UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

ROGER HARUKI YAMAKAWA

Polimorfismo HLA e MICA em candidatos ao transplante renal da região Norte/Noroeste do Estado do Paraná

Maringá
2017

ROGER HARUKI YAMAKAWA

Polimorfismo HLA e MICA em candidatos ao transplante renal da região Norte/Noroeste do Estado do Paraná

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ciências da Saúde
Área de concentração: Saúde Humana

Orientador: Prof.ª Dr.ª Sueli Donizete Borelli

Maringá
2017

FOLHA DE APROVAÇÃO

ROGER HARUKI YAMAKAWA

Polimorfismo HLA e MICA em candidatos ao transplante renal da região Norte/Noroeste do Estado do Paraná.

Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ciências da Saúde pela Comissão Julgadora composta pelos membros:

COMISSÃO JULGADORA

Profª. Drª. Sueli Donizete Borelli
Universidade Estadual de Maringá (Presidente)

Profª. Drª. Maria Angélica Ehara Watanabe
Universidade Estadual de Londrina

Profª. Drª. Maria Dalva Barros Carvalho
Universidade Estadual de Maringá

Prof. Dr. Jorge Juarez Vieira Teixeira
Universidade Estadual de Maringá

Profª. Drª. Sueli de Oliveira Silva Lautenschlager
Universidade Estadual de Maringá

Aprovada em:
Local de defesa:

DEDICATÓRIA(S)

Dedico este trabalho a todos aqueles que contribuíram para sua realização.

AGRADECIMENTO(S)

A Deus.

Aos meus familiares, Alcírio Harumio Yamakawa, Luzia Norico Wakai Yamakawa e Melina Ayumi Yamakawa, pela confiança, dedicação, carinho e incentivo durante toda minha vida.

À minha namorada, Patrícia Keiko Saito, pela amizade, paciência e companheirismo.

À minha orientadora, Profª. Drª. Sueli Donizete Borelli, por sua dedicação, competência e profissionalismo, essenciais ao desenvolvimento dessa pesquisa, a quem tenho grande admiração, carinho e respeito.

A todos que, direta ou indiretamente, ajudaram em meu trabalho, minha sincera e mais profunda gratidão.

EPÍGRAFE

"A semente do bem se origina no sentimento fraterno de querer alegrar ou favorecer os semelhantes."

Mokiti Okada

Polimorfismo HLA e MICA em candidatos ao transplante renal da região Norte/Noroeste do Estado do Paraná

RESUMO

O sistema HLA (*Human Leukocyte Antigen*) é um importante marcador de sobrevida do enxerto. Embora a importância do HLA-A, -B e -DR no transplante de órgãos sólidos seja conhecida há muitos anos, o papel do HLA-C, HLA-DQ e MICA (*major histocompatibility complex MHC class I chain-related gene A*) no transplante foi recentemente documentado. Neste estudo, avaliamos as frequências alélicas e haplotípicas de MICA; HLA-A, -B, -C, -DRB1, -DQA1 e -DQB1 em 346 pacientes, de diferentes etnias, com doença renal crônica (DRC), candidatos ao transplante renal da região Norte/Noroeste do Estado do Paraná, Sul do Brasil. Também foi avaliado o desequilíbrio de ligação entre MICA e HLA-B nas mesmas amostras. As frequências alélicas e haplotípicas de MICA e HLA-B encontradas nos pacientes renais foram comparadas com frequências encontradas em indivíduos saudáveis da mesma região do Brasil. A tipificação HLA e MICA foi realizada pelo método de reação em cadeia da polimerase-sequência específica de oligonucleotídeos (PCR-SSO), associado à tecnologia Luminex. Os 346 participantes foram classificados de acordo com o grupo étnico (189 caucasianos, 98 mestiços, 50 negros e 9 orientais). Um total de 19 grupos alélicos MICA, 20 HLA-A, 29 HLA-B, 14 HLA-C, 13 HLA-DRB1, 6 HLA-DQA e 5 HLA-DQB1 foram identificados. Os grupos alélicos mais frequentes foram *MICA*008*, *HLA-A*02*, *B*35*, *C*07*, *DRB1*04*, *DQA1*01* e *DQB1*03*. Diferenças significativas ($p<0.05$) foram observadas na frequência dos grupos alélicos *MICA*009*, *MICA*010*, *HLA-A*24*, *A*68, B*52, DRB1*09, C*03, C*07* e *DQA1*03* entre os grupos étnicos. O haplótipo extendido mais comum no total de amostras foi o *HLA-A*01-C*07-B*08-MICA*008-DRB1*03-DQA1*05-DQB1*02* (2.4%). Os haplótipos *MICA-HLA-B* mais comuns foram: *MICA*009-B*51* (7,8%), *MICA*004-B*44* (6,1%) e *MICA*002-B*35* (5,6%). Como esperado devido à proximidade do *loci* MICA e HLA-B, a maioria dos haplótipos mostraram forte desequilíbrio de ligação. Pacientes renais e indivíduos saudáveis da mesma região do Brasil mostraram diferenças estatisticamente significativas em seus polimorfismos MICA. O grupo alélico *MICA*027* foi mais frequente em pacientes renais (Pc=0,018; OR: 3,4; IC 95%: 1,5-7,7), enquanto o grupo alélico *MICA*019* foi mais frequente em indivíduos saudáveis (Pc<0,01; OR: 0,0; IC 95%: 0,0-0,5). Foi demonstrado uma contribuição étnica nas amostras analisadas, com evidências de que a população brasileira é formada por uma mistura de etnias, e que a contribuição HLA e MICA

pode ser determinada pela etnia predominante de cada região, uma vez que os alelos de origem européia se apresentaram em maior número que os demais. No entanto, foi encontrado uma série de outros grupos alélicos, que podem resultar da contribuição de alelos de diferentes populações ameríndias ou européias, africanas e asiáticas que colonizaram a região. O conhecimento da diversidade HLA e MICA em pacientes com DRC, potenciais candidatos ao transplante de uma região e sua comparação com indivíduos saudáveis pode favorecer um melhor entendimento da relação entre antígenos do MHC e desenvolvimento da doença renal. Finalmente, nossos dados poderão ser úteis como referência clínica preliminar para melhor compreensão dos mecanismos envolvidos na rejeição de aloenxertos associados aos polimorfismos HLA e MICA na população brasileira.

Palavras-chave: Alelos; Brasil; Antígenos de Histocompatibilidade classe I; Antígenos de Histocompatibilidade classe II; Antígenos HLA-A; Antígenos HLA-B; Antígenos HLA-C; Antígenos HLA-D; Transplante renal; Desequilíbrio de ligação; Polimorfismo Genético; Complexo Principal de Histocompatibilidade; Genética populacional.

HLA and MICA polymorphism in renal transplant candidates in North/ Northwest of Parana State

ABSTRACT

The HLA (Human leukocyte antigen) system is an important marker of graft survival. While the importance of HLA-A, -B, and -DR in solid-organ transplantation has been known for many years, the role of HLA-C, HLA-DQ and MICA (major histocompatibility complex MHC class I chain-related gene A) in the transplantation has recently been documented. In this study, we evaluated the allelic and haplotype frequencies of MICA; HLA-A, -B, -C, -DRB1, -DQA1 and -DQB1 in 346 patients, of different ethnicities, with chronic kidney disease (CKD), renal transplant candidates from Northern/Northwestern of Parana State, Southern Brazil. HLA and MICA typing were performed using the polymerase chain reaction-sequence specific primer method (PCR-SSO), combined with the Luminex technology. The allelic frequencies of MICA and the linkage disequilibrium with HLA-B alleles were studied in the same samples. The 346 participants were classified according to ethnic group (189 caucasians, 98 mestizos, 50 blacks and 9 orientals). A total of 19 MICA, 20 HLA-A, 29 HLA-B and 14 HLA-C, 13 HLA-DRB1, 6 HLA-DQA and 5 HLA-DQB1 allele groups were identified. The most frequent allele groups were *MICA*008*, *HLA-A*02, B*35, C*07, DRB1*04, DQA1**01 and *DQB1*03.* Significant differences ($p<0.05$) were observed in *MICA*009, MICA*010, HLA-A*24, A*68, B*52, DRB1*09, C*03, C*07* and *DQA1*03* allele group frequencies between ethnic groups. The most common extended haplotype in the total samples was *HLA-A*01-C*07-B*08-MICA*008-DRB1*03-DQA1*05-DQB1*02 (*2.4%). The most common haplotypes were *MICA*009-B*51* (7.8%), *MICA*004-B*44* (6.1%) and *MICA*002-B*35* (5.6%). As expected from the proximity of the MICA and HLA-B loci, most haplotypes showed strong LD. Renal patients and healthy subjects in the same region of Brazil showed statistically significant differences in their MICA polymorphisms. The *MICA*027* allele group was more frequent in renal patients (Pc=0.018; OR: 3.4; CI 95%: 1.5-7.7), while the *MICA*019* allele group was more frequent in healthy subjects (Pc<0.01; OR: 0.0; CI 95%: 0.0-0.5). The MICA; HLA-A ,-B, -C, -DRB1, -DQA1, -DQB1 alleles and haplotypes frequencies were studied in 346 renal-transplant candidates. An ethnic contribution was shown in the analyzed samples, with evidence that the Brazilian population is composed of a mixture of ethnicities, and that the HLA and MICA contribution can be determined by the predominant ethnicity of each region, since the alleles of European origin presented in greater number than the others. However, a number of other allelic groups have

been found, which may result from the contribution of alleles from different Amerindian or European, African and Asian opopulations that colonized the region. Knowledge of HLA and MICA diversity in patients with CKD, potential candidates for transplantation in a region and its comparison with healthy individuals may favor a better understanding of the association between MHC antigens and the development of renal disease. Finally, our data could be useful as a preliminary clinical reference for better understanding of the mechanisms involved in the allograft rejection associated with HLA and MICA polymorphisms in the Brazilian population.

Keywords: Alleles; Brazil; Histocompatibility antigens class I; Histocompatibility Antigens Class II; HLA-A Antigens; HLA-B Antigens; HLA-C Antigens; HLA-D Antigens; Gene Frequency, Haplotypes; Kidney transplantation; Linkage Disequilibrium; Polymorphism, Genetic; Major Histocompatibility Complex; Population genetics.

LISTA DE ILUSTRAÇÕES

Tese elaborada e formatada conforme as normas da ABNT (Capítulo I) e das publicações científicas (Capítulo II): Plos One (artigo 1) disponível em:
< http://journals.plos.org/plosone/s/submission-guidelines > e
Human Immunology (artigo 2)
disponível em:
<https://www.elsevier.com/journals/human-immunology/0198-8859/guide-for-authors >

SUMÁRIO

1. CAPÍTULO I

1.1. INTRODUÇÃO

O primeiro sucesso no transplante de órgãos ocorreu em 1954, na cidade de Boston, Estados Unidos (EUA), onde foi realizado um transplante de rim, entre gêmeos idênticos. Este fato foi um marco para o tratamento da doença renal crônica (DRC) terminal (MURRAY, 2011). O fator mais importante no transplante de órgãos continua sendo a compatibilidade entre doador e receptor. Essa compatibilidade, atualmente, é definida por meio da tipagem sanguínea e, principalmente, pela tipagem HLA (*Human Leucocyte Antigen*). Quanto mais semelhantes forem os alelos HLA do doador e do receptor, melhores serão os resultados. Por este motivo, laboratórios de histocompatibilidade e centrais de transplante que, atendem estes pacientes, mantêm um banco de dados com as tipagens HLA, seguindo o que preconiza a política nacional de transplantes de órgão e tecidos[1]. Esses bancos de dados, por garantirem uma consulta rápida da tipagem HLA de doadores e receptores favorecem a melhor distribuição de órgãos.

O transplante renal é o tratamento de escolha para uma parcela significativa dos pacientes com DRC. Esta opção possibilita a melhoria da qualidade de vida e sobrevida desses pacientes, quando comparado àquele mantido continuamente em diálise (CARVALHO et al., 2012). No entanto, esta é uma forma de tratamento que ainda se encontra longe da perfeição, uma vez que órgãos transplantados podem ser perdidos por diversas causas (FADILI; HABIB ALLAH; LAOUAD, 2013; FIDLER et al., 2013; MEHRA et al., 2013; PÉREZ-GUTIÉRREZ et al., 2013).

Os transplantes de órgãos e tecidos têm o objetivo de suprir a deficiência da função de um órgão. Cada tipo de transplante tem suas próprias dificuldades clínicas e cirúrgicas, mas, de modo geral, a principal barreira no sucesso dos transplantes é a própria resposta imune do receptor frente ao enxerto. Exceto quando o doador e o receptor sejam geneticamente idênticos, os antígenos do enxerto, invariavelmente, estimulam uma reação imunológica no receptor, ativando vários mecanismos de imunidade celular e humoral. O reconhecimento do tecido transplantado como estranho é determinado principalmente pelos genes polimórficos do complexo principal de

[1] Lei nº9.434/97, Lei nº10.211/01, Lei nº8.080/90 e Lei nº8.142/90.

histocompatibilidade (CPH ou MHC, do inglês: *Major Histocompatibility Complex*) (ABBAS, 2015).

Nos seres humanos, o sistema HLA foi assim denominado por ter sido descrito inicialmente na superfície de leucócitos humanos (DAUSSET, 1958). As moléculas clássicas do sistema HLA compreendem as moléculas classe I (HLA-A, -B e -C) e classe II (HLA-DR, -DQ e -DP). Tais moléculas são glicoproteínas altamente polimórficas, distribuídas em diferentes frequências na população e que diferem entre si quanto à distribuição em tecidos e funções (ABBAS, 2015). Por estarem presentes em todas as células do organismo, tais moléculas funcionam como aloantígenos, sendo considerado um potente marcador de sobrevida dos transplantes, vindo a apresentar um papel de destaque entre os sistemas biológicos envolvidos no processo de rejeição (OTTEN et al., 2012; FIDLER et al., 2013, MEHRA et al., 2013; ABBAS, 2015).

Uma nova família de genes não clássicos do MHC de classe I, denominada MIC, (do inglês, *MHC class I chain-related genes*) tem sido estudada no contexto dos transplantes. A primeira descrição de anticorpos induzidos por peptídeos derivados de MIC aconteceu em 1996, a partir de experimentos realizados com coelhos imunizados e também pela análise com marcação fluorescente de diversos tipos de tecidos (LEELAYUWAT et al., 1996). Mais tarde, estudos realizados com receptores renais, apresentando rejeição aguda ou crônica, demonstraram a presença de anticorpos contra as moléculas MIC provenientes do aloenxerto (HANKEY et al., 2002).

Anticorpos específicos contra MIC também foram detectados, juntamente com anticorpos anti-HLA, em pacientes sensibilizados aguardando um transplante renal, em pacientes já transplantados e em amostras obtidas a partir de enxertos rejeitados. Estes dados sugerem fortemente a participação de MIC na patogênese da rejeição de órgãos (ZOU et al., 2006).

Vários estudos demonstram o efeito dos anticorpos anti-HLA na sobrevida do enxerto, no entanto, embora sejam responsáveis por uma grande parte dos danos causados, eles não explicam por si só as perdas de enxertos (SUMITRAN-HOLGERSSON, 2008; STASTNY et al., 2009; ZOU; STASTNY, 2011).

1.2. SISTEMA HLA

O sistema HLA foi descoberto em 1958, quando Jean Dausset observou a capacidade do soro de pessoas politransfundidas aglutinar leucócitos de outros

indivíduos, descrevendo assim, a primeira molécula HLA que, foi chamada de MAC (atualmente HLA-A*02) (DAUSSET, 1958; PORTO; PONTES, 2007). Em 1963, Van Rood e Van Leeuwen descobriram o primeiro loco do sistema de antígenos leucocitários humanos, chamado de 4 "*Four*" (atualmente HLA-B) (VAN ROOD; VAN LEEUWEN, 1963). Posteriormente, surgiram evidências de novos sistemas antigênicos análogos, associados ao HLA-C (SOLHEIN et al.,1973) e HLA-DR (DUQUESNOY; MARRARI; ANNEN, 1979). As investigações levaram ao reconhecimento, em 1983, dos genes que codificam os isotipos HLA-DR, -DQ e DP. Atualmente, a estrutura e função dos genes do sistema HLA, já são conhecidos detalhadamente (STEINMETZ; HOOD, 1983; BJORKMAN et al., 1987).

O Sistema HLA está inserido nos genes do MHC, localizado no braço curto do cromossomo 6, na posição p21.3 (LAMM, 1974, SENGER et al., 1993). O MHC é didaticamente divido em 3 regiões: região de classe I, II e III. Cada região de classe I e II é constituída de diversos locos, os quais contêm genes que codificam os antígenos ou moléculas HLA de classe I e II. Tais moléculas são glicoproteínas altamente polimórficas, que diferem entre si quanto à distribuição em tecidos, estrutura e funções (ABBAS et al., 2015). Os genes da região de classe I codificam as moléculas clássicas HLA-A, -B e -C, e as moléculas não clássicas HLA-E, -F e –G. Enquanto que os genes da região de classe II codificam as moléculas clássicas HLA-DR, -DQ e –DP (JANEWAY et al., 2002; ABBAS, 2015).

A estrutura das moléculas do sistema HLA de Classe I (Figura 1) consiste em uma cadeias polipeptídicas, ligadas de forma não covalentes, à cadeia α (ou cadeia pesada) de 44 a 47 kDa e, a cadeia β2-microglobulina, uma subunidade de 12 kDa, não codificada pelo MHC. Os segmentos aminoterminais α1 e α2 interagem para formar uma plataforma de oito fitas, em estrutura β-pregueada, nas quais se apoiam duas α-hélices paralelas. Esta estrutura forma a fenda de ligação de peptídeos, nas moléculas de Classe I, sendo a região onde se concentra o polimorfismo da molécula, devido às substituições nucleotídicas, nos éxons 2 e 3. As moléculas de MHC da Classe II são compostas de duas cadeias polipeptídicas, ligadas de forma não-covalente, uma cadeia α com 32 a 34 kDa e uma cadeia β de 29 a 32 kDa. Ao contrário das moléculas de Classe I, ambas as cadeias das moléculas de Classe II são codificadas por genes MHC polimórficos (ABBAS, 2015).

