UNIVERSIDADE FEDERAL DO PARANÁ

MATILDE RISTI

EFEITO DOS POLIMORFISMOS *MICA, NKG2D E HLA-G* E NÍVEIS PLASMÁTICOS DE sMICA E sHLA-G NO PROGNÓSTICO E EPISÓDIOS DE REJEIÇÃO EM PACIENTES TRANSPLANTADOS RENAIS

EFFECT OF *MICA, NKG2D* AND *HLA-G* POLYMORPHISMS AND sMICA AND sHLA-G PLASMA LEVELS ON PROGNOSIS AND REJECTION EPISODES IN RENAL TRANSPLANT PATIENTS

CURITIBA 2017

MATILDE RISTI

EFEITO DOS POLIMORFISMOS MICA, NKG2D E HLA-G E NÍVEIS PLASMÁTICOS DE sMICA E sHLA-G NO PROGNÓSTICO E EPISÓDIOS DE REJEIÇÃO EM PACIENTES TRANSPLANTADOS RENAIS

:EFFECT OF *MICA, NKG2D* AND *HLA-G* POLYMORPHISMS AND sMICA AND sHLA-G PLASMA LEVELS ON PROGNOSIS AND REJECTION EPISODES IN RENAL TRANSPLANT PATIENTS

Tese apresentada ao Programa de Pós-Graduação em Genética da Universidade Federal do Paraná, como requisito parcial para a obtenção grau acadêmico de Doutor.

Orientadora: Profª. Drª. Maria da Graça Bicalho
Corientadora: Profª. Drª. Sueli Borrelli

CURITIBA 2017

ERRATA:

RISTI, M., **EFEITO DOS POLIMORFISMOS *MICA, NKG2D* E *HLA-G* E NÍVEIS PLASMÁTICOS DE sMICA E sHLA-G NO PROGNÓSTICO E EPISÓDIOS DE REJEIÇÃO EM PACIENTES TRANSPLANTADOS RENAIS**: EFFECT OF *MICA, NKG2D* AND *HLA-G* POLYMORPHISMS AND sMICA AND sHLA-G PLASMA LEVELS ON PROGNOSIS AND REJECTION EPISODES IN RENAL TRANSPLANT PATIENTS. 2017. Tese de doutorado (Doutorado em Genética), Universidade Federal do Paraná, Curitiba, 2017.

Errata			
Folha	Linha	Onde se lê	Leia-se
5	3,4,5	**EFEITO DOS POLIMORFISMOS MICA-129 E MICA A5.1 E NÍVEIS PLASMÁTICOS DE sMICA NO PROGNÓSTICO E EPISÓDIOS DE REJEIÇÃO EM PACIENTES TRANSPLANTADOS RENAIS** : EFFECT OF MICA-129 AND MICA A5.1 POLYMORPHISMS AND sMICA PLASMA LEVELS ON PROGNOSIS AND REJECTION EPISODES IN RENAL TRANSPLANT PATIENTS	**EFEITO DOS POLIMORFISMOS MICA, NKG2D E HLA-G E NÍVEIS PLASMÁTICOS DE sMICA E sHLA-G NO PROGNÓSTICO E EPISÓDIOS DE REJEIÇÃO EM PACIENTES TRANSPLANTADOS RENAIS**:EFFECT OF *MICA, NKG2D* AND *HLA-G* POLYMORPHISMS AND sMICA AND sHLA-G PLASMA LEVELS ON PROGNOSIS AND REJECTION EPISODES IN RENAL TRANSPLANT PATIENTS

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
Setor CIÊNCIAS BIOLÓGICAS
Programa de Pós-Graduação GENÉTICA

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em GENÉTICA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de MATILDE RISTI intitulada: Efeito dos polimorfismos MICA-129 e MICA A5.1 e níveis plasmáticos de sMICA no prognóstico e episódios de rejeição em pacientes transplantados renais, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 31 de Agosto de 2017.

RENATE VON LINSINGEN
Avaliador Externo

ENILZE MARIA DE SOUZA FONSECA RIBEIRO
Avaliador Interno

Lupe Furtado Alle
Coordenadora do PPG-GEN/UFPR
Matrícula 200309

ALEXANDRA CRISTINA SENEGAGLIA
Avaliador Externo

ANGELICA BEATE WINTER BOLDT*
Avaliador Interno

MARIA DA GRAÇA BICALHO
Presidente da Banca Examinadora

* A membro da banca Angelica Beate Winter Boldt participou via videoconferência

I dedicate this work to my family and my boyfriend, for always supporting me and encouraging my studies and my professional growth.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my advisor prof. Maria da Graça Bicalho who welcomed me in the LIGH laboratory, allowing me to develop my project. I would also like to thank Prof. Maria Luiza Petzl-Erler and Prof. Enilze Ribeiro for encouraging me and always giving me constructive criticism. I'm further extending my gratitude to Prof. Ana Claudia Bonatto and Prof. Valeria Sperandio Roxo for helping me and giving me useful suggestions, and to Prof. Sueli Borelli for being an incredibly generous host in Maringà and for giving me the opportunity to extend our research in her laboratory.

I would like to thank Dr. Eduardo Antonio for the help provided by his laboratory.

My sincere thanks also go to Dr. Carolina Maria Pozzi and Dr. Fabiana Loss De Contieri, and all the nurses and patients of the Evangelic Hospital of Curitiba who participated in our project.

My deepest thanks are dedicated to all my labmates: my "big sister" Georgia, my "little unicorn" Bruna, Samuel, Renata, Suelen, Soraya, Rafaela, Kadu, Juliana, Mariana, Michelle, Ana Claudia and all those who work in LIGH. Vanessa earned a special place in my heart for her role as a colleague but, most of all, as a true friend.

I am grateful to this country and the UFPR University that welcomed me and it allowed me to study and know beautiful people.

And finally, last but by no means least, I am also grateful to my family: my aunt and uncle who gave me hospitality here in Curitiba, my granny for her never-ending love and care, my cousins and their children for support me, my cousin's wife Kate who always helped me with love, English lessons and biological advices, and my patient, caring and supportive boyfriend Steve.

All of this wouldn't have been possible if it wasn't for my family!

RESUMO

O rim é o órgão mais frequentemente transplantado no mundo. O transplante renal está associado a melhorias significativas na qualidade de vida e na longevidade de pacientes com falência irreversível desse órgão.

Mesmo havendo histocompatibilidade HLA entre paciente e doador, terapia com imunossupressores é ainda necessária para minimizar os efeitos de anticorpos que teriam como alvo imune antígenos de histocompatibilidade secundários (MiHAs) presentes no órgão transplantado e que poderiam desencadear a rejeição e perda de enxerto. Pouco se conhece sobre o papel destes antígenos MiHAS, especialmente a molécula MICA, como indutor e alvo de resposta imune. Portanto, foi realizado uma revisão sobre o papel de MICA e anticorpos anti-MICA no transplante renal. A continuidade deste estudo se deu com a investigação de MICA e HLA-G, ambas moléculas com função imunomoduladora antagônica na resposta imune. MICA tem sido considerado como um MiHAs e potencial alvo de resposta imune mediada por anticorpos, enquanto HLA-G é descrito como um inibidor da resposta imune. Genótipos *MICA*, *NKG2D* e *HLA*-G de pacientes transplantados foram avaliados. Nosso estudo teve como principal objetivo desenvolver um Modelo de Pontuação de Risco (MPR) para avaliar pacientes que apresentam um risco maior ou menor de desenvolver a disfunção de aloenxerto renal. Outros objetivos foram: avaliar a relevância de genótipos e fenótipos de *MICA* (sMICA) e *HLA-G* (sHLA-G) para o prognóstico dos pacientes transplantados e comparar os níveis solúveis de sHLA-G e sMICA entre os grupos de pacientes transplantados (n=67), pacientes renais crônicos (n=32) e controles saudáveis (n=79). As amostras sanguíneas para análise de plasma foram coletadas no pré-transplante e até três meses após o transplante renal. Utilizou-se a técnica ELISA para avaliação dos níveis plasmáticos de sMICA e sHLA-G. As genotipagens de *MICA*, *NKG2D* e *HLA*-G foram realizadas por PCR-SSOP, RT-PCR e SBT, respetivamente. Esses resultados foram relacionados com 40 variáveis clínicas obtidas dos pacientes transplantados. Dos pacientes renais crônicos e controles saudáveis foram avaliados os genótipos e os níveis de sHLA-G e sMICA, os quais foram comparados com aqueles dos pacientes transplantados. Na construção do MPR estruturado a partir de todas as variáveis não invasivas (demográficas, clínicas, genéticas), aquelas que se mostraram mais fortemente associadas ao risco de disfunção do aloenxerto renal foram: presença de anticorpos HLA doador específico (DSAs), transplantes e transfusões sanguíneas precedentes ao transplante monitorado, abortos prévios, uso de ATG em terapia de indução, gênero masculino e idade dos doadores superior à 55 anos. Interessantemente, na criação do MPR os genótipos *MICA* e *HLA*-G não se mostraram como variáveis relevantes para o aprimoramento do modelo. No entanto, o papel antagônico anteriormente hipotetizado, entre MICA e HLA-G foi evidenciado quando genótipos portadores do alelo MICA A5.1 mostraram-se associados a fenótipos alto produtores de sMICA e baixa de sHLA-G, enquanto aqueles genótipos portadores de HLA-G*01:04P mostraram- se associados com a alta produção de sHLA-G e baixa de sMICA. Estes achados demonstram a presença de potenciais genótipos-fenótipos MICA-HLA-G característicos a serem encontrados em pacientes com maior ou menor risco imunológico em desenvolver rejeição.

Palavras chaves: MICA. HLA-G. NKG2D. transplante renal.

ABSTRACT

The kidney is the most frequently transplanted organ in the world, and this treatment is associated to significant improvements in the quality of life and longevity of patients with end-stage renal disease. Even when there's HLA histocompatibility between patient and donor, a standard immunosuppressive therapy is still needed to reduce the antibody effects that target the MiHAs (minor histocompatibility antigens) present in the transplanted organ, which could be responsible for rejection. Very little is known on MiHAs' function, especially in regards to the MICA molecule. This is the reason why a review on *MICA* and anti-MICA antibodies in renal transplant was written. This review led to a further research to better understand MICA and HLA-G's roles, two molecules with an antagonist immunoregulatory function. From literature it emerged that MICA has been treated as a MiHA and as a possible target of an antibody-mediated immune response. Meanwhile, HLA-G has been described as an inhibitor of the immune response. In our research, *MICA*, *NKG2D* and *HLA-G* genotypes of kidney transplant patients were taken into account.

The target of our study was to find a Risk Score Model (RSM) to evaluate patients exhibiting higher or lower risk for allograft dysfunction development. Other focus points of our study were: to investigate the role of *MICA* (sMICA) and *HLA-G* (SHLA-G) genotypes and phenotypes in the prognosis of kidney transplanted patients and compare the levels of soluble HLA-G and soluble MICA between groups of transplanted patients (n=67), chronic renal patients (n=32) and control group (n=79). Plasma samples were collected in the time that went from before transplant up to 3 months after transplant. Soluble HLA-G and MICA levels were detected by ELISA, while MICA, NKG2D and HLA-G genotyping was performed by PCR-SSOP, RT-PCR and SBT, respectively.

Chronic renal disease patients and controls were evaluated for sHLA-G and sMICA plasma levels, comparing them with those of transplanted patients. The Risk Score Model is composed of 40 non-invasive variables (demographic, clinical, treatment, genetic) obtained from transplanted patients. The ones which showed to be more strictly correlated to the risk of malfunctions of the transplanted organ were: total donor-specific antibodies (DSA), DSA positive against selected donors, previous transplant, previous blood transfusion and previous abortion, use of ATG in induction therapy, male gender and donors' age higher than 50 years.

It's noteworthy that, during the creation of the RSM, *MICA* and *HLA-G* genotypes didn't appear to be especially relevant variables for the model. Nevertheless, the antagonistic role of MICA and HLA-G has resulted in the association of *MICA-A5.1* genotypes with high production of soluble MICA and a low one of soluble HLA-G, while the *HLA-G*01:04P* genotype was connected with a high production of sHLA-G and a low one of sMICA. These results prove the presence of potentially characteristic MICA-HLA genotypes/phenotypes, which can be found in patients with higher/lower risk of rejection.

Keywords: MICA. HLA-G. NKG2D. Kidney transplant.

INDEX:

1.0 INTRODUCTION

There's a strong relationship between allograft loss and HLA antibodies (TERASAKI, 2003). This isn't the only cause though, as multiple studies have shown that non-HLA antigens can also be involved in graft failure, even when the patients had good HLA matches and were subjected to standard immunosuppressive therapy (BAHRAM et al., 1994).

Solid organ transplantation has been one of the most remarkable and dramatically therapeutic advances in medicine of the past 60 years. This field has progressed initially from what can be accurately called a ‘‘clinical experiment’’ to routine and reliable practice, which has proven to be clinically effective, life-saving and cost effective when compared with non-transplantation management strategies of both chronic and acute end stage organ failures (SAYEGH and CARPENTER, 2004; STARZL, 2000; SUTHANTHIRAN and STROM, 1994). This remarkable evolution stems from a serial confluence of: cultural acceptance; legal and political evolution to facilitate organ donation, procurement and allocation; technical and cognitive advances in organ preservation, surgery, immunology, immunosuppression; and management of infectious diseases. The history of organ transplantation has also been laced with pure serendipitous discoveries, tragic accidents, unfulfilled promises, abandoned paths, and incidents or practices that have produced legal or ethical quandaries. These features all combine to make the field a dynamic work in progress.

1.1.1 HISTORY OF TRANSPLANTATION

By the early twentieth century, successful transplantation of non-visceral tissues such as human skin and cornea had already been reported (CHICK, 1988; ZIRM, 1989). The most important developments in this period include: experimentation with organ transplantation in animal models; attempted (but failed) kidney transplantation in humans; and observational but seminal discoveries pertaining to the timing, clinical manifestations, and immunologic mechanisms of allograft rejection in immunosuppressive naïve recipients (CHICK, 1988).

Rapid advances in experimental and clinical surgical skills, including vascular anastomotic methods from the late nineteenth century into the early twentieth century, forged a knowledge and technical skills base upon which experimental visceral organ transplantation, principally kidney, could be performed in animals (CARREL, 1963). World War I and the Great Depression initially slowed advances in transplantation knowledge and experimentation. The field was reinvigorated with the increased need for skin allografts for severe burns and other battle injuries. Success, though, continued to be limited by rejection (ONO, 2004).

Peter Medawar, a British surgeon who was assigned to investigate the mechanisms of skin allograft rejection, showed that serial full-thickness skin allografts in cattle were rejected more vigorously. He also found that skin grafts between monozygotic (fraternal) twins promptly thrived and were tolerated; this finding supported the concept that allograft rejection was an immunologic phenomenon with the classic immune properties of sensitization, memory and tolerance, all concepts, which have stood to the present day (BILLINGHAM et al., 1953; ONO, 2004). These findings, coupled with subsequent experiments in rabbits that demonstrated that allograft rejection was modified with the administration of corticosteroids harnessed from the adrenal glands, formed the rationale for ultimately targeting the host immune system of allograft recipients (BILLINGHAM et al., 1953).

Peter Bent Brigham Hospital documented the first successful transplantation of a kidney between living identical twin brothers (Ronald and Richard Herrick). The recipient, Richard, had been supported on an artificial kidney machine invented in Holland and modified at the Peter Bent Brigham Hospital. The procedure was both a surgical and immunologic success as the recipient survived 8 years with intact renal allograft function and no evidence of rejection before succumbing to cardiovascular disease (MERRIL, 1960; MERRILL et al., 1956).

In 1960, the first renal transplantation managed with only pharmacologic immunosuppression (cyclophosphamide and methotrexate) was a female recipient of her mother's kidney (GOODWIN et al., 1963). Recovery of the recipient's bone marrow was accompanied by intermittent rejection managed with prednisone; however, the recipient expired after 143 days. In a series of ten kidney transplantations in the early 1960s that were immunosuppressed with either 6-mercaptopurine or azathioprine, there was only one 6 month survivor (MURRAY et al., 1962).

Landmark studies performed by Dr. Thomas Starzl in the early 1960s, while he was at the University of Colorado, showed that very high doses of prednisone (200 mg/d) added to azathioprine were able to reverse renal allograft rejection and induce host tolerance whereby the subsequent required immunosuppressant dose was diminished over time (STARZL et al., 1963).

In 1967, in a remarkable paper titled ''Death After Transplantation'' (HILL et al., 1967), Starzl summarized the outcome of the first 125 organ recipients at the University of Colorado. This patients group was immunosuppressed with a variable combination of irradiation, splenectomy, thymectomy, high-dose corticosteroids, and azathioprine. The first 60 reported deaths demonstrated a remarkably high rate of opportunistic bacterial, fungal, viral and protozoal infections, often multiple, many of which were undetected and untreated ante-mortem. The dominant pathogen was cytomegalovirus, an invasive disease that was present on 50% of autopsies (MURRAY et al., 1962).

Nevertheless, further advances in immunosuppressive pharmacology, customized to solid organ transplantation needs, were taking place. In 1966, the development of polyclonal anti-lymphocyte globulin (ALG) was synthesized from the serum of horses inoculated with human leukocytes. ALG supplanted the limited practice of thoracic duct drainage to achieve lymphocyte depletion and was used only in a minority of kidney and liver recipients as part of a ‘‘triple regimen’’ with steroids and azathioprine. It was the class precursor of future soluble anti-lymphocyte and anti-thymocyte polyclonal and monoclonal preparations, which became valuable adjuncts for the management of refractory rejection and, at some centers, for immunosuppressive induction (STARZL et al., 1963).

1.1.2 TRANSPLANTATION IN BRAZIL

Brazil is a country with over 190 000 000 inhabitants and a public health system managed by the government (SILVA et al., 2011) (IBGE, <http://www.ibge.gov.br>). The state of Paranà (Brazil) in 2016 had 543 kidney transplantations, making it one of Brazil's states with the highest number of kidney transplants per year. In 2015 Brazil was the second largest country in the absolute number of kidney transplants (n = 5648) (ABTO <http://www.abto.org.br>). The still increasing number of transplants performed every year, along with more efficient regulatory and sanitary analysis, organized clinical research programs and reduction in regional performance disparities will eventually further increase the participation of Brazil in trials worldwide (SILVA et al., 2011).

The development of transplant programs in Brazil was linked to the implementation of an integrated system for the treatment of patients with end-stage kidney diseases. From 1976 to 1986, the number of patients undergoing dialysis increased from 500 to 9000 while the number of kidney transplants increased from 729 to 820. This disproportional growth was due to more attractive reimbursements and relative procedure simplicity of dialysis over transplantation. In 1986 the Brazilian Association for Organ and Tissue Transplantation (ABTO) was founded and started taking decisive actions to influence the development of future policies to increase the number of transplants in the country (PEGO-FERNANDES and GARCIA, 2010; SILVA et al., 2011).

In 1997 the National Transplant System (SNT) was created to coordinate and develop the process of recovery and distribution of tissues, organs and parts taken from the human body for therapeutic purposes. SNT is an integrated and decentralized system composed by the National Centre for Notification, Procurement and Distribution of Organs (CNNCDO), located at Brasilia airport, and by state Centres for Notification, Procurement and Distribution of Organs (CNCDO) who organize organ procurement, allocation and waiting lists. Organ and tissue procurement

services (SPOT) and regional hospitals coordinate the procedures of organ procurement and recovery (PEGO-FERNANDES and GARCIA, 2010).

1.2.0 KIDNEY TRANSPLANTATION

Chronic kidney disease (CKD) is associated with debilitating consequences and a reduction in life expectancy, and commonly progresses to end-stage renal disease (ESRD), in which renal replacement therapy is required to prevent death from uremic complications. Dialysis and transplantation are the two treatment options for ESRD. There are mainly two different forms of maintenance dialysis in existence: Haemodialysis (HD) (the patient's blood is taken out of the body through a complex set of tubes, run through a filter called dialyzer, and returned back to the patient) and Peritoneal Dialysis (PD) (a dialysis technique that uses the patient's peritoneum as a filter) (SINNAKIROUCHENAN and HOLLEY, 2011). Compared with dialysis, transplantation is associated with a significant improvement in quality of life and in overall longevity (CURRY, 2004).

1.3.0 TRANSPLANT IMMUNOLOGY

The immune system response to allografting is a multistep process that involves both the adaptive and innate immune systems. In the adaptive immune system, T-cells become activated on recognition of alloantigens presented by donor and host antigen-presenting cells (APCs) (AUCHINCLOSS and SULTAN, 1996). T-cell activation results in the activation and recruitment of other cell types, and in T-cell maturation to effector cells, which induce tissue destruction and the production of cytokines (HALL, 1991).

One of the most important functions of the innate immune system is the activation of the adaptive immune system through a process known as antigen presentation. Allograft injury, induced by the re-perfusion response, initiates an innate immune response by activating innate immune cells (such as donor-derived and recipient-derived toll-like receptor–bearing dendritic cells and innate lymphocytes, natural killer cells, dendritic cells, and macrophages) as well as humoral factors (complement, natural IgM antibodies) (LAND, 2007). These innate immune cells act as inflammatory cells promoting rejection by directly damaging the graft. Alternatively, the acute innate intragraft inflammatory response can initiate and expand the adaptive alloimmune system, because the innate inflammatory cells act as APCs to the different major histocompatibility complex (MHC) antigens. Innate immune cells can also regulate differentiation of T effector cells by the virtue of their cytokine production, thus affecting the nature and strength of the rejection response (LIU and LI, 2010).

Most of the immune targets in an allograft are the polymorphic human leukocyte antigens (HLA) molecules. Class I HLA antigens are expressed in all nucleated cells, such as tubular cells, and interacts with $CD8^+$ T-cells. HLA class II molecules are expressed on activated cells and on APCs. T cells are activated by graft antigens either directly through cross-linking with HLA molecules or more commonly, indirectly, *via* interacting with APCs that are processing donor antigens. This interaction requires the engagement of the cell receptor and accessory activation molecules. T-cell activation results in expression of T-cell activation markers, secretion of cytokines such as interleukin 2 (IL-2), leading to activation and mobilization of $CD4^+$ and $CD8^+$ cells into the graft, and creates the conditions prompting graft infiltration (HEEGER and DINAVAHI, 2012). Activated T-cells can also help B cells, which in response to antigenic stimulation are triggered into differentiation to antibody-producing plasma cells and memory cells (BLOOM et al., 2009). Antibody-mediated rejection (ABMR) is triggered when enough circulating antibodies to allograft antigens are present or produced. Antigen-antibody interaction and deposition in the graft to complements activation and triggers of an inflammatory response is centred on endothelial surfaces of the peritubular and glomerular capillaries (STEGALL et al., 2012). In addition, T-cell activation induces activation of cytokines and other inflammatory mediators, both within the interstitium and around blood vessels. This activation results in up regulation of vascular endothelial adhesion molecules, which attract inflammatory cells into the vascular space. Graft infiltrating cells recognize class I alloantigens on donor tubular cells, whereas vascular endothelial cells express both class I and class II MHC antigens. Inflammatory activation of the endothelium increases the intensity of class II expression and may also accentuate the expression of other alloantigens, such as endothelial-monocyte antigens, and other polymorphic alloantigen systems, which can be particularly important in the evolution of vascular rejection (DELVES and ROITT, 2000).

1.4.0 REJECTION CLASSIFICATION

Based on the timing of its occurrence, rejection was classified as hyperacute, acute, and chronic. Although these terminologies have been replaced by more histologic nomenclature, they still provide a useful guide to the cause and progression of rejection. Hyperacute rejection started immediately after perfusion and was related to the presence of high levels of antidonor antibodies, the deposition of which on the vascular endothelium led to complement activation and intravascular thrombosis. Advances in antibody detection by single antigen beads have almost eliminated this type of rejection. Acute rejection described a rejection usually occurring early after the transplant and characterized by lymphocytic infiltration, whether in the tubules (acute cellular

rejection) or the blood vessels (acute vascular rejection (AVR)) (GABER et al., 1992). Chronic rejection was believed to be related to chronic slow antibody deposition, leading to progressive vascular sclerosis of the allograft (GABER et al., 1992).

1.5.0 HLA (HUMAN LEUKOCYTE ANTIGENS)

The principal antigenic barrier to transplantation is a series of molecules, which are polypeptide products of a closely linked cluster of genes known as the major histocompatibility complex (MHC) or human leukocyte antigens (HLA) in humans. The MHC is polymorphic between individuals and segregates in families in a Mendelian codominant fashion (SUTHANTHIRAN and STROM, 1994). In humans, the HLA is located on the short arm of chromosome 6 and occupies a large segment of DNA, extending about 3500 kilobases (kb) (FIGURE 1) (ABBAS A.K, 2012).

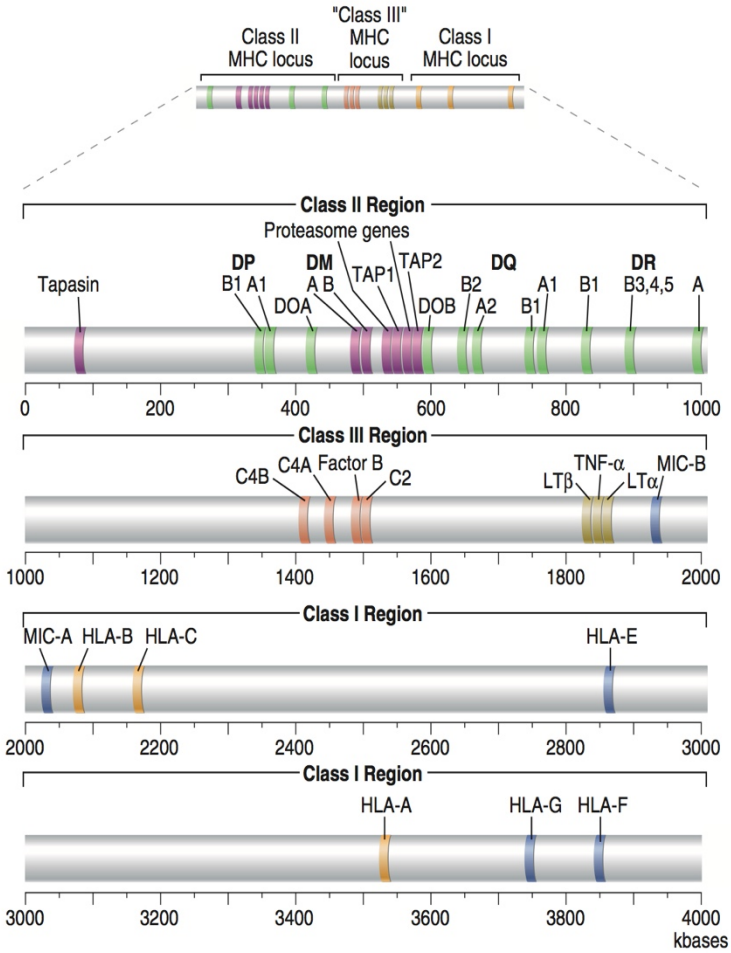


FIGURE 1 - THE GENES LOCATED WITHIN THE HUMAN MHC LOCUS ARE ILLUSTRATED.
SOURCE: (Abbas A.K., 2012)

The classical human class I *HLA* genes were first defined by serologic approach (antibody binding). There are three class I *MHC* genes called *HLA-A*, *HLA-B,* and *HLA-C*, which encode three class I MHC molecules with the same names (ABBAS A.K, 2012). However, another interesting group exists, called non-classical HLA I class, or class Ib molecules. The HLA class Ib antigens, HLA-E, -F and -G, share some characteristics with the class I antigens, but also differ from them in a range of ways (CAROSELLA et al., 1999); in addition, a new family of highly glycosylated MHC-encoded molecules, the MHC class I chain-related (MIC) genes, has been identified. The MIC molecules possess a low degree of homology to other MHC class I encoded genes and interact with both T-cell and natural killer (NK)-cell receptors (BAHRAM, 2000).

Class II *MHC* genes were first identified by use of assays in which T-cells from one individual were activated by cells of another individual. There are three class II *HLA* gene loci called *HLA-DP, HLA-DQ,* and *HLA-DR*. Each class II MHC molecule is composed of a heterodimer of α and β polypeptides, and the DP, DQ, and DR loci each contain separate genes designated A or B, encoding α and β chains, respectively (ABBAS A.K, 2012). Class III *MHC* genes include genes encoding proteins involved in the immune system; the complement genes *C4, C2* and *Bf* (factor B), tumor necrosis factor alpha (*TNF-α*), tumour necrosis factor beta (*TNF-β*) (lymphotoxin) and heat-shock protein (*HSP70*) genes. Genes with no obvious association with the immune system have also been identified in this region. Apart from the complement components, this region also contains two genes coding for the steroid hormone, 21-hydroxylase or CYP21. The *CYP21A* gene is frequently deleted in specific *HLA* haplotypes, particularly the extended haplotype *HLA-A1, B8, DR3, SCO1* in Western Caucasians (MEHRA et al., 2001). This haplotype is known to be associated with insulin-dependent (type 1) diabetes mellitus (MEHRA et al., 2001).

The nomenclature of the HLA locus takes into account the enormous level of polymorphism identified by serologic and molecular methods. Thus, based on modern molecular typing, individual alleles may be called *HLA-A*02:001*, referring to the 01 subtype of *HLA-A2*, or *HLA-DRB1*04:001*, referring to the 01 subtype of the DR4 allele in the B1 gene, and so on (ABBAS A.K, 2012).

1.5.1 HLA-A, HLA-B, HLA-C

HLA-A, B and C molecules are heterodimeric glycoproteins consisting of an MHC-encoded α or heavy chain of about 44kDa and a non MHC-encoded light chain (β_2-microglobulin) of 12 kDa molecular weight (FIGURE 2). The α chain is approximately 350 amino acid residues long and can be divided into three functional regions: external, transmembrane and intracytoplasmic. The extracellular portion of the heavy chain is folded into three globular domains (α_1, α_2 and α_3), each of

which contains stretches of about 90 amino acids encoded by separate exons. While the α_1 and α_2 domains take part in antigen binding (antigen-binding domains), the α_3 domain is essentially conserved. The transmembrane region (23–25 amino acid residues) spans the lipid bilayer of the plasma membrane while the cytoplasmic region (30–32 amino acid residues) contains elements responsible for association with the cytoskeleton (BJORKMAN et al., 1987).

The β_2-microglobulin is a soluble protein encoded in humans by a gene located on chromosome 15; it associates non-covalently with the heavy α chain. Cells lacking β_2-microglobulin are deficient in the expression of MHC class I molecules, indicating its important role in the transport of these molecules to the cell surface. Recently, X-ray crystallography of the HLA-A2 molecule has provided an exciting leap forward in our understanding of the three-dimensional structure of the MHC (BJORKMAN et al., 1987). It has become clear that the membrane-proximal structures (α_3 and β_2-micro-globulin) are folded to form immunoglobulin-like domains, and thus MHC molecules are considered to be part of the immunoglobulin gene superfamily. The α_3 and β_2-microglobulins are responsible for imparting general shape to the class I molecule by providing a platform for top-lying polymorphic domains. Furthermore, the α_3 domain contains binding sites for the α chain of the CD8 glycoprotein, which is important for the recognition of antigens by cytotoxic T-cells. It is now also clear, that the region distal from the membrane is formed by the α_1 and α_2 domains, each consisting of four β strands forming a platform which constitutes the antigen-binding site. Thus, the two α helixes form the sides of a cleft whose floor is formed by a plane of eight antiparallel β-pleated sheets. The dimension of this cleft (2.5 × 1.0 × 1.1 nm) is large enough to accommodate peptides ranging from 10 to 15 amino acid in length, depending on how the peptide is folded (BJORKMAN et al., 1987).

The peptide translocation from the cytosol into the lumen of the ER is accomplished by the transporter associated with antigen processing (TAP). TAP is a member of the ABC transporter family and is a heterodimeric multimembrane-spanning polypeptide consisting of TAP1 and TAP2. The two subunits form a peptide binding site and two ATP binding sites that face the cytosol. TAP binds peptides on the cytoplasmic side and translocates them under ATP consumption into the lumen of the ER. The MHC class I molecule is then, in turn, loaded with peptides in the lumen of the ER (KOOPMANN et al., 2000).

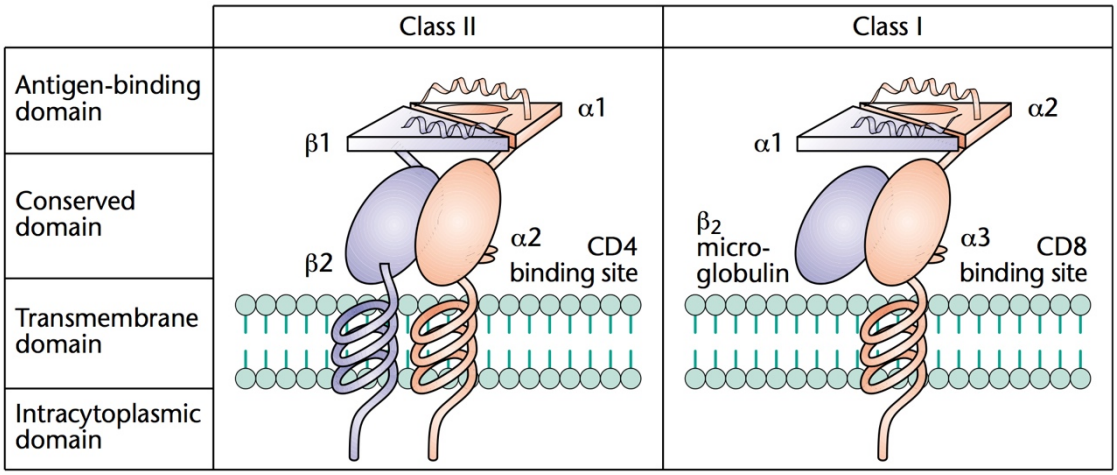


FIGURE 2 - BIOCHEMICAL STRUCTURE AND THREE-DIMENSIONAL FOLDING OF HUMAN LEUCOCYTE ANTIGEN (HLA) CLASS I AND II MOLECULES.
The two antigen-binding domains in class I molecules are contributed by the heavy α chain, whereas both α and β chains contribute the same in the class II molecule.
SOURCE: (MEHRA, 2001).

1.5.2 HLA-DR, HLA-DQ, HLA-DP

Structurally, the class II molecules are similar to class I molecules and are expressed as heterodimers on the cell surface with one heavy α chain (molecular weight 34 kDa) and one β chain (molecular weight 29kDa) of integral membrane glycoproteins (FIGURE 2). Three-dimensional structural differences between the two include an altered position of the immunoglobulin-like β_2 domain relative to that of the α_3 domain of class I HLA, and considerable changes in the peptide-binding site.

The DR region contains multiple, highly polymorphic β genes and only one invariant α gene. The conventional serologically defined DR molecules (DR1–DR18) are coded by the *DRB1* gene, whereas the DR52 and DR53 specificities are encoded by the *DRB3* and *DRB4* genes respectively. *DRB2, DRB6, DRB7, DRB8* and *DRB9* are pseudogenes without a first exon. The DQ subregion contains five genes, *DQA1, DQA2, DQB1, DQB2* and *DQB3*, of which *DQA2, DQB2* and *DQB3* are not known to be expressed. In contrast, both DQA1 and DQB1 are functional and polymorphic, expressing four different types of DQ molecules by different 'cis' and 'trans' combinatorial events (BJORKMAN et al., 1987). The DP subregion contains two α and two β genes, with *DPA2* and *DPB2* being pseudogenes. *DPB1* shows extensive polymorphism, while *DPA1* displays limited polymorphism (BJORKMAN et al., 1987).

1.5.3 HLA-E, HLA-F

HLA-E consists of 8 exons. Of these, the first encodes the leader peptide sequence, exons 2, 3 and 4 encode the MHC immunoglobulin-like α domains 1, 2, and 3, respectively, exon 5 encodes the transmembrane domain and exons 6 and 7 encode the cytoplasmic tail. Similar to HLA-G, HLA-E forms a complex with β2 microglobulin (GRIMSLEY et al., 2002). *HLA-F* consists of 8 exons and is organized in a similar way as *HLA-E*. Thus, the first *HLA-F* exon encodes the leader peptide, exons 2, 3, and 4 the MHC immunoglobulin-like α domains, exon 5 the transmembrane domain and exons 6 and 7 the cytoplasmic tail. Three splicing variants of HLA-F have been described so far, leading to the synthesis of HLA-F isoforms that differ from each other in the length of the cytoplasmic tail. Similar to other HLA molecules, HLA-F can form a complex with β2 microglobulin (COUPEL et al., 2007).

HLA-E is expressed on the surface of the trophoblastic cells (during pregnancy) as well as following organ transplantation, viral infection, inflammatory conditions, and autoimmune diseases. Transcription of HLA-E has been detected in almost all cell types, although expression of the corresponding protein on the cell surface is mainly restricted to endothelial cells, T and B lymphocytes, monocytes, and macrophages. Interestingly, while cells of the immune system express HLA-E at high levels, endothelial cells exhibit reduced levels of HLA-E on their surface, much lower than those of most MHC class Ia genes. Only when endothelial cells are exposed to pro-inflammatory cytokines such as tumor necrosis factor α (TNFα), IL-1β and IFNγ, do they produce and display on their surface increased levels of HLA-E (WADA et al., 2004).

Some evidence indicates that HLA-G could act in concert with HLA-E in immune regulation. Thus, it has been proposed that some HLA-G isoforms stimulate the expression of HLA-E on the cell surface (WADA et al., 2004). HLA-E binds killer cell lectin-like receptor subfamily C, member 1 (KLRC1, an activatory receptor best known as NKG2A) and KLRC2 (an inhibitory receptor best known as NKG2C) on NK cells, T lymphocytes and macrophages (WADA et al., 2004). Interestingly, several groups have observed an interaction between peptide-loaded HLA-E molecules and the T-cell receptor (TCR) of CD8+ T cells, and CD160 (LI et al., 2001).

HLA-F is expressed by peripheral blood B cells, B-cell lines as well as by all tissues containing B cells (adult tonsils, thymus, bladder, skin, fetal liver, and major sites of B-cell development) (Le bouteiller P, 1996). HLA-F has also been detected in embryonic tissues, including the extravillous trophoblasts invading maternal deciduas, and in spermatozoids (LE BOUTEILLER and LENFANT, 1996). The expression of HLA-F by trophoblasts correlates with protection of the fetus from destruction by the maternal immune system, an observation that also applies to HLA-G and -E.

Gobin and co-workers performed a study on non-classical *HLA* promoter regions and found that HLA-F transcription can be induced by IFNγ (GOBIN and VAN DEN ELSEN, 2000).

1.5.4 HLA G

HLA-G gene

HLA-G sequence had 86% structure and sequence similarity with the consensus sequence of classical HLA-class I genes. The main difference resides in the presence of a stop codon in exon 6 (Figure 3A), which generates a shorter HLA-G protein compared with classical HLA-class I molecules (CAROSELLA et al., 2015).

This gene polymorphism is very limited, actually only 54 alleles are listed for HLA-G gene encoding 18 proteins, and 2 of them are null alleles (HLA-ALLES <http://hla.alleles.org/nomenclature/stats.html>).

Large populations studies revealed that the 5'-regulatory region presents a total of 68 haplotypes, 9 of which account for more than 93–95% of all haplotypes found. Prom-1 and Prom-2 (31% and 30%, respectively) are the most represented haplotypes, and belong to the two main lineages (CAROSELLA et al., 2015).

