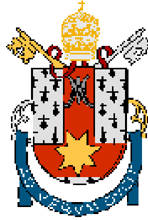

**Pontifícia Universidade Católica do Rio Grande do Sul**
**Faculdade de Biociências**
**Programa de Pós-Graduação em Biologia Celular e Molecular**

# Variantes polimórficas 2029C>T e 2258G>A no gene que codifica para o TLR2 humano: avaliação de uma população brasileira e revisão sistematizada.

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**Porto Alegre**
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**Dissertação apresentada como requisito para obtenção do grau de Mestre pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Pontifícia Universidade Católica do Rio Grande do Sul.**

**Porto Alegre**
**2009**

**Aos meus pais,**
**fontes de sabedoria e amor incondicional.**

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À Deus, por me conceder a vida e pelas suas carinhosas bênçãos.

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"[...] acho que só há um caminho para a ciência — ou para a filosofia: encontrar um problema, ver a sua beleza e apaixonarmo-nos por ele; casarmo-nos com ele, até que a morte nos separe — a não ser que obtenhamos uma solução. Mas ainda que encontremos uma solução poderemos descobrir, para nossa satisfação, a existência de toda uma família de encantadores, se bem que talvez difíceis, problemas-filhos, para cujo bem-estar poderemos trabalhar, com uma finalidade em vista, até ao fim dos nossos dias".
- Karl Popper

"A coisa mais bela que o homem pode experimentar é o mistério. É essa emoção fundamental que está na raiz de toda ciência e toda arte".
- Albert Einstein

"A maturidade do homem consiste em haver reencontrado a seriedade que tinha nas brincadeiras de quando era criança".
- Friedrich Nietzsche

**RESUMO**

O Toll-like receptor 2 (TLR2) é um receptor de reconhecimento de microorganismos que identifica um amplo repertório de padrões moleculares associados a patógenos. Entre os polimorfismos já descobertos no gene que codifica para o TLR2, dois SNPs (*single nucleotide polimorphism*) (supposed-2029C>T e 2258G>A) podem estar relacionados com a redução da ativação do NF-kB e, consequentemente, com o aumento do risco às doenças infecciosas. O objetivo deste estudo foi investigar os SNPs supposed-2029C>T e 2258G>A do TLR2 em 422 pacientes criticamente doentes oriundos de uma população do sul do Brasil (295 com sepse e 127 sem sepse), e realizar uma revisão sistematizada de 33 trabalhos publicados sobre esses SNPs conduzindo um estudo de avaliação de qualidade com um sistema de escores. Entre os pacientes admitidos no estudo foi encontrado apenas um heterozigoto (0,2%; 1/422) para o SNP supposed-2029C>T e nenhum para o SNP 2258G>A (0%; 0/422). Assim, não foi possível identificar qualquer aplicabilidade clínica entre esses SNPs do TLR2 nos pacientes críticos do sul do Brasil. A revisão sistematizada detectou que os atuais trabalhos com tais SNPs do TLR2 apresentam conclusões controversas e contraditórias resultantes de estudos com uma ampla variedade de critérios de qualidade. Os resultados sugerem que, quando analisados individualmente, os SNPs supposed-2029C>T e 2258G>A não são bons candidatos para os trabalhos que buscam por aplicações clínicas diretas entre genótipo e fenótipo. Esforços futuros para aumentar o conhecimento e para realizar análises simultâneas com outros polimorfismos poderão revelar efeitos mais efetivos do TLR2 na susceptibilidade às doenças infeciosas.

Palavras Chave: TLR2; 2029C>T; 2258G>A; Arg677Trp; Arg753Gln

**ABSTRACT**

Toll-like receptor 2 (TLR2) is a recognition receptor for the widest repertoire of pathogen-associated molecular patterns. Two polymorphisms of TLR2 could be linked to reduced NF-kB activation and to increased risk of infection (supposed-2029C>T and 2258G>A). We investigated the supposed-2029C>T and 2258G>A TLR2 polymorphisms in 422 critically ill patients with European origin from southern Brazil (295 with sepsis and 127 without sepsis), and we reviewed of 33 studies with these polymorphisms conducting a quality assessment with a score system. Among our patients we found only one heterozygote (1/422) for the supposed-2029C>T and none of 2258G>A (0/422) SNP. We have failed to find a clinical application of supposed-2029T and 2258A allele analysis in our southern Brazilian population. Our review detected that current TLR2 SNP assays had very controversial and contradictory results derivate from reports with a variety of quality criteria of investigation. We suggest that, if analyzed alone, the supposed-2029C>T and 2258G>A TLR2 SNP are not good candidates for genetic markers in studies that search for direct or indirect clinical applications between genotype and phenotype. Future efforts to improve the knowledge and to provide other simultaneous genetic markers might reveal a more effective TLR2 effect on the susceptibility to infections diseases.

Key Words: TLR2; 2029C>T; 2258G>A; Arg677Trp; Arg753Gln

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# LISTA DE ABREVIATURAS E SIGLAS

˚C: Graus Celsius
A: Adenina
APACHE II: *Acute Phsysoilogy and Chronic Health Evaluation*
APS: *Acute Physiology Score*
bpm: batimentos por minuto
C: Citosina
CD14: *Cluster of Differenciation* 14
cels/mm³: células por milímetro cúbico
CMV: Citomegalovírus.
DNA: Deoxyribonucleic Acid.
G: Guanina
IKK: *Inhibitor of nuclear factor-kB*.
IL: Interleucina
IRAK: IL-1R-*associated kinase*
irpm: impulso respiratório por minuto
LPS: Lipopolissacarídeos
MAP Kinases: *Mitogen activated protein*
mmHg: milímetros de Mercúrio
MODS: *Multiple Organ Dysfunction Syndrome*
MOF: *Multiple Organ Failure*
MyD88: *Myeloid Differentiation Factor*
NEMO: *NF-kB essential modulator*.
NF-kB: *Nuclear factor kB*.
PaCO2: pressão parcial de Dióxido de Carbono
PAMP: *Pathogen-associated molecular patterns*
PRR: *Pattern Recognition Receptor*
SIRS: Síndrome da Resposta Inflamatória Sistêmica
SNP: *Single Nucleotide Polymorphism*.
SOFA: *Sequential Organ Failure Assessment*
T: Timina
TAB: *TAK1-binding protein*
TAK1: *Transforming-growth factor-β-activated kinase*
TIR: *Toll Interleucine Receptor*
TLR: *Toll-like Receptor*
TNF-α: *Tumor Necrosis Factor alpha*
TRAF6: *Tumor-necrosis-factor receptor-associated factor 6*
UBC13: *Ubiquitin conjugating enzyme* 13
UEV1A: *ubiquitin conjugating enzyme* E2 *variant* 1
UTI: Unidade de Terapia Intensiva
UTIG-HSL-PUCRS: Unidade de Terapia Intensiva Geral do Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul.

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# 1 FUNDAMETAÇÃO TEÓRICA E OBJETIVOS

## 1.1 FUNDAMENTAÇÃO TEÓRICA

### 1.1.1 Pacientes críticos internados na UTI

Os pacientes admitidos nas Unidades de Terapia Intensiva (UTI) representam entre 5% e 15% de todos os pacientes hospitalizados, mas o custo do tratamento destes pacientes é seis vezes maior do que os dos demais [Martin *et al.*, 2005; Ylipalosaari *et al.*, 2006]. Os pacientes internados nas UTIs são caracterizados por apresentarem um quadro patológico crítico e complexo decorrente de fragilidades fisiológicas graves e um risco de infecção de cinco a 10 vezes maior do que os pacientes de outras alas hospitalares [Vincent *et al.*, 2002; Vosylius *et al.*, 2003]. A alta incidência de infecção na UTI tem sido associada com a permanência prolongada destes pacientes na unidade, com a evolução da severidade da disfunção orgânica e com o aumento da taxa de mortalidade [Vosylius *et al.*, 2003].

Nos últimos 20 anos, vários instrumentos de medida de predição de risco têm sido aplicados aos pacientes críticos de UTIs na tentativa de reconhecer as melhores estratégias terapêuticas. A avaliação do quadro crítico, nos dias de hoje, é principalmente realizada através de instrumentos que analisam a disfunção de órgãos e sistemas através do monitoramento diário de seu estado fisiológico. O escore SOFA (*Sequentical Organ Failure Assessment*) avalia diariamente a condição de seis sistemas orgânicos (respiratório, renal, hepático, hematopoiético, cardiovascular e neurológico), independentemente da terapia a qual o paciente está sendo submetido [Vincent *et al.*, 1998]. Outro escore utilizado é o APACHE II (*Acute Physiology and Chronic Health Evaluation*), um sistema de classificação que usa pontuação baseada em 12 medidas fisiológicas, idade e mais as doenças anteriores para prover uma medida geral da severidade do quadro clínico do paciente. O APACHE II é um sistema útil e de confiança para classificar os pacientes da UTI: o aumento do APS (*Acute Physiology Score*), que é o escore fisiológico, é associado

ao aumento do risco de morte. Este sistema é indicado para ser usado nos primeiros momentos, como na emergência ou no momento de admissão na UTI [Knaus *et al*., 1985].

### 1.1.2 Sepse

A sepse é uma condição bastante freqüente em UTIs com um alto índice de mortalidade. Nos Estados Unidos foram estimados aproximadamente 750.000 casos de sepse por ano com a mortalidade em torno de 28,6% [Angus *et al*., 2001; O'Brien *et al*., 2007]. Na Europa, mais de 35% dos pacientes da UTI tiveram sepse com a mortalidade chegando aos 27% [Vincent *et al*., 2006]. No Brasil, um estudo de Silva e colaboradores [Silva *et al*., 2004] mostrou que a incidência de sepse é de 61.4 a cada 1.000 paciente por dia com a mortalidade chegando a 33.9%.

A sepse é atualmente vista como uma complexa desregulação da inflamação que surge quando o hospedeiro é incapaz de conter a infecção. Esta desregulação afeta múltiplos órgãos através de efeitos em células endoteliais, epiteliais e imunes que levam a um dano irreversível (Figura 1) [Buras *et al*., 2005].

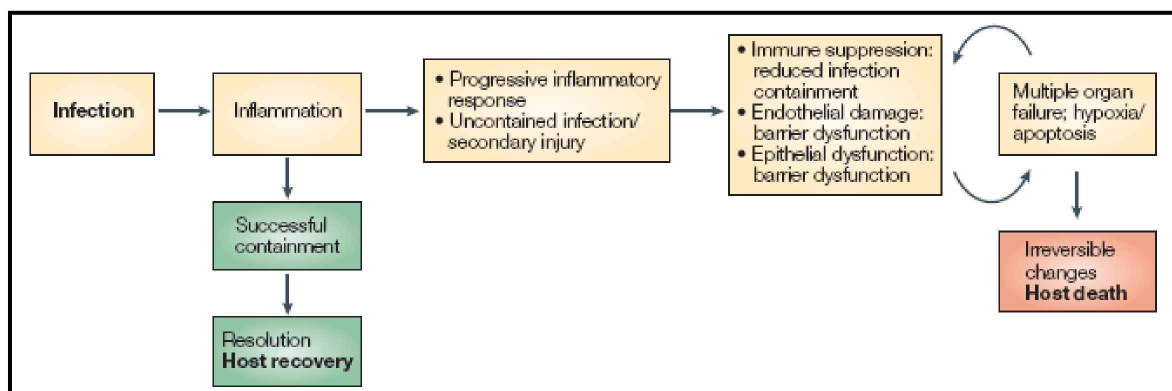


**Figura 1:** Patogênese da sepse. Extraído de Buras *et al*, 2005.

No ano de 1992, foram estabelecidos conceitos que podem ser aplicados em pacientes nos diferentes estágios da sepse. O conceito de SIRS (*Systemic Inflammatory Response Syndrome*) (Figura 2) foi postulado como Síndrome da Resposta Inflamatória Sistêmica. A SIRS é diagnosticada quando são manifestados dois ou mais dos critérios a seguir: (I) Febre, temperatura corporal >38ºC ou

hipotermia, temperatura corporal <36ºC; (II) Taquicardia, freqüência cardíaca >90 bpm; (III) Taquipnéia, freqüência respiratória >20 irpm ou $PaCO_2$ <32 mmHg; (IV) Leucocitose ou leucopenia, Leucócitos >12.000 cels/$mm^3$ ou <4.000 cels/$mm^3$, ou presença de >10% de neutrófilos de formas jovens (bastões) [Bone *et al.*, 1992] .

A resposta inflamatória sistêmica está associada a um grande número de condições clínicas. Além das infecções que podem produzir a SIRS, causas patológicas não infecciosas podem incluir pancreatites, isquemia, traumas múltiplos e lesão nos tecidos, choque hemorrágico e administração exógena de alguns mediadores do processo inflamatório como o fator de necrose tumoral e outras citocinas. Uma freqüente complicação da SIRS é o desenvolvimento de uma disfunção sistêmica dos órgãos, incluindo condições clínicas como lesão aguda nos pulmões, choque, falha renal e Síndrome da Disfunção Múltipla dos Órgãos (MODS; *Multiple Organ Dysfunction Syndrome*) [Bone *et al.*, 1992].

A sepse é definida como a SIRS com um processo infeccioso confirmado. A infecção se origina pela presença de microorganismos ou pela invasão destes organismos normalmente em tecidos estéreis do hospedeiro [Bone *et al.*, 1992]. A ocorrência de sepse por bactérias Gram-positivas é estimada em 30% a 50%, por Gram-negativa em 25% a 30%, por fungos e parasitas em 1% a 3% e vírus em 2% a 4% [Annane *et al.*, 2005]. A sepse severa é conceituada como sepse associada à disfunção orgânica, hipoperfusão tecidual que inclui acidose lática, oligúria e alteração aguda do nível mental [Bone *et al.*, 1992]. O choque séptico é uma complicação da sepse caracterizado por uma hipotensão refratária, a qual é a principal causa dos óbitos. Se a hipotensão ou hipoperfusão induzidas pela sepse são refratárias à ressuscitação volêmica adequada e, se há necessidade subseqüente de administração de agentes vasopressores, as complicações circulatórias podem levar à Falência de Múltiplos Órgãos (MOF; *Multiple Organ Failure*) ou à MODS. A falência de múltiplos órgãos é uma alteração tão severa na função orgânica que sua homeostasia não pode ser mantida sem intervenção terapêutica artificial [Levy *et al.*, 2003].

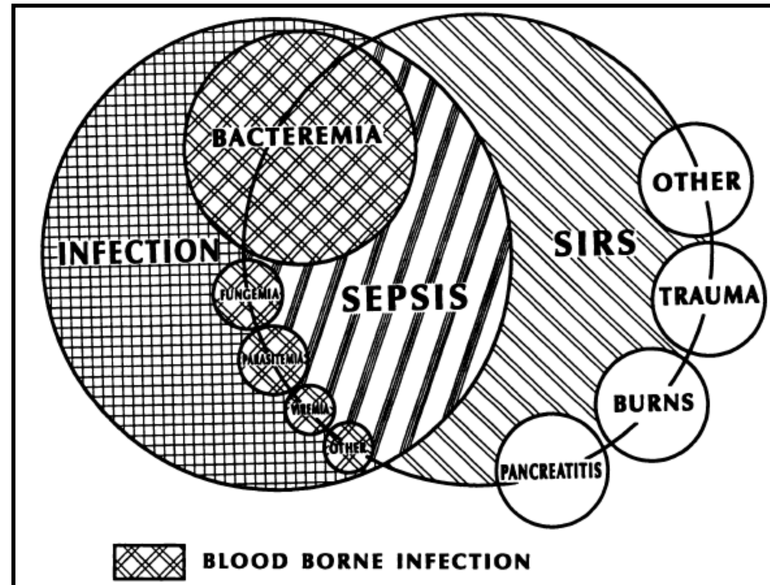


**Figura 2:** Inter-relação entre SIRS, sepse e infecção. Extraído de Bone *et al*, 1992.

No quadro de choque séptico, a vasodilatação periférica é evidenciada e há excessiva presença de agentes pró-inflamatórios que, juntos, acentuam ainda mais a disfunção e a falência de múltiplos órgãos. Detectam-se evidências de disfunção muito antes da falência de órgãos, resultado da reação inflamatória pela massiva liberação de citocinas. A resposta sistêmica à infecção é mediada através das citocinas derivadas de macrófagos que alvejam os receptores da extremidade-órgão em resposta a ferimento ou infecção [Bone *et al*., 1997]. Tal resposta inflamatória à infecção ou ao ferimento é uma reação altamente conservada e regulada do organismo. A liberação concomitante de agentes pró-inflamatórios e anti-inflamatórios mantém a homeostasia do organismo. A reação anti-inflamatória pode ser maior e algumas vezes mais longa que a pró-inflamatória; o objetivo disto é diminuir a síntese de agentes pró-inflamatórios, conservando o equilíbrio homeostático [Bone *et al*., 1997].

Sendo a sepse uma condição freqüente no âmbito da terapia intensiva, que cursa com elevada mortalidade e com tratamento com custo econômico elevado, sua abordagem é de interesse direto do sistema de saúde. O estudo da sepse deve contribuir para os levantamentos epidemiológicos e pautar-se numa abordagem direcionada para o conhecimento dos mecanismos moleculares e celulares que desencadeiam as variações fisiopatológicas. Este conhecimento básico poderá contribuir para a modulação da seqüência de eventos que culmina nos desfechos

desfavoráveis. Conhecer as bases genéticas de tais eventos é, portanto, fundamental.

### 1.1.3 Imunidade Inata

A primeira linha de defesa contra infecção é provida pelo sistema imune inato, o qual reconhece classes genéricas de moléculas produzidas por vários microorganismos patogênicos [Hollingsworth *et al.*, 2007]. A imunidade inata, ao contrário da imunidade adaptativa que se molda ao agente infeccioso, desempenha um papel fundamental nos processos infecciosos e inflamatórios uma vez que provoca uma resposta inflamatória generalista, na qual certas células (macrófagos, monócitos, granulócitos e células dendríticas) detêm o agente invasor impedindo que ele se espalhe [Zweigner *et al.*, 2001].