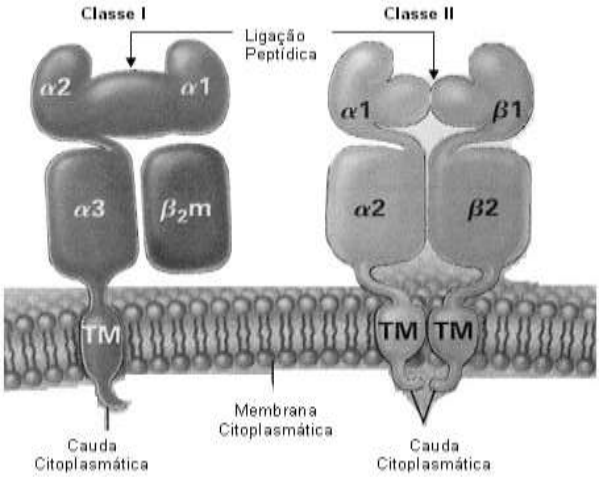

Figura 1. Estrutura das moléculas HLA de Classe I e II (MHC Classe I e MHC Classe II). A molécula HLA de Classe I possui três domínios extracelulares classificados em α1, α2 e α3, um segmento transmembrana (TM) e uma pequena cauda citoplasmática. A molécula β2 microglobulina (β2m) está particularmente ligada ao domínio α3 fora da célula. A molécula HLA de Classe II possui uma cadeia α e uma β, as quais possuem dois domínios extracelulares cada uma, α1 e α2, e β1 e β2, respectivamente, além de um segmento transmembrana e uma porção citoplasmática. Adaptado de Klein (2000).

As moléculas HLA de Classe I são expressas, na maioria das células humanas nucleadas, e são as principais responsáveis pela apresentação de antígenos endógenos. Quando há uma infecção viral ou alteração de proteínas da própria célula, as proteínas citosólicas são degradadas no proteossomo, formando peptídeos capazes de se ligar às moléculas HLA de Classe I, resultando em um complexo na superfície celular. Os linfócitos T CD8+ reconhecem esse complexo, e desencadeiam uma resposta que, resulta na eliminação da célula (DOHERTY; ZINKERNAGEL, 1975; ABBAS, 2015).

As moléculas HLA de Classe II são expressas principalmente nos macrófagos e células dendríticas. Essas moléculas estão envolvidas na apresentação dos antígenos às células T CD4+, ativando-as. As células apresentadoras de antígenos (APCs) interiorizam as proteínas extracelulares nos endossomas que, por ação de enzimas, são clivadas. Após o processamento, essas proteínas são ligadas ao MHC de Classe II, formando um complexo que migra até a superfície celular, para ser reconhecido pelos linfócitos T CD4+. A partir desse reconhecimento, serão secretadas citocinas que, desencadeiam as cascatas de reações imunológicas (MAGALHÃES; BOHLKE; NEUBARTH, 2004; ABBAS, 2015).

Entre os locos de Classe I e os locos de Classe II existe uma porção não relacionada, ocupada por genes que, desempenham outras funções do sistema imune, e

por outros sem função definida. Esses locos são denominados como região MHC de Classe III. Nessa região, estão presentes os locos que codificam proteínas do complemento, genes de citocinas, o gene da enzima 21-hidroxilase (CYP 21B); gene do citocromo P-450, bem como alguns genes de proteínas de choque térmico (HSP) (JANEWAY et al., 2002; ABBAS; 2015).

O sistema HLA tem como característica principal o seu polimorfismo, sendo considerado o conjunto de genes mais polimórfico entre todos os locos expressos no genoma humano. Atualmente, já foram identificados milhares de alelos diferentes para o HLA de Classe I e II (DORAK et al., 2006; EBI, 2016; ALLELE FREQUENCIES, 2016).

1.2.1. SISTEMA HLA E TRANSPLANTE DE ÓRGÃOS

Pelo fato do sistema HLA ser composto de moléculas, encontradas na superfície dos leucócitos, e em quase todas as células de tecidos; e seus genes serem extremamente polimórficos e co-dominantes (JANEWAY et al., 2002), a compatibilidade dos antígenos HLA do doador e do receptor torna-se essencial. Estudos evidenciaram que o número de incompatibilidades HLA (ou seja, número de antígenos no doador diferentes do receptor) pode estar associado à menor sobrevida do enxerto (OPELZ; DÖHLER, 2007). A maior compatibilidade HLA, entre doador e receptor, favorece a tolerância do sistema imune, aumentando a sobrevida do enxerto renal, tanto em transplante intervivos, quanto em doador falecido (OPELZ; DOHLER, 2007).

Os anticorpos anti-HLA, presentes no soro do receptor, também representam um sério fator de risco para o transplante, podendo gerar um fenômeno chamado de Rejeição Mediada por Anticorpos (AMR) e, consequente perda do enxerto (TERASAKI; OZAWA, 2004; GLOOR et al., 2008).

Sem dúvida o transplante de tecidos e órgãos é uma alternativa terapêutica para uma variedade de doenças. No entanto, uma grande limitação, para o sucesso do transplante, é a resposta imune do receptor ao tecido ou órgão do doador. Quando um órgão ou tecido alogênico é transplantado em um receptor, este é reconhecido como estranho pelo seu sistema imune. O reconhecimento de antígenos transplantados, como próprios ou não-próprios, é determinado principalmente pelas moléculas HLA, por serem altamente imunogênicas. A resposta imune ao enxerto inicia-se com uma fase de sensibilização, onde os linfócitos reativos aos antígenos de histocompatibilidade

realizam o reconhecimento de antígenos estranhos, posteriormente proliferam e diferenciam-se em células efetoras; e uma fase efetora, na qual ocorre o ataque do sistema imune contra o enxerto, podendo ocorrer de forma humoral ou celular (ABBAS et al., 2015).

Em especial, a resposta imune humoral aos aloantígenos HLA desempenha um papel importante na rejeição, seja por anticorpos pré-formados (rejeição hiperaguda) ou formados após o transplante (rejeições agudas ou crônicas) (ABBAS et al., 2015). Indivíduos que desenvolvem anticorpos anti-HLA são denominados sensibilizados e representam um número expressivo dentro da lista de espera em um centro de transplante (SAITO et al., 2014).

Além das moléculas HLA, outras moléculas, presentes no organismo humano, estão envolvidas no estudo da compatibilidade entre doador e receptor. Recentemente uma nova família de genes não clássicos do MHC de classe I, os genes MICA, tem se destacado no contexto dos transplantes.

1.3. GENES RELACIONADOS A CADEIAS MHC DE CLASSE I (MIC)

Os genes MIC (genes relacionados às cadeias MHC de classe I) constituem uma segunda linhagem de genes não clássicos do MHC de classe I que foram descritos por Bahram em 1994 (BAHRAM et al., 1994). Estudos identificaram sete genes MIC (A ao G), dos quais MICA e MICB são funcionalmente genes expressos, enquanto MICC, MICD, MICE, MICF e MICG são pseudogenes (BAHRAM, 2000). O gene MICA (*MHC class I chain-related gene A*) (Figura 2) está localizado no cromossomo 6 aproximadamente 46,4 kb distantes do HLA-B em direção ao centrômero (MORALES-BUENROSTRO; ALBERÚ, 2008).

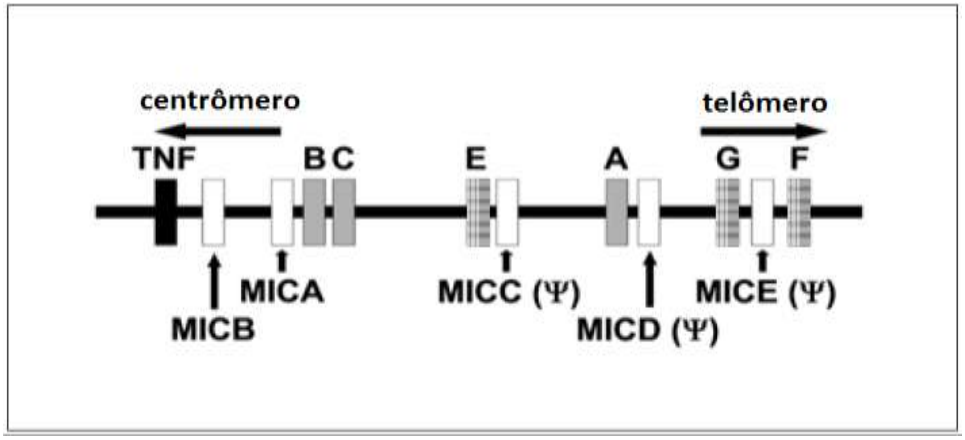

Figura 2 – Mapa da região do MHC Classe I humano. Os genes clássicos do MHC Classe I (HLA-A, -B e –C) estão indicados como caixas cinzas, os genes não clássicos do MHC Classe I (HLA-E, -F e –G) estão indicados como caixas quadriculadas, os membros da família MIC estão representados como caixas brancas e, o gene TNF, está como caixa preta. O símbolo "Ψ" indica alguns dos pseudogenes MIC. Adaptado de Zwirner (2006).

Os genes MICA e MICB codificam proteínas de membrana, que funcionam como ligantes para o receptor NKG2D (C-Type lectin-like activating immunoreceptor), expresso em células NK, células γδ, αβ e células T $CD8^{+}$. A molécula MICA é formada por uma cadeia α, com aproximadamente 383 aminoácidos, com 3 domínios extracelulares (α1, α2, α3) (BAHRAM et al., 1994). Ao contrário de seus clássicos HLA classe I homólogos, não está associada a uma cadeia de β2-microglobulina, nem parece apresentar peptídeos ligados em sua fenda (Figura 3)(GROH et al., 1996). As moléculas MICA são expressas principalmente na superfície do epitélio intestinal (GROH et al., 1996) células endoteliais, queratinócitos, fibroblastos (ZWIRNER; DOLE; STASTNY, 1999) e células estressadas, tais como células tumorais (GROH et al., 1999). Vários estudos demonstraram que a expressão de MICA desempenha um papel na eliminação tumoral. A interação MICA-NKG2D, associado à proteína adaptadora de membrana DAP10, parece potencializar a atividade anti-tumoral inata das células NK, mesmo na presença de moléculas HLA de classe I, além de promover respostas antígeno específicas de células T (BAUER et al., 1999 e WU et al., 1999).

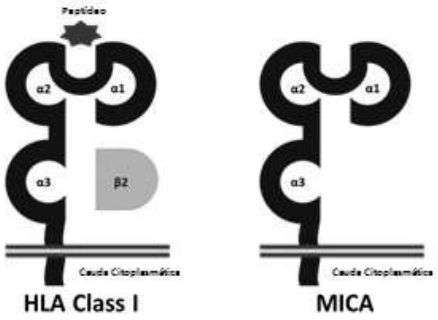

Figura 3 – Diagrama da comparação entre as estruturas das moléculas HLA de Classe I e MICA. Adaptado de *Atlas of Genetics and Cytogenetics in Oncology and Haematology* (2014).

A expressão de MICA está portanto relacionada com vários tumores epiteliais e sugerem uma função potencial na resposta imune de vigilância tumoral, sendo reconhecida como importante marcador em situações de "estresse" no epitélio (GROH et al., 1996; GROH et al., 1999).

A grande proximidade dos genes MICA e HLA-B no cromossomo, favorece um alto grau de desequilíbrio de ligação entre seus alelos. Diversos estudos demonstram a formação de haplótipos mais frequentes como: HLA-B51 com MICA006 e MICA009; MICA008 com HLA-B07 e -B08; MICA002 com HLA-B35 e –B53 entre outros (KOMATSU-WAKUI et al., 1999; MENDONZA-RINCON et al., 1999; FODIL et al., 1999).

A associação de alelos MICA e HLA pode estar envolvida com a sucetibilidade à algumas doenças, assim como sugerem alguns estudos sobre a doença de Behçet (MIZUKI et al., 1997, MIZUKI et al., 1999), espondiloartropatias (TSUCHIYA et al.,1998), diabetes (GAMBELUNGHE et al., 2001), uveíte anterior aguda (GOTO et al., 1998) e doença celíaca (LOPEZ-VAZQUEZ et al., 2002). O alto grau de desequilíbrio de ligação entre os locus HLA, possivelmente, é resultado da seleção natural e eventos demográficos (MEYER et al., 2006).

A expressão de MICA também tem sido relatada em rejeições de aloenxertos de rim e pâncreas (HANKEY et al., 2002; ZOU; STASTNY, 2011). Existem também evidências de anticorpos anti-MICA sendo detectados em receptores de aloenxerto renal. Isto sugere que a expressão de MICA pode desempenhar um papel importante na sobrevivência de transplantes de órgãos (HANKEY et al., 2002; ZWINER et al., 2000). É provável que o polimorfismo de genes MICA possa ser alvo de anticorpos específicos e de células T nos órgãos transplantados ou na doença do enxerto contra o hospedeiro (ZWIRNER; DOLE; STASTNY, 1999).

1.3.1. **ANTICORPORPOS ANTI-MICA E TRANSPLANTE RENAL**

A evolução na terapia imunossupressora tem reduzido em grande escala os episódios de rejeição aguda a transplantes. Ela garante um melhor resultado de enxerto em curto prazo, mas não tem modificado a sobrevida em longo prazo no transplante renal. A rejeição crônica é definida como a deterioração do tecido transplantado, ocorrendo meses ou anos após a enxertia e representa a principal ameaça à sobrevida em longo prazo do aloenxertos (NICKNAM et al., 2004). Se o receptor possuir um sistema imune competente, invariavelmente, a transplantação resultará em alguma forma de rejeição. Para evitar ou retardar esse processo pode-se tornar o enxerto menos imunogênico com a diminuição das diferenças aloantigênicas e, desta forma, selecionar

o melhor doador para um determinado receptor (MAGALHÃES; BÖHLKE; NEUBARTH, 2004).

Diversos estudos têm demonstrado que MICA representa um papel relevante nos processos imunes e em transplantes (BAHRAM et al., 1994, ANGASWAMY et al, 2010). Sua diversidade genética e sua expressão na superfície de células endoteliais sugerem a participação desta molécula nos processos de rejeições agudos e crônicos de aloenxertos (ZWIRNER; DOLE; STASTNY, 1999).

A importância dos genes MICA na rejeição de enxertos foi sugerida por Zwirner e colaboradores (2000), onde anticorpos para os vários produtos MICA foram detectados em soros de pacientes que haviam rejeitado o aloenxerto renal. Terasaki (2003), por meio de um estudo para determinar os fatores imunológicos e não imunológicos que levam a falha do enxerto, observou-se que do total de 60% de falhas do enxerto que ocorriam em transplantes com doador falecido ao longo de dez anos, 38% das falhas eram devido a reações imunológicas contra fatores não-HLA, 18% das falhas eram devido aos fatores HLA e 43% foram atribuídos a fatores não imunológicos.

Estudos preliminares com um pequeno número de pacientes submetidos ao transplante renal indicaram que anticorpos anti-MICA detectados após o transplante podem estar associado com rejeição de aloenxertos e que estes anticorpos são produzidos no decorrer da imunização em mulheres grávidas e no transplante renal (YU et al., 2011). Estes anticorpos também ocorrem mais frequentemente em pacientes que rejeitaram o enxerto (MIZUTANI et al., 2006).

Zou e colaboradores (2006), em um estudo envolvendo a detecção de anticorpos anti-MICA, no soro de pacientes em lista de espera para o transplante renal, no soro de pacientes transplantados (em um período de quatro anos) e no eluato, obtido à partir da nefrectomia, sugeriram que incompatibilidades MICA entre doador e receptor levam à produção de anticorpos anti-MICA e que os anticorpos anti-MICA alelo específicos encontrados no eluato, podem estar envolvidos na patogênese da rejeição do enxerto renal.

Outro estudo realizado por Zou e colaboradores (2007), observou que, dos 1910 receptores renais, anticorpos anti-MICA foram detectados em 217 pacientes (11,4%) os quais se encontravam associados aos processos de rejeição. Foi também observado neste estudo que a taxa de sobrevida do enxerto no período de um ano foi de 88,3 ±

2,2% entre os receptores que apresentavam anticorpos anti-MICA, em comparação com 93,0 ± 0,6% entre os receptores sem anticorpos anti-MICA (p=0.01). Entre os receptores do primeiro transplante renal, a taxa de sobrevida foi menor entre os pacientes com a presença de anticorpos MICA (87.8 ± 2.4%) do que entre os pacientes sem anticorpos MICA (93.5 ± 0,6%, p= 0.005). Além disso, foi constatado que a associação da sensibilização às moléculas MICA com a diminuição da taxa de sobrevida do enxerto foi mais evidente mesmo nos transplantes de rins com boa compatibilidade HLA.

Diversos outros estudos também evidenciaram a presença de anticorpos anti-MICA em transplantes renais e na rejeição de enxertos (COX et al., 2011; LI et al., 2012; RODRÍGUEZ et al., 2012; SEYHUN et al., 2012; YU et al, 2012). Li e colaboradores (2012) sugeriram que anticorpos anti-MICA estão associados com a disfunção do enxerto renal a longo prazo em receptores renais. Seyhun e colaboradores (2012) confirmaram que anticorpos anti-HLA e anti-MICA podem ser prejudiciais no pós-transplante, promovendo processos de rejeição e representando uma importante causa de falência do enxerto renal.

1.4. JUSTIFICATIVA

Durante as últimas décadas, estudos demostram a importância do HLA na rejeição do aloenxerto e o efeito que os aloanticorpos HLA representam na sobrevida do órgão transplantado. Com certeza, a grande variabilidade e o papel central desempenhado pelos antígenos HLA justificam esta atenção. No entanto, embora sejam responsáveis por uma grande parte das rejeições, o sistema HLA não é o único envolvido nesse processo. O endotélio representa um importante alvo da resposta imune em transplantes e, por expressar moléculas não-HLA envolvidas na rejeição, merecem uma investigação e uma discussão mais aprofundada. Semelhante ao HLA, o gene MICA também é polimórfico, no entanto, dados sobre MICA em diferentes populações não tem sido bem explorados como os descritos para o HLA.