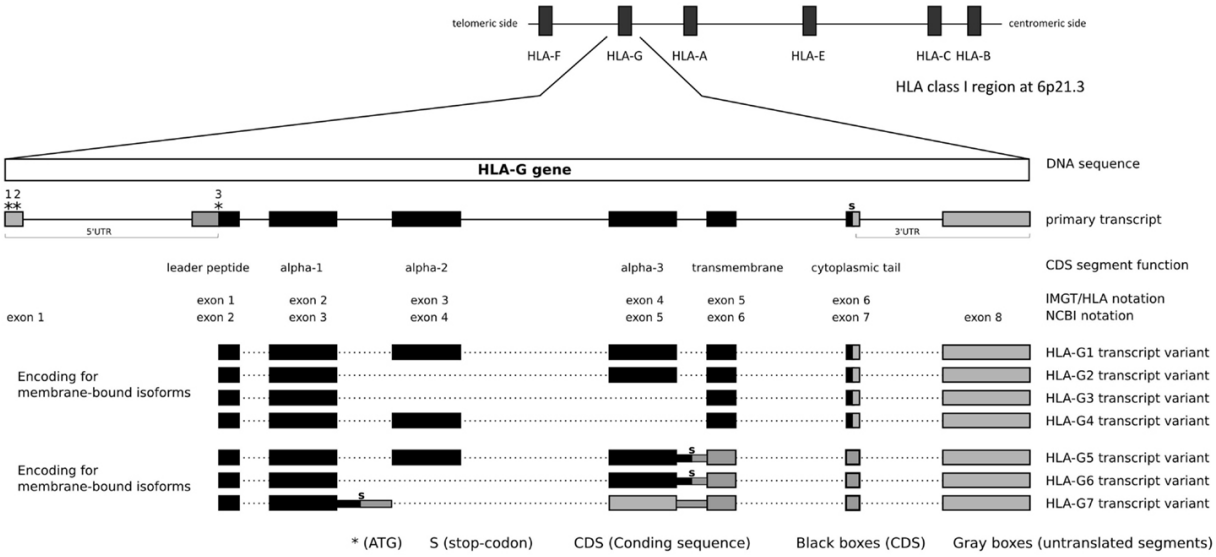


FIGURE 3A - *HLA-G* GENE STRUCTURE AND TRANSCRIPTS.
In this thesis it is considered the IMGT notation.
SOURCE: (CASTELLI et al., 2014)

HLA-G protein

The external part of the structure of the HLA-G molecule consists of three parts: the α_1, α_2 and α_3 domains (encoded by exons 2–4) (figure 3A); α_1 and α_2 contribute to the peptide-binding cleft.

The crystal structure of HLA-G has been determined in 2012 (HOWANGYIN et al., 2012). Interestingly, the candidate binding-site for leukocyte Ig-like receptor 1 (LIR-1 or ILT2) and LIR-2

(or ILT4) inhibitory receptors is on the α_3 domain of HLA-G, and this domain is structurally distinct from the α_3 domain of classical MHC class I molecules. Nevertheless, HLA-G can bind peptides (CLEMENTS et al., 2005).

The alternative splicing of the primary transcript of the gene leads to the production of membrane-bound (mHLA-G) or soluble proteins (sHLA-G), but the HLA-G molecule can also be found in the extracellular vesicle (CAROSELLA et al., 2015). *HLA-G* can be expressed in seven different isoforms, including four membrane-bound (HLA-G1, G2, G3, G4) and three soluble (HLA-G5, G6, G7) proteins, resulting from alternative splicing of the HLA-G primary transcript (FIGURE 3B) (CAROSELLA et al., 2015; CLEMENTS et al., 2005).

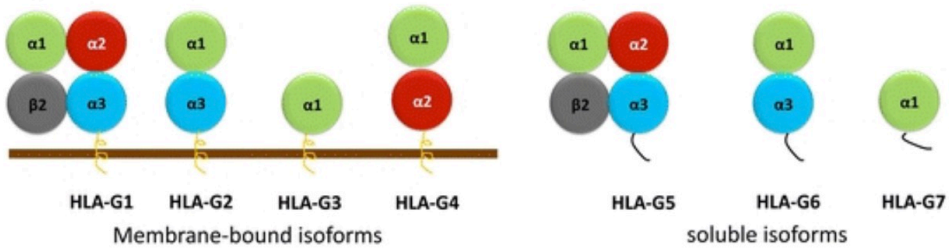


FIGURE 3B - ALTERNATIVE SPLICING OF HLA-G PRIMARY TRANSCRIPT YIELDS 7 ISOFORMS. Excision of one or two exons encoding globular domain generates truncated isoforms, and translation of intron 4 or intron 2 yield secreted isoforms that lack the trans membrane domain.
SOURCE: (DONADI et al., 2011) .

The HLA-G1 isoform encoded by full-length mRNA associates with β_2-microglobulin and has a structure similar to that of classical HLA class I molecules. The HLA-G2 isoform, encoded by a transcript lacking exon 3, was found to be present at the cell surface in association with β_2-microglobulin. Alternatively, this protein might also be present as a truncated molecule not associated with β_2-microglobulin. The HLA-G3 isoform, encoded by a transcript lacking exons 3 and 4, may have a protein structure with only one extracellular domain. The HLA-G4 isoform, encoded by a transcript lacking exon 4, may be present at the cell surface as a two-extracellular domain protein not associated with β_2-microglobulin. The presence of a stop codon in intron 4 deletes the transmembrane domain of the HLA-G5 and -G6 molecule, resulting in the production of soluble proteins that may have a structure similar to the extracellular domains of HLA-G1 and HLA-G2, respectively (HOWANGYIN et al., 2012).

HLA-G molecules can be found as monomers or dimers. The dimers are created by two unpaired cysteine residues (Cys42 and Cys147 in α_1 and α_2 domain respectively) that can form intermolecular disulfide bonds (GONEN-GROSS et al., 2010). HLA-G dimers bind immunoglobulin-like transcript (ILT) receptors with higher affinity and slower dissociation rates than monomers (SHIROISHI et al., 2006). HLA-G heterodimers have not been described yet, but

they may exist since all isoforms can dimerize and all of them interact through cysteine 42 of their α_1 domain (HOWANGYIN et al., 2012).

HLA-G expression

HLA-G molecule can be "high" and "low" expressed, the *HLA-G*01:04:0x* alleles-group had significantly increased sHLA-G levels, followed by the *G*01:01:01:0x* and the *G*01:01:02:0x* groups which were very similar. In contrast, the *G*01:01:03:0x* group or the "null" allele *G*01:05N* presented significantly reduced sHLA-G levels (CAROSELLA et al., 2015). The HLA-G regulatory regions was investigated as well, in particular the HLA-G 3'-UTR, by examining the association of 3'-UTR polymorphic sites with plasma or serum sHLA-G levels. It was found that the UTR-1 was a high producer, while UTR-5 and UTR-7 were lower producers. UTR-2, UTR-3, UTR-4, and UTR-6 have all been classified as medium producers (MARTELLI-PALOMINO et al., 2013). Others studies specifically focused on the 14 bp indel in the HLA-G 3'-UTR and showed associations with the 14 bp insertion and low levels of sHLA-G (MARTELLI-PALOMINO et al., 2013).

Regarding the HLA-G expression at the level of tissues or cells, this molecule was initially identified in cytotrophoblasts, where it acts to protect the fetus from rejection by the maternal immune system (XIAO et al., 2013). The expression of HLA-G is restrictive although HLA-G mRNA has been detected in many different tissues. In general, HLA-G protein expression has been also observed on and by the trophoblast cells in the placenta, on and by certain immune cells (in most cases monocytes), mesenchymal stem cells, erythroid and endothelial precursors cells, in the thymus, cornea, bronchial epithelial cells and pancreas. However, HLA-G protein expression can sometimes be observed in muscle fibers and in liver biliary and renal tubular epithelial cells and HLA-G can be detected in serum/plasma from both women and men (CAROSELLA et al., 2015; HVIID, 2006).

Under pathologic conditions, mHLA-G can be expressed in antigen-presenting cells with activated cytokines, virus infected cells, and in inflammatory diseases. Some studies have reported that the expression of sHLA-G in patients with leukemia was associated with an unfavorable outcome, and that HLA-G^+ tumor cells provide a novel escape mechanism for tumors (HVIID, 2006).

In addition, it was observed that mHLA-G and sHLA-G can be expressed and secreted in the peripheral blood of live kidney transplantation recipients. This result suggests that sHLA-G is the main isoform of HLA-G in the peripheral blood of live kidney transplant recipients, and that it might be directly involved in the acceptance of kidney transplants (HVIID, 2006).

HLA-G ligands

HLA-G inhibits both natural killer (NK) and cytotoxic T lymphocyte (CTL)-mediated cytolysis through binding to inhibitory receptors (HVIID, 2006). The HLA-G molecule can interact with immunoglobulin-like transcript 2 (ILT2 or LILRB1), which is expressed by T and B-lymphocytes, NK cells, monocytes/macrophages, and dendritic cells, and with ILT4 (LILRB2), which is expressed only by myeloid cells (MORANDI et al., 2016). Both ILT2 and ILT4 contain immunoreceptor tyrosine-based inhibitory receptor motifs (ITIMs) in their cytoplasmic tails that inhibit cellular responses by recruiting phosphatases such as SHP-1 (Src-homology 2 domain-containing tyrosine phosphatase 1) (CAROSELLA et al., 2015). In addition HLA-G can interact with KIR2DL4 expressed by NK cells and CD160 expressed by T lymphocytes, NK cells, and endothelial cells (MORANDI et al., 2016). KIR2DL4 is not a classical inhibitory receptor (ADRIAN CABESTRE et al., 1999), authors reported its inhibitory function using cell lines while others reported its activatory one (RAJAGOPALAN, 2010). In fact KIR2DL4, unlike all other KIRs, has both activating and inhibitory signaling domains (MORADI et al., 2015).

The role of HLA-G in reducing graft rejection may be due to its direct interaction with ILT2 and ILT4. The functional consequences of this interactions include the inhibition of cytotoxic activity of $CD8^+$ T cells and NK cells, $CD4^+$ T-cell alloproliferation, cell cycle progression of alloreactive T cells, and APC maturation. Furthermore, HLA-G plays an indirect tolerogenic role by inducing immunosuppressive DCs and T cells (REBMANN et al., 2009).

1.6.0 MIC

Bahram et al. first discovered *MIC* genes in 1994 as a second lineage of mammalian MHC I genes (BAHRAM et al., 1994). The MIC molecules possess a low degree of homology to other MHC class I encoded genes and interact with both T-cell and natural killer (NK)-cell receptors (BAHRAM, 2000). Functional evidence has indicated that MIC molecules, unlike the structurally related MHC class I and CD1 (cluster of differentiation 1), are not associated with peptide or other ligands (STEINLE et al., 2001). The *MIC* gene family consists of seven members (*MICA*−*MICG*) (Figure 4), five of which are pseudogenes, and two, *MICA* and *MICB,* which are functional (COLLINS, 2004; ZWIRNER et al., 2006). *MIC* genes are highly conserved and are present in humans, primates, dogs, goats, cows and pigs, but not in mice or rats (BAHRAM et al., 1994). This absence suggested that *H2-Q*, *-M*, and *-T* loci in mice could be evolutionary counterparts of *MIC* genes, compensating for the absence of *MIC* in the mouse genome (FODIL et al., 1996). A research in 2001 described how the retinoic acid-early (RAE-1) molecule functions as ligand for natural killer group 2 member D ligand (NKG2D), suggesting it could act as *MICA*'s counterpart in mice (STEINLE et al., 2001).

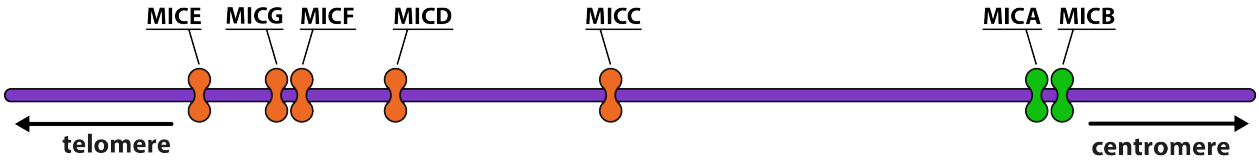


FIGURE 4 - REPRESENTATION OF MIC GENES.
The functional genes are represented in green, the pseudogenes in orange.
SOURCE: (RISTI and BICALHO, 2017)

These two functional genes (*MICA* e *MICB*) in humans share 84% sequence identity (STEINLE et al., 2001) and are polymorphic. In January of 2017 the number of *MICA* alleles reached 106 while MICB has been reported as having 42 alleles (HLA-ALLELES <http://hla.alleles.org/nomenclature/stats.html>).

Some individuals, with *HLA-B*48*-containing haplotypes, have shown evidence of gene deletion in the *MICA* e *MICB* regions (*MICA* e *MICB* null); this demonstrates that this mutation is not lethal (KOMATSU-WAKUI et al., 1999).

The *MIC* genes are transcribed in keratinocytes, endothelial cells, monocytes, fibroblasts, epithelial cell lines and epithelial tissues (BAHRAM, 2000; ZWIRNER et al., 1998). Besides, the MIC protein is only expressed on the cell surface of freshly isolated endothelial cells, fibroblasts (ZWIRNER et al., 1999) and gastric epithelium (GROH et al., 1996), and the gene is not usually transcribed in $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ cells (ZWIRNER et al., 1999).

The MIC protein acts as a ligand for natural killer cells, γδ T cells and αβ $CD8^+$ T cells, which express NKG2D (natural killer group 2 member D ligand) (BAUER et al., 1999).

In terms of evolution of *MICA* and *MICB* genes, it is known that *MICA* diverged into two lineages called LI and LII, and gene duplication of *MICA* LII evolved into *MICB* genes. As noted by Luo *et al.* (LUO et al., 2014), polymorphism of *MICA* promoters is likely to have occurred by point mutations and probably existed before allelic divergence in the coding regions as different alleles can have the same promoter. By contrast, *MICB* promoter and coding diversification may have occurred at the same time (COX et al., 2014; LUO et al., 2014).

1.6.1 MICA GENE AND ALTERNATIVE SPLICING

The *MICA* gene, located 46.4 kilobases centromeric to *HLA−B* on the short (p) arm of chromosome 6 at position 21.33, has a molecular structure similar to *HLA* class I genes (BAHRAM et al., 1994).

MICA has six exons separated by five introns (FIGURE 5): exon 1 encodes one leader peptide, exons 2 to 4 encode three extracellular globular domains, exon 5 encodes one

transmembrane domain and exon 6 encodes a cytoplasmic tail (CHOY and PHIPPS, 2010; ZWIRNER et al., 2006). The first exon is followed by an intron of 6840 bp, which is unusually large for a class I gene. The remainder of the *MICA* gene shows an organization quite similar to that of conventional class I genes, except for the presence of a relatively long intron following the transmembrane exon and the fusion of the cytoplasmic tail and 3 untranslated sequences on exon six (BAHRAM et al., 1996).

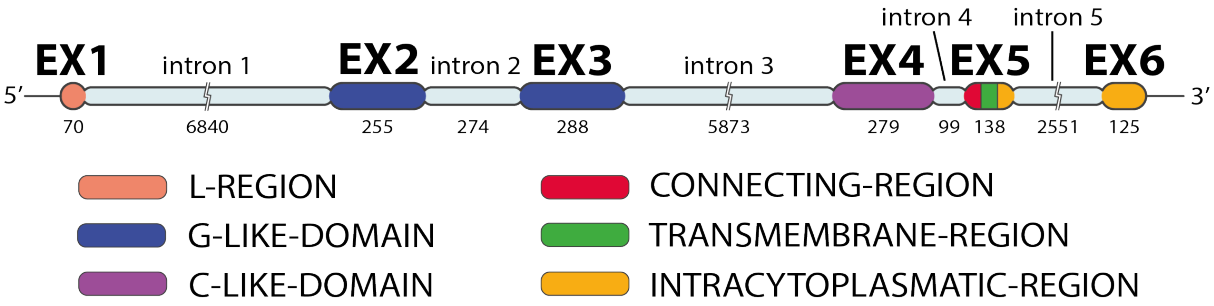


FIGURE 5 - REPRESENTATION OF MICA GENE SHOWING ITS 6 EXONS AND 5 INTRONS.
SOURCE: (RISTI and BICALHO, 2017)

MICA is regulated by a promoter including putative heat shock elements similar to those of *HSP 70* genes, suggesting that the transcription of *MICA* is induced under stress conditions and thereby the MICA protein functions as an indicator of cell stress (GROH et al., 1996; KATSUYAMA et al., 1999). The first intron of the *MICA* gene contains an NFkB-binding site that binds p65 (RelA)/p50 heterodimers and p50/p50 homodimers of the NFkB transcription factor family. The role of the proximal –130-bp NFkB site was reported as necessary and sufficient for transcriptional transactivation of *MICA* in the response to TNFα in primary ECs (CHAUVEAU et al., 2014).

MICA, along with *MICB*, has been shown to differ in the transcriptional control regions from conventional HLA class I genes, as *MICA/B* genes lack the prototypic MHC class I gene regulatory elements composed of the SXY module (LUO et al., 2014).

The *MICA* 5' promoter regions have 12 known possible haplotypes (P1-P12), including a null haplotype (*MICA*-P12) due to a deletion of the entire *MICA* gene (COX et al., 2014; LUO et al., 2014). All of the 12 variable sites were in *linkage disequilibrium* (LD), forming two basic haplotypes represented by Promoter-7 (P7) and Promoter-10 (P10), respectively. P7 appeared to be an ancestral haplotype, Promoter-4 (P4) may have probably arisen from P7 through a cytosine substitution at position 309, while Promoter-9 (P9) appears to be derived from P7 through a single

mutation from cytosine to guanine at position 80; Promoter-10 (P10), the other basic haplotype in MICA 5' promoter, was characterized by a unique insertion of a14 bp sequence at position 41 (LUO et al., 2014). Another study with the IHW (International Histocompatibility Workshop) cells line showed that the most frequent MICA promoter haplotype is *MICA*-P7. Some *MICA* alleles were found to be always associated with MICA-P7, such as *MICA*001*, **009:01* and **010:01*. However, other *MICA* alleles had a more heterogeneous promoter and allele combination. For example, *MICA*002:01* was found to have MICA-P3, P4 or P7 promoters, *MICA*008:01/04* could have MICA-P1, P6 or P7 promoters and *MICA*007:01* had either MICA-P7 or P11 promoter sequences (COX et al., 2014).

A nucleotide substitution of consensus C to G was found within the activator protein 1 (AP-1) transcription factor-binding sites (TFBSs) of *MICA* promoter P5, which was only found in combination with allele *MICA*012:01* in the Cox *et al.* study with IHW (International Histocompatibility Workshop) cells and in Chinese Han subjects (COX et al., 2014; LUO et al., 2014).

Furthermore, the *MICA* gene presents an alternative splicing that leads to the formation of four mRNA isoforms. Two of them are described by Zou *et al*. (MICA isoform 1 and 2) and they did not appear to be tissue specific (ZOU and STASTNY, 2002), the others two are only described on NCBI site (NCBI <https://www.ncbi.nlm.nih.gov>).

1.6.2 MICA polymorphism

Polymorphisms of the *MICA* gene in the exons' region lead to the formation of 106 alleles (HLA-ALLELES <http://hla.alleles.org>), while a variable number of GCT repetitions in the *MICA* exon 5 constitute five different alleles (MICA-A4, A5, A5.1, A6, and A9) (MIZUKI et al., 1997), and lastly MICA Val/Met dimorphism (rs1051792) at nucleotide 454 (G/A) leads to an amino acid substitution from valine (Val) to methionine (Met) at position 129 in the α_2 domain of the MICA protein (ISERNHAGEN et al., 2015; ISERNHAGEN et al., 2016).

MICA polymorphisms in the exons' region:

MICA polymorphism has limited variation across specific racial groups. A single group of three alleles (*MICA*008, MICA*002* and *MICA*004*), for example, accounts for more that 50% of the allele frequencies commonly found in several Caucasoid populations (MARIN et al., 2004; MURO et al., 2012). *MICA*008,* is the most frequent allele in Caucasian, Brazilians, Japanese, Korean, Turkish, Thai, Moroccan, African-American, Chinese Han, Chinese Mongolian and Chinese Tujia populations, whereas *MICA*002* is the most frequent allele in several Amerindian

populations (South Americans Indians) (; KOMATSU-WAKUI et al., 1999; MARIN et al., 2004; MURO et al., 2012; WANG et al., 2016).

The following paragraph will describe in details the most frequent *MICA* alleles for each population.

In a Japanese population study by Katsuyama *et al.*, the most frequent allele was *MICA*008* with the gene frequency of 30.8%. *MICA*009* was the second most frequent (16.5%), while the rarest one was *MICA*007* (1.2%). *MICA* alleles displayed strong LD with HLA-B antigens, *MICA*008* with *HLA-B*07*, *HLA-B*48, HLA-B*60* and *HLA-B*61*; *MICA*009* with *HLA-B*51* and *HLA-B*52*; *MICA*002* with *HLA-B*35, HLA-B*39*, *HLA-B*58* and *HLA-B*67*; *MICA*004* with *HLA-B*44; MICA*007* with *HLA-B*13* and *B*27*; *MICA*010* with *HLA-B*46*, *HLA-B*62* and *HLA-B*48*; *MICA*012* with *HLA-B*54*, *HLA-B*55*, *HLA-B*56* and *HLA-B*59*; *MICA*019* and *HLA-B*70*, *HLA-B*71* and *HLA-B*62*. Tight linkage between *MICA* alleles in extracellular exons and GCT repeat polymorphisms in the TM exon was observed: *MICA*002/A4*, *MICA*004/A6*, *MICA*007/A4*, *MICA*008/A5* or *A5.1*, *MICA*010/A5*, *MICA*011/A6*, *MICA*018*/A4. Among 8 serologically *HLA-B*48* homozygous individuals, four were found to represent this *MICA null* allele as assessed by no polymerase chain reaction (PCR) amplification using MICA-specific primers, while the remaining four possessed the intact *MICA* gene with *MICA*008* or *MICA*010* (KATSUYAMA et al., 1999). In another study on the Japanese population a 100-kb deletion including the *MICA* gene was found. This deletion (*MICA null* allele) has been reported to be associated with *HLA-B*48:01*. Interestingly, this *MICA* deletion is accompanied by a *MICB null* allele, *MICB*009N* (; KOMATSU-WAKUI et al., 1999).

In the Korean population study by Pyo *et al.* the common alleles were *MICA*008* (24.4%), *MICA*010* (18.3%), and *MICA*002* (17.8%). In contrast, the *MICA*007* (3.3%) was relatively infrequent. In the GCT repeat polymorphism, the *A5* allele was the most common in the Korean population, followed by the *A6* allele. In Koreans, as with the Japanese, the *MICA*008* (*MICA*008:01* and *MICA*027*) allele was primarily associated with *HLA-B*60, B*61*, and *B*7* alleles. In Korean population it has also been identified the association between *MICA*008* (*MICA*008:01*) and *HLA-B*13* (PYO et al., 2003). These frequencies are confirmed by subsequent Baek *et al* study with specific primer extension (ASPE) on microarrays (BAEK et al., 2013).

The Chinese population studied by Zhu is the Han population (ZHU et al., 2009), the one studied by Wang is the Mongolian population (WANG et al., 2016) and the one studied by Wang is the Tujia population (WANG et al., 2016; WANG et al., 2016). In the Chinese Han sample fourteen *MICA* alleles were found in the population, with *MICA*008:01/04* having the highest frequency of 27.0%, but its frequency was lower than that in the Caucasian population. *MICA*002* and

*MICA*010* were also found in the Zhejiang Han population, which showed higher frequency than that in the Caucasian population, and the *MICA*049* allele (3.5%) was first identified in the Zhejiang Han population (ZHU et al., 2009). In the Chinese Mongolian population *MICA*008:01* (21.2%), *MICB*005:02* (48.1%) and *HLA-B*51:01* (7.91%) were the most common alleles. *MICA-129 Val* e *MICA-129 Met* percentage was 65,82% and 28,48%, respectively (WANG et al., 2016). In the Chinese Tujia population, *MICA*008:04* (29.41%), *MICA*A5* (29.68%), *MICA A5.1* (29.68%) and *MICB*005:02* (39.57%) were the most frequent ones (WANG et al., 2016; WANG et al., 2016). In this last population *MICB*005:02-MICA*019* (13.10%) and *MICB*002-MICA*008:04* (9.89%) were found to be the most common two-locus haplotypes (WANG et al., 2016; WANG et al., 2016) and in the Chinese Han the frequency distribution of the MICA-STR allele in the Zhejiang Han population was similar to that in the Japanese and Korean and the other Han population, but it was different from the one in the Caucasian population (ZHU et al., 2009). In the Mongolian population, the most frequent haplotypes were *HLA-B*51:01-MICA*009:01* (7.28%), *HLA-B*58:01-MICB*008* (6.96%), *MICA*010-MICB*005:02* (13.92%) and *HLA-B*58:01-MICA*002:01-MICB*008* (6.96%). *HLA-B-MICA* haplotypes such as *HLA-B*50:01-MICA*009:02* were associated with a single *MICB* allele. Some *HLA-B-MICA* haplotypes were associated with multiple *MICB* alleles, including *HLA-B*51:01-MICA*009:01*. Finally, it was confirmed in this population the existence of a recently recognized *MICA* allele, *MICA*073*, whose ethnic origin had not been previously described (WANG et al., 2016).

In the Northeastern Thai population, *MICA*008, *010, *002* and **019* were highly predominant with allele frequencies of 21.4%, 18.2%, 17.6% and 15.3%, respectively. *MICA*010* and **019* are different from *MICA*008* by double- and single-nucleotide substitutions. This could mean they were originally *MICA*008*, and then they it split in to two new alleles that remained in Thai population. Interestingly, *MICA*052*, which is identical to *MICA*007* except for a single-nucleotide substitution at nucleotide position 751 (G to C), was detected only in this population, but not in the Japanese or Caucasian population, while *MICA*007* was not present in the Thai population (ROMPHRUK et al., 2001).

In the North American Caucasian population, *MICA*008* accounted for 291 of the 550 alleles (53%) and hence was the single most common allele in this study's population. The overall frequency of *MICA*002* was 13%, followed by *MICA*004* (7%) and *MICA*010* (5%). *MICA*008* was found in strong LD with four different *HLA-B* alleles: *B*08:01*, *B*07:02*, *B*44:02*, and *B*40:01*. *MICA*004* and **010* were significantly associated with *HLA-B*44:03*. Hence, for at least one HLA-B antigen, *HLA-B*44*, association with MICA appeared to segregate with different *HLA-B* alleles (PETERSDORF et al., 1999).

In the study on the European-Americans and African-Americans population, *MICA*002:01, *004*, and **008:01* were the three most common alleles and the only ones with frequency greater than 10%. Among European-Americans, *MICA*008:01* had the highest allele frequency (43.05%), followed by *MICA*002:01* (14.1%). Instead, looking at the African-Americans, *MICA*002:01, *004*, and **008:01*, account for 25.37%, 19.09%, and 26.61% of the gene frequency, respectively. The two dominant *MICA* alleles **002:01* and **008:01* are both associated with several *HLA-B* alleles. *MICA*002:01* is associated with *HLA-B*35, B*53* and *B*58* related subtypes while *MICA*008:01* is associated with *HLA-B*07, B*08, B*13, B*37, B*40* and *B*44:02* alleles. Both populations share most LD relationships between *MICA* and *HLA-B*, only a smaller proportion of the haplotypes is apparently population specific. *HLA-B*51:01* and *B*52:01,* for example*,* are associated with *MICA*004* in African-Americans, while with *MICA*009:01* (an allele closely related to *MICA*004*) and, to a lesser frequency, with *MICA*010* in European-Americans. In this populations study six individuals who have one copy of *HLA-B*48:01* have been included. Gao *et al.'s* study, with the typing methods used, has shown that *HLA-B*48:01* is associated with the *MICA null* allele (GAO et al., 2006).

Two more studies on African-American populations point out similar frequencies of the most common *MICA* alleles: *MICA*008*, *MICA*004*, and *MICA*002*. Tian *et al.*'s data showed gene frequencies of 28.2%, 26.4%, and 25.5%, respectively, while Zhang *et al.* showed gene frequencies of 26.9%, 18.7% and 27.8% respectively (TIAN et al., 2001; ZHANG et al., 2003).

In Tian *et al.'s* study, *HLA-B*35:01*, *HLA-B*42:01* and *HLA-B*08:01* are exclusively linked to *MICA*002*, *MICA*004* and *MICA*008*, respectively. *HLA-B*07:02* is associated with *MICA*008; B*44:03* with *MICA*004 (*this–allelic associations have been previously reported in Caucasians too); *HLA-B*57:03* with both *MICA*004* and *MICA*008*; *HLA-B*49:01* occurred with both *MICA*002* and *MICA*004*; *HLA-B*52:01* with *MICA*009* and *MICA*004*; *HLA-B*53:01* is associated with *MICA*002* and *MICA*018*; *HLA-B*15:03* with *MICA*008* and *MICA*019*; *HLA-B*18:01* with *MICA*001* and *MICA*018* (TIAN et al., 2001). In Zhang *et al.*'s study *MICA*002:01* has associations with *HLA-B*35, B*53, B*58*, and *B*42*, and *MICA*004* with *HLA-B*42, B*49, B*51, B*52*, and *B*07* (ZHANG et al., 2003). These *MICA-HLA-B* associations suggest that *HLA-B-MICA* combinations on certain haplotypes in African-Americans are more complex than in other populations. Additionally in the Tian *et al* study *HLA-A*30:01-MICA*004* and *MICA*004-HLA-DRB1*03:02* were found to be in LD, and the LD between *HLA-A*01:01* and *MICA*008* was found to be secondary to the strong association between *HLA-A*01:01* and *HLA-B*08:01* (TIAN et al., 2001).

In the Moroccan population, *MICA* allele frequencies were assessed in 82 unrelated healthy individuals from a Moroccan Berber population named Metalsa. *MICA*008:01* was the most frequently allele detected, followed by *MICA*004* and *MICA*009:02*. *MICA*009:02* is associated with *B*50:02-C*06:02*, also found associated to *DRB1*04:06* in this population, where this haplotype was one of the most common. *MICA*004* was mainly associated to *B*44:03:01-C*04:01:01* or *C*02:02*, whereas *MICA*008:01* was associated with *B*44:02-C*05:01* or *B*08:01-C*07:01* (PIANCATELLI et al., 2005).

152 subjects in the Tian *et al.* study have been analyzed, from the Yoruba (n=74), Efik (n=32), and Igbo (n=46) tribes of southern Nigeria, 39 nationwide African-American stem cell donors, and 60 African-American individuals residing in the metropolitan Boston area. *MICA*002:01, *004,* and **008:01* were commonly observed in all groups (sub-Saharan African ancestry), with combined gene frequencies ranging from 71.6 to 80.4%. In three groups (Boston African-American, Yoruba, and Igbo) the most common haplotype was *HLA-B*53:01–MICA*002:01* whereas n the nationwide African-American and Efik populations, the most frequent haplotype was *HLA-B*42:01– MICA*004* and *HLA-B*15:03–MICA*008:01*, respectively. Pairwise comparisons revealed that overall *HLA-B–MICA* haplotype frequencies in the Efik and Igbo tribes were significantly different from each other and from both African-American groups (TIAN et al., 2003).

In the South American Indians population's study by Zhang *et al*. three tribes were analyzed, Toba, Wichi and Terena, while Oliveira *et al.* studied three Guarani groups (Guarani Ñandeva, Guarani Kaiowá, Guarani M'byá) and two Kaingang populations (Rio das Cobras, Ivaí). In all of these populations, with the exception of the Kaingang, the *MICA* locus is dominated by three alleles: *MICA*002:01*, *MICA*027* (external domain sequence like *MICA*008/TM* allele *A5*) and *MICA*010* (OLIVEIRA et al., 2008; ZHANG et al., 2002). For the Kaingang populations, the highest frequencies were observed for *MICA*002:01*, *MICA*010* and MICA*020, representing 84% of the alleles (OLIVEIRA et al., 2008). Zhang *et al.*'s data showed that *MICA*002:01* was the most frequent allele, with a gene frequency of about 35% in Toba, 48% in Wichi and 45% in Terena (ZHANG et al., 2002). Oliveira *et al.*'s study showed that *MICA*002:01* was the most common allele with a frequency of 38% and 64%, respectively in the Guarani Ñandeva and Kaiowá populations, whereas for Guarani M'byá it was *MICA*027* with 40%. In the Kaingang populations *MICA*002:01* was the most frequent in both Rio das Cobras (44%) and Ivaí (36%). Interestingly, *MICA*020*, which differs from *MICA*002:01* only by the transmembrane STR, was almost twice more common in Ivaí (21%) than in Rio das Cobras (12%), compensating the frequency difference of allele *MICA*002:01* (OLIVEIRA et al., 2008). These frequencies are very different than North

American Caucasians. For the Toba, Wichi and Terena populations most of the HLA-B-MICA relationships were absolute except for *MICA*008*, which was associated with two TM genotypes: *A5.1* in *MICA*008:01* and *A5* in *MICA*027*. *MICA*002:01* allele was accompanied by *A9*, which was also associated with *MICA*011*, *MICA*017* and *MICA*047*. Similarly, *MICA*004* was combined with *A6* and the latter was also seen with *MICA*009:01* and *MICA*009:02*. *MICA TM A4* was seen together with *MICA*001*, *MICA*007:01*, *MICA*012:01* and *MICA*018*. *A5* was found in *MICA*010*, *MICA*016* and *MICA*019*, in addition to *MICA*027* (ZHANG et al., 2002). In the Guarani and Kaingang the STR-MICA alleles *A5* and *A9* occurred at high frequencies (>20%) in all five Amerindian populations (OLIVEIRA et al., 2008). In the Toba, several *MICA* alleles have associations with different *HLA-B* alleles: *MICA*002:01* has associations with *B*35:05* and *B*39:03*; *MICA*027 (*008/A5)* with *B*40:02* and *B*48:03*; and *MICA*010* with *B*15:07* and *B*52:01*. In the Wichi tribe, *MICA*027* is associated with both *B*40:02* and *B*40:35* and *MICA*10* has association with different *B*15* group alleles: *B*15:08* and *B*15:20.* And finally *MICA*027 (MICA*008/TM A5)*, which has the same sequence as *MICA*008:01* except for the TM region, has a strong association with *B*40:02* in the Wichi, and with *B*40:02* and *B*48:03* in the Toba. Interestingly, in Toba, Wichi and Terena, some *MICA* alleles have associations with different *HLA-B* group alleles. For example, *MICA*010* is significantly associated with *B*15:07* in the Toba, but with *B*15:08* and *B*15:20* in the Wichi (ZHANG et al., 2002). In the Guarani and Kaingang populations *A5* is associated with *HLA-B*40* and *A9* with *HLA-B*39. HLA-B*51:04*, occurred exclusively with *A5* and probably with *MICA*010* and *HLA-C*15:02:01*. Conversely, MICA*010 and *MICA*002:01* appeared with more than one *HLA-B* allele. In these population the deletion of the *MICA* gene was found in the same haplotype as *HLA-B*48* in all populations (OLIVEIRA et al., 2008).

The subjects of the Brazilian population studies by Marin *et al.* and Ribas *et al.,* were the Brazilian population of São Paulo city and the State of Paraná, respectively (MARIN et al., 2004) (RIBAS et al., 2008). In the São Paulo population, as in most of the other populations, *MICA*008, MICA*002* and *MICA*004* were found to be the most frequent alleles (MARIN et al., 2004), while in the state of Paraná's population, alleles *MICA*008:01*, *MICA*002:01* and *MICA*009:01* were the most common and accounted for more than 55% of the total allelic frequency (RIBAS et al., 2008). In the Marin *et al.* study *MICA*008* was found to be in association with *HLA-B*8, HLA-B*13, HLA-B*37*, *HLA-B*60* and *HLA-B*72*. Nevertheless, due to the low frequency of studied alleles, these values were not significant, but they observed a new haplotype: *MICA*008-HLA-B*37*. Both *MICA*006* and *MICA*009* have been described as being in linkage disequilibrium with *HLA-B*51* (MARIN et al., 2004). Even in the Ribas *et al.* study *MICA*009:01-B*51:01* are

associated and this haplotype shows the highest frequency in this population, followed by *MICA*002:01-B*35* and *MICA*008:01-B*07*. In this study, *MICA*008:01* is associated with *HLA-B*07, B*08, B*44, B*15, B*13, B*40* (from the most frequent to least frequent respectively). *MICA*002:01* is associated with *HLA-B*35, B*39, B*38, B*58*, while *MICA*009:01* is associated with *HLA-B*51*, *B*35 and B*50* (RIBAS et al., 2008).

In conclusion, it's noteworthy that very few *HLA-B* alleles showed multiple associations with *MICA*, including some of the most common *B* alleles, after removing the low-frequency haplotypes from consideration. The differences in these populations of LD patterns of *MICA* and *HLA-B* alleles might suggest that haplotype diversity may be a selective pressure response to the parasite and pathogens endemic to a specific area.

In fact, pathogen-driven selection of *HLA* alleles, especially those of *HLA-B* locus, has been suggested in previous studies (ARNAIZ-VILLENA et al., 1996; BAEK et al., 2013; DE VRIES et al., 1979). Such factors may also have played a role in diversifying MICA (BAUER et al., 1999; ZHU et al., 2009).

GCT repetitions:

The *MICA* exon 5 holds short tandem repeats (STR) (MIZUKI et al., 1997). These STRs showed a variable number of trinucleotide GCT repeats that encode 4, 5, 6, 7, 9 or 10 Alanine (A, Ala). These microsatellite alleles were designated as *A4, A5, A6, A7, A8, A9* and *A10*. There is also an *A5.1* allele that contains five triplet repeats of GTC plus an additional guanine nucleotide insertion (GGCT) (FIGURE 6). This insertion leads to a frameshift and results in a stop codon and a premature termination. The *A4, A5, A6, A7, A8, A9, A10* and *A5.1* sizes are, respectively, 179 bp, 182 bp, 185 bp, 194 bp, and 183 bp (GAMBELUNGHE et al., 2007; MIZUKI et al., 1997; PYO et al., 2003).

The *A5.1* variant affects MICA subcellular localization, membrane anchorage, surface expression and the release of soluble MICA (sMICA) molecules (ASHIRU et al., 2013; SUEMIZU et al., 2002; TONNERRE et al., 2016). This *MICA A5.1* genetic variant is associated with 11 alleles: *MICA*008, *023, *028, *053, *058, *070, *073, *080, *082, *085, *087* (hla.alleles.org).

Suemizu et al. demonstrated, in polarized epithelial cells, that the full-length MICA protein is sorted to the basolateral membrane, while the A5.1 allele is aberrantly transported to the apical surface. They also performed a site-directed mutagenesis that identified that the cytoplasmic tail-encoded leucine-valine dihydrophobic tandem is a basolateral sorting signal. Therefore, the authors affirmed that the physiological location of MICA in the epithelial cells can be controlled by its cytoplasmic tail. This situation implies an impairment for the A5.1 homozygous individuals that

may compromise the immunological surveillance exerted by NK and T lymphocytes on epithelial malignancies (SUEMIZU et al., 2002).

Tonnerre et al. in 2013 made another important finding on the role of *MICA A5.1*. The authors demonstrated that a mismatch between donor and recipient for the *MICA A5.1* mutation could be a risk factor for long-term transplant survival; due to enhanced anti-MICA sensitization of kidney transplant recipients (TONNERRE et al., 2013).

