa pelo *Streptococcus* do Grupo A	
Cultura faríngea ou teste rápido pra antígenos streptococicos positivos	
Títulos elevados do Título de anticorpos anti-*Streptococcus*	

1.2 EPIDEMIOLOGIA

A incidência, gravidade e mortalidade da Febre Reumática declinaram dramaticamente nos países desenvolvidos durante o século 20. Notavelmente isto ocorreu antes da introdução de antibióticos na prática clinica. Em 1862, a Dinamarca apresentava cerca de 250 novos casos por 100.000 habitantes. Este número caiu para cerca de 100 por 100.000 habitantes em 1962. A partir de 1950, os antibiótico aceleraram este declínio até a marca de 0,23 a 1,88 novos casos por 100.000 habitantes, verificada em 1980. Três notáveis exceções a este declínio são as populações Neo-zelandeza, Havaiana e Maiori, de ancestralidade predominante polinésia, onde a incidência permanece cerca de 13,4 por 100.000 habitantes (Ayoub, 2001).

Nos países em desenvolvimento a febre reumática (FR) continua sendo um grande fardo econômico e social.

No mundo, estima-se que ainda hoje ocorram a cada ano perto de 500.000 novos casos de febre reumática, determinando uma prevalência de mais de 15 milhões de casos de cardite reumática. Cerca de 233.000 pessoas morrem todos os anos em conseqüência desta moléstia. (Carapetis *et al.*, 2005). Na América Latina, *21.000* casos de febre reumática aguda ocorrem anualmente. Os dados no Brasil são escassos. Em 2002, 5.000 casos novos foram reportados (IBGE).

1.3 FISIOPATOLOGIA

A patogênese da FR é complexa, e nela tanto fatores genéticos como ambientais parecem atuar (Ayoub, 2001). Estima-se que cerca de 0,3% a 3,0% dos indivíduos infectados por cepas de *Streptococcus* sabidamente reumatogênicas vão efetivamente desenvolver FR (Ayoub, 2001; Zabriskie, 1985), e aproximadamente um a dois terços deles desenvolverão cardite reumática (Gray *et al.*, 1952; Guilherme *et al.*, 2007; Wilson & Schweitzer, 1954). A susceptibilidade à FR não é completamente compreendida, mas padrões de agregação familiar (Morris *et al.*, 1993; Spagnuolo & Taranta, 1968) e a maior concordância entre gêmeos idênticos em relação a gêmeos não idênticos sugerem influência genética (Hirsch *et al.*, 1996). Da mesma forma, as semelhanças no padrão de apresentação clínica entre irmãos sugerem que as diferentes manifestações da doença podem apontar sua proximidade genética (Hirsch *et al.*, 1996).

Por outro lado, é bem conhecido que a FR é uma doença mediada pelo sistema imune, na qual citoquinas pró-inflamatórias têm importante papel. Uma produção exacerbada de interleucina-1 (IL-1) parece ser um evento precoce entre as anormalidades imunológicas observadas na FR, e é seguida pela produção de interleucina-2 (IL-2) (Spagnuolo & Taranta, 1968). A produção de IL-1 e Interleucina-2 (IL-2) por células mononucleares está exacerbada em pacientes com FR aguda quando comparada a pacientes com faringite streptococica ou FR crônica latente (Spagnuolo & Taranta, 1968).

O antagonista do receptor de IL-1 (IL1-RA) é um inibidor competitivo endógeno do receptor da IL-1. Ele se liga a este receptor sem induzir a cascata intracelular que sinaliza as ações pró-inflamatórias da IL-1 (Figura 1).

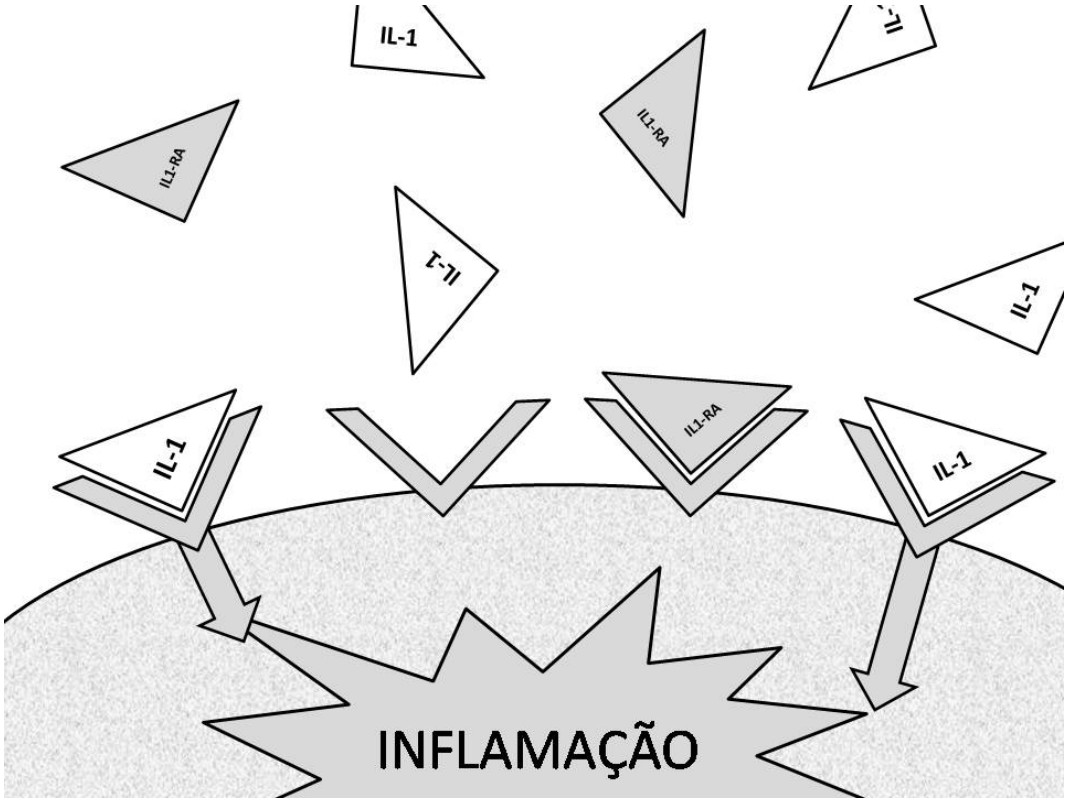
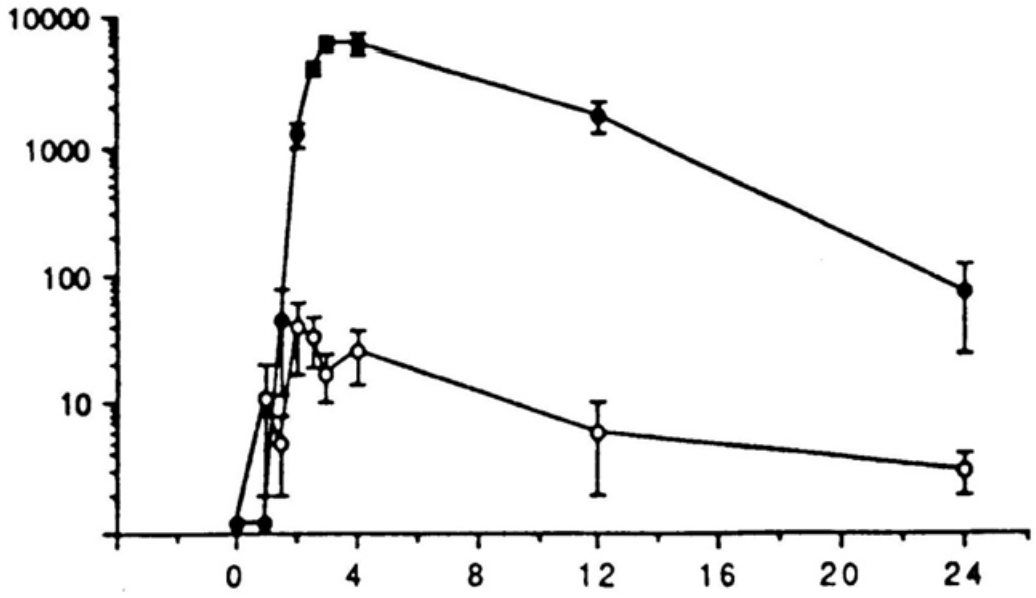