Tendo em vista a participação dos sistemas HLA e MICA como antígenos de histocompatibilidade principal e secundário, respectivamente e, considerando-se suas funções fisiológicas, relacionadas à resposta imune em diversas doenças e processos inflamatórios, torna-se importante a pesquisa da frequência de seus antígenos na população. No caso dos pacientes renais crônicos, esses dados favorecem um melhor

entendimento da relação entre os antígenos do MHC ao desenvolvimento da doença renal, além de contribuir no planejamento de distribuição de órgãos, de acordo com o perfil genético de cada região.

1.5. OBJETIVOS

1.5.1. Geral

Analisar os polimorfismos HLA (-A, -B e –C, -DRB1, - DQA1 e -DQB1) e MICA em pacientes com doença renal crônica à espera de um transplante.

1.5.2. Específicos

- Determinar as frequências de alelos HLA classe I (HLA-A, -B e -C) e classe II (HLA-DRB1, -DQA1 e -DQB1);
- Determinar as frequências de alelos MICA;
- Verificar a conformidade das frequências genotípicas observadas em relação às expectativas em equilíbrio de Hardy-Weinberg;
- Comparar as frequências alélicas e haplotípicas MICA e HLA-B entre indivíduos com DRC e indivíduos saudáveis da mesma região;
- Verificar o desequilíbrio de ligação entre MICA e HLA-B;
- Estimar os haplótipos HLA mais frequentes;
- Comparar as frequências alélicas e haplotípicas HLA entre os grupos étnicos.

1.6. REFERÊNCIAS

ABBAS, A.K.; LICHTMAN, A.H.; PILLAI, S. **Imunologia Celular e Molecular**. 8ª. ed. Rio de Janeiro: Elsevier, 2015. 552p.

ATLAS OF GENETICS AND CYTOGENETICS IN ONCOLOGY AND HAEMATOLOGY. MICA (MHC class I polypeptide-related sequence A). Disponível em: http://atlasgeneticsoncology.org/Genes/MICAID41364ch6p21.html. Acesso em 02 de setembro de 2017.

ALLELE FREQUENCIES. Allele frequency net 2015 update. Disponivel em: http://www.allelefrequencies.net/default.asp. Acesso em 02 de agosto de 2016.

ANGASWAMY, N.; SAINI, D.; RAMACHANDRAN, S.; NATH, D. S.; PHELAN, D.; HACHEM, R.; TRULOCK, E.; PATTERSON, G. A.; MOHANAKUMAR, T. Development of antibodies to human leukocyte antigen precedes development of antibodies to major histocompatibility class I-related chain A and are significantly associated with development of chronic rejection after human lung transplantation. **Human Immunology**. New York, v. 71, n. 6, p. 560-565, 2010.

BAHRAM, S. MIC genes: from genetics to biology. **Advances in Immunology**, New York, v. 76, p. 1-60, 2000.

BAHRAM, S.; BRESNAHAN, M.; GERAGHTY, D. E.; SPIES, T. A second lineage of mammalian major histocompatibility complex class I genes. Proceedings of the National **Academy of Sciences of the United States of America**, Washington, v. 91, n. 14, p. 6259-6263, 1994.

BAUER, S.; GROH, V.; WU, J.; STEINLE, A.; PHILLIPS, J. H.; LANIER, L. L.; SPIES, T. Activation of NK Cells and T Cells by NKG2D, a Receptor for Stress-Inducible MICA. **Science**, Washington, n. v. 285, n. 5428, p. 727-729, 1999.

BJORKMAN, P. J.; SAPER, M. A.; SAMRAOUI, B.; BENNETT, W. S.; STROMINGER, J. L.; WILEY, D. C. Structure of the human class I histocompatibility antigen, HLA-A2. **Nature**, London, v. 329, n. 6139, p. 506-512, 1987.

CARVALHO, M. T. V.; BATISTA, A. P. L.; ALMEIDA, P. P.; MACHADO, D. M.; AMARAL, E. O. Qualidade de vida dos pacientes transplantados renais do Hospital do Rim. **Motricidade**, Santa Maria da Feira, v. 8, n. Supl. 2, p. 49-57, 2012.

COX, S. T.; STEPHENS, H. A.; FERNANDO, R.; KARASU, A.; HARBER, M.; HOWIE, A. J.; POWIS, S.; ZOU, Y.; STASTNY, P.; MADRIGAL, J. A.; LITTLE, A. M. Major histocompatibility complex class I-related chain A allele mismatching, antibodies, and rejection in renal transplantation. **Human Immunology**, New York, v. 72, n. 10, p. 827-834, 2011.

DAUSSET, J. Iso-leuko-antibodies. **Acta Haematology**, Basel, v. 20, p. 156-166, 1958.

DOHERTY, P. C.; ZINKERNAGEL, R. M. A biological role for the major histocompatibility antigens. **Lancet**, London, v. 305, p. 1406-1409, 1975.

DORAK, M. T.; SHAO, W.; MACHULLA, H. K.; LOBASHEVSKY, E. S.; TANG, J.; PARK, M. H.; KASLOW, R. A. Conserved extended haplotypes of the major histocompatibility complex: further characterization. **Genes and Immunity**, Basingstoke, v.7, p. 450-467, 2006.

DUQUESNOY, R. J.; MARRARI, M.; ANNEN, K. Identification of an HLA-DR-associated system of B-cell alloantigens. **Transplantation Proceedings**, New York, v. 11, n. 4, p. 1757-1760, 1979.

EBI. European Bioinformatics Institute. IMGT/HLA Database. Disponivel em: http://www.ebi.ac.uk/imgt/hla/stats.html. Acesso em 02 de agosto de 2016.

FADILI, W.; HABIB ALLAH, M.; LAOUAD, I. Chronic renal allograft dysfunction: risk factors, immunology and prevention. **Arab Journal of Nephrology and Transplantation**, Khartoum, v. 6, n. 1, p. 45-50, 2013.

FIDLER, S. J.; IRISH, A. B.; LIM, W.; FERRARI, P.; WITT, C.S.; CHRISTIANSEN, F. T. Pre-transplant donor specific anti-HLA antibody is associated with antibody-mediated rejection, progressive graft dysfunction and patient death. **Transplant Immunology**, Dunto Grenn, v. 28, n. 4, p. 148-153, 2013.

FODIL, N.; PELLET, P.; LALOUX, L.; HAUPTMANN, G.; THEODOROU, I.; BAHRAM, S. MICA haplotypic diversity. **Immunogenetics**, Berlin, v. 49, n. 6, p. 557-560, 1999.

GAMBELUNGHE, G.; GHADERI, M.; TORTOIOLI, C.; FALORNI, A.; SANTEUSANIO, F.; BRUNETTI, P.; SANJEEVI, C. B.; FALORNI, A.; UMBRIA, TYPE 1 DIABETES REGISTRY. Two distinct MICA gene markers discriminate major

autoimmune diabetes types. **The Journal of clinical endocrinology and metabolism**, Philadelphia, v. 86, n. 8, p. 3754-3760, 2001.

GLOOR, J.; COSIO, F.; LAGER, D. J.; STEGALL, M. D. The spectrum of antibody-mediated renal allograft injury: implications for treatment. **American Journal of Transplantation**, Copenhagen, v. 8, p. 1367-73, 2008.

GOTO, K.; OTA, M.; ANDO, H.; MIZUKI, N.; NAKAMURA, S.; INOUE, K.; YABUKI, K.; KOTAKE, S.; KATSUYAMA, Y.; KIMURA, M.; INOKO, H.; OHNO, S. MICA gene polymorphisms and HLA-B27 subtypes in Japanese patients with HLA-B27-associated acute anterior uveitis. **Investigative ophthalmology & visual science**, St. Louis, v. 39, n. 3, p. 634-637, 1998.

GROH, V.; BAHRAM, S.; BAUER, S.; HERMAN, A.; BEAUCHAMP, M.; SPIES, T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. **Proceedings of the National Academy of Sciences of the United States of America**, Washington, v. 93, n. 22, p. 12445-12450, 1996.

GROH, V.; RHINEHART, R.; SECRIST, H.; BAUER, S.; GRABSTEIN, K. H.; SPIES, T. Broad tumor-associated expression and recognition by tumor-derived γδ T cells of MICA and MICB. **Proceedings of the National Academy of Sciences of the United States of Ameri**ca, Washington, v. 96, n. 12, p. 6879-6884, 1999.

HANKEY, K. G.; DRACHENBERG, C. B.; PAPADIMITRIOU, J. C.; KLASSEN, D. K.; PHILOSOPHE, B.; BARTLETT, S. T.; GROH, V.; SPIES, T.; MANN, D. L. MIC expression in renal and pancreatic allografts. **Transplantation**, Baltimore, v. 73, n. 2, p 304-306, 2002.

JANEWAY, C.A.; TRAVERS, P.; WALPORT, M.; SHLOMCHIK, M. **Imunobiologia**. 5ª ed. Porto Alegre; Artmed, 2002.

KLEIN J; SATO A. The HLA system. First of two parts. **The New England journal of medicine**, Boston, v. 343, n. 10, p. 702-709, 2000.

KOMATSU-WAKUI, M.; TOKUNAGA, K.; ISHIKAWA, Y.; KASHIWASE, K.; MORIYAMA, S.; TSUCHIYA, N.; ANDO, H.; SHIINA, T.; GERAGHTY, D. E.; INOKO H.; JUJI, T. MIC-A polymorphism in Japanese and a MIC-A-MIC-B null haplotype. **Immunogenetics**, Berlin, v. 49, n. 7-8, p. 620-628, 1999.

LAMM, L.U. Assignment of the major histocompatibility complex to chromosome no. 6 in a family with a pericentric inversion**. Human Heredity**, Basel, v. 24, p. 273-284, 1974.

LEELAYUWAT, C.; HOLLINGSWORTH, P.; PUMMER, S.; LERTMEMONGKOLCHAI, G.; THOM, G.; MULLBERG, J.; WITT, C.; KAUFMAN, J.; DEGLI-ESPOSTI, M. A.; CASMAN, D.; DAWKINS, R. Antibody reactivity profiles following immunization with diverse peptides of the PERB11 (MIC) family. **Clinical and Experimental Immunology**, Oxford, v. 106, p. 568-576, 1996.

LI, Z.; LUO, M.; QIU, J.; LIU, Y.; FAN, Y.; JAHR, F. M.; CAI, J.; TERASAKI, P. I. Detection of antibodies against major histocompatibility complex class I-related chain A in long-term renal graft recipients. **Experimental and Clinical Transplantation**, Ankara, v. 10, n. 3, p. 239-242, 2012.

LOPEZ-VAZQUEZ, A.; RODRIGO, L.; FUENTES, D.; RIESTRA, S.; BOUSOÑO, C.; GARCIA-FERNANDEZ, S.; MARTINEZ-BORRA, J.; GONZALEZ, S.; LOPEZ-LARREA, C. MHC class I chain related gene A (MICA) modulates the development of coeliac disease in patients with the high risk heterodimer DQA1*0501/DQB1*0201. **Gut**, London, v. 50, n. 3, p. 336-340, 2002.

MAGALHÃES, P. S. C.; BOHLKE, M.; NEUBARTH, F. Complexo principal de histocompatibilidade (MHC): codificação genética, bases estruturais e implicações clinicas. **Revista de Medicina da UCPEL Pelotas**, Pelotas, v. 2, p. 54-59, 2004.

MAGALHÃES, P. S. C.; BÖHLKE, M.; NEUBARTH, F. Complexo Principal de Histocompatibilidade (MHC): codificação genética, bases estruturais e implicações clínicas. **Revista de Medicina da Universidade Católica de** Pelotas, Pelotas, v. 2, n.1, p. 54-59, 2004.

MEHRA, N. K.; SIDDIQUI, J.; BARANWAL, A.; GOSWAMI, S.; KAUR, G. Clinical relevance of antibody development in renal transplantation. **Annals of the New York Academy of Sciences**, New York, v. 1283, n. 30-42, 2013.

MENDOZA-RINCON, J.; ARGÜELLO, J. R.; PÉREZ-RODRÍGUEZ, M.; MCWHINNIE, A.; MARSH, S. G.; FISCHER, G.; MADRIGAL, J. A. Characterization of the MICA polymorphism by sequence-specific oligonucleotide probing. **Immunogenetics**, Berlin, p. 49, n. 6, p. 471-478, 1999.

MEYER, D.; SINGLE, R. M.; MACK, S. J.; ERLICH, H. A.; THOMSON, G. Signatures of demographic history and natural selection in the human major histocompatibility complex Loci. **Genetics**. Austin, v. 173, n. 4, p. 2121-2142, 2006.

MIZUKI, N.; OTA, M.; KATSUYAMA, Y.; YABUKI, K.; ANDO, H.; GOTO, K.; NAKAMURA, S.; BAHRAM, S.; OHNO, S.; INOKO, H. Association analysis between the MIC-A and HLA-B alleles in Japanese patients with Behçet's disease. **Arthritis and rheumatism**, Atlanta, v. 42, n. 9, p. 1961-1966, 1999.

MIZUKI, N.; OTA, M.; YABUKI, K.; KATSUYAMA, Y.; ANDO, H.; PALIMERIS, G. D.; KAKLAMANI, E.; ACCORINTI, M.; PIVETTI-PEZZI, P.; OHNO, S.; INOKO, H. Localization of the pathogenic gene of Behçet's disease by microsatellite analysis of three different populations. **Investigative ophthalmology & visual science**, St. Louis, v. 41, n. 12, p. 3702-3708, 2000.

MIZUTANI, K. ; TERASAKI, P. I. ; SHIH, R. N. ; PEI, R. ; OZAWA, M.; LEE, J. Frequency of MIC Antibody in Rejected Renal Transplant Patients without HLA Antibody. **Human Immunol**ogy, New York, v. 67, n. 3, p. 223-229, 2006.

MORALES-BUENROSTRO, L. E.; ALBERÚ., J. Anti–major histocompatibility complex class I-related chain A antibodies in organ transplantation **Transplantation Reviews**, Copenhagen, v. 22, p. 27–38, 2008.

MURRAY, J. E. Ronald Lee Herrick Memorial: June 15, 1931-December 27, 2010. **American Journal of Transplantation**, Copenhagen, v. 11, n. 3, p. 419, 2011.

NICKNAM, M. H.; TORKASHVAND, A.; GHODS, A.; AMIRZARGAR, A. A.; AMIRKHANI, A.; KHOSRAVI, F.; NIKBIN, B. Evaluation of Anti- HLA Class I Antibodies in Chronic Rejection of Kidney Transplantation. **Iranian Journal of Allergy**, Asthma, and Immunology, Teheran, v. 3, n. 2, p. 65-69, 2004.

OPELZ, G.; DÖHLER, B. Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. **Transplantation**, Baltimore, v. 84, n. 2, p. 137-143. 2007.

OTTEN, H. G.; VERHAAR, M. C.; BORST, H. P.; HENÉ, R. J.; VAN ZUILEN, A. D. Pretransplant donor-specific HLA class-I and -II antibodies are associated with an increased risk for kidney graft failure. **American Journal of Transplantation**, Copenhagen, v. 12, n. 6, p. 1618-1623, 2012.

PÉREZ-GUTIÉRREZ, A.; MORALES-BUENROSTRO, L. E.; VILATOBÁ-CHAPA, M.; MENDOZA-DE-LA-GARZA, A.; VEGA-VEGA, O.; GABILONDO-PLIEGO, B.; ALBERÚ, J. Risk factors in the development of delayed graft function in deceased donor kidney transplant recipients and their impact on patient and graft survival. **Revista de Investigación Clínica, México**, v. 65, n. 2, p. 109-115. 2013.

PORTO, L. C. M. S.; PONTOS, L. F. S. **Estudos de associação HLA x doenças: extratos do I Simposio Brasileiro**. Rio de Janeiro: EdUERJ; 2007.

RODRÍGUEZ FERRERO, M. L.; ARROYO, D.; PANIZO, N.; VICARIO, J. L.; BALAS, A.; ANAYA, F. Monitoring of circulating antibodies in a renal transplantation

population: preliminary results. **Transplantation Proceedings**, New York, v. 44, n. 9, p. 2548-2550, 2012.

SAITO, P.K.;YAMAKAWA, R.H.; APARECIDA, E.P.; DA SILVA JÚNIOR, W.V.; BORELLI, S.D. Evaluation of the humoral immune response to human leukocyte antigens in Brazilian renal transplant candidates. **PloS one**, San Francisco, v. 9, n. 6, p. e100270, 2014.

SENGER, G., RAGOUSSIS, J., TROWSDALE, J. AND SHEER, D. Fine mapping of the MHC class II region within 6p21 and evaluation of probe ordering interphase fluorescence in situ hybridization. **Cytogenetics and Cell Genetics**, Basel, v. 61, p. 49-53, 1993.

SEYHUN, Y.; OZDILLI, K.; OGUZ, F.; KARAHAN, G.; ONAL, E.; TURKMEN, A.; ELDEGEZ, U.; NANE, I.; ÇALIŞKAN, Y..; BAKKALOGLU, H.; CARIN, M. Human leukocyte antigen and major histocompatibility complex class I-related chain A antibodies after kidney transplantation in Turkish renal transplant recipients. **Transplantation Proceedings**, New York, v. 44, n. 6, p. 1660-1666, 2012.

SOLHEIM, B. G.;BRATLIE, A.; SANDBERG, A.; STAUB-NIELSEN, L.; THORSBY E. Further evidence of a third HL-A locus. **Tissue Antigens**, Copenhagen, v. 3, p. 439–453, 1973.

STASTNY, P.; ZOU, Y.; FAN, Y.; IN, Z.; LAVINGIA, B. The emerging issue of MICA antibodies: antibodies to MICA and other antigens of endothelial cells. **Contributions to Nephrology**, Switzerland, v. 162, p. 99-106, 2009.