Uma função importante para a resposta imune inata é de recrutar mais células fagocitárias e moléculas efetoras para o local da infecção, através da liberação de uma bateria de citocinas e de outros mediadores inflamatórios que têm profundos efeitos sobre os fatos subseqüentes. As citocinas, cuja síntese também é estimulada quando os macrófagos reconhecem constituintes microbianos, são chamadas freqüentemente de monocinas, uma vez que são elaboradas principalmente por células da linhagem monócito-macrófago; as monocinas compreendem um grupo estruturalmente diferenciado de moléculas e incluem as interleucinas IL-1 (*interleukin 1 receptor)*, IL-6 (*interleukin 6 receptor)*, IL-8 (*interleukin 8 receptor)*, IL-12 (*interleukin 12 receptor)* e o fator de necrose tumoral (TNF-α), todos apresentam importantes efeitos locais e sistêmicos. Os efeitos combinados desses mediadores contribuem para as reações locais contra a infecção na forma de resposta inflamatória [Bevilacqua, 1993; Downey, 1994; Springer, 1994].

As respostas inflamatórias são caracterizadas pela dor, rubor, calor e pelo tumor no sítio de uma infecção. A primeira dessas alterações reside num aumento do diâmetro vascular, levando a um aumento do volume sanguíneo local. Uma vez que a infecção se dissemine para a corrente circulatória, os mesmos mecanismos através dos quais o TNF-α continha a infecção local com tanta eficiência, tornam-se ineficazes. A sepse é, portanto, acompanhada pela liberação de TNF-α pelos

macrófagos no fígado, no baço e em outros órgãos. A liberação sistêmica de TNF-α causa vaso-dilatação e perda do volume plasmático, devido a um aumento da permeabilidade vascular, conduzindo ao choque [Cohen J, 2002].

No choque séptico, a coagulação intravascular disseminada é igualmente deflagrada pelo TNF-α, levando à formação de microtrombos e ao consumo de proteínas de coagulação, de modo que o paciente perde a capacidade de coagular o sangue de maneira apropriada. Tal condição leva com freqüência à falência de órgãos vitais tais como os rins, o fígado, o coração e os pulmões, que são rapidamente comprometidos pela insuficiência da perfusão normal (formação de microtrombos) [Cohen J, 2002; O'Shea *et al.*, 2002; Matsuda *et al.*, 2006].

O reconhecimento de padrões moleculares associados à patógenos (*Pathogen-associated molecular patterns;* PAMPs) por receptores de reconhecimento padrão (*pattern recognition receptor;* PRR), e a subseqüente ativação de citocinas, são características importantes envolvidas durante o quadro séptico. Este reconhecimento de componentes de bactérias pelo sistema imune inato é um evento chave para o início da resposta inflamatória, a qual é necessária para deter os microorganismos invasores. No entanto, uma resposta inflamatória incontrolada pode ser a causa de disfunções orgânicas, hipotensão ou choque séptico [Zweigner *et al.*, 2001; Werling *et al.*, 2003; Nakada *et al.*, 2005].

### 1.1.4 Receptores Toll-Like (TLR)

Os TLRs, também conhecidos como PRR, têm um papel essencial no início da imunidade inata reconhecendo uma grande variedade de PAMPs de bactérias, protozoários, vírus e fungos, tornando-se indispensável na ligação da imunidade inata com a imunidade adquirida [Texereau *et al.*, 2005].

Os TLRs compreendem uma família de, pelo menos, 12 proteínas de membrana do tipo I com um domínio extracelular rico em repetições de leucina (Leu) e um domínio citoplasmático homólogo ao receptor da interleucina I (*interleukin 1 receptor*; IL-1R) chamado de Toll-interleucina-1 (*toll interleucine receptor*; TIR) [Akira *et al.*, 2001; Sandor *et al.*, 2005; Trinchieri *et al.*, 2007].

A família dos TLRs é expressa em várias células imunes, incluindo macrófagos, células dendríticas, células B, tipos específicos de células T e até mesmo em células não imunes como fibroblastos e células epiteliais [30]. Nestas células, alguns TLRs estão expressos na superfície celular (TLRs 1, 2, 4, 5, e 6), enquanto outros são expressos na membrana de vesículas ou organelas intracelulares (TLRs 3, 7, 8, e 9) [Akira *et al.*, 2006; Trinchieri *et al.*, 2007].

O nome Toll é derivado de uma seqüência homóloga da *Drosophilla spp.,* o gene toll, descoberto em 1996. Este gene seria relacionado com a formação do eixo ventral dorsal, assim como com a resposta imune a infecção fungal. A similaridade do domínio citoplasmático do toll de Drosophila e o receptor IL-1 de mamíferos impeliram a bisca por receptores ortólogos, subseqüentemente levando a descoberta do Toll humano [Akira *et al.*, 2001; Takeda *et al.*, 2003; Sandor *et al.*, 2005; O'Neill, 2005; Pandey *et al.*, 2006].

### 1.1.5 Toll-like Receptor 2

Cada TLRs têm a capacidade de reconhecer tipos específicos de PAMPs. O TLR2 possui um lugar especial entre os membros da família de TLRs humanos, sendo o receptor capaz de reconhecer o maior repertório de PAMPs de uma grande variedade de patógenos. Estes incluem lipoproteínas de patógenos tais como, bactérias Gram-negativas, Micoplasma e espiroquetas, peptideoglicanos e ácido lipoteicóico de bactérias Gram-positivas, lipoarabinomannan de micobactérias, glicoinositolfosfolipídios de *Trypanosoma Cruzi*, **"***phenol-soluble modulin*" de *Staphylococcus epidermidis*, zimosano de fungos, glicolipídeos de *Treponema maltophilum* e porinas que constituem outras membranas de *Neisseria* [Takeda *et al.*, 2003; Texereau *et al.*, 2005].

Este largo repertório de reconhecimento é principalmente o resultado da habilidade do TLR2 em se ligar a outros membros da família TLRs, em particular com TLR1 e TLR6, o que confere discriminação entre os diferentes tipos de componentes microbianos. TLR1 e TLR2 se heterodimerizam, resultando em dímeros que sensibilizam lipopeptídeos bacterianos triacilados. TLR2 heterodimeriza

com TLR6 que reconhece lipopeptídeos bacterianos diacilados [Takeda *et al.*, 2003; Texereau *et al.*, 2005; O'Neill *et al.*, 2007].

Tem sido demonstrado que o TLR2 é um receptor para LPS [Kirschning *et al.*, 1998; Yang *et al.*, 1998] e para CD14 [Schwandner *et al.*, 1999; Muta *et al.*, 2001; Iwaki *et al.*, 2005]. O TLR2 pode reconhecer diferentes tipos de LPS como *Leptospira interrogan* [Werts *et al.*, 2001] e *Porphyromonas gingivalis* [Hirschfeld *et al.*, 2001]. Entretanto, o TLR2 liga-se ao LPS com baixa afinidade, assim o TLR2 requer CD14 a fim de aumentar a sensibilidade ao LPS [Kirschning *et al.*, 1998; Muta *et al.*, 2001]. Além disso, o reconhecimento do LPS pelo TLR2 pode se diferir do reconhecimento do LPS feito pelo TLR4 (Toll-like Receptor 4). A explicação desta diferenciação no reconhecimento feito pelo TLR2 e TLR4 está na variação da composição molecular e da conformação tridimensional do Lipídio A (componente do LPS). Quando o lipídio A na molécula do LPS assume uma forma cônica, o TLR4 pode ser ligado. Uma conformação cilíndrica do lipídio A causa indução de citocinas através do TLR2. Se o LPS assumir uma conformação intermediária, a estimulação pode ocorrer através do TLR4 ou TLR2-TLR1 (Figura 3) [Netea *et al.*, 2002].

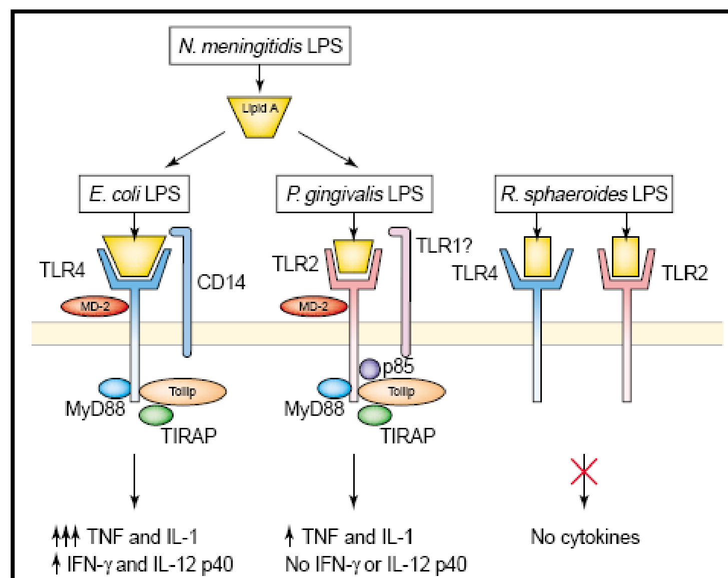


**Figura 3**: Hipótese da conformação tridimensional do lipídio A do LPS, permitindo sua ligação com diferentes TLRs. Extraído de Netea *et al*, 2002.

A sinalização do TLR2 (Figura 4) é feita por uma via dependente de MyD88 (*Myeloid Differentiation Factor*). A estimulação do TLR causa a associação do MyD88, o qual por sua vez recruta IRAK4 (*IL-1R-associated kinase 4*), assim permitindo a associação do IRAK1, o qual é fosforilado. TRAF6 (*tumour-necrosis-*

*factor receptor- associated factor 6*) também é recrutado para o complexo do receptor pela associação com o IRAK1 fosforilado. IRAK1 e TRAF6, então se dissociam do receptor e formam um complexo com TAK1 (*transforming-growth factor-β-activated kinase),* TAB1 *(TAK1-binding protein 1*) e TAB2 na membrana plasmática, o que induz a fosforilação do TAB2 e TAK1. O IRAK1 é degradado na membrana plasmática e o complexo remanescente (consistindo de TRAF6, TAK1, TAB1 e TAB2) desloca-se para o citosol, onde este se associa com ubiquitinas ligases UBC13 (*ubiquitin-conjugating enzyme 13*) e UEV1A (*ubiquitin-conjugating enzyme E2 variant 1*). Este processo, leva a ubiquitinação de TRAF6, o que induz a ativação de TAK1 que, em resposta, fosforila "*mitogen-activated protein (MAP) kinases*" e o complexo IKK (*inhibitor of nuclear factor-κB (IκB)-kinase complex*), o que consiste de IKK-α, IKK-β e IKK-γ (também conhecidos como IKK1, IKK2 e o modulador essencial "*nuclear factor kB*" (NF-kB), NEMO, respectivamente). O complexo IKK então fosforila IkB, que leva a sua ubiquitinização e subseqüente degradação. Isto permite o NF-kB translocar para o núcleo e induzir a expressão de genes alvo [Akira *et al.*, 2004].

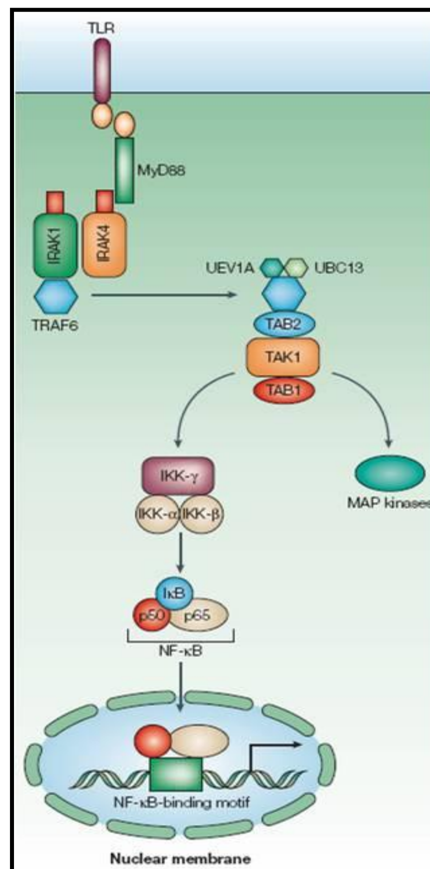


**Figura 4:** Cascata de sinalização realizada pelo TLR2. Extraído de Akira *et al*, 2004.

### 1.1.6 O Gene TLR2

O gene que codifica para o TLR2 foi mapeado no cromossomo 4 (Figura 5), *locus* 4q31.3-32. Este gene é composto por 3 éxons, sendo que o primeiro e o segundo éxons são não-codificantes e a região aberta de leitura é localizada no éxon 3. (GenBank – NM_003264 – em anexo) [Chaudhary *et al.*, 1998; Rock *et al.*, 1998; Texereau *et al.*, 2005].

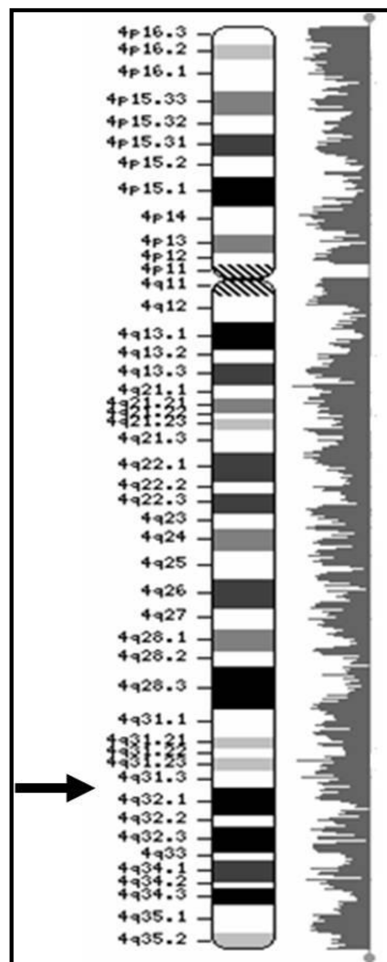


**Figura 5:** Mapa do cromossomo 4 que contém o *locus* gênico para o TLR2. A seta indica o *lócus* estudado. [NCBI: Map Viewer, em http://www.ncbi.nlm.nih.gov,acessado em 28 de setembro de 2007].

Texereau e colaboradores [2005] indicaram as principais alterações polimórficas descritas no gene do TLR2 (Figura 6). Mais tarde, Merx *et al* [2007] também caracterizaram alguns polimorfismos importantes (Figura 7).

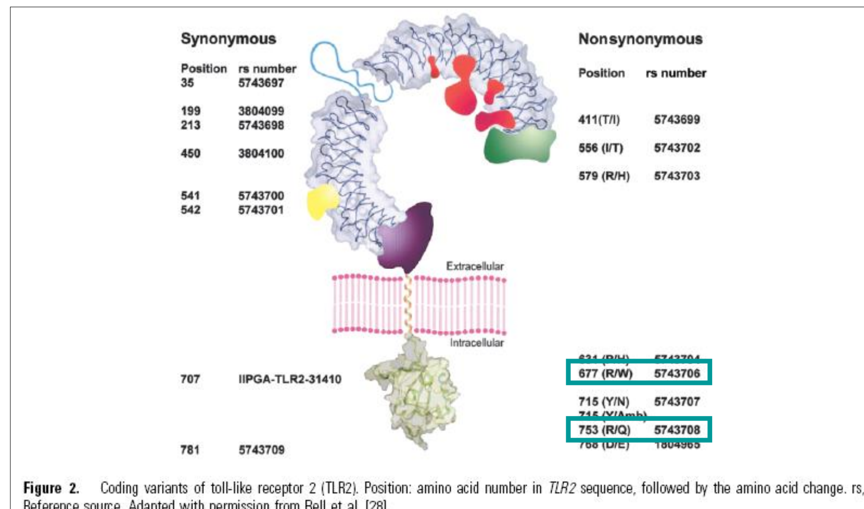


**Figure 2.** Coding variants of toll-like receptor 2 (TLR2). Position: amino acid number in *TLR2* sequence, followed by the amino acid change. rs, Reference source. Adapted with permission from Bell et al. [28].

**Figura 6:** Principais variantes polimórficas do gene do TLR2 ilustradas por Texereau *et al*, 2005. Em destaque nos quadros estão os SNPs estudados neste trabalho.

Table 1. Database results of investigated SNPs in the human TLR2 gene

| Database results cDNA (nt position) | gDNA (nt position) | refSNP_ID | SNP (amino acid position) | Heterozygosity frequency |
|---|---|---|---|---|
| 597T>C | 817T>C | rs3804099 | Asn199Asn | 0.486 |
| 639G>C | 859G>C | rs5743698 | Leu213Leu | 0.021 |
| 1232C>T | 1452C>T | rs5743699 | Thr411Ile | 0.008 |
| 1350T>C | 1570T>C | rs3804100 | Ser450Ser | 0.239 |
| 1623C>T | 1843C>T | rs5743700 | Phe541Phe | 0.044 |
| 1626C>G | 1846C>G | rs5743701 | Leu542Leu | 0.044 |
| 1667T>C | 1887T>C | rs5743702 | Ile556Thr | n.a. |
| 1736G>A | 1956G>A | rs5743703 | Arg579His | 0.008 |
| 1892C>A | 2112C>A | rs5743704 | Pro631His | 0.035 |
| 2121T>C | 2341T>C | rs5743705 | Phe707Phe | n.a. |
| 2143T>G | 2341T>G | rs5743707 | Tyr715stop | 0.004 |
| 2143/45TAT>AAA | 2363/65 TAT>AAA | n.a. | Tyr715Lys | CHIP + innate immunity |
| 2258G>A | 2478G>A | rs5743708 | Arg753Gln | 0.028 |
| 2304G>T | 2524G>T | rs1804965 | Glu768Asp | n.a. |
| 1339C>T | 1559C>T | | Arg447stop | n.a. |

SNP information about human TLR2 obtained from human genetic database research. cDNA, coding DNA; gDNA, genomic DNA; het.frequency, heterozygosity frequency; Het, heterozygote; Hom, homozygote; n.a., not available.