Figura 1. Interleucina-1, seu receptor e antagonista

A concentração plasmática da interleucina-1 cresce precocemente após o estímulo inflamatório. Paralelamente aos seus efeitos pró-inflamatórios, a IL-1 estimula a secreção de seu antagonista principalmente por células mononucleares, iniciando uma alça de retroalimentação negativa que ajuda a sinalizar o término da resposta imune (gráfico 1). A razão IL1-RA/IL-1 é importante na determinação da intensidade e duração da resposta inflamatória (Hirsch *et al.*, 1996).

Gráfico 1. Concentrações plasmáticas (eixo verticas, pg/ml) de IL-1β (o) e IL-1ra (•) ao longo do tempo (eixo horizontal, horas), após endotoxemia experimental (Granowitz *et al.*, 1991)



No segundo *intron* do gene codificador do IL-1ra existe uma seqüência de repetições tandem de 86 pares de bases. O número de vezes que esta seqüência é repetida varia de 2 a 6 (alelos 1 a 5). A freqüência destes alelos varia entre os diferentes grupos étnicos e geográficos, mas em todas as populações estudadas o alelo 1 (A1) foi sempre o mais comum, seguido pelo 2 (A2). Juntos, os demais alelos compreendem menos de 1% na maioria das populações. A prevalência de homozigotos para o IL1RN*2 é tipicamente menor que 10% (Tabela 2).

Tabela 2. Alelos do IL1RN segundo o número de repetições em tandem de 86pb no íntron 2 e sua prevalência aproximada na população (Langdahl *et al.*, 2000)

Alelo	No. Repetições em Tandem *Intron* 2	Prevalência Aproximada de Homozigotos
1	4	~49%
2	2	< 10%
3	3	< 1%
4	5	< 1%
5	6	< 1%

Apesar de diversos polimorfismos terem sido descritos para o gene codificador do IL1-RA *(IL1RN*) (Clay *et al.*, 1996; Langdahl *et al.*, 2000), a maioria está em desequilíbrio de ligação, de forma a que o estudo de um único polimorfismo, a repetição em tandem de 86-pb no segundo *intron* do IL1RN é suficiente para avaliar apropriadamente a variação alélica do gene (Langdahl *et al.*, 2000) (Figura 2).

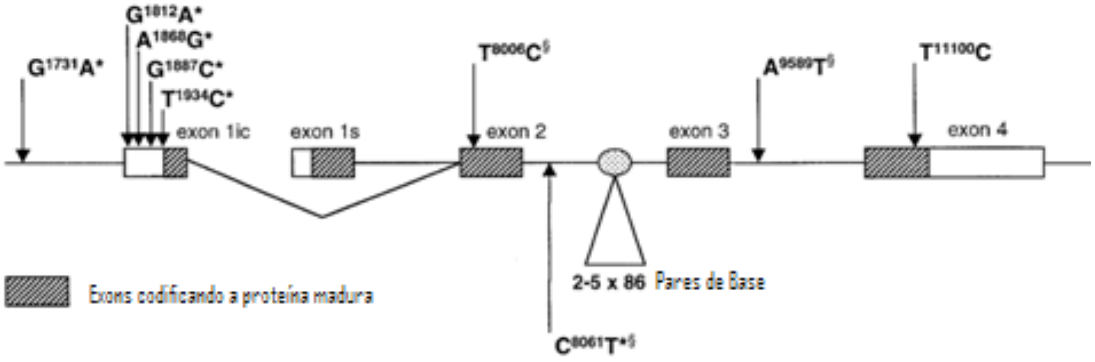


Figura 2. Estrutura do IL1RN com os diversos polimorfismos descritos (Langdahl *et al.*, 2000)

Tem sido aceito que o número de repetições afeta a produção celular de IL1-RA, e que a variação alélica pode influenciar a concentração plasmática de IL1-RA e a resposta imune. A maioria dos estudos associa o alelo 2 a uma resposta imune exacerbada e o alelo 1 a um estado imunológico relativamente brando (Witkin *et al.*, 2002). De fato, o A2 tem sido relacionado a um número de doenças inflamatórias e autoimunes, bem como a uma maior resistência a infecções (Witkin *et al.*, 2002).

Considerando que a FR é uma doença inflamatória autoimune desencadeada por uma infecção bacteriana, avaliamos o polimorfismo do IL1RN com o intuito de determinar possível relevância na susceptibilidade à FR e suas manifestações clínicas.

2. OBJETIVOS

a. Avaliar o polimorfismo do IL1RN como possível marcador de susceptibilidade à febre reumática,

b. Avaliar a presença do polimorfismo do IL1RN com as manifestações clínicas da febre reumática e gravidade de doença.

3. MÉTODOS

3.1 PARTICIPANTES

Foram estudados 84 pacientes consecutivos com diagnóstico de FR que preencheram os critérios de Jones (Tabela 1) e tiveram o diagnóstico confirmado por pelo menos 3 anos de segmento ambulatorial na divisão de Reumatologia Pediátrica da Universidade de São Paulo, Brasil.