STEINMETZ, M.; HOOD, L. Genes of the major histocompatibility complex in mouse and man. **Science**, Washington, v. 222, n. 4625, p. 727-733, 1983.

SUMITRAN-HOLGERSSON, S. Relevance of MICA and other non-HLA antibodies in clinical transplantation. **Current Opinion in Immunology**, England, v. 20, p. 607–613, 2008.

TERASAKI, P. I. Deduction of the fraction of immunologic and non-immunologic failure in cadaver donor transplants. **Clinical Transplants**, Los Angeles, p. 449-452, 2003.

TERASAKI, P. I.; OZAWA, M. Predicting kidney graft failure by HLA antibodies: a prospective trial. **American Journal of Transplantation**, Copenhagen, v. 4, n. 3, p. 438-443, 2004.

TSUCHIYA, N.; SHIOTA, M.; MORIYAMA, S.; OGAWA, A.; KOMATSU-WAKUI, M.; MITSUI, H.; GERAGHTY, D. E.; TOKUNAGA, K. MICA allele typing of HLA-B27 positive Japanese patients with seronegative spondylarthropathies and healthy individuals: differential linkage disequilibrium with HLA-B27 subtypes.**Arthritis and rheumatism**, Atlanta, v. 41, n. 1, p. 68-73, 1998.

VAN ROOD, J. J.; VAN LEEUWEN, A. Leukocyte grouping. A method and its application. **Journal of Clinical Investigation**, New York, v. 42, p. 1382-1390, 1963.

WU, J.; GROH, V.; SPIES, T. T Cell Antigen Receptor Engagement and Specificity in the Recognition of Stress-Inducible MHC Class I-Related Chains by Human Epithelial γδ T Cells. **The Journal of Immunology**, Baltimore, v. 169, p. 1236–1240, 2002.

WU, J.; SONG, Y. ; BAKKER, A. B. ; BAUER, S.; SPIES, T.; LANIER, L. L.; PHILLIPS, J. H. An Activating Immunoreceptor Complex Formed by NKG2D and DAP10. **Science**, Washington, n. 5428, v. 285, p. 730-732, 1999.

YU, L. X.; WANG, G.; FU, S. J.; XIAO, L. L.; XU, J.; DU, C. F. Anti-MICA antibodies: risk factors for sensitization and the impact on renal transplantation outcomes. Nan fang yi ke da xue xue bao. **Journal of Southern Medical University**, China, v. 31, n. 4, p. 615-618, 2011.

YU, L.; ZHANG, X.; LUO, M.; XIAO, L.; XU, J.; DU, C.; LIU, R. Impact of MICA antibodies on acute graft rejection early after kidney transplantation. Nan Fang Yi Ke

Da Xue Xue Bao, **Journal of Southern Medical University**. China, v. 32, n. 5, p. 651-654, 2012.

ZOU, Y.; HEINEMANN, F. M.; GROSSE-WILDE, H.; SIRECI, G.; WANG, Z.; LAVINGIA, B.; STASTNY, P. Detection of Anti-MICA Antibodies in Patients Awaiting Kidney Transplantation, during the Post-transplant Course, and in Eluates from Rejected Kidney Allografts by Luminex Flow Cytometry. **Human Immunology**, New York, v. 67, n. 3, p. 230–237, 2006.

ZOU, Y.; STASTNY, P. Antibodies against major histocompatibility complex class I-related chain A in transplant recipients. **Chinese Medical Journal (Engl)**, Beijing, v. 124, n. 5, p. 764-770, 2011.

ZOU, Y.; STASTNY, P.; SÜSAL, C.; DÖHLER, B.; OPELZ, G. Antibodies against MICA antigens and kidney-transplant rejection. **The New England Journal of Medicine**, Boston, v. 357, n. 13, p. 1293-300, 2007.

ZWIRNER, N. W.; DOLE, K.; STASTNY, P. Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes, and monocytes. **Human Immunology**, New York, v. 60, n. 4, p. 323-30, 1999.

ZWIRNER, N. W.; FUERTES, M. B.; GIRART, M. V.; DOMAICA, C. I.; ROSSI, L. E. Immunobiology of the human MHC class I chain-related gene A (MICA): from transplantation immunology to tumor immune escape. **Inmunología**, Madrid, v. 25, n. 1, p. 25-38, 2006.

ZWIRNER, N. W.; MARCOS, C. Y.; MIRBAHA, F.; ZOU, Y.; STASTNY, P. Identification of MICA as a new polymorphic alloantigen recognized by antibodies in sera of organ transplant recipients. **Human Immunology**, New York, v. 61, n. 9, p. 917-924, 2000.

2. CAPÍTULO II

2.1.ARTIGO 1: "MICA diversity and linkage disequilibrium with HLA-B alleles in renal-transplant candidates in southern Brazil"

PLOS ONE

RESEARCH ARTICLE

MICA diversity and linkage disequilibrium with *HLA-B* alleles in renal-transplant candidates in southern Brazil

Roger Haruki Yamakawa[1☯], Patrícia Keiko Saito[1☯], Geórgia Fernanda Gelmini[2☯], José Samuel da Silva[2☯], Maria da Graça Bicalho[2☯], Sueli Donizete Borelli[1☯*]

1 Department of Basic Health Science, Universidade Estadual de Maringá – Maringá, Paraná, Brazil, **2** Laboratório de Imunogenética e Histocompatibilidade, Department of Genetics, Universidade Federal do Paraná – Curitiba, Paraná, Brazil

☯ These authors contributed equally to this work.
* sueliborelli@gmail.com

OPEN ACCESS

Citation: Yamakawa RH, Saito PK, Gelmini GF, da Silva JS, Bicalho MdG, Borelli SD (2017) *MICA* diversity and linkage disequilibrium with *HLA-B* alleles in renal-transplant candidates in southern Brazil. PLoS ONE 12(4): e0176072. https://doi.org/10.1371/journal.pone.0176072

Editor: Qing Song, Morehouse School of Medicine, UNITED STATES

Received: November 22, 2016

Accepted: April 5, 2017

Published: April 18, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abstract

The major histocompatibility complex (MHC) class I chain-related gene A (*MICA*) is located centromerically to the human leukocyte antigen (*HLA)-B*. The short distance between these loci in the MHC indicates the presence of linkage disequilibrium (LD). Similarly to the *HLA*, the *MICA* is highly polymorphic, and this polymorphism has not been well documented in different populations. In this study, we estimated the allelic frequencies of *MICA* and the linkage disequilibrium with *HLA-B* alleles in 346 renal-transplant candidates in southern Brazil. *MICA* and *HLA* were typed using the polymerase chain reaction-sequence-specific primer method (PCR-SSO), combined with the Luminex technology. A total of 19 *MICA* allele groups were identified. The most frequent allele groups were *MICA* 008* (21.6%), *MICA* 002* (17.0%) and *MICA* 004* (14.8%). The most common haplotypes were *MICA* 009-B* 51* (7.8%), *MICA* 004-B* 44* (6.06%) and *MICA* 002-B* 35* (5.63%). As expected from the proximity of the *MICA* and *HLA-B* loci, most haplotypes showed strong LD. Renal patients and healthy subjects in the same region of Brazil showed statistically significant differences in their *MICA* polymorphisms. The *MICA* 027* allele group was more frequent in renal patients (Pc = 0.018, OR: 3.421, 95% CI: 1.516–7.722), while the *MICA* 019* allele group was more frequent in healthy subjects (Pc = 0.001, OR: 0.027, 95% CI: 0.002–0.469). This study provided information on the distribution of *MICA* polymorphisms and linkage disequilibrium with *HLA-B* alleles in Brazilian renal-transplant candidates. This information should help to determine the mechanisms of susceptibility to different diseases in patients with chronic kidney disease, and to elucidate the mechanisms involved in allograft rejection associated with *MICA* polymorphisms in a Brazilian population.

Introduction

The major histocompatibility complex (MHC) class I chain-related gene A (*MICA*) is one of the highly polymorphic genes located in the human MHC [1–3] and is located 46 kb centromerically from the human leukocyte antigen *(HLA)-B*. The short distance separating *MICA* from *HLA-B* in the MHC indicates the presence of linkage disequilibrium between these loci [1, 2, 4].

Several studies have demonstrated the role of *MICA* polymorphism in a large number of diseases, and the immune response against *MICA* antigens may correlate with acute and chronic rejection of various organs, including renal transplants [5–9]. Similarly to the known involvement of preformed antibodies against the *HLA* antigens in acute and chronic rejection of a graft [8, 10–12], many studies have shown the importance of MICA alloantibodies in the rejection of various organs [13, 14].

The Brazilian population is one of the most ethnically diverse in the world [15], which may impede the search for a matching, unrelated donor. Previous studies have reported some ethnic differences in the distribution of *MICA* polymorphisms, similar to those found for *HLA* polymorphisms [16–18]. However, *MICA* polymorphisms in different populations have not been as well documented as those of *HLA*. To our knowledge, no studies in Brazil have investigated the *MICA* allelic diversity and linkage disequilibrium with *HLA-B* in renal-transplant candidates.

To fill this gap, we evaluated the *MICA* diversity and linkage disequilibrium with *HLA-B* alleles in renal-transplant candidates in a population in southern Brazil.

Materials and methods

Samples

The study included a total of 346 patients (female/male: 135/211) with chronic kidney disease who were renal-transplant candidates and were registered at two regional transplant centers, the Central Regional de Transplantes Norte/Londrina (CRTN/Londrina) and the Central Regional de Transplantes Noroeste/Maringá (CRTNO/Maringá), in northern and northeastern Paraná, respectively, in the period from July 2010 to March 2011. Inclusion criteria were patients with current data (active patients and potential recipients), on dialysis for at least 60 days and to give informed consent to participate in the study. Age over 18 years was considered as exclusion criteria. The study was approved by the Ethics Committee of the Universidade Estadual de Maringá (Protocol No. 333/2011). All procedures followed Resolution 196/1996 of the Brazilian Health Council, which rules on research involving humans. All procedures were explained to each subject, and written informed consent was obtained from each subject.

DNA extraction and HLA-B and MICA typing

To perform the *HLA-B* and *MICA* typing, about 5 mL of blood was collected by venipuncture in vacuum tubes (Vacutainer, Becton and Dickson, Oxford, UK) containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Then, we extracted the genomic DNA by the separation-column method, using the Biopur kit for DNA extraction (Biometrix, Curitiba, Paraná, Brazil), following the manufacturer's protocol. After adjusting the DNA concentration, obtained by the optical-density method, we amplified the DNA using polymerase chain reaction-sequence specific primers (PCR-SSO) combined with Luminex technology. The genomic DNA was amplified using biotinylated sequence-specific primers for *HLA-B* and *MICA* in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA),

followed by hybridization with complementary probes for DNA, conjugated with microspheres (beads) labeled with different fluorochromes to identify complementary sequences of the amplified DNA, using the LABType kit (One Lambda, Inc., Canoga Park, CA, USA), following the manufacturer's protocol. After hybridization, the results were read using the flow cytometry platform LABScan™100 (One Lambda, Inc.), followed by analysis using the program HLA Fusion version 2.0 (One Lambda, Inc.). The results showed low-medium resolution.

Comparison of the results with published data

In the *HLA-B* and *MICA* comparisons, this study used as control the data published by Ribas et al. (2008)[17], since their study was carried out in the same region of Brazil.

Statistical analyses

The Arlequin software package version 3.11 [19] was used to calculate the allele and haplotype frequencies and to assess the Hardy-Weinberg equilibrium. The haplotype frequencies were estimated using the expectation-maximization algorithm (maximum-likelihood method) as included in Arlequin 3.11. The values for relative linkage disequilibrium (LD) between pairs of *MICA* and *HLA-B* allele groups and their level of significance (p values) were determined with the same software package. The overall comparison of *HLA* and *MICA* allelic frequencies between renal patients and healthy subjects [17] was performed with a G-test, and individual comparisons were performed using Fisher's exact test. Statistically significant differences ($P \leq 0.05$) were corrected by the Bonferroni method for multiple comparisons (Pc). Odds ratios (OR) and 95% confidence intervals (CI) were calculated.

Results

The *MICA* and *HLA*-B allelic frequencies are shown in Tables 1 and 2, respectively. A total of 29 *HLA*-B and 19 *MICA* allele groups were identified. The most common allele groups were *HLA-B*35* (10.5%), *HLA-B*44* (9.9%), *HLA-B*51* (9.6%), *MICA*008* (21.6%), *MICA*002* (17.0%) and *MICA*004* (14.8%).

The *MICA* allele distribution was in Hardy-Weinberg equilibrium ($p>0.05$), the observed heterozygosity was 82.0% and the expected heterozygosity was 87.9%. In contrast, the observed and expected heterozygosity of the *HLA-B* allelic distribution differed significantly ($p = 0.009$): the observed heterozygosity was 90.7% and the expected, 94.1%.

The overall comparison of *MICA* allele frequencies between renal patients and healthy subjects indicated a significant difference between the groups ($p<0.0001$). In individual comparisons, the *MICA*027* allele was more frequent in renal patients (Pc = 0.018, OR: 3.421, 95% CI: 1.516–7.722), while the *MICA*019* allele was more frequent in the healthy population (Pc = 0.001, OR: 0.027, 95% CI: 0.002–0.469). The *MICA*008* allele was more frequent in healthy subjects; however, with the Bonferroni correction, no statistically significant difference was apparent.

The *HLA-B* allele frequencies did not differ significantly between renal patients and healthy subjects.

The result for haplotype inference showed a total of 77 haplotypes, of which 23 had a frequency greater than 1%. Table 3 shows the frequencies and linkage disequilibrium (LD) values for all haplotypes with a frequency greater than 1% in both studies. The supplementary table (S1 Table) presents a graphical view of the LD parameters and frequencies for all haplotypes characterized in our study.

Table 1. Allele group frequencies of *MICA* in all samples (n = 346), and comparison with healthy subjects.

MICA	Renal	%	Healthy subjects (Ribas et al., 2008)[17]	%	*p*	p_c	*OR* (95% CI)
**001*	9	1.30%	4	0.98%	0.436	1	
**002*	118	17.05%	72	17.65%	0.437	1	
**004*	103	14.88%	47	11.52%	0.068	1	
**006*	4	0.58%	3	0.74%	0.480	1	
**007*	17	2.46%	13	3.19%	0.385	1	
**008*	150	21.68%	108	26.47%	0.047	1	
**009*	95	13.73%	53	12.99%	0.401	1	
**010*	46	6.65%	29	7.11%	0.469	1	
**011*	21	3.03%	19	4.66%	0.122	1	
**012*	9	1.30%	4	0.98%	0.436	1	
**015*	7	1.01%	3	0.74%	0.457	1	
**016*	23	3.32%	7	1.72%	0.079	1	
**017*	14	2.02%	7	1.72%	0.455	1	
**018*	32	4.62%	16	3.92%	0.349	1	
**019*	0	0.00%	10	2.45%	0.000	0.001	0.027 (0.002–0.469)
**021*	0	0.00%	1	0.25%	0.371	1	
**027*	39	5.64%	7	1.72%	0.001	0.018	3.421 (1.516–7.722)
**044*	1	0.14%	0	0.00%	0.629	1	
**045*	1	0.14%	0	0.00%	0.629	1	
**046*	1	0.14%	0	0.00%	0.629	1	
**049*	0	0.00%	3	0.74%	0.051	1	
**052*	2	0.29%	0	0.00%	0.396	1	

P = P-value; Pc = P-value adjusted for multiple comparisons; OR: odds ratio; CI: confidence interval.
Healthy subjects described by Ribas et al., (2008) [17].

https://doi.org/10.1371/journal.pone.0176072.t001

The most frequent haplotype was *MICA*009-B*51* (7.8%), followed by *MICA*004-B*44* (6.0%) and *MICA*002-B*35* (5.6%). The analysis of linkage disequilibrium (LD) showed that 8 haplotypes had a relative LD value (D') of 1.

No statistically significant difference was observed in the frequency of those *MICA–HLA-B* haplotypes with a frequency greater than 1%, in both studies.

Discussion

The vast majority of studies have addressed the *MICA* polymorphism of an entire population, using apparently healthy subjects [16–18, 20].

The Brazilian population has wide genetic heterogeneity, composed of a mix of ethnic groups, a result of immigration from several countries during the colonization of Brazil, resulting in an interracial mixture of Europeans, Africans, Amerindians and Asians [15]. The samples evaluated in this study are from the north/northeastern region of Paraná, in southern Brazil. The southern region, including the state of Paraná, was colonized largely by European immigrants in the 19th century, and presently has a high proportion of Caucasians. However, people of Amerindian and African descent are also frequent in the population [21].

In the current study, a significant difference between the observed and expected heterozygosity was observed for *HLA-B*. Despite a high degree of heterozygosity (90.7%), the number of heterozygous individuals was lower than expected. This difference can be explained by the composition of our sample, i.e. non-healthy individuals. This deviation from Hardy-Weinberg

Table 2. Allele group frequencies of *HLA-B* in all samples (n = 346), and comparison with healthy subjects.

HLA-B	Renal	%	Healthy subjects (Ribas et al., 2008)[17]	%
**07*	45	6.50%	28	6.86%
**08*	41	5.92%	27	6.62%
**13*	11	1.59%	9	2.21%
**14*	22	3.18%	26	6.37%
**15*	59	8.53%	45	11.03%
**18*	34	4.91%	21	5.15%
**27*	21	3.03%	11	2.70%
**35*	73	10.55%	40	9.80%
**37*	9	1.30%	4	0.98%
**38*	23	3.32%	10	2.45%
**39*	24	3.47%	18	4.41%
**40*	36	5.20%	13	3.19%
**41*	13	1.88%	6	1.47%
**42*	15	2.17%	5	1.23%
**44*	69	9.97%	46	11.27%
**45*	14	2.02%	6	1.47%
**46*	1	0.14%	0	0.00%
**47*	1	0.14%	1	0.25%
**48*	3	0.43%	2	0.49%
**49*	22	3.18%	9	2.21%
**50*	17	2.46%	6	1.47%
**51*	67	9.68%	42	10.29%
**52*	14	2.02%	8	1.96%
**53*	15	2.17%	4	0.98%
**54*	2	0.29%	0	0.00%
**55*	7	1.01%	3	0.74%
**56*	1	0.14%	2	0.49%
**57*	18	2.60%	7	1.72%
**58*	15	2.17%	9	2.21%

Healthy subjects described by Ribas et al., (2008)[17].

https://doi.org/10.1371/journal.pone.0176072.t002

proportions may also be related to the pathological condition itself, whether or not it is related to genetic causes; or because we did not exclude individuals who had some degree of kinship to each other [22, 23].