**Figura 7**: Caracterização dos principais polimorfismos do TLR2 segundo Merx *et al*, 2007. Em destaque no quadro está um dos SNPs estudados neste trabalho.

Este mesmo estudo identificou uma nova mutação do TLR2 no aminoácido de posição 447 (Arg447stop) que resulta da substituição de um nucleotídeo (SNP), de uma citosina (C) para uma timina (T) na base 1339 (1339C>T) formando um *stop codon* prematuro. Os doadores heterozigotos para este novo polimorfismo exibiram reduzida expressão de citocinas pró-inflamatórias IL-6 e TNF-alfa. Segundo os autores, são necessárias mais investigações para elucidar o papel desta mutação na suscetibilidade a inflamações e doenças infecciosas, assim como a sua freqüência em outras populações [Merx *et al*, 2007].

Yim, *et al* [2004] encontraram uma repetição polimórfica de dinucleotídeos (GT)n de cerca de 100pb após o sítio de início da tradução no íntron 2 do TLR2. O número de repetições GT nos indivíduos variou de 12 para 28, e a distribuição da

extensão do alelo foi significativamente diferente entre os grupos raciais. De acordo com os autores, este microsatélite polimórfico funcional pode ser importante na patologia de doenças infecciosas e inflamatórias. Este mesmo polimorfismo já foi associado com Câncer Colorretal [2006] e Artrite Reumatóide [2006].

Dentre todos os polimorfismos relacionados ao gene que codifica para o TLR2, somente dois deles têm sido mais estudados nos últimos anos e têm sido supostamente relacionados à redução da ativação do NF-kB e ao aumento do risco de infecção, confirmando modelos animais que sugerem que uma sinalização defeituosa do TLR2 é um fator causal para o aumento na susceptibilidade a doenças bacterianas [Texereau *et al.*, 2005].

O primeiro polimorfismo consiste em uma substituição de uma citosina (C) por uma timina (T) no nucleotídeo 2029 (2029C>T) do gene humano do TLR2, o qual resulta na modificação protéica de uma Arginina por um Triptofano no aminoácido 677 (Arg677Trp). Foi primeiramente relacionado com pacientes com Lepra Lepromatosa [Kang *et al.*, 2001; Kang *et al.*, 2004] e posteriormente com a suscetibilidade à Tuberculose [Ben-Ali *et al.*, 2004].

O segundo polimorfismo foi descrito como a substituição de uma guanina (G) para uma adenina (A) no nucleotídeo 2258 (2258G>A) gerando a substituição de uma Arginina por uma Glutamina no resíduo 753 (Arg753Gln) da proteína TLR2. Este SNP foi primeiramente descrito por Lorenz *et al* [2000], que identificaram que esta mutação ocorria em 3% da população testada. O estudo de Lorenz *et al* [2000], afirma que, *in vitro* o polimorfismo 2258G>A não afeta a habilidade do TLR2 em responder ao LPS, mas afeta na habilidade do TLR2 em responder a peptídeos bacterianos. Além disso, este mesmo estudo verificou se a ocorrência da mutação em pacientes com choque séptico infectados com bactérias Gram-positivas era mais freqüente do que em pacientes infectados por bactérias Gram-negativas. Foram encontrados, em 91 casos e 73 controles estudados, 22 pacientes com choque séptico causado por bactérias Gram-positivas. Destes, dois pacientes carregavam a mutação 2258G>A e também apresentavam infecção por estafilococos. Assim, de acordo com o estudo, a mutação 2258G>A pode ser um fator de risco para o desenvolvimento de choque séptico após a infecção por bactérias Gram-positivas sendo que uma das sugestões apresentadas pelos autores é a necessidade de ampliar o número de amostras [Lorenz *et al.*, 2000].

Schröder *et al* [2003] publicaram que o alelo 2258A ocorria em 9,4% da população estudada (caucasóide), o que era uma porcentagem significativamente maior do que a encontrada anteriormente por Lorenz *et al* [2000]. Os autores ainda salientam que o fato de o alelo 2258A estar presente em uma maior freqüência em caucasóides faz deste um alvo para estudos que correlacionem este polimorfismo com a incidência de doenças infecciosas [Schröder *et al., 2003*].

Além de ter sido primariamente identificado como um possível risco para o desenvolvimento de choque séptico por bactéria Gram-positiva, o polimorfismo 2258G>A do TLR2 têm sido estudado ao longo dos anos sendo relacionado a outras doenças: Ogus *et al* [2004] sugeriram que o alelo 2258A pode ter influência no aumento da suscetibilidade para o desenvolvimento e severidade da Tuberculose. Hamann *et al* [2005] relataram que um significativo aumento da freqüência do alelo 2258A foi encontrado em pacientes que desenvolveram Reestenose. Ainda, Schröder *et al* [2005] indicaram que o alelo 2258A do TLR2 pode proteger do desenvolvimento do estado tardio da Doença de Lyme e Berdeli *et al* [2005] verificaram que avaliação do polimorfismo 2258G>A contribui para o diagnóstico da patogênese da Febre Reumática Aguda em crianças.

Já Kijpittayarit *et al* [2007] mencionam que 2258A possivelmente é associado com a replicação e a doença do citomegalovírus (CMV) após transplante de fígado. Eid e colaboradores [2007] demonstraram haver relação entre o alelo 2258A e a necessidade de transplante de fígado decorrente de infecção pelo vírus da Hepatite C (HCV). Este mesmo alelo também foi associado a infecções bacterianas recorrentes por Kutukculer e colegas [2007]. Outro estudo ainda evidenciou a ligação do alelo 2258A e o alto risco de infecção do trato urinário por bactérias Gram-positivas [Tabel *et al*., 2007].

A literatura científica trás certos estudos com associações significaticas para os SNPs 2029C>T e 2258G>A do TLR2. Mas, há um número ainda maior de estudos sem associação. Alguns autores como Moore *et al* [2004], Sánchez *et a*l [2004], Emingil *et al* [2007] e Berdeli *et al* [2007], entre outros que estudaram o SNP 2258G>A e não obtiveram associação, ainda apresentam uma baixa freqüência do alelo mutante em suas populações. Outros autores como Mockenhaup *et al* [2006], Ryu *et al* [2006], Yoon *et al* [2006], Cheng *et al* [2007] e Zafra *et al* [2008] demonstram a total ausência dos alelos mutantes. A maioria dos estudos do polimorfismo 2029C>T também não apresenta o alelo mutante em suas amostras.

## 1.2 JUSTIFICATIVA

Em razão destas inconsistências nos resultados dos estudos destes SNPs do TLR2, buscamos verificar a presença destes polimorfismos do gene que codifica para o TLR2 em indivíduos do Sul do Brasil. Nossa amostra é composta por sujeitos com alto grau de risco para infecção, dado que estão criticamente doentes e internados em uma Unidade de Terapia Intensiva. Caso as variantes alélicas do TLR2 sejam responsáveis por conferir maior susceptibilidade aos quadros infecciosos, será possível identificá-las na amostra escolhida para estudo. Nosso foco foi investigar se há associações com a susceptibilidade à sepse, choque séptico e/ou mortalidade. Havendo ou não associação positiva, nossa intenção com esse trabalho é realizar um estudo aprofundado com a literatura disponível para compreender a real aplicação clínica da avaliação genética dos SNPs 2029C>T e 2258G>A do gene TLR2.

## 1.3 OBJETIVOS

1. Investigar a ocorrência dos polimorfismos 2029C>T e 2258G>A em uma população do sul do Brasil.
2. Realizar um estudo associativo entre os alelos 2029T e 2258A do gene do TLR2 e a suscetibilidade à sepse e/ou aos desfechos críticos a partir da sepse.
3. Realizar uma revisão sistemática dos trabalhos que estudaram os polimorfismos 2029C>T e 2258G>A do gene do TLR2 em diferentes populações e com diferentes desfechos fenotípicos.

## 2 ARTIGO CIENTÍFICO

Very low frequencies of TLR2 supposed-2029T and 2258A (rs5743708) mutant alleles in southern Brazilian critically ill patients: would it be a lack of worldwide accepted clinical applications of TLR2 variants?

Periódico Escolhido: International Journal of Immunogenetics.

***TITLE OF THE PAPER***

VERY LOW FREQUENCIES OF TLR2 SUPPOSED-2029T AND 2258A (RS5743708) MUTANT ALLELES IN SOUTHERN BRAZILIAN CRITICALLY ILL PATIENTS: WOULD IT BE A LACK OF WORLDWIDE ACCEPTED CLINICAL APPLICATIONS OF TLR2 VARIANTS?

**Running Head**

A discuss about TLR2 supposed-2029T and 2258A mutant alleles.

**Keywords:**

TLR2; 2029C>T; 2258G>A; Arg677Trp; Arg753Gln

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***SUMMARY***

Toll-like receptor 2 (TLR2) is a recognition receptor for the widest repertoire of pathogen-associated molecular patterns. Two polymorphisms of TLR2 could be linked to reduced NF-kB activation and to increased risk of infection (supposed-2029C>T and 2258G>A). We investigated the supposed-2029C>T and 2258G>A TLR2 polymorphisms in 422 critically ill patients with European origin from southern Brazil (295 with sepsis and 127 without sepsis), and we reviewed of 33 studies with these polymorphisms conducting a quality assessment with a score system. Among our patients we found only one heterozygote (1/422) for the supposed-2029C>T and none of 2258G>A (0/422) SNP. We have failed to find a clinical application of supposed-2029T and 2258A allele analysis in our southern Brazilian population. Our review detected that current TLR2 SNP assays had very controversial and contradictory results derivate from reports with a variety of quality criteria of investigation. We suggest that, if analyzed alone, the supposed-2029C>T and 2258G>A TLR2 SNP are not good candidates for genetic markers in studies that search for direct or indirect clinical applications between genotype and phenotype. Future efforts to improve the knowledge and to provide other simultaneous genetic markers might reveal a more effective TLR2 effect on the susceptibility to infections diseases.

***INTRODUCTION***

Toll-like receptors (TLRs) are key regulators of both innate and adaptive immunity based on their function as recognition receptors for pathogen-associated molecular patterns (PAMPs) which are unique to microbes and essential for their survival (Akira *et al.*, 2006; Carpenter & O'Neill, 2007). TLRs are expressed on immune cells such as macrophages, dendritic, B, and T cells and on some non-immune cells, such as epithelial (skin, respiratory, intestinal and genitourinary tracts), endothelial, and smooth muscle cells. Different TLRs have variable expression: TLRs 1, 2, 4, 5, 6, and 10 are expressed on the cell surface, while TLRs 3, 7, 8, and 9 are expressed intracellularly (Becker & O'Neill, 2007). The cell surface TLRs are characterized by the presence of an extracellular leucine-rich repeat (LRR) and an intracellular Toll/IL-1 receptor (TIR) domain. The LRR is found on a diverse number of proteins with ligand recognition and signal transduction properties, and is separated from the trans-membrane region by a LRR C-terminal domain. The TIR domain is required for intracellular signaling and spans about 200 amino acids with varying degrees of sequence similarity among TLR family members. Three particular boxes are highly conserved among TLR family members: box 1 being the signature sequence of the family whereas boxes 2 and 3 containing amino acids critical for signaling (Akira & Takeda, 2004; Carpenter & O'Neill, 2007; Trinchieri & Sher, 2007).

TLR2 (Reactome-UniProt: O60603) has a special place among the 10 members of the human TR family. Of all of the mammalian TLRs and, perhaps, of all pathogen recognition receptors, TLR2 is capable of detecting the widest repertoire of pathogen-associated molecular patterns (Texereau *et al.*, 2005). The variety of microorganisms that TLR2 recognizes are lipoproteins from Gram-negative bacteria, *Mycoplasma* and spirochetes, peptidoglycan and lipoteichoic acid from Gram-positive bacteria, lipoarabinomannan from mycobacteria, glycoinositolphospholipids from *Trypanosoma cruzi*, modulin from *Staphylococcus epidermidis*, zymosan from fungi, glycolipids from *Treponema maltophilum*, and porins that constitute the outer membrane of *Neisseria* (Takeda *et al.*, 2003). One aspect of TLR2 ligand recognition involves cooperation with other TLR family members, particularly with TLR6 and TLR1 for discrimination among different microbial components (Takeda *et al.*, 2003). To enable the innate system to recognize numerous structures of lipopeptides in

various pathogens, distinct acylation patterns are recognized by either TLR2/TLR1 and TLR2/TLR6, the former heterodimer only recognizes triacylated lipopeptides whereas the latter, diacylated lipopeptides. There is evidence that the charge of the amino acids, especially the charge of the C-terminal amino acids, may determine the signaling through TLR2/TLR1 or TLR2/TLR6 (Buwitt-Beckmann *et al.*, 2006). Reactome data base (http://www.reactome.org/) demonstrates that these two triggers release the same activation cascade through MyD88.

Only two of the nonsynonymous nucleotide polymorphisms (SNPs) (one of these is just a supposed polymorphism) of the cytoplasmic domain of TLR2 have been linked to reduced NF-kB activation and to increased infection risk, confirming animal models that suggest that defective TLR2 signaling is a causative factor for increased susceptibility to bacterial disease (Texereau *et al.*, 2005). The first is the suggested C>T substitution at position 2029 from the start codon. Located in exon 3, this 2029C>T SNP results in replacement of a conserved arginine residue by tryptophan at position 677 (Arg677Trp protein mutation). As this arginine residue is highly conserved at the C-terminal of TLR family, its mutation is likely to affect the signaling function of the molecule (Kang & Chae, 2001). Nevertheless, Malhotra *et al* (2005) proposes that it is not a true polymorphism because a C is fixed at the authentic (functional) TLR2 exon 3 sequence, but a T occupies the same position in a pseudo-exon 3 upstream to the TLR2 gene. Both regions (exon 3 and pseudo-exon 3) are simultaneously recognized by primers, and the double amplification is suggestive of an erroneous 2029C>T SNP suggestion and an apparent heterozygosis in the populations.

The second, and more proper, SNP is a G>A change at 2258 from the start codon of the TLR2 gene (2258G>A; rs5743708) which causes an arginine to glutamine substitution at residue 753 (Arg753Gln). Since this arginine is conserved between mice and humans and is part of a highly conserved stretch of amino acids at the C-terminus of TLR2, its change would have important phenotypical effects (Lorenz *et al.*, 2000). The HapMap (http://www.hapmap.org/) shows the 2258G>A nucleotide substitution as polymorphic just in the European Caucasian population, with a frequency of the 2258A allele is 5%, being 2258G fixed in the oriental and black populations.

Based on the above, it would be expected that polymorphic alterations on TLR2 codifying sequence could cause a hyporesponsive TLR2 pathway and affect the susceptibility to severe infections. Thus, we tested whether the supposed-2029T and 2258A rare alleles of TLR2 would be outnumbered in patients with sepsis when compared to matching individuals without sepsis in a well-characterized population of 422 white critically ill patients. We also performed a refined review on TLR2 and a discussion about the controversial nature of these mutations on different diseases and populations so to analyze whether the supposed-2029T and 2258A rare alleles identification would have clinical applications.

## *MATERIAL AND METHODS*

### The Patients' Study

The patients' study was an observational, hospital-based cohort study of subjects admitted to the medical and surgical Intensive Care Unit (ICU) of the São Lucas Hospital, of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Brazil, between January 1$^{st}$, 2004, and December 31$^{st}$, 2006. Patients were not eligible if they were diagnosed with HIV-infection, taking immunosuppressive drugs, pregnant or lactating, or from non-European ancestry. The Research Ethics Committee of the institution (protocols #03-01732, and #07-1500; REC Tel.: 55 51 3320 3345) approved this study. A total of 422 white critically ill adult patients from southern Brazil (225 males and 197 females) admitted to the ICU were included in this study. The organ dysfunction and failure were evaluated using the Sequential Organ Failure Assessment (SOFA) score (Vincent *et al.*, 1998) obtained during the first seven days from the ICU admission and also on days 15 and 29. The patients' follow-up was extended up to the total time they stayed in the hospital from the ICU admission to a maximum of 242 days. We monitored the patients daily during their entire ICU and post-ICU (hospital) stay, measuring sepsis and septic shock occurrences until the discharge of the hospital or death. For diagnosis of sepsis and septic shock we used the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Criteria (ACCP/SCCM, 1992). For illness severity evaluation we used the Acute Physiology and Chronic Health Evaluation II (APACHE-II) score obtained on the ICU admission day (Knaus *et al.*, 1985). Clinical endpoints of the study were discharge from the

hospital (survivors) or death (non-survivors). Mortality was measured in days until death. For those patients with multiple ICU admission during the study period, only data from the first entrance was considered.