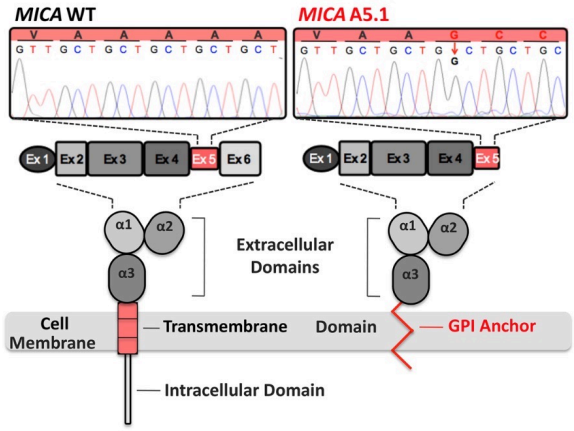


FIGURE 6 - A SCHEMATIC REPRESENTATION OF EXONS AND PROTEIN DOMAIN DISTRIBUTION FOR FULL-LENGTH MICA WILD TYPE (WT) AND THE MICA A5.1 MUTANT.
SOURCE: (TONNERRE et al., 2016).

MICA-129Val/Met dimorphism

An amino acid substitution from valine (Val) to methionine (Met) at position 129 in the α_2 MICA domain has been reported to affect the binding avidity of a MICA receptor: NKG2D. This substitution is in the position 454 (G/A) of the *MICA* gene (rs1051792) and divides *MICA* alleles into two groups (BOUKOUACI et al., 2009; ISERNHAGEN et al., 2015; LOPEZ-HERNANDEZ et al., 2010; STEINLE et al., 2001; ZHAO et al., 2011).

The MICA-129Val/Met–NKG2D high/low affinity has not been completely understood yet.

An experiment conducted by Steinle *et al.* in 2001, constructed four hybrid sequences, mutually exchanging three segments of *MICA*04* and *MICA*07* cDNAs. In this study Steinle *et al.* demonstrated that the reduced binding affinity of NKG2D for MICA*04 (met) *versus* MICA*01 (val) was not due to post-translational modifications but instead it could be attributed to amino acid differences between these variants. Soluble forms of NKG2D (sNKG2D) showed that substitution of valine 129 by methionine conferred high NKG2D binding affinity to MICA*04. Conversely, substitution of methionine 129 with valine drastically reduced binding of NKG2D to MICA*07. The side chain of methionine 129 is partially buried and forms hydrophobic interactions with glutamine 136, alanine 139, and methionine 140, its replacement by valine likely affects NKG2D

binding indirectly by a conformational change. This indicates that the 129 position may affect the NKG2D binding indirectly, perhaps by association with a conformational change (STEINLE et al., 2001). However a study performed by Isernhagen et al. in 2016 showed that NKG2D-MICA affinity changes with the membrane MICA (mMICA) expression intensity (ISERNHAGEN et al., 2016). In fact, a lower mMICA-129Met expression triggers more NKG2D signals compared with -129Val. Meanwhile, a higher expression of mMICA-129Val evokes more NKG2D signals compared to the MICA-129 Met variant, which in this case downregulates NKG2D, leading to impaired functions (FIGURE 7) (ISERNHAGEN et al., 2015; ISERNHAGEN et al., 2016).

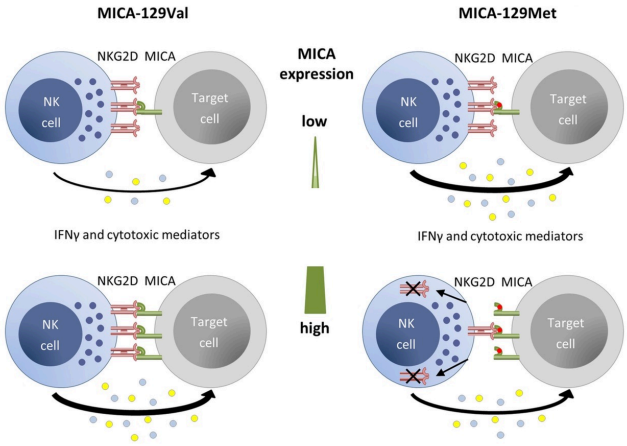


FIGURE 7 - SUMMARY OF FUNCTIONAL EFFECTS OF MICA-129 VARIANTS DEPENDING ON EXPRESSION INTENSITY.
SOURCE: (ISERNHAGEN et al., 2016).

When MICA-129Met binds NKG2D with higher avidity in respect to the other isoform, this bond triggers more NK cell cytotoxicity and interferon-γ release. On $CD8^+$ T cells the MICA-129Met mediated a rapid co-stimulation and activation of these cells in combination with a limited CD3 mediated signals, but also a rapid downregulation of NKG2D, whereas those with a valine bound NKG2D with low avidity (ISERNHAGEN et al., 2015; ISERNHAGEN et al., 2016).

The allele frequency for MICA-129Val/Met is 0,21 (Met) and 0,79 (Val) for the European population; 0,3 (Met) and 0,7 (Val) for the Han population; 0,24 (Met) and 0,76 (Val) for the Japanese population; 0,5 (Met) and 0,5 (Val) for the Yoruba population (1000 GENOMES <http://www.internationalgenome.org>).

In the Gao et al. study, the frequencies of MICA-129Met *versus* -129Val NKG2D binders were analyzed in two populations, European-Americans and African-Americans, and the results were 32/60% and 44/56% respectively. European-Americans showed a lower ratio of strong to weak NKG2D binding alleles than African-Americans resulting from their extremely high frequency of the valine 129 (weak binding) allele *MICA*008:01* (43%) (GAO et al., 2006). Another study by Zhang et al. genotyped the North American population: 692 Caucasians and 32 African-

Americans. The percentage of *MICA-129 Met/Met* was 30% for the African-Americans and 12% for the Caucasian, the percentage of *MICA-129Val/Met* was 43% for the African Americans and 40% for the Caucasian, and the percentage of *MICA-129 Val/Val* was 27% for the African-Americans and 48% for the Caucasian (p value < 0.0001). This study confirmed the Gao et al. study because they related a significant difference of *MICA129* genotype distribution between Caucasian and African-Americans, despite the low number of individuals of this last population. Compared to Caucasians, African-Americans had higher incidence of -*129Met/Met*, similar percentage of -*129Met/Val* and lower incidence of -*129Val/Val*. Interestingly the *MICA-129Met* allele was more frequently distributed among the *MICA* and *HLA-B* haplotype shared in both populations (ZHANG et al., 2013).

The rs1051792 SNP (*MICA-129Val/Met*) has also been associated with the risks for nasopharyngeal carcinoma, hepatitis B virus-induced hepatocellular carcinoma, ulcerative colitis, the severity of chronic Chagas heart disease, and autoimmune diseases, including ankylosing spondylitis, rheumatoid arthritis, inflammatory bowel disease, lupus erythematosus, type I diabetes, and psoriatic disease (ISERNHAGEN et al., 2015; TONG et al., 2013; ZHAO et al., 2011).

In 2015, it has been observed that *MICA-129Met* alelles increased the risk of experiencing acute graft-versus-host disease (aGVHD). This effect could be the consequence on NKG2D signaling by the *MICA-129Met* variant (ISERNHAGEN et al., 2015). Another recent study of Isernhagen *et al.* showed that the expression of plasma membrane MICA (pmMICA) was higher in a panel of 16 tumor cell lines (P = 0.0699) and 13 melanoma cell lines (P = 0.0429) carrying the *MICA-129Val/Val* genotype, whereas *MICA-129Val* homozygous melanoma cell lines released more soluble MICA (sMICA) by shedding (P = 0.0015) (ISERNHAGEN et al., 2016). It's interesting to note that the MICA-129 variant not only reflects the strength of bond with NKG2D but also the expression of sMICA. The researchers transfected a MICA-negative melanoma cell line (Malme) with expression constructs for MICA, which differed only at position 129, and obtained cell clones expressing both MICA variants at similar intensities. Thanks to this experiment the researchers noticed a higher rate of intracellularly retained MICA-129Met variants. If expressed at the cell surface, the MICA-129Met isoform was more susceptible to shedding. Both processes appear to limit the cell surface expression of MICA-129Met variants that have a high binding avidity to NKG2D (ISERNHAGEN et al., 2016).

In addition to this, MICA-129 dimorphism has shown signs suggesting that it may directly affect plasma membrane expression and shedding, and these functional effects might contribute to the numerous disease associations which have been reported for this polymorphism (ISERNHAGEN et al., 2016).

1.7.0 MICA POLYPEPTIDE

MICA encodes for a polypeptide (FIGURE 8) of 383 amino acids that is expressed on the surface of different cells and resembles the domain organization of the α chain of MHC class I molecules (ZWIRNER et al., 1998). The structure of the MICA molecule is inherently flexible, particularly in the area linking the α_3 domain to the α_1 and α_2 platform, which raised the possibility that the MICA molecule might reconfigure when interacting with its receptor (LI et al., 1999). MICA is a highly glycosylate membrane-anchored cell surface protein (GROH et al., 1996). Interestingly, complexes with unglycosylated MICA appeared to be less stable than those incorporating glycosylated MICA (STEINLE et al., 2001).

MICA has an immunoglobulin (Ig) domain similar to the α_1/α_2 and the $\alpha_1/\alpha_2/\alpha_3$ domains, respectively, of MHC class I proteins (LI et al., 2001). The initially hypothesized role of MICA in peptide binding and antigen presentation based on domain similarity was dismissed because, unlike the classical class I molecules, MICA does not bind β_2-microglobulin (β_2-m) and is independent of any transporter-associated protein (TAP). In addition, attempts to identify peptides bound to MICA have been unsuccessful (CHOY and PHIPPS, 2010; GROH et al., 1996). In the crystal structure of MICA, the platform consisted of four distinct α helices arranged in an eight-stranded anti-parallel β sheet. These helices in MICA corresponded roughly to the two helices that define the peptide-binding groove in peptide-binding MHC class I proteins and homologs (LI et al., 2001). Even if there are many similarities between the structures of MICA and classical class I molecules, MICA is evidently not a peptide/antigen-presenting molecule (CHOY and PHIPPS, 2010).

MICA is preferentially concentrated in lipid rafts (cholesterol and sphingolipid-rich plasma membrane microdomains). Like other proteins associated with lipid rafts, MICA is S-acylated (two juxtaposed cysteines encoded by exon 5, codons 33-34 to 39-40 depending on the microsatellite allele). *In vitro* mutation in the S-acylation site, in which the cysteine codon at position 39 of an A10 allele is replaced by a stop codon, leads to a truncated form of MICA that is unable to activate NK cells (ELEME et al., 2004).

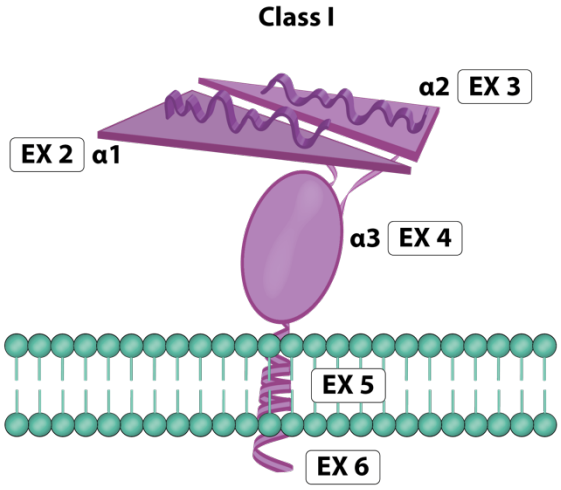


FIGURE 8 - MICA MOLECULE. Exon 1 encodes one leader peptide, exons 2 to 4 encode three extracellular globular domains, exon 5 encodes one transmembrane domain and exon 6 encodes a cytoplasmic tail.
SOURCE: (RISTI and BICALHO, 2017).

MICA acts as a ligand for NK cells, γδ T cells and αβ $CD8^+$ T cells, which express a common activating NK cell receptor NKG2D (BAUER et al., 1999; CHOY and PHIPPS, 2010; SUAREZ-ALVAREZ et al., 2009). NKG2D recognizes the human MICA protein in conjunction with a transmembrane signaling adaptor protein, DNAX-activation protein (DAP10) (BAUER et al., 1999; CHOY and PHIPPS, 2010). MICA molecule can also be recognized by $_{\delta\gamma}$T cells with the TCR variable region $V_\delta1$ (; BAUER et al., 1999; LI et al., 2005; WU et al., 2002; XU et al., 2011).

Both types receptors types, $V_\delta1$TCR and NKG2D, when expressed on the $V_\delta1$ cells surface, can simultaneously recognize and bind to MICA.

There are close associations between the tissue distribution of $V_\delta1$ cells and the physiological expression of MICA molecules, as MICA affects $V_\delta1$ cells' lineage development (LI et al., 2005). $V_\delta1$ T cells have been proposed to respond not to a diversity of microbial antigens, but to unique ''stress antigens'' that are markers of cell infection or transformation. Such being the case, all known antigens of $V_\delta1$ T cells, including MICA, are consistent with this characteristic. According to works by two groups (ADAMS et al., 2005; SHIN et al., 2005), δ chain alone can recognize and bind to MICA *in vitro*, which indicates that the δ chain plays a more important role in interacting with MICA than the γ chain does, the $V_\delta1$-TCR directly recognizes MICA, it doesn't need an antigen presentation and the $V_\delta1$-TCR displays the limited diversity recognition. In fact, the molecule basis is the exit of "putative binding box" motif within CDR3 region, which derives from germ line-encoding residues. Finally, the V_δ gene fragment that encodes CDR1 and CDR2 correlates mainly with tissue origin (ADAMS et al., 2005; LI et al., 2005; SHIN et al., 2005).

In Vδ1 γδ cells the strength of the binding between TCR and MICA is weaker than that between NKG2D and MICA. However, although weak, TCR:MICA complexes, after they are

formed, show unusual stability, with long complex half-lives, and both receptors compete for binding to MIC ligands (XU et al., 2011). Xu *et al* suggested that the first interactions at the point of contact could be dominated by NKG2D:MIC binding events, before the longer-lived γδ TCR:MIC complexes take over (XU et al., 2011).

1.8.0 MICA EXPRESSION

MICA molecules are expressed on thymic medulla and gastrointestinal epithelium, tumors of different origin, for example epithelial tumors (including lung, breast, kidney, ovary, prostate, and colon carcinomas), some melanomas and T cell leukemia cellular lines, freshly isolated human endothelial cells and fibroblasts, human keratinocytes, monocytes, human epithelial and fibroblast cell lines (BAUER et al., 1999; GROH et al., 1996; KATSUYAMA et al., 1999; MURO et al., 2012; ROMPHRUK et al., 2001; STEINLE et al., 2001). Additionally, surface expression of MICA on corneal epithelium has been identified at low levels (GROH et al., 1996; MURO et al., 2012).

Some debate exists over whether *MIC* gene products are found in monocytes. This might reflect variations in the specificity, avidity, characterization and availability of the various MIC-specific antisera and monoclonal antibodies (Abs) that have been produced, as well as variability in the high level of glycosylation of *MIC* product (GROH et al., 1999; LEELAYUWAT et al., 1996; ZWIRNER et al., 1998). Finally, MICA is not expressed by T or B resting cells, but PHA (phytohemagglutinin)-activated $CD4^+$ and $CD8^+$ T cell blasts can express it (MOLINERO et al., 2002; MURO et al., 2012; ZWIRNER et al., 1998). Also, direct stimulation through CD3 or CD28 in the presence of autologous APC induced transcriptional activation of the MICA gene and the expression of MICA protein on the cell surface of activated T cells, demonstrating clearly that signaling through these receptors actually participates in MICA expression (MOLINERO et al., 2002).

Unlike MHC class I promoters, the MICA gene lacks the IFN-γ-responsive element. IFN-γ does not regulate the expression of MICA (ZWIRNER et al., 2006) but is regulated by promoter heat-shock elements and strongly induced upon cell stress. For this reason MICA may function as an indicator of cell stress (ROMPHRUK et al., 2001), but the expression of MICA by proliferating T lymphocytes and its detection in different cell lines and tumors suggest that MICA could be a cell proliferation sensor as well (MOLINERO et al., 2002). An increase of MICA expression on the cell surface could possibly enhance antigen-specific cytotoxic T-lymphocyte (CTL)-mediated cytotoxicity and activate NK cells (ZOU et al., 2002).

Immature dendritic cells (DCs) do not express MICA, but IFN-α and IL-15, while promoting DC maturation, induce surface expression of MICA (ZWIRNER et al., 2006). In fact

MICA antigens have been found to be expressed on the surface of endothelial cells, keratinocytes, and monocytes and DC cells by flow cytometry, western blotting and immunoprecipitation using polyclonal rabbit antibodies against MICA (LUO et al., 2013).

An important factor for cell surface expression of MICA is the intact *N*-glycosylation machinery; 2-deoxy D-Glucose (2DG) inhibit MIC surface expression by blocking cellular *N*-glycosylation (MELLERGAARD et al., 2014) but there is no indication of a relationship of alternative splicing with surface expression and the lack of surface expression of MICA in different cells (ZOU and STASTNY, 2002).

The Mellergaard et al. study showed that Asn^8 glycosylation is essential for cell surface expression of the full-length *MICA*018* allele, but the *MICA*008* allele is, however, not affected by changes in *N*-glycosylation. The regulation of MICA*018 surface expression by *N*-glycosylation is critically dependent on expression of a threonine residue at position 24. MICA*018 surface expression was thus not affected by altered *N*-glycosylation when position 24 was changed to alanine, the residue present in MICA*008. Both position Asn^8 and Thr^{24} are located in the α_1 domain of *MICA*. Several *MICA* alleles *(*001, *012, *018, *060, and *061*) possess a threonine residue at position 24, and it is likely that surface expression of these alleles will depend on *N*-glycosylation, although this requires experimental verification. MICA *N*-glycosylation and Thr^{24} showed no significant influence for binding to the NKG2D receptor. Tunicamycin and 2DG inhibit *N*-glycosylation in the ER compartment and block MICA*018 surface expression. Also, the immunoevasin U21 inhibits surface expression of MICA*018, and this inhibition was rescued by substitution of threonine 24. Interestingly, the (Human Herpesvirus-7) HHV7-U21 protein also retained MICA*008; however, this was likely not through a direct change in MICA*008 *N*-glycosylation, but possibly through a novel post-translational regulatory mechanism (MELLERGAARD et al., 2014).

In pathological conditions such as malignancy, virus infection, hypoxia, and heat shock, MICA expression is up-regulated and recognized by the NKG2D activating receptors, promoting the cytolysis of virus infected or transformed cells (LUO et al., 2014). For example an oxidative stress with H_2O_2 can induce MICA expression (GROH et al., 1999). The oxidative stress is a common feature of chronic renal failure, the reactive oxygen species (ROS) trigger the up-regulation of MICA and have been implicated in the down-regulation of NKG2D in NK cells from healthy individuals as well (PERALDI et al., 2009). Besides, cytomegalovirus (CMV) infection can induce MICA expression (STEINLE et al., 2001), and the accumulation of hypoxia-inducible factor-1 (HIF-1α) can up-regulates MICA expression on human cardiomyocytes during hypoxia/reoxygenation, inducing IFNγ secretion and NK cell cytotoxicity (LUO et al., 2010).

There have been reports, for HIV infection**,** of higher levels of soluble MICA in the serum of chronically infected individuals compared with healthy controls and HIV-1 controllers (ESTESO et al., 2014). In line with these pathological conditions, it has been shown that the treatment of glioma cells with endogenous micro-RNA miR-93 decreased the expression of MICA, with a specific interaction in the 3'UTR region of the *MICA* gene (CODO et al., 2014).

Still on the miRNA subject, the aim of the Stern-Ginossar et al. study was to determine which of the predicted 3' UTR–binding human micro-RNAs (miRNA) were able to repress MICA and MICB expression. They investigated miRNA representatives of each family (miR-20a, miR-93, miR106b, miR-302d, miR-372, miR-373 and miR-520d) in HeLa and DU145 cells, which had high expression of MICA and MICB, respectively. Analysis of cells transduced with the various human microRNAs showed that expression of both *MICA* and *MICB* was decreased by all human microRNAs tested except miR-302d, which had no effect, and miR-372, which showed only a modest effect. Under normal conditions, these cellular miRNAs maintain MICA and MICB protein expression under a certain threshold. Furthermore, during short-term stresses such as heat shock and viral infection, the amount of miRNAs did not significantly change, but the elevation in *MICA* and *MICB* mRNA expression probably exceeded the miRNAs repression activity, resulting in the observed expression of MICA and MICB protein (STERN-GINOSSAR et al., 2008). Stern-Ginossar *et al.* suggested that these cellular miRNAs set up a threshold for MICA and MICB protein expression. This post-transcriptional regulation of miRNAs has several possible advantages. MICA and MICB could be already transcribed and thus, upon stress, a rapid induction of MICA/B expression could be achieved. Also this type of regulation can potentially prevent the undesired effects in which small fluctuations in the amounts of *MICA* and *MICB* mRNA would lead to NKG2DL expression and self-destruction and ensure that only 'real' stress will result in recognition and elimination by immune cells (STERN-GINOSSAR et al., 2008).

1.8.1 MICA SHEDDING

A particular type of MICA expression is its shedding. Shedding of MICA molecules primarily depends on a proteolytic process involving multiple enzymes, including a disintegrin and metalloproteinase (ADAM): ADAM9, ADAM10, ADAM17, some members of the matrix metalloproteinases (MMP) family: MMP9 and MMP14, and the disulfide isomerase Erp5 (CAMODECA et al., 2016; HUERGO-ZAPICO et al., 2012; SALIH et al., 2003; ZINGONI et al., 2015). The ADAMs are a family of transmembrane proteins involved in cell adhesion and proteolysis. They are zinc metalloproteinases characterized by a metalloproteinase domain responsible for proteolytic activity and a disintegrin domain that interacts with integrins

(CAMODECA et al., 2016). The ERp 5 is a protein disulfide isomerase family A, member 6 (also named PDIA6) (SALIH et al., 2003). ERp5 binds and cleaves MICA α_3 domain, as well as ADAM10 or ADAM17 (HUERGO-ZAPICO et al., 2012). The cleaved MICA then interacts with NKG2D, which, in turn, induces the internalization and degradation of the receptor and stimulates the population expansion of immunosuppressive $NKG2D^{+}CD4^{+}$ T cells (GROH et al., 2006). Another level of complexity is reflected by the fact that MICA displays a high degree of polymorphism leading to an allele-dependent shedding, as demonstrated by the allelic variant MICA*008 released in association with exosomes (ZINGONI et al., 2015).

MICA shedding is associated with the tumor escape (WALDHAUER et al., 2008). In recent years, several selective ADAM inhibitors, especially against ADAM-10 and ADAM-17, have been shown to synergize with existing therapies in reducing tumor cell growth. The majority of these synthetic inhibitors use hydroxamate as the zinc-binding group (ZBG) and are designed to interact with the subsites S1'-S3' of the MMP-like catalytic site of ADAMs (CAMODECA et al., 2016).

It has also been discovered, in the tumor research field, that the matrix metalloproteinase 9 (MMP9) is a cell surface-associated type I collagen-degrading MMP, which is highly expressed and secreted by invasive human osteosarcoma cells. Several reports have indicated that inhibition of MMP9 expression and activity decreases cell migration and invasion by human and murine osteosarcoma cells. The Sun et al. study showed that the MMP9 inhibitor I, a specific inhibitor for MMP9, increased membrane-anchored MICA expression and decreased sMICA release in a dose-dependent manner without inhibiting MICA mRNA transcription or cell growth. These results suggest a role for MMP9 in MICA shedding. Sun et al. speculate that MMP9 might hydrolyse MICA on the osteosarcoma cell surface releasing soluble MICA and ultimately undermine NKG2D–MICA-mediated immune surveillance. This suggests that MMP9, aside from playing an important role in the invasion, metastasis and angiogenesis processes, may also have a role in MICA shedding in osteosarcoma cells, allowing these cells to evade immune attack (SUN et al., 2011). Interestingly, the Ashiru et al. study observed no reduction in soluble MICA shedding on HeLa and A375 cells after treatment with metalloproteinase inhibitors. One explanation for this discrepancy could be that the extensive polymorphism of MIC molecules affects the shedding process. Full-length, and therefore membrane-resident, MICA*008 proteins can be released from cells in exosomes. In contrast, the *MICA*019* allele, which is almost identical to MICA*008 in the extracellular domains but has transmembrane and cytoplasmic tail domains typical of most MICA molecules, was only found in cell culture supernatant as a soluble truncated molecule. Despite this difference in mechanism of shedding, both soluble MICA*019 and exosomal MICA*008 molecules are able to trigger downregulation of NKG2D from the NK cell surface (ASHIRU et al., 2010).

Another disease associate with MICA shedding is the human cytomegalovirus (HCMV). The HCMV-infected cells are associated with a reduced expression of the endogenous metalloprotease inhibitor tissue inhibitors of metalloproteinase 3 (TIMP3). Esteso et al. demonstrate that the HCMV-encoded microRNA US25-2-3p is able to modulate TIMP3 expression. These observations may explain previous reports documenting increased levels of a range of ADAM17 substrates in the serum of patients with CMV disease. In these experiments, it has been shown that the miRUS25-2-3p can directly regulate TIMP3 expression, and that the expression of miRUS25-2-3p in U373/MICA*019 cells leads to an increase in the release of soluble MICA molecules (ESTESO et al., 2014).

In a Hepatite B virus (HBV) infected cells study in vitro, a compound library was screened and identified putative regulators of MICA shedding were identified having the potential to enhance the immune reaction by simultaneously increasing cell surface MICA levels and decreasing soluble MICA levels. Kishikawa et al. identified molsidomine and metergoline molecules as suppressors of MICA protein shedding, as they decreased the levels of soluble MICA in the culture media and increased cell surface MICA levels in the cell lysate. However, the molecular mechanisms for this modulation are currently unknown. It is known that molsidomine is a vasodilator and metergoline is a serotonin antagonist; however, it is difficult to speculate on how these compounds are directly involved in the regulation of MICA shedding based on their currently known actions (KISHIKAWA et al., 2015).

1.9.0 MICA AND DISEASES

MICA is an interesting molecule, and its relevance is not associated only with kidney transplantation. In fact, there are many diseases that have been associated with a specific *MICA* allele or a specific MICA protein expression.

MICA alleles could be associated with ankylosing spondylitis, Behçet's disease, crohn's disease, psoriasis, psoriatic arthritis, insulin-dependent diabetes mellitus, systemic lupus erithematosus, rheumatoid arthritis, Addison's disease, primary sclerosing-cholangitis, Kawasaki disease, coronary aneurysm in Kawasaki disease, ulcerative colitis, leprosy, kervical neoplasia, HIV type, inflammatory bowel disease, chronic hepatitis B and C, chlamydia trachomatis, idiopathic pulmonary fibrosis, graft-versus-host disease, nasopharyngeal cancer, bronchiolitis obliterans syndrome, cholangiocarcinoma, large granular lymphocyte leukemia, acute graft-versus-host disease, hepatocellular carcinoma, BK polyomavirus. MICA protein expression can also be associated with gastric cancer, gastric cancer patients who have received a D2 gastrectomy, osteosarcomas, breast cancer, non-small cell lung cancer and urothelial carcinoma.

(Box 1).

MICA alleles/haplotype and related diseases				
Disease	**Allele association**	**Ethnic group**	**Risk or not**	**References**
Ankylosing spondylitis	MICA-A4	Caucasoid (European)	Risk	(GOTO et al., 1997)
	MICA*007:01	Caucasoid (USA)	Risk	(ZHOU et al., 2014)
	MICA-A4	Caucasoid (European)	None	(YABUKI et al., 1999)
	MICA-A4	Caucasoid (Sardinian)	Risk	(RICCI-VITIANI et al., 2000)
	MICA*007 and MICA*010 (HLA-B*27)	Oriental (Japanese)	Risk	(TSUCHIYA et al., 1998)
	MICA*007:01, MICA*019	Oriental (Chinese)	Risk	(ZHOU et al., 2014)
	MICA-129	(Algeria)	Risk	(AMROUN et al., 2005)
Behcet's disease	MICA-A6	Oriental (Japanese)	Risk	(MIZUKI et al., 1997)
	MICA*009 and A6	Middle Eastern (Palestinian)	Risk	(WALLACE et al., 1999)
	MICA*009	Oriental (Japanese)	Risk	(MIZUKI et al., 1999)
	MICA-A6	Caucasoid (Greek)	Risk	(YABUKI et al., 1999)
	MICA-A6	Oriental (Korean)	Risk	(PARK et al., 2002)
	MICA-A6	Caucasoid (Italian)	Risk	(PICCO et al., 2002)
	MICA-A6	Caucasoid (Italian)	None	(SALVARANI et al., 2001)
	MICA-A6	Caucasoid (Spanish)	None	(GONZÁLEZ-ESCRIBANO et al., 1999)
	MICA-A6	Middle Eastern (Iranian)	None	(MIZUKI et al., 2002)
	MICA*009	Caucasoid (European)	Risk	(HUGHES et al., 2005)
	HLA-B51	Caucasoid (Greek, Italian) and Oriental (Japanese)	Risk	(MIZUKI et al., 2000)
	MICA*006 and *009	Brazil	Risk	(MARIN et al., 2004)
	MICA*009 (HLA-B*51)	Turkish	Risk	(MIZUKI et al., 2007)
	MICA*019	Caucasoid (Spanish)	Risk	(MUNOZ-SAA et al., 2006)
Crohn's disease	MICA-A5.1	Tunisian	Protective	(MUNOZ-SAA et al., 2006) (KAMOUN et al.,

				2013)
Psoriasis / Psoriatic Arthritis	MICA*002 and A9	Caucasoid (Spanish)	Risk	(GONZALEZ et al., 1999)
	MICA-A9	Caucasoid (Spanish)	Risk	(QUEIRO et al., 2012)
	PERB11.1*06	Caucasoid (Australian)	Risk	(TAY et al., 2000)
	MICA-A5.1	Oriental (Korean)	Risk	(CHOI et al., 2000)
	MICA-A9	Caucasoid (European), Oriental (Asian), Jewish	Risk	(SONG et al., 2014)
	MICA-A9	European	Risk	(AMROUN et al., 2005)
	MICA-A9	Jewish	Risk	(GONZALEZ et al., 2001)
	MICA-A9	Oriental (Chinese)	None	(CHANG et al., 2003)
	MICA-A9	European	None	(OKADA et al., 2014)
	MICA-129 Met allele	Caucasoid (North America)	Risk	(POLLOCK et al., 2013)
Insulin-dependent diabetes mellitus	MICA-129 Val/val	Algerian	Risk	(RAACHE et al., 2012)
	MICA-A4	Oriental (Korean)	Risk	(PARK et al., 2001)
	MICA-A6	Oriental (Korean)	Protective	(PARK et al., 2001)
	MICA-A9	Oriental (Taiwanese)	Risk	(LEE et al., 2000)
	MICA-A5.1	Caucasoid (European)	None (children) and Risk (adults)	(GAMBELUNGHE et al., 2007)
	MICA-A4 and A5.1	Oriental (Japanese)	Risk	(KAWABATA et al., 2000)
	MICA-A6	Oriental (Japanese)	Protective	(KAWABATA et al., 2000)
Systemic Lupus Erythematosus	MICA-A5 and A5.1	Caucasoid (Italian)	Risk	(GAMBELUNGHE et al., 2005)
	MICA-A9	Caucasoid (Italian)	Protective	(GAMBELUNGHE et al., 2005)
	MICA-A5.1	Caucasoid (Czech)	Risk	(FOJTIKOVA et al., 2011)
	MICA-129Met and A9	Oriental (Japanese)	Risk	(YOSHIDA et al., 2011)
Rheumatoid Arthritis	MICA-A6	Spanish	Protective	(MARTINEZ et al., 2001)
	MICA-A6	Caucasian	Protective	(SINGAL et al., 2001)

	MICA-A6	Caucasoid (French and German)	None	(KIRSTEN et al., 2009)
	MICA-129	Caucasoid (French and German)	Unknown	(KIRSTEN et al., 2009)
Addison's disease	MICA-A5.1 and MICB-CA-25	Caucasoid (Italian)	Risk	(GAMBELUNGHE et al., 1999)
	MICA-A6	Caucasoid (Italian)	Protective	(GAMBELUNGHE et al., 1999)
	MICA-A5.1/A5.1 (or neighboring gene)	Caucasoid (North America)	Risk	(PARK et al., 2002)
Primary sclerosing-cholangitis	MICA*002	Caucasoid (English)	Protective	(NORRIS et al., 2001)
	MICA-A5.1	Caucasoid (Norwegian)	Risk	(WIENCKE et al., 2001)
	MICA 5.1 allele	Caucasoid (Northern Italy)	None	(HOV et al., 2010)
Kawasaki disease	MICA-A5.1	Oriental (Japanese)	Risk	(HUANG et al., 2000)
Coronary aneurysm in Kawasaki disease	MICA-A4	Oriental (Japanese)	Protective	(HUANG et al., 2000)
	MICA-A5	Oriental (Japanese)	Risk	(HUANG et al., 2000)
Ulcerative colitis	MICA-A4	Caucasoid (Spanish)	Protective	(MARTINEZ-CHAMORRO et al., 2016)
	MICA-A6	Oriental (Japanese)	Risk	(SUGIMURA et al., 2001)
	MICA-A5.1	Caucasoid (Spanish)	Protective	(FDEZ-MORERA et al., 2003)
	MICA-A5.1	Oriental (Chinese)	Risk	(DING et al., 2005), (LU et al., 2009)
	MICA-A6	Tunisian	Risk	(KAMOUN et al., 2013)
	MICA-A6	Japanese	None	(SEKI et al., 2001)
	MICA-A7	Japanese	Risk	(SUGIMURA et al., 2001)
	MICA-129Val	Chinese	Risk	(ZHAO et al., 2011)
	MICA*007	Caucasian (European)	Risk	(ORCHARD et al., 2001)
Leprosy	MICA*10 and *027	Brazil	Protective	(SACRAMENTO et al., 2012)
	MICA*5A5.1	Southern Indian	Risk	(TOSH et al., 2006)
Cervical neoplasia	MICA-A4 and A5	Caucasoid (Northern European)	Protective	(CHEN et al., 2013)

	MICA-A5.1	Caucasoid (Northern European)	Risk	(CHEN et al., 2013)
	MICA-A4, A5, A5.1, A6 and A9	Caucasoid (Northern European)	None	(GHADERI et al., 1999)
HIV type1	MICA*017 (rs199503730)	France	Protective	(LE CLERC et al., 2014)
	Any of MICA variants	European American and African American	None	(GAO et al., 2006)
Inflammatory bowel disease	MICA-129val/met	Murcians (Spanish)	Protective	(LOPEZ-HERNANDEZ et al., 2010)
	MICA-129met/met	Murcians (Spanish)	Risk	(LOPEZ-HERNANDEZ et al., 2010)
Chronic hepatitis B and C	rs2596542 (5' flanking region of MICA)	Oriental (Japanese)	Risk	(KUMAR et al., 2012)
Chlamydia trachomatis	MICA*008	Oriental (Chinese)	Negative correlation with C. trachomatis IgG antibodies	(MEI et al., 2009)
Idiopathic pulmonary fibrosis	MICA*001/*00201	Mexican	Risk	(AQUINO-GALVEZ et al., 2009)
Graft-versus-host disease	MICA-129val	Caucasoid (French)	Risk in combination with sMICA level below 80 pg/mL	(BOUKOUACI et al., 2009)
	MICA-129Met	Caucasoid (German)	Protective	(ISERNHAGEN et al., 2015)
Nasopharyngeal cancer	MICA-129 val/val	Tunisian	Risk	(DOUIK et al., 2009)
Bronchiolitis obliterans syndrome	Abs to MICA*001 and *009	North American	Risk	(ANGASWAMY et al., 2010)
Cholangiocarcinoma	MICA 5.1	Scandinavian	Protective	(MELUM et al., 2008)
Large granular lymphocyte leukemia	MICA 5.1/MICA *00801	USA	Risk	(VINY et al., 2010)
Hepatocellular Carcinoma	MICA (rs2596542) AA genotype	Oriental (Chinese)	Risk	(LI et al., 2016)
BK polyomavirus	MICA A5.1	Caucasoid (French)	Protective	(TONNERRE et al., 2016)
MICA protein expression and related diseases				
Disease	**MICA expression**	**Ethnic group**	**Risk**	**References**
Gastric cancer	Overexpression of MICA	Chile	Risk	(RIBEIRO et al., 2016)
Gastric cancer patients who have received a D2 gastrectomy	Overexpression of MICA	Oriental (Chinese)	Protective	(CHEN et al., 2016)
Osteosarcomas	sMICA	Oriental (Chinese)	Risk	(LU et al., 2008)
Breast cancer	High sMICA level	Iran	Risk	(ROSHANI et al., 2015)
*Non-small cell lung cance*r	High sMICA level	Oriental (Chinese)	Risk	(WANG et al., 2015)

Urothelial carcinoma	High sMICA level	Oriental (Chinese)	Risk	(ZHAO et al., 2015)
Cholangiocarcinoma	High MICA expression and NKG2DL	Oriental (Japanese)	Protective	(TSUKAGOSHI et al., 2016)

BOX 1 - MICA AND ASSOCIATED DISEASES

Primary sclerosing cholangitis (PSC) is often complicated by the development of cholangiocarcinoma (CCA) (MELUM et al., 2008). In the Norris et al. study there was no increase of *MICA*007* frequency in PSC patients with inflammatory bowel disease (IBD), but the researchers found a highly significant association between PSC and *MICA*008* allele in 2 independently collected sets of northern European Caucasoid PSC patients (NORRIS et al., 2001). But *MICA*008* is the most frequent allele in this population. In the Norris et al. study they also identified a significantly lower frequency of the *MICA*002* allele in patients compared with controls. This suggests that the *MICA*002* allele is associated with protection from disease, and since one copy of the allele is sufficient to prevent PSC, the resistance allele may be dominant (NORRIS et al., 2001). In the Melum et al. study *MICA A-5.1* was genotyped in 365 Scandinavian (Norwegian) PSC patients and 368 healthy controls. The *5.1* allele and the *MICA*008* allele, are generally considered to be in LD. CCA was found in 49 of the PSC patients (13.6%) and *MICA 5.1* allele frequency showed an increase in PSC patients without CCA. This seems to play a protective effect (MELUM et al., 2008).

In the Behçet's disease (BD) study by Marin et al*., MICA*006* appears to be associated with the disease, but always in the presence of *HLA-B51*. *MICA*009*, however, was not associated with this disease (MARIN et al., 2004).

MICA polymorphisms may be an important factor in the risk of acute graft-versus-host disease (aGVHD) following stem cell transplantation (TIAN et al., 2001). The Boukouaci et al. study found that the cumulative incidence of chronic GVHD (cGVHD) accounting for the different combinations of *MICA-129* genotypes (val/val, met/val, or met/met) and serum levels of sMICA (80 pg/mL and 80 pg/mL) showed that each of these risk factors raised the risk of cGVHD in an additive manner. Patients bearing the *val/val* genotype together with serum sMICA level greater than 80pg/mL were at the highest risk of developing cGVHD (93% at 20 months), whereas the lower risk was observed for those with *MICA-129 met/met* or *met/val* in combination with sMICA level below 80 pg/mL (23% at 20 months; *P* 0.001). So, the *MICA-129 val* in an allele dose-dependent manner increased the risk of cGVHD, suggesting that the effect is recessive. Instead, the *MICA-129 met* allele was associated with a high incidence of relapse. Furthermore, an increase of sMICA in post-transplantation conditions in the patients is associated with a higher incidence of

cGVHD independent of preexisting history of aGVHD as well as MICA-129 genotype. They also found that the presence of MICA Abs before transplantation was correlated with low levels of sMICA after transplantation and consequently with a low incidence of cGVHD, strongly suggesting a neutralizing effect of MICA antibodies on sMICA (BOUKOUACI et al., 2009). On the contrary, in the Park et al. study a significant association of *MICA* genotype and the incidence of aGVHD were not observed. This may be caused by the small sample size of this study (PARK et al., 2016).