Os critérios de exclusão compreenderam história de doenças que pudessem causar dano cardíaco ou valvar (hipertensão, endocardite, doença de Chagas, uso de drogas ilícitas endovenosas), doenças psiquiátricas ou neurológicas, artrite idiopática juvenil, artrite reumatóide, lúpus e outras doenças inflamatórias.

Dados sobre as manifestações clínicas da FR foram obtidos por questionário padronizado e revisão extensa de prontuários. Cardite foi definida como sopro cardíaco novo detectado por médico treinado, com a correspondente estenose ou regurgitação valvar ao ecocardiograma. Artrite foi definida como dor, edema, calor, eritema ou limitação de movimento em grandes articulações, presenciados por reumatologista. Coréia foi definida como movimentos de tronco e/ou extremidades, rápidos, involuntários e despropositados, associados ou não a fraqueza muscular e labilidade emocional (1992).

Todos os pacientes foram submetidos à ecocardiografia transtorácica em pelo menos duas ocasiões durante período de inatividade da doença. Cardite reumática foi graduada em grave ou moderada/leve e definida como grave se acompanhada de sintomas de insuficiência cardíaca congestiva (ICC), lesão valvar grave ao ecocardiograma, ou indicação de cirurgia valvar, esta última de acordo com as diretrizes do American College of Cardiology/American Heart Association, 1998 (Bonow *et al.*, 1998). Cardite foi considerada leve/moderada na ausência de lesões valvares com repercussão hemodinamicamente significativas ou sintomas cardíacos.

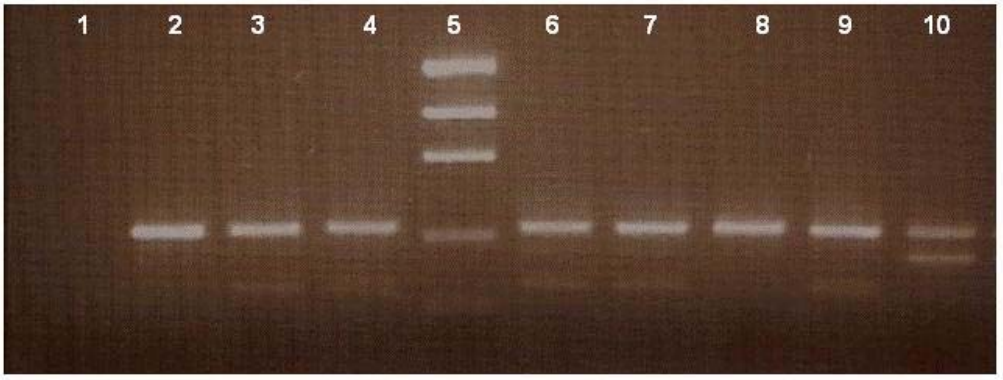
Raça foi definida de acordo com a cor referida para os ascendentes de cada participante até a segunda geração, de acordo com método previamente validado para a população brasileira (Fuchs *et al.*, 2002). A presença de afro-descendentes e caucasianos, ou de pardos entre os ascendentes, determinou a classificação como pardo. Na ausência de informações sobre os avôs, raça foi determinada pela raça referida para os pais. Descendentes das outras raças foram excluídos.

Oitenta e quatro doadores de sangue saudáveis do Hospital Bandeirantes, São Paulo, Brasil, com idade superior a 35 anos (com o intuito de minimizar a possibilidade de incluírem indivíduos que viessem a desenvolver FR) foram convidados a participar como controles. O critério de exclusão foi história de doença crônica ou recorrente.

O estudo foi aprovado por comissão de ética local, e os pacientes (ou seus responsáveis legais) e controles assinaram consenso informado.

3.2 DETERMINAÇÃO GENOTÍPICA DO IL1RN

DNA genômico foi extraído de leucócitos periféricos por meio do kit *GFX (Genomic Blood DNA Purification Kit* - Amersham Bioscience, UK*)*, segundo especificações do fabricante. A genotipagem foi realizada por meio de análise do número de repetições em tandem de 86pb no Segundo *intron* do IL1RN, de acordo com Tarlow et al. (Tarlow *et al.*, 1993), utilizando-se sonda genética específica (5'-ctc agc aac act cct at-3' and 5'-tcc tgg tct gca ggt aa-3'). Os produtos da amplificação genética por PCR (*polymerase chain reaction*) foram corados com brometo de etídio e visualizados em gel de agarose a 1% (Figura 3)



Linha 1: controle negativo
Linhas 2 e 8: genótipo A1/A1
Linhas 3, 4, 6, 7 e 9: genótipo A1/A2
Linha 10: genótipo A1/A4

Alelos	No Rep	No PB
A2	2	240
A4	3	325
A1	4	410
A3	5	500
A5	6	595

Figura 3. Exemplo de visualização em gel de eletroforese do produto da amplificação do IL1RN por PCR

3.3 ANÁLISE ESTATÍSTICA

Os dados foram analisados através do software SPSS 13.0 (SPSS Inc.). Foram calculadas a freqüência dos alelos (número de ocorrências do alelo na população estudada dividido pelo número total de alelos) e a taxa de porte (proporção de indivíduos que possuem uma ou mais cópias do alelo investigado). O equilibrio de Hardy-Weinberg foi testado para o polimorfismo do IL1RN usando o teste qui-quadrado. Para verificar a existência de associação entre as medidas observadas e a presença dos alelos foram também empregados testes qui-quadrado de homogeneidade (Agresti, 1990). Para cruzamentos de medidas incluindo menos de cinco indivíduos, usou-se o teste exato de Fisher. Um nível de significância de 5% foi considerado estaticamente positivo.

4. RESULTADOS

4.1 CARACTERÍSTICAS DOS PARTICIPANTES

A idade dos pacientes variou de 7 a 41 anos (média de 18,6 anos), e a duração média de doença foi 9,4 anos (3-22). A distribuição racial nos 84 pacientes revelou clara predominância de caucasianos (44/52,4%) e pardos (38/45,2%) com uma pequena representação de afro-descendentes (2/2,4%).

Cardite foi observada em 67 (79,8%) pacientes. A maioria (79,1%) foi classificada como leve/moderada e 14 (20,9%) casos foram classificados como graves (Tabela 3). Todos os pacientes com cardite grave apresentaram sintomas de ICC e dez deles também com indicação cirúrgica. Lesão multivalvular (MVL) foi observada em 26 (38,8%) pacientes: em um, 4 válvulas; em dois, 3 válvulas; e em vinte e três, 2 válvulas estavam acometidas. Os três pacientes com envolvimento de 3 ou 4 válvulas tinham doença cardíaca grave segundo os critérios adotados. A válvula mitral estava comprometida em 66 pacientes (98,5%), a aórtica em 20 (29,8%), a tricúspide em 9 (13,4%) e a pulmonar em 1 (1,5%).