A total of 19 alleles of *MICA* were found in this study. Similar numbers of alleles were observed in Brazilian Caucasians [17], Afro-Americans and Euro-Americans [24], Moroccans [25] and in Murcia, Spain [20]. Although 19 *MICA* alleles were detected, a large number of these alleles were found with a frequency of only 1%. In addition, *MICA*008*, *MICA*002*, *MICA*004* and *MICA*009* together comprised more than 67% of the allelic distribution, and these alleles are also common in other populations [16, 17, 20, 24, 25].

The *MICA* allelic diversity found in this study is similar to the levels found by Marin et al. (2006) [16] and Ribas et al. (2008) [17] in samples from healthy Brazilian subjects. *MICA*008* was the most frequent allele group, similar to findings in other Caucasian populations [5, 17, 20, 26, 27]. However, we also found a series of other alleles from different European, African and Asian populations that colonized the region [20, 28, 29].

Table 3. *MICA–HLA-B* haplotype frequencies and relative LD values (D') for haplotypes with a frequency exceeding 1% in all samples (n = 346), and comparison with healthy subjects.

Haplotype	Renal				Healthy subjects (Ribas et al., 2008)[17]			
	n	%	D'	p LD	n	%	D'	p LD
*MICA*009-B*51*	54	7.80%	0.775	0	33	8.09%	0.75	0
*MICA*004-B*44*	42	6.07%	0.540	0	24	5.88%	0.47	0
*MICA*002-B*35*	39	5.64%	0.439	0	25	6.13%	0.54	0
*MICA*008-B*07*	38	5.49%	0.801	0	25	6.13%	0.86	0
*MICA*008-B*08*	36	5.20%	0.844	0	24	5.88%	0.85	0
*MICA*010-B*15*	36	5.20%	0.762	0	24	5.88%	0.81	0
*MICA*018-B*18*	26	3.76%	0.803	0	15	3.68%	0.93	0
*MICA*002-B*39*	24	3.47%	1.000	0	18	4.41%	1.00	0
*MICA*002-B*38*	23	3.32%	1.000	0	10	2.45%	1.00	0
*MICA*008-B*44*	22	3.18%	0.130	0.0301	20	4.90%	0.26	0.0017
*MICA*004-B*49*	21	3.03%	0.947	0	9	2.21%	1.00	0
*MICA*016-B*35*	21	3.03%	0.903	0	7	1.72%	1.00	0
*MICA*011-B*14*	20	2.89%	0.951	0	19	4.66%	1.00	0
*MICA*008-B*15*	18	2.60%	0.113	0.0852	9	2.21%	20.12	0.6207
*MICA*007-B*27*	16	2.31%	0.939	0	11	2.70%	1.00	0
*MICA*004-B*42*	15	2.17%	1.000	0	5	1.23%	1.00	0
*MICA*002-B*58*	14	2.02%	0.920	0	9	2.21%	1.00	0
*MICA*017-B*57*	13	1.88%	0.927	0	7	1.72%	1.00	0
*MICA*004-B*41*	12	1.73%	0.910	0	6	1.47%	1.00	0
*MICA*009-B*50*	12	1.73%	0.659	0	5	1.23%	0.81	0
*MICA*008-B*40*	11	1.59%	0.113	0.1842	7	1.72%	0.38	0.0158
*MICA*009-B*35*	10	1.45%	-0.002	0.9938	6	1.47%	0.01	0.8052
*MICA*008-B*13*	9	1.30%	0.768	0	8	1.96%	0.85	0
*Other***	160	23.12%			82	20.10%		

**Haplotypes with frequency below 1%;

D' = Relative linkage disequilibrium value; Only haplotypes in attraction (D' = 1 and D'<1) are shown. Healthy subjects described by Ribas et al., (2008)[17].

https://doi.org/10.1371/journal.pone.0176072.t003

As expected from the short distance between the *HLA-B* and *MICA* loci, a significant linkage disequilibrium was observed. The largest number of *MICA–HLA-B* haplotypes was found in Caucasian populations, such as *MICA*009-B*51*, *MICA*004–B*44* and *MICA*002–HLA-B*35* [7, 20, 26, 30–32]. However, these haplotypes were also found in Asian [18] and African population [29].

More typically, a single *MICA* allele is associated with several *HLA-B* alleles, whereas a few *HLA-B* alleles are associated with some *MICA* alleles [17]. In this study, the most common allele groups (*MICA*008*, *-*002*, and *-*004*) had several associations with *HLA-B*: *MICA*008* was associated with *HLA-B*15*, *-*07*, *-*44*, *-*08*, *-*13*, *-*40*, *-*37* and *-*40*; *MICA*002* was associated with *HLA-B*15*, *-*39*, *-*35*, *-*58* and *-*43*; and *MICA*004* was associated with *HLA-B*44*, *-*42*, *-*41*, *-*49* and *-*48*. In contrast, *HLA-B* alleles had associations with *MICA*, such as *HLA-B*35* with *MICA*002*, *MICA-*16*, *-*46* and **-52*. In addition, most *HLA-B* alleles, such as the *B*07* and **08* allele group, had only a single *MICA* association (*MICA-*008*).

This association may indicate a different evolutionary history of the *MICA* gene from classical *HLA*; the common alleles *MICA* are very old, predating major branches of the *HLA-B* alleles [24]. In vitro, the *MICA* allelic diversity may affect ligand binding between the MICA

(strong or weak binders) and the NK-cell receptor NKG2D, affecting natural killer-cell activation and the modulation of T-cell responses [20, 24, 33, 34]. According to Gao et al. (2006) [24], future studies of the capacity of MICA to interact with NK-cell receptors across populations may provide information for population-based studies of diseases.

The frequencies of the MICA and HLA-B allele groups reported in this study were compared with those published by Ribas et al. [17], since we adopted the similar criteria used in that study. Our enrolled patients and the bone-marrow volunteer donors selected by Ribas et al. [17] came from the same geographic area (the same State) and showed the same pattern of ethnicity, predominantly Caucasians [21].

The present results are very similar to those found by Ribas et al. (2008) [17]. Among 23 haplotypes with a frequency above 1%, 20 had significant values of attraction in both studies. *MICA*008-B*15* and *MICA*009-B*35* did not show any significant values, so they are in linkage equilibrium. A discordant result was found for *MICA*008-B*40*, which showed a significant result only in the study by Ribas et al. (2008) [17]. *MICA*002-B*39*, *MICA*002-B*38* and *MICA*004-B*42* had D' = 1 in both studies, making it possible to determine the *MICA* allele merely by knowing the *HLA-B* allele. Among the significant results, 7 haplotypes showed D' = 1 in the study of Ribas et al. (2008) [17], and D'<1 in our renal patients. These results suggest that despite the strong linkage disequilibrium observed in the majority of frequent haplotypes in our population, the linkage is not absolute, and as the sample size increases, the number of "D' = 1" values may decrease.

Another interesting result, as observed in the S1 Table, is the haplotype repulsion, indicating that 36 haplotypes that would be expected if the sample were in linkage equilibrium were not observed in this sample. Also, 8 haplotypes were found with significantly lower frequencies than expected. A very unusual combination expected for our population was found in haplotype *MICA*045-B*47*, the only combination that showed a correlation equal to 1 ($r^2 = 1$, D' = 1, $p = 0.0000$), i.e., a perfect correlation between two rare alleles (both 0.14%). This could be explained by inferring a recent in-migration of the family of the individual who provided the sample, with no direct association with the overall population. Although Ribas et al. (2008) [17] did not list samples with a frequency less than 1%, one can deduce that these values would not have appeared in that study, because the *MICA*045* allele was not observed and the *HLA-B*47* allele was observed, so the correlation is not absolute.

Comparison of *MICA–HLA-B* haplotype frequencies showed no significant difference between the haplotypes with a frequency above 1% in both studies. Notably, the *MICA*027-B*40* and *MICA*002-B*53* haplotypes, found in this study in frequencies of 3.32% and 2.02%, respectively, could be candidates for common haplotypes; however, we could not determine the exact frequencies that were observed in the healthy population.

Considering that several studies have suggested associations of the *HLA* haplotype with various diseases, the calculation of LD parameters was used for comparison with the results of health subjects also in the state of Paraná found by Ribas et al. (2008) [17], and not with the aim of determining the population structure.

In conclusion, the *MICA* allelic diversity in our population is similar to those of other Caucasian populations worldwide. However, we found a series of other allele groups, which may result from the contribution of alleles from different European, African or Amerindian populations that colonized the region. This study expands our knowledge of the distribution of *MICA* polymorphisms and linkage disequilibrium with *HLA-B* alleles, helping to elucidate possible associations with different diseases in patients with chronic kidney disease. Finally, our data could be useful as a preliminary clinical reference for better understanding of the mechanisms involved in the allograft rejection associated with *MICA* polymorphisms in the Brazilian population.

Supporting information

S1 Table. Graphic representation of the linkage disequilibrium between MICA and HLA-B alleles. The values shown in the fields are haplotype frequencies and in brackets the correlation index r2. 0% frequency was not represented. P values <0.05 were colored. Haplotypes in attraction with D' = 1 were colored in dark red. Haplotypes in attraction with D'<1 were colored in bright red. Haplotypes in repulsion with D' = -1 were colored in dark blue. Haplotypes in repulsion with D'>-1 were colored light blue.
(DOCX)

Acknowledgments

We express our gratitude to all the patients involved in this study.

Author Contributions

Conceptualization: RHY PKS GFG JSS MGB SDB.

Data curation: RHY PKS GFG JSS MGB SDB.

Formal analysis: RHY PKS GFG JSS MGB SDB.

Funding acquisition: RHY PKS GFG JSS MGB SDB.

Investigation: RHY PKS GFG JSS MGB SDB.

Methodology: RHY PKS GFG JSS MGB SDB.

Project administration: RHY PKS GFG JSS MGB SDB.

Resources: RHY PKS GFG JSS MGB SDB.

Software: RHY PKS GFG JSS MGB SDB.

Supervision: RHY PKS GFG JSS MGB SDB.

Validation: RHY PKS GFG JSS MGB SDB.

Visualization: RHY PKS GFG JSS MGB SDB.

Writing – original draft: RHY PKS GFG JSS MGB SDB.

Writing – review & editing: RHY PKS GFG JSS MGB SDB.

References

1. Bahram S, Bresnahan M, Geraghty DE, Spies T. A second lineage of mammalian major histocompatibility complex class I genes. Proc Natl Acad Sci U S A. 1994; 91(14):6259–63. Epub 1994/07/05. PMID: 8022771
2. Leelayuwat C, Townend DC, Degli-Esposti MA, Abraham LJ, Dawkins RL. A new polymorphic and multicopy MHC gene family related to nonmammalian class I. Immunogenetics. 1994; 40(5):339–51. Epub 1994/01/01. PMID: 7927538
3. Stephens HA. MICA and MICB genes: can the enigma of their polymorphism be resolved? Trends Immunol. 2001; 22(7):378–85. Epub 2001/06/29. PMID: 11429322
4. Bahram S, Spies T. The MIC gene family. Res Immunol. 1996; 147(5):328–33. Epub 1996/06/01. PMID: 8876061
5. Gonzalez S, Brautbar C, Martinez-Borra J, Lopez-Vazquez A, Segal R, Blanco-Gelaz MA, et al. Polymorphism in MICA rather than HLA-B/C genes is associated with psoriatic arthritis in the Jewish population. Hum Immunol. 2001; 62(6):632–8. Epub 2001/06/08. PMID: 11390038

6. Collins RW. Human MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation. Eur J Immunogenet. 2004; 31(3):105–14. Epub 2004/06/09. https://doi.org/10.1111/j.1365-2370.2004.00457.x PMID: 15182323
7. Hughes EH, Collins RW, Kondeatis E, Wallace GR, Graham EM, Vaughan RW, et al. Associations of major histocompatibility complex class I chain-related molecule polymorphisms with Behcet's disease in Caucasian patients. Tissue Antigens. 2005; 66(3):195–9. Epub 2005/08/17. https://doi.org/10.1111/j.1399-0039.2005.00465.x PMID: 16101830
8. Zou Y, Stastny P, Susal C, Dohler B, Opelz G. Antibodies against MICA antigens and kidney-transplant rejection. N Engl J Med. 2007; 357(13):1293–300. Epub 2007/09/28. https://doi.org/10.1056/NEJMoa067160 PMID: 17898098
9. Kaimen-Maciel DR, Reiche EM, Borelli SD, Morimoto HK, Melo FC, Lopes J, et al. HLA-DRB1* allele-associated genetic susceptibility and protection against multiple sclerosis in Brazilian patients. Mol Med Rep. 2009; 2(6):993–8. Epub 2009/11/01. https://doi.org/10.3892/mmr_00000204 PMID: 21475933
10. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. Lancet. 1966; 2(7465):662–5. Epub 1966/09/24. PMID: 4162350
11. Lee PC, Terasaki PI, Takemoto SK, Lee PH, Hung CJ, Chen YL, et al. All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. Transplantation. 2002; 74(8):1192–4. Epub 2002/11/20. https://doi.org/10.1097/01.TP.0000031249.33030.FB PMID: 12438971
12. Terasaki PI, Ozawa M. Predictive value of HLA antibodies and serum creatinine in chronic rejection: results of a 2-year prospective trial. Transplantation. 2005; 80(9):1194–7. Epub 2005/11/30. PMID: 16314785
13. Clatworthy MR, Espeli M, Torpey N, Smith KG. The generation and maintenance of serum alloantibody. Curr Opin Immunol. 2010; 22(5):669–81. Epub 2010/10/12. https://doi.org/10.1016/j.coi.2010.08.018 PMID: 20932734
14. Kafetzi ML, Boletis JN, Melexopoulou CA, Tsakris A, Iniotaki AG, Doxiadis II. Clinical evaluation of the endothelial tie-2 crossmatch in ABO compatible and ABO incompatible renal transplants. Hum Immunol. 2013; 74(11):1425–30. Epub 2013/06/25. https://doi.org/10.1016/j.humimm.2013.06.003 PMID: 23792052
15. Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A. 2003; 100(1):177–82. Epub 2003/01/02. https://doi.org/10.1073/pnas.0126614100 PMID: 12509516
16. Marin ML, Savioli CR, Yamamoto JH, Kalil J, Goldberg AC. MICA polymorphism in a sample of the Sao Paulo population, Brazil. Eur J Immunogenet. 2004; 31(2):63–71. Epub 2004/04/17. https://doi.org/10.1111/j.1365-2370.2004.00446.x PMID: 15086345
17. Ribas F, Oliveira LA, Petzl-Erler ML, Bicalho MG. Major histocompatibility complex class I chain-related gene A polymorphism and linkage disequilibrium with HLA-B alleles in Euro-Brazilians. Tissue Antigens. 2008; 72(6):532–8. Epub 2008/11/13. https://doi.org/10.1111/j.1399-0039.2008.01142.x PMID: 19000131
18. Sohn YH, Cha CH, Oh HB, Kim MH, Choi SE, Kwon OJ. MICA polymorphisms and haplotypes with HLA-B and HLA-DRB1 in Koreans. Tissue Antigens. 2010; 75(1):48–55. Epub 2009/11/10. https://doi.org/10.1111/j.1399-0039.2009.01396.x PMID: 19895570
19. Excoffier L, Laval G, Schneider S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 2005; 1:47–50. Epub 2005/01/01.
20. Lucas D, Campillo JA, Lopez-Hernandez R, Martinez-Garcia P, Lopez-Sanchez M, Botella C, et al. Allelic diversity of MICA gene and MICA/HLA-B haplotypic variation in a population of the Murcia region in southeastern Spain. Hum Immunol. 2008; 69(10):655–60. Epub 2008/08/23. https://doi.org/10.1016/j.humimm.2008.07.011 PMID: 18718856
21. Probst CM, Bompeixe EP, Pereira NF, de OD MM, Visentainer JE, Tsuneto LT, et al. HLA polymorphism and evaluation of European, African, and Amerindian contribution to the white and mulatto populations from Parana, Brazil. Hum Biol. 2000; 72(4):597–617. Epub 2000/10/26. PMID: 11048789
22. Crow JF. Eighty years ago: the beginnings of population genetics. Genetics. 1988; 119(3):473–6. Epub 1988/07/01. PMID: 3042506
23. Wigginton JE, Cutler DJ, Abecasis GR. A note on exact tests of Hardy-Weinberg equilibrium. Am J Hum Genet. 2005; 76(5):887–93. Epub 2005/03/25. https://doi.org/10.1086/429864 PMID: 15789306
24. Gao X, Single RM, Karacki P, Marti D, O'Brien SJ, Carrington M. Diversity of MICA and linkage disequilibrium with HLA-B in two North American populations. Hum Immunol. 2006; 67(3):152–8. Epub 2006/05/16. https://doi.org/10.1016/j.humimm.2006.02.009 PMID: 16698437

25. Piancatelli D, Del Beato T, Oumhani K, El Aouad R, Adorno D. MICA polymorphism in a population from north Morocco, Metalsa Berbers, using sequence-based typing. Hum Immunol. 2005; 66(8):931–6. Epub 2005/10/12. https://doi.org/10.1016/j.humimm.2005.06.008 PMID: 16216678