Regarding the bronchiolitis obliterans syndrome (BOS) after human lung transplantation, the Angaswamy et al. study showed that the development of Abs to both MICA and HLA was strongly associated with the development of BOS, suggesting a synergistic effect. Patients who developed both HLA and MICA Abs developed BOS earlier than those that had only anti-HLA or anti-MICA Abs alone (ANGASWAMY et al., 2010).

The Li et al. study on hepatocellular carcinoma (HCC), discovered a statistical significance in *AA* genotype distribution of *MICA* rs2596542 polymorphism between case and control groups ($P<0.05$), and *AA* genotype was positively correlated with the onset risk of HCC (OR=2.90, 95% CI=1.10–7.67). Finally, the A allele of rs2596542 conferred higher risk for HCC than the G allele (LI et al., 2016).

1.10.0 NKG2D GENE

The NKG2D (natural killer group 2 member D ligand) gene, also known as *KLRK1* (killer cell lectin-like receptor subfamily member 1), is a member of a family of C-type lectin-like receptors called CD94/NKG2 (LI et al., 2001). NKG2D displays limited sequence similarity with his family of NCRs (NK cell surface receptors) and CD94. NKG2D forms homodimers, rather than heterodimers, with CD94, as do other NKG2 NCRs (LI et al., 2001). *NKG2D* is highly evolutionarily conserved and is located on chromosome 6 in mice and on a syntenic region of human chromosome 12 (HO et al., 1998; LI et al., 2001; VANCE et al., 1997). The human *NKG2D* gene, located on the NK complex, has 10 exons (GLIENKE et al., 1998). From exons 2 to 4 it encodes the intracellular/transmembrane domain, followed by exons 5 to 8 that encode the ligand-binding ectodomain (UHRBERG et al., 1997). In the human *KLRK1(NKG2D)*, it is flanked on the centromeric side by *KLRD1* (*CD94*) and on the telomeric side by the cluster of *KLRC4 (NKG2F), KLRC3 (NKG2E), KLRC2 (NKG2C),* and *KLRC1 (NKG2A)* genes (BURGESS et al., 2006). This gene has limited polymorphism, and the mouse ortholog, Klrk1, has limited polymorphism too (HO et al., 1998). *NKG2D* appeared to be highly conserved during evolution because orthologs of *KLRK1* are present in the genome of all mammals, as well as in marsupials (BAUER et al., 1999; JAMIESON et al., 2002).

NKG2D expression is subject to post-transcriptional regulation by splicing variation (UHRBERG et al., 1997). In mice, NKG2D isoforms generated by alternative mRNA splicing include long (NKG2D-L) and short (NKG2D-S) variants (DIEFENBACH et al., 2002; GILFILLAN et al., 2002). The human *NKG2D* gene is expressed from at least 3 distinct alleles, and several isoforms at the mRNA level have been described, including an alternatively spliced variant that introduces a nonsense mutation resulting in a protein that lacks the entire extracellular ligand binding domain (SHUM et al., 2002). The Karimi et al. study was the first example of a dominant negative alternative splice variant. They characterized a novel splice variant that encodes a truncated form of NKG2D ($NKG2D^{TR}$) in $CD8^+$ T cells and NK cells. This $NKG2D^{TR}$ encodes a truncated receptor lacking the ligand-binding ectodomain, which acts as a dominant negative regulator of NKG2D function by down-regulating full-length NKG2D ($NKG2D^{FL}$) surface expression through intracellular retention. This $NKG2D^{TR}$ thus acts as a negative regulator of NKG2D-mediated function (KARIMI et al., 2014).

1.11.0 NKG2D POLYMORPHISM: HNK1 AND LNK1 HAPLOBLOCKS

Eight single nucleotide polymorphisms, most of which are located in the *NKG2D* (*KLRK1*) gene region within an annotated region called the natural killer gene complex (*NKC*) on 12p13.2–p12.3, have been found to closely associate with natural cytotoxic activity. These SNPs form two haploblocks, named *NKG2D* hb-1 and hb-2. *NKG2D* hb-1 generates two major haplotype alleles: low-activity-related *LNK1* and high-activity-related *HNK1* (HAYASHI et al., 2006; IMAI et al., 2012; UCISIK-AKKAYA and DORAK, 2009). Hayashi et al. conducted a case-control study in the Saitama Prefecture (Japan), between 1986 and 1990, who showed that *LNK1* and *HNK1* have a significant association with natural cytotoxic activity and, in addition, the haplotype *HNK1*/*HNK1* revealed a decreased risk of cancer in all sites compared with *LNK1*/*LNK1* (HAYASHI et al., 2006).

Another study on a Japanese population demonstrated that the *HNK1* haplotype is associated, in the peripheral blood, with a greater activity of NK cells and, at the same time, with a lower prevalence of cancers originating from epithelial cells. This study showed an association between the NKG2D-*HNK1* haplotype in unrelated donors of HLA- matched myeloablative bone marrow transplants (haplotype frequency, 61%) and a significantly reduced transplant-related mortality and better overall survival for their recipients with standard-risk disease (ESPINOZA et al., 2009).

An interesting study by Imai et al. genotyped 732 A-bomb survivors in Hiroshima, Japan, from 2005 to 2008. They selected the 8 SNPs which constituted NKG2D haplotype blocks, hb-1

and hb-2, and they found that one SNPs (rs1049174) constituting the NKG2D hb-1 haplotypes may be involved in transcription regulation of NKG2D. Genotypes from rs1049174 in the noncoding region showed the closest association with NKG2D expression on NK cells ($p = 0.003$ for trend) compared with the other four SNPs. But they did not find a significant effect of past A-bomb radiation exposure on expression levels of NKG2D, or the NKG2D haplotype-expression relationship on NK and $CD8^+$ T cells. Also there was no significant radiation effects on the percentages of NK and CD8 T cells, so it is likely that radiation might not significantly affect these immune cell populations (IMAI et al., 2012).

1.12.0 NKG2D POLYPEPTIDE

NKG2D is a transmembrane-anchored receptor expressed as a disulfide-linked homodimer on the cell surface (LI et al., 2001). The folding of NKG2D is similar to other C-type lectin–like NK receptor domains (NKDs). The secondary structure elements of canonical C-type lectins include two β sheets and two α helices, but the folding of NKG2D is more similar to that of CD94, which includes only one of the α helices (corresponding to α_1 in NKG2D) (LI et al., 2001). The major difference between the structures of NKG2D and true C-type lectins involve the NH_2-terminal disulfide bond-containing subdomain (which consists of the NH_2-terminal arms and loops and β strands 1 and 2 in NKG2D), which is very similar to a comparable structure in CD94 (LI et al., 2001).

In humans each NKG2D homodimer (FIGURE 9) associates with two homodimers of DAP10 to form a hexameric structure (GARRITY et al., 2005) which can signal by recruitment of phosphatidylinositol 3-kinase (STEINLE et al., 2001).

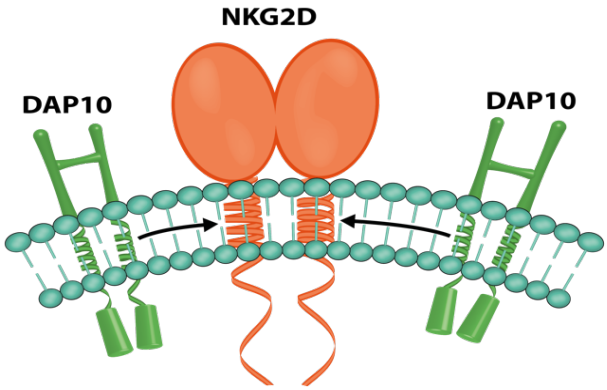


FIGURE 9 - NKG2D AND DAP10.
SOURCE: (RISTI and BICALHO, 2017)

In mice, the NKG2D-S isoform pairs with DAP12 in addition to DAP10 (DIEFENBACH et al., 2002; GILFILLAN et al., 2002) and there is no evidence that NKG2D forms heterodimers with CD94 in humans as it does in mice (VANCE et al., 1997).

Human NKG2D ligands (NKG2DLs) are MICA and MICB, and a group of glycosylphosphatidylinositol (GPI)-bound surface molecules including UL16 binding protein (ULBP)-1, -2, -3 and -4 (ZWIRNER et al., 2006), RAET1G (or ULBP5) and RAET1L (or ULBP6) (BACON et al., 2004; STEINLE et al., 2001). NKG2DLs differ in the way they are attached to the membrane. Human ULBP1, ULBP2, ULBP3, and ULBP6 and mouse retinoic acid-early Rae-1α-ε and H60c are attached to the cell surface membrane via glycosylphosphatidylinositol (GPI) anchors while human MICA, MICB, ULPB4, and ULBP5 and mouse H60a and H60b are transmembrane proteins and have cytoplasmic tails of varying length and sequences. It has been suggested that the membrane anchorage of NKG2D ligands might impact their affinity for lipid rafts (ELEME et al., 2004).

NKG2D receptor's signals transduction is mediated by the associated DAP10 dimer (FIGURE 10) (GARRITY et al., 2005) because NKG2D lacks a tyrosine-based inhibitory motif in its cytoplasmic tail (BAUER et al., 1999; GROH et al., 2001). The DAP10 dimer carries a pair of aspartic acid residues close to the center of the transmembrane (TM) domains, and a conserved arginine in the TM sequence of NKG2D is required for assembly with the DAP10 dimer (GARRITY et al., 2005). DAP10 does not have any ITAMs (GROH et al., 2001), but forms a disulfide-linked homodimer and its cytoplasmic domain carries a YINM motif (UPSHAW et al., 2006) that contains a YxxM motif (GARRITY et al., 2005) and a YxNx motif (UPSHAW et al., 2006) that bind, when phosphorylated, the p85 subunit of phosphatidylinositol-3 kinase (BAUER et al., 1999; GARRITY et al., 2005) or the adaptor Grb2 (UPSHAW et al., 2006) respectively. Because these two binding sites overlap, a single DAP10 chain will bind either p85 or Grb2, but not both (UPSHAW et al., 2006). The YxxM motif is also present in the cytoplasmic domain of CD28, and the phosphatidylinositol-3 kinase pathway is critical for the costimulatory function of this molecule in T cells (GARRITY et al., 2005). The Garrity et al. data demonstrate that the two basic TM residues interacts with a separate signaling dimer, and that the structural motif responsible for assembly is remarkably similar to the TCR–CD3 complex (GARRITY et al., 2005).

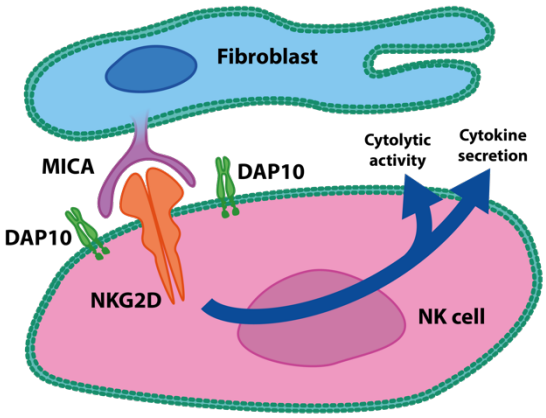


FIGURE 10 - NKG2D AND DAP10

SOURCE: Matilde Risti (2016)

The downstream consequences of the recruitment of Grb2 versus p85 after DAP10 cross-linking, however, are distinct. A DAP10 construct containing a mutated p85-binding site induces phosphorylation of SLP-76 (SH2 domain—containing leukocyte protein of 76 kDa), Vav1 and PLC-γ2, whereas a DAP10 mutant unable to bind Grb2, but able to recruit p85, fails to induce phosphorylation of these substrates, so in this pathway Vav1 is upstream of SLP-76 and PLC-γ2 (UPSHAW et al., 2006). The recruitment of Grb2 and p85 requires tyrosine phosphorylation of DAP10. There's two possible candidates for this phosphorylation, one might be by a Src family kinase, and the other may be the kinase Jak3 (HORNG et al., 2007). The events that occur downstream of PI3K and Grb2-Vav1 in the NKG2D signaling pathway in activated NK cells are less well characterized. In cultured human NK cell lines, stimulation with trimeric soluble recombinant NKG2D ligands (for example, ULBP-1 or ULBP-2) elicits phosphorylation of the kinases Jak2, Stat5, Akt, MEK1/2 and Erk, but not the kinases Jnk or p38 (SUTHERLAND et al., 2002).

It's interesting to report an Antoun et al. study that performed the first comparative population analysis of polymorphism within the *NKG2DL* gene family and observed significant differences in the frequency of several SNPs. They determined the frequency of 25 single nucleotide polymorphisms (SNPs) in the promoter and coding regions of genes of the *RAET1/ULBP* cluster in 223 Euro-Caucasoid, 60 Afro-Caribbean, and 52 Indo-Asian individuals to determine *NKG2DL* allele and haplotype frequencies within these populations. They showed differences in the frequency of NKG2DL SNPs and haplotypes among the three ethnic groups, and certain haplotypes were observed almost exclusively in Afro-Caribbean compared with the Euro-Caucasoid and Indo-Asian populations. Interestingly, the variation was focused within the RAET1E (*ULBP4*), *RAET1L*, and *ULBP3* genes, whereas the *ULBP1*, *ULBP2* and *RAET1G (ULBP5)* genes were highly

conserved. These findings suggest that individual *NKG2DL* alleles have been subject to divergent selective pressures during the migration of *Homo sapiens* (ANTOUN et al., 2010).

1.13.0 NKG2D EXPRESSION

NKG2D is a type II cell surface glycoprotein with a molecular weight of ~42 kDa, and is expressed by all human NK cells, δγ T lymphocytes, αβ $CD8^+$ T lymphocytes (ZWIRNER et al., 2006), interferon-producing killer DC (SEILER et al., 2007), invariant NKT cells (iNKT) cells and a small subset of effector or memory $CD4^+$ T cells (BAUER et al., 1999; JAMIESON et al., 2002; SAEZ-BORDERIAS et al., 2006). NK cell surface receptors (NCRs) belong to two structural families: the killer cell immunoglobulin-like receptors (KIRs), type I transmembrane glycoproteins containing one to three tandem immunoglobulin domains in the extracellular moiety, and the homo- and heterodimeric type II transmembrane glycoproteins containing C-type lectin–like NK receptor domains (NKDs) (LI et al., 2001).

Expression of NKG2D on NK cells and $CD8^+$ T cells can be modulated by cytokines due to their effects on transcription and posttranscriptional processing of NKG2D and DAP10. In humans, IL2, IL7, IL12 (PARK et al., 2011), IL15 and IFN-α (FERNANDEZ-SANCHEZ et al., 2013) upregulate NKG2D expression, whereas TGFβ (CODO et al., 2014; CRANE et al., 2010; FERNANDEZ-SANCHEZ et al., 2013), IFNβ1 (MUNTASELL et al., 2010), and IL21 (BURGESS et al., 2006) IL-4, IL-12, and IFN-γ (FERNANDEZ-SANCHEZ et al., 2013) downmodulate NKG2D. This downregulation is attributed to the overexposure to soluble or membrane-bound NKG2DLs, which promotes the internalization and subsequent degradation of the receptor or catabolites produced on macrophage activation (reactive oxygen species and L-kynurenine) (FERNANDEZ-SANCHEZ et al., 2013).

This is a possible explanation for the mechanism behind the oxidative stress, which is a common feature of chronic renal failure. ROS trigger the up-regulation of MICA and down-regulated NKG2D in NK cells *in vitro* study (PERALDI et al., 2009).

Also the availability of DAP10 is a decisive factor in NKG2D surface expression and the miRNAs expression can downregulate NKG2D expression in NK cells, damping its cytotoxic function (FERNANDEZ-SANCHEZ et al., 2013).

The Fernandez-Sanchez et al. research has shown for the first time that epigenetic mechanisms, such as DNA methylation and histone modifications, are involved in the regulation of NKG2D expression. They analyzed a region located around the translation initiation site between -992 and +263 positions of the *NKG2D* gene which including 11 CpG sites and they found, in a region localized between the positions -992 and -255, the greatest differences in the DNA

methylation patterns. The DNA methylation profile in *DAP10* gene was also analyzed, but no differences were found. Regarding *NKG2D*, they discovered that the acetylation of histone H3 lysine 9 acetylation H3K9 is important for correct NKG2D expression in NK and $CD8^+$ T cells, while DNA demethylation may be associated with an increased expression of NKG2D in $CD4^+$ T cells. $CD4^+$ T lymphocytes and T cell lines (Jurkat and HUT78) had a DNA methylation, while NKG2D-positive cells ($CD8^+$ T lymphocytes, NK cells and NKL cell line) had an unmethylated *NKG2D* gene and high levels of histone H3 lysine 9 acetylation (H3K9Ac).

It was observed that the histone acetyltransferase (HAT) inhibitor, Curcumin, reduced H3K9Ac levels in the *NKG2D* gene, downregulate NKG2D transcription and leads to a marked reduction in the lytic capacity of NKG2D-mediated NKL cells (FERNANDEZ-SANCHEZ et al., 2013).

Another interesting study by Karimi et al. discovered, in primary human NK and $CD8^+$ T cells, a novel splice variant of human NKG2D that encodes a truncated receptor lacking the ligand-binding ectodomain ($NKG2D^{TR}$). Overexpression of this truncated isoform severely attenuated cell killing and IFNγ release mediated by full-length NKG2D ($NKG2D^{FL}$). A specific knockdown of a $NKG2D^{TR}$ isoform enhanced NKG2D-mediated cytotoxicity, suggesting that $NKG2D^{TR}$ is a negative regulator of $NKG2D^{FL}$. At the biochemical level they demonstrated that $NKG2D^{TR}$ bound to DAP10 and interfered with the DAP10-$NKG2D^{FL}$ interaction. In addition, $NKG2D^{TR}$ formed heterodimers with $NKG2D^{FL}$ and negatively modulated $NKG2D^{FL}$ preventing its surface expression. So $NKG2D^{TR}$ constitutes a novel mechanism for regulation of NKG2D-mediated function in human $CD8^+$ T cells and NK cells (KARIMI et al., 2014).

Unlike $CD8^+$ T cells, TCR-mediated activation is not sufficient to induce NKG2D expression on $CD4^+$ T cells, and the factors responsible for induction of NKG2D on $CD4^+$ T cells are still unknown (KARIMI et al., 2014).

Saez-Borderias et al. provided the first evidence that a subset of HCMV (human CMV)-specific $CD4^+$ T cells displays NKG2D. CMV up-regulates the expression of MICA on infected cells, which provides the necessary co-stimulation for virus-specific CD28-deficient cytotoxic T cells through the engagement of NKG2D. This data suggested that $CD4^+NKG2D^+$ cells expanding in HCMV-stimulated cultures correspond to virus-specific memory T cells that have acquired NKG2D while losing CD28 (SAEZ-BORDERIAS et al., 2006).

1.14.0 NKG2D AND DISEASES

Just like the MICA gene, NKG2D is associated with several diseases as well. *NKG2D* alleles could be associated with cancer, colorectal cancer, mortality of bone marrow transplants,

aerodigestive tract cancer, primary scleorising cholangitis, lupus erythematosus, chronic hepatitis B, cholangiocarcinoma (Box 2). NKG2D expression could be associated with rheumatoid arthritis, type 1 diabetes, celiac disease, injury of the small intestine induced by poly (IC), breast cancer, cholangiocarcinoma, celiac disease, autoimmune encephalitis and multiple myeloma cells (Box 2).

Espinoza et al. genotyped, for *rs1049174,* 145 recipients with hematologic malignancies and their unrelated donors who were part of the Japan Marrow Donor Program (JMDP). They discriminated a single locus featuring a G-C substitution to distinguish between the *HNK1* (G) and *LNK1* (C) haplotypes of the *NKG2D* gene. The haplotype frequencies of *LNK1/LNK1*, *HNK1/LNK1* and *HNK1/HNK1* were respectively 43%, 42% and 15% in donors and 35%, 45% and 20% in recipients. The presence of the *HNK1* haplotype in the donors resulted in better overall survival (hazard ratio, 0.44; 95% confidence interval, 0.23 to 0.85; p=0.01 and transplant-related mortality (hazard ratio, 0.42; 95% confidence interval, 0.21 to 0.86; p=0.02). The researchers showed an association between the *NKG2D-HNK1* haplotype in unrelated donors of HLA-matched myeloablative bone marrow transplants and a significantly reduced transplant-related mortality, and better overall survival for their recipients with standard-risk disease (ESPINOZA et al., 2009).

Another study on the Japanese population analyzed 502 patients at the Aichi Cancer Center Hospital (ACCH) who were histologically diagnosed as having aerodigestive tract cancer. Controls were outpatients who never suffered any form of cancer nor had any prior history of neoplasia. Furue et al. found a protective effect of G allele in the NKG2D polymorphism (rs1049174), among people who never smoked (OR 0.35; 95% CI 0.15–0.84) and those who never drank alcohol (0.42; 0.19–0.94). On the contrary, increased risks were observed for G allele among heavy smokers (5.92; 3.23– 10.85) and heavy drinkers (4.13; 2.29–7.47). Interactions between NKG2D genotype and lifestyle exposure were statistically significant (interaction p 5 0.001 for smoking, 0.005 for drinking) (FURUE et al., 2008). In the Melmum et al. study they genotyped seven single nucleotide polymorphisms (SNPs) covering the *NKG2D* gene (rs1049174, rs2255336, rs11053781, rs12819494, rs728010, rs2617165, and rs2617167) in 365 Scandinavian (Norwegian) primary sclerosing cholangitis patients and 368 healthy controls. Primary sclerosing is a disease often complicated by the development of cholangiocarcinoma. The latter was found in 49 of the primary sclerosing patients (13.6%), and the diagnosis was confirmed by histology in all patients except for one. This data showed an increase in allele frequencies for two SNPs, rs11053781 and rs2617167, in patients with primary sclerosing cholangitis with cholangiocarcinoma compared to patients that only had primary sclerosing (MELUM et al., 2008). The Jensen et al. study analyzed nine HCMV seropositive and six HCMV seronegative healthy blood donors from the Blood Bank of the Copenhagen University Hospital (Denmark) and the Basel Swiss Blood Center. NKG2D expression

was normally absent from $CD4^+$ T cells, however some expression was detected on certain virus-specific $CD4^+$ T cells. In this case they tested 14 pathogens, and NKG2D cell surface expression was solely observed on human cytomegalovirus (HCMV) HCMV-primed $CD4^+$ T-cells. They observed a correlation between CD94 and NKG2D expression in the $CD4^+$ T-cells following HCMV stimulation. However, knock-down of CD94 did not affect NKG2D cell surface expression or signaling. In addition, they showed that NKG2D is recycled at the cell surface of activated $CD4^+$ T-cells, whereas it is produced *de novo* in resting $CD4^+$ T-cells (JENSEN et al., 2012).

The Ulbar et al. data suggests NKG2D-dependent reciprocal cross-talk between $CD34^+$ cells and allogeneic NK cells. NK cells participate in several hematopoietic stem cells (HSCs) diseases, suggesting a direct interaction with $CD34^+$ HSCs, and the NK cells can be directly activated by $CD34^+$ (ULBAR et al., 2016). Regarding the mouse models, an example of NKG2D function is reported in bone marrow transplantation. Treatment with a neutralizing antibody to NKG2D prevented rejection of parental BALB/c bone marrow in (C57BL/6 BALB/c) F_1 recipients and allowed engraftment of allogeneic BALB.B bone marrow in C57BL/6 recipients. NK cells reject the bone marrow cells expressing the NKG2D ligand, Rae-1, and the blockade with a neutralizing non-depleting NKG2D monoclonal antibody prevents rejection (OGASAWARA et al., 2005).

Another example using a mouse model is the Ito et al. study. They transferred CD4 T cells, which recognize alloantigens only through the indirect pathway and mediate rejection by a NK cell-dependent route. Inflammation induced by indirectly primed $CD4^+$ T cells leads to the upregulation of NKG2D ligands in the allografts. These $CD4^+$ T cells recruit and trigger activation of NK cells through the interactions of the NKG2D-activating receptor with its ligands on donor cells. These results showed that NKG2D blocking significantly prolonged survival but did not induce a permanent acceptance, probably because of the involvement of other activating NK receptors (ITO et al., 2008).

Disease	Allele association	Risk	Ethnic group	References
Colorectal cancer	LNK1/LNK1	Risk	Japanese	(FURUE et al., 2008)
	LNK1/HNK1 and HNK1/HNK1	Protective		
Cancer	HNK1/HNK1	Protective	Japanese	(HAYASHI et al., 2006)
	LNK1/LNK1	Risk		
Mortality of Bone Marrow Transplants	HNK1 haplotype	Protective	Japanese	(ESPINOZA et al., 2009)
Aerodigestive tract cancer	HNK1 haplotype	Protective	Japanese (never smokers and drinkers)	(FURUE et al., 2008)
	HNK1 haplotype	Risk	Japanese (heavy smokers and drinkers)	
Primary sclerosing	rs11053781 (G/G)	Risk	Scandinavian	(MELUM et al., 2008)

cholangitis	rs2617167 (A/A)	Risk		
Lupus erythematosus	rs2255336 (G/G)	Risk	German and Spanish	(KABALAK et al., 2010)
Chronic hepatitis B	rs2617160 (T/T)	Risk	Han Chinese population	(MA et al., 2010)
	rs2717160 (T/A)	Protective		
Cholangiocarcinoma	rs11053781 (A allele)	Protective	Norwegian	(MELUM et al., 2008)
	rs2617167 (G allele)	Protective		
Disease	Risk / Activation pathway		Ethnic group/species	References
Rheumatoid Arthritis	NKG2D activation pathway participates in the development of immune-mediated disorders.		North-American/Caucasoid, humans	(GROH et al., 2003)
Celiac Disease	NKG2D activation pathway participates in the development of immune-mediated disorders.		North-American/Caucasoid, humans	(MERESSE et al., 2004)
Type 1 Diabetes	NKG2D activation pathway participates in the development of immune-mediated disorders.		Sweden, Basque. Humans	(BILBAO et al., 2002; GONZALEZ et al., 2001)
	NKG2D activation pathway doesn't participate in the development of this disorder.		Mouse	(GUERRA et al., 2013)
Injury of the small intestine induced by poly(I:C)	NKG2D activation pathway doesn't participate in the development of this disorder.		Mouse	(GUERRA et al., 2013)
Breast cancer	High risk with low NKG2D expression		Iranian, humans	(ROSHANI et al., 2015)
Autoimmune encephalitis (EAE)	Low risk with NKG2D deficient expression		Mice	(GUERRA et al., 2013)
Cholangiocarcinoma	Low risk with high NKG2D expression		Japanese, humans	(TSUKAGOSHI et al., 2016)
Multiple myeloma cells	NKG2D expression is associated with risk		Cell lines	(SORIANI et al., 2009; WU et al., 2012)
Type 1 diabetes	Aberrant NKG2D signaling is associated with risk		USA, humans	(QIN et al., 2011)

BOX 2 - NKG2D AND ASSOCIATED DISEASE

1.15.0 MICA-NKG2D

The crystal structure of the MICA-NKG2D complex has revealed that NKG2D binds as a homodimer to one molecule of MICA. One of the NKG2D molecules binds mostly to the α1 domain of MICA, while the other NKG2D molecule binds mostly to the α2 domain of MICA (LI et al., 2001; ZWIRNER et al., 2006).

The NKG2D homodimer overlays MICA diagonally in a similar way as the αβTCR overlays the MHC class I molecules. The central section of the α2 domain of MICA (residues 152-161), disordered in the crystal structure of isolated MICA, is ordered when bound to NKG2D and forms part of the interface between these 2 molecules (ZWIRNER et al., 2006).

MICA glycosylation was not essential but enhanced complex formation with NKG2D. In fact bacterially produced MICA formed less stable complexes than MICA derived from insect cells, suggesting that glycosylation enhanced the interaction with NKG2D. However, of the three N-linked glycosylation sites in the MICA $\alpha_1\alpha_2$ domain Asn8, Asn56, and Asn102, none is present in MICB which also bound strongly to NKG2D. This suggesting that they may have an accessory but not a pivotal role in the MIC-NKG2D interaction. Likewise, the glycosylation state of NKG2D, which was purified from insect cell supernatant or refolded from bacterial inclusion bodies, had no substantial effect on complex formation (STEINLE et al., 2001).

The MICA-NKG2D system is a versatile ligand-receptor pair since NKG2D can act as primary receptor or costimulatory molecule during anti-tumor immune responses, infection or autoimmunity (ZWIRNER et al., 2006). For example it has been shown that endothelial MICA triggered an activating signal in allogeneic polyclonal NK cells through the immunoreceptor NKG2D that may accounted for a significant part in endothelial cells lysis by allogeneic NK cells. In coculture assays in vitro, endothelial MICA interacting with NKG2D provided an immunosuppressive pathway by downregulating NKG2D on the NK cell surface (CHAUVEAU et al., 2014).

The Boukouaci et al. data suggested that the endocytosis of NKG2D receptor, upon binding to sMICA, is considerably more rapid than the replenishment of cell surface NKG2D by de novo synthesis. They also found that sMICA down regulates NKG2D receptor on $CD8^+$ T cells. sMICA up-regulates the IFNγ production only by cytokines-activated-NK cells, while it has no effect on non-activated cells, triggering of NKG2D alone was not sufficient to induce NK cell IFN-γ production. The researchers demonstrated that the sMICA molecules upregulate IFN-γ expression by interleukin-12/interleukin-18-activated CD3 $CD56^+$ natural killer cells, witnessing the pro-inflammatory effect of soluble MICA (BOUKOUACI et al., 2013).

In the Zhang et al. study on the mouse model, they showed that Sirt6 deficient animals show an aging-like phenotype, while male transgenic mice of Sirt6 show increased longevity. Sirt6 is a member of the class III histone deacetylase family and it has been demonstrated to increase NKG2D ligand expression in macrophages and endothelial cells, leading to NK cell activation and increased levels of inflammatory cytokines in NK cells. This *in vivo* evidence proved that Sirt6 is involved in atherosclerosis development. Sirt6 heterozygous mice showed exacerbated atherosclerosis and exhibited more features of instable atherosclerotic plaques than wild-type mice (ZHANG et al., 2016).

Another study with the mouse model found that Lewis rat hearts transplanted into BALB/c mice developed typical acute rejection (AR) in 6 days. The level of severity of xenograft rejection

increased with time, from 2 to 6 days. In the same way the MICA protein and MICA mRNA reached their highest value after 6 hours. The prevalence of anti-MICA was significantly higher among those with severe acute rejection. However, sMICA was significantly increased during AR cat 2 hours, then gradually decreased, and reached the lowest value after 6 hours (YU et al., 2015).

1.15.1 NKG2D-MICA, SMICA, TUMORS AND ACCEPTANCE

It seems to be impossible but in a certain way, tumor and transplant are correlated. In fact they are both the result of not-self acceptance. Aside from this, cancer can take advantage of the compromised immunological condition that comes from transplantation.

This theory that cancer may arise under conditions of reduced immune capacity is supported by observations in humans with immune deficiencies such as those that occur following organ transplantations (KIM et al., 2007). Kidney transplantation increases the risk of cancer both in the short and in the long term, consistent with the theory that an impaired immune system allows carcinogenic factors to act (KIM et al., 2007). In the Piselli et al. study they analyzed 7217 kidney transplant recipients and follow-up for an average of 5.2 years. During the period of observation, 395 cancers were diagnosed in 382 recipients. For all cancers and for solid tumors, the risk steadily increased with time after transplant: 4.8% after 5 years and 9.9% after 10 years for all cancers, and 3.1% and 7.2% for solid tumors. They found that immunosuppression was the reason of an elevated risk for a large spectrum of cancers (PISELLI et al., 2013). Another study by Kaufmann et al. performed statistical analysis on 376 kidney transplant recipients screened for non-melanoma skin cancers (NMSC), the most common malignancies in kidney transplant recipients. NMSC developed in 23.67% of individuals with a squamous cell carcinomas (SCC) / basal cell carcinomas (BBC) ratio of 2.15:1 and an age-standardized incidence ratio (IR) of 2.71 cases (95% CI: 1.97-3.46) per 100 patients/year. Calcineurin inhibitors were associated with higher NMSC incidence (IRR = 2.81; 95% CI: 1.1-7.01; $p = 0.03$), while no difference was seen with the mammalian target of rapamycin (mTOR) inhibitors (KAUFMANN et al., 2016).

MICA expression has been observed in different epithelial and non-epithelial tumor cell lines and freshly isolated tumors of different histotypes like lung, breast, kidney, ovary, prostate, colon carcinomas, melanomas and acute myeloid leukemia, some T-cell acute lymphoblastic leukemia and multiple myeloma cells (ZWIRNER et al., 2006). Upon engagement by MICA or others NKG2DLs, NKG2D delivers an activating signal that results in the release of lytic enzymes (perforin or granzymes) and antitumor cytokines like tumor necrosis factor α (TNFα) (FERRARINI et al., 2002; GONZALEZ et al., 2008). In cancer, the MIC–NKG2D interaction should activate antitumor NK and T cell responses, but NKG2D is reduced in both $CD8^+$ tumor-infiltrating T cells

and peripheral blood T cells when associated with circulating tumor-derived soluble MICA (sMICA) (CHOY and PHIPPS, 2010).

MICA, MICB, and ULBPs can be cleaved (and hence released in the extracellular milieu) by ERp5 as well as by ADAM10 and ADAM17 (FERRARINI et al., 2002; GONZALEZ et al., 2008; KAISER et al., 2007; ZOCCHI et al., 2012). ADAMs are cell surface enzymes involved in cell adhesion and proteolysis. A prototypical ADAM comprises an N-terminal pro-domain that maintains the enzyme in a latent state, followed by metalloproteinase, disintegrin, cysteinrich, and EGF-like domains, a transmembrane region and a cytoplasmic tail of variable length (RZYMSKI et al., 2012).

The cleaved MICA then interacts with NKG2D, which, in turn, induces the internalization and degradation of the receptor and stimulates the population expansion of immunosuppressive $NKG2D^{+}CD4^{+}$ T cells (CHOY and PHIPPS, 2010). The shedding of MICA by tumors constitutes an immune escape mechanism that makes the cytotoxic cells "blind" to the presence of MICA on the tumor cells and that explains the low levels of surface MICA on highly aggressive, end-stage human tumors (ZWIRNER et al., 2006). For this reason soluble NKG2DLs bind to NKG2D but they are unable to deliver activating signals (FERRARINI et al., 2002; GONZALEZ et al., 2008). The overexpression of these sheddases has been reported in patients with multiple myeloma and other tumors, including Hodgkin's and non-Hodgkin's lymphomas (ZOCCHI et al., 2012).

Studies reported shedding of MICA A5.1 proteins into exosomes as a mechanism of immune escape used by some tumor cells lines (ASHIRU et al., 2010). The Ashiru et al. study showed that MICA molecules could be released from a tumor cell line in more than one way. For example *MICA*008* was released from cells as a membrane-anchored molecule in exosomes, but the alterative for MICA molecules was to be released by proteolysis. The way MICA was secreted on exosomes could be an alternative pattern for immune evasion because an NK cell exposed to MICA*008 containing exosome triggers a marked loss of cytotoxic function (ASHIRU et al., 2010).

The overexpression of microRNAs binding MICA or MICB 3'UTR could be a mechanism of tumorigenesis, and downregulate MICA and MICB expression. They are also evidence that microRNAs are involved in an immunoevasion mechanism and it would probably be useful at early stages of tumor growth (STERN-GINOSSAR et al., 2008).

The tumor-released sMICA plays a very important role for a strong decrease in NKG2D cytotoxicity of activated haploidentical dNK cells in an *in vivo* study on pediatric patients suffering for neuroblastoma, as it was demonstrated that soluble NKG2D ligands drastically reduce the cytotoxicity of activated dNK (KLOESS et al., 2010). In the breast cancer field the Roshani et al.

study demostrated a direct association between serum sMICA levels, detected by ELISA, and the stage of the disease. They also found an inverse relationship for level of expression and presentation of NKG2D receptors on the NK peripheral blood cells (ROSHANI et al., 2015). Bargostavan et al. on 2016 confirmed that the MICA/B mean serum levels were significantly elevated in patients compared with healthy individuals ($p<0.05$). They also found that there wasn't a significant difference between the control group and the patients for the matrix metallopeptidase 9 (MPP9) polymorphisms (-1562 C/T) ($p>0.05$) (BARGOSTAVAN et al., 2016). The Zhao et al. study reported that for the urothelial carcinoma patients the sMICA levels were significant higher than in the control group ($P < 0.05$) (ZHAO et al., 2015). Zingoni et al. demonstrated that genotoxic agents used in the therapy of human multiple myeloma (MM) (doxorubicin and melphalan) promoted the proteolytic cleavage of MICB or MICA*019 molecules, but they didn't affect the MICA*008 shedding. This allele is frequent in caucasian populations. They also found that the ADAM10 expression is upregulated by chemotherapeutic treatment (ZINGONI et al., 2015). In the gastric cancer field Chen et al. demonstrated that, in patients who had D2 gastrectomy, the high tumor MICA expression was a predictive biomarker of their positive response to Cytokine-induced killer (CIK) cells therapy to adjuvant chemotherapy. In general, patients who have high MICA levels have a Disease-free survival (DFS) and Overall Survival (OS) longer time than patients who had low MICA levels (CHEN et al., 2016). Instead, Shiraishi et al. demostrated the inverse correlation between MMP-9 expression and MICA, MICB, ULBP-2, and ULBP-3 expression in a panel on *in vitro* culture of gastric cancer (SHIRAISHI et al., 2016). Finally Chen et al. discovered that polypeptides extracted from scorpion venom (PESV) could up regulated MICA expression in HepG2 (human liver cancer cell line) cells, suggesting that it might benefit tumor cell identification by NK. The PESV treatment in C57BL/6 mice that had been injected with H22 (hepatocarcinoma) cell line in the left liver, demonstrated that the average survival time was significantly higher than the control group (H22 tumor-bearing mice without PESV). They also found that PESV could up-regulated NKG2D expression in both liver and spleen. These findings showed that PESV could up-regulate the NKG2D-MICA pathway, inducing more cytotoxic granules for killing tumor cells (CHEN et al., 2016).

1.16.0 MICA AND HLA-G IN KIDNEY TRANSPLANT

A thorough review of the MICA and NKG2D role in the renal transplant can be read in the manuscript "MICA and NKG2D: is there an impact on kidney transplant outcome?" (RISTI and BICALHO, 2017), annexed in the results.