As outras manifestações da FR observadas foram: artrite em 54 (64,3%), coréia em 38 (45,2%), eritema marginado em 9 (10.7%), nódulos subcutâneos em 8 (1,2%), artralgia em 63 (75,0%), e febre em 58 (69,0%). Dezessete (20,2%) apresentaram apenas manifestações não cardíacas da FR (Tabela 3).

Tabela 3. Características Clínicas dos pacientes com Febre Reumática

Envolvimento Cardíaco		**Manifestações Não-Cardíacas**	
Cardite (%)	67 (79,8)	Poliartrite (%)	54 (64,3)
Cardite Grave (%)	14 (20,9)	Coréia (%)	38 (45,2)
Indicação de Cirurgia Valvar (%)	10 (11,9)	Eritema Marginado (%)	9 (10,7)
Insuficiência Cardíaca (%)	14 (20,9)	Nódulos Subcutâneos (%)	8 (9,5)
Lesão Multivalvar (%)	26 (38,8)	Artralgia (%)	63 (75)
		Febre (%)	58 (69)

4.2 RESULTADOS DA GENOTIPAGEM

Oitenta e quatro pacientes com FR e 84 controles foram submetidos à análise dos alelos. Um total de 82 pacientes e 78 controles foi avaliado do ponto de vista genotípico, uma vez que apenas dois pacientes e cinco controles portavam o alelo A3, e 1 controle portava o A4 (genótipo A1/A4). Os genótipos A1/A3 e A1/A4 foram muito raros para que conclusões estatísticas fossem tiradas. Estas freqüências alélicas e genotípicas encontradas não estão em concordância com o equilíbrio genético de Hardy-Weinberg.

A taxa de porte para os alelos 1 e 2 foram comparáveis nos pacientes e controles (p= 0,188; p=0,106, respectivamente) (Tabela 4). Cinqüenta e oito pacientes (70,7%) foram homozigotos para o alelo 1 (A1/A1), cinco (6,1%) foram homozigotos para o alelo 2 (A2/A2), e dezenove (23,2%)

possuíam o genótipo A1/A2. Dos 78 controles analisados, 44 (56,4%) foram homozigotos para o alelo 1 (A1/A1), 10 (12,8%) foram homozigotos para o alelo 2 (A2/A2), e 24 (30,8%) apresentaram o genótipo A1/A2. Não houve diferença entre pacientes e controles em relação à freqüência genotípica (p=0,131) (Tabela 4).

Tabela 4. Taxa de porte para os alelos do *IL1RN* e freqüência genotípica em pacientes com Febre Reumática e controles normais

		Febre Reumática	**Controles**	**P**
Alelo N (%)		**N=84**	**N=84**	
Alelo 1				
	Presente	79 (94,0)	73 (86,9)	0,188
	Ausente	5 (6,1)	11 (13,1)	
Alelo 2				
	Presente	24 (28,6)	35 (41,7)	0,106
	Ausente	60(71,4)	49 (58,3)	
Genótipos N (%)		N=82	N=78	
	A1/A1	58 (70,7)	44 (56,4)	
	A1/A2	19 (23,2)	24 (30,8)	0,131
	A2/A2	5 (6,1)	10 (12,8)	

A avaliação das diferentes manifestações clínicas da FR revelou uma menor taxa de porte e uma menor freqüência para o A1 entre os portadores de cardite grave do entre aqueles que não apresentaram essa manifestação

(OR= 0,11, 95% IC: 0,016 – 0,72, p=0,031 e OR=0,32, 95% CI: 0,129 – 0,783, respectivamente) (Tabela 5).

Tabela 5. Taxa de porte dos alelos e freqüência genotípica para o *IL1RN* em relação ao envolvimento cardíaco (Cardite e Cardite Grave) em pacientes com Febre Reumática

	Cardite			**Cardite Grave**		
	Presente	**Ausente**	**P**	**Presente**	**Ausente**	**P**
Taxa de porte dos Alelos n=84 - n (%)						
Alelo 1						
Presente	62 (92,5)	17 (100,0)	0,578	11 (78,6)	68 (97,1)	**0,031**
Ausente	5 (7,5)	0 (0,0)		3 (21,4)	2 (2,9)	
Alelo 2						
Presente	20 (29,9)	4 (23,5)	0,767	7 (50,0)	17 (24,3)	0,101
Ausente	47 (70,1)	13 (76,5)		7 (50,0)	53 (75,7)	
Freqüência dos alelos n=168						
A1	106	31	0,451	18	119	**0,016**
A2	25	4		10	21	
Genótipos n=82 - n (%)						
A1/A1 n(%)	45 (69,2)	13 (76,5)		7 (50,0)	51 (75,0)	
A1/A2 n(%)	15 (23,1)	4 (23,5)	0,494	4 (28,6)	15 (22,1)	**0,021**
A2/A2 n(%)	5 (7,7)	0 (0,0)		3 (21,4)	2 (2,9)	

Semelhantemente, uma distribuição genotípica desigual (A1/A1 vs. A1/A2 vs. A2/A2; p=0,021) foi observada neste grupo (Tabela 6), sendo que

a proporção de pacientes com cardite grave foi menor nos pacientes com genótipo A1/A1 (7 em 58) do que nos pacientes com genótipo A2/A2 (3 em 5) (OR= 0,092, 95% IC: 0,013 – 0,65, p=0,017). Nenhuma outra manifestação clínica foi associada a porte de alelo ou freqüência genotípica (Tabela 6).

Tabela 6. Taxa de porte e freqüência genotípica para o *IL1RN* em relação às manifestações não cardíacas nos pacientes com Febre Reumática

	Poliartrite			**Coréia**		
	Presente	**Ausente**	**P**	**Presente**	**Ausente**	**P**
Taxa de porte dos Alelos n=84 – n (%)						
Alelo 1						
Presente	52 (96,3)	35 (92,1)	0,343	35 (92,1)	44 (95,7)	0,654
Ausente	2 (3,7)	3 (7,9)		3 (7,9)	2 (4,3)	
Alelo 2						
Presente	13 (24,1)	12 (31,6)	0,313	12 (31,6)	12 (26,1)	0,633
Ausente	41 (75,9)	26 (68,4)		26 (68,4)	34 (73,9)	
Freqüência dos alelos n=168						
A1	92	60	0,314	60	77	0,539
A2	15	15		15	14	
Genótipo n=82 - n (%)						
A1/A1 n(%)	40 (75,0)	25 (68,0)		25 (68,0)	33 (73,3)	
A1/A2 n(%)	11 (20,8)	9 (24,3)	0,336	9 (24,3)	10 (22,3)	0,748
A2/A2 n(%)	2 (3,8)	3 (8,1)		3 (8,1)	2 (4,4)	

5. DISCUSSÃO

Este é o primeiro estudo que demonstrou a influência do polimorfismo do IL1RN em uma população brasileira de pacientes com FR, e encontrou uma associação negativa entre cardite grave (CG) e taxa de porte do A1 e o genótipo A1/A1. O resultado contrasta com os achados de Settin e cols. que demonstraram associação positiva entre o genótipo A1/A1, FR e lesão multivalvar (MVL) em 50 crianças egípcias (Settin *et al.*, 2007). O critério usado para a definição de cardite grave pode, em parte, ser responsabilizado por esta diferença. Em nosso trabalho, lesão valvar grave, com repercussões hemodinâmicas significativas, insuficiência cardíaca congestiva ou indicação cirúrgica foram necessárias para definir cardite grave, o que provavelmente selecionou uma doença cardíaca mais agressiva.