26. Petersdorf EW, Shuler KB, Longton GM, Spies T, Hansen JA. Population study of allelic diversity in the human MHC class I-related MIC-A gene. Immunogenetics. 1999; 49(7–8):605–12. Epub 1999/06/17. PMID: 10369917

27. Munoz-Saa I, Cambra A, Pallares L, Espinosa G, Juan A, Pujalte F, et al. Allelic diversity and affinity variants of MICA are imbalanced in Spanish patients with Behcet's disease. Scand J Immunol. 2006; 64(1):77–82. Epub 2006/06/21. https://doi.org/10.1111/j.1365-3083.2006.01780.x PMID: 16784494

28. Katsuyama Y, Ota M, Ando H, Saito S, Mizuki N, Kera J, et al. Sequencing based typing for genetic polymorphisms in exons, 2, 3 and 4 of the MICA gene. Tissue Antigens. 1999; 54(2):178–84. Epub 1999/09/17. PMID: 10488745

29. Tian W, Boggs DA, Uko G, Essiet A, Inyama M, Banjoko B, et al. MICA, HLA-B haplotypic variation in five population groups of sub-Saharan African ancestry. Genes Immun. 2003; 4(7):500–5. Epub 2003/10/11. https://doi.org/10.1038/sj.gene.6364017 PMID: 14551603

30. Bolognesi E, Dalfonso S, Rolando V, Fasano ME, Pratico L, Momigliano-Richiardi P. MICA and MICB microsatellite alleles in HLA extended haplotypes. Eur J Immunogenet. 2001; 28(5):523–30. Epub 2002/03/08. PMID: 11861819

31. Reinders J, Rozemuller EH, Otten HG, van der Veken LT, Slootweg PJ, Tilanus MG. HLA and MICA associations with head and neck squamous cell carcinoma. Oral Oncol. 2007; 43(3):232–40. Epub 2006/07/22. https://doi.org/10.1016/j.oraloncology.2006.03.003 PMID: 16857416

32. Cambra A, Munoz-Saa I, Crespi C, Serra A, Etxagibel A, Matamoros N, et al. MICA-HLA-B haplotype diversity and linkage disequilibrium in a population of Jewish descent from Majorca (the Balearic Islands). Hum Immunol. 2009; 70(7):513–7. Epub 2009/04/15. https://doi.org/10.1016/j.humimm.2009.04.005 PMID: 19364518

33. Steinle A, Li P, Morris DL, Groh V, Lanier LL, Strong RK, et al. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics. 2001; 53 (4):279–87. Epub 2001/08/09. PMID: 11491531

34. Zhang Y, Stastny P. MICA antigens stimulate T cell proliferation and cell-mediated cytotoxicity. Hum Immunol. 2006; 67(3):215–22. Epub 2006/05/16. https://doi.org/10.1016/j.humimm.2006.02.014 PMID: 16698445

Supporting Information

S 1 Table. Graphic representation of the Linkage Disequilibrium between MICA and HLA-B alleles. The values shown in the fields are haplotype frequencies and in brackets the correlation index r2. 0 % frequency was not represented. P values <0.05 were colored. Haplotypes in attraction with D'=1 were colored in dark red. Haplotypes in attraction with D'<1 were colored in bright red. Haplotypes in repulsion with D'=-1 were colored in dark blue. Haplotypes in repulsion with D'>-1 were colored light blue.

Supplementary Table 1: Graphic representation of the Linkage Disequilibrium between *MICA* and *HLA-B* alleles.

Locus			*MICA**																		
	Alleles		*01*	*02*	*04*	*06*	*07*	*08*	*09*	*10*	*11*	*12*	*15*	*16*	*17*	*18*	*27*	*44*	*45*	*46*	*52*
	Freq.		*1.30 %*	*17.05 %*	*14.88 %*	*0.58 %*	*2.46 %*	*21.68 %*	*13.73 %*	*6.65 %*	*3.03 %*	*1.30 %*	*1.01 %*	*3.32 %*	*2.02 %*	*4.62 %*	*5.64 %*	*0.14 %*	*0.14 %*	*0.14 %*	*0.29 %*
HLA-B*	*07*	*6.50 %*		(0.01)	0.29 % (0.01)			5.49 % (0.16)	(0.01)				0.14 % (0)				0.58 % (0)				
	08	*5.92 %*		(0.01)	0.14 % (0.01)			5.20 % (0.16)	(0.01)								0.58 % (0)				
	13	*1.59 %*		0.14 % (0)			0.14 % (0)	1.30 % (0.03)													
	14	*3.18 %*	0.14 % (0)	(0.01)	0.14 % (0)			(0.01)			2.89 % (0.86)										
	15	*8.53 %*		0.14 % (0.02)	(0.02)			2.60 % (0)	(0.01)	5.20 % (0.44)				0.29 % (0)			0.29 % (0)				
	18	*4.91 %*	1.16 % (0.2)	(0.01)	(0.01)			(0.01)	(0.01)							3.76 % (0.6)					
	27	*3.03 %*		(0.01)			2.31 % (0.71)	0.29 % (0)								0.14 % (0)	0.29 % (0)				
	35	*10.55 %*		5.64 % (0.11)	(0.02)			0.14 % (0.03)	1.45 % (0)	(0.01)				3.03 % (0.24)		(0.01)	(0.01)			0.14 % (0.01)	0.14 % (0)
	37	*1.30 %*						1.30 % (0.05)													
	38	*3.32 %*		3.32 % (0.17)	(0.01)			(0.01)													
	39	*3.47 %*		3.47 % (0.17)	(0.01)			(0.01)	(0.01)												
	40	*5.20 %*		0.14 % (0.01)	(0.01)			1.59 % (0)	(0.01)							0.14 % (0)	3.32 % (0.35)				
	41	*1.88 %*			1.73 % (0.09)										0.14 % (0)						
	42	*2.17 %*			2.17 % (0.13)			(0.01)													
	44	*9.97 %*		(0.02)	6.07 % (0.18)			3.18 % (0.01)	0.14 % (0.01)	(0.01)							0.58 % (0)				
	45	*2.02 %*						(0.01)	1.16 % (0.03)				0.87 % (0.36)								
	46	*0.14 %*								0.14 % (0.02)											
	47	*0.14 %*																	0.14 % (1)		
	48	*0.43 %*			0.14 % (0)			0.29 % (0.01)													
	49	*3.18 %*		(0.01)	3.03 % (0.17)			(0.01)			0.14 % (0)										
	50	*2.46 %*			0.43 % (0)			0.14 % (0)	1.73 % (0.07)	0.14 % (0)											
	51	*9.68 %*		(0.02)	0.29 % (0.01	0.58 % (0.0		0.14 % (0.03	7.80 % (0.4)	0.58 % (0)						0.14 % (0)	(0.01)				0.14 % (0.0

					)	5)		)															1)
	52	2.02 %						(0.01)	1.45 % (0.06)	0.58 % (0.02)													
	53	2.17 %		2.02 % (0.09)				(0.01)								0.14 % (0)							
	54	0.29 %										0.29 % (0.22)											
	55	1.01 %			0.14 % (0)							0.87 % (0.57)											
	56	0.14 %										0.14 % (0.11)											
	57	2.60 %		0.14 % (0)	0.29 % (0)			(0.01)							1.88 % (0.66)	0.29 % (0)							
	58	2.17 %		2.02 % (0.09)				(0.01)										0.14 % (0.07)					

The values shown in the fields are haplotype frequencies and in brackets the correlation index r^2. 0 % frequency was not represented. P values <0.05 were colored. Haplotypes in attraction with D'=1 were colored in dark red. Haplotypes in attraction with D'<1 were colored in bright red. Haplotypes in repulsion with D'=-1 were colored in dark blue. Haplotypes in repulsion with D'>-1 were colored light blue.

2.2.ARTIGO 2: "MICA genetic polymorphism and HLA-A, C, B, MICA, DRB1, DQA1, DQB1 haplotypic diversity in renal transplant candidates, southern Brazil"

MICA genetic polymorphism and HLA-A, C, B, MICA, DRB1, DQA1, DQB1 haplotypic diversity in renal transplant candidates, southern Brazil

Roger Haruki Yamakawa[1], Patrícia Keiko Saito[1],Sueli Donizete Borelli[1,*].

Affiliations:

[1]Department of Basic Health Sciences, Universidade Estadual de Maringá - Maringá, PR, Brazil.

Corresponding author for proofs and reprints:

Sueli Donizete Borelli

Universidade Estadual de Maringá (UEM)

Departamento de Ciências Básicas da Saúde

Laboratório de Imunogenética

Av. Colombo, 5790, Zona 07

CEP: 87020-900 - Maringá, Paraná, Brazil

Avenida Colombo 5790, CEP: 87020-900, Maringá, Paraná, Brazil.

Phone: + 55 44 3011-5388

Fax: + 55 44 3011-5388

E-mail: sueliborelli@gmail.com

Short title: HLA and MICA diversity in renal transplant candidates

Conflicts of Interest

The authors declare that there is no conflict of interest

ABSTRACT

The HLA polymorphisms and recently, MICA polymorphisms, have been shown to be associated with large number of diseases and allograft rejection. The frequencies of MICA and HLA-A,-B,-C,-DRB1,-DQA1,-DQB1 alleles and haplotypes were studied in 346 renal transplant candidates in a population from southern Brazil. Participants were classified according to ethnic group (189 caucasians, 98 mestizos [mixed race], 50 blacks and 9 orientals). The MICA and HLA typing was performed using the polymerase chain reaction-sequence specific primer method (PCR-SSO), combined with the Luminex technology. A total of 19 MICA, 20 HLA-A, 29 HLA-B and 14 HLA-C, 13 HLA-DRB1, 6 HLA-DQA and 5 HLA-DQB1 allele groups were identified. The most frequent allele groups were *MICA*008, HLA-A*02, B*35, C*07, DRB1*04, DQA1*01* and *DQB1*03*. Significant differences ($p<0.05$) were observed in *MICA*009, MICA*010, HLA-A*24, A*68, B*52, DRB1*09, C*03, C*07* and *DQA1*03* allele group frequencies between ethnic groups. The most common extended haplotype in the total samples was *HLA-A*01-C*07-B*08-MICA*008-DRB1*03-DQA1*05-DQB1*02* (2.4%). An ethnic contribution was shown in the analyzed samples, with evidence that the Brazilian population is composed of a mixture of ethnicities. The data from this study showed the distribution of the MICA and HLA alleles and haplotypes in renal transplant candidates from southern Brazil.

Keywords: Gene Frequency; haplotypes; histocompatibility antigens class I; Histocompatibility Antigens Class II; HLA antigens; population genetics.

1. INTRODUCTION

Although the classical human leukocyte antigens (HLA) loci (HLA-A, -B, -C, -DR, -DQ and -DP) are the major genetic determinants to organ transplantation success, recent studies in kidney transplantation have demonstrated the roles of MHC class I chain-related gene A (MICA) [1, 2]. The genes of the HLA and MICA are located on the short arm of chromosome 6. The genes of HLA class I (A, B, and C) and class II (DR, DQ, DP) encode molecules that participate in antigen presentation to T cells and MICA genes encode surface glycoproteins strongly implicated in innate immunity [1, 3].

The HLA system is an important marker of graft survival , and is the main factor among the biological systems involved in graft rejection [4, 5]. While the importance of HLA-A, -B, and -DR in solid-organ transplantation has been known for many years [5, 6]. T, the role of HLA-C, -DQ and MICA in the transplantation has recently been documented [2, 7, 8]. The HLA polymorphisms and recently, MICA polymorphisms, have been shown to be associated with large number of diseases and allograft rejection [1, 4, 9-11].

For historical reasons, the Brazilian population is one of the most mixed in the world [12], which may difficult the search for a donor with the best immunological compatibility. The research of HLA genetical profile is important for studies of the ethnic influence, genetic pattern and origin of populations [13]. Previous studies have shown ethnic differences in the distribution of *MICA* polymorphisms, likewise, HLA polymorphisms [14, 15]. However, the information available for *MICA* is not been well documented as HLA polymorphisms [15, 16].

In Brazil, the frequencies of HLA and MICA alleles may vary according to the predominant ethnic group of each region [13, 14, 17-19]. However, to our knowledge,

very few studies in Brazil have investigated the HLA alleles and haplotype diversity in renal transplant candidates [10] and no study evaluated the HLA-C, -DQA1, -DQB1 and MICA loci.

In this study, we evaluated the frequencies of the MICA and HLA-A, -B, -C, -DRB1, -DQA1, -DQB1 alleles and haplotypes in renal transplant candidates in the north/northwestern region of Paraná state in southern Brazil.

2. MATERIAL AND METHODS

2.1 Samples

The study was conducted with 346 blood samples of patients with chronic kidney disease, renal transplant candidates, who were registered at two regional transplant centers, the Central Regional de Transplantes Norte/Londrina (CRTN/Londrina) and the Central Regional de Transplantes Noroeste/Maringá (CRTNO/Maringá), in northern and northwestern Paraná, respectively, in the period from July 2010 to March 2011. We included only patients with updated data (active patients and potential recipients). Patients were divided into four ethnic groups: Caucasian (n = 189), mestizo (mixed race/mixed of Europeans, Africans and Amerindians individuals; n = 98), black (n = 50) and oriental (n=9). The ethnic group was reported by Transplant Centers, using self-definition as the criterion for determining the ethnic group, based on the method used by the official census of Brazil (Brazilian Institute of Geography and Statistics national census survey). This study was approved by the Ethics Committee of the Universidade Estadual de Maringá (protocol no. 333/2011). All procedures followed Resolution 466/2012 of the Brazilian Health Council which rules on research work on humans.

2.2 DNA extraction, MICA and HLA typing

To perform the HLA and MICA typing, about 5 mL of blood was collected by venipuncture into vacuum tubes (Vacutainer, Becton and Dickson, Oxford, UK) containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Then, we extracted the genomic DNA by the separation column method, using the commercial kit for DNA extraction Biopur (Biometrix, Curitiba, Paraná, Brazil), following the manufacturer's protocol. After adjusting the DNA concentration, obtained by the optical density method, we amplified the DNA using polymerase chain reaction-sequence specific primers (PCR-SSO) combined with Luminex technology. The genomic DNA was amplified using biotinylated sequence-specific primers for MICA, HLA class I (HLA-A, -B, -C) and class II (-DRB1, -DQA1, -DQB1) in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), followed by hybridization with complementary probes for DNA, conjugated with microspheres (beads) labeled with different fluorochromes to identify complementary sequences of the amplified DNA, using the commercial kit LABType (One Lambda, Inc., Canoga Park, CA, USA), following the manufacturer's protocol. After hybridization, the results were read using the flow cytometry platform LABScanTM100 (One Lambda, Inc.), followed by analysis using the program HLA Fusion version 2.0 (One Lambda, Inc.). The results showed low-medium resolution.

2.3 Statistical analyses

The Arlequin program version 3.11 [20] was used to calculate the allele and haplotype frequencies and to assess the Hardy-Weinberg equilibrium. The haplotype frequencies were estimated using the expectation-maximization algorithm (maximum likelihood method). The Statistica 7.0 and R programs were used to calculate the comparison

among the allele frequencies of the ethnic groups. Significant differences among the allele frequencies in these groups were calculated using the Fisher exact test with Bonferroni correction for multiple comparisons. The *p* values were considered significant when less than 0.05. The Odds Ratio (OR) was used to estimate the risk.

3. RESULTS

Among the 346 renal transplant candidates, 211 (60.98%) were males. The predominant ethnic group was Caucasian (54.6%), followed by mestizo (28.3%) and black (14.4%).

The MICA, HLA-A, -C, -DRB1, -DQA1 and -DQB1 loci were in Hardy-Weinberg equilibrium ($p > 0.05$). For the MICA locus, the observed heterozygosity was 82.0% and the expected heterozygosity was 87.9% ($p = 0.07$). For the HLA, in the A locus, the observed heterozygosity was 86.9% and the expected heterozygosity was 88.6% ($p = 0.52$); for the C locus, these values were 89.3% and 88.6% respectively ($p = 0.65$); for the DRB1 locus, 87.5% and 89.8% ($p = 0.16$); for the DQA1 locus, 69.0% and 72.6% ($p = 0.66$) and for the DQB1 locus, 74.5% and 76.4% ($p = 0.42$). A significant difference was observed only for the B locus between the observed and expected heterozygosity ($p <0.01$), meaning; only the B locus was not in Hardy-Weinberg equilibrium ($p < 0.05$). In the B locus, the observed heterozygosity was 90.7% and the expected, 94.1%.

Table 1, 2 and 3 shows the frequencies of the MICA, HLA class I (-A, -B and -C) and class II (-DRB1, -DQA1 and -DQB1) alleles in the total sample and the comparison among the ethnic groups. A total of 19 MICA, 20 HLA-A, 29 HLA-B, 14 HLA-C, 13 HLA-DRB1, 6 HLA-DQA1 and 5 HLA-DQB1 allele groups were identified. The most frequent allele groups MICA locus were: *MICA*008, MICA*002*, *MICA*004* and for each HLA locus were: *HLA-A*02, A*24, A*01, B*35, B*44, B*51, C*07, C*04, C*03,*

*C*12, DRB1*04, DRB1*11, DRB1*13, DQA1*01, DQA1*05, DQA1*03, DQB1*03, DQB1*05, DQB1*02* and *DQB1*06.* There was good agreement in the distribution of allele frequencies among the three groups. Significant differences ($p < 0.05$) were observed in *MICA*009*, *MICA*010*, *HLA-A*24, A*68, B*52, DRB1*09, C*03, C*07* and *DQA1*03* allele group frequencies between ethnic groups.

Table 4 shows the most common HLA-MICA extended haplotypes. The *HLA-A*01-C*07-B*08-MICA*008-DRB1*03-DQA1*05-DQB1*02* was the most common for the total samples (2.4%), Caucasian (2.9%), mestizo (1.0%) and black (4.0%) and *A*24-C*12-B*52-MICA*009-DRB1*15-DQA1*01-DQB1*06* was the most common in the oriental group (2.1%).