Genomic DNA were isolated from leucocytes by standard procedures and maintained at -20°C (Lahiri & Nurnberger, 1991). A 340 bp PCR product was designed to encompass both SNPs under study using forward primer TLR2-F 5'-GCC-TAC-TGG-GTG-GAG-AAC-CT-3' and reverse primer TLR2-R 5'-GGC-CAC-TCC-AGG-TAG-GTC-TT-3' (Invitrogen-Life Technologies, São Paulo, SP, Brazil). In this genotyping, we applied primers described by Schröder *et al* (2003) that are different of the PP1-primer reported by Malhotra *et al* (2005) which amplified both exon 3 and pseudo-exon 3 identifying in 100% of individuals the presence of supposed-2029T nucleotide. Each reaction was performed in 25µl containing 10-50 ng DNA, 0,12 µM each primer, 0,2 mM dNTP, 1,5 mM $MgCl_2$ and 1 U Taq polymerase in Taq 1x Buffer (LGC Biotecnologia, Cotia, SP, Brazil). The reactions were carried in a TC-412__thermocycler (Barloworld Scientific Ltd, Stone, Staffordshire, United Kingdom) as follows: 95°C for 10 minutes; 35 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 30 s; and 72°C for 5 minutes for final extension. The amplified PCR products were genotyped by the method of direct sequencing (BigDye TerminatorCycle Sequencing Kit and ABI PRISM 3100 Avant Genetic Analyzer, Perkin-Elmer, Foster City, Calif., USA) using the forward primer, and the results were confirmed by sequencing with reverse primer. At least 10% of the samples were subjected to a second, independent PCR-sequencing re-analysis analysis cycle to confirm the genotypes. In order to confirm that the 340bp PCR amplified product really represented the targeted product, we performed a latest sequence analysis in MegaBase 1000 capillary DNA sequencer (Amersham Biosciences UK Ltd, Chalfont St Giles, Bucks, UK); also using the designed primers (forward and reverse). Each sequence obtained was submitted to an online BLASTn alignment (at http://www.ncbi.nlm.nih.gov/BLAST/), and we found consensus with the *Homo sapiens* toll-like receptor 2 (TLR2) gene, promoter region (GenBank accession NM_ 003264) and the sequence exported from chromatogram file. The alignment view was performed in ClustalX program (version 1.8, as described in Thompson *et al* (1997) in multiple alignment modes, with sequences loaded in FASTA format. Blank control wells were always used to test contamination of the PCR reagents. All the

personnel involved in patient care were blind to the selection process and genotyping results.

### The Review Assessment

### Search Strategy

A review of the literature was performed in the PubMed database covering all papers published from 1998 to 2008. Terms used for search were a combination of the following keywords: TLR2, polymorphism, 2029C>T, 2258G>A, Arg677Trp, and/or Arg753Gln. Additionally, the reference lists of the selected papers were used to identify studies that were not be collected in the database. We included articles that were available in English and studies that reported disease and/or association with the TLR2 polymorphisms were also included (Clark & Baudoin, 2006; Geng *et al.*, 2008; Shi *et al.*, 2008; Flores *et al.*, 2008).

### Study Quality Assessment

We used a 5-point scoring classes based on previous criteria adopted from published recommendations on the assessment of the quality of genetic association studies. The criteria and scores are described in detail below; we scored as 1 if present or 0 if absent (Clark & Baudoin, 2006). These independent criteria were included in the Figure 1 (Results section), and the total score (0 to 5) were applied in Tables 1 and 2 (Results section).

*Control Group:* Score 1: When the Control Group was fully described and/or the work was clear if more than one group was used or if control data was reused from a previous study (Bird *et al.*, 2001; Clark & Baudoin, 2006). Score 0: When the Control Group was absent.

*Hardy-Weinberg Equilibrium*: Score 1: When the Hardy-Weinberg equilibrium was tested (the Hardy-Weinberg equilibrium is a state in which allele frequencies in a population tend to remain the same from generation to generation unless acted on by outside influences) (Hattersley & McCarthy, 2005; Clark & Baudoin, 2006). In this category we also discriminated studies which the sample was in Hardy-Weinberg equilibrium or not. Score 0: When the Hardy-Weinberg equilibrium test was not cited.

*Blinding*: Score 1: When the study had cited that genotyping was blind to the clinical status of the patient (Clark & Baudoin, 2006). Score 0: When the blinding strategy was not cited.

*Control for Confounders*: Score 1: When the study tests were adjusted to confounding variables and/or it was executed multiple comparisons to test for more than one association in this study and/or it repeated in an independent group of cases and controls (Vitali & Randolph, 2005). Score 0: When the control for confounders was not used.

*Duplicated Genotyping*: Score 1: When duplicate genotyping were performed on the same assay or on another assay (Hattersley & McCarthy, 2005). Score 0: When the duplicate genotyping was not performed.

**Frequency Calculation**

The allelic frequencies of both SNPs studied were calculated based on the following formula: the number of rare allele divided for the total number of alleles, for each polymorphism.

**Presence of Association**

We considered as statistically significant the association between genotype/allele and phenotype when the study presented a $P<0.05$ in, at least, a non-adjusted analysis.

## ***RESULTS***

**The Patients' Study**

Table 1 show clinical and the demographic data of our sample of critically ill patients. Four hundred and twenty two patients were investigated: data about age, gender, APACHE-II and SOFA scores, length of stay in ICU and ICU plus hospital, septic shock occurrence, cause of admission, and mortality rates are shown in patients with or without sepsis. The incidence of sepsis in ICU patients was 69.9% (295/422) from whom 71.5% of them had septic shock (211/295). The frequency of septic shock in ICU patients was 50.0% (211/422). Sepsis was positively associated

with older age, higher APACHE-II and SOFA-1 scores, longer ICU stay, and medical admission causes (all *P*<0.01). Accordingly, mortality rates were higher in septic than non-septic patients (*P*<0.001).

The main causes of admission to ICU were Medical Sepsis (35%; 147/420), followed by Medical Respiratory (24.5%; 103/420), and Surgical Abdominal (11.4%; 48/420). Microbiological results from blood sample of patients with sepsis (n=295) were as follows: 55.1% (162/295) with no focus identified, 21.8% (64/295) with only Gram-negative bacteria; 6.8% (20/295) with only Gram-positive bacteria; 9.9% (29/295) with both Gram-negative and Gram-positive bacteria; 1.4% (4/295) with fungal infection; and 5.1% (15/295) with fungal and bacterial infection. The anatomical distribution of the primary site of infection in septic patients was as follows: 65.7% (194/295) pulmonary; 22.8% (67/295) abdominal; 4.1% (12/295) urinary; 1.7% (5/295) central nervous system; 1.7% (5/295) skin; and 2% (6/295) from another site.

The genotypic and allelic frequencies in our sample to supposed-2029C>T and 2258G>T SNPs were: 2029CC=0.998 (421/422), 2029CT=0.002 (1/422), and 2029TT=0; 2029C=0.99 (843/844) and 2029T=0.01 (1/844); 2258GG=1 (422/422), 2258GA and 2258AA=0; 2258G=1 (844/844); 2258A=0. The phenotypes (sepsis, shock septic, organ dysfunction, or mortality) were not related to genotypes because the very low frequency of the supposed-2029T allele and null frequency of 2258A allele. The frequencies apparently did not differ from the values expected by the Hardy-Weinberg model. As only one mutant allele was identified to supposed-2029C>T SNP in our sample, we show the amplified sequences to subsequent potential analysis (Fig. 1).

**The Review Assessment**

We have compiled 33 reports published from 1998 to 2008 regarding the supposed-2029C>T and/or 2258G>A TLR2 SNPs. The TLR2 SNP investigations have grown up in the last ten years (Fig. 2) especially in 2007 when it was published 27.8% (5/18) and 39.3 (11/28) of the reports on the supposed-2029C>T and 2258G>A SNPs. Tables 2 and 3 present our compilation with the main characteristics and findings of each study, as follows: methods, population, and number of cases and controls, frequencies of genotypes and alleles, the study quality scores. In the

last columns, it was noticed the end points and the statistical data of association tests (when applicable).

The majority of TLR2 SNP studies was concentrated in three regions of the world: Europe (45.4%; 15/33) (mainly in Germany); Turkey (24.2%; 8/33), and South Korea (12.1%; 4/33). Other few reports are originated from US (6.0%; 2/33), Colombia, India, Tunisia, and Taiwan (all 3.0%; 1/33 each).

The supposed-2029T allele was null (0%) on Europe, but in Tunisia it was found in 47% of case and 15% of control subjects (Ben-Ali *et al.*, 2004), and in Korea, it was observed in 6%, and later, in 17% of case individuals (Kang & Chae, 2001; Kang *et al.*, 2004). The frequency of 2258A allele was from 1% to 9% in Europe and it was higher in three reports from Turkey [14% (Ogus *et al.*, 2004), 23% (Kutukculer *et al.*, 2007) and 46% (Berdeli *et al.*, 2005)]. In the two studies from the US, 2258A allele frequency was reported as 9% (Eid *et al.*, 2007; Kijpittayarit *et al.*, 2007) and in a report from Colombia both mutant alleles are null (Zafra *et al.*, 2008). In order to better visualize the TLR2 mutant allele frequencies around the word, we showed it in the maps on Figure 3.

Most reports revealed no statistically significant associations between mutant alleles and phenotypes: 16.7% (3/18) to supposed-2029C>T and 42.8% (12/28) to 2258G>A SNP. All studies (100%; 3/3) to supposed-2029C>T and 41.7% (5/12) to 2258G>A SNP were performed with less than 200 subjects (Fig. 4). We detected four works (1/3 to supposed-2029C>T and 3/12 to 2258G>A SNPs) that report a statistically significant association but did not cite the *P* value (Tables 2 and 3).

None of the set of 33 TLR2 SNP reports covered the five points to scoring classes; only 11.1% (2/18) to supposed-2029C>T and 6.9% (2/28) to 2258G>A SNP comprised four points, (i.e., in these works four of five points were present), but majorly the reports enclosed two of the 5-point scoring classes (Fig. 5). In Figure 6 there was a detailed comparison among all studies according the 5-point scoring classes to quality assessment. A control group was included in 94.4% (17/18) of the studies concerning the supposed-2029C>T and 78.6% (22/28) to 2258G>A SNP studies. Around 22% (4/18) to supposed-2029C>T and 35% (10/28) to 2258G>A of investigations mentioned the Hardy–Weinberg equilibrium testing. The blinding strategy was reported in just three of studies (9.1%; 3/33). Only 11.1% (2/18) to supposed-2029C>T and 28.6% (8/28) to 2258G>A performed the adjusted analysis

to confounding variables, and around 60% (11/18) to supposed-2029C>T and 40% (11/28) to 2258G>A achieved the standard for reproducibility with a second confirmatory assay (Fig. 6).

In both SNP analyses the most used laboratory method for genotyping (around 65%) was PCR followed by restriction fragment length polymorphism (RFLP) examination (Fig. 7). When this PCR-RFLP technique is used it is recommended to perform duplicate assays (with the same or other method) to detect discrepancy between results (Hattersley & McCarthy, 2005), and ideally, when >10% of samples genotyped are inconsistent the assays should be redone. We showed that the duplicate strategy was employed in 75% (to supposed-2029C>T SNP) and 52.6% (to 2258G>A SNP) of the PCR+RFLP assays performed (Fig. 7).

## ***DISCUSSION***

It is well known that TLR2 is an important cellular receptor responsible for the recognition of a large range of pathogens and activation of inflammatory cytokines production, having an essential role in the innate and acquired immunity. Polymorphic mutations on its codifying sequence could produce a hyporesponsive TLR2 pathway and affect susceptibility to infections. Thus, investigations about TLR2 mutant alleles have a biological plausibility, due to a possible causal effect for TLR2 in the pathogenesis of the infectious diseases. Since the most studied TLR2 SNPs have been the supposed-2029C>T and the 2258G>A, we investigated these two polymorphisms in 422 critically ill patients to test whether the mutant alleles would affect the critically ill patients' susceptibility or outcome and we found that these alleles are rare (supposed-2029T) or null (2258A) in our population.

To discuss our results and to inquire about their potential clinical applications, we performed a review with 33 reports. We observed that TLR2 SNP investigations have grown up especially in 2007 and they were basically distributed in three regions around the world: Europe, Turkey, and South Korea, with the American and African continents with just a few studies. In face of these few reports, we assume that the literature do not expose the actual repertoire of TLR2 genotyping performed worldwide since assays concerning null or rare alleles are more likely to remain unpublished. Additionally, failure in detecting significant association may have inhibited new investigations.

Initial studies usually yield suggestive rather conclusive results since they are likely to overestimate the true effect size (Hattersley & McCarthy, 2005). Although an adequate sample size and power calculations are necessary to assure conclusions and exclude false negative results, this was a rare finding among the TLR2 reports analyzed: the TLR2 SNP studies did not mention the power calculation to establish the sample size. The concept of power is closely related to the two types of statistical errors: the Type I error, named α (i.e. the probability of rejecting a true hypothesis), and the Type II error, named β (i.e., the probability of accepting a false hypothesis). Power is defined as 1 - β, i.e., the probability of rejecting a false hypothesis, or the probability of not making a Type II error (Sluis *et al.*, 2008). The basic aim of a power study is to determine the sample size "N", which is required to achieve adequate power, given a chosen α and a particular effect size. Regarding a hypothetic very high frequency of mutant TLR2 alleles (case: mutant allele=0.20; control: mutant allele=0.01) in a case-control study, we calculated that at least 10,000 subjects would be necessary to achieve 80% power to obtain a *P*-value of 0.05 in studies involving infectious diseases. Therefore, due to the impossibility of obtaining such a large sample, we recognize the importance of small sample size investigations since they are pioneer and because further systematic meta-analysis could be evaluating the impact of these individual reports.

Approximately 35% of the 33 TLR2 papers had less than 200 subjects and among this group was observed around 50% of cases with positive associations between mutant allele and phenotype. All studies with supposed-2029C>T SNP and more than 200 subjects (61%; 11/18) had no statistically association. And, even that 43% (12/28) of 2258G>A studies had met significant association, in the largest study (with >600 subjects) was not found any association. A study with more than 600 subjects tend to be more robust than one with less, and an inefficiency sampling strategy can be compensated for an increases in sample size. Although there are some possible problems with very large samples (as the misclassification of outcome or the population heterogeneity), we would like alarm for the risk of these studies with <200 that found association have incurred in type II error.

Altogether, it is noteworthy that most studies (83%; 15/18) did not find any association between the supposed-2029C>T TLR2 SNP and illnesses, probably due to the concurrence of sample size or the rarity of this allele Nonetheless, Ben-Ali *et al*

(2004) exposed a strong positive association with Tuberculosis (*P*<0.0001), and Kang *et al* (2001) with Lepromatous Leprosy (without *P* value). As Kang *et al* (2001), three other reports assured significant association despite not showing statistical parameters (Lorenz *et al.*, 2000; Kang & Chae, 2001; Merx *et al.*, 2007; Woehrle *et al.*, 2008) that would be essential information in association studies since it confirms results and warrants credibility to the data. Statistical imprecision as the absence of the *P*-value and power calculation, lack of Hardy-Weinberg equilibrium testing, or multiple statistical analyses, make a pool of inaccurate TLR2 SNP studies.

Although the supposed-2029T allele is rare in many world populations (0% in Europe), in Tunisia, Ben-Ali *et al* (2004) found this allele to be present in 47% of cases and 15% of controls, and in Korea, Kang *et al* found it in 6%, and later, in 17% of their cases (Kang & Chae, 2001; Kang *et al.*, 2004) It is possible that the frequency of this SNP will be altered in small populations or in subgroups of particular populations (small, culturally isolated communities that are mostly closed breeding groups) that have been exposed to founder effect (that occurs when a small amount of people have many descendants surviving after a number of generations) or to specific environmental or lifestyle factor pressures. Interbreeding causes a random change in genotypic frequencies, particularly if the population is very small (if the population is small, Hardy-Weinberg may be violated). In such cases, the frequency of an allele may begin to drift toward higher or lower values. The result for a population is often high frequencies of specific alleles inherited from the few common ancestors who first had them. Genetic drift produces changes, but there is no guarantee that the novel population will be more fit than the original one. Changes by drift are aimless and not adaptive, and thus a possible explanation why the supposed-2029T allele, which cause susceptibility to infectious diseases, is increased in Tunisia and Korea. Additionally, if supposed-2029T allele was a protective allele, its higher frequency would reflect selective pressures having favored the transmission of this allele in their antecedents, and would be expected to be more common in specific populations. Although this possible explanation, we can not discard the likelihood of laboratorial inaccuracy. Clark *et al* (2006) showed that a combination of methodological and analytical problems is likely to explain the failure to replicate results: technical troubles with the methods of identifying the mutant allele, for example, may lead to false-positive and inconsistent conclusions.

The most used laboratory method to TLR2 genotyping was the non-automatized PCR-RFLP technique which has some advantages such as being cheap and do not require sophisticate equipments. However, when compared to other methods, it needs intensive work and the results can be delayed and inexact (Table 4). Automated sequencing and Real-Time PCR have elevated cost and require highly trained technicians and support, but provide advanced precision and sensitivity, and smaller contamination risk because they have minimized manipulation. Even so, automated sequencing and Real-Time PCR were used on fewer instances.

Regarding technical inconsistencies, in 2005 Malhotra *et al* (2005) advised that 2029-C>T is not a true polymorphism because the authentic TLR2 exon 3 sequence and a mutant pseudo-exon 3 upstrean of TLR2 gene are simultaneously recognized by primers, and the double amplification generate the erroneous 2029C>T SNP suggesting an apparent heterozygosis in the populations. In our genotyping, we applied primers described by Schröder *et al* (2003) that are different of the PP1-primer reported by Malhotra *et al* (2005) which amplified both exon 3 and pseudo-exon 3 identifying in 100% of individuals the presence of supposed-2029T nucleotide. With our primers we tried not to amplify the pseudo-exon 3, but we found (confirming by two different sequencing systems) one supposed-2029T allele in a unique heterozygote patient (Fig. 1). This could reveal that either the Schröder *et al* (2003) primer sequences' recognized the pseudo-exon 3 or that the supposed-2029C>T SNP really exists. It is important to note that we studied a population with European origin (Caucasian), and the sample of Malhotra *et al* (2005) was from India.