Contudo, Chou e colaboradores não encontraram correlações entre os genótipos do IL1RN, FR ou MVL em 115 crianças chinesas de Taiwan (Chou *et al.*, 2005). Estas disparidades ilustram a idéia de que a função de um gene é dependente do contexto no qual está inserido e que um mesmo gene pode ter diferentes expressões em diferentes raças. Esforços para associar FR com Antígenos Leucocitários Humanos (HLA) chegaram a diferentes resultados em diferentes populações (Guilherme *et al.*, 2007) (tabela 7). Em alguns estudos, as associações variaram entre raças mesmo dentro de uma população especifica (Genc *et al.*, 2002; Rider *et al.*, 2000; Tountas *et al.*, 1999; Witkin *et al.*, 2001).

A observação de que o A1 e o genótipo A1/A1 desempenham papel protetor contra cardite grave está em concordância com relatos prévios segundo os quais o A1 foi associado à uma resposta imune mais branda e o A2 à uma resposta mais agressiva (Witkin *et al.*, 2002). Guilherme e colaboradores propuseram um modelo para a fisiopatologia da FR onde o mimetismo molecular inicia uma reação cruzada entre peptídeos derivados de *Streptococcus* do grupo A (principalmente resíduos N-terminais de aminoácidos da proteína-M) e proteínas cardíacas, que leva ao dano cardíaco inicial. A destruição de tecidos do coração a leva à apresentação de antígenos usualmente escondidos, ao recrutamento e expansão policlonal de linfócitos e à exacerbação e perpetuação da autoimunidade e da subseqüente destruição tecidual (Guilherme & Kalil, 2004). (Figura 4)

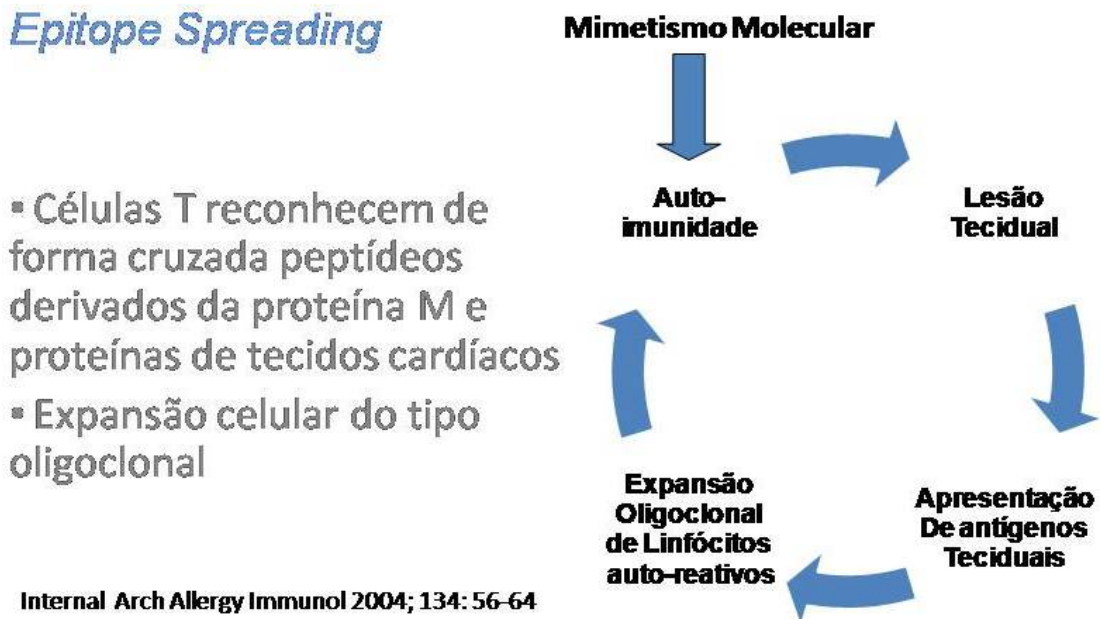


Figura 4. Modelo para a Fisiopatologia da febre reumática segundo Guilherme & Kalil

Neste modelo, a gravidade e perpetuação da reação autoimune tem um papel central, em conjunto com o mimetismo molecular. De fato, diversos dados sugerem que pacientes com FR apresentam uma resposta imune mais intensa e duradora. Os linfócitos de pacientes com febre reumática aguda têm reatividade celular exacerbada *in vitro* quando comparados àqueles de pacientes com gromerulonefrite pós-streptococica aguda, e esta reatividade tem seu pico de 1 a 6 meses após a infecção e dura até 2 anos (Jeremias *et al.*, 1999). Quando estimuladas, células mononucleares derivadas de sangue periférico (PBMC) de pacientes com febre reumática aguda produzem maiores quantidades de IL-1 e IL-2 que PBMC de controles normais, pacientes com faringite estreptocócica (FE) ou cardite reumática crônica (CRC), e esta produção exagerada persiste após 48 semanas. As PBMC de pacientes com febre reumática aguda e cardite reumática aguda (CRA) também expressam maiores proporções de receptores de IL-2 (IL-2r), também conhecido como CD25. A IL-1 é considerada um amplificador da produção de IL-2 e IL-2r, e os autores especulam que a IL-1 é o principal fator responsável pela persistência do processo inflamatório (Morris *et al.*, 1993). Em outro estudo, as concentrações plasmática de IL-1 e IL-2 foram maiores na febre reumática aguda do que na FE, CRC ou em controles sadios, e a produção de IL-2 na febre reumática aguda e CRC correlacionaram-se diretamente com uma porcentagem aumentada de linfócitos CD4+ (T *helper*) e CD25+ (ativados) no sangue periférico (Narin *et al.*, 1995). Os fatores responsáveis por esta imunidade exacerbada não são conhecidos. A análise do perfil de citoquinas *in cito* de células mononucleares infiltradas nos