Focusing instead on HLA-G and MICA, there are actually few papers that are considering them in the context of kidney transplant and, in general, that are considering these two molecules together. In Qui et al.'s study they found that sHLA-G was negatively associated with allograft failure from chronic rejection and sHLA-G had a negative relationship with HLA IgG antibodies production. Regarding MICA, the authors only investigated MICA antibody (Ab). 35.9% (23/64) of all Ab^+ MICA sera detected positive for sHLA-G, while the remaining 64.1% (41/64) was negative (p = NS)(QIU et al., 2006). Interestingly, when MICA antibodies are present there are few chances of detecting sHLA-G. In another study, Racca et al. didn't find any correlation between MICA mRNA levels and the graft state. Still, they measured HLA-G1 mRNA expression levels in biopsies from patients with favorable outcome after acute rejection, and this data suggests the participation of such isoform in immune mechanisms that operate to overcome the rejection process. The mechanism by which HLA-G could be induced remains to be elucidated (RACCA et al., 2009). As evidenced, no manuscript emerges from literature that relates MICA and the HLA-G molecules in the serum with kidney transplant.

There are others manuscripts that relate HLA-G and MICA in other contests; for example in cervical cancer patients sHLA-G did not show any influence in the survival, regardless of histology. However, patients with adenocarcinoma that has shown high sMICA levels have an increased disease-free survival and disease-specific survival (SAMUELS et al., 2015). In hepatocellular carcinoma tissues it was found that the expressions of HLA-G, HLA-E, MICA and MICB were up-regulated (CHEN et al., 2011), and in the M8 melanoma cell line the expression of MICA triggered NK cell tumor lysis and HLA-G1 co-expression mediated the inhibition of NK cytotoxicity by mitigating the MICA activating signal. HLA-G1 and MICA expression in a tumor cell line appear to be a powerful way to turn off NK cells, supporting the emerging idea that the balance between positive and negative NK cytolysis signals critically influences tumor progression (MENIER et al., 2002). In light of these discoveries there is a clear, but still unexplored, correlation between HLA-G and MICA, especially in the area of kidney transplantation, a field in which the immunological balance of the transplant receptor is extremely delicate and complex.

1.17.0 THERAPEUTIC PERSPECTIVES

In regards to therapeutic perspectives for tumors it might be useful to adopt screening strategies that look for drugs or compounds that can inactivate ERp5 and soluble MICA (CHOY and PHIPPS, 2010). It could be possible to block ERp5 with peptides that simulate the MICA α_3 domain, hindering the interaction between the enzyme and its substrate (WANG et al., 2009).

Pharmacological inhibitors for ADAM10 and ADAM17 with variable selectivity have already been developed (NUTI et al., 2010). Increased methylation of the promoter of the endogenous metalloprotease inhibitor (TIMP3) is known to mediate downmodulation of TIMP3 expression in cancer (ESTESO et al., 2014) so it may be possibly used in a therapeutic way.

Regarding transplantations, sMICA showed a negative association with acute rejection in heart transplantation and may be a good predictor of transplant outcomes (YU et al., 2015).

Antibodies against MICA could be used as therapeutics because they could reduce levels of circulating soluble MICA, antagonize immune suppression and stimulate antitumor cytotoxicity (CHOY and PHIPPS, 2010). It's also important to follow MICA antibody screenings for donors and patients, especially those who have undergone re-transplantion, because they could identify the risk for graft dysfunction and may influence the management of transplant survival (COX et al., 2011). In the Narayan et al. study they found that MICA Abs are not detected using current crossmatch procedures, and the quantification and monitoring of MICA MFI (mean florescent intensity) levels could change the way allograft rejection is dealt with in hospitals and should be the focus of large randomized, prospective trials (NARAYAN et al., 2011).

Regarding the HLA-G molecule, studies in vitro and in vivo demonstrate that this molecule is involved in the development of immune tolerance against allogeneic antigens. If these data will be confirmed, the next step should be to establish the therapeutic role of HLA-G. To this end functionally active HLA-G structures have to be produced with good manufacturing practice production conditions and used to favour a successful transplantation (REBMANN et al., 2014).

2.0.0 JUSTIFICATION OF STUDY

Success in clinical transplantations depends on HLA matching, immunosuppressive drugs and technical skill. However, even if HLA matching were accurate it would not prevent graft rejection, since if it were possible to match donor and recipient at every locus of the HLA, some tissue incompatibility would still remain (IMAI et al., 2012). Currently used immunosuppressors block the immune system in a non-specific way. Considerable research work has been focused on achieving an effective mechanism to specifically prevent this allorecognition without affecting the host immune system, thus contributing to allograft tolerance (OPELZ, 2005).

At present time increasing the success rate of transplants avoiding rejections is still a challenge. However, many factors influence patients' prognostic. Thus, it is important identify the main variables able to predict rejection. The exploration of MICA and HLA-G, a non-classical MHC-I molecule, can be useful in predicting the transplant outcome. Their expression exhibits a tissue and cell specific regulation that plays a relevant role in immunoregulation. HLA-G has been associated with an immunosuppression response, while MICA with a stress immune response.

Considering the described opposite immunomodulation effects of MICA and HLA-G, it is important to investigate the cluster levels of both molecules (sMICA and sHLA-G) as well as MICA, NKG2D (MICA receptor) and HLA-G genotypes in kidney transplanted patients.

In recent years the main goal has been to look for other approaches to monitor in a noninvasive manner the immune response (MALYSZKO and MALYSZKO, 2017). For these reasons, molecules research, that are easily measured, widely available and cost-effective (how they may be sMICA and sHLA-G plasma levels), are very important to improve clinical diagnostics. Furthermore, we developed a multivariate study evaluating 40 noninvasive variables in order to select the most relevant ones. This is an important approach when we consider the transplant outcome, as it is nearly impossible to select few variables for a complexity of an immune transplant outcome.

3.0.0 OBJECTIVES

3.1.0 GENERAL OBJECTIVE:

Investigate the relationship between HLA-G and MICA molecules and other noninvasive variables on kidney transplantation, and create a risk model for the kidney transplant immunological risk.

3.2.0 SPECIFIC OBJECTIVES:

1. Bibliographic review of the MICA and NKG2D genes, and their relevance in kidney transplantation.
2. Selection of the most important non-invasive variables that allow us to create a risk model for the kidney transplant immunological risk.
3. Studying the phenotype-genotype association between the *MICA* and *HLA-G* genes and their plasma levels.
4. Biological significance evaluation of soluble MICA and HLA-G (sMICA, sHLA-G) levels variation in the transplant patients' plasma (from pre- to post-transplant), and in both patients with chronic kidney disease population and control patients.
5. Solving MICA's ambiguities by using its linkage disequilibrium with the HLA-B gene.

4.0.0 MATERIALS AND METHODS

The Ethics Committee of the Hospital and of the Federal University of Paraná, Brazil approved this study (Ethics Committee approval number: 53627315.0.0000.0102), and all participants signed a voluntary and informed consent form (TCLE) (Appendix 1: TCLE for transplanted patients, Appendix 2: TCLE for chronic patients, Appendix 3: TCLE for control group).

4.1.0 TRANSPLANT PATIENTS

A total of 67 patients undergoing kidney transplantation at the Kidney Transplant Sector of the Hospital "Universitàrio Evangélico do Paranà" (Curitiba, Paraná, Brazil) from 2012 to 2013 were included in this study. Patients underwent transplantation with allografts from both living and deceased donors. Pairs of donors and recipients were selected and characterized according to the data obtained from medical records.

The blood samples from each transplanted patient who underwent a transplant have been categorized into five periods, according to the time of collection:
PRE period: The blood sample has been collected a few days before transplantation
Period 1: The blood sample has been collected 1st week after transplantation
Period 2: The blood sample has been collected 2nd week after transplantation
Period 3: The blood sample has been collected 1st month after transplantation
Period 4: The blood sample has been collected 2-3 months after transplantation

Some clarification should be made about the data being collected. For example, the type of donor was used to determine whether the donation was made during life or after death of the donor. The degree of relationship was considered from the first to the fourth degree, all others were considered to have no relationship with or degrees of relationship unknown. The disease may include the basal glomerular diseases, kidney disease, kidney failure, high blood pressure, diabetes, cancer, etc. The typing of HLA determines the match of the *HLA* (matches, designated with M) of the loci of *HLA-A, HLA-B* and *HLA-DRB1*, as regards the compatibility of the donor recipient. HLA-identical pairs of donor and recipient are the same groups of alleles for the three HLA loci analyzed (equivalent to 6 M) and zero represents the incompatibility or zero mismatches (MM). The pairs of *HLA* haploidentical who have some shared haplotypes are represented as 3-5 M, while couples with different *HLA* are represented from 2 M to 0 M.

4.2.0 CHRONIC KIDNEY DISEASE PATIENTS AND CONTROL GROUP

We also recruited 32 chronic kidney disease patients never transplanted during their routine

checks at the at the Kidney Transplant Sector of the Hospital “Universitàrio Evangélico do Paranà” from 2013 to 2015 and 79 control subjects who did not have kidney disease during LIGH (Immunogenetics and Histocompatibility Laboratory, Federal University of Paraná) sensibilisation campaigns for organ and bone marrow donation from 2014 to 2016.

For the chronic patients and control group the collect of blood sample was only one, after they agreed at the free and inform consent (TCLE). Individuals form control group have been selected according to the data obtained from a survey form (Appendix 4).

4.3.0 SAMPLING

Blood samples have been assembled at the Laboratory of Immunogenetics and Histocompatibility (LIGH) of the Genetics Department, Federal University of Paraná, UFPR (Brazil).

Transplanted patients' blood samples were obtained before transplantation and serially drawn up to 3 months after transplants. The blood samples were drawn at the same time as the routine samples for clinical tests, such as DSA detection and serum creatinine.

The blood sample of transplanted patients, chronic patients and control group was collected in ethylene-diamine-tetra-acetic acid (EDTA) tubes; after this, the samples were centrifugated at 1000g for 15 minutes. The plasma obtained was stored at -80°C and buffy coats at -20°C until use.

In total, 67 transplanted patients were genotyped for MICA, NKG2D and HLA-G gene and a subgroup of 28 patients (due to problems in blood collection quality) was measured for sHLA-G and sMICA plasma levels. 32 chronic patients and 79 individuals from the control group were genotyped for MICA, NKG2D and HLA-G gene, and their sHLA-G and sMICA plasma levels were measured.

4.4 MICA AND HLA-G ANALYSES

4.4.1 MICA AND HLA-G GENOTYPING

Peripheral blood DNA extraction was carried out with the salting out technique, described by Lahiri and Nurnberger (LAHIRI and NURNBERGER, 1991).

For the characterization of the MICA gene, the Polymerase Chain Reaction - Sequence Specific Probes oligonucleotide (PCR - SSOP) method was used, through the Kit LABType MICA (One Lambda Inc.). This method uses the LUMINEX ® technology and the reverse typing by SSO DNA. This technology consists of a multi-analysis system that works with color-coded microspheres of different wavelengths, which are analyzed in an advanced fluorimeter flow. Microspheres are discriminated by color, and each colored microsphere contains on its surface a

specific oligonucleotide probe. For this reason, different microspheres can be used on a single tube, replacing the conventional format of multi-well. All the kit LABType MICA procedures was conducted in accordance with the manufacturer's instructions.

On the LUMINEX ® technology the target region of the DNA was amplified by the oligonucleotides initiators biotinylated. The specific LABType kit for the PCR contains a Dmix (buffer, dNTPs, $MgCl_2$) and a solution with the oligonucleotides initiators for the hybridization of the specific exon or intron of each gene. The PCR was performed with a PerkinElmen 9600 thermocycler. Each labeled PCR product was denatured, and held as simple strand through the use of tampons. The PCR product in simple strand was re-hybridized with DNA complementary probes (sequence-specific oligonucleotide probes), conjugated to fluorescent microspheres. After the beads washing, the biotinylated DNA (from the sample) was labeled with a R-Phycoerythrin/Streptavidin (SAPE). The reactions was read using a flow fluorimeter (LABScanTM 100), which employs the LUMINEX ® technology, in which a red laser with a wavelength of 633nm recognizes the color of the beads and another green laser with wavelength of 532nm recognizes the intensity of the fluorescence emitted from the phycoerythrin present on the surface of each microsphere, detecting if the bead was labeled with SAPE, and thus determining the positivity. The analyses were performed with the HLA Fusion software. MICA A5.1 and MICA-129 are investigated with specific beads.

DNA samples were genotyped by sequence-based typing (SBT) for the *HLA-G* gene, using the following primers amplified in a single reaction: i) exon 2 (5′:GGGTCGGGCGGGTCTCAA, used to perform amplification and sequencing reactions, and 3′:TCCGTGGGGCATGGAGGT, used just to perform amplification reaction; ii) exon 3 (5′:CCCAGACCCTCTACCTGGGAGA, used just to perform amplification reaction, and 3′:CTCTCCTTGTGCTAGGCCAGGCTG, used to perform amplification and sequencing reactions; and iii) exon 4 (5′:CCATGAGAGATGCAAAGTGCT, used to perform amplification and sequencing reactions, and 3′:TGCTTTCCCTAACAGACATGAT, used just to perform amplification reaction), as adapted from previous work (NARDI et al., 2012). All sequences were obtained using ABI Prism Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA) and analyzed through *ABI Prism SeqScape* (Applied Biosystems) software, based on the official 53 alleles listed in the International Immunogenetics Information System (IMGT). The resolution analysis was performed on typing results at the second field of resolution and genotypes were also separated according to their P group, which concerns IMGT genetic division that is based on exon 2 and 3 variations from *HLA-G* gene (ROBINSON et al., 2015).

4.4.2 SOLVING MICA GENOTYPE AMBIGUITIES

MICA genotype was performed using the PCR-SSOP method, amplifying exclusively exons 2, 3, 4 and 5 according to the manufacture descriptions. This methodology can generate ambiguities at an allelic level, which were resolved using MICA's linkage disequilibrium with HLA-B with the tools available on The Allele Frequency Net Database <http://www.allelefrequencies.net>.

We also develop another strategy to resolve the ambiguities generated by the PCR-SSOP methodology. Instead of using Sequence Specific Primers (SSP) we propose to solve ambiguities via the classic Sanger sequencing method. MICA typing by PCR-SSP involves amplifying a target DNA sequence with pairs of primers studied so as to be perfectly complementary only to a single allele or a group of alleles. Instead we designed primers that pair with DNA sequences that are common to all known alleles. These primers capable of discriminating nucleotide variations at exons 2, 3, 4 and 5 of the MICA gene are:

- MICA_3F557-CTATCACGCTATGCATGCAGA,
- MICA_3R553 -TAGTTCCTGCAGGCAGTCTGC,
- MICA_2R305-CTTCTTTCTGGTCCTTGATAT,
- MICA_4F779-GGGGGATGTCCTGCCTGATGG,
- MICA_4R759-CCATCAGGCAGGACATCCCCC,
- MICA_5F915-AAAGTGCTGGTGCTTCAGAGT.

4.4.3 SOLUBLE MICA AND HLA-G MEASUREMENTS

A soluble form of MICA (sMICA) was detected in pre and post-transplant sera from all patients and controlled by using a customized sandwich immunoassay according to the manufacturer's protocol (R&D Systems®).

The Capture Antibody was diluted to the working concentration in PBS without carrier proteins. Immediately a 96-well microplate has been coated with 100 µL per well of the diluted Capture Antibody. The plate was sealed and incubated overnight at room temperature. Each one was aspirated and washed with Wash Buffer. The plates were blocked by adding 300 µL of Reagent Diluent to each well, and they were incubated at room temperature.

100 µL of sample or standards in Reagent Diluent were added, per well. The plate was then covered with an adhesive strip and incubated for 2 hours at room temperature.

100 µL of the Detection Antibody, diluted in Reagent Diluent with NGS, were added to each well. The plate was then covered with a new adhesive strip and incubated for 2 more hours at room temperature.

100 µL of the working dilution of Streptavidin-HRP were added to each well. The plate was covered and incubated for 20 minutes at room temperature.

100 µL of Substrate Solution were added to each well and incubated at room temperature. Finally 50 µL of Stop Solution were added to each well, then the optical density of each well was immediately determined using a microplate reader set to 450 nm.

Soluble HLA-G was measured by ELISA in plates of 96 wells coated with the capture antibody MEM-G/9 and monoclonal anti-human β2-microglobulin antibody labeled with horseradish peroxidase for detection, as provided by the sHLA-G Kit from Exbio (Czech Republic). This reaction recognizes sHLA-G1 and HLA-G5 isoforms. In the same way, sMICA was measured by ELISA according to the manufacturer's protocol from the DuoSet MICA ELISA kits (R & D Systems, Minneapolis, MN, USA). After several washes, plasma samples and calibrators were added to each well (100mL) in duplicate and the plates were incubated for 16 hours. The labeling was performed with the sHLA-G (Exbio Kit) and the optical densities were measured at 450 nm using ELX-800 Universal Microplate Reader (Bio-Tek Instruments Inc., Winooski, VT, USA). The final concentration was determined by optical density compared to the standard curves. The mouse monoclonalantibodies (mAb) used was MEM-G/9, which is specific for HLA-G molecules associated with β2-microglobulin. It recognizes not only membrane-bound HLA-G1 isoform, but also its soluble counterpart sHLA-G1 and the secreted isoform HLA-G5.

Measurements that fell at or below the minimum detectable dose specific to each kit were considered not detectable (sHLA-G under 6.25 ng/mL; sMICA under 31.25 pg/mL).

4.5.0 NGK2D ANALYSES

The NGK2D gene was typed using the allelic discrimination TaqMan® method with an RT-PCR.

The concentration of DNA is 20ng/ul for the PCR realization. For this step the TaqMan® universal Master Mix (Applied Biosystems) was used. This Master Mix comes with the DNA polymerase (Ampli Taq Gold Dna polymerase) and other useful solutions for PCR.

For SNP genotyping, specific probes were used (product number C_9345347_10, Applied Biosystems), with a 20X dilution in a 1X TE buffer. The probes detected a G-C replacement for the discrimination of allelics haplotypes HNK1 and LNK1. Each probe TaqMan® SNP genotyping assay consists of two primers for the polymorphic sequence of interest amplification and two TaqMan probes (TaqMan® MGB probes) for the distinction of the alleles.

Thermal cycling started with a 2 minutes long incubation at 50°C, followed by a first denaturation step of 10 minutes at 95°C, and then by 45 cycles of 15 seconds at 92°C and finally 1

minute at 60°C. After PCR was completed, plates were brought to room temperature, to be read in a Realplex (Eppendorf) where fluorescence data from the endpoint was acquired (at 60°C for 2 minutes).

5.0.0 RESULTS

5.1.0 REVIEW: “MICA and NKG2D: is There an impact on Kidney Transplant Outcome?”

frontiers in Immunology

REVIEW
published: 27 February 2017
doi: 10.3389/fimmu.2017.00179

MICA and NKG2D: Is There an Impact on Kidney Transplant Outcome?

*Matilde Risti and Maria da Graça Bicalho**

LIGH – Immunogenetics and Histocompatibility Laboratory, Federal University of Paraná, Curitiba, Brazil

This paper aims to present an overview of MICA and natural killer group 2 member D (NKG2D) genetic and functional interactions and their impact on kidney transplant outcome. Organ transplantation has gone from what can accurately be called a “clinical experiment” to a routine and reliable practice, which has proven to be clinically relevant, life-saving and cost-effective when compared with non-transplantation management strategies of both chronic and acute end-stage organ failures. The kidney is the most frequently transplanted organ in the world (transplant-observatory[1]). The two treatment options for end-stage renal disease (ESRD) are dialysis and/or transplantation. Compared with dialysis, transplantation is associated with significant improvements in quality of life and overall longevity. A strong relationship exists between allograft loss and human leukocyte antigens (HLA) antibodies (Abs). HLA Abs are not the only factor involved in graft loss, as multiple studies have shown that non-HLA antigens are also involved, even when a patient has a good HLA matche and receives standard immunosuppressive therapy. A deeper understanding of other biomarkers is therefore important, as it is likely to lead to better monitoring (and consequent success) of organ transplants. The objective is to fill the void left by extensive reviews that do not often dive this deep into the importance of MICA and NKG2D in allograft acceptance and their partnership in the immune response. There are few papers that explore the relationship between these two protagonists when it comes to kidney transplantation. This is especially true for the role of NKG2D in kidney transplantation. These reasons give a special importance to this review, which aims to be a helpful tool in the hands of researchers in this field.

Keywords: transplantation, kidney, allograft, MICA, MICA-129, NKG2D, LNK1, HNK1

OPEN ACCESS

Edited by:
Gianfranco Pittari,
Hamad Medical Corporation, Qatar

Reviewed by:
Nicolas Dulphy,
Paris Diderot University, France
Myra Coppage,
University of Rochester Medical Center, USA

****Correspondence:***
Maria da Graça Bicalho
ligh@ufpr.br

Specialty section:
This article was submitted to Alloimmunity and Transplantation, a section of the journal Frontiers in Immunology

Received: *29 November 2016*
Accepted: *07 February 2017*
Published: *27 February 2017*

Citation:
Risti M and Bicalho MDG (2017) MICA and NKG2D: Is There an Impact on Kidney Transplant Outcome? Front. Immunol. 8:179. doi: 10.3389/fimmu.2017.00179

INTRODUCTION

Genetic diversity is the hallmark of MHC genes (1). The main antigenic barrier to transplantation is molecules, which are polypeptide products of a cluster of genes known, in humans, as human leukocyte antigens (HLA). In addition, a family of highly glycosylated MHC-encoded molecules, the *MHC class I chain-related (MIC)* genes, has been identified (2) as a second lineage of mammalian MHC I genes, which could constitute an antigenic barrier to transplantation as well (3). The MIC molecules possess a low degree of homology to other *MHC class I* encoded genes and interact with

[1]http://www.transplant-observatory.org.

both T-cell and natural killer (NK)-cell receptors (2). MIC proteins act as ligands for NK cells, γδ T cells, and αβ $CD8^+$ T cells, which express natural killer group 2 member D (NKG2D) ligand (4). The importance of the MICA protein in kidney transplantation has been acknowledged in recent years, and the role they play in graft rejection has been intensely pursued.

MICA GENE: STRUCTURE, POLYMORPHISMS, AND FUNCTION

The *MIC* gene family consists of seven members (*MICA*–*MICG*) (**Figure 1**), five of which are pseudogenes, and two, *MICA* and *MICB*, of which are functional (5, 6). *MICA* and *MICB* are the most divergent members of the human MHC-encoded class I genes identified to date, having an average of 19, 25, and 35% similarity in the extracellular α1, α2, and α3 domains, respectively, to those of other MHC α-polypeptides (7).

The *MICA* gene is located 46.4 kb centromeric to *HLA–B* on the short (p) arm of chromosome 6 at position 21.33 (3).

MICA and *MICB* have been shown to differ in the transcriptional control regions from common HLA class I genes. *MICA/B* genes lack the prototypic MHC class I gene promoter regulatory elements, the SXY module [heterotrimeric X-box-binding factor—regulatory factor X; X2-box-binding factor—cyclic-AMP-responsive-element-binding protein; Y-box-binding factor—nuclear transcription factor Y (NF-Y); and an as-yet-unidentified S-box-binding factor]. In contrast, the regulatory promoter module of *MICA/B* contains heat shock elements resembling those of HSP70 genes, a CCAAT box that binds to nuclear transcription factor Y (NF-Y), and a GC box that binds to Sp1, Sp3, and Sp4 transcription factors (8).

There are 12 known possible haplotypes of *MICA* 5′ promoter regions, including a null haplotype due to a deletion of the entire *MICA* gene (*MICA*-P12), which are more densely distributed in both ends compared to the central portion of 5′ promoter (8, 9).

MICA has six exons separated by five introns (**Figure 2**): exon 1 encodes the leader peptide, exons 2–4 encode three extracellular globular domains, exon 5 encodes the transmembrane domain, and exon 6 encodes the cytoplasmatic tail (6, 10). An intron of 6,840 bp follows exon 1 and is unusually large for a class I gene. The remainder of the *MICA* gene has a quite similar organization to classical class I genes, except for the presence of a relatively long intron 5 and the fusion of the cytoplasmic tail and 3′ UTR sequence in a single last exon (11).

It is considered that *MICA* gene has a codominant expression, and the presence of heat shock elements within the promoter suggests that MICA transcription is indued under stress conditions, and that therefore the MICA protein functions as an indicator of cell stress (11–13). The first intron of the *MICA* gene contains an NFkB-binding site that binds p65 (RelA)/p50 heterodimers and p50/p50 homodimers of the NFkB transcription factor family. The role of the proximal −130 bp NFkB site was reported as necessary and sufficient for transcriptional transactivation of *MICA* in response to TNFα in primary endothelial cells (ECs) (14).

Gene transcription isoforms are mRNAs transcribed from the same locus that differs in their transcription start sites and/or untranslated regions or protein coding DNA sequences (CDSs) also producing different protein isoforms. The alternative splicing of *MICA* leads to the formation of four isoforms. Two of them were described by Zou and Stastny (15) (MICA isoforms 1 and 2), and they did not appear to be tissue specific.

MICA isoform 1 (*1*001*) is the longest isoform, derived from the *MICA*001* allele. *MICA* isoform 2 (*1*008:01*) is a variant isoform derived from the *MICA*008:01* allele that contains a four-nucleotide insertion (rs9279200), which causes a frameshift mutation and subsequent truncation of the CDS, compared to isoform 1 (allele *MICA*001*) (15). The other two isoforms of *MICA*, isoforms 3 and 4, are described only in the *ncbi.nih.gov/gene*[2] website. *MICA* isoform 3 is, like isoform 2, encoded by the *MICA*008:01* allele; however, it is shorter than isoform 2 at the N-terminus, containing an alternate 5′ exon, differences in the 5′ UTR, and lacking a portion of the 5′ coding region, with translation being initiated from a downstream in frame start codon. *MICA* isoform 4 contains an alternate 5′ exon and uses an alternate splice site in an internal exon. It differs in the 5′ UTR, lacks a portion of the 5′ coding region, and initiates translation from an alternate start codon, compared to variant 1 (*MICA*008:01 allele*). Isoform 4 has a distinct and shorter N-terminus, compared to isoform 2.

The *MIC* genes are transcribed in keratinocytes, ECs, fibroblasts, monocytes, epithelial cell lines and epithelial tissues of cell lines, and freshly isolated cells (2, 16) and are not usually transcribed in $CD4^+$ T cells, $CD8^+$ T cells, and $CD19^+$ cells (17). MIC protein is only expressed on the cell surface of freshly isolated ECs, fibroblasts (17), and gastric epithelium (12). MIC protein acts as a ligand for NK cells, γδ T cells, and αβ $CD8^+$ T cells, which express NKG2D ligand (NKG2DL) (4).

[2]https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=DetailsSearch&Term=100507436#.

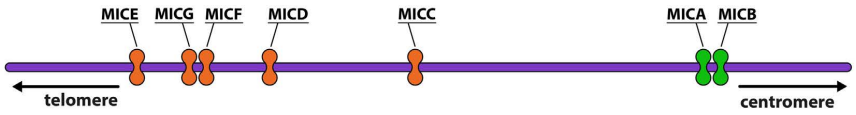


FIGURE 1 | Representation of *MHC class I chain-related (MIC)* genes. The functional genes are represented in green and the pseudogenes are in orange (image by Matilde Risti).

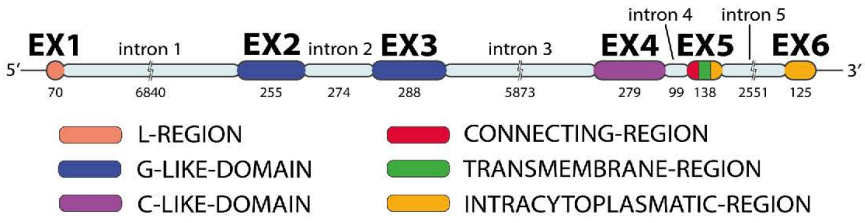


FIGURE 2 | *MICA* gene exon–intron organization. The *MICA* gene has five introns and six exons (image by Matilde Risti).

MICA Polymorphism and -129Met/Val Dimorphism

Bahram et al. (3) first described *MICA*01* to *MICA*05* alleles with a total of 18 nucleotide substitutions resulting in 14 amino acid changes in the final polypeptide. Fodil et al. (7) described the alleles, *MICA*06* to *MICA*16*, with nine nucleotide substitutions and eight amino acids changes. One year later, Mizuki et al. (18) showed a variable number of trinucleotide GCT repeats that encode 4, 5, 6, 7, 9, or 10 alanine (A, Ala). The short tandem repeats or microsatellite alleles were labeled as *A4*, *A5*, *A6*, *A7*, *A8*, *A9*, and *A10*. There is also an *A5.1* allele that contains five triplet repeats of GCT plus an additional guanine nucleotide insertion (GGCT). This insertion causes a frameshift mutation leading to a premature intradomain stop codon within the transmembrane region, which deletes the MICA cytoplasmic tail. The A4, A5, A6, A7, A8, A9, A10, and A5.1 sizes are, respectively, 179, 182, 185, 194, and 183 bp (18–20). At the time of writing (October 2016) hla.alleles.org[3] reports 105 *MICA* alleles, 2 of which considered null, result in 82 different MICA proteins. All *MICA* alleles from **001* to **087* producing different proteins and their nucleotides variations on exons 2–6 are shown in **Table 1**.

Several studies have documented *MICA* allele frequencies within different populations (**Figure 3**), and the frequency distribution varies between them. For example, the same group of three alleles (*MICA*008*, *MICA*002*, and *MICA*004*) accounts for more than 50% of the allele frequencies commonly found in several Caucasoid populations (21–24) but at the same time *MICA*027*'s frequency is extremely different in a comparison between the South American Indian and Caucasoid populations (25). Single high-frequency *MICA* alleles are each associated with more than one different *HLA*-B allele, but this pattern is not reciprocal. Most specific *HLA-B* alleles, including *B*07:02* and *B*08:01* variations, are usually linked to a single *MICA* allele. This pattern suggests that the *MICA* alleles had an earlier origin than major branches of *HLA-B* alleles (26).

The evolutionary history of *HLA-B* alleles is recognizable in the linkage relationship between *HLA-B* and *MICA* genes. The high degree of sequence similarity between three *HLA* alleles (*B*35*, *B*53*, and *B*58*) indicates that they were all generated from the same progenitor allele, and the observation that globally they are all linked to the *MICA*002* allele further supports

TABLE 1 | Nucleotide variations on exons 2–6 for *MICA* alleles from **001* to **087*.

EXON 2 α1		
CODON 6	CTG (LEU)	CGC (ARG)
		CTC (PRO)
CODON 14	TGG (TRP)	GGG (GLY)
CODON 23	CTC (LEU)	GTT (LEU)
CODON 24	ACT (THR)	GCT (VAL)
CODON 26	GTA (VAL)	GGA (GLY)
CODON 36	TGT (CYS)	TAT (TYR)
CODON 38	AGG (ARG)	AGC (SER)
CODON 39	CAG (GLN)	TAG (Stop)
CODON 55	GGA (GLY)	GGC (GLY)
CODON 56	AAT (ASN)	AAC (ASN)
CODON 64	AGA (ARG)	AAG (ARG)
CODON 69	AAC (ASN)	AAT (ASN)
EXON 3 α2		
CODON 90	CTC (LEU)	TTC (PHE)
CODON 91	CAG (GLN)	CGG (ARG)
CODON 93	ATT (ILE)	ATG (MET)
CODON 102	AAC (ASN)	AGC (SER)
CODON 105	AAG (ARG)	AAG (LYS)
CODON 112	TAC (TYR)	TAT (TYR)
CODON 114	GGG (GLY)	AGG (ARG)
CODON 122	CTG (LEU)	GTG (VAL)
CODON 124	ACT (THR)	TCT (SER)
CODON 125	AAG (LYS)	GAG (GLU)
CODON 129	ATG (MET)	GTG (VAL)
CODON 130	CCC (PRO)	TCC (SER)
CODON 139	GCC (ALA)	GCA (ALA)
CODON 142	GTC (VAL)	ATC (ILE)
CODON 151	ATG (MET)	GTG (VAL)
CODON 156	CAC (HIS)	CTC (LEU)
		CGC (ARG)
CODON 169	CGG (ARG)	TGG (TRP)
CODON 173	AAA (LYS)	GAA (GLU)
CODON 174	TCC (SER)	TCT (SER)
CODON 175	GGC (GLY)	AGC (SER)
		GGT (GLY)
CODON 176	GTA (VAL)	ATA (ILE)
CODON 181	ACA (THR)	AGA (ARG)
EXON 4 α3		
CODON 190	CGC (ARG)	TGC (CYS)
CODON 191	AGC (SER)	AGT (SER)
CODON 193	GCC (ALA)	GCA (ALA)
CODON 198	ATT (ILE)	ATC (ILE)
CODON 205	TCT (SER)	TCC (SER)
CODON 206	GGC (GLY)	AGC (SER)

(Continued)

[3]http://hla.alleles.org/nomenclature/stats.html.

TABLE 1 | Continued

EXON 4 α3		
CODON 208	TAT (TYR)	TGT (CYS)
CODON 210	TGG (TRP)	CGG (ARG)
CODON 213	ACA (THR)	ATA (ILE)
CODON 215	AGC (SER)	ACC (THR)
CODON 221	GTA (VAL)	CTA (LEU)
CODON 230	TGG (TRP)	TCG (SER)
CODON 244	TGG (TRP)	TGA (Stop)
CODON 247	AAC (THR)	ACT (THR)
CODON 250	TGC (CYS)	CGC (ARG)
CODON 251	CAA (GLN)	CGA (ARG)
CODON 253	GAG (GLU)	AAG (LYS)
CODON 254	GAG (GLU)	AAG (LYS)
CODON 256	AAG (ARG)	AGT (SER) AAG (LYS)
CODON 265	GGG (GLY)	AGG (ARG)
CODON 268	AGC (SER)	GGC (GLY)
CODON 269	ACT (THR)	ATT (ILE)
CODON 271	CCT (PRO)	GCT (ALA)
EXON 5 TM		
CODON 295	CGT (ALA)	GCGT
CODON 304	TAT (TYR)	TAC (TYR)
CODON 306	CGT (ARG)	TGT (CYS)
EXON 6		
CODON 350	GAT (ASP)	GCT (ALA)
CODON 354	ACT (THR)	GCT (ALA)
CODON 359	GGC (GLY)	GGT (GLY)
CODON 360	GCC (ALA)	ACC(THR)

*Codons are shown in the first column. The second column shows the triplets and their corresponding amino acids in the consensus sequence (MICA*001). The third column lists that triplet's possible variations in other alleles compared with the consensus sequence. Amino acid substitutions in MICA on the three external protein domains (exons 2–4), on the transmembrane domain TM (exon 5) and carboxy-terminal cytoplasmic tail (exon 6). The G nucleotide insertion is represented in red in the exon 5 TM.*

this conclusion. Specific *MICA* alleles also tend to associate with serological HLA-B groups. A rare exception can be found in *B*44*, whose two subgroups *B*44:02* and *B*44:03* have exclusive associations with *MICA*008* and *MICA*004* (26).

The *MICA-129Val/Met* dimorphism, caused by an SNP (rs1051792) at nucleotide position 454 (G>A) of the *MICA* gene is of particular interest. The substitution of valine (Val) for methionine (Met) at position 129 in the α2 domain of the MICA protein has been reported to affect NKG2D binding avidity (36–40). This dimorphism divides the *MICA* alleles into two groups (**Table 2**). In 2015, it has been observed that *MICA-129Met* alelles increased the risk of experiencing acute graft-versus-host disease. This effect could be the consequence on NKG2D signaling by MICA-129Met variant (40). In addition to this, it has been shown that the MICA-129 dimorphism may directly affect plasma membrane expression and shedding of MICA, and these functional effects might contribute to the numerous disease associations (41).

MICA Molecule

MICA is a highly glycosylated membrane-anchored cell surface protein composed of 383 amino acids (12). Unglycosylated MICA appeared less stable than those incorporating glycosylated MICA (36). Its expression has been reported on the surface of different cells and resembles the domain organization (**Figure 4**) of the α chain of MHC class I molecules (16, 42). MICA α chain does not bind β2-microglobulin and is independent of any transporter-associated protein. Attempts to identify peptides bound to MICA have been unsuccessful (10, 12). The crystal structure of MICA shows four distinct α helices arranged in an eight-stranded antiparallel β sheet. These helices in MICA roughly correspond to the two helices that define the peptide-binding groove in peptide-binding MHC class I proteins and homologs (42).

MICA is generally concentrated in lipid rafts and is *S*-acylated, similar to other lipid rafts-associated proteins. *In vitro* mutation of the S-acylation site, replacing a cysteine residue with a stop codon at aminoacid position 39, yields a truncated form of MICA, unable to activate NK cells (43).

The MICA molecule interacts with NK cells, γδ T cells, and αβ $CD8^+$ T cells, which express NKG2D, a common activating NK cell receptor (4, 10, 44). NKG2D recognizes the human MICA protein in conjunction with a transmembrane signaling adaptor protein, DNAX-activation protein (DAP10) (4, 10).

It is noteworthy that the MICA molecule can also be recognized by γδ T cells with the TCR variable region $V_δ1$ (4, 45–47).

Both types of receptors, $V_δ1$TCR and NKG2D, can simultaneously recognize and bind to MICA on a $V_δ1$ cell surface. There is close association between the tissue distribution of $V_δ1$ cells and the physiological expression of MICA, as MICA affects $V_δ1$ cell lineage development (46). In $V_δ1$ $_γδ$T cells, the strength of the binding between TCR and MICA is weaker than that between NKG2D and MICA. Although weak, TCR:MICA complexes show unusual stability after they are formed, with long half-lives. TCR and NKG2D receptors compete for binding to MIC ligands, and it has been suggested that initial interactions at the point of contact may be dominated by NKG2D:MIC binding events, which then give way to longer-lived γδ TCR:MIC complexes (47).

Conclusions on MICA

The *MICA* gene is polymorphic, and it is in linkage disequilibrium with *HLA-B* genes. The MICA protein is expressed on the cell surface, and it is possibly the proteolytic cleavage of the $α_3$ domain which in turn releases soluble MICA (sMICA). The MICA molecule does not present a peptide in its groove and can interact with the NKG2D receptor, which is the focus of the following paragraphs.

NKG2D OR *KILLER CELL LECTIN-LIKE RECEPTOR K1 (KLRK1)* GENE: STRUCTURE, POLYMORPHISMS, AND FUNCTION

NKG2D gene, also known as KLRK1, is located in the natural killer complex (NKC) on chromosome 12 (42, 48, 49). Human *NKG2D* (**Figure 5**) has 10 exons (50). Exons 2–4 encode the intracellular/transmembrane domain; exons 5–8 encode the ligand-binding ectodomain, which is a membrane-bound domain protruding into extracellular space (50, 51). *NKG2D* has a low number of nucleotide variations (48). *NKG2D* appears to be conserved

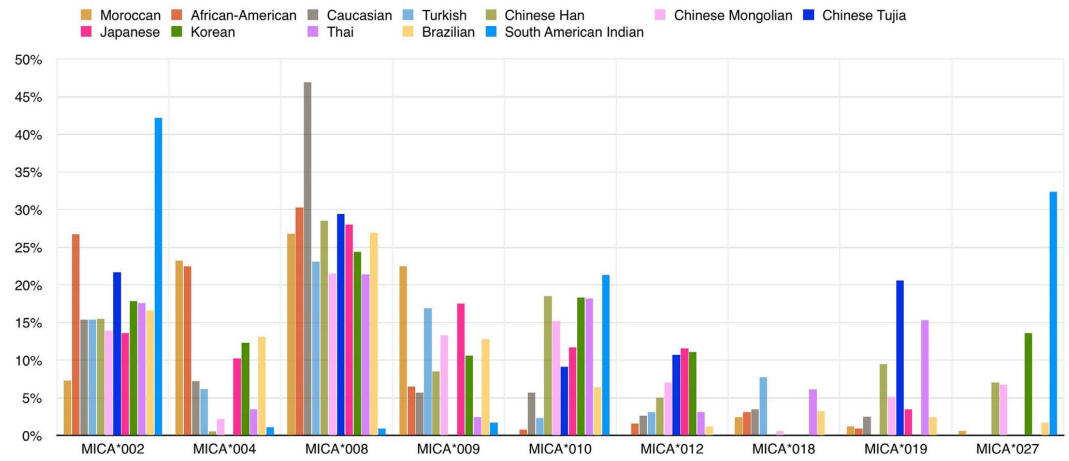


FIGURE 3 | Frequencies (%) of common *MICA* alleles within 12 populations. The allele frequencies of nine *MICA* alleles are shown for 12 populations: Caucasoid (21–23), Korean (19), North-Eastern Thai (27), Japanese (13, 28), African-American (21, 29), South American Indian (25), Moroccan (30), Turkish (31), Brazilian (24, 32), Chinese Mongolian (33), and Chinese Tujia (34, 35) (image by Matilde Risti).