In a unique previous study from Colombia both mutant alleles are null (Zafra *et al.*, 2008). In Europe the 2258A allele frequency ranged from 1% to 9% but, interestingly, it was higher in three singular reports from Turkey [14% (Ogus *et al.*, 2004), 23% (Kutukculer *et al.*, 2007) and 46% (Berdeli *et al.*, 2005)]. Once more, apart from experimental or statistics inaccuracy, it would be possible due to different genetic background in these populations or singular patterns of lifestyle risk factors. Other flaws were also detected in 2258G>A TLR2 SNP reports. In one paper a mistake about the 2258G>A SNP (Arg753Gln) description: it changed the G allele to T allele (as 2258T>A) and in a following moment it changed the A allele to C (as 2258C>T) (Labrum *et al.*, 2007). It was a small error but it can be a source of additional technical and interpretation mistakes.

In general, the set of 33 TLR2 reports examined presented some limitations such as the lack of well-designed strategies for analysis, which precluded precise significant final conclusions.

In summary, our genotyping results do not support a clinical application for the supposed-2029T and 2258A alleles in the southern Brazilian population. Our review detected that current TLR2 SNP assays had very controversial and contradictory results derivate from reports with a variety of quality criteria of investigation. We suggest that, if analyzed alone, the supposed-2029C>T and 2258G>A TLR2 SNP are not good candidate for genetic markers in studies that search for direct clinical applications. Future efforts to improve the knowledge and to provide other simultaneous genetic markers might reveal a more effective TLR2 effect on the susceptibility to infectious diseases.

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## *REFERENCES*

ACCP/SCCM: American College of Chest Physicians/Society of Critical Care Medicine (1992) Consensus Conference: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Critical Care Medicine*, 20, 864.

Akira, S. & Takeda, K. (2004) Toll-Like receptor signalling. *Nature Reviews. Immunology*, 4, 499.

Akira, S., Uematsu, S. & Takeuchi, O. (2006) Pathogen recognition and innate immunity. *Cell,* 124, 783.

Bacanli, A., Sallakci, N., Yavuzer, U., Alpsoy, E. & Yegin, O. (2006) Toll-like receptor 2 Arg753Gln gene polymorphism in Turkish patients with Behçet's disease. *Clinical and Experimental Dermatology*, 31, 699.

Becker, C.E. & O'Neill, L.A. (2007) Inflammasomes in inflammatory disorders: The role of TLRs and their interactions with NLRs. *Seminars in Immunopathology*, 29, 239.

Ben-Ali, M., Barbouche, M.R., Bousnina, S., Chabbou, A. & Dellagi, K. (2004) Toll-Like Receptor 2 Arg677Trp Polymorphism Is Associated with Susceptibility to Tuberculosis in Tunisian Patients. *Clinical and Diagnostic Laboratory Immunology*, 11, 625.

Berdeli, A., Celik, H.A., Ozyürek, R., Dogrusoz, B. & Aydin, H.H. (2005) TLR-2 gene Arg753Gln polymorphism is strongly associated with acute rheumatic fever in children. *Journal of Molecular Medicine*, 83, 535.

Berdeli, A., Emingil, G., Han Saygan, B., Gürkan, A., Atilla, G., Köse, T. & Baylas, H. (2007) TLR2 Arg753Gly, TLR4 Asp299Gly and Thr399Ile gene polymorphisms are not associated with chronic periodontitis in a Turkish population. *Journal of Clinical Periodontology*, 34, 551.

Bird, T.D., Jarvik, G.P. & Wood, N.W. (2001) Genetic association studies: Genes in earch of diseases. *Neurology*, 57, 1153-4.

BLAST. Basic Local Alignment Search. 2008 [cited 2009 Jan 19]. Available from: http://www.ncbi.nlm.nih.gov/BLAST

Buwitt-Beckmann, U., Heine, H., Wiesmüller, K.H., Jung, G., Brock, R., Akira, S. & Ulmer, A.J. (2006) TLR1- and TLR6-independent recognition of bacterial lipopeptides. *The Journal of Biological Chemistry*, 281, 9049.

Carpenter, S. & O'Neill, L.A. (2007) How important are Toll-like receptors for antimicrobial responses? *Cellular Microbiology*, 9, 1891.

Cheng, P.L., Eng, H.L., Chou, M.H., You, H.L. & Lin, T.M. (2007) Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population. *Translational Research: The Journal of Laboratory and Clinical Medicine*, 150, 311.

Clark, M.F. & Baudoin, S.V. (2006) A systematic review of the quality of genetic association studies in human sepsis. *Intensive Care Medicine*, 32, 1706.

Düzgün, N., Duman, T., Haydardedeoğlu, F.E. & Tutkak, H. (2007) The lack of genetic association of the Toll-like receptor 2 (TLR2) Arg753Gln and Arg677Trp polymorphisms with rheumatic heart disease. *Clinical Rheumatology*, 26, 915.

Eid, A.J., Brown, R.A., Paya, C.V. & Razonable, R.R. (2007) Association Between Toll-Like Receptor Polymorphisms and the Outcome of Liver Transplantation for Chronic Hepatitis C Virus.*Transplantation*, 84, 511.

Emingil, G., Berdeli, A., Baylas, H., Saygan, B.H., Gürkan, A., Köse, T. & Atilla, G. (2007) Toll-Like Receptor 2 and 4 Gene Polymorphisms in Generalized Aggressive Periodontitis. *Journal of Periodontology*, 78, 1968.

Flores, C., Yanes, M.M.P. & Villar, J. (2008) A quality assessment of genetic association studies supporting susceptibility and outcome in acute lung injury. Critical Care, 12, R130.

Folwaczny, M., Glas, J., Török, H.P., Limbersky, O. & Folwaczny, C. (2004) Toll-like receptor (TLR) 2 and 4 mutations in periodontal disease. *Clinical and Experimental Immunology*, 135, 330.

Geng, J., Zhang, Y.W., Huang, G.C. & Chen, L.B. (2008) XRCC1 genetic polymorphism Arg399Gln and gastric cancer risk: A meta-analysis. *World Journal of Gastroenterology,* 14, 6733.

Graaf, C.A., Netea, M.G., Drenth, I.P., te Morsche, R.H., van der Meer, J.W. & Kullberg, B.J. (2003) Candida-specific interferon-gamma deficiency and Toll-like receptor polymorphisms in patients with chronic mucocutaneous candidiasis. *The Netherlands Journal of Medicine,* 61, 365.

Hamann, L., Gomma, A., Schröder, N.W., Stamme, C., Glaeser, C., Schulz, S., Gross, M., Anker, S.D., Fox, K. & Schumann, R.R. (2005) A frequent toll-like receptor (TLR)-2 polymorphism is a risk factor for coronary restenosis. *Journal of Molecular Medicine*. 83, 478.

HapMap. International HapMap Project. 2008. [cited 2009 Jan 20]. Available from: http://www.hapmap.org/

Härtel, Ch., Finas, D., Ahrens, P., Kattner, E., Schaible, T., Müller, D., Segerer, H., Albrecht, K., Möller, J., Diedrich, K. & Göpel, W. (2004) Genetic Factors in Neonatology Study Group. Polymorphisms of genes involved in innate immunity: Association with preterm delivery. *Molecular Human Reproduction*, 10, 911.

Hattersley, A.T. & McCarthy, M.I. (2005) What makes a good genetic association study? Lancet, 366, 1315.

Jelavić, T.B., Barisić, M., Drmic Hofman, I., Boraska, V., Vrdoljak, E., Peruzović, M., Hozo, I., Puljiz, Z. & Terzić, J. (2006) Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. *Clinical Genetics*, 70, 156.

Kang, T.J. & Chae, G.T. (2001) Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunology and Medical Microbiology*, 31, 53.

Kang, T.J., Yeum, C.E., Kim, B.C., You, E.Y. & Chae, G.T. (2004) Differential production of interleukin-10 and interleukin-12 in mononuclear cells from leprosy patients with a Toll-like receptor 2 mutation. *Immunology*, 112, 674.

Kijpittayarit, S., Eid, A.J., Brown, R.A., Paya, C.V. & Razonable, R.R. (2007) Relationship between Toll-Like Receptor 2 Polymorphism and Cytomegalovirus Disease after Liver Transplantation. *Clinical Infectious Diseases*, 44, 1315.

Knaus, W.A., Draper, E.A., Wagner, D.P. & Zimmerman, J.E. (1985) APACHE II: A severity of disease classification system. *Critical Care Medicine*, 13, 818.

Krediet, T.G., Wiertsema, S.P., Vossers, M.J., Hoeks, S.B., Fleer, A., Ruven, H.J. & Rijkers, G.T. (2007) Toll-like Receptor 2 Polymorphism Is Associated With Preterm Birth. *Pediatric Research*, 62, 474.

Kutukculer, N., Yeniay, B.S., Aksu, G. & Berdeli, A. (2007) Arg753Gln Polymorphism of the Human Toll-like Receptor-2 Gene in Children with Recurrent Febrile Infections. *Biochemical Genetics*, 45, 507.

Labrum, R., Bevan, S., Sitzer, M., Lorenz, M. & Markus, H.S. (2007) Toll Receptor Polymorphisms and Carotid Artery Intima-Media Thickness. *Stroke*, 38, 1179.

Lahiri, D.K. & Nurnberger, J.I. Jr. (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Research,* 19, 5444.

Lorenz, E., Mira, J.P., Cornish, K.L., Arbour, N.C. & Schwartz, D.A. (2000) A novel polymorphism in the Toll-Like receptor 2 gene and its potential association with Staphylococcal infection. *Infection and Immunity*, 68, 6398.

Malhotra, D., Relhan, V., Reddy, B.S. & Bamezai, R. (2005) TLR2 Arg677Trp polymorphism in leprosy: Revisited. *Human Genetics,* 116, 413.

Merx, S., Neumaier, M., Wagner, H., Kirschning, C.J. & Ahmad-Nejad, P. (2007) Characterization and investigation of single nucleotide polymorphisms and a novel TLR2 mutation in the human TLR2 gene. *Human Molecular Genetics*. 16, 1225.

Mockenhaupt, F.P., Cramer, J.P., Hamann, L., Stegemann, M.S., Eckert, J., Oh, N.R., Otchwemah, R.N., Dietz, E., Ehrhardt, S., Schröder, N.W., Bienzle, U. & Schumann, R.R. (2006) Toll-like receptor (TLR) polymorphisms in African children: Common TLR-4 variants predispose to severe malaria. *Proceedings of the National Academy of Sciences of the United States of America,* 103, 177.

Moore, C.E., Segal, S., Berendt, A.R., Hill, A.V. & Day, N.P. (2004) Lack of Association between Toll-Like Receptor 2 Polymorphisms and Susceptibility to Severe Disease Caused by *Staphylococcus aureus. Clinical and Diagnostic Laboratory Immunology*, 11, 1194.

Ogus, A.C., Yoldas, B., Ozdemir, T., Uguz, A., Olcen, S., Keser, I., Coskun, M., Cilli, A. & Yegin, O. (2004) The Arg753Gln polymorphism of the human Toll-like receptor 2 gene in tuberculosis disease. *The European Respiratory Journal,* 23, 219.

Reactome. A Curated Knowledgebase of Biologicakl Pathways. 2008 [cited 2009 Jan 20]. Available from: http://reactome.org

Ryu, Y.J., Kim, E.J., Koh ,W.J., Kim, H., Kwon, O.J. & Chang J.H. (2006) Toll-Like Receptor 2 Polymorphisms and Nontuberculous Mycobacterial Lung Diseases. *Clinical and Vaccine Immunology*, 13, 818.

Sánchez, E., Orozco, G., López-Nevot, M.A., Jiménez-Alonso, J. & Martín, J. (2004) Polymorphisms of toll-like receptor 2 and 4 genes in rheumatoid arthritis and systemic lupus erythematosus. *Tissue Antigens*, 63, 54.

Schröder, N.W., Diterich, I., Zinke, A., Eckert, J., Draing, C., von Baehr, V., Hassler, D., Priem, S., Hahn, K., Michelsen, K.S., Hartung, T., Burmester, G.R., Göbel, U.B., Hermann, C. & Schumann, R.R. (2005) Heterozygous Arg753Gln Polymorphism of

Human TLR-2 Impairs Immune Activation by *Borrelia burgdorferi* and Protects from Late Stage Lyme Disease. *Journal of Immunology*, 175, 2534.

Schröder, N.W., Hermann, C., Hamann, L., Göbel, U.B., Hartung, T. & Schumann, R.R. (2003) High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. *Journal of Molecular Medicine*, 81, 368.

Shi, J., Gershon, E.S. & Liu, C. (2008) Genetic associations with schizophrenia: Meta-analyses of 12 candidate genes. *Schizophrenia Research*, 104, 96.

Sluis, S., Dolan, C.V., Neale, M.C. & Posthuma, D. (2008) Power Calculations Using Exact Data Simulation: A Useful Tool for Genetic Study Designs. *Behavior Genetics*, 38, 202.

Tabel, Y., Berdeli, A. & Mir, S. (2007) Association of TLR2 gene Arg753Gln polymorphism with urinary tract infection in children. *International Journal of Immunogenetics*, 34, 399.

Takeda, K., Kaisho, T. & Akira, S. (2003) Toll-Like receptors. *Annual Review of Immunology*, 21, 335.

Texereau, J., Chiche, J.D., Taylor, W., Choukroun, G., Comba, B. & Mira, J.P. (2005) The importance of Toll-Like receptor 2 polymorphisms in severe infections. *Clinical Infectious Diseases,* 41, S408.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research,* 25, 4876.

Trinchieri, G. & Sher, A. (2007) Cooperation of Toll-like receptor signals in innate immune defence. *Nature Reviews. Immunology*, 7, 179.

Vincent, J.L., de Mendonça, A., Cantraine, F., Moreno, R., Takala, J., Suter, P.M., Sprung, C.L., Colardyn, F. & Blecher, S. (1998) Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: Results of a multicenter, prospective study. *Critical Care Medicine*, 26, 1793.

Vitali, S.H. & Randolph, A.G. (2005) Assessing the quality of case-control association studies on the genetic basis of sepsis. *Pediatric Critical Care Medicine*, 6, S74.

Woehrle, T., Du W., Goetz, A., Hsu, H.Y., Joos, T.O., Weiss, M., Bauer, U., Brueckner, U.B. & Schneider, E.M. (2008) Pathogen specific cytokine release reveals an effect of TLR2 Arg753Gln during Candida sepsis in humans. *Cytokine*, 41, 322.

Yoon, H.J., Choi, J.Y., Kim, C.O., Park, Y.S., Kim, M.S., Kim, Y.K., Shin, S.Y., Kim, J.M. & Song, Y.G. (2006) Lack of Toll-like Receptor 4 and 2 Polymorphisms in Korean Patients with Bacteremia. *Journal of Korean Medical Science*, 21, 979.

Zafra, G., Flórez, O., Morillo, C.A., Echeverría, L.E., Martín, J. & González, CI. (2008) Polymorphisms of toll-like receptor 2 and 4 genes in Chagas disease. *Memórias do Instituto Oswaldo Cruz*, 103, 27.

## FIGURE LEGENDS

**Figure 1**: Genotyping analysis of the supposed-2029C>T TLR2 SNP (into the box) via sequencing in an ABI PRISM 3100 Avant Genetic Analyzer, Perkin-Elmer, USA (A) and in a MegaBase 1000 capillary DNA sequencer, Amersham Biosciences, UK (B). Both were sequenced by forward and reverse primers.

**Figure 2:** Distribution of TLR2 studies by the time: from 1998 to 2008. Number of studies with supposed-f 2029C>T (gray line) and with 2258G>A (black line) SNP.

**Figure 3:** Distribution and frequencies of TLR2 supposed-2029C>T (A) and 2258G>A (B) studies around the World.

**Figure 4**: Sample size in TLR2 2029C>T (gray bars) and 2258G>A (black bars) reports. Inside each bar is a striped box that represents the percents of studies that observed positive association with mutant alleles, where there is no striped box the percents is zero.

**Figure 5**: Quality criterion points in TLR2 2029C>T (gray bars) and 2258G>A (black bars) reports. For description of quality criterion points and scores see Materials and Methods section.

**Figure 6**: Quality criterion categories in TLR2 2029C>T (gray bars) and 2258G>A (black bars) reports. For description of quality criterion categories and scores see Materials and Methods section.

**Figure 7**: Methods used to perform TLR2 2029C>T (gray bars) and 2258G>A (black bars) genotyping. Inside each bar is a striped box that represents the percents of studies that have duplicated analysis, where there is no striped box the percents is zero. PCR: Polymerase Chain Reaction; RFLP: Restriction fragment length polymorphism; SSCP: Single Strand Conformation Polymorphism; Seq.: Automatic Sequencing by Sanger or Enzymatic Method (pyrosequencing); Real-Time: Real-

Time PCR; ARMS: Amplification Refractory Mutation System; SSP: Sequence Specific Primer.