tecidos cardíacos e a análise da produção *in vitro* de citoquinas por linhagens de células T também infiltradas em tecidos cardíacos (válvulas *versus* átrio), ambas derivadas de pacientes com cardite reumática grave em suas fases aguda e crônica, sugeriu que a regressão da inflamação miocárdica não depende apenas da ação da interleucina 10 (IL-10), a interleucina regulatória predominante. A diferença observada nos tecidos derivados do átrio e das válvulas foram especulativamente atribuídas a células T auto-reativas imigrantes, quimiocinas de ação local e a diferentes expressões de moléculas de adesão. Embora a IL-1, ou seu antagonista, não tenham sidos testados, a predominância de interferon-gama (IFN-γ) e do fator de necrose tumoral alfa (TNF-α) no coração sugerem que uma resposta tipo Th1 poderia mediar a doença reumática cardíaca. A produção de interleucina 4 (IL-4), uma citoquina tipo Th2, pareceu exercer um papel protetor (Guilherme *et al.*, 2004). O fato de que, no presente estudo, o IL1RN tenha influenciado a cardite grave e não as demais manifestações da FR está em concordância com a hipótese de que apenas a cardite grave seja mediada por uma resposta Th1, com um predomínio de IL-1, IL-2 e TNF-α na fase aguda da doença. Artrite, coréia e as formas mais brandas de cardite parecem mediadas por uma resposta imune predominantemente do tipo Th2 (IL-4, IL-5, IL-6, IL-10 e IL-13)

Combinando análise imuno-histoquímica e morfologia comparativa, Fraser e colaboradores dividiram a progressão dos nódulos de Aschoff em três estágios: presença de macrófagos isolados, acumulação dos primeiros linfócitos Ts e, finalmente, o acúmulo de linfócitos B. Eles concluíram que a

secreção macrofágica de IL-1 e TNF-α é necessária para a ativação e agregação de linfócitos T e B, o que sugere que macrófagos chegariam à cena da lesão reumática antes dos linfócitos. IL-2 é usualmente expressa posteriormente e foi encontrada apenas em agregados linfóides (Fraser *et al.*, 1997).

Combinado nossos resultados com os referidos dados sobre a FR, é possível postular um modelo no qual a imunidade cruzada inicial entre peptídeos estreptocócicos e proteínas cardíacas iniciariam um processo autoimune no coração que de outra forma seria auto-limitado se fatores genéticos e ambientais não determinassem uma reação imune mais agressiva, duradora e voltada para o tipo Th1. Os fatores levando para essa característica reação imune parecem agir precocemente no curso do processo inflamatório e também de forma importante localmente, a níveis parácrinos e autócrinos. Sendo que os monócitos e macrófagos são as primeiras células a chegar ao sítio inflamatório, é plausível que as citoquinas secretadas por estas células determinem o padrão da reação autoimune subseqüente através da promoção de moléculas de adesão, recrutamento e da ativação de linfócitos e promoção da apresentação de antígenos. A IL-1 e seu antagonista, o IL1-ra, são secretados precocemente por macrófagos intra-lesionais e a proporção entre estas duas citoquinas é importante para determinação da agressividade manutenção ou extinção da resposta imune (Hirsch *et al.*, 1996). O polimorfismo do *IL1RN* influencia esta proporção e é provavelmente um dos fatores genéticos que influenciam a gravidade da doença cardíaca reumática.

6. CONCLUSÃO

Nossos dados indicam que o polimorfismo do *IL1RN* influencia a gravidade da manifestação cárdica em pacientes brasileiros com febre reumática.

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8. APÊNDICES

8.1 ARTIGO PARA PUBLICAÇÃO

Interleukin-1 receptor antagonist gene (IL-1RN) polymorphism as a possible contributing factor for the severity of Rheumatic Carditis in a Brazilian cohort

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Abstract

Aims: To evaluate the *IL-1RN* polymorphism as a possible marker for Rheumatic Fever (RF) susceptibility or disease severity.

Methods: The genotypes of 84 RF patients (Jones criteria) and 84 normal race-matched controls were determined through the analysis of the number of 86-bp tandem repeats in the second intron of *IL-1RN*. The DNA was extracted from peripheral-blood leukocytes and amplified with specific primers. Clinical manifestations of RF were obtained through a standardized questionnaire and an extensive chart review. Carditis was defined as new onset cardiac murmur that was perceived by a trained physician with corresponding valvae regurgitation or stenosis on echocardiogram. Carditis was classified as severe in the presence of congestive heart failure or upon the indication for cardiac surgery. The statistical association among the genotypes, RF and its clinical variations was determined.

Results: The presence of allele 1 and the genotype A1/A1 were found less frequently among patients with severe carditis when compared to patients without this manifestation (OR= 0.11, p=0.031; OR= 0.092, p=0.017). Neither allele 1 nor allele 2 were associated with the presence of RF (p=0.188; p=0.106), overall carditis (p=0.578 and p=0.767), polyarthritis (p=0.343 and p=0.313) and chorea (p=0.654 and p=0.633).

Conclusion: In the Brazilian population, the polymorphism of the IL-1ra gene is a relevant factor for rheumatic heart disease severity.

Key words: IL1RN; Gene Polymorphism; Rheumatic Fever; Rheumatic Heart Disease.

1. Introduction

Rheumatic Fever (RF) is a late consequence of a pharyngeal infection by Group A *Streptococcus*. RF is still a great economical and social burden in developing countries, where the incidence remains close to 500,000 new cases each year [1]. The pathogenesis of RF is complex and mediated through both genetic and environmental factors [2]. It is estimated that only 0.3 to 3% of individuals who are known to be infected by rheumatogenic streptococcus will develop RF [2, 3] and that one- to two-thirds of these individuals will develop rheumatic carditis [4-6]. The factors that lead to the susceptibility to RF are not well understood, but patterns of familial aggregation [7, 8] and the stronger matching between identical over non-identical twins, both argue in favor of a genetic influence [9]. In the same way, the similarities of RF manifestation in siblings may represent their genetic proximity [9].

On the other hand, it is well known that RF is an immune-mediated disease in which pro-inflammatory cytokines play an important role. Exacerbated Interleukin-1 (IL-1) production seems to be an early event in the immunological abnormalities that are observed in RF, which is subsequently followed by the production of Interleukin-2 (IL-2) [9]. The production IL-1 and IL-2 by peripheral mononuclear cells is exacerbated in acute RF patients when compared with *Streptococcal* pharyngitis or chronic latent RF patients [9].