4. Discussion

A significant number of HLA and MICA diversity studies have been conducted in different regions, using healthy subjects, but none has involved the HLA-A ,-B, -C, -DRB1, -DQA1, -DQB1, MICA loci and combinations [13, 14, 17-19, 21-27].

Recent studies have shown ethnic differences in the MICA frequencies [14, 15].

However, the information available for *MICA* polymorphisms is still insufficient. To our knowledge, our study provides the first data of the MICA and HLA-A, -B, -C, -DRB1, -DQA1, -DQB1 alleles and haplotypes frequencies in Brazilian renal transplant candidates.

In the current study, a significant difference between the observed and expected heterozygosity was observed only for HLA-B. The B locus was not in Hardy-Weinberg equilibrium ($p < 0.05$) and showed a high degree of heterozygosity (90.7%), even the number of heterozygous individuals was lower than expected. This difference can be

explained by the number of samples, difficulties in the identification of HLA, pathological condition itself, natural forces (such as a selective advantage or recent racial admixture, natural selection, or gene flow due to migration) or because we did not exclude individuals who had some degree of kinship to each other [28].

The Brazilian population is characterized by a wide mix of ethnic groups, a result of immigration from several countries during the colonization of Brazil, over a period of five centuries, resulting in an interracial mixture of Europeans, Africans, Amerindians and Asians [12].

The investigation of HLA and MICA alleles and haplotypes indicate the origin of the populations in a particular country or region [13-15], and assist in studies of susceptibility to disease [9-11].

This state was colonized largely by Europeans, and now has a high proportion of Caucasians, but Amerindian and African descents are also frequent in the population [13]. These influences can be observed in our study, which show a high contribution of HLA alleles of European origin, such as *HLA-A*02, B*35* and *-B*44* [14, 29-31], as well as the occurrence of alleles of African origin, such as HLA-*A*30* and *B*15* [32-34].

Because of its biological function and high degree of polymorphism, the HLA system has been increasingly studied as a genetic marker involved in susceptibility to different diseases [9, 10]. The *HLA-A*74* and *HLA-DRB1*11* allele groups, in the current study, showed a frequency of 1.0% and 13.0%, respectively, and were positively associated with chronic renal disease, like a study of Brazilian patients awaiting a kidney transplant [35].

The overall comparison among the allele frequencies of all HLA loci investigated and the ethnic groups of samples of the current study, revealed more similarities than

differences. The general distribution of the HLA-A, -B, and -DRB1 loci is similar to that found in other studies in southern Brazil, where the health populations studied were also described as mostly Caucasian [19, 26].

The MICA allelic diversity found in our study is similar to that found in Brazil by Marin et al. (2004) [18] and Ribas et al. (2008) [14]. The *MICA*008*, *MICA*002* and *MICA*004* allele group was the most common observed and are common in several populations [14, 36].

Only certain HLA allele groups exhibited significant differences in frequency among ethnic groups. *HLA-A*24*, *B*52*, *DRB1*09*, *C*03*, *DQA1*03* showed a significant difference between oriental and others ethnic groups; *HLA-A*68*, between black and Caucasian and between black and mestizo; and *HLA-C*07* between Caucasian and mestizo groups. This indicates that these alleles may be characteristic of a particular ethnic group in the population or their origin. However, allele groups that generally have a high frequency in Caucasian, such as *HLA-A*02, HLA-B*35, B*44, B*51, -C*04, -DRB1*11, -DRB1*04, -DQA1*05* and *-DQB1*03* showed similar, higher frequencies in Caucasians, blacks and mestizos, and therefore showed no significant differences among the three groups.

Other differences are the highest allelic frequencies of *MICA*009* and *MICA*010* in orientals, when compared with blacks and Caucasians. These MICA alleles are common in Asian populations of China, Japan, Korea and Thailand [15, 37-39].

The similarities found in HLA and MICA allele frequencies among the four study groups reflect the mixing of ethnic groups in the population, and the difficulty to define the ethnicity of each individual, based on self-definition and using only the physical evaluation and skin color. The official census of Brazil (Brazilian Institute of Geography and Statistics [IBGE] national census) also uses the criteria of self-definition

based in skin color to determine the ethnicity of respondents. However, HLA and MICA diversity studies may provide knowledge of the true ancestry and the degree of mixing of a population, and are therefore important in populations with a wide mix of ethnic groups, such as in Brazil.

Two studies with samples of populations from five continents found differences among HLA alleles, and these differences increased in populations that had a high degree of mixing of ethnic groups [40, 41]. However, in agreement with our results, a study of five different Brazilian populations, with samples composed of both Caucasians and blacks, also showed more similarities than differences in the frequencies of HLA alleles [21].

The HLA and MICA allele frequencies found in mestizos were not intermediate between blacks and caucasians, it seems like the mestizo ethnic group was formed by mixing different populations of Europeans, Africans, or Amerindians and who colonized the region.

The haplotype frequencies differed according to the reported ethnic group. The *HLA-A*01-C*07-B*08-MICA*008-DRB1*03-DQA1*05-DQB1*02* was the most common for the total samples (2.4%), Caucasians (2.9%), mestizos (1.0%) and blacks (4.0%). This haplotype was not found in orientals. The *A*24-C*12-B*52-MICA*009-DRB1*15-DQA1*01-DQB1*06* haplotype was more common in orientals (2.1%), and did not occur in Caucasians, mestizos and blacks. Haplotypes found in mestizos may be the result of the contribution of HLA alleles from different European, African or Amerindian populations [13].

The present results are in agreement with other studies conducted in southern Brazil, where the haplotype *HLA-A*01-B*08-DRB1*03* was more frequent in the total sample [19, 26]. This haplotype was also the most common in caucasians (2.7%) and African-

Brazilians (1.5%) in a study of HLA frequency in Paraná [27]. In Americans of European descent, the haplotypes *HLA-A*01-B*08-C*07-DRB1*03* (4.3%) [31] and *DRB1*03-DQA1*05-DQB1*02* (13.1%) [42] were the most frequent. The *HLA-A*01-B*08-C*07-DRB1*03-DQB1*02* haplotype was found in Caucasian populations from the Balearic Islands of Ibiza, Majorca and Minorca, with frequencies of 6.1%, 5.7% and 0.8% respectively [43]; and also in Caucasians from Albania (3.1%)[44].

Because of the proximity between MICA and HLA-B genes, their alleles tend to be in linkage disequilibrium. More commonly, a single MICA allele is associated with several HLA-B alleles or allelic groups rather than the opposite [14]. The *B*08-MICA*008* haplotype shows the highest frequency in our study, and it is also common in Caucasian populations [14, 45]. The *B*52-MICA*009* haplotype was described in our oriental group and it is also common in asians [15] and Euro-caucasians populations [46].

In the current study, the haplotype frequencies were estimated based on the MICA and HLA alleles identified in the sample. The distribution of haplotypes provides important information on the ethnic influence and ancestry of the populations under study. In the context of organ transplants, this knowledge may assist in the search for a donor with the best immunological compatibility in organ or tissue transplantation [19].

In conclusion, an ethnic contribution was shown in the analyzed samples, with evidence that the Brazilian population is composed of a mixture of ethnicities, and that the HLA and MICA contribution can be determined by the predominant ethnicity of each region, since the alleles of European origin presented in greater number than the others. The data from this study document the diversity of alleles and haplotypes MICA, HLA class I (HLA-A, -B and -C) and class II (HLA-DRB1, DQA1 and -DQB1) in renal transplant candidates from southern Brazil. These data will be important for understanding the

biology of the distribution of these alleles in our population, as well as in studies of susceptibility to different diseases, in patients with chronic kidney disease.

Acknowledgments

We thank the all patients who participated in this study.

References

[1] Zou Y, Stastny P, Susal C, Dohler B, Opelz G: Antibodies against MICA antigens and kidney-transplant rejection. N Engl J Med 2007;357:1293.

[2] Sanchez-Zapardiel E, Castro-Panete MJ, Castillo-Rama M, Morales P, Lora-Pablos D, Valero-Hervas Det al. : Harmful effect of preformed anti-MICA antibodies on renal allograft evolution in early posttransplantation period. Transplantation 2013;96:70.

[3] Abbas AK, Lichtman AH, Pillai S: Imunologia celular e molecular. Elsevier; 2008.

[4] Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O: Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. Lancet 1966;2:662.

[5] Opelz G, Dohler B: Effect of human leukocyte antigen compatibility on kidney graft survival: comparative analysis of two decades. Transplantation 2007;84:137.

[6] Wujciak T, Opelz G: Evaluation of HLA matching for CREG antigens in Europe. Transplantation 1999;68:1097.

[7] Tambur AR, Leventhal JR, Friedewald JJ, Ramon DS: The complexity of human leukocyte antigen (HLA)-DQ antibodies and its effect on virtual crossmatching. Transplantation 2010;90:1117.

[8] Tran TH, Dohler B, Heinold A, Scherer S, Ruhenstroth A, Opelz G: Deleterious impact of mismatching for human leukocyte antigen-C in presensitized recipients of kidney transplants. Transplantation 2011;92:419.

[9] Kaimen-Maciel DR, Reiche EM, Borelli SD, Morimoto HK, Melo FC, Lopes Jet al. : HLA-DRB1* allele-associated genetic susceptibility and protection against multiple sclerosis in Brazilian patients. Mol Med Rep 2009;2:993.

[10] Yamakawa RH, Saito PK, da Silva Junior WV, de Mattos LC, Borelli SD: Polymorphism of leukocyte and erythrocyte antigens in chronic kidney disease patients in southern Brazil. PLoS One 2014;9:e84456.

[11] Yabuki K, Mizuki N, Ota M, Katsuyama Y, Palimeris G, Stavropoulos Cet al. : Association of MICA gene and HLA-B*5101 with Behcet's disease in Greece. Invest Ophthalmol Vis Sci 1999;40:1921.

[12] Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD: Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A 2003;100:177.

[13] Probst CM, Bompeixe EP, Pereira NF, de ODMM, Visentainer JE, Tsuneto LTet al. : HLA polymorphism and evaluation of European, African, and Amerindian contribution to the white and mulatto populations from Parana, Brazil. Hum Biol 2000;72:597.

[14] Ribas F, Oliveira LA, Petzl-Erler ML, Bicalho MG: Major histocompatibility complex class I chain-related gene A polymorphism and linkage disequilibrium with HLA-B alleles in Euro-Brazilians. Tissue Antigens 2008;72:532.

[15] Sohn YH, Cha CH, Oh HB, Kim MH, Choi SE, Kwon OJ: MICA polymorphisms and haplotypes with HLA-B and HLA-DRB1 in Koreans. Tissue Antigens 2010;75:48.

[16] Stephens HA: MICA and MICB genes: can the enigma of their polymorphism be resolved? Trends Immunol 2001;22:378.

[17] Nigam P, Dellalibera E, Mauricio-da-Silva L, Donadi EA, Silva RS: Polymorphism of HLA class I genes in the Brazilian population from the Northeastern State of Pernambuco corroborates anthropological evidence of its origin. Tissue Antigens 2004;64:204.

[18] Marin ML, Savioli CR, Yamamoto JH, Kalil J, Goldberg AC: MICA polymorphism in a sample of the Sao Paulo population, Brazil. Eur J Immunogenet 2004;31:63.

[19] Bortolotto AS, Petry MG, da Silveira JG, Raya AR, Fernandes SR, Neumann Jet al. : HLA-A, -B, and -DRB1 allelic and haplotypic diversity in a sample of bone marrow volunteer donors from Rio Grande do Sul State, Brazil. Hum Immunol 2012;73:180.

[20] Excoffier L, Laval G, Schneider S: Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online 2005;1:47.

[21] Trachtenberg A, Jobim LF, Kraemer E, Salzano FM, Moraes ME, Moraes JRet al.: The HLA polymorphism in five Brazilian populations. Ann Hum Biol 1988;15:213.

[22] Moraes ME, Fernandez-Vina M, Salatiel I, Tsai S, Moraes JR, Stastny P: HLA class II DNA typing in two Brazilian populations. Tissue Antigens 1993;41:238.

[23] Braun-Prado K, Vieira Mion AL, Farah Pereira N, Culpi L, Petzl-Erler ML: HLA class I polymorphism, as characterised by PCR-SSOP, in a Brazilian exogamic population. Tissue Antigens 2000;56:417.

[24] Louzada-Junior P, Smith AG, Hansen JA, Donadi EA: HLA-DRB1 and -DQB1 alleles in the Brazilian population of the northeastern region of the state of Sao Paulo. Tissue Antigens 2001;57:158.

[25] Williams F, Nascimento E, Middleton D: HLA-A and -B alleles in a population from Belo Horizonte, Brazil. Human Immunology 2004;65:866.

[26] Ruiz TM, da Costa SM, Ribas F, Luz PR, Lima SS, da Graca Bicalho M: Human leukocyte antigen allelic groups and haplotypes in a brazilian sample of volunteer donors for bone marrow transplant in Curitiba, Parana, Brazil. Transplant Proc 2005;37:2293.

[27] Bardi MS, Jarduli LR, Jorge AJ, Camargo RB, Carneiro FP, Gelinski JRet al. : HLA-A, B and DRB1 allele and haplotype frequencies in volunteer bone marrow donors from the north of Parana State. Rev Bras Hematol Hemoter 2012;34:25.

[28] Chen JJ, Hollenbach JA, Trachtenberg EA, Just JJ, Carrington M, Ronningen KSet al. : Hardy-Weinberg testing for HLA class II (DRB1, DQA1, DQB1, and DPB1) loci in 26 human ethnic groups. Tissue Antigens 1999;54:533.

[29] Silva Carvalho A: HLA-A, B and C markers in the Portuguese population. Tissue Antigens 1983;21:39.

[30] Piazza A, Olivetti E, Griffo RM, Rendine S, Amoroso A, Barbanti Met al. : The distribution of HLA antigens in Italy. Gene Geogr 1989;3:141.

[31] Mack SJ, Tu B, Lazaro A, Yang R, Lancaster AK, Cao Ket al. : HLA-A, -B, -C, and -DRB1 allele and haplotype frequencies distinguish Eastern European

Americans from the general European American population. Tissue Antigens 2009;73:17.

[32] Lulli P, Mangano VD, Onori A, Batini C, Luoni G, Sirima BSet al. : HLA-DRB1 and -DQB1 loci in three west African ethnic groups: genetic relationship with sub-Saharan African and European populations. Hum Immunol 2009;70:903.

[33] Paximadis M, Mathebula TY, Gentle NL, Vardas E, Colvin M, Gray CMet al. : Human leukocyte antigen class I (A, B, C) and II (DRB1) diversity in the black and Caucasian South African population. Hum Immunol 2012;73:80.

[34] Assane AA, Fabricio-Silva GM, Cardoso-Oliveira J, Mabunda NE, Sousa AM, Jani IVet al. : Human leukocyte antigen-A, -B, and -DRB1 allele and haplotype frequencies in the Mozambican population: a blood donor-based population study. Hum Immunol 2010;71:1027.

[35] Crispim JC, Mendes-Junior CT, Wastowski IJ, Palomino GM, Saber LT, Rassi DMet al. : HLA polymorphisms as incidence factor in the progression to end-stage renal disease in Brazilian patients awaiting kidney transplant. Transplant Proc 2008;40:1333.

[36] Collins RW, Stephens HA, Clare MA, Vaughan RW: High resolution molecular phototyping of MICA and MICB alleles using sequence specific primers. Hum Immunol 2002;63:783.

[37] Komatsu-Wakui M, Tokunaga K, Ishikawa Y, Kashiwase K, Moriyama S, Tsuchiya Net al. : MIC-A polymorphism in Japanese and a MIC-A-MIC-B null haplotype. Immunogenetics 1999;49:620.

[38] Romphruk AV, Naruse TK, Romphruk A, Kawata H, Puapairoj C, Kulski JKet al. : Diversity of MICA (PERB11.1) and HLA haplotypes in Northeastern Thais. Tissue Antigens 2001;58:83.

[39] Gong W, Fan L, Yang J, Xu L, Yao F: [Analysis on polymorphism in exons 2,3 and 4 of the MICA gene in three different Chinese populations]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2002;19:336.

[40] Middleton D, Williams F, Meenagh A, Daar AS, Gorodezky C, Hammond Met al. : Analysis of the distribution of HLA-A alleles in populations from five continents. Hum Immunol 2000;61:1048.

[41] Williams F, Meenagh A, Darke C, Acosta A, Daar AS, Gorodezky Cet al. : Analysis of the distribution of HLA-B alleles in populations from five continents. Hum Immunol 2001;62:645.

[42] Klitz W, Maiers M, Spellman S, Baxter-Lowe LA, Schmeckpeper B, Williams TMet al. : New HLA haplotype frequency reference standards: high-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans. Tissue Antigens 2003;62:296.

[43] Crespi C, Mila J, Martinez-Pomar N, Etxagibel A, Munoz-Saa I, Priego Det al. : HLA polymorphism in a Majorcan population of Jewish descent: comparison with Majorca, Minorca, Ibiza (Balearic Islands) and other Jewish communities. Tissue Antigens 2002;60:282.

[44] Sulcebe G, Sanchez-Mazas A, Tiercy JM, Shyti E, Mone I, Ylli Zet al. : HLA allele and haplotype frequencies in the Albanian population and their relationship with the other European populations. Int J Immunogenet 2009;36:337.

[45] Zhang Y, Lazaro AM, Lavingia B, Stastny P: Typing for all known MICA alleles by group-specific PCR and SSOP. Hum Immunol 2001;62:620.

[46] Cambra A, Munoz-Saa I, Crespi C, Serra A, Etxagibel A, Matamoros Net al. : MICA-HLA-B haplotype diversity and linkage disequilibrium in a population of Jewish descent from Majorca (the Balearic Islands). Hum Immunol 2009;70:513.

Table 1 - MICA allele frequencies for renal-transplant candidates and comparisons among ethnic groups.