**TABLES LEGENDS**

**Table 1:** Clinical and demographic data of critically ill patients.

**Table 2:** Characteristics of 2029C>T TLR2 studies.

**Table 3:** Characteristics of 2258G>A TLR2 studies.

**Table 4:** Some characteristics of the main methods used on TLR2 previously published reports.

**Table 1.** Clinical and demographic data of critically ill patients.

| Variables | All Patients | With sepsis | Without sepsis | *P-value** |
|---|---|---|---|---|
| Frequency$^{\dagger}$ | 422 (100) | 295 (69.9) | 127 (30.1) | - |
| Age (years)$^{\ddagger}$ | 57 (38.5-71.5) | 60 (42-72) | 53 (32.5-69.5) | 0.056$^{MW}$ |
| Male$^{\dagger}$ | 225 (53.3) | 151 (51.2) | 72 (57.6) | 0.229 $^{MW}$ |
| APACHE II Score$^{\S}$ | 19.37(7.7) | 20.73 (7.1) | 16.12 (7.9) | 0.000$^{ST}$ |
| SOFA-1 Score$^{\ddagger}$ | 6 (4-9) | 8 (5-10) | 4 (3-6) | 0.000$^{MW}$ |
| SOFA-2 Score$^{\ddagger}$ | 6 (4-9) | 7 (5-10) | 4 (3-7) | 0.000$^{MW}$ |
| SOFA-3 Score$^{\ddagger}$ | 6 (3-9) | 7 (4-9) | 4 (3-7) | 0.000$^{MW}$ |
| SOFA-4 Score$^{\ddagger}$ | 6 (4-8) | 6 (4-9) | 4 (2-7) | 0.000$^{MW}$ |
| SOFA-5 Score$^{\ddagger}$ | 5 (3-8) | 6 (4-9) | 3.5(3-6) | 0.000$^{MW}$ |
| SOFA-6 Score$^{\ddagger}$ | 5 (3-7) | 6 (3-8) | 4(2-6) | 0.000$^{MW}$ |
| SOFA-7 Score$^{\ddagger}$ | 5 (3-8) | 6 (3-8) | 4 (2-6) | 0.000$^{MW}$ |
| SOFA-15 Score$^{\ddagger}$ | 5 (3-8) | 6 (3-8) | 3(1.25-5) | 0.001$^{MW}$ |
| SOFA-29 Score$^{\ddagger}$ | 5 (3-8) | 6 (3-8.75) | 3 (3-4.25) | 0.014$^{MW}$ |
| ICU LOS (days)$^{\ddagger}$ | 14 (8-24) | 15 (8-27) | 11 (5.5-17) | 0.000$^{MW}$ |
| ICU+H LOS (days)$^{\ddagger}$ | 37 (22-58) | 37.5 (22-59.25) | 35 (19-53) | 0.177$^{MW}$ |
| Medical Admission | 352 (83.8) | 255 (86.7) | 96 (76.8) | 0.000$^{X2}$ |
| Surgical Admission | 68 (16.2) | 39 (13.3) | 29 (23.2) | |
| Septic Shock$^{\dagger}$ | 212 (50.6) | 211 (71.5) | 1 (0.8) | 0.000 $^{MW}$ |
| ICU mortality$^{\dagger}$ | 131 (31.0) | 117 (39.7) | 13 (10.4) | 0.000 $^{MW}$ |
| ICU+H mortality$^{\dagger}$ | 185 (44.2) | 154 (52.4) | 30 (24.2) | 0.000 $^{MW}$ |
| Supposed-2029T TLR2 allele | 1 (0.2) | 0 (0.0) | 1 (0.8) | - |
| 2258A TLR2 allele | 0 (0.0) | 0 (0.0) | 0 (0.0) | |

†: n (%); ‡: median (IQR); §: mean (SD); SD: Standard Deviation of the mean; IQR: Interquartile Range; ST: Student's *t*-test; MW: Mann-Whitney *U*-test; X2: Pearson Chi-Square test; *: *P*-value describes a comparison between patients with sepsis and without sepsis; LOS: length of stay; HW: Pearson Chi-Square test for Hardy-Weinberg equilibrium.

**Table 2.** Characteristics of 2029C>T TLR2 SNP studies.

| Ref | Met | Population | Cases N | 2029 CC N | 2029 CT N | 2029 TT N | 2029 T N | Controls N | 2029 CC N | 2029 CT N | 2029 TT N | 2029 T N | S | HW Eq | End Point | 2029T and end point association | *P* | OR (95%CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Occident** | | | | | | | | | | | | | | | | | | |
| 1 | 1 | Netherlands | 7 | 7 | 0 | 0 | 0 | 200 | 200 | 0 | 0 | 0 | 1 | No | Chronic Mucocutaneous Candidiasis | No | ns | ns |
| 2 | 1 | Germany | - | - | - | - | - | 319 | 319 | 0 | 0 | 0 | 2 | No | * | * | ns | ns |
| 3 | 1 | Germany | 122 | 122 | 0 | 0 | 0 | 122 | 122 | 0 | 0 | 0 | 2 | No | Periodontal Disease | No | ns | ns |
| 4 | 1 | Spain | 346 | 346 | 0 | 0 | 0 | 199 | 199 | 0 | 0 | 0 | 2 | No | RA/SLE | No | ns | ns |
| 5 | 3 | Tunisia | 33 | 2 | 31 | 0 | 0.47 | 33 | 23 | 10 | 0 | 0.15 | 2 | Yes | Tuberculosis | Yes | <0.0001 | NI |
| 6 | 1 | Germany | 290 | 290 | 0 | 0 | 0 | 290 | 290 | 0 | 0 | 0 | 2 | No | Malaria | No | ns | ns |
| 7 | 3 | Germany | 325 | 325 | 0 | 0 | 0 | - | - | - | - | - | 0 | No | Different pattern microbial defense | No | ns | ns |
| **Orient** | | | | | | | | | | | | | | | | | | |
| 8 | 2 | Korea | 86 | 76 | 10 | 0 | 0.06 | 45 | 45 | 0 | 0 | 0 | 1 | No | Lepromatous Leprosy | yes | NI | NI |
| 9 | 2 | Korea | 15 | 10 | 5 | 0 | 0.17 | 10 | 10 | 0 | 0 | 0 | 1 | No | Alteration of Cytokines/Poor Cellular Immune Response | Yes | 0.05 | NI |
| 10 | 1 | Turkey | 61 | 61 | 0 | 0 | 0 | 207 | 207 | 0 | 0 | 0 | 2 | No | Rheumatic fever | No | ns | ns |
| 11 | 3 | India | 286 | 286 | 0 | 0 | 0 | 183 | 183 | 0 | 0 | 0 | 2 | No | * | * | ns | ns |
| 12 | 1 | Korea | 80 | 80 | 0 | 0 | 0 | 84 | 84 | 0 | 0 | 0 | 2 | No | NTM Lung Diseases | No | ns | ns |
| 13 | 1 | Korea | 154 | 154 | 0 | 0 | 0 | 179 | 179 | 0 | 0 | 0 | 2 | No | Bacteremia | No | ns | ns |
| 14 | 1 | Turkey | 90 | 90 | 0 | 0 | 0 | 155 | 155 | 0 | 0 | 0 | 4 | Yes | Aggressive Periodontitis | No | ns | ns |
| 15 | 1 | Turkey | 83 | 83 | 0 | 0 | 0 | 106 | 106 | 0 | 0 | 0 | 4 | Yes | Chronic Periodontitis | No | ns | ns |
| 16 | 1 | Turkey | 85 | 85 | 0 | 0 | 0 | 141 | 141 | 0 | 0 | 0 | 1 | No | Rheumatic Heart Disease | No | ns | ns |
| 17 | 4 | Taiwan | 200 | 200 | 0 | 0 | 0 | - | - | - | - | - | 3 | Yes | Viral infection | No | ns | ns |
| 18 | 1 | Turkey | 52 | 52 | 0 | 0 | 0 | 91 | 91 | 0 | 0 | 0 | 2 | No | Recurrent Febrile Infections | No | ns | ns |

Ref: References = 1: Graaf *et al.*, 2003; 2: Schröder *et al.*, 2003; 3: Folwaczny *et al.*, 2004; 4: Sánchez *et al.*, 2004; 5: Ben-Ali *et al.*, 2004; 6: Mockenhaupt *et al.*, 2006; 7: Woehrle *et al.*, 2008; 8: Kang & Chae, 2001; 9: Kang *et al.*, 2004; 10: Berdeli *et al.*, 2005; 11: Malhotra *et al.*, 2005; 12: Ryu *et al.*, 2006; 13: Yoon *et al.*, 2006; 14: Emingil *et al.*, 2007; 15: Berdeli *et al.*, 2007; 16: Düzgün *et al.*, 2007; 17: Cheng *et al.*, 2007; 18: Kutukculer *et al.*, 2007. Met: Laboratorial Method = 1: RFLP; 2: SSCP; 3: Sequencing; 4: Real-Time; 5: ARMS or SSP-PCR; S: 5-point Scoring Classes; HW Eq: Hardy-Weinberg Equilibrium; P: P value; OR: Odds Ration; * No association study; ns: Not Significant; NI: Not informed; RA: Rheumatoid Arthritis; SLE: Systemic Lupus Erythematosus; NTM: Nontuberculous Mycobacterial.

**Table 3:** Characteristics of 2258G>A TLR2 SNP studies.

| Ref | Met | Population | Cases N | 2258 GG N | 2258 GA N | 2258 AA N | 2258 A N | Controls N | 2258 GG N | 2258 GA N | 2258 AA N | 2258 A N | S | HW Eq. | End Point | 2258G and end point association | *P* | OR (95%CI) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Occident** | | | | | | | | | | | | | | | | | | |
| 1 | 3 | France | 91 | 89 | 2 | 0 | 0.02 | 73 | 73 | 0 | 0 | 0 | 2 | No | Septic Shock | Yes | NI | NI |
| 2 | 1 | Germany | - | - | - | - | - | 319 | 289 | 30 | 0 | 0.05 | 2 | No | * | * | 0.022 | NI |
| 3 | 1 | UK | 420 | 400 | 20 | 0 | 0.02 | 696 | 664 | 32 | 0 | 0.02 | 2 | No | Disease by *S. aureus* | No | ns | ns |
| 4 | 1 | Germany | 122 | - | - | - | 0.02 | 122 | - | - | - | 0.04 | 2 | No | Periodontal Disease | No | ns | ns |
| 5 | 1 | Spain | 346 | 340 | 6 | 0 | 0.01 | 199 | 197 | 2 | 0 | 0.01 | 2 | No | RA/SLE | No | ns | ns |
| 6 | 1 | Germany | 1398 | - | - | - | 0.03 | - | - | - | - | - | 3 | Yes | Preterm Delivery | No | ns | ns |
| 7 | 4 | Germany | 104 | 90 | 13 | 1 | 0.07 | 102 | 97 | 5 | 0 | 0.02 | 2 | No | PTCA Patients with and without Restenosis | Yes | 0.014 | 3.093 (1.103-8.676) |
| 8 | 4 | Germany | 388 | 359 | 27 | 2 | 0.04 | 189 | 173 | 15 | 1 | 0.04 | 2 | No | Astherosclerosis | No | ns | ns |
| 9 | 1 | Germany | 155 | 146 | 9 | 0 | 0.03 | 349 | 307 | 42 | 0 | 0.06 | 3 | Yes | Lyme Disease | Yes | 0.0369 | 0.459 (0.21-0.95) |
| 10 | 1 | Croatia | 89 | - | - | - | ** | 88 | - | - | - | ** | 2 | Yes | Câncer Colorretal | No | ns | ns |
| 11 | 1 | Germany | 290 | 290 | 0 | 0 | 0 | 290 | 290 | 0 | 0 | 0 | 1 | No | Malaria | No | ns | ns |
| 12 | 4 | USA | 92 | 80 | 7 | 5 | 0.09 | - | - | - | - | - | 1 | No | Outcome of liver Transplantation for Chronic Hepatitis C Virus | Yes | 0.007 | 5.20 (1.65-13.9) |
| 13 | 4 | USA | 92 | 80 | 7 | 5 | 0.09 | - | - | - | - | - | 1 | No | Citomegalovirus Disease after Liver Transplantation | Yes | 0.08 | 1.91 (0.91-3.40) |
| 14 | 3 | Germany | 106 | 96 | 10 | 0 | 0.05 | - | - | - | - | - | 0 | No | Hypo-responsiveness | Yes | NI | NI |
| 15 | 5 | Netherlands | 524 | - | - | - | 0.03 | - | - | - | - | - | 1 | Yes | Preterm Birth | Yes | <0.02 | NI |
| 16 | 3 | UK | 2955 | - | - | - | 0.48 | - | - | - | - | - | 3 | Yes | CAIMT | No | ns | ns |
| 17 | 1 | Colombia | 275 | 275 | 0 | 0 | 0 | 200 | 200 | 0 | 0 | 0 | 1 | No | Chagas Disease | No | ns | ns |
| 18 | 3 | Germany | 325 | 308 | 17 | 0 | 0.03 | - | - | - | - | - | 0 | No | DPMD | Yes | NI | NI |
| **Orient** | | | | | | | | | | | | | | | | | | |
| 19 | 5 | Turkey | 151 | 124 | 13 | 14 | 0.14 | 116 | 107 | 7 | 2 | 0.05 | 2 | Yes | Tuberculosis | Yes | 0.022 | 6.04 (2.01-20.08) |
| 20 | 1 | Turkey | 61 | 5 | 56 | 0 | 0.46 | 207 | 186 | 21 | 0 | 0.05 | 2 | No | Rheumatic fever | Yes | 0.0001 | 100 (32-320) |
| 21 | 1 | Turkey | 83 | 82 | 1 | 0 | 0.01 | 95 | 93 | 2 | 0 | 0.01 | 1 | No | Behçet's disease | No | ns | ns |
| 22 | 1 | Korea | 80 | 80 | 0 | 0 | 0 | 84 | 84 | 0 | 0 | 0 | 2 | No | NTM Lung Diseases | No | ns | ns |
| 23 | 1 | Korea | 154 | 154 | 0 | 0 | 0 | 179 | 179 | 0 | 0 | 0 | 2 | No | Bacteremia | No | ns | ns |
| 24 | 1 | Turkey | 90 | 83 | 7 | 0 | 0.04 | 155 | 136 | 19 | 0 | 0.06 | 4 | Yes | Aggressive Periodontitis | No | ns | ns |
| 25 | 1 | Turkey | 83 | 72 | 11 | 0 | 0.07 | 106 | 92 | 14 | 0 | 0.07 | 4 | Yes | Chronic Periodontitis | No | ns | ns |
| 26 | 1 | Turkey | 85 | 85 | 0 | 0 | 0 | 141 | 138 | 3 | 0 | 0.01 | 1 | No | Rheumatic Heart Disease | No | ns | ns |
| 27 | 4 | Taiwan | 200 | 200 | 0 | 0 | 0 | - | - | - | - | - | 3 | Yes | Viral infection | No | ns | ns |
| 28 | 1 | Turkey | 52 | 34 | 12 | 6 | 0.23 | 91 | 82 | 9 | 0 | 0.05 | 2 | No | Recurrent Febrile Infections | Yes | 0.007 | 6.3 (1.33-29.8) |
| 29 | 1 | Turkey | 124 | 91 | 33 | | ** | 116 | 104 | 12 | | ** | 3 | Yes | Urinary Tract Infection | Yes | 0.001 | 3.14 (1.53-6.44) |

Ref: Reference = 1: Lorenz *et al*., 2000; 2: Schröder *et al*., 2003; 3: Moore *et al*., 2004; 4: Folwaczny *et al*., 2004; 5: Sánchez *et al*., 2004; 6: Härtel *et al*., 2004; 7: Hamann *et al*., 2005; 8: Hamann *et al*., 2005; 9: Schröder *et al*., 2005; 10: Jelavić *et al*., 2006; 11: Mockenhaupt *et al*., 2006; 12: Eid *et al*., 2007; 13: Kijpittayarit *et al*, 2007; 14: Merx *et al*., 2007; 15: Krediet *et al*., 2007; 16: Labrum *et al*., 2007; 17: Zafra *et al*., 2008; 18: Woehrle *et al*., 2008; 19: Ogus *et al*., 2004; 20: Berdeli *et al*., 2005; 21: Bacanli *et al*., 2006; 22: Ryu *et al*., 2006; 23: Yoon *et al*., 2006; 24: Emingil *et al*., 2007; 25: Berdeli *et al*., 2007; 26: Düzgün *et al*., 2007; 27: Cheng *et al*., 2007; 28: Kutukculer *et al*., 2007; 29: Tabel *et al*., 2007. Met: Laboratorial Method = 1: RFLP; 2: SSCP; 3: Sequencing; 4: Real-Time; 5: ARMS or SSP-PCR; S: 5-point Scoring Classes; HW Eq: Hardy-Weinberg Equilibrium; P: P value; OR: Odds Ration; * No association study; ** Calculate was not possible; ns: Not Significant; NI: Not informed; UK: United Kingdom, USA: United States of America; RA: Rheumatoid Arthritis; SLE: Systemic Lupus Erythematosus; NTM: Nontuberculous Mycobacterial; CAIMT: Carotid Artery Intima-Media Thickness; DPMD: Different pattern microbial defense; PTCA: Percutaneous Transluminal Coronary Angioplasty

**Table 4:** Some characteristics of the main methods used on TLR2 previously published reports.

| Method | Positive Points | Negative Points |
|---|---|---|
| PCR-RFLP | Low cost | Intensive work |
| | Multiple analysis at the same time | Delayed process |
| | Large application | Low sensitivity |
| | Detects small portions of the DNA | Not automatized process |
| | Universal equipments | Intensive manipulation |
| | Universal reagents | |
| | Easy routine to operators | |
| Real Time PCR | Faster and Precise | High cost |
| | High sensitivity | Specific equipments |
| | High production | Specific reagents |
| | Quantifies the product | highly training operators |
| | Automatized process | Specific support necessary |
| | Reduced manipulation | Contamination risk with mRNA |
| | Reduced contamination | |
| | Real time results | |
| Automatized Sequencing | Specific and precise | High cost |
| | High precision and sensitivity; | Delayed process |
| | Reduced manipulation | Specific equipments |
| | Reduced contamination | Specific reagents |
| | High sensitivity | Highly training operators |
| | High production | Specific support necessary |
| | Automatized process | |