TABLE 2 | Dimorphism 129 Val/Met divides *MICA* alleles into two groups.

Dimorphism 129 val/met divides *MICA* alleles in two groups	
ATG (Met)	**GTG (Val)**
*MICA*001*, ****002***, **007*, **011*, ****012***, **014*, **015*, **017*, ****018***, **020*, **023*, **025*, **026*, **029*, **030*, **031*, **032*, **034*, **035*, **036*, **037*, **038*, **039*, **040*, **041*, **042*, **043*, **045*, **046*, **047*, **050*, **051*, **052*, **055*, **059*, **060*, **061*, **068*, **072*, **075*, **078*, **079*, **081*, **083*, **084*, **086*	*MICA**, ****004***, **005*, **006*, ****008***, ****009***, ****010***, **013*, **016*, ****019***, **022*, **024*, ****027***, **028*, **033*, **044*, **048*, **049*, **053*, **054*, **056*, **057*, **058*, **062*, **063*, **064*, **065*, **066*, **067*, **069*, **070*, **073*, **074*, **076*, **077*, **080*, **082*, **085*, **087*

The most frequent alleles present in **Figure 3** *are shown in bold. The MICA alleles shown are from MICA*001 to MICA*087.*[4] *"MICA*003:01" label has never been assigned to any sequence. MICA*021 sequence was renamed MICA*012:03 in August 2007. The sequence originally labeled MICA*071 was proven to contain errors and to be identical to MICA*017 (March 2013) (see text footnote 3).*

[4]http://hla.alleles.org/alleles/classo.html.

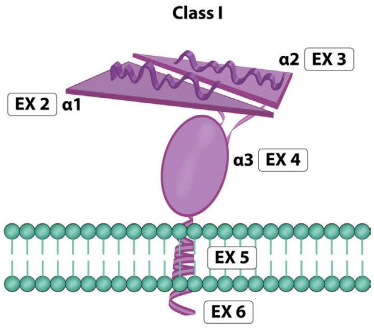


FIGURE 4 | MICA molecule. Exon 1 encodes one leader peptide, exons 2–4 encode three extracellular globular domains, exon 5 encodes one transmembrane domain, and exon 6 encodes a cytoplasmic tail (image by Matilde Risti).

during evolution, with orthologs of *KLRK1* are present in the genome of all mammals, as well as in marsupials (4, 52).

Human *NKG2D* is expressed from at least three distinct alleles, and several gene transcription isoforms have been described, including an alternatively spliced variant that introduces a non-sense mutation resulting in a protein isoform that lacks the entire extracellular ligand-binding domain (53).

Hayashi et al. (54) evaluated the SNPs in the NKC gene region. They selected 20 SNPs with a >10% higher frequency in Caucasoid or Japanese populations (**Table 3**); these SNPs covered *CD94*, *NKG2D*, *NKG2F*, *NKG2E*, *NKG2A*, and *Ly49* genes. They selected 8 out of the 20 SNPs that were closely associated with natural cytotoxic activity, having P values <0.001. All these SNPs are located in the NKG2D gene region, except for rs1983526 that is located in the promoter region of the NKG2A gene. These eight SNPs were split into two groups: group 1 (rs1049174, rs2617160, rs2617170, rs2617171, and rs1983526) and group 2 (rs2255336, rs2246809, and rs2617169). All the SNP combinations of group 1/group 1 and group 2/group 2 revealed a strong linkage disequilibrium, with r^2 values >0.9, whereas group 1/group 2 combinations showed much weaker linkage disequilibrium, with r^2 values <0.5. This indicates that the five group 1 and three group 2 SNPs belong to two different haplotype blocks (NKG2D hb-1 and hb-2), each of which generates two major haplotypes associated with low

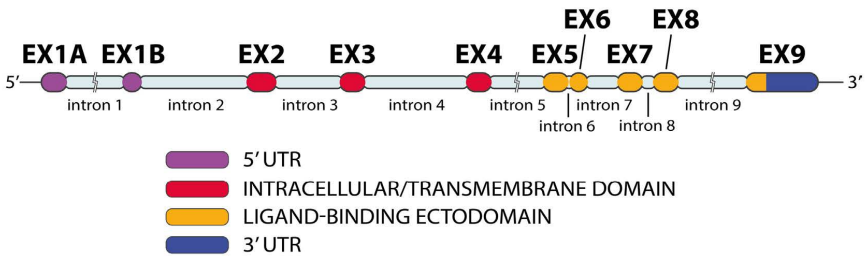


FIGURE 5 | *NKG2D* gene exon–intron organization. The *NKG2D* gene has 10 exons and 9 introns (image by Matilde Risti).

TABLE 3 | 20 SNPs selected by Hayashi et al. in their study (54).

SNP ID	Variation	SNP ID	Variation	NKG2D hb-1	Low	High
rs3759272	G>T	rs2617170	T>C	**rs1049174**	C	G
rs2537752	T>A	rs2617171	C>G	rs2617170	C	T
rs1049174	G>C	rs1971939	C>G	rs2617171	C	G
rs2255336	A>G	rs1915319	A>G	rs1983526	C	G
rs2294148	G>A	rs4763525	G>A	rs2617160	T	A
rs2049796	A>C	rs3003	C>T	**NKG2D hb-2**	**Low**	**High**
rs2617160	A>T	rs1983526	C>G	rs2255336	G	A
rs7972757	A>G	rs10772285	G>C	rs2246809	G	A
rs2246809	A>G	rs1915325	G>A	rs2617169	T	A
rs2617169	T>A	rs2607893	C>T			

Blue fields belong to group 1 and green ones represent group 2. Each of the different haplotype blocks (NKG2D hb-1 and hb-2) is split in low and high natural cytotoxic activity haplotypes. hb-1 and hb-2 may be successfully predicted knowing only rs1049174 (in bold).

(LNK) and high (HNK) natural cytotoxic activity phenotypes (**Table 3**) (54).

A separate study on a European population confirmed that the NKG2D region haplotype associated with increased cancer susceptibility in the Japanese population also exists in Europeans at similar frequency. Therefore, the conclusions of the original study may also be applicable to this population (55).

NKG2D: HNK1 and LNK1 Haploblocks

Several studies have demonstrated that high and low natural cytotoxic activity haplotype alleles (HNK1 or LNK1) belonging to NKG2D haplotype blocks 1 (hb-1) may be successfully predicted by only a single SNP (dbSNP: rs1049174) (54, 56, 57).

A study on Japanese individuals demonstrated that the *HNK1* haplotype is associated with a greater activity of NK cells in the peripheral blood and a lower prevalence of cancers originating from epithelial cells (58). Espinoza et al. showed an association between the *NKG2D-HNK1* haplotype (haplotype frequency, 61%) in bone marrow donors and a significantly reduced transplant-related mortality and better overall survival for unrelated donors of HLA-matched myeloablative bone marrow recipients with standard-risk disease (58).

The rs1049174 distribution for 25 populations (**Figure 6**) is reported on the 1,000 genomes website.[5] HNK is reported to be associated with the rs1049174 (G) allele, and LNK with rs1049174 (C) (54, 56).

NKG2D Protein

The NKG2D is a member of a C-type lectin-like family receptor called CD94/NKG2 (42). Despite its inclusion in the NKG2 family, NKG2D displays only limited sequence similarity to other members of the NKG2 family of NK cell surface receptors (NCRs) and CD94 and forms homodimers, rather than heterodimers, with CD94, as do other NKG2 NCRs (42).

Natural killer group 2 member D is a transmembrane-anchored receptor expressed as a disulfide-linked homodimer on the cell surface, with a molecular weight of ~42 kDa (42).

In humans, each NKG2D homodimer (**Figure 7**) associates with two DAP10 homodimers to form a hexameric structure (59), which can signal by recruitment of phosphatidylinositol 3-kinase (36).

Human NKG2DLs are MICA and MICB, and a group of glycosylphosphatidylinositol-bound surface molecules including UL16 binding protein(ULBP)-1, -2, -3, and -4 (6), RAET1G (or ULBP5), and RAET1L (or ULBP6) (60), which share about 25% identical amino acids in their $\alpha_1\alpha_2$ domains that are variably scattered throughout the aligned sequences without discernible patterns of sequence conservation (36).

Signals triggered by the NKG2D receptor are transmitted through the associated DAP10 dimer (**Figure 7**) (59) because NKG2D lacks a tyrosine-based inhibitory motif in its cytoplasmic tail (4, 61).

Natural killer group 2 member D is expressed by all human NK cells, γδ T lymphocytes, αβ $CD8^+$ T lymphocytes (6), interferon-producing killer DC (62), invariant NKT cells cells, and a small subset of effector or memory $CD4^+$ T cells (4, 52, 63). Expression of NKG2D on NK cells and $CD8^+$ T cells can be modulated by cytokines due to their effects on transcription and posttranscriptional processing of *NKG2D* and *DAP10*. In

[5]http://browser.1000genomes.org/Homo_sapiens/Variation/Population?db=core;r=12:10524865-10525865;v=rs1049174;vdb=variation;vf=750969.

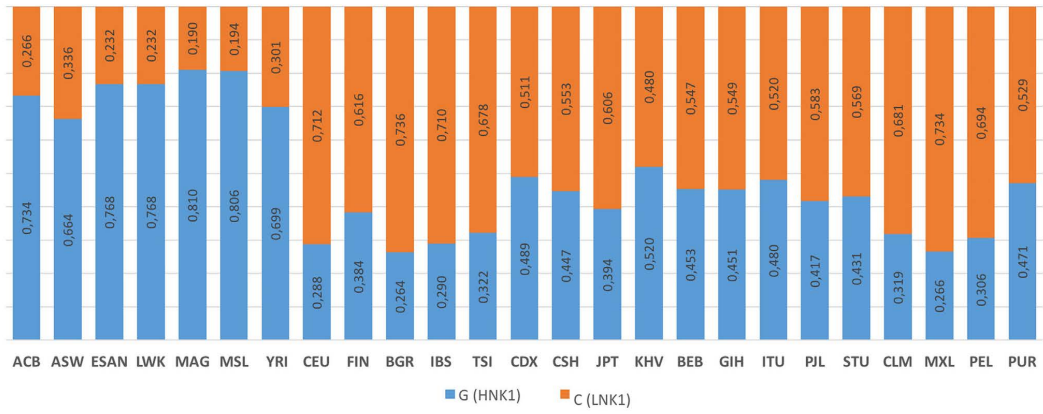


FIGURE 6 | 1000 Genomes frequency for the G>C alleles (NKG2D hb-1) (see Footnote 5). The population represented are African Caribbean in Barbados (ACB), African Ancestry in Southwest US (ASW), Esan in Nigeria (ESAN), Luhya in Webuyu Kenya (LWK), Mandinka in Degambia (MAG), Mende in Sierra Leone (MSL), Yoruba in Ibadan Nigeria (YRI), Utah Residence with Northern and Western European Ancestry (CEU), Finnish in Finland (FIN), British in England and Scotland (BGR), Iberian populations in Spain (IBS), Toscani in Italy (TSI), Chinese Dai in Xishuangbanna, China (CDX), Han Chinese in Bejing, China (CSH), Japanese in Tokyo, Japan (JPT), Kinh in Ho Chi Minh City Vietnam (KHV), Bengali in Bangladesh (BEB), Gujarat Indian in Houston Texas (GIH), Indian Telegu in the UK (ITU), Punjabi in Lahore Pakistan (PJL), Srilankan Tamil in the UK (STU), Colombian in Medellin Colombia (CLM), Mexican Ancestry in Los Angeles, California, USA (MXL), Peruvian in Lima Peru (PEL), and Puerto Rican in Puerto Rico (PUR).

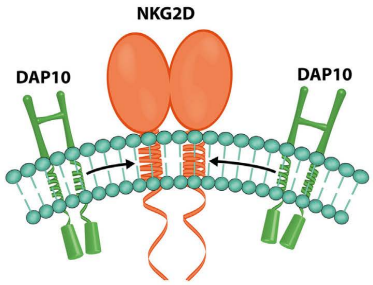


FIGURE 7 | NKG2D and DAP10. Representation of the hexametric structure formed by one NKG2D and two DAP10 homodimers (image by Matilde Risti).

humans, IL-2, IL-7, IL-12 (64), IL-15, and IFN-α (65) upregulate *NKG2D* expression, whereas TGFβ (65–67), IFNβ1 (68), and IL-21 (69), IL-4, IL-12, and IFNγ (65) downmodulate *NKG2D*. This downregulation can also be attributed to the overexposure to soluble or membrane-bound NKG2DLs, which promote the internalization and subsequent degradation of the receptor or catabolites produced on macrophage activation [reactive oxygen species (ROS) and L-kynurenine] (65). This is a possible explanation of the mechanism of oxidative stress, which is a common feature of chronic renal failure. ROS trigger the upregulation of MICA and downregulation of NKG2D in NK cells *in vitro* (70). DAP10 availability is also a decisive factor in NKG2D surface expression, and miRNAs can downregulate NKG2D expression in NK cells, reducing its cytotoxic effect (65).

Fernandez-Sanchez et al. (65) have shown for the first time that epigenetic mechanisms are involved in the regulation of NKG2D expression. They analyzed the region around the translation initiation site of the *NKG2D* gene (which included 11 CpG sites between −992 and +263 positions), and they found the greatest differences in DNA methylation patterns between the positions −992 and −255. These CpGs were highly methylated in Jurkat, HUT78 cell lines and $CD4^+$ T cells, partially methylated in $CD8^+$ T lymphocytes and NK cells, and fully demethylated in NK cells lines. They discovered that the acetylation of histone H3 lysine 9 (H3K9) is important for correct NKG2D expression in NK and $CD8^+$ T cells, while DNA demethylation may be associated with an increased expression of NKG2D in $CD4^+$ T cells. The DNA methylation profile of *DAP10* gene was also analyzed, but no differences were found. $CD4^+$ T lymphocytes and T cell lines (Jurkat and HUT78) had a DNA methylation; instead NKG2D-positive cells ($CD8^+$ T lymphocytes, NK cells, and NKL cell line) had an unmethylated *NKG2D* gene and high levels of histone H3 lysine 9 acetylation (H3K9Ac). It was observed that the histone acetyltransferase inhibitor, curcumin, reduced H3K9Ac levels in the *NKG2D* gene, downregulated NKG2D transcription, and led to a marked reduction in the NKG2D-mediated lytic capacity of NK cell lines (65).

Another interesting study by Karimi et al. (71) of human primary NK and $CD8^+$ T cells discovered a novel splice variant of human NKG2D that encodes a truncated receptor lacking the ligand-binding ectodomain ($NKG2D^{TR}$). Overexpression of this truncated isoform severely attenuated cell killing and

IFNγ release mediated by full-length NKG2D ($NKG2D^{FL}$). A specific knockdown of an $NKG2D^{TR}$ isoform enhanced NKG2D-mediated cytotoxicity, suggesting that $NKG2D^{TR}$ is a negative regulator of $NKG2D^{FL}$. At the biochemical level, it was demonstrated that $NKG2D^{TR}$ bound to DAP10 and interfered with the DAP10–$NKG2D^{FL}$ interaction. In addition, $NKG2D^{TR}$ formed heterodimers with $NKG2D^{FL}$ and negatively modulated $NKG2D^{FL}$ preventing its surface expression. Therefore, $NKG2D^{TR}$ constitutes a mechanism for regulation of NKG2D-mediated function in human $CD8^+$ T cells and NK cells (71).

Unlike $CD8^+$ T cells, TCR-mediated activation is not sufficient to induce NKG2D expression on $CD4^+$ T cells, and the factors responsible for induction of NKG2D on $CD4^+$ T cells are still unknown (71).

Saez-Borderias et al. (63) provided the first evidence that a subset of human cytomegalovirus (HCMV)-specific $CD4^+$ T cells displays NKG2D. Their data suggest that $CD4^+NKG2D^+$ cells expanding in HCMV-stimulated cultures correspond to virus-specific memory T cells that have acquired NKG2D while losing CD28 (63).

Conclusions on NKG2D

The *NKG2D* gene can be split into two haploblocks: *HNK1* and *LNK1* (high and low cytotoxic activity related). The NKG2D protein is a homodimer associated with two DAP10 molecules and can interact with MICA. In NK cells, the NKG2D protein is an activation receptor which is able by itself to trigger cytotoxicity. This is the main reason why it is interesting to study the relationship between MICA and NKG2D in depth in the following paragraphs.

MICA LIGAND AND ITS RECEPTOR NKG2D: FUNCTIONAL INTERACTIONS

The crystal structure of the MICA–NKG2D complex shows that NKG2D binds to one MICA molecule as a homodimer. One of the NKG2D molecules binds mostly to the α1 domain of MICA, while the other binds mostly to the α2 domain (6). The contact between these two molecules creates a small pocket (roughly 6 Å wide × 6 Å thick × 14 Å long) (42).

The NKG2D homodimer overlays MICA diagonally in way that resembles αβTCR overlaying MHC I molecules. The central section of the MICA α2 domain is disordered when MICA is crystallized in isolated form, but it becomes ordered when MICA is bound to NKG2D and forms part of the interface between the two molecules (6).

MICA glycosylation was not essential, but it enhanced complex formation with NKG2D. Likewise, the glycosylation state of NKG2D had no substantial effect on complex formation (36).

MICA–NKG2D is considered a versatile ligand–receptor pair. As a matter of fact, NKG2D can act as a primary receptor or costimulatory molecule during infections, autoimmunity, or antitumor immune responses (6). For example, it has been shown that endothelial MICA triggeres an activating signal in allogeneic polyclonal NK cells through the immunoreceptor NKG2D, which may have account for a significant part in EC lysis by allogeneic NK cells. *In vitro* coculture assays show that interaction of endothelial MICA with NKG2D provides an immune suppressive pathway by downregulating NKG2D on the NK cell surface (14).

Boukouaci et al. (72) suggested that endocytosis of the NKG2D receptor, upon binding to sMICA, is considerably more rapid than the replenishment of cell surface NKG2D by *de novo* synthesis. The same authors also found that sMICA down regulates NKG2D receptor expression on $CD8^+$ T cells. sMICA upregulates the IFNγ production only by cytokines-activated NK cells, while it has no effect on non-activated cells. The researchers demonstrated that sMICA upregulates IFNγ expression by IL-12/IL-18-activated CD3 $CD56^+$ NK cells, demonstrating the pro-inflammatory effect of sMICA (72). A study with a mouse model found that Lewis rat hearts transplanted into BALB/c mice developed typical acute rejection (AR) in 6 days. The severity of xenograft rejection increased with time, from 2 to 6 days. Also increasing over time, the MICA protein and MICA mRNA reached their highest value after 6 h. The prevalence of anti-MICA was significantly higher among mice with severe AR. However, sMICA was significantly increased during AR at 2 h, then gradually decreased, and reaching its lowest value after 6 h (73).

MICA–NKG2D AND KIDNEY TRANSPLANT

In the last few decades, the role of MICA and NKG2D in kidney transplants has emerged (**Table 4**). The involvement of NK cells was discovered in 1995 when some indirect evidence was reported during rejection of kidney transplants. Accumulation of $CD56^+$ NK cells expressing granzyme in kidney biopsies of patients undergoing AR suggested a role of their cytolytic activity in kidney-allograft rejection (74). Over the years, the association between NK cells and the mechanisms of microcirculation injury during antibody-mediated rejection (AMR) in kidney transplants has become increasingly evident. The researchers proposed that donor-specific antibodies (DSA) were able to bind to the endothelium and to recruit NK cells that produce IFNγ and trigger antibody (Ab)-dependent cellular cytotoxicity (75).

NK Cells and Kidney Damage in Mice and Cell Lines

Natural killer group 2 member D-ligand engagement delivers a strong dominant activating signal that overrides the inhibitory signal delivered by self-MHC class I, thus activating NKG2D-expressing cells, resulting in innate and adaptive immunity activation (113).

Zhang et al. (116) reported a study on ischemia/reperfusion injury (IRI) on mice and discovered the capacity of NK cells to injure renal tubular epithelial cells *in vitro*. *In vivo* data supported the hypothesis that NK cells interact with tubular epithelia through NKG2D/Rae-1 interaction to mediate kidney damage following IRI.

Luo et al. (89) performed an *in vitro* study on human renal proximal tubular epithelial cell line (HK-2). They discovered that hypoxia-inducible factor-1-α (HIF-1α) plays a very important role in upregulating MICA expression and enhancing NK cell cytotoxicity toward target cells during hypoxia/reoxygenation in HK-2 cells. HIF is a heterodimer consisting of an α-subunit (HIF-1α) and a β-subunit (HIF-1β), the HIF-1β protein is constitutively

TABLE 4 | Relevant published work regarding NKG2D, MICA, and kidney transplants.

Reference	Summary	MICA biomarker
Relevant published works regarding MICA and transplants		
Zwirner et al. (76)	Several patients had specific antibodies (Abs) against MICA. Most of them were detected in serum samples collected at different times after organ rejection	Yes
Hankey et al. (77)	MHC class I chain-related expression was documented in allografted kidneys and pancreas. Expression of MICB was observed in epithelial cells in allografted kidney and pancreas that showed histologic evidence of rejection and/or cellular injury	Yes
Opelz (78)	This work showed that non-HLA immunity contributed substantially to long-term kidney transplant failure. The targets for Abs causing late rejections could be called minor histocompatibility antigens	Yes
Mizutani et al. (79)	Patients who rejected transplants had anti-HLA and anti-MICA Abs more frequently than those with functioning grafts. These Abs found in the peripheral circulation were not necessarily donor-specific, but their association with failure was consistent with a causality hypothesis	Yes
Amezaga et al. (80)	Anti-MICA Abs were not detected pretransplant nor posttransplant in patients receiving a compatible graft. Anti-MICA Abs were detected posttransplant acute antibody-mediated rejection in patients receiving an incompatible graft	Yes
Mizutani et al. (81)	Anti-HLA and anti-MICA Abs were present independently on a more frequent basis in patients with failed grafts than those with functioning grafts	Yes
Panigrahi et al. (82)	Patients who developed both anti-HLA and anti-MICA Abs rejected their grafts more frequently than those having either of these Abs	Yes
Zou et al. (83)	Pre-sensitization of kidney transplant recipients against MICA antigens had been associated with an increased frequency of graft loss and might contribute to allograft loss among recipients who were well matched for HLA	Yes
Seiler et al. (62)	Unlike previous reports, in this work the researchers could not detect elevated MICA mRNA levels in kidney biopsies derived from patients undergoing acute rejection (AR) or chronic allograft nephropathy. In contrast, they observed a strong NKG2D mRNA induction during renal-allograft rejection, which was verified by immunohistology in kidney biopsies	No
Suarez-Alvarez et al. (84)	Anti-MICA Abs were detected in 17.6% of the patients and correlated with the development of AR. The presence of anti-MICA Abs could be an important marker for diagnosis because of their contribution to the outcome of the graft, regardless of presence of anti-HLA Abs	Yes
Alvarez-Marquez et al. (85)	At the time of the biopsy, 21% patients had only anti-HLA I Abs, 15.8% had anti-GSTT1 Abs, 10.5% had anti-HLA II Abs, and 10.5% had anti-MICA Abs. Besides anti-HLA Abs, donor-specific Abs against MICA and GSTT1 antigens could be responsible for the occurrence of Ab-mediated kidney graft rejection	Yes
Racca et al. (86)	This work did not show a correlation between MICA expression and renal graft state. The state of kidney allograft could be measured by using HLA-G1 isoforms, but not MICA mRNA levels, as markers	No
Lemy et al. (87)	The comparison between anti-MICA Abs$^+$ and anti-MICA Abs$^-$ patients showed that the incidence of AR episodes during the first year was similar in both groups. MICA Abs did not adversely affect renal graft outcomes	No
Li et al. (88)	Anti-MICA Abs were detected in 11 of the 15 transplant patients, irrespective of interval acute graft rejection. Also, integrative genomics predicted localization of the MICA antigen on the glomerulus in the kidney. MICA localization may explain both immunoregulatory and pathogenic roles for MICA after transplantation	Yes
Luo et al. (89)	HIF-1α plays a very important role in upregulating MICA expression and enhancing natural killer (NK) cell cytotoxicity toward target cells during hypoxia/reoxygenation in HK-2 cells. Their results demonstrated that hypoxia/reoxygenation-promoted MICA expression on HK-2 cells is through a HIF-1 pathway	Yes
Cox et al. (90)	Anti-MICA and anti-HLA Abs significantly associated with AR and anti-MICA donor-specific antibodies (DSA) and anti-HLA DSA correlated with decreased graft function by univariate and multivariate analysis. The researchers concluded that mismatching for MICA epitopes in renal transplantation is a mechanism leading to production of MICA Abs that associate with AR and graft dysfunction	Yes
Narayan et al. (91)	Case report: this case demonstrated that donor-specific anti-MICA Abs could be associated with both acute antibody-mediated rejection (AMR) and type IIA acute cellular rejection and emphasized the necessity of treating both humoral and cellular components of the rejection	Yes
Yao et al. (92)	The authors proved that Anti-MICA Abs$^+$ rate was significantly higher in sensitized recipients and it had significant effect on the recovery of allograft function in early postoperative period. Protein A immunoadsorption plays an important role in decreasing preexisting Abs, especially the anti-MICA Abs	Yes

(*Continued*)

TABLE 4 | Continued

Reference	Summary	MICA biomarker
Zhang et al. (93)	Anti-MICA Abs were present in 28.9% of patients and they were associated with renal-allograft deterioration. The researchers concluded that, besides anti-HLA Abs, the presence of posttransplant anti-MICA Abs was associated with poor graft outcome and increased the risk of graft failure	Yes
Lemy et al. (94)	Anti-MICA Abs$^+$ patients were more frequently anti-HLA Abs sensitized and regrafted. Four-year death-censored graft survival was not different between MICA$^+$ and MICA$^-$ patients. These data did not support an independent pathogenic role for MICA in long-term renal graft injury	No
Li et al. (95)	The levels of the peak mean fluorescence intensity of MICA Abs in patients with impaired renal function were significantly higher than those in normal renal function controls. They also concluded that some MICA Abs might be more important than others in mediating graft rejection	Yes
Seyhun et al. (96)	Anti-HLA class II and anti-MICA Abs$^+$ were only important predictors of graft failure when present together with anti-HLA I Abs$^+$. Patients who developed anti-HLA Abs alone or both anti-HLA Abs and anti-MICA Abs rejected their grafts more frequently than Abs$^-$ recipients	Yes
Rodriguez Ferrero et al. (97)	They compared patients with versus without preformed circulating antibodies (circulating anti-MICA Abs and anti-HLA Abs), and they did not observe a significant difference in graft survival or renal function at 3-month follow-up	No
Solgi et al. (98)	This research supported the idea that monitoring of anti-HLA and anti-MICA Abs as well as soluble CD30 levels early after transplant had predictive value for early and late allograft dysfunctions and the presence of these factors was detrimental to graft function and survival	Yes
Akgul et al. (99)	In this study, the scientist observed the role of anti-HLA II Abs in the development of chronic active AMR and in long-term allograft survival. It is observed that anti-MICA and anti-GSTT1 Abs showed no effect on rejection mechanisms	No
Chaudhuri et al. (100)	Anti-MICA and anti-HLA Abs appeared in approximately 25% of unsensitized pediatric patients, placing them at greater risk for acute and chronic rejection with accelerated loss of graft function	Yes
Ding et al. (101)	When comparing patients with acute graft rejection against recipients with stable renal functions, the researchers highlighted a significantly higher positivity rate of anti-MICA Abs. The status of anti-MICA Abs can predict the occurrence and treatment outcomes of AR, and affect the long-term survival of the renal grafts	Yes
He et al. (102)	By following transplantation recipients during follow-ups, anti-HLA and anti-MICA Abs expression was proven to have a predictive value for early and late allograft dysfunction. The presence of donor-specific Ab is detrimental to graft function and graft survival	Yes
Jin et al. (103)	They observed the prevalence of panel-reactive antibody (PRA) and anti-MICA Abs to be increased among Ptc, albeit not significantly different from C4d AR. These results implied that Ptc could be an early indicator of AR	Yes
Li et al. (104)	CD19$^+$ B cells and CD19$^+$CD27$^+$ memory B-cell subsets were detected from peripheral blood mononuclear cells obtained from six anti-MICA-sensitized kidney recipients. Kidney recipients had a higher percentage of CD19$^+$CD27$^+$ B cells compared with healthy controls. This study thus showed that B cells may be stimulated to secrete Abs	Yes
Sanchez-Zapardiel et al. (105)	The researchers detected that pretransplantation sensitization against anti-MICA and anti-HLA Abs were independent events. Preformed anti-MICA Abs independently increase risk for kidney rejection and enhance the deleterious effect of PRA$^+$ status early after transplantation	Yes
Tonnerre et al. (106)	The researcher found that individual carrying *MICA A5.1/MICA A5.1* had 10-fold higher levels of *MICA* mRNA and MICA proteins at the endothelial cell surface. They also demonstrate a significant association between D/R MICA A5.1 mismatch and anti-MICA alloimmunization, particularly when donors carry the A5.1 mutation. They concluded that A5.1 mutation is an immunodominant factor and a potential risk factor for transplant survival	Yes
Zhang et al. (107)	5 years after transplantation, the frequencies of *de novo* anti-HLA and anti-MICA Abs were 25.8 and 12%, while 26.5% of patients had proteinuria. All of these factors have been associated with poor graft survival	Yes
Sapak et al. (108)	The researchers did not prove a complete correlation between the recipient anti-MICA Abs specificities and MICA antigens of the donor. They assumed that anti-MICA Ab induction occurred not only due to the allogeneic stimulation itself but also due to other factors that needed to be elucidated	No
Ming et al. (109)	Case report: the patient's HLA alloantibodies were not specific to the first kidney donor, but the MICA alloantibodies were. This indicates the importance of MICA virtual crossmatch in the process of selection for the kidney donor if the recipient is sensitized.	Yes
Xu et al. (110)	Serum anti-HLA II Abs, anti-MICA Abs, and anti-HLA plus MICA Abs all statistically increased in renal-transplanted recipients	Yes

(*Continued*)

TABLE 4 | Continued

Reference	Summary	MICA biomarker
Cai et al. (111)	Transplant recipients had Abs against denatured HLA class I, II, and MICA antigens. However, only C1q-fixing Abs were associated with graft failure, which was related to AMR	Yes (only for c1q-fixing denaturated MICA Abs)
Sanchez-Zapardiel et al. (112)	Occasionally, preformed anti-MICA Abs may be cytotoxic by activating and fixing complement. This could lead to a reduced function in early kidney grafts	Yes
Relevant published works regarding NKG2D and transplant		
Feng et al. (113)	Ischemia/reperfusion injury (IRI) caused mRNA expression of Rae-1 and protein expression of Rae-1 in ischemic kidneys. This study suggested that the expression of the NKG2D ligand, Rae-1, may play a potential role in innate immunity associated with IRI	
Zheng et al. (114)	The absence of enhancement of NKG2D expression in the kidney in AN in immunodeficient mice suggested that the populations expressing NKG2D were likely to be CD8 or γδ T cells, which were not present in the immunodeficient mice, rather than macrophages, which were present and activated in both models of AN	
Seiler et al. (62)	Unlike previous reports, in this paper, the researchers could not detect elevated MICA mRNA levels in kidney biopsies derived from patients undergoing AR or chronic allograft nephropathy. In contrast, they observed a strong mRNA induction of NKG2D during renal-allograft rejection, which could be verified by immunohistology in kidney biopsies	
Hadaya et al. (115)	The results of this paper have shown an expansion of the NKG2D$^+$ NK cell population during acute cytomegalovirus (CMV) infection (after kidney transplantation), which decreased over time to a level very similar to that of the control group. This suggests that the NKG2D receptor could play a similar role in NK and CD4$^+$ T cells	
Zhang et al. (116)	In this study, the researchers demonstrated for the first time that NK cells could induce kidney TEC death *in vitro* and that NKG2D and Rae-1 interactions played a critical role in this killing in mice	
Shabir et al. (117)	Cytotoxic CD4$^+$ CD28^{null} cell is an important biomarker for and potential mediator of adverse events after kidney transplantation. NKG2D represents an integral component of CMV immunosurveillance and immunoevasion and was upregulated on CD4$^+$ CD27$^-$ CD28^{null} cells isolated from patients of this study. The researchers proposed it as an important component of the cytotoxic effects (either protective or pathogenic) of these cells	

"Yes" and "No" labels have been used if, in the studies analyzed, MICA has been valued as a possible biomarker ("Yes") or not ("No").

present, while HIF-1α has a unique O_2-dependent degradation domain, which leads to its degradation under normoxia conditions. The authors speculate that HIF-1α upregulates the surface expression of MICA on grafts during renal IRI, causing NK cells cytotoxicity against the organ (89).

Possible Causes of End-stage Renal Disease

A 2009 study of the possible causes of end-stage renal disease (ESRD) (70), while note directly related to kidney transplants, inevitably reported findings of consequences for kidney transplantation. Peraldi et al. evaluated seven patients with ESRD that were treated with peritoneal dialysis, and not with the hemodialysis procedure; NKG2D expression on NK cells was significantly decreased in these patients compared to healthy donors, indicating that reduction in NKG2D expression was independent of the dialysis procedure and linked with chronic renal failure. The authors also discovered that oxidative stress in presence of increased ROS production is one of the most significant consequences of chronic renal failure, alone or in concert with other mediators, and it seems to decrease the NKG2D levels on NK cells in ESRD and to favor the upregulation of MICA expression (70).

Anti-MICA Abs and Rejection

Some mechanisms have been proposed for MIC-mediated organ rejection. MICA antigens expressed in the allograft could induce the generation of anti-MICA Abs, which in turn might injure cells in the presence of complement.

This section contains no works that focus solely on NKG2D since most of the manuscripts are almost exclusively conserved with anti-MICA Abs: NKG2D is often just a side note; its presence and the link with MICA are given.

MICA-Sensitized Kidney Recipients and Higher Percentage of CD19$^+$CD27$^+$B Cells

CD19$^+$CD27$^+$ B cells are the subset of memory B cells that have the potential ability to secrete Abs. Li et al. (104) assessed the serum from 68 long-term survival kidney recipients and found 11 subjects who were MICA positive. They analyzed six MICA-sensitized kidney transplant recipients and six healthy volunteers who did not receive a transplant (control group). Healthy controls had a higher percentage of CD19$^+$CD27$^-$ in PBMCs than transplant patients, while the percentage of CD19$^+$CD27$^+$ in B cells was higher in transplant patients. The MICA-sensitized transplant patients had a significantly lower average percentage of CD19$^+$ B cells in PBMC than healthy controls (3.58 ± 0.80 versus 8.53 ± 1.04%; $P < 0.01$). These results suggest that CD19$^+$CD27$^+$ B cells from sensitized patients have the potential ability to secrete Abs. In the same study, PBMC cells were isolated and cultured and stimulated with different molecules [toll-like receptor-9 ligand ODN-2006 CpG, PMA, B-cell activating factor (BAFF), CD40 ligand (CD40L), human recombinant IL-2 (rhuIL-2), rhuIL-10,

rhuIL-4, rhuIL-21, CD40L, and BAFF] including MICA antigens. After stimulation, B cells from healthy controls and transplant patients had a lower percentage of apoptosis than non-stimulated cells. The average percentage of apoptosis cells from transplant patients was significantly higher than from healthy controls, and the IgM production (the first Ab produced by B cells after antigen stimulation) was higher in stimulated B cells from transplant patients than from healthy controls. The authors speculate that the B-cell population may be compromised by the transplant because patients are under immunosuppressive regimens, which may alter the apoptosis of B stimulated cells compared with healthy controls. The same study also performed an *in vitro* study with drugs and found that bortezomib and mycophenolic acid could inhibit B-cell Ab secretion (104).

MICA Abs

Hankey et al. (77) first reported that MICA and MICB expression on epithelial cells in transplanted kidneys and pancreases with histological evidence of rejection and cellular injury played a role in allograft rejection. The study showed that in a healthy kidney there was no immunochemical evidence of MIC expression. In contrast, the majority of biopsies with histologic proof of rejection or acute tubular necrosis (ATN) showed MICA positive staining of the tubular epithelium in the proximal and distal tubules. For this reason, it was concluded that alloantibodies against MICA might play a role in allograft rejection.

Zwirner et al. (76) found that several patients who had undergone a kidney transplant had specific Abs against MICA, and most of them were detected in serum samples collected at different times after organ rejection. However, these Abs were not directed against the alleles expressed by the patients, and it was speculated that if the presence of MICA Abs was probably caused by multiple blood transfusions received by the patients while awaiting a transplant, or resulting from a pregnancy or a previous transplant (76).

Lemy et al. (87) analyzed the MICA Abs from 494 controls and 597 patients with chronic kidney disease. They found a three times higher prevalence of MICA Abs in patients with chronic kidney disease when compared with controls (14.9 versus 4.7%). Nevertheless, they speculated that even if the increase in MICA Abs prevalence among patients affected by chronic kidney disease was probably related to previous renal transplantation and transfusions. Logistic regression analysis and analysis of chronic kidney disease patients who have not been subjected to transfusions and renal transplantations suggest that the increase of urea (and other nitrogenous waste) in the blood is connected to an increase of MICA immunization. The authors also reported that MICA Abs were more frequent in men than in women, despite pregnancy being an independent risk factor for the development of MICA Abs (87). This finding is in sharp contrast with other published work. The fact that nearly one-third of MICA chronic kidney disease stage V patients have never experienced any identifiable immunizing event indicates that there must be other causes for MICA sensitization. At the same time, one-fifth of the same patients showed the presence of autoreactive MICA IgG Abs, distinctly rare with respect to HLA Abs. The authors showed that patients with MICA Abs had a somewhat better overall graft survival than MICA Abs$^-$ patients. Finally, Lemy et al. found in MICA Abs$^+$ and MICA Abs$^-$ patients a similar incidence of AR episodes during the first year (10.2 versus 12.8%), as well as similar levels of proteinuria and creatinine (87).

Another study of MICA Abs screened 147 recipients with end-stage renal disease; 82 of these patients were Abs$^+$ (55.8%). Forty patients had both anti-HLA and anti-MICA, 33 had only anti-HLA, and 9 only anti-MICA Abs in the posttransplant period. The authors found that patients who developed HLA alone, or both HLA and MICA Abs, rejected their grafts more frequently than Abs$^-$ recipients. The rates of HLA class I, class II, or both Abs$^+$ were greater in the rejection patients than the non-rejection patients ($P = 0.011$, 0.037, and 0.0275, respectively). So the authors speculated that HLA class II and MICA Abs$^+$ were the only important predictors of graft failure when both of them were present with HLA class I Abs$^+$ (96).