The Interleukin-1 receptor antagonist (IL-1ra) is an endogenous competitive inhibitor of IL-1 that acts by linking to the IL-1 receptor without initiating the intracellular cascade that leads to the inflammatory effects of the interleukins. The IL-1ra/IL-1 ratio is important in evaluating the intensity and duration of the inflammatory response [10].

Although many polymorphisms have been described for the IL-1ra gene (*IL1RN*) [11, 12], the majority are in linkage disequilibrium with each other, such that a single polymorphism, the 86-bp tandem repeat in the second intron of *IL1RN*, is sufficient to evaluate the allelic variation of the gene [12]. It has been accepted that the number of repeats affects the cellular production of IL-1ra, and each allele may particularly influence the plasma concentration of IL-1ra and its immune response. The majority of studies associate A2 with an exacerbated inflammatory activity and A1 with a relatively mild inflammatory state [13]. In fact, A2 has been related to a number of inflammatory and autoimmune diseases as well as to a greater resistance to infections [13].

Considering that RF is an inflammatory autoimmune disease that is triggered by a bacterial infection, we have evaluated the *IL-1RN* polymorphism and its possible relevance to the susceptibility to RF and its clinical manifestations.

2. Materials and Methods

2.1. Subjects

The study panel consisted of 84 consecutive chronic RF patients who fulfilled the Jones criteria and had their RF diagnosis confirmed by a follow-up after at least 3 years at the Pediatric Rheumatology Unit and Rheumatology Division, University of São Paulo, School of Medicine, Brazil. Exclusion criteria were a history of disease that may cause heart or valvular damage (such as hypertension, endocarditis, Chagas' disease and intravenous illicit drug use), neurological and psychiatric illnesses, juvenile idiopathic arthritis, rheumatoid arthritis and lupus and other inflammatory diseases.

Clinical manifestations of RF were obtained through a standardized questionnaire and an extensive chart review. Carditis was defined as new onset cardiac murmur that was perceived by a trained physician with corresponding valvae regurgitation or stenosis on echocardiogram. Arthritis was defined as tenderness, swelling, heat, redness or limitation of motion in the large joints, as determined by a rheumatologist. Chorea was defined as purposeless, involuntary, rapid movements of the trunk and/or extremities that may or may not be associated with muscle weakness and emotional lability [14].

All patients underwent transthoracic echocardiography on at least two occasions during periods of inactive disease, confirming or not the rheumatic heart disease. Heart rheumatic disease was graded as severe or mild/moderate. Carditis was defined as severe if accompanied by symptoms of congestive heart failure (CHF), a severe valvular lesion on echocardiography or the indication for valvular surgery, with the latter based upon the American College of Cardiology/American Heart Association 1998 Guidelines for the Management of Patients With Valvular Heart Disease [15]. Carditis was considered mild/moderate in patients without significant valvular hemodynamic impairment or heart symptoms.

Race was defined based on the self-reported race of ascendants until the second generation by each participant, as previously validated for the Brazilian population [16]. The presence of both Caucasian and African ascendants in a patient's heritage determined them as 'mixed race'. In the absence of information about the grandparents, race was determined by the race of the parents. Descendants of other races were excluded.

Eighty-four race-matched, healthy blood donors older than 35 years (to minimize the possibility of including individuals who were yet to show susceptibility to RF) from Hospital Bandeirantes (HB), São Paulo, Brazil, were invited to participate as controls. The exclusion criterion was a history of chronic or recurrent disease.

The study was approved by the Local Ethical Committee, and patients and controls (or their respective legal responsible) signed a consent form.

2.2. L-1RN Genotyping

Genomic DNA was extracted from peripheral-blood leucocytes using the *GFX Genomic Blood DNA Purification Kit* (Amersham Bioscience, Little Chalfont, UK), following specifications of the manufacturer. Genotyping was performed through the analysis of the number of 86-bp tandem repeats in *IL-1RN* intron 2, according to Tarlow et al. [17], using standard primers (5'-ctc agc aac act cct at-3' and 5'-tcc tgg tct gca ggt aa-3'). Ethidium bromide-stained polymerase chain reaction (PCR) products were visualized on a 1% agarose gel.

2.3. Statistical analysis

Data were analyzed using SPSS 13.0 software (SPSS Inc, Chicago, USA.). Allele frequencies (number of occurrences of the investigated allele in the population divided by the total number of alleles) and carriage rate (proportion of individuals who have at least one copy of the investigated allele) were calculated. Hardy-Weinberg equilibrium was tested for the IL1RN polymorphism using the X^2 – test. The X^2 statistic was also used to verify the existence of an association between the analyzed parameters and the presence of the alleles [18]. For 2X2 tables of data that included less than five individuals per cell, Fisher's exact test was used. A 5% significance level was considered to indicate statistical significance.

3. Results

3.1. Patient characteristics

The age of RF patients varied from 7 to 41 years old (median = 18.6), and the median disease duration was 9.4 years (3-22). Race distribution in 84 RF patients revealed a clear predominance of Caucasians (44/52.4%) and mixed race (38/45.2%), with a small representation of African descendants (2/2.4%).

Carditis was observed in 67 (79.8%) patients. The majority (79.1%) of carditis was graded as mild/moderate, and 14 (20.9%) cases were graded as severe (Table 1). The severe carditis cases all had CHF symptoms, with ten of those also indicated for surgery. Multivalvar damage was observed in 26 (38.8%) patients, with 1 patient having 4 damaged valves, 2 patients 3 damaged valves and 23 patients having 2 damaged valves. The three patients with three- or four-valve involvement had severe cardiac disease, as determined by the adopted criteria. The mitral valve was compromised in 66 patients (98.5%), the aortic valve in 20 (29.8%), the tricuspid valve in 9 (13.4%) and the pulmonary valve in one (1.5%).

The other clinical manifestations that were observed in RF were 54 (64.3%) patients with polyarthritis, 38 (45.2%) with chorea, 9 (10.7%) with erythema marginatum, 8

(1.2%) with subcutaneous nodules, 63 (75.0%) with arthralgia and 58 (69.0%) with fever. Seventeen (20.2%) had solely non-cardiac manifestations of RF (Table 1).

3.2. Genotyping Results

For the allelic analysis, a total of 84 RF patients and 84 controls were evaluated. For genotype purposes, a total of 82 patients and 78 controls were evaluated, since two patients and five controls carried the A3 allele and one control carried an A4 allele (A1/A4 genotype). The genotypes A1/A3 and A1/A4 were too rare to draw statistical conclusions. These allele and genotype frequencies are not in accordance with Hardy-Weinberg of genetic equilibrium.