HLA	fa TOTAL	fa C	fa M	fa B	fa O	C X M		C X B		C X O		M X B		M X O		B X O	
						p-value	OR(CI95%)	p-value	OR(CI95%)	p-value	OR(CI95%)	p-value	OR(CI95%)	p-value	OR(CI95%)	p-value	OR(CI95%)
MICA*001	0.013	0.015	0.010	0.01	-	ns		ns		ns		ns		ns		ns	
MICA*002	0.170	0.158	0.173	0.21	0.166	ns		ns		ns		ns		ns		ns	
MICA*004	0.148	0.134	0.178	0.19	-	ns		ns		ns		ns		ns		ns	
MICA*006	0.005	0.005	-	-	-	ns		ns		ns		-	-	-	-	-	-
MICA*007	0.024	0.023	0.025	0.03	-	ns		ns		ns		ns		ns		ns	
MICA*008	0.216	0.230	0.183	0.26	0.055	ns		ns		ns		ns		ns		ns	
MICA*009	0.137	0.142	0.147	0.06	0.333	ns		ns		ns		ns		ns		0.049	7.6 (1.7;33.9)
MICA*010	0.066	0.055	0.076	0.04	0.333	ns		ns		0.010	8.4 (2.4;27.3)	ns		ns		0.014	11.6 (2.4;64.3)
MICA*011	0.030	0.031	0.030	0.03	-	ns		ns		ns		ns		ns		ns	
MICA*012	0. 013	0.010	0.020	-	0.055	ns		ns		ns		ns		ns		ns	
MICA*015	0.010	0.007	0.015	0.01	-	ns		ns		ns		ns		ns		ns	
MICA*016	0.033	0.034	0.030	0,04	-	ns		ns		ns		ns		ns		ns	
MICA*017	0.020	0.021	0,020	0.02	-	ns		ns		ns		ns		ns		ns	
MICA*018	0.046	0.060	0.035	0.02	-	ns		ns		ns		ns		ns		ns	
MICA*027	0.056	0.060	0.045	-	0.055	ns		ns		ns		ns		ns		ns	
MICA*044	0.001	-	0.005	0.06	-	ns		ns	-	-	-	ns		ns		ns	
MICA*045	0.001	0.002	-	-	-	ns		ns		ns		-	-	-	-	-	-
MICA*046	0.001	-	-	0.01	-	-	-	ns		-	-	ns		-	-	ns	
MICA*052	0.002	0.002	-	0.01	-	ns		ns		ns		ns		-	-	ns	

fa, allele frequencies; C, Caucasian; M, mestizo; B, black; O, oriental; OR, Odds ratio; CI, Confidence Interval;

ns – not significant; -. Did not occur in this group.

Table 2 - HLA class I (-A, -B and -C) allele frequencies for renal-transplant candidates and comparisons among ethnic groups.

HLA	fa TOTAL	fa C	fa M	fa B	fa O	C X M		C X B		C X O		M X B		M X O		B X O	
						p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)
A*01	0.101	0.111	0.096	0.09	-	ns		ns		ns		ns		ns		ns	
A*02	0.255	0.253	0.290	0.2	0.222	ns		ns		ns		ns		ns		ns	
A*03	0.098	0.097	0.127	0.06	-	ns		ns		ns		ns		ns		ns	
A*11	0.049	0.044	0.066	0.04	-	ns		ns		ns		ns		ns		ns	
A*23	0.052	0.060	0.035	0.06	-	ns		ns		ns		ns		ns		ns	
A*24	0.108	0.103	0.091	0.09	0,5	ns		ns		<0.01	8.6 (2.8;26.1)	ns		<0.001	9.7 (3.0;31.7)	<0.01	9.8 (2.7;36.9)
A*25	0.023	0.026	0.025	0.01	-	ns		ns		ns		ns		ns		ns	
A*26	0.053	0.047	0.056	0.04	0.222	ns		ns		ns		ns		ns		ns	
A*29	0.034	0.047	0.020	0.02	-	ns		ns		ns		ns		ns		ns	
A*30	0.054	0.044	0.061	0.09	-	ns		ns		ns		ns		ns		ns	
A*31	0.030	0.031	0.025	0.03	0.055	ns		ns		ns		ns		ns		ns	
A*32	0.020	0.026	0.015	0.01	-	ns		ns		ns		ns		ns		ns	
A*33	0.027	0.029	0.010	0.06	-	ns		ns		ns		ns		ns		ns	
A*34	0.010	0.002	0.015	0.03	-	ns		ns		ns		ns		ns		ns	
A*36	0.004	-	0.010	0.01	-	ns		ns		-	-	ns		ns		ns	
A*66	0.008	0.007	0.015	-	-	ns		ns		ns		ns		ns		-	-
A*68	0.053	0.050	0.015	0,15	-	ns		0.032	3.3 (1.5;7.2)	ns		<0.001	11.3 (3.1;62.3)	ns		ns	
A*69	0.001	0.002	-	-	-	ns		ns		ns		-	-	-	-	ns	
A*74	0.010	0.005	0.020	0,01	-	ns		ns		ns		ns		ns		ns	
A*80	0.002	0.005	-	-	-	ns		ns		ns		-	-	-	-	-	-
B*07	0.065	0.068	0.051	0.09	-	ns		ns		ns		ns		ns		ns	
B*08	0.059	0.079	0.030	0.05	-	ns		ns		ns		ns		ns		ns	
B*13	0.015	0.013	0.025	0.01	-	ns		ns		ns		ns		ns		ns	
B*14	0.031	0.034	0.030	0.03	-	ns		ns		ns		ns		ns		ns	
B*15	0.085	0.068	0.096	0.09	0.277	ns		ns		ns		ns		ns		ns	
B*18	0.049	0.066	0.035	0.02	-	ns		ns		ns		ns		ns		ns	
B*27	0.030	0.034	0.030	0.02	-	ns		ns		ns		ns		ns		ns	
B*35	0.105	0.105	0.107	0.1	0.111	ns		ns		ns		ns		ns		ns	
B*37	0.013	0.018	0.005	0.01	-	ns		ns		ns		ns		ns		ns	
B*38	0.033	0.031	0.035	0.03	0.055	ns		ns		ns		ns		ns		ns	
B*39	0.034	0.031	0.025	0.07	-	ns		ns		ns		ns		ns		ns	
B*40	0.052	0.044	0.045	0.08	0.111	ns		ns		ns		ns		ns		ns	
B*41	0.018	0.021	0.015	0.02	-	ns		ns		ns		ns		ns		ns	
B*42	0.021	0.007	0.040	0.04	-	ns		ns		ns		ns		ns		ns	

B*44	0.099	0,103	0.096	0.11	-	ns		ns		ns		ns		ns		
B*45	0.020	0.021	0.025	0.01	-	ns		ns		ns		ns		ns		
B*46	0.001	-	-	-	0.055	-	-	-	-	ns		-	-	ns		ns		
B*47	0.001	0.002	-	-	-	ns		ns		ns		-	-	-	-	-	-	
B*48	0.004	0.002	0.005	0.01	-	ns		ns		ns		ns		ns		ns		
B*49	0.031	0.039	0.025	0.02	-	ns		ns		ns		ns		ns		ns		
B*50	0.024	0.021	0.040	0.01	-	ns		ns		ns		ns		ns		ns		
B*51	0.096	0.105	0.102	0.05	0.111	ns		ns		ns		ns		ns		ns		
B*52	0.020	0.013	0.020	0.01	0.222	ns		ns		<0.01	20.8 (3.7;108.9)	ns		ns		0.049	26.9 (2.4;1392.9)	
B*53	0.021	0.015	0.020	0.05	-	ns		ns		ns		ns		ns		ns		
B*54	0.002	0.002	-	-	0.055	ns		ns		ns		-	-	ns		ns		
B*55	0.010	0.005	0.020	0.01	-	ns		ns		ns		ns		ns		ns		
B*56	0.001	0.002	-	-	-	ns		ns		ns		-	-	-	-	-	-	
B*57	0.026	0.021	0.035	0.03	-	ns		ns		ns		ns		ns		ns		
B*58	0.021	0.015	0.030	0.03	-	ns		ns		ns		ns		ns		ns		
C*01	0.037	0.037	0.040	0.01	0.166	ns		ns		ns		ns		ns		ns		
C*02	0.066	0.063	0.076	0.07	-	ns		ns		ns		ns		ns		ns		
C*03	0.095	0.066	0.107	0,14	0.333	ns		ns		0.017	7.0 (2.0;22.3)	ns		ns		ns		
C*04	0.147	0.153	0.147	0.14	0.055	ns		ns		ns		ns		ns		ns		
C*05	0.041	0.042	0.040	0.05	-	ns		ns		ns		ns		ns		ns		
C*06	0.091	0.092	0.112	0.06	-	ns		ns		ns		ns		ns		ns		
C*07	0.225	0.275	0.158	0.19	0.111	0.025	2.0 (1.3;3.3)	ns		ns		ns		ns		ns		
C*08	0.033	0.034	0.020	0.05	0.055	ns		ns		ns		ns		ns		ns		
C*12	0.095	0.100	0.076	0.09	0.222	ns		ns		ns		ns		ns		ns		
C*14	0.026	0.021	0.035	0.02	0.055	ns		ns		ns		ns		ns		ns		
C*15	0.052	0.050	0.056	0.06	-	ns		ns		ns		ns		ns		ns		
C*16	0.046	0.042	0.056	0.05	-	ns		ns		ns		ns		ns		ns		
C*17	0.037	0.021	0.061	0.06	-	ns		ns		ns		ns		ns		ns		
C*18	0.004	-	0.010	0.01	-	ns		ns		-	-	ns		ns		ns		

fa, allele frequencies; C, Caucasian; M, mestizo; B, black; O, oriental; OR, Odds ratio; CI, Confidence Interval; ns – not significant; -. Did not occur in this group.

Table 3 - HLA class II (-DRB1, -DQA1 and -DQB1) for renal-transplant candidates and comparisons among ethnic groups.

HLA	fa TOTAL	fa C	fa M	fa B	fa O	C X M		C X B		C X O		M X B		M X O		B X O	
						p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)	p-value	OR(IC95%)
DRB1*01	0.104	0.095	0.112	0.14	-	ns		ns		ns		ns		ns		ns	
DRB1*03	0.108	0.116	0.096	0.12	-	ns		ns		ns		ns		ns		ns	
DRB1*04	0.132	0.132	0.117	0.15	0.222	ns		ns		ns		ns		ns		ns	
DRB1*07	0.114	0.140	0.091	0.08	-	ns		ns		ns		ns		ns		ns	
DRB1*08	0.050	0.042	0.051	0.08	0.055	ns		ns		ns		ns		ns		ns	
DRB1*09	0.015	0.010	0.010	0.01	0.222	ns		ns		0.002	25.9 (4.4;155.4)	ns		0.006	26.6 (3.5;316.1)	0.023	26.9 (2.4;1392.9)
DRB1*10	0.021	0.026	0.020	0.01	-	ns		ns		ns		ns		ns		ns	
DRB1*11	0.130	0.132	0.137	0.13	-	ns		ns		ns		ns		ns		ns	
DRB1*12	0.010	0.002	0.025	0.01	-	ns		ns		ns		ns		ns		ns	
DRB1*13	0.119	0.126	0.137	0.08	-	ns		ns		ns		ns		ns		ns	
DRB1*14	0.046	0.044	0.040	0.04	0.166	ns		ns		ns		ns		ns		ns	
DRB1*15	0.104	0.087	0.107	0.12	0.333	ns		ns		ns		ns		ns		ns	
DRB1*16	0.041	0.042	0.051	0.03	-	ns		ns		ns		ns		ns		ns	
DQA1*01	0.407	0.394	0.433	0.39	0.5	ns		ns		ns		ns		ns		ns	
DQA1*02	0.119	0.142	0.102	0.09	-	ns		ns		ns		ns		ns		ns	
DQA1*03	0.144	0.129	0.137	0.17	0.388	ns		ns		0.043	4.2 (1.3;12.7)	ns		ns		ns	
DQA1*04	0.053	0.044	0.066	0.07	-	ns		ns		ns		ns		ns		ns	
DQA1*05	0.264	0.277	0.255	0.26	0.111	ns		ns		ns		ns		ns		ns	
DQA1*06	0.010	0.010	0.005	0.02	-	ns		ns		ns		ns		ns		ns	
DQB1*02	0.199	0.235	0.173	0.15	-	ns		ns		ns		ns		ns		ns	
DQB1*03	0.333	0.317	0.321	0.4	0.444	ns		ns		ns		ns		ns		ns	
DQB1*04	0.065	0.058	0.071	0.06	0.166	ns		ns		ns		ns		ns		ns	
DQB1*05	0.202	0.193	0.224	0.22	0.055	ns		ns		ns		ns		ns		ns	
DQB1*06	0.199	0.195	0.209	0.17	0.333	ns		ns		ns		ns		ns		ns	

fa, allele frequencies; C, Caucasian; M, mestizo; B, black; O, oriental; OR, Odds ratio; CI, Confidence Interval; ns – not significant; -. Did not occur in this group.

Table 4 - The most common HLA-A-C-B-MICA-DRB1-DQA1-DQB1 haplotype in renal-transplant candidates and in the different ethnic groups.

Haplotypes HLA-A-C-B-MICA-DRB1-DQA1-DQB1							f Total	f Caucasian	f Mestizo	f Black	f Oriental
*A*01*	*C*07*	*B*08*	*MICA*08*	*DRB1*03*	*DQA1*05*	*DQB1*02*	0.024	0.029	0.010	0.040	-
*A*26*	*C*12*	*B*38*	*MICA*02*	*DRB1*13*	*DQA1*01*	*DQB1*06*	0.008	0.007	-	-	-
*A*02*	*C*04*	*B*35*	*MICA*16*	*DRB1*03*	*DQA1*05*	*DQB1*02*	0.007	0.007	-	-	-
*A*02*	*C*07*	*B*07*	*MICA*08*	*DRB1*01*	*DQA1*01*	*DQB1*05*	0.007	0.005	0.005	-	-
*A*24*	*C*12*	*B*52*	*MICA*09*	*DRB1*15*	*DQA1*01*	*DQB1*06*	0.007	-	-	-	0.222
*A*24*	*C*07*	*B*08*	*MICA*08*	*DRB1*03*	*DQA1*05*	*DQB1*02*	0.007	0.005	-	-	-
*A*03*	*C*07*	*B*07*	*MICA*08*	*DRB1*15*	*DQA1*01*	*DQB1*06*	0.007	0.007	-	-	-
*A*33*	*C*08*	*B*14*	*MICA*11*	*DRB1*01*	*DQA1*01*	*DQB1*05*	0.007	0.005	-	-	-
*A*68*	*C*03*	*B*40*	*MICA*27*	*DRB1*04*	*DQA1*03*	*DQB1*03*	0.007	0.002	-	0.010	-
*A*11*	*C*04*	*B*35*	*MICA*02*	*DRB1*01*	*DQA1*01*	*DQB1*05*	0.007	0.010	-	-	-

-. Did not occur in this group.

3. CAPÍTULO III

3.1. CONCLUSÕES

Os alelos HLA classe I e classe II dos pacientes com doença renal crônica foram determinados, sendo os mais frequentes: HLA-A*02, B*35, C*07, DRB1*04, DQA1*01 e DQB1*03, alelos comuns em outros estudos com populações européias.

Quanto a frequência dos alelos MICA, o MICA*008 foi o mais comum na população investigada e em outros povos pelo mundo.

Todos os alelos estudados estavam em equilíbrio de Hardy-Weinberg com excessão do HLA-B, sugerindo interferência do tipo de amostra.

A comparação global dos alelos MICA, entre indivíduos com DRC e indivíduos saudáveis da mesma região, apresentou diferença estatisticamente significativa, sendo o MICA*019 mais frequente nestes o MICA*027 naqueles. A mesma comparação realizada com HLA-B não apresentou diferença. O haplótipo MICA*009-HLA-B*51 foi o mais comum nos dois gupos. Como esperado devido à proximidade do loci MICA e HLA-B, a maioria dos haplótipos mostraram forte desequilíbrio de ligação. O conhecimento do perfil desses marcadores em pacientes com DRC, potenciais candidatos ao transplante de uma região e sua comparação com indivíduos saudáveis pode favorecer um melhor entendimento da relação entre antígenos do MHC e desenvolvimento da doença renal.

Nesse trabalho foram comparadas as frequências alélicas e haplotípicas HLA e MICA entre os grupos étnicos. Foi demonstrado uma contribuição étnica nas amostras analisadas, com evidências de que a população brasileira é formada por uma mistura de etnias, e que a contribuição HLA e MICA é determinada pela etnia predominante de cada região, uma vez que os alelos de origem européia se apresentaram em maior número que os demais (HLA-A*01- C*07-B*08-MICA*008-DRB1*03-DQA1*05-DQB1*02). No entanto, foi encontrado uma série de outros grupos alélicos, que podem resultar da contribuição de alelos de diferentes populações ameríndias ou européias, africanas e asiáticas que colonizaram a região.

Nossos dados podem ser úteis como referência clínica preliminar para melhor compreensão dos mecanismos envolvidos na rejeição de aloenxertos associados aos polimorfismos HLA e MICA na população brasileira, além de contribuir com parâmetros epidemiológicos no planejamento de distribuição de órgãos, de acordo com o perfil genético de cada região.

3.2.PERSPECTIVAS FUTURAS

O antígeno MICA, desde seu primeiro relato, tem sido alvo de constantes estudos e discussões. Isso se deve por ser uma molécula que carrega fortes indícios de associação na patogênese de diversas doenças, imunogenicidade e indução de aloanticorpos em transplantes, presença chave em células cancerígenas e outras diversas áreas relevantes para a evolução da espécie humana. Em virtude dessas características, sua participação no desenvolvimento da doença renal precisa ser ainda melhor investigado.

O conhecimento do polimorfismo HLA, na população de candidatos ao transplante, poderá contribuir em estudos de associação e suscetibilidade a diversas doenças às quais os pacientes portadores de DRC são acometidos.

O estudo de antígenos de histocompatibilidade, principal e sencundário, na DRC porderá auxiliar na compreensão da doença, melhor seleção no transplante de órgãos e evolução do enxerto.