# FIGURES

## FIGURE 1

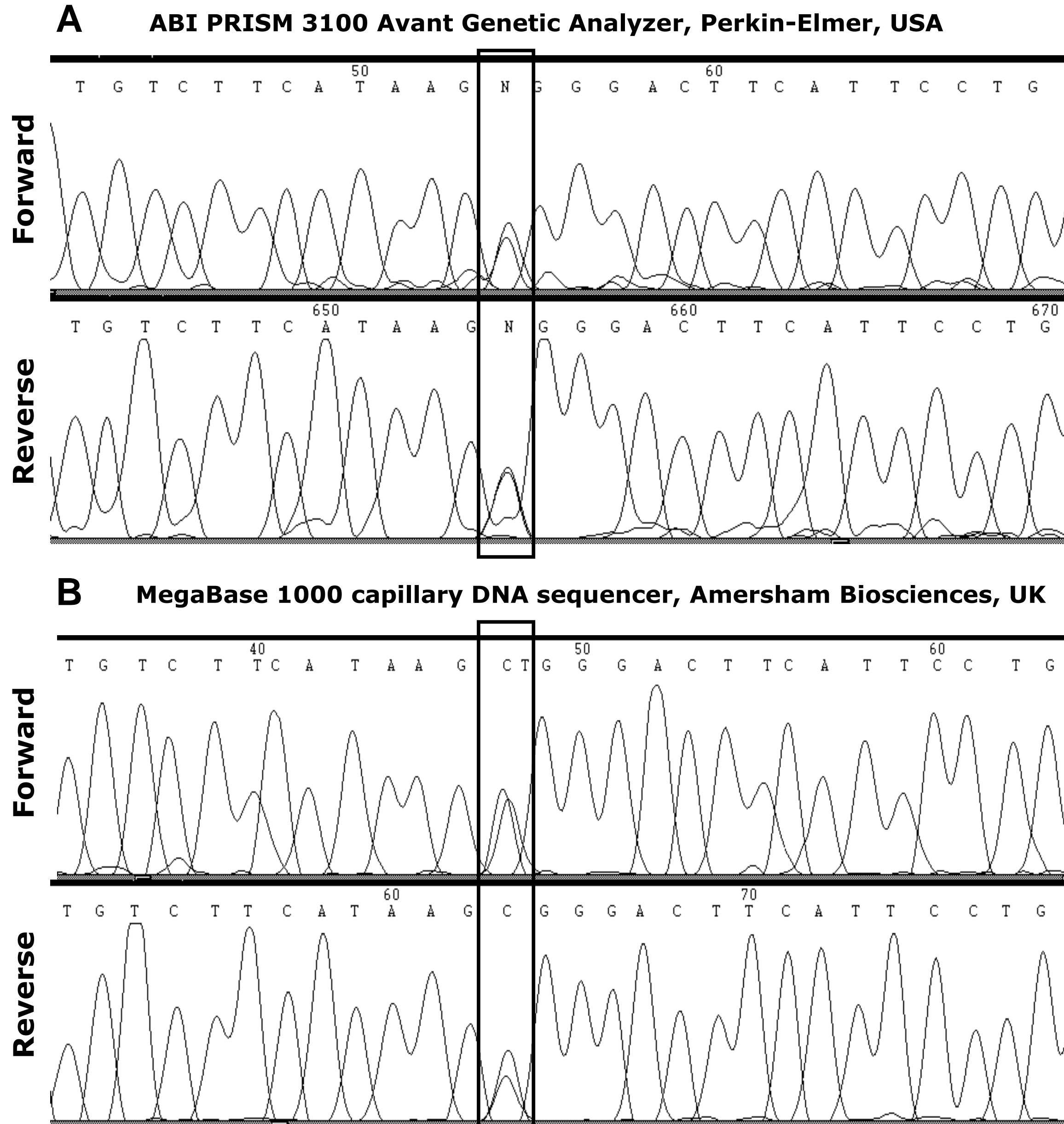


FIGURE 2

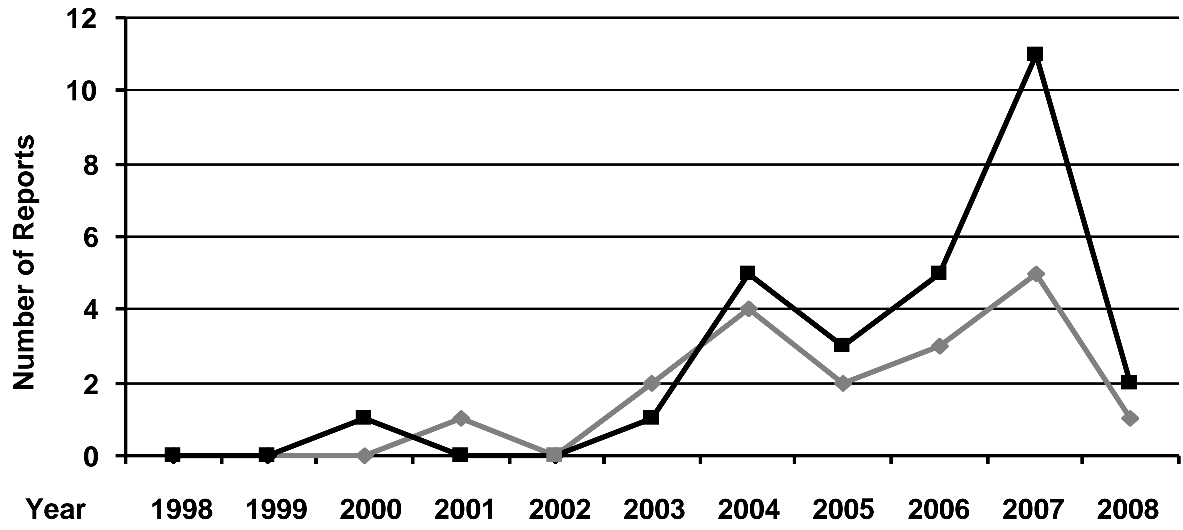


**FIGURE 3**

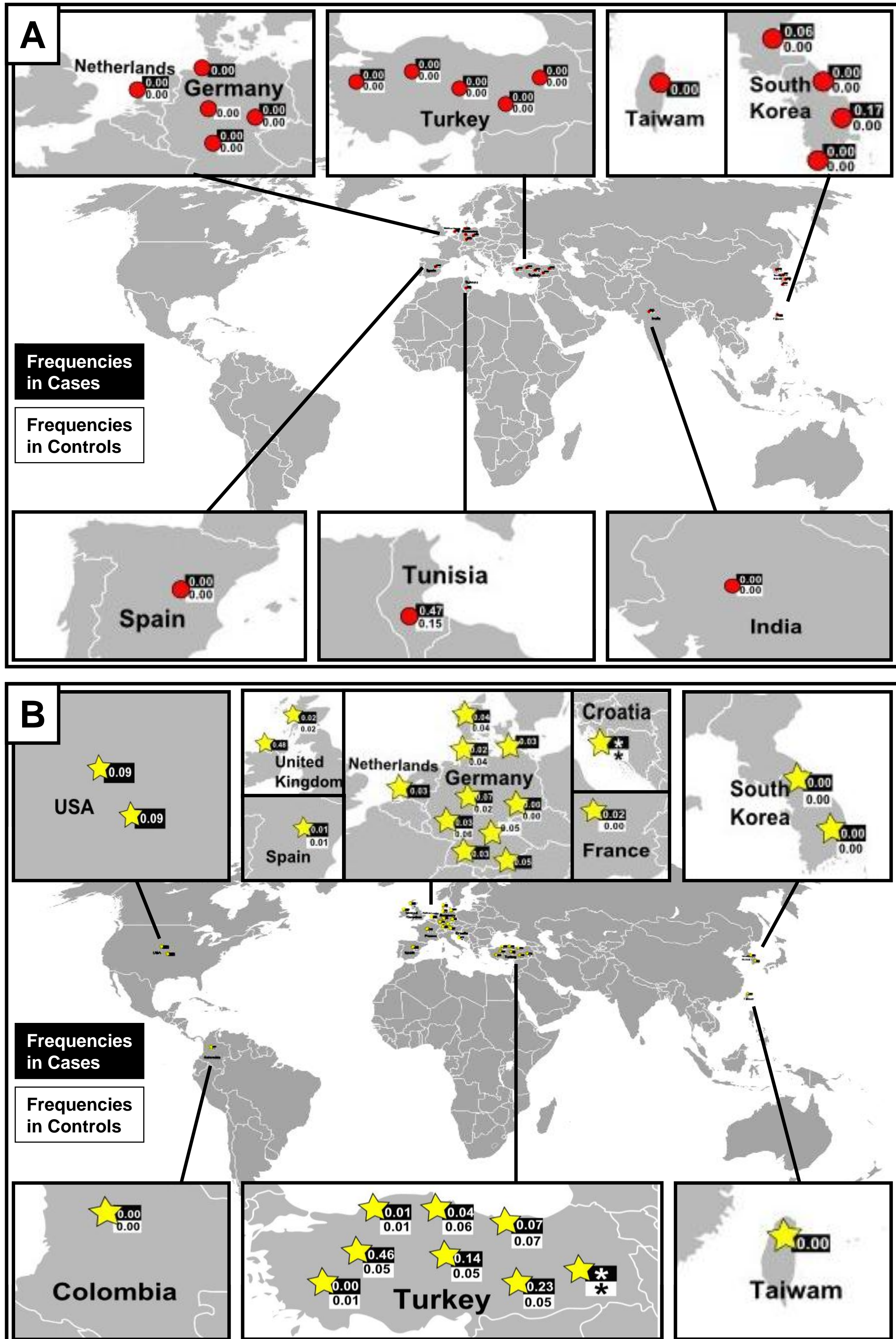


**FIGURE 4**

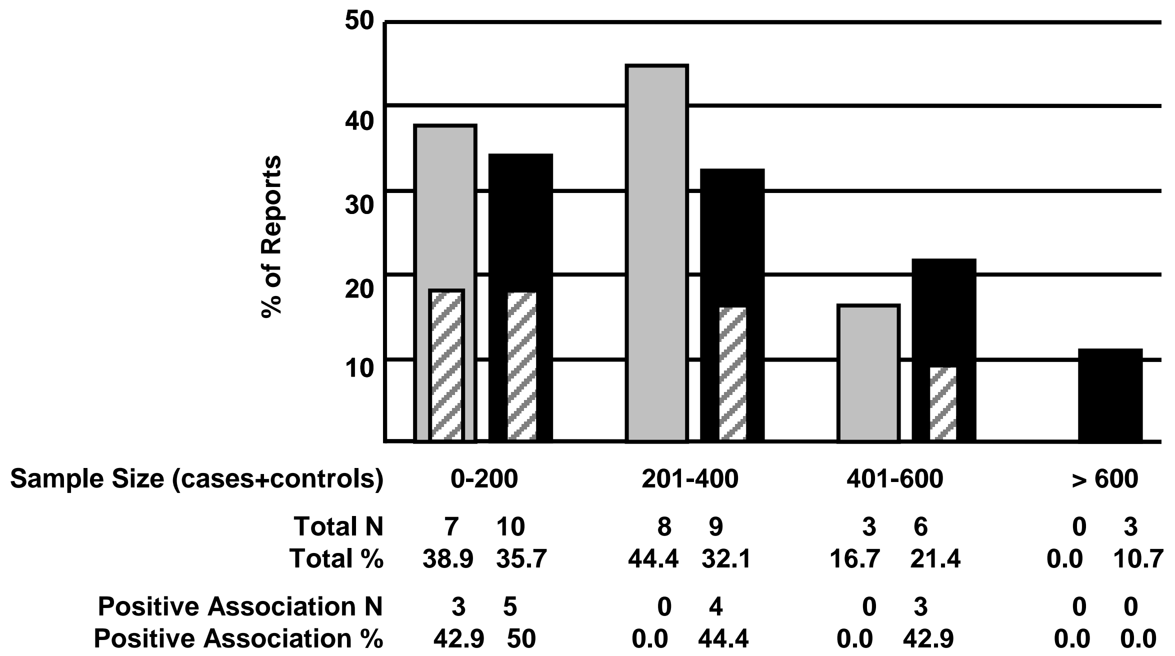


**FIGURE 5**

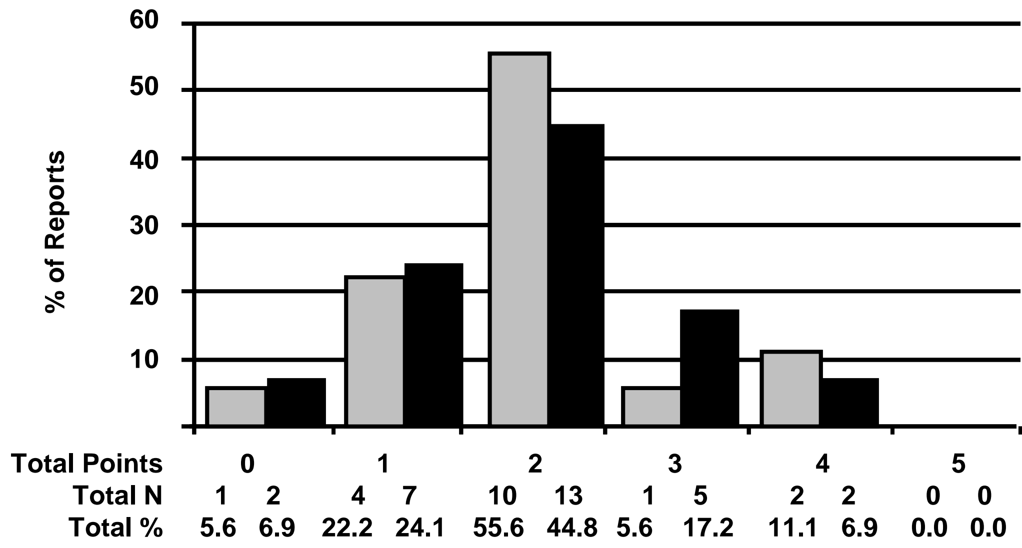


FIGURE 6

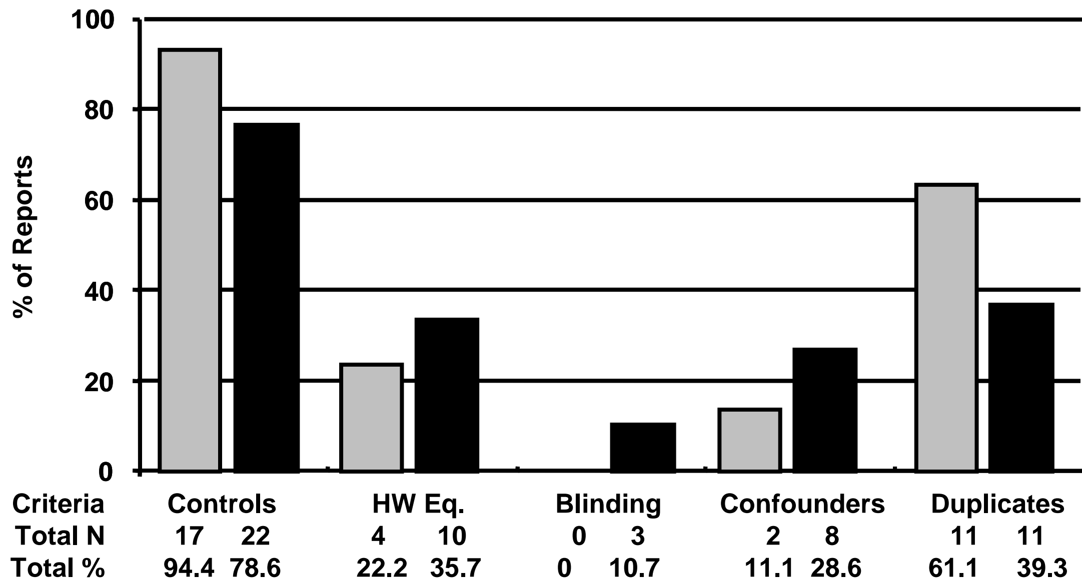
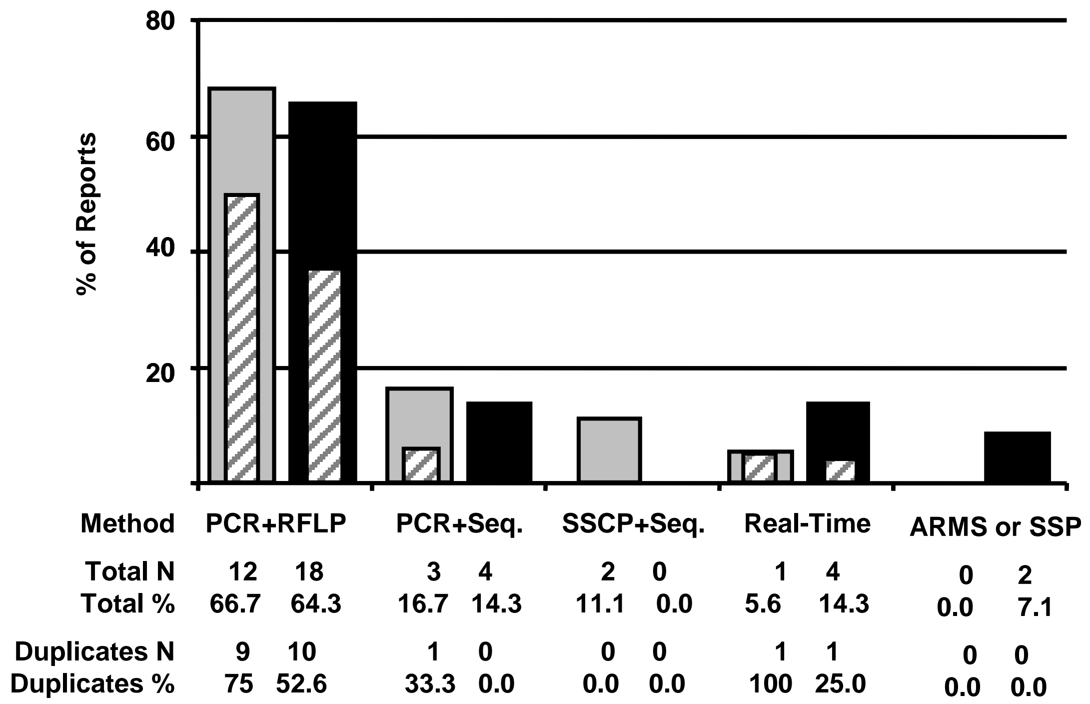


**FIGURE 7**



# 3 CONSIDERAÇÕES FINAIS

A identificação de genótipos não é capaz isoladamente de predizer um fenótipo final dado que as características complexas, como a sepse, são sempre reguladas pela ação conjunta das características genéticas individuais sob as influências ambientais externas. Além destas influências ambientais externas, é fundamental ressaltar também a importância da relação parasito-hospedeiro e as características genéticas dos próprios microrganismos.