The *1L-1RN* carriage rate for allele 1 (94 vs. 86.9%, p=0.188) and allele 2 (28.6 vs. 41.7%, p=0.106) was comparable in the RF and controls. Fifty-eight patients (70.7%) were homozygous for allele 1 (A1/A1), five (6.1%) were homozygous for allele 2 (A2/A2) and 19 (23.2%) were heterozygous for alleles 1 and 2 (A1/A2). From the 78 controls who were analyzed, 44 (56.4%) were homozygous for A1, 10 (12.8%) were homozygous for A2 and 24 (30.8%) were heterozygous for A1 and A2. No significant difference was observed between the patients and controls with regard to genotype frequency (p=0.131).

The evaluation of the different clinical manifestations showed that the A1 allele was less carried (OR= 0.11, 95% CI: 0.016 – 0.72, p=0.031) and less frequent (OR=0.32, 95% CI: 0.129 – 0.783, p=0.016) in patients with severe carditis than in patients without this manifestation. Accordingly, an uneven genotype distribution (A1/A1 vs. A1/A2 vs. A2/A2; p=0.021) was observed in this group, with severe carditis being less frequent among those patients with the A1/A1 genotype (7 out of 58) than in those with the A2/A2 genotype (3 out of 5) (OR= 0.092, 95% CI: 0.013 – 0.65, p=0.017). No other clinical manifestation was associated with allele carriage or genotype frequency (Table 3).

4. Discussion

This is the first study that demonstrates the influence of an *IL-1RN* polymorphism in RF for a Brazilian population, with a negative association being found between severe carditis and the A1 carriage rate and the A1/A1 genotype. This is in contrast to the findings of Settin *et al*., who demonstrated a positive association between the A1/A1 genotype, RF and multivalvar lesions (MVL) in 50 Egyptian children [19]. The criteria for the definition of severe carditis may, in part, account for this difference. In our work, severe valvar lesions with significant hemodynamic repercussions,

congestive heart failure or surgery indication was necessary to define severe carditis, which probably selected for a more aggressive heart disease.

However, Chou *et al.* found no statistical correlation between the *IL-1RN* genotypes, RF or MVL in 115 Chinese children from Taiwan [20]. These disparities illustrate the idea that the function of a gene is dependent on its genomic context and that the same gene may have different expression patterns in different races. Efforts directed towards associating RF with specific Human Leukocyte Antigens (HLA) have led to different results in different populations [4]. In some studies, the associations varied between races within a specific population [21-24].

The observation that A1 and A1/A1 have a protective effect against severe carditis is in agreement with previous reports in which the A1 allele was associated with a milder immune response and the A2 allele with a more aggressive response [13]. Guilherme *et al.* proposed a model of RF physiopathology in which molecular mimicry triggers a cross-reaction between group A Streptococcus peptides (mainly the N-terminal amino acid residues of the M-protein) and heart proteins, which leads to initial heart damage, tissue destruction, the presentation of usually hidden heart antigens, polyclonal lymphocyte recruitment and expansion and an enhancement and perpetuation of autoimmunity and its subsequent tissue destruction [25]. In this model, the severity and perpetuation of the autoimmune reaction plays a central role, along with molecular mimicry. In fact, several observations suggest that RF patients have a more severe and long-lasting immune reaction. The lymphocytes of acute RF (ARF) patients have exacerbated in vitro cellular reactivity as compared to those patients with acute post-streptococcal glomerulonephritis; this reactivity peaks at 1 to 6 months after onset and lasts for at least 2 years [26]. When stimulated, the peripheral blood mononuclear cells (PBMC) of ARF patients produced larger amounts of IL-1 and IL-2 than do PBMCs from normal controls, streptococcal pharyngitis (SP) patients or chronic rheumatic heart disease (CRHD) patients. This IL-2 overproduction persists after 48 weeks. The PBMCs of ARF and acute rheumatic heart disease (ARHD) patients also express higher proportions of IL-2 receptors (CD25). IL-1 is considered to be an amplifier of IL-2 and IL-2r production, and the authors speculated that IL-1 is the major factor that is responsible for the persistent inflammatory process [26]. In another study, the plasma concentration of IL-1 and IL-2 were higher in ARF than in SP, CRHD or normal control samples, and the production of IL-2 in ARF and CRHD directly correlated with increased percentages of CD4+ (T helper) and CD25+ cells in the peripheral blood [27].

The factors behind this exacerbated immunity in RF are not known. The analysis of the in situ cytokine profile of heart-infiltrating mononuclear cells and the cytokine pattern produced in vitro by heart-infiltrating T-cell lines (HILs) from the heart tissue (valvar versus atrium) of severe RHD patients in the acute or chronic phases of the disease suggested that Th1-type cytokines could mediate RHD. The production of IL-4 (a Th2-type cytokine) seemed to play a protective role [28]. In the same way, the fact that, in this study, IL-1RN influenced severe carditis but not the other manifestations of RF is in agreement with the hypothesis that only severe carditis is mainly mediated through a Th-1 immune response, with a preponderance of IL-1, IL-

2 and TNF-α being available in the acute phase of the disease. Arthritis, chorea and the milder forms of carditis seem to be associated with a preponderance of the Th-2 type immune response (IL-4, IL-5, IL-6, IL-10 and IL-13) [2, 29].

Combining immuno-histochemical analysis with comparative morphology, Fraser *et al*. divided the progression of the Aschoff nodules into three chronologic stages, which were macrophages only, accumulation of first T lymphocytes and finally the accumulation of B lymphocytes. They concluded that IL-1 and TNF-α secretion in macrophages is required for T and B lymphocyte activation and aggregation, which suggests that the macrophages arrive at the scene of rheumatic injury prior to lymphocytes. IL-2 is usually expressed later and was found only in the lymphoid aggregates [30].

Combining our results with the previous RF data , it is possible to postulate a model in which the initial immune cross-reaction between *Streptococcus* peptides and heart proteins triggers an autoimmune process in the heart that would otherwise be self-limited, if genetic and environmental factors did not determine a stronger, longer and more Th1-type oriented immune reaction. The factors leading to this characteristic immune reaction seem to act early in the course of the inflammation process, as well as locally, at the paracrine and autocrine level. Since monocytes and macrophages are the first cells to arrive at the inflammation site, it is plausible that the cytokines that are secreted by these cells determine the pattern of the subsequent immune reaction through the promotion of adhesion molecules, lymphocyte recruitment and activation and the promotion of antigen presentation. IL-1 and its antagonist, IL-1ra, are secreted early by intra-lesional macrophages, and the proportion of these two cytokines is important for the determination of the maintenance or extinction of the immune response [10]. The *IL-1RN* polymorphism influences this proportion and is likely to be one of the genetic factors that influence the severity of rheumatic heart disease.

In conclusion, our data indicate that this *IL-1RN* polymorphism may influence the severity of the heart manifestation in Brazilian RF patients.

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