In a retrospective study, Solgi et al. (98) analyzed sera samples of 40 living unrelated donor kidney recipients, looking at anti-HLA and anti-MICA Abs and the levels of soluble CD30 (sCD30) and sMICA. They found that patients with pre- and posttransplant HLA Abs had a higher incidence of AR episodes ($P = 0.01$ and $P = 0.02$), more graft loss ($P = 0.001$), and lower graft survival during a mean follow-up of 3 years. This group of patients also had higher levels of sCD30 and serum creatinine and decreased contents of sMICA early after transplantation, as compared to the patients without HLA Abs. Anti-MICA Abs were observed in 8/40 (20%) and 5/40 (12.5%) of all patients pre- and posttransplant, respectively. HLA and MICA Abs were both found in two out of four cases with graft loss. In a comparison of transplant rejecting to functioning graft groups, sCD30 levels increased at day 14 ($P = 0.001$), while sMICA levels were insignificantly lower in the first group (98).

Chaudhuri et al. (100) studied the evolution of humoral immunity in low-risk pediatric patients during the first 2 years after renal transplantation. They correlated the presence of serum anti-HLA DSA and serum MICA Abs with clinical outcomes and histology (the biopsies were performed at 0, 6, 12, and 24 months). They found anti-HLA Abs in 22% of patients, 6% of which were donor-specific, while 6% developed anti-MICA Abs. Three percent of patients developed *de novo* Abs to both HLA and MICA. The presence of *de novo* Abs was associated with significantly higher risks for AR ($P = 0.02$), chronic graft injury ($P = 0.02$), and decline in graft function ($P = 0.02$). Graft function was monitored by the difference between creatinine clearances. Anti-MICA and -HLA Abs were found in 25% of unsensitized pediatric patients. This was correlated with a greater risk of acute and chronic rejection (100).

Zhang et al. (107) associated the presence of *de novo* MICA Abs and proteinuria with graft failure, after renal transplantation. They investigated 275 patients without preexisting anti-HLA and -MICA Abs. Five years after renal transplantation, 25.8% showed *de novo* anti-HLA Abs, 12% showed *de novo* anti-MICA Abs, and 26.5% proteinuria. *De novo* anti-HLA Abs were associated with increased proteinuria after transplantation (relative risk, 3.12). Anti-HLA Abs and proteinuria were both associated with poor 5-year graft survival ($P = 0.027$ and $P = 0.006$, respectively). Patients with *de novo* anti-MICA Abs were also apt to have

proteinuria. The authors concluded that *de novo* anti-HLA and -MICA Abs and proteinuria are all associated with poor graft survival (107).

Pretransplant Panel-Reactive Abs and Preexistent Circulating Abs

Opelz (78) studied the influence of pretransplant panel-reactive antibody (PRA) status on the long-term outcome of kidney grafts from HLA-A, -B, and -DR, identical sibling donors. In over 10 years of follow-up, he discovered that non-HLA-directed immunity and Abs against HLA had a similar influence for the long-term results for kidney recipients with PRA. Opelz suggested that the targets for Abs causing late rejections could be the so-called minor histocompatibility antigens (78).

Sanchez-Zapardiel et al. (105) studied 727 transplanted patients and showed that the effect of anti-MICA Abs occurs independently of the presence of anti-HLA Abs. Pacients were categorized into four groups according to the presence (+) or absence (−) of anti-HLA and anti-MICA Abs: HLA^+MICA^+ ($n = 27$); HLA^-MICA^- ($n = 510$); HLA^+MICA^- ($n = 165$), and HLA^-MICA^+ ($n = 25$). A notable difference was observed 3 months after transplantation, when HLA^-MICA^+ patients had a graft rejection rate of 8% compared with 2% in HLA^-MICA^- patients. The patients were also grouped according to the presence of preexisting anti-HLA Abs, as measured by % PRA (PRA^+ or PRA^-): PRA^+MICA^+ ($n = 7$), PRA^-MICA^- ($n = 610$), PRA^+MICA^- ($n = 65$), and PRA^-MICA^+ ($n = 45$). The incidence of rejection was found to be superior in PRA^+MICA^- cohort versus PRA^-MICA^- patients (24 months after transplantation), but allograft rejection rate was the highest when comparing PRA^+MICA^+ patients with PRA^-MICA^- patients 3 months after transplantation, a finding which was repeated at 6 months (105). This work is of interest because it performed a comparative study on the effects of anti-MICA and anti-HLA Abs on kidney transplants.

The Rodriguez Ferrero et al.'s (97) study included 22 recipients of kidney transplantations from deceased donors, and no differences between patients that showed preexistent circulating antibodies (CA) and those that did not were reported. In regards to the incidence of AR episodes, the only factor associated with CA was re-transplantation. So the authors concluded that CA monitoring is important for highly sensitized renal transplants, but they did not observe a difference in graft survival or renal function in the first 3-month follow-up (97).

Cd4 Deposition and C1q-Fixing Abs

A study of patients with acute antibody-mediated rejection (AAMR), who had MICA*008 Ab, showed that the presence of anti-MICA Abs and the deposition of C4d in biopsies performed at the time of AAMR was associated with the detection of DSA or Abs against HLA (80). The observation that the control group of 30 patients with long-term functioning grafts did not have anti-MICA*008 Abs provided indirect evidence of the importance of anti-MICA Abs in chronic rejection. Furthermore, all patients receiving an allograft fully matched at MICA had functioning grafts (80). It is also important to mention that MICA Abs are able to activate complement in *in vitro* experiments (80).

Alvarez-Marquez et al. (85) selected 58 patients that underwent a kidney biopsy because of primary non-function, delayed graft function or acute dysfunction of a previously functional graft, suspected by oliguria, increase of serum creatinine levels, or proteinuria. At the time of the transplant, all patients showed negative complement-dependent cytotoxicity crossmatches. Researchers demonstrated that 80% of a group of 19 patients with clinically evident graft dysfunction and with C4d deposition in kidney biopsies had Abs directed against donor-specific HLA class I, class II, MICA, or GSTT1 (glutathione-*S*-transferase T1) antigens (85).

In the Li et al.'s (88) study, a human ProtoArray platform was used to study 37 serum samples from 15 renal transplant patients (pediatric and young adult) with ($n = 10$) and without ($n = 5$) AR, and seven normal controls. To test serum Abs, they used a ProtoArray containing 5,056 non-redundant human proteins expressed in a baculovirus system, purified from insect cells and printed in duplicate onto a nitrocellulose-coated glass slide. Moreover, all patients were primary transplant recipients, and the biopsies were graded by the Banff classification. The authors found that the mean immune response signal in posttransplant patient serum showed an increase in anti-MICA Abs when compared with healthy normal controls ($n = 7$), but anti-MICA Abs signal intensity was unrelated to the sampling time interval post-transplantation. Mean MICA Abs signal intensity was higher in transplant patients with $C4d^+AR$ (121.4) versus $C4d^-AR$ (4.3), so a correlation between high MICA Abs levels and $C4d^+$ graft rejection $r = 0.54$ ($P = 0.039$) was observed. On ProtoArray, each gene on the cDNA platform was compared between a specific kidney compartment versus all other compartments, by a two-unpaired class comparison and a multi-class comparison. The signal intensity of anti-MICA Abs ranked in the top 15 for glomerulus, so the MICA antigen was found to have a 2.7-fold higher expression in the glomerulus when compared to the other 6 normal kidney compartments. Cytoplasmic granular staining for MICA in normal and stable transplanted kidneys was observed solely in podocytes within glomeruli. In AR, in addition to the persisting glomerular staining, the infiltrating mononuclear lymphocytes also showed strong positive staining for MICA. So the authors demonstrated that Ab responses in patients are modulated by MICA after transplantation in patients, irrespective of graft rejection (88).

Another study correlates Cd4 deposition and creatinine levels. Ding et al. (101) evaluated serum anti-MICA Abs before and after kidney transplant, and they also examined PRA, serum creatinine, urine, graft ultrasound, lymphocyte subsets, and the pathology of graft biopsy. The study was split into two parts. In the first part, patients with AR were grouped into $MICA^+$, $MICA^-$ ($P < 0.05$) and control groups. There were a significantly higher number of anti-MICA Abs positive patients with acute graft rejection compared with stable renal functions patients (control group).

Two to three days after the occurrence of AR, the anti-MICA Abs level increased gradually. Anti-rejection treatment had no effect on anti-MICA Abs but lowered serum creatinine to a normal level. In the second part, the authors analyzed chronic graft rejection patients. The number of anti-MICA Ab positive patients was significantly higher than those with stable renal

function ($P < 0.05$), and the serum creatinine levels were significantly higher in MICA$^+$ than in MICA$^-$ cases ($P < 0.05$). The authors also found that graft biopsy of all MICA$^+$ cases showed C4d deposition (101).

Jin et al. (103) studied 53 cases of AR that showed C4d deposition in the peritubular capillaries, 50 cases of ARs without C4d deposition, 30 with peritubular capillaries alone, 28 with ATN, and 78 patients with surveillance biopsies (control group). The authors observed that the prevalence of PRA and anti-MICA Abs was increased among the peritubular capillaries alone group (30.0 and 43.3%, respectively), albeit not significantly different from the group with C4d$^+$ AR (49.1 and 39.6%, respectively). They also observed that the immunophenotype of infiltrating T lymphocytes and serum Abs (85.9% of control biopsies presented) had a regulatory phenotype while in the peritubular capillaries cohort, 93.3% of biopsies showed the cytotoxic phenotype. These results showed that peritubular capillaries in biopsy specimens from patients with early renal-allograft dysfunction could be an indicator of AR, especially acute humoral rejection (103).

Cai et al. (111) collected samples from 975 kidney transplant recipients, and they tested for C1q-fixing Abs against denatured HLA class I, class II, and MICA antigens. Among 169 patients who lost renal grafts, 44% had c1q-fixing Abs against denatured HLA/MICA antigens, which was significantly higher in patients with functioning renal transplants (25%). They concluded that C1q-fixing Abs were significantly associated with graft failure caused by AMR (72.73%) and they affirmed that only c1q-fixing Abs were associated with graft failure and AMR (111).

MICA Allele Epitopes and Eplets

Regarding the anti-MICA Abs, Duquesnoy et al. (118) developed an eplet-based version of the HLA-Matchmaker algorithm as a tool to assess the epitope specificity of these Abs. A repertoire of 38 potentially immunogenic *MICA* eplets was selected (based on MICA structure molecular viewing and the amino acid sequence differences between *MICA* alleles). These eplets are based on a functional epitope structure (a configuration of amino acids within a 3 Å radius of an Ab accessible polymorphic residue on the molecular surface). In this study, the eplet frequencies were calculated from *MICA* allele frequencies in 1,245 European-Americans and 605 African-Americans. Many eplets are shared by very similar groups of *MICA* alleles. For instance, the combination of eplets called CMGWS "supereplet" is composed by 36C, 129M, 206GW, and 215S epitopes and shared by the same group of *MICA* alleles (*A*001*, *A*002*, *A*007*, *A*011*, *A*012*, *A*015*, *A*017*, *A*018*, *A*021*, *A*030*, *A*041*, *A*043*, *A*045*, *A*046*, *A*047*, *A*014*, *A*020*, *A*023*, *A*026*, *A*029*, *A*036*, *A*040*, *A*050*, *A*052*, and *A*055*). The random chance that these eplets are a mismatch is 20.1% in African-Americans and 24.0% in European-Americans. Alternatively, the combination of eplets named AYVE "supereplet" is composed by 25AY, 129V, and 173E and was shared by another group of *MICA* alleles (*A*004*, *A*006*, *A*008*, *A*009*, *A*010*, *A*016*, *A*019*, *A*024*, and *A*044*). The random chance of their being a mismatch is 28.2% in African-Americans and 20.1% in European-Americans (118).

Panigrahi et al. (82, 119) analyzed the presence of Abs against MICA*001, MICA*002, MICA*004, MICA*008, and MICA*009 in serum samples of 185 patients transplanted with live related donor kidneys. Sixteen percent of all recipients developed anti-MICA Abs during the posttransplant period, 83% of the patients whose grafts eventually failed had both anti-HLA and anti-MICA Abs as compared to 29% patients who had only anti-MICA Abs, and 11% of those without any of the Abs (HLA or MICA) (82, 119).

Analysis of anti-MICA*001, MICA*002, MICA*004, MICA*008, and MICA*009 Abs in serum samples from 1,910 kidney recipients showed that a correlation between the presence of anti-MICA Abs and the reduced in kidney-allograft survival was not influenced by the simultaneous presence of Abs against HLA (120). In this study, decreased renal-allograft survival is associated with anti-MICA Abs formed before transplantation. It was also found that patients with Abs against MICA before transplantation did not received more transfusions than patients without such Abs, in contrast with the Zwirner et al.'s study (76). So the authors speculate that cross-reactivity with substances from the environment may play a role in priming the immune system, facilitating anti-MICA Ab production (120).

Suarez-Alvarez et al. (84) screened 284 kidney transplant sera for anti-MICA Abs and mapped the epitopes of MICA by screening a library of synthetic overlapping peptides from the extracellular domains of the protein against the sera from kidney transplant patients with anti-MICA Abs. Anti-MICA Abs were detected in 50 of 284 patients (17.6%), and they correlated with the development of AR. The authors found that nine regions were reactive with anti-MICA Abs. Five epitopes were located in constant regions (II, III, IV, VI, and IX) and were present in all *MICA* alleles, while the other four regions (I, V, VII, and VIII) mapped to variable sites of polymorphic amino acids among the different alleles products of MICA. In particular, regions V, VII, and VIII were the regions with the highest amino acid variability. Three polymorphic residues, 173 (E/K), 175 (S/G), and 181 (R/T), had determined allele-specific epitopes. The aminoacid 208Y and 213T, instead, contributed in the cross-reactivity among alleles (84).

Cox et al. (90) identified MICA IgG Abs directed against MICA*001, *002, *004, *007, *008, *009, *012, *017, *018, *019, and *027. Analysis of 116 healthy control subjects revealed only one subject with anti-MICA Abs (0.9%) and five subjects (4%) with anti-HLA class II Abs, while in a subgroup of 227 transplant recipients and their donors the coproduction of Abs to HLA and MICA significantly associated with acute cellular rejection (ACR). Analysis of patients with AAMR established strong associations with the presence of Abs against HLA class I and II, but not anti-MICA. By aligning *MICA* allele profiles present in the subgroup of 227 renal graft recipients and their respective donors, it was possible to establish the precise position of amino acid mismatches that correlate strongly with MICA Ab production. Mismatching at residues 36, 129, 173, 175, 213, and 251 showed the strongest association with anti-MICA Ab production in transplant recipients, while 91, 125, 156, and 221 residues were also mismatched between recipients and donors, but were not significantly associated with anti-MICA Ab production. There are two immunodominant motifs: MICA-G1 is characterized by residues 36 cysteine (C), 129 methionine (M), 173 lysine (K), 206

glycine (G), 210 tryptophan (W), and 215 serine (S). Alternatively MICA-G2 epitopes share residues 36 tyrosine (Y), 129 valine (V), 173 glutamic acid (E), 206 serine (S), 210 arginine (R), and 215 threonine (T). The majority of these recipients (10 out of 17 individuals, 59%) developed *de novo* donor-specific anri-MICA Abs posttransplantation, and there was a significant association of graft dysfunction with the presence of anti-MICA DSA alone after 2 years. In conclusion, it was discovered that mismatching *MICA* alleles lead to the development of anti-MICA Abs in some renal graft recipients, and the presence of anti-MICA DSA was independently associated with decreased glomerular filtration rate (eGFR) and poorer graft outcome (90).

Tonnerre et al. (106) went beyond the usual studies of anti-MICA Abs and focused on searching for a specific allele that could lead to a poorer outcome. The authors performed a study that showed that the MICA*008 (A5.1) molecule is a major antigenic determinant and target for recipient sensitization of kidney transplant patients. *MICA A5.1* is associated with four alleles: **023*, **028*, **053*, and **008*. The authors divided primary EC cultures from transplant donors in *MICA A5.1* homozygous, heterozygous, and control. The MICA surface expression was significantly higher on ECs from *A5.1/A5.1* donors than from controls. The *MICA A5.1* allele also leads to a reduction of sMICA and an increase in the MICA level in exosomes in ECs. Anti-MICA (A5.1) Abs intensities in the sera of recipients with anti-MICA Abs were not higher than intensities observed for other anti-MICA (control) Abs. However, when tested on EC cultures expressing physiologic levels of membrane-bound MICA, the sera only bound to ECs from *MICA A5.1* donors. This seemed to show that anti-MICA Abs bind ECs' targets in an allele-specific manner.

In fact, the combination of the donor carrying *MICA A5.1* and the recipient having a non-*MICA A5.1* allele was overrepresented in the group of MICA-sensitized patients compared with the group of non-immunized recipients (106).

Sapak et al. (108) concluded that anti-MICA Abs could not be responsible for the rejection if they were not directly detected in the transplanted graft. In the sera of 124 renal recipients, the authors found only 22 patients positive for anti-MICA Abs. The most frequent anti-MICA Abs were directed against *MICA*018* and *MICA*001*. *MICA*008* had the highest gene frequency (31%), followed by *MICA*002* (14%). Comparing *MICA* allele profiles of donors and anti-MICA Ab epitopes of their respective recipients, Sapak et al. found a match in only in 9 donor–recipient pairs (41%) while the sera of the other 13 patients was negative for Abs against graft MICA molecules, but positive for Abs against other MICA antigens. The majority (59%) of anti-MICA Abs in patients were not donor-specific, so the authors suggested that anti-MICA Ab induction was not caused by renal graft allogeneic stimulation but was also probably stimulated by other still unknown immune mechanisms (108).

Sanchez-Zapardiel et al. (112) studied 727 kidney recipients. They found that PRA$^+$MICA$^+$ recipients exhibited a longer time to reach optimal serum creatinine level after transplantation ($P = 0.005$) had the lowest eGFR at 3 months and PRA$^+$MICA$^+$ status independently increased the risk for chronic kidney disease stage 5 at month 3. Pretransplant anti-MICA Abs were poly-specific; anti-AYVE supereplet reactivity was higher in HLA$^+$MICA$^+$ versus HLA$^-$MICA$^+$ patients and superior than anti-CMGWS supereplet within HLA$^+$MICA$^+$ patients. The authors also found that some preformed anti-MICA Abs might bind complement, using the C1q Luminex assay. Sanchez-Zapardiel et al. analyzed 13 anti-MICA$^+$ pretransplant sera that were positive for the C1q binding assay and one of them (serum 3) exclusively recognized the AYVE supereplet with a strong reactivity against MICA*027 antigen. The authors concluded that these preformed anti-MICA Abs are able to mediate cell death by fixing and activating the complement cascade. So they speculated that the anti-MICA Abs might contribute to worse early kidney graft function (112).

Correlation between Anti-MICA Abs and Creatinine Levels or Estimated Glomerular Filtration Rate (eGRF) or Death-Censored Graft Survival (DCGSs)

Yao et al. (92) included 29 sensitized recipient patients who had undergone living-related donor renal transplantation between 2007 and 2009. They found a statistical difference in postoperative serum creatinine levels within 1 week between anti-MICA Ab-positive (135.4 ± 21.4 mol/L) and anti-MICA Ab-negative groups (108.6 ± 31.6 mol/L), but no significant difference between the two groups at discharge. To decrease the preexisting Abs (mainly IgG, IgM, and IgE), all recipients were treated with protein A immunoadsorptions, and this therapy was effective in decreasing anti-MICA Abs (92).

Zhang et al. (93) studied patients receiving primary kidney transplants (all from deceased donors) between 2004 and 2007. No significant association was found between the presence of anti-MICA and -HLA Abs, nor between the presence of anti-MICA Abs and 1-year graft survival rate. However, during the follow-up period, eGFR decreased 24.0 ± 3.4% in the anti-MICA Abs positive group, while it decreased only 8.4 ± 3.0% in anti-MICA Abs negative patients. A strong correlation between the production of anti-MICA Abs and renal impairment was also found. For these reasons, the authors concluded that patients with anti-MICA Abs had a more rapid deterioration of graft function, compared to those without anti-MICA Abs (93).

In another study that did not recognize MICA as a biomarker, sera from 779 kidney transplant recipients was tested with two single-antigen flow bead assays 1 year after transplantation. Thirteen of the 779 patients were lost to follow-up, 50 had lost their graft, and 33 died with a functioning graft. The prevalence of anti-MICA Abs was 5.3% at 1-year posttransplantation, and that MICA$^+$ patients were more frequently HLA sensitized and regrafted. However, 4-year DCGSs were not different between MICA$^+$ and MICA$^-$ patients (97 versus 94%, $P = 0.28$), and 4- and 8-year survival rates were similar in MICA$^+$ and MICA$^-$ patients. Thus, the hypothesis of an independent pathogenic role for MICA in long-term renal graft injury was not supported, and the authors questioned the utility of monitoring anti-MICA Abs posttransplant with single-antigen flow bead assays (94).

MICA Abs in Case Study

Narayan et al.'s (91) case study focused on a 14-year-old girl with branchiooto renal syndrome who underwent re-transplantation

with an HLA crossmatch-negative deceased donor kidney. She lost her first kidney transplant to chronic rejection at the age of 10 and underwent allograft nephrectomy. She was highly sensitized, and to improve her chances for transplantation, she underwent desensitization with high-dose IVIG and rituximab. When she received a deceased donor renal transplant, the pretransplant anti-HLA Ab testing showed no anti-donor HLA Abs. The patient maintained good allograft function until postoperative day 10 when she presented with fever and anuric renal failure. The only Ab found was donor-specific anti-MICA Ab, specifically directed against MICA*012 protein. Evaluation of the pretransplant serum revealed preformed anti-MICA*012 Abs with levels that were elevated both before transplant and at the time of rejection. Anti-MICA Abs levels declined with the initiation of plasmapheresis and IVIG and correlated well with normalization of renal function and resolution of ACR and AMR. The authors speculated that the sensitization to the MICA*012 protein was caused by prior sensitization from the first renal transplant or previous infections or transfusions. The conclusion of their research is that donor-specific anti-MICA Abs can be associated with both AMR and Banff type IIA ACR and may require treatment with plasmapheresis (91).

Ming et al. (109) studied a patient who suffered early aggressive AMR in the presence of DSA against MICA after her first renal transplant. The researchers found that anti-MICA–DSA in recipient serum could bind MICA-G1 antigens expressed in the cultured human umbilical cord vein endothelial cells (HUVECs). The recipient serum was cytotoxic to these HUVECs, but not against HUVECs that did not express MICA-G1 antigens in the presence of complement. The researchers discovered that the patient had been sensitized to MICA antigens and HLA, before transplantation, and the HLA alloantibodies were not specific to the first kidney donor, but the MICA alloantibodies were. In light of this discovery, the second renal transplant was with a negative MICA virtual crossmatch, and it was successful (109).

microRNA and mRNA's Analysis

Seiler et al. (62) showed that an elevated NKG2D mRNA expression in biopsy material was correlated with the severity of AR and detected NKG2D$^+$ cells located in clusters around tubules in biopsies derived from patients diagnosed with acute and chronic rejection. The expression of *NKG2D* mRNA was also detected in urinary sediments obtained 2–3 days before the AR episode. However, significant levels of MICA mRNA were not detected in the patient groups analyzed (62). For the first time, the focus was on the importance of the role of the NKG2D molecule, which is responsible for MICA signal transduction.

Another controversial paper regarding the role of MICA is the Racca et al.'s study (86), in which the authors obtained peripheral blood samples from 29 renal-transplanted patients (19 men). They classified patients it into three groups: AR group (9 patients with acute grade I/II allograft rejection), chronic rejection group (10 patients with chronic allograft rejection), and stable evolution group (10 patients with clinically stable allograft evolution). The authors observed that MICA mRNA levels in peripheral blood mononuclear cells showed similar expression levels in all groups evaluated and in the control group. They also found similar levels of MICA expression in a comparison of biopsy specimens from AR and nephrotoxic ATN patients. They did not find a correlation between MICA expression and renal graft state (86). It is interesting to note that the MICA expression in biopsies did not have a healthy control group, while expression of MICA mRNA may be a posttranscriptional control that modules MICA expression on the cell surface. The Racca et al.'s (86) study still represents an interesting opportunity to discuss the role of MICA as a biomarker.

Xue et al. (110) studied miR-338-5p, a microRNA downregulated in AMR renal allografts, and negatively correlated with BAFF. This molecule plays an important role in the differentiation, development, and proliferation of B lymphocytes. BAFF could be released in a soluble form (sBAFF) after cleavage and would bind to BAFF receptor. The receptor-associated factor 3 is a sort of adaptor for the BAFF–BAFF-R connection, it is implicated in a signal transduction, and it appeared to be a candidate target for miR-338-5p. In the study, 49 follow-up renal-transplanted recipients and a healthy control group were examined, and it was found that anti-HLA II Ab, anti-MICA Ab, and anti-HLA + MICA mixed Abs were all statistically increased in recipients. Serum miR-338-5p was significantly downregulated in renal-transplanted recipients compared with healthy volunteers and was inversely correlated with sBAFF. The authors speculate that miR-338-5p may regulate the BAFF signal, and they suggested that sBAFF was significantly negatively correlated with anti-MICA Abs (110).

Cytomegalovirus (CMV) and Polyomavirus and Transplantation

Cytomegalovirus infection is the most common viral complication after renal transplantation and solid organ transplantation in general. One hundred ninety-six recipients who underwent kidney transplantation during the past 6 years were assessed with at follow-up of at least 12 months. In this study, it was shown that the activating receptor NKG2D was expressed in a significantly higher number of NK cells at day 0 and day 20 compared to day 180 ($P = 0.01$ and $P = 0.003$, respectively) and compared to the control group ($P = 0.0003$ and $P = 0.0004$, respectively) (121). This finding suggests a possible mechanism for the activation of NKG2D that goes beyond organ rejection, but it is closely related. In fact, in the Hadaya et al. (121) study, it was shown that an expansion of the NKG2D$^+$ NK cell population occurred during acute CMV infection which decreased over time to a level very similar to that of the control group.

An interesting study that involved NKG2D, performed by Shabir et al. (117), demonstrated that CD4$^+$CD28^{null} T cell expansion is driven by latent CMV infection inflammation. The immune surveillance of CMV may have an unwanted consequence in the development of endothelial injury, which was proven to be mediated by CD4$^+$CD27$^-$CD28^{null} cells in *in vitro* experiments. NKG2D was upregulated on CD4$^+$CD27$^-$CD28^{null} cells isolated from patients in this study and might have an important component of the cytotoxic effects of these cells. In fact, CD4$^+$CD28^{null} cells were found predominantly in CMV-seropositive patients, and expanded in the posttransplantation period, and expressed markers of cytotoxicity (NKG2D and perforin) and endothelial homing (CX3CR1). Isolated CD4$^+$CD27$^-$CD28^{null} cells

previously exposed only to CMV-derived antigens showed signs of endothelial damage and apoptosis, and this effect was mitigated by NKG2D-blocking Ab. They concluded that the increase in $CD4^+CD28^{null}$ cell frequencies was associated with delayed graft function and lower eGRF at end follow-up, and this could be mediated by NKG2D (117).

Another study by Tonnerre et al. (122) investigated the implication of MICA in BK polyomavirus (BKPyV) reactivation in a cohort of 144 transplant donor/recipient pairs including recipients with no reactivation (control). BKPyV is frequently reactivated in kidney transplant recipients receiving an immunosuppressive regimen and is associated with nephropathy (BKPyVAN) and graft rejection. They investigated the impact of the *MICA A5.1* mutation on recipient BKPyV reactivation, and they found that recipients carrying a *non-MICA A5.1 (nA5.1)* genotype transplanted with a kidney from a donor carrying the *A5.1 MICA* variant had a lower risk of BKPyV reactivation ($P = 0.0148$). So they speculated that *MICA A5.1* could be a protective allele toward BKPyV infection (122). Interestingly, these researchers also found that the donor (A5.1)–recipient (nA5.1) combination was overrepresented in the group of MICA-sensitized patients, but in the latter, MICA A5.1 seemed to be a protective factor for a virus related to graft rejection (106).

CONCLUSION

Since the *MICA* gene was first described, it has been the subject of many studies aiming to comprehend its immunobiology and the role it plays in fine-tuning the innate and adaptive immune response. MICA appears to be involved in transplant rejection, immune response against viruses and intracellular bacteria, inflammation, homeostasis of epithelia, immune response against tumors, and tumor immune escape mechanisms. However, there remain a number of open issues to be addressed surrounding MICA's functions and roles. Developing and implementing typing strategies for *MICA* alleles may increase the chance for positive outcomes in solid organ transplantation by allowing better matching. MICA's biological function is achieved through its interaction with the NKG2D receptor. This activating receptor and its ligands are deeply involved in the outcomes of transplanted grafts, in fact, the overexpression of NKG2DLs could be involved in rejection episodes and can contribute to graft loss (44).

Various studies have shown that anti-MICA Abs, binding to MICA molecules expressed at the endothelial allograft cell surface, may have relevance to kidney transplantation outcome (81, 106). However, it is important to note that some studies, such as that of Lemy et al. (87), where the presence of anti-MICA Abs do not show adverse effects in renal graft outcomes (87). Also, *MICA* mRNA level analysis in blood mononuclear cells did not show a correlation between *MICA* expression and renal graft state (86). Seiler et al. (62) did not find an enhancement of mRNA expression levels of MICA in kidney biopsies from patients undergoing AR or chronic allograft nephropathy, but they observed increased *NKG2D* expression. In an interesting study performed by Sapak et al. (108), 41% of the detected anti-MICA Abs were donor-specific, but an astonishing 36% were anti-MICA Abs against self-MICA antigens and several patients (27%) produced both (108).

Regarding NKG2D, there are studies that report that it is possible to prolong graft survival and to prevent CD28-independent rejection of cardiac allografts after blocking NKG2D (123).

We can conclude that the role of MICA and NKG2D in transplant outcome is not yet clear; MICA-mediated rejection probably is not just a reaction to the MICA non-self protein. The stress condition following a transplant causes a general inflammatory status in the recipient. This could increase MICA production, thus activating the response *via* the NKG2D receptor. The clinical impact of these interactions will remain unclear until further studies are performed.

AUTHOR CONTRIBUTIONS

MR: planning and organizing structure of the review; research and analysis of the papers; wrote the review; and planning and creation of figures and tables. MB: planning and organizing structure of the review and contributions to the sections writing/critical review of the manuscript.

FUNDING

This work was funded by LIGH-FUNPAR ALLIANCE.

REFERENCES

1. Suthanthiran M, Strom TB. Renal transplantation. *N Engl J Med* (1994) 331(6):365–76. doi:10.1056/nejm199408113310606
2. Bahram S. MIC genes: from genetics to biology. *Adv Immunol* (2000) 76:1–60. doi:10.1016/S0065-2776(01)76018-X
3. 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.
4. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science* (1999) 285(5428):727–9.
5. 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. doi:10.1111/j.1365-2370.2004.00457.x
6. Zwirner NW, Fuertes MB, Girart MaV, Domaica CI, Rossi LE. Immunobiology of the human MHC class I chain-related gene A (MICA): from transplantation immunology to tumor immune escape. *Immunologia* (2006) 25(1):25–38.
7. Fodil N, Laloux L, Wanner V, Pellet P, Hauptmann G, Mizuki N, et al. Allelic repertoire of the human MHC class I MICA gene. *Immunogenetics* (1996) 44(5):351–7.
8. Luo J, Tian W, Pan F, Liu X, Li L. Allelic and haplotypic diversity of 5'promoter region of the MICA gene. *Hum Immunol* (2014) 75(4):383–8. doi:10.1016/j.humimm.2013.12.010
9. Cox ST, Madrigal JA, Saudemont A. Diversity and characterization of polymorphic 5' promoter haplotypes of MICA and MICB genes. *Tissue Antigens* (2014) 84(3):293–303. doi:10.1111/tan.12400
10. Choy MK, Phipps ME. MICA polymorphism: biology and importance in immunity and disease. *Trends Mol Med* (2010) 16(3):97–106. doi:10.1016/j.molmed.2010.01.002

11. Bahram S, Mizuki N, Inoko H, Spies T. Nucleotide sequence of the human MHC class I MICA gene. *Immunogenetics* (1996) 44(1):80–1.
12. 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. *Proc Natl Acad Sci U S A* (1996) 93(22):12445–50.
13. 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.
14. Chauveau A, Tonnerre P, Pabois A, Gavlovsky PJ, Chatelais M, Coupel S, et al. Endothelial cell activation and proliferation modulate NKG2D activity by regulating MICA expression and shedding. *J Innate Immun* (2014) 6(1):89–104. doi:10.1159/000351605
15. Zou Y, Stastny P. Alternatively spliced forms of MICA and MICB lacking exon 3 in a human cell line and evidence of presence of similar RNA in human peripheral blood mononuclear cells. *Immunogenetics* (2002) 54(9):671–4. doi:10.1007/s00251-002-0496-y
16. Zwirner NW, Fernández-Viña MA, Stastny P. MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes. *Immunogenetics* (1998) 47(2):139–48.
17. Zwirner NW, Dole K, Stastny P. Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes, and monocytes. *Hum Immunol* (1999) 60(4):323–30.
18. Mizuki N, Ota M, Kimura M, Ohno S, Ando H, Katsuyama Y, et al. Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behcet disease. *Proc Natl Acad Sci U S A* (1997) 94(4):1298–303.
19. Pyo CW, Hur SS, Kim YK, Choi HB, Kim TY, Kim TG. Distribution of MICA alleles and haplotypes associated with HLA in the Korean population. *Hum Immunol* (2003) 64(3):378–84. doi:10.1016/S0198-8859(02)00826-1
20. Gambelunghe G, Brozzetti A, Ghaderi M, Candeloro P, Tortoioli C, Falorni A. MICA gene polymorphism in the pathogenesis of type 1 diabetes. *Ann N Y Acad Sci* (2007) 1110:92–8. doi:10.1196/annals.1423.011
21. Zhang Y, Han M, Vorhaben R, Giang C, Lavingia B, Stastny P. Study of MICA alleles in 201 African Americans by multiplexed single nucleotide extension (MSNE) typing. *Hum Immunol* (2003) 64(1):130–6. doi:10.1016/S0198-8859(02)00743-7
22. 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.
23. Norris S, Kondeatis E, Collins R, Satsangi J, Clare M, Chapman R, et al. Mapping MHC-encoded susceptibility and resistance in primary sclerosing cholangitis: the role of MICA polymorphism. *Gastroenterology* (2001) 120(6):1475–82. doi:10.1053/gast.2001.24041
24. Marin ML, Savioli CR, Yamamoto JH, Kalil J, Goldberg AC. MICA polymorphism in a sample of the São Paulo population, Brazil. *Eur J Immunogenet* (2004) 31(2):63–71. doi:10.1111/j.1365-2370.2004.00446.x
25. Zhang Y, Lazaro AM, Zou Y, Lavingia B, Moraes EM, Moraes RJ, et al. MICA polymorphism in South American Indians. *Immunogenetics* (2002) 53(10–11):900–6. doi:10.1007/s00251-001-0426-4
26. 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. doi:10.1016/j.humimm.2006.02.009
27. Romphruk AV, Naruse TK, Romphruk A, Kawata H, Puapairoj C, Kulski JK, et al. Diversity of MICA (PERB11.1) and HLA haplotypes in Northeastern Thais. *Tissue Antigens* (2001) 58(2):83–9. doi:10.1034/j.1399-0039.2001.580203.x
28. Komatsu-Wakui M, Tokunaga K, Ishikawa Y, Kashiwase K, Moriyama S, Tsuchiya N, et al. MIC-A polymorphism in Japanese and a MIC-A-MIC-B null haplotype. *Immunogenetics* (1999) 49(7–8):620–8.
29. Tian W, Boggs DA, Ding WZ, Chen DF, Fraser PA. MICA genetic polymorphism and linkage disequilibrium with HLA-B in 29 African-American families. *Immunogenetics* (2001) 53(9):724–8. doi:10.1007/s00251-001-0392-x
30. 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. doi:10.1016/j.humimm.2005.06.008
31. Mizuki N, Meguro A, Tohnai I, Gul A, Ohno S, Mizuki N. Association of major histocompatibility complex class I chain-related gene A and HLA-B alleles with Behcet's disease in Turkey. *Jpn J Ophthalmol* (2007) 51(6):431–6. doi:10.1007/s10384-007-0473-y
32. 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. doi:10.1111/j.1399-0039.2008.01142.x
33. Wang W, Tian W, Zhu F, Liu X, Li L, Wang F. MICA, MICB polymorphisms and linkage disequilibrium with HLA-B in a Chinese Mongolian population. *Scand J Immunol* (2016) 83(6):456–62. doi:10.1111/sji.12437
34. Wang YJ, Zhang NJ, Chen E, Chen CJ, Bu YH, Yu P. MICA/B genotyping of Tujias from Zhangjiajie, Hunan Province, China. *Hum Immunol* (2016) 77(4):340–1. doi:10.1016/j.humimm.2016.01.014
35. Wang YJ, Zhang NJ, Chen E, Chen CJ, Bu YH, Yu P. Allele polymorphism and haplotype diversity of MICA/B in Tujia nationality of Zhangjiajie, Hunan Province, China. *Hum Immunol* (2016) 77(5):411–7. doi:10.1016/j.humimm.2016.03.005
36. 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. doi:10.1007/s002510100325
37. Boukouaci W, Busson M, Peffault de Latour R, Rocha V, Suberbielle C, Bengoufa D, et al. MICA-129 genotype, soluble MICA, and anti-MICA antibodies as biomarkers of chronic graft-versus-host disease. *Blood* (2009) 114(25):5216–24. doi:10.1182/blood-2009-04-217430
38. Lopez-Hernandez R, Valdes M, Lucas D, Campillo JA, Martinez-Garcia P, Salama H, et al. Association analysis of MICA gene polymorphism and MICA-129 dimorphism with inflammatory bowel disease susceptibility in a Spanish population. *Hum Immunol* (2010) 71(5):512–4. doi:10.1016/j.humimm.2010.02.003
39. Zhao J, Jiang Y, Lei Y, Zou K, Wang C, Huang S, et al. Functional MICA-129 polymorphism and serum levels of soluble MICA are correlated with ulcerative colitis in Chinese patients. *J Gastroenterol Hepatol* (2011) 26(3):593–8. doi:10.1111/j.1440-1746.2010.06524.x
40. Isernhagen A, Malzahn D, Viktorova E, Elsner L, Monecke S, von Bonin F, et al. The MICA-129 dimorphism affects NKG2D signaling and outcome of hematopoietic stem cell transplantation. *EMBO Mol Med* (2015) 7(11):1480–502. doi:10.15252/emmm.201505246
41. Isernhagen A, Schilling D, Monecke S, Shah P, Elsner L, Walter L, et al. The MICA-129Met/Val dimorphism affects plasma membrane expression and shedding of the NKG2D ligand MICA. *Immunogenetics* (2016) 68(2):109–23. doi:10.1007/s00251-015-0884-8
42. Li P, Morris DL, Willcox BE, Steinle A, Spies T, Strong RK. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nat Immunol* (2001) 2(5):443–51. doi:10.1038/87757
43. Eleme K, Taner SB, Onfelt B, Collinson LM, McCann FE, Chalupny NJ, et al. Cell surface organization of stress-inducible proteins ULBP and MICA that stimulate human NK cells and T cells via NKG2D. *J Exp Med* (2004) 199(7):1005–10. doi:10.1084/jem.20032194
44. Suarez-Alvarez B, Lopez-Vazquez A, Baltar JM, Ortega F, Lopez-Larrea C. Potential role of NKG2D and its ligands in organ transplantation: new target for immunointervention. *Am J Transplant* (2009) 9(2):251–7. doi:10.1111/j.1600-6143.2008.02526.x
45. 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 gamma delta T cells. *J Immunol* (2002) 169(3):1236–40. doi:10.4049/jimmunol.169.3.1236
46. Li J, Cui L, He W. Distinct pattern of human Vdelta1 gammadelta T cells recognizing MICA. *Cell Mol Immunol* (2005) 2(4):253–8.
47. Xu B, Pizarro JC, Holmes MA, McBeth C, Groh V, Spies T, et al. Crystal structure of a gammadelta T-cell receptor specific for the human MHC class I homolog MICA. *Proc Natl Acad Sci U S A* (2011) 108(6):2414–9. doi:10.1073/pnas.1015433108
48. Ho EL, Heusel JW, Brown MG, Matsumoto K, Scalzo AA, Yokoyama WM. Murine Nkg2d and Cd94 are clustered within the natural killer complex and are expressed independently in natural killer cells. *Proc Natl Acad Sci U S A* (1998) 95(11):6320–5.