Ao longo deste trabalho buscamos verificar se a presença dos alelos raros de dois polimorfismos do TLR2 poderia acorrer com maior freqüência em pacientes com sepse do que em pacientes sem sepse. Após nossas análises, constatamos que estes polimorfismos do TLR2 ocorrem com uma freqüência muito baixa ou nula em nossa população não possuindo efeito determinante na sepse. Inicialmente, havíamos confirmado que o HapMap mostrava uma freqüência de 5% do alelo A do SNP 2258G>A na população de caucasianos europeus, sendo o alelo G fixado na população oriental e negra. Levando em consideração a origem étnica, a população do sul do Brasil é composta por características genéticas particulares, com a maioria de indivíduos de origem Européia (com ancestralidade portuguesa, italiana, espanhola e alemã) e uma pequena parte da população de origem africana contribuindo com seu patrimônio genético.

Estudos de associação genética são importantes para identificar e analisar variantes que podem ser um forte fator de suscetibilidade a doenças. No entanto, realizando uma avaliação da qualidade dos estudos dos polimorfismos do TLR2, nós encontramos algumas deficiências em relação a características importantes de trabalhos associativos como: falta de informação quanto ao equilíbrio de Hardy-Weinberg, ao estudo cego, às análises estatísticas e quanto a duplicação dos estudos. O método utilizado na genotipagem e o tamanho da amostragem, os quais são também importantes na confiabilidade do resultado, foram, na maioria dos estudos, realizados por técnicas menos precisas (PCR-RFLP) e com número não muito alto de indivíduos (<400). Tais fatores conjuntamente devem ser a explicação para inconsistências encontradas em relação aos resultados: enquanto muitos

artigos não apresentam associação nenhuma entre alelos raros e fenótipos em suas populações, outros encontram fortes associações.

Analisando nossos resultados e levando em consideração nossa revisão e análise dos estudos com variantes alélicas no TLR2, sugerimos que os SNPs 2029C>T e 2258G>A não são bons candidatos a estudos de associação genética quando estudados isoladamente. Esforços futuros na busca de marcadores genéticos com aplicações clínicas deveriam ser direcionados a estudar simultaneamente outros SNPs a fim de identificar seus efeitos modificantes na susceptibilidade às doenças infecciosas.

# REFERÊNCIAS

Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001 Aug;2(8):675-80.

Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004 Jul;4(7):499-511.

Akira S, Uematsu S, Takeuchi O. Pathogen Recognition and Innate Immunity. Cell. 2006 Feb;124(4):783-801.

Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001 Jul;29(7):1303-10.

Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet. 2005 Jan;365(9453):63-78.

Ben-Ali M, Barbouche MR, Bousnina S, Chabbou A, Dellagi K. Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clin Diagn Lab Immunol. 2004 May;11(3):625-6.

Berdeli A, Celik HA, Ozyürek R, Dogrusoz B, Aydin HH. TLR-2 gene Arg753Gln polymorphism is strongly associated with acute rheumatic fever in children. J Mol Med. 2005 Jul;83(7):535-41.

Berdeli A, Emingil G, Han Saygan B, Gürkan A, Atilla G, Köse T, et al. TLR2 Arg753Gly, TLR4 Asp299Gly and Thr399Ile gene polymorphisms are not associated with chronic periodontitis in a Turkish population. J Clin Periodontol. 2007 Jul;34(7):551-7.

Bevilacqua MP. Endothelial leukocyte adhesion molecules. Annu Rev Immunol. 1993;11:767-804.

Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992 Jun;101(6):1644-55.

Bone RC, Grodzin CJ. Sepsis: A New Hypothesis for Pathogenesis of the Disease Process. Chest. 1997 Jul;112(1):235-43.

Buras JA, Holzmann B, Sitkovsky M. Animal Models of Sepsis: Setting the Stage. Nat Rev Drug Discov. 2005 Oct;4(10):854-65.

Chaudhary PM, Ferguson C, Nguyen V, Nguyen O, Massa HF, Eby M, et al. Cloning and characterization of two Toll/Interleukin-1 receptor-like genes TIL3 and TIL4: evidence for a multi-gene receptor family in humans. Blood. 1998 Jun;91(11):4020-7.

Cheng PL, Eng HL, Chou MH, You HL, Lin TM. Genetic polymorphisms of viral infection-associated Toll-like receptors in Chinese population. Transl Res. 2007 Nov;150(5):311-8.

Cohen J. The immunopathogenesis of sepsis. Nature. 2002 Dec;420(6917):885-91.

Downey GP. Mechanism of leukocyte motility and chemotaxis. Curr Opin Immunol. 1994 Feb;6(1):113-24.

Eid AJ, Brown RA, Paya CV, Razonable RR. Association between toll-like receptor polymorphisms and the outcome of liver transplantation for chronic hepatitis C virus. Transplantation. 2007 Aug;84(4):511-6.

Emingil G, Berdeli A, Baylas H, Saygan BH, Gürkan A, Köse T, et al. Toll-like receptor 2 and 4 gene polymorphisms in generalized aggressive periodontitis. J Periodontol. 2007 Oct;78(10):1968-77.

Hamann L, Gomma A, Schröder NW, Stamme C, Glaeser C, Schulz S, et al. A frequent toll-like receptor (TLR)-2 polymorphism is a risk factor for coronary restenosis. J Mol Med. 2005 Jun;83(6):478-85.

Hirschfeld M, Weis JJ, Toshchakov V, Salkowski CA, Cody MJ, Ward DC, et al. Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. Infect Immun. 2001 Mar;69(3):1477-82.

Hollingsworth JW, Whitehead G, Berman KG, Tekippe EM, Gilmour MI, Larkin JE, et al. Genetic basis of murine antibacterial defense to streptococcal lung infection. Immunogenetics. 2007 Sep;59(9):713-24.

Iwaki D, Nishitani C, Mitsuzawa H, Hyakushima N, Sano H, Kuroki Y. The CD14 region spanning amino acids 57-64 is critical for interaction with the extracellular Toll-like receptor 2 domain. Biochem Biophys Res Commun. 2005 Mar;328(1):173-6.

Jelavić TB, Barisić M, Drmic Hofman I, Boraska V, Vrdoljak E, Peruzović M, et al. Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. Clin Genet. 2006 Aug;70(2):156-60.

Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. FEMS Immunol Med Microbiol. 2001 Jul;31(1):53-8.

Kang TJ, Yeum CE, Kim BC, You EY, Chae GT. Differential production of interleukin-10 and interleukin-12 in mononuclear cells from leprosy patients with a Toll-like receptor 2 mutation. Immunology. 2004 Aug;112(4):674-80.

Kijpittayarit S, Eid AJ, Brown RA, Paya CV, Razonable RR. Relationship between Toll-like receptor 2 polymorphism and cytomegalovirus disease after liver transplantation. Clin Infect Dis. 2007 May;44(10):1315-20.

Kirschning CJ, Wesche H, Merrill Ayres T, Rothe M. Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. J Exp Med. 1998 Dec;188(11):2091-7.

Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: A severity of disease classification system. Crit Care Med. 1985 Oct;13(10):818-29.

Kutukculer N, Yeniay BS, Aksu G, Berdeli A. Arg753Gln polymorphism of the human toll-like receptor-2 gene in children with recurrent febrile infections. Biochem Genet. 2007 Aug;45(7-8):507-14.

Lee EY, Yim JJ, Lee HS, Lee YJ, Lee EB, Song YW. Dinucleotide repeat polymorphism in intron II of human Toll-like receptor 2 gene and susceptibility to rheumatoid arthritis. Int J Immunogenet. 2006 Jun;33(3):211-5.

Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference.Crit Care Med. 2003 Apr;31(4):1250-6.

Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect Immun. 2000 Nov;68(11):6398-401.

Martin CM, Hill AD, Burns K, Chen LM. Characteristics and outcomes for critically ill patients with prolonged intensive care unit stays. Crit Care Med. 2005 Sep;33(9):1922-7.

Matsuda N, Hattori Y. Systemic Inflammatory Response Syndrome (SIRS): Molecular Pathophysiology and Gene Therapy. J Pharmacol Sci. 2006 Jul;101(3):189-98.

Merx S, Neumaier M, Wagner H, Kirschning CJ, Ahmad-Nejad P. Characterization and investigation of single nucleotide polymorphisms and a novel TLR2 mutation in the human TLR2 gene. Hum Mol Genet. 2007 May;16(10):1225-32.

Mockenhaupt FP, Cramer JP, Hamann L, Stegemann MS, Eckert J, Oh NR, et al. Toll-like receptor (TLR) polymorphisms in African children: Common TLR-4 variants predispose to severe malaria. Proc Natl Acad Sci U S A. 2006 Jan;103(1):177-82.

Moore CE, Segal S, Berendt AR, Hill AV, Day NP. Lack of association between Toll-like receptor 2 polymorphisms and susceptibility to severe disease caused by Staphylococcus aureus. Clin Diagn Lab Immunol. 2004 Nov;11(6):1194-7.

Muta T, Takeshige K. Essential roles of CD14 and lipopolysaccharide-binding protein for activation of toll-like receptor (TLR)2 as well as TLR4 Reconstitution of TLR2- and TLR4-activation by distinguishable ligands in LPS preparations. Eur J Biochem. 2001 Aug;268(16):4580-9.

Nakada TA, Hirasawa H, Oda S, Shiga H, Matsuda KI, Nakamura M et al. Influence of Toll-Like Receptor 4, CD14, Tumor Necrosis Factor, and Interleukine-10 Gene Polymorphisms on Clinical Outcome in Japanese Critically Ill Patients. J Surg Res. 2005 Dec;129(2):322-8.

Netea MG, van Deuren M, Kullberg BJ, Cavaillon JM, Van der Meer JW. Does the shape of lipid A determine the interaction of LPS with Toll-like receptors? Trends Immunol. 2002 Mar;23(3):135-39.

O'Brien JM, Ali NA, Aberegg SK, Abraham E. Sepsis. Am J Med. 2007 Dec;120(12):1012-22.

O'Neill LA. Estopim Imunológico. Scientific American Brasil 2005; 68-75.

O'Neill LAJ, Bowie AG. The family of five: TIR domain containing adaptors in Toll-like receptor signaling. Nat Rev Immunol. 2007 May;7(5):353-64.

O'Shea JJ, Ma A, Lipsky P. Cytokines and Autoimmunity. Nat Rev Immunol. 2002 Jan;2(1):37-45.

Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I, et al. The Arg753GLn polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. Eur Respir J. 2004 Feb;23(2):219-23.

Pandey S, Agrawal DK. Immunobiology of Toll-like receptors: emerging trends. Immunol Cell Biol. 2006 Aug;84(4):333-41.

Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to Drosophila Toll. Proc Natl Acad Sci U S A. 1998 Jan;95(2):588-93.

Ryu YJ, Kim EJ, Koh WJ, Kim H, Kwon OJ, Chang JH. Toll-like receptor 2 polymorphisms and nontuberculous mycobacterial lung diseases. Clin Vaccine Immunol. 2006 Jul;13(7):818-9.

Sánchez E, Orozco G, López-Nevot MA, Jiménez-Alonso J, Martín J. Polymorphisms of toll-like receptor 2 and 4 genes in rheumatoid arthritis and systemic lupus erythematosus. Tissue Antigens. 2004 Jan;63(1):54-7.

Sandor F, Buc M. Toll-like Receptors. I. Structure, Function and Their Ligands. Folia Biol (Praha). 2005;51(5):148-57.

Schröder NW, Diterich I, Zinke A, Eckert J, Draing C, von Baehr V, et al. Heterozygous Arg753Gln polymorphism of human TLR-2 impairs immune activation by Borrelia burgdorferi and protects from late stage Lyme disease. J Immunol. 2005 Aug 15;175(4):2534-40.

Schröder NW, Hermann C, Hamann L, Göbel UB, Hartung T, Schumann RR. High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. J Mol Med. 2003 Jun;81(6):368-72.

Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J Biol Chem. 1999 Jun;274(25):17406-9.

Silva E, Pedro MA, Sogayar ACB, Mohovic T, Silva CLO, Janiszewski M, et al. Brazilian Sepsis Epidemiological Study (BASES study). Crit Care. 2004 Aug;8(4):R251-60.

Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multi - step paradigm. Cell. 1994 Jan;76(2):301-14.

Tabel Y, Berdeli A, Mir S. Association of TLR2 gene Arg753Gln polymorphism with urinary tract infection in children. Int J Immunogenet. 2007 Dec;34(6):399-405.

Takeda K, Kaisho T, Akira S. Toll-Like Receptors. Annu Rev Immunol. 2003;21:335-76.

Texereau J, Chiche JD, Taylor W, Choukroun G, Comba B, Mira JP. The Importance of Toll-Like Receptor 2 Polymorphisms in Severe Infections. Clin Infect Dis. 2005 Nov;41 Suppl 7:S408-15.

Trinchieri G, Sher A. Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol. 2007 Mar;7(3):179-90.

Vincent JL, Carlet J, Opal SM. Sepsis: The magnitude of the problem. The Sepsis Text. 1ª Ed. Dordrecht: Kluwer Academy Publisher; 2002.

Vincent JL, Mendonca A, Cantraine F, Moreno R, Takala J, Suter PM, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: Results of a multicenter, prospective study. Crit Care Med. 1998 Nov;26(11):1793-800.

Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: Results of the SOAP study. Crit Care Med. 2006 Feb;34(2):344-53.

Vosylius S, Sipylaite J, Ivaskevicius J. Intensive care unit acquired infection: a prevalence and impact on morbidity and mortality. Acta Anaesthesiol Scand. 2003 Oct;47(9):1132-7.

Werling D, Jungi TW. TOLL-like receptors linking innate and adaptive immune response. Vet Immunol Immunopathol. 2003 Jan;91(1):1-12.

Werts C, Tapping RI, Mathison JC, Chuang TH, Kravchenko V, Saint Girons I, et al. Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. Nat Immunol. 2001 Apr;2(4):346-52.

Yang RB, Mark MR, Gray A, Huang A, Xie MH, Zhang M. Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. Nature. 1998 Sep;395(6699):284-8.

Yim JJ, Ding L, Schäffer AA, Park GY, Shim YS, Holland SM. A microsatellite polymorphism in intron 2 of human Toll-like receptor 2 gene: functional implications and racial differences. FEMS Immunol Med Microbiol. 2004 Mar;40(2):163-9.

Ylipalosaari P, Ala-Kokko TI, Laurila J, Ohtonen P, Syrja¨la¨ H. Community- and hospital-acquired infections necessitating ICU admission: Spectrum, co-morbidities and outcome. J Infect. 2006 Aug;53(2):85-92.

Yoon HJ, Choi JY, Kim CO, Park YS, Kim MS, Kim YK, et al. Lack of Toll-like receptor 4 and 2 polymorphisms in Korean patients with bacteremia. J Korean Med Sci. 2006 Dec;21(6):979-82.

Zafra G, Flórez O, Morillo CA, Echeverría LE, Martín J, González CI. Polymorphisms of toll-like receptor 2 and 4 genes in Chagas disease. Mem Inst Oswaldo Cruz. 2008 Feb;103(1):27-30.

Zweigner J, Gramm HJ, Singer OC, Wegscheider K, Schumann RR. High concentrations of lipopolysaccharide-binding protein in serum of patients with severe

sepsis or septic shock inhibit the lipopolysaccharide response in human monocytes. Blood. 2001 Dec;98(13):3800-8.

# ANEXOS

# ANEXO A – APROVAÇÃO PELO COMITÊ DE ÉTICA EM PESQUISA DA PUCRS

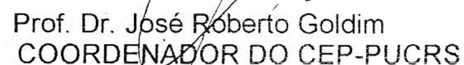
Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
COMITÊ DE ÉTICA EM PESQUISA

Ofício 1500/07-CEP                                        Porto Alegre, 10 de dezembro de 2007.

Senhor(a) Pesquisador(a):

O Comitê de Ética em Pesquisa da PUCRS apreciou e aprovou seu protocolo de pesquisa registro CEP 07/04025, intitulado: "**Estudo das variantes polimórficas 2029C> T (Arg677Trp) e 2258G>A (Arg753Gln) do gene que codifica para o TLR2 humano em pacientes críticos internados em uma Unidade de Tratamento Intensivo (UTI)**".

Sua investigação está autorizada a partir da presente data.



Relatórios parciais e final da pesquisa devem ser entregues a este CEP.

Atenciosamente,

Prof. Dr. José Roberto Goldim
COORDENADOR DO CEP-PUCRS

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# ANEXO B – COMPROVANTE DE SUBMISSÃO AO PERIÓDICO: INTERNATIONAL JOURNAL OF IMMUNOGENETICS.



INTERNATIONAL JOURNAL OF IMMUNOGENETICS

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Thank you for submitting your manuscript to *International Journal of Immunogenetics*.

Manuscript ID: IJIG-Jan-09-0012

Title: VERY LOW FREQUENCIES OF TLR2 SUPPOSED-2029T AND 2258A (RS5743708) MUTANT ALLELES IN SOUTHERN BRAZILIAN CRITICALLY ILL PATIENTS: WOULD IT BE A LACK OF WORLDWIDE ACCEPTED CLINICAL APPLICATIONS OF TLR2 VARIANTS?

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