49. Vance RE, Tanamachi DM, Hanke T, Raulet DH. Cloning of a mouse homolog of CD94 extends the family of C-type lectins on murine natural killer cells. *Eur J Immunol* (1997) 27(12):3236–41. doi:10.1002/eji.1830271222
50. Glienke J, Sobanov Y, Brostjan C, Steffens C, Nguyen C, Lehrach H, et al. The genomic organization of NKG2C, E, F, and D receptor genes in the human natural killer gene complex. *Immunogenetics* (1998) 48(3):163–73.
51. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, et al. Human diversity in killer cell inhibitory receptor genes. *Immunity* (1997) 7(6):753–63.
52. Jamieson AM, Diefenbach A, McMahon CW, Xiong N, Carlyle JR, Raulet DH. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. *Immunity* (2002) 17(1):19–29. doi:10.1016/S1074-7613(02)00333-3
53. Shum BP, Flodin LR, Muir DG, Rajalingam R, Khakoo SI, Cleland S, et al. Conservation and variation in human and common chimpanzee CD94 and NKG2 genes. *J Immunol* (2002) 168(1):240–52. doi:10.4049/jimmunol.168.1.240
54. Hayashi T, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the NKG2D haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. *Cancer Res* (2006) 66(1):563–70. doi:10.1158/0008-5472.CAN-05-2776
55. Ucisik-Akkaya E, Dorak MT. A study of natural killer cell lectin-like receptor K1 gene (KLRK1/NKG2D) region polymorphisms in a European population sample. *Tissue Antigens* (2009) 73(2):177–83. doi:10.1111/j.1399-0039.2008.01181.x
56. Furue H, Matsuo K, Kumimoto H, Hiraki A, Suzuki T, Yatabe Y, et al. Decreased risk of colorectal cancer with the high natural killer cell activity NKG2D genotype in Japanese. *Carcinogenesis* (2008) 29(2):316–20. doi:10.1093/carcin/bgm260
57. Furue H, Kumimoto H, Matsuo K, Suzuki T, Hasegawa Y, Shinoda M, et al. Opposite impact of NKG2D genotype by lifestyle exposure to risk of aerodigestive tract cancer among Japanese. *Int J Cancer* (2008) 123(1):181–6. doi:10.1002/ijc.23456
58. Espinoza JL, Takami A, Onizuka M, Sao H, Akiyama H, Miyamura K, et al. NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies. *Haematologica* (2009) 94(10):1427–34. doi:10.3324/haematol.2009.008318
59. Garrity D, Call ME, Feng J, Wucherpfennig KW. The activating NKG2D receptor assembles in the membrane with two signaling dimers into a hexameric structure. *Proc Natl Acad Sci U S A* (2005) 102(21):7641–6. doi:10.1073/pnas.0502439102
60. Bacon L, Eagle RA, Meyer M, Easom N, Young NT, Trowsdale J. Two human ULBP/RAET1 molecules with transmembrane regions are ligands for NKG2D. *J Immunol* (2004) 173(2):1078–84. doi:10.4049/jimmunol.173.2.1078
61. Groh V, Rhinehart R, Randolph-Habecker J, Topp MS, Riddell SR, Spies T. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. *Nat Immunol* (2001) 2(3):255–60. doi:10.1038/85321
62. Seiler M, Brabcova I, Viklicky O, Hribova P, Rosenberger C, Pratschke J, et al. Heightened expression of the cytotoxicity receptor NKG2D correlates with acute and chronic nephropathy after kidney transplantation. *Am J Transplant* (2007) 7(2):423–33. doi:10.1111/j.1600-6143.2006.01625.x
63. Saez-Borderias A, Guma M, Angulo A, Bellosillo B, Pende D, Lopez-Botet M. Expression and function of NKG2D in CD4+ T cells specific for human cytomegalovirus. *Eur J Immunol* (2006) 36(12):3198–206. doi:10.1002/eji.200636682
64. Park YP, Choi SC, Kiesler P, Gil-Krzewska A, Borrego F, Weck J, et al. Complex regulation of human NKG2D-DAP10 cell surface expression: opposing roles of the gammac cytokines and TGF-beta1. *Blood* (2011) 118(11):3019–27. doi:10.1182/blood-2011-04-346825
65. Fernandez-Sanchez A, Baragano Raneros A, Carvajal Palao R, Sanz AB, Ortiz A, Ortega F, et al. DNA demethylation and histone H3K9 acetylation determine the active transcription of the NKG2D gene in human CD8+ T and NK cells. *Epigenetics* (2013) 8(1):66–78. doi:10.4161/epi.23115
66. Crane CA, Han SJ, Barry JJ, Ahn BJ, Lanier LL, Parsa AT. TGF-beta downregulates the activating receptor NKG2D on NK cells and CD8+ T cells in glioma patients. *Neuro Oncol* (2010) 12(1):7–13. doi:10.1093/neuonc/nop009
67. Codo P, Weller M, Meister G, Szabo E, Steinle A, Wolter M, et al. MicroRNA-mediated down-regulation of NKG2D ligands contributes to glioma immune escape. *Oncotarget* (2014) 5(17):7651–62. doi:10.18632/oncotarget.2287
68. Muntasell A, Magri G, Pende D, Angulo A, Lopez-Botet M. Inhibition of NKG2D expression in NK cells by cytokines secreted in response to human cytomegalovirus infection. *Blood* (2010) 115(25):5170–9. doi:10.1182/blood-2009-11-256479
69. Burgess SJ, Marusina AI, Pathmanathan I, Borrego F, Coligan JE. IL-21 down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells. *J Immunol* (2006) 176(3):1490–7. doi:10.4049/jimmunol.176.3.1490
70. Peraldi MN, Berrou J, Dulphy N, Seidowsky A, Haas P, Boissel N, et al. Oxidative stress mediates a reduced expression of the activating receptor NKG2D in NK cells from end-stage renal disease patients. *J Immunol* (2009) 182(3):1696–705. doi:10.4049/jimmunol.182.3.1696
71. Karimi MA, Aguilar OA, Zou B, Bachmann MH, Carlyle JR, Baldwin CL, et al. A truncated human NKG2D splice isoform negatively regulates NKG2D-mediated function. *J Immunol* (2014) 193(6):2764–71. doi:10.4049/jimmunol.1400920
72. Boukouaci W, Al-Daccak R, Dulphy N, Lauden L, Amokrane K, Fortier C, et al. Soluble MICA-NKG2D interaction upregulates IFN-gamma production by activated CD3-CD56+ NK cells: potential impact on chronic graft versus host disease. *Hum Immunol* (2013) 74(12):1536–41. doi:10.1016/j.humimm.2013.08.281
73. Yu R, Xu S, Wang Y, Cai H, Xu P. Role of MICA expression, anti-MICA antibodies and serum MICA during acute rejection in a rat-to-mouse cardiac transplantation model. *Int J Clin Exp Pathol* (2015) 8(11):14514–20.
74. Kummer JA, Wever PC, Kamp AM, ten Berge IJ, Hack CE, Weening JJ. Expression of granzyme A and B proteins by cytotoxic lymphocytes involved in acute renal allograft rejection. *Kidney Int* (1995) 47(1):70–7.
75. Hidalgo LG, Sis B, Sellares J, Campbell PM, Mengel M, Einecke G, et al. NK cell transcripts and NK cells in kidney biopsies from patients with donor-specific antibodies: evidence for NK cell involvement in antibody-mediated rejection. *Am J Transplant* (2010) 10(8):1812–22. doi:10.1111/j.1600-6143.2010.03201.x
76. Zwirner NW, Marcos CY, Mirbaha F, Zou Y, Stastny P. Identification of MICA as a new polymorphic alloantigen recognized by antibodies in sera of organ transplant recipients. *Hum Immunol* (2000) 61(9):917–24. doi:10.1016/S0198-8859(00)00162-2
77. Hankey KG, Drachenberg CB, Papadimitriou JC, Klassen DK, Philosophe B, Bartlett ST, et al. MIC expression in renal and pancreatic allografts. *Transplantation* (2002)73(2):304–6. doi:10.1097/00007890-200201270-00029
78. Opelz G. Non-HLA transplantation immunity revealed by lymphocytotoxic antibodies. *Lancet* (2005) 365(9470):1570–6. doi:10.1016/S0140-6736(05)66458-6
79. Mizutani K, Terasaki P, Rosen A, Esquenazi V, Miller J, Shih RN, et al. Serial ten-year follow-up of HLA and MICA antibody production prior to kidney graft failure. *Am J Transplant* (2005) 5(9):2265–72. doi:10.1111/j.1600-6143.2005.01016.x
80. Amezaga N, Crespo M, Lopez-Cobos M, Millan MA, Vinas O, Sole M, et al. Relevance of MICA antibodies in acute humoral rejection in renal transplant patients. *Transpl Immunol* (2006) 17(1):39–42. doi:10.1016/j.trim.2006.09.016
81. Mizutani K, Terasaki P, Bignon JD, Hourmant M, Cesbron-Gautier A, Shih RN, et al. Association of kidney transplant failure and antibodies against MICA. *Hum Immunol* (2006) 67(9):683–91. doi:10.1016/j.humimm.2006.06.002
82. Panigrahi A, Gupta N, Siddiqui JA, Margoob A, Bhowmik D, Guleria S, et al. Post transplant development of MICA and anti-HLA antibodies is associated with acute rejection episodes and renal allograft loss. *Hum Immunol* (2007) 68(5):362–7. doi:10.1016/j.humimm.2007.01.006
83. 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. doi:10.1056/NEJMoa067160
84. Suarez-Alvarez B, Alonso-Arias R, Bravo-Mendoza C, Lopez-Vazquez A, Ortega T, Baltar JM, et al. Identification of epitopes and immunodominant regions on the MICA protein defined by alloantibodies from kidney

transplant patients. *Transplantation* (2009) 88(3 Suppl):S68–77. doi:10.1097/TP.0b013e3181afeb7a
85. Alvarez-Marquez A, Aguilera I, Gentil MA, Caro JL, Bernal G, Fernandez Alonso J, et al. Donor-specific antibodies against HLA, MICA, and GSTT1 in patients with allograft rejection and C4d deposition in renal biopsies. *Transplantation* (2009) 87(1):94–9. doi:10.1097/TP.0b013e31818bd790
86. Racca AL, Veaute CM, Bailat AS, Gaite L, Arriola M, Hajos SE, et al. Expression of HLA-G and MICA mRNA in renal allograft. *Transpl Immunol* (2009) 21(1):10–2. doi:10.1016/j.trim.2009.01.002
87. Lemy A, Andrien M, Wissing KM, Ryhahi K, Vandersarren A, Racape J, et al. Major histocompatibility complex class 1 chain-related antigen a antibodies: sensitizing events and impact on renal graft outcomes. *Transplantation* (2010) 90(2):168–74. doi:10.1097/TP.0b013e3181e228f7
88. Li L, Chen A, Chaudhuri A, Kambham N, Sigdel T, Chen R, et al. Compartmental localization and clinical relevance of MICA antibodies after renal transplantation. *Transplantation* (2010) 89(3):312–9. doi:10.1097/TP.0b013e3181bbbe4c
89. Luo L, Lu J, Wei L, Long D, Guo JY, Shan J, et al. The role of HIF-1 in up-regulating MICA expression on human renal proximal tubular epithelial cells during hypoxia/reoxygenation. *BMC Cell Biol* (2010) 11:91. doi:10.1186/1471-2121-11-91
90. Cox ST, Stephens HA, Fernando R, Karasu A, Harber M, Howie AJ, et al. Major histocompatibility complex class I-related chain A allele mismatching, antibodies, and rejection in renal transplantation. *Hum Immunol* (2011) 72(10):827–34. doi:10.1016/j.humimm.2011.05.004
91. Narayan S, Tsai EW, Zhang Q, Wallace WD, Reed EF, Ettenger RB. Acute rejection associated with donor-specific anti-MICA antibody in a highly sensitized pediatric renal transplant recipient. *Pediatr Transplant* (2011) 15(1):E1–7. doi:10.1111/j.1399-3046.2010.01407.x
92. Yao QC, Wang W, Li XB, Yin H, Zhang XD. Expression characteristics of major histocompatibility complex class I-related chain A antibodies and immunoadsorption effect in sensitized recipients of kidney transplantation. *Chin Med J (Engl)* (2011) 124(5):669–73. doi:10.3760/cma.j.issn.0366-6999.2011.05.006
93. Zhang M, Lu FM, Qu LX, He J, Yuan XN, Gu Y. Serum major-histocompatibility-complex class I-related chain A antibody detection for the evaluation of graft dysfunction in renal allograft recipients. *Chin Med J (Engl)* (2011) 124(14):2127–31. doi:10.3760/cma.j.issn.0366-6999.2011.14.009
94. Lemy A, Andrien M, Lionet A, Labalette M, Noel C, Hiesse C, et al. Posttransplant major histocompatibility complex class I chain-related gene A antibodies and long-term graft outcomes in a multicenter cohort of 779 kidney transplant recipients. *Transplantation* (2012) 93(12):1258–64. doi:10.1097/TP.0b013e31824fd8f1
95. Li Z, Luo M, Qiu J, Liu Y, Fan Y, Jahr FM, et al. Detection of antibodies against major histocompatibility complex class I-related chain A in long-term renal graft recipients. *Exp Clin Transplant* (2012) 10(3):239–42. doi:10.6002/ect.2011.0103
96. Seyhun Y, Ozdilli K, Oguz F, Karahan G, Onal E, Turkmen A, et al. Human leukocyte antigen and major histocompatibility complex class I-related chain A antibodies after kidney transplantation in Turkish renal transplant recipients. *Transplant Proc* (2012) 44(6):1660–6. doi:10.1016/j.transproceed.2012.04.018
97. Rodriguez Ferrero ML, Arroyo D, Panizo N, Vicario JL, Balas A, Anaya F. Monitoring of circulating antibodies in a renal transplantation population: preliminary results. *Transplant Proc* (2012) 44(9):2548–50. doi:10.1016/j.transproceed.2012.09.072
98. Solgi G, Furst D, Mytilineos J, Pourmand G, Amirzargar AA. Clinical relevance of pre and post-transplant immune markers in kidney allograft recipients: anti-HLA and MICA antibodies and serum levels of sCD30 and sMICA. *Transpl Immunol* (2012) 26(2–3):81–7. doi:10.1016/j.trim.2011.12.002
99. Akgul SU, Oguz FS, Caliskan Y, Kekik C, Cagatay P, Turkmen A, et al. The effect of anti-human leukocyte antigen, anti-major histocompatibility complex class 1 chain-related antigen a, and anti-glutathione transferase-T1 antibodies on the long-term survival of renal allograft. *Transplant Proc* (2013) 45(3):890–4. doi:10.1016/j.transproceed.2013.02.097
100. Chaudhuri A, Ozawa M, Everly MJ, Ettenger R, Dharnidharka V, Benfield M, et al. The clinical impact of humoral immunity in pediatric renal transplantation. *J Am Soc Nephrol* (2013) 24(4):655–64. doi:10.1681/ASN.2012070663
101. Ding X, Jiao P, Wang X, Xue W, Tian P, Li Y, et al. [Significance of MICA antibody monitoring in management of acute and chronic rejection after renal transplantation]. *Nan Fang Yi Ke Da Xue Xue Bao* (2013) 33(10):1427–31. doi:10.3969/j.issn.1673-4254.2013.10.04
102. He J, Li C, Yuan XN, Zhang JL, Li Y, Wei XD, et al. Anti-human leukocyte antigens and anti-major histocompatibility complex class I-related chain A antibody expression in kidney transplantation during a four-year follow-up. *Chin Med J (Engl)* (2013) 126(15):2815–20. doi:10.3760/cma.j.issn.0366-6999.20131143
103. Jin J, Xu Y, Wang H, Huang H, He Q, Wu P, et al. Peritubular capillaritis in early renal allograft dysfunction is an indicator of acute rejection. *Transplant Proc* (2013) 45(1):163–71. doi:10.1016/j.transproceed.2012.08.018
104. Li Z, Wen X, Wang Y, Luo M, Qiu J. Effect of mycophenolic acid and bortezomib on purified human B cells: an in vitro study of long-term functionally stable MICA-sensitized renal recipients. *Exp Clin Transplant* (2013) 11(6):482–8. doi:10.6002/ect.2012.0281
105. Sanchez-Zapardiel E, Castro-Panete MJ, Castillo-Rama M, Morales P, Lora-Pablos D, Valero-Hervas D, et al. Harmful effect of preformed anti-MICA antibodies on renal allograft evolution in early posttransplantation period. *Transplantation* (2013) 96(1):70–8. doi:10.1097/TP.0b013e3182943506
106. Tonnerre P, Gerard N, Chatelais M, Poli C, Allard S, Cury S, et al. MICA variant promotes allosensitization after kidney transplantation. *J Am Soc Nephrol* (2013) 24(6):954–66. doi:10.1681/ASN.2012080814
107. Zhang LW, Peng ZG, Xian WH, Cui XQ, Sun HB, Li EG, et al. Association of de novo human leukocyte antigen and major histocompatibility complex class I chain-related gene-A antibodies and proteinuria with graft survival 5 years after renal transplantation. *Transplant Proc* (2013) 45(9):3249–53. doi:10.1016/j.transproceed.2013.08.029
108. Sapak M, Chrenova S, Tirpakova J, Zilinska Z, Durmanova V, Shawkatova I, et al. Donor non-specific MICA antibodies in renal transplant recipients. *Immunobiology* (2014) 219(2):109–12. doi:10.1016/j.imbio.2013.08.006
109. Ming Y, Hu J, Luo Q, Ding X, Luo W, Zhuang Q, et al. Acute antibody-mediated rejection in presence of MICA-DSA and successful renal re-transplant with negative-MICA virtual crossmatch. *PLoS One* (2015) 10(5):e0127861. doi:10.1371/journal.pone.0127861
110. Xu H, Ma X, He X, Dong P, Xue D, Zhang Y, et al. Serum miR-338-5p, soluble B-cell-activating factor, allo-antibodies, and renal transplantation. *Transplant Proc* (2015) 47(2):337–42. doi:10.1016/j.transproceed.2014.11.030
111. Cai J, Terasaki PI, Zhu D, Lachmann N, Schonemann C, Everly MJ, et al. Complement-fixing antibodies against denatured HLA and MICA antigens are associated with antibody mediated rejection. *Exp Mol Pathol* (2016) 100(1):45–50. doi:10.1016/j.yexmp.2015.11.023
112. Sanchez-Zapardiel E, Castro-Panete MJ, Mancebo E, Morales P, Laguna-Goya R, Morales JM, et al. Early renal graft function deterioration in recipients with preformed anti-MICA antibodies: partial contribution of complement-dependent cytotoxicity. *Nephrol Dial Transplant* (2016) 31(1):150–60. doi:10.1093/ndt/gfv308
113. Feng L, Cheng F, Ye Z, Li S, He Y, Yao X, et al. The effect of renal ischemia-reperfusion injury on expression of RAE-1 and H60 in mice kidney. *Transplant Proc* (2006) 38(7):2195–8. doi:10.1016/j.transproceed.2006.06.013
114. Zheng G, Zheng L, Wang Y, Wu H, Kairaitis L, Zhang C, et al. NK cells do not mediate renal injury in murine adriamycin nephropathy. *Kidney Int* (2006) 69(7):1159–65. doi:10.1038/sj.ki.5000244
115. Hadaya K, de Rham C, Bandelier C, Bandelier C, Ferrari-Lacraz S, Jendly S, et al. Natural killer cell receptor repertoire and their ligands, and the risk of CMV infection after kidney transplantation. *Am J Transplant* (2008) 8(12):2674–83. doi:10.1111/j.1600-6143.2008.02431.x
116. Zhang ZX, Wang S, Huang X, Min WP, Sun H, Liu W, et al. NK cells induce apoptosis in tubular epithelial cells and contribute to renal ischemia-reperfusion injury. *J Immunol* (2008) 181(11):7489–98. doi:10.4049/jimmunol.181.11.7489
117. Shabir S, Smith H, Kaul B, Pachnio A, Jham S, Kuravi S, et al. Cytomegalovirus-associated CD4(+) CD28(null) cells in NKG2D-dependent glomerular

endothelial injury and kidney allograft dysfunction. *Am J Transplant* (2016) 16(4):1113–28. doi:10.1111/ajt.13614
118. Duquesnoy RJ, Mostecki J, Hariharan J, Balazs I. Structurally based epitope analysis of major histocompatibility complex class I-related chain A (MICA) antibody specificity patterns. *Hum Immunol* (2008) 69(12): 826–32. doi:10.1016/j.humimm.2008.10.003
119. Panigrahi A, Gupta N, Siddiqui JA, Margoob A, Bhowmik D, Guleria S, et al. Monitoring of anti-HLA and anti-major histocompatibility complex class I related chain A antibodies in living related renal donor transplantation. *Transplant Proc* (2007) 39(3):759–60. doi:10.1016/j.transproceed.2007.02.009
120. Zou Y, Stastny P, Süsal C, Döhler B, Opelz G. Antibodies against MICA antigens and kidney-transplant rejection. *N Engl J Med* (2007) 357(13):1293–300. doi:10.1056/NEJMoa067160
121. Hadaya K, de Rham C, Bandelier C, Ferrari-Lacraz S, Jendly S, Berney T, et al. Natural killer cell receptor repertoire and their ligands, and the risk of CMV infection after kidney transplantation. *Am J Transplant* (2008) 8(12):2674–83. doi:10.1111/j.1600-6143.2008.02431.x
122. Tonnerre P, Gerard N, Gavlovsky PJ, Mazalrey S, Hourmant M, Cheneau ML, et al. MICA mutant A5.1 influences BK polyomavirus reactivation and associated nephropathy after kidney transplantation. *J Infect Dis* (2016) 214(5):807–16. doi:10.1093/infdis/jiw168
123. McNerney ME, Lee KM, Zhou P, Molinero L, Mashayekhi M, Guzior D, et al. Role of natural killer cell subsets in cardiac allograft rejection. *Am J Transplant* (2006) 6(3):505–13. doi:10.1111/j.1600-6143.2005.01226.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.2.0 ORIGINAL MANUSCRIPT: "A novel risk score model for the prognosis of kidney allograft dysfunction indicates the influence of specific MICA and HLA-G genotypes".

Submitted to the "Clinical Experimental Immunology" journal.

Corresponding author: Drª Maria da G. Bicalho.

Phone number: +33 41 3361 1729; email address: mgbicalho@gmail.com.

A novel risk score model for the prognosis of kidney allograft dysfunction indicates the influence of specific *MICA* and *HLA-G* genotypes

Running title: A novel risk score model for kidney transplant outcome

Matilde Risti*†, Vanessa Hauer*†, Geórgia F. Gelmini*, Bruna L.M. Miranda*, Carolina M. Pozzi‡, Ibrahim A. Sadissou**, Eduardo A. Donadi**, Maria da G. Bicalho*

*Laboratory of Immunogenetics and Histocompatibility-LIGH, Department of Genetics, Federal University of Paraná, Curitiba, Brazil

†Authors contributed equally to the work.

‡Hospital Universitário Evangélico de Curitiba, Alameda Augusto Stellfeld, 1908 Curitiba, 80.730-150, Brazil

** Departamento de Clínica Médica, Divisão de Imunologia Clínica, Faculdade de Medicina de Ribeirão Preto, Brazil

Considering that MICA and HLA-G present immunomodulatory properties, they may be putative useful biomarkers in predicting the outcome of a kidney transplant. We evaluated *MICA* (and *MICA* receptor *NKG2D*) and *HLA-G* genotypes and quantified soluble MICA and HLA-G levels in patients from pre-transplant up to three months after kidney transplantation, analyzing more than 40 clinical variables. Soluble HLA-G and MICA levels were detected by ELISA, while *MICA, NKG2D* and *HLA-G* genotyping was performed by PCR-SSOP, qRT-PCR and SBT, respectively. We implemented a Risk Score Model to evaluate patients exhibiting higher or lower risk for allograft dysfunction development, studying more than 40 demographic / clinical / treatment / genetic variables. Major variables associated with risk for kidney allograft dysfunction included total donor-specific antibodies (DSA), DSA positive against selected donors, previous transplant, previous blood transfusion and previous abortion, use of ATG in induction therapy, male gender and donors' age. Even with the use of the most powerful model, we did not show evidence of an association between *HLA-G*, *MICA* or *NKG2D* genotypes and the risk of developing allograft dysfunctions. Notwithstanding, isolated *MICA* (*MICA5.1* at double or single doses) and HLA-G (*G*01:04P* at single or double doses) genotypes revealed an association with differential phenotypes associated with high or low MICA and HLA-G producers.

Key words: Kidney transplantation; sMICA; sHLA-G.

[1] *Grant support* - This work was supported by the research fund of FUNPAR- LIGH.

[2] Abbreviations - MICA: MHC class I chain-related gene A; sMICA: soluble MICA; sHLA-G: soluble HLA-G; TX patients: transplanted patients; CKD patients: not transplanted patients with kidney chronic disease; PRA: panel reactive antibody; DSA: donor specific antibody; ATG: anti-thymocyte globulin.

[3] Corresponding address - Laboratório de Imunogenética e Histocompatibilidade, Departamento de Genética, Universidade Federal do Paraná, R. Cel. Francisco H. dos Santos S/N, Centro Politécnico – Jardim das Américas, CP 19071, CEP 81.531.980, Curitiba, PR, Brazil. Fax: +55 41 3266 2042.

.

Introduction

Epidemiological, clinical and etiopathogenetic variables are important variables to be studied, envisaging successful transplantation outcome. Donor-specific antibodies (DSAs) play a causative role on the development of antibody-mediated rejection in solid organ transplantation [1]. Pre-transplant DSAs are routinely evaluated to improve discrimination of sensitized risk patients prior to solid organ transplantation [2], and panel reactive antibodies (PRA) report possible DSAs exhibiting the potential risk to react against antigens of either a putative or a specific donor [3, 4]. Other events associated with patient sensitization include previous transplants [5, 6], pregnancies that may induce the production of *de novo* DSAs in titers even higher than the pre-transplant tests [7-9] and blood transfusions [10]. In all of these situations, alloimmunization may be a consequence of humoral immune response primarily against classical and non-classical HLA antigens [11].

Much attention has been devoted to the role of classical histocompatibility antigens in solid organ transplatation; however, little attention has been payed to the role of non-classical histocompatibility class I genes and molecules [12-14]. Among these markers, HLA-G and MICA (major histocompatibility complex class I chain-related gene A) are usually produced in pathological conditions associated with immunosuppression and inflammatory responses, respectively [14]. Both molecules may be expressed on cell membranes as well as soluble isoforms (sHLA-G and sMICA). At least seven HLA-G isoforms have been described; the membrane-bound HLA-G1 and the secreted soluble HLA-G5 represent the most widely investigated isoforms [15, 16]. Cell surface HLA-G1 can also be proteolytic cleaved by matrix metalloproteinase-2 producing sHLA-G1 [17-20]. Similarly, sMICA is produced via multiple enzyme cleavage, including disintegrin and metalloproteinase [21-25].

MICA molecules are expressed on the thymic medulla and the gastrointestinal epithelium, on freshly isolated human endothelial cells and fibroblasts, human keratinocytes, monocytes, and also on human epithelial and fibroblast cell lines [26-31]. In pathological conditions, MICA has been found in many epithelial tumors but not in the corresponding non-affected cells, supporting the hypothesis that stress induces MICA expression [13]. HLA-G is expressed on

regulatory T-cells, tolerogenic dendritic cells [32], and endothelial cells (in the maternal-fetal interface and in heart-transplanted patients) [33]. Considering that the expression of HLA-G in transplanted specimens has been associated with better graft survival [34-38], and since HLA-G expression in autoimmune disorders has also been associated with bettter prognosis [39, 40], HLA-G has a beneficial effect in these situations, inhibiting the ongoing immune responses. The immunomodulatory role of HLA-G is mainly performed by the interaction with inhibitory receptors such as the leukocyte Ig-like receptor family, the leukocyte Ig-like receptor subfamily B member 1 (LILRB1), LILRB2 and the killer cell Ig-like receptor 2DL4 (KIR2DL4) [39]. Soluble HLA-G can induce apoptosis of $CD8^+$T and NK cells, inhibit B-cell proliferation, differentiation and Ig secretion [41], and may participate in allograft tolerance through cell cycle inhibition of alloreactive T-cells [42-44].

The best described interaction of MICA occurs with natural killer group 2 member D ligand (NKG2D), inducing receptor internalization and degradation [29]. The NKG2D receptor is expressed on NK, γδ T-cells, and αβ $CD8^+$ T-cell membranes [29]. The interaction of NKG2D with its ligands is transmitted through the associated DAP10 dimer [45], and that can culminate in a cytotoxic activity of NK cells, and the expansion of immunosuppressive $NKG2D^+CD4^+$T cells [46]. The *NKG2D* gene has two haploblocks: hb1 and hb2. The first haploblock (hb1) can be divided into high and low natural cytotoxic activity haplotype (*HNK1* and *LNK1*, respectively). *HNK1* (described by the main dbSNP: rs1049174-G) is associated with a greater activity of NK cells in the peripheral blood and a lower predominance of cancers originating from epithelial cells [47, 48].

Important genetic variation sites at the coding region of *HLA-G* and *MICA* may also influence phenotypes. The number of GCT repetitions at exon 5 of the *MICA* gene may constitute five different alleles (*MICA-A4*, *A5*, *A5.1*, *A6*, and *A9*). The *A5.1* allele alone has five GCT repetitions and one insertion, which leads to frameshift mutation and originates a truncated protein with a differential expression and cellular localization [49]. The *MICA-129 Val/Met* polymorphism (dbSNP: rs1051792) at nucleotide 454 (G>A) leads to a Valine (Val) to Methionine substitution [50] at amino acid position 129 in the α2 domain of the MICA protein [48]. The *MICA-129 Val/Met* variant can affect NKG2D binding

avidity, which leads to an alteration in immune response mediated by NK cells, associated with a differential MICA expression [51].

Four *HLA-G* coding haplotypes are the most frequently observed in worldwide populations, yielding full-length HLA-G molecules [52]. Although many HLA isoforms possess immunomodulatory effects, proteins generated by the *G*01:01P* (composed of the *G*01:01, G*01:06, G*01:08* and *G*01:18* allelic groups), *G*01:03P* (composed of the *G*01:03* allelic group) and *G*01:04P* (composed of the *G*01:04* allelic group) may produce differential effects. The *G*01:04P* allelic group has already been associated with: i) increased levels of sHLA-G expression in kidney-transplanted patients without acute rejection [53], ii) predisposition to squamous intraepithelial lesions and cervical cancer [54], and iii) susceptibility to HIV-1 infection [55]. The *G*01:03* allelic group was associated with lower sHLA-G plasma levels and a reduced risk of vertical transmission of HIV-1 [56]. In contrast, the *G*01:01P* group exhibit alleles, partly associated with intermediate HLA-G expression as *G*01:01:08*, and also with a low expression such as *G*01:06, G*01:01:01:02* and *G*01:01:01:03* alleles [38, 53, 57]. Despite these findings, many efforts should be made in order to clarify the associations between genotypes and HLA-G phenotypes.

Considering the already described opposite immunomodulatory effects of MICA and HLA-G, and the scarcity of data regarding the role of these molecules in kidney transplantation, in the present study we evaluated sMICA and sHLA-G levels and *MICA, NKG2D* and *HLA-G* genotypes in kidney-transplanted patients. These analyses have been performed for noninvasive variables that passed through a statistic selection step to achieve the best model capable to discriminate the high or low risk of developing rejection episodes in pre-transplant patients. In recent years, the main goal for transplantation outcome has been driven to non-invasively monitor the immune response [58] in a noninvasive manner. Considering that, research on biomarkers, which are easily measured, widely available and cost-effective are very important to improve clinical diagnostics.

Materials and Methods

Patients

We studied 178 individuals recruited from 2012 to 2016, stratified into the following groups: i) 67 patients undergoing kidney transplantation (TX) in the Renal Transplant Sector at Hospital Universitário Evangélico do Paraná (Curitiba, Brazil); ii) 32 patients exhibiting chronic kidney disease (CKDs) and not transplanted from the same hospital, and iii) 79 control subjects (without kidney disease) recruited through a voluntary donation campaign (**Figure 1**). This study followed the Helsinki Declaration and was approved by the Ethics Committees of the University Hospital and of the Federal University of Paraná, Brazil (Ethics Committee approval number: 53627315.0.0000.0102). All participants signed a voluntary and informed consent form. Transplanted patients were clinically monitored from pre-transplantation up to three months after transplantation, and blood collection was performed at pre-transplant, first and second weeks, and first and second/third months post-transplant (Figure 1).

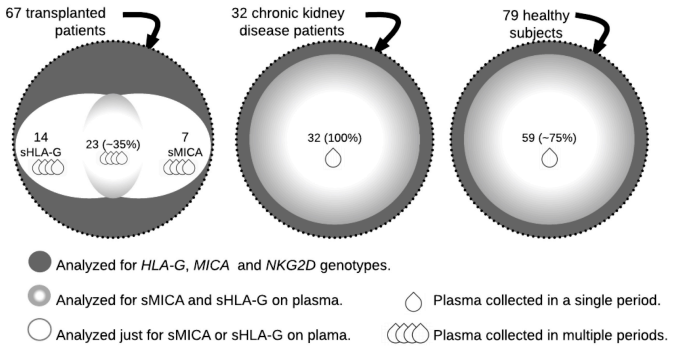


Figure 1 - Workflow of sample analyzed in this study (Image by Vanessa Hauer & Matilde Risti, 2017).

The main clinical and demographic profiles of selected participants are shown in **Table 1**, and we considered each collected piece of data as a variable to submit through regression logistic univariate analysis in the first selection step. For all patients, the pre-transplant immunosuppression was based on intravenous corticosteroid administration (methylprednisolone, 500 mg), as shown in **Table 2,** and it continued at the post-transplant period according to the immunosuppression protocol. Patients with increased immunological risk for allograft rejection

underwent an induction therapy with anti-thymocyte globulin (ATG, 1 to 1.5 mg/kg/day), initiated a few hours before surgery and lasting 5 to 10 days thereafter. The treatment with calcineurin inhibitor was discontinued during induction, and resumed three days before the end of the induction therapy.

Table 1 - Patient demographic and clinical data.

Characteristics	All TX patients (N=67)	TX high risk patients (N=36)	TX low risk patients (N=31)	CKD patients (N=32)	Control group (N=59)
Total men (%)	58.20	71.40	22.20	56.30	33.90
Age (years as mean - SD)	45.90 (13.82)	46.71 (48.00)	43.72 (15.52)	49.90 (12.00)	42.00 (13.40)
Living donor (%)	32.80	28.60	44.44	----	----
Donors age (years as mean - SD)	43.94 (12.59)	45.10 (13.75)	40.78 (8.18)	----	----
Underlying diseases (%)					
Hypertension	92.50	91.80	94.40	83.90	20.30
Diabetes	17.90	16.30	22.20	35.50	3.40
Dyslipidemias	9.00	6.10	16.70	32.30	3.40
Glomerulonephritis	41.80	40.80	44.40	32.30	0.00
Pyelonephritis	7.50	10.20	0.00	6.50	0.00
Polycystic kidney	10.40	12.20	5.50	6.30	0.00
Chronic kidney failure	89.60	91.80	83.30	74.20	0.00
Hypertensive nephropathy	14.90	16.30	11.11	15.60	0.00
Others	44.80	44.90	44.44	67.70	27.10
Previous transplants (%)	16.40	22.40	0.00	0.00	0.00
Previous blood transfusion (%)	44.80	51.00	27.80	29.00	----
Multiparous women (%)	20.90	16.30	33.33	37.50	39.0
Number of abortions (%)	9.00	8.20	11.11	6.30	6.80
Detection of DSA as PRA >30% (%)	44.80	61.20	0.00	23.10	----
Detection of donor-positive DSA in pre-transplant (%)	11.90	16.30	0.00	----	----
Induction therapy with ATG	23.90	32.70	0.00	----	----
Time on dialysis before transplantation in months (mean - SD)	46 (42)	52 (42)	29.83 (40)	----	----
Glomerular Filtration Rate (mean - SD)	8.02 (7.02)	7.96 (7.85)	8.17 (4.27)	----	----
Cold ischemia time – hours : minutes (mean - SD)	11:21 (9:06)	10:46 (9:02)	9:21 (9:26)	----	----
Detection of allograft dysfunction (%)	67.20	81.60	27.80	----	----
Kidney allograft loss (%)	10.40	15.20	0.00	----	----
***MICA-129* Val/Met genotypes (%)**					
MICA-129 Met/Met	16.40	14.30	22.20	12.50	16.90
MICA-129 Val/Val	38.80	36.70	44.40	62.50	35.60
MICA-129 Val/Met	44.80	49.00	33.30	25.00	47.50
***MICA A.51* genotypes (%)**					
MICA A5 / A5.1	4.50	4.20	5.60	16.10	10.20
MICA A5.1/-	37.90	37.50	38.90	29.00	32.20
MICA -/-	57.60	58.30	55.60	54.80	57.60
***NKG2D hb1* genotypes (%)**					
HNK1/LNK1	49.30	49.00	50.00	40.60	49.20
LNK1/LNK1	34.30	32.70	38.90	46.90	40.70
HNK1/HNK1	16.40	18.40	11.10	12.90	10.20
HLA-G genotypes - P groups (%)					
*G*01:01P/G*01:01P*	73.10	75.50	66.70	50.00	71.20
*G*01:01P/G*01:03P*	4.50	2.00	11.10	21.90	6.80
*G*01:01P/G*01:04P*	14.90	18.40	5.60	28.10	28.80
*G*01:03P/G*01:03P*	1.50	2.00	0.00	0.00	0.00
*G*01:03P/G*01:04P*	3.00	0.00	11.10	0.00	0.00
*G*01:04P/G*01:04P*	3.00	2.00	5.60	0.00	0.00
High producers of sMICA(%) (no detection (%))	23.33 (40.00)^{A}	18.18 (36.36)^{C}	37.50 (50.00)^{E}	31.25 (50.00)^{G}	5.08 (66.10)^{H}
High producers of sHLA-G (%) (no detection (%))	51.35 (2.70)^{B}	53.57 (3.57)^{D}	44.44 (0.00)^{F}	25.00 (12.50)^{G}	0.00 (79.66)^{H}

Percentages were determined for the total sample in each group, (N)= 30^{A}; 37^{B}; 22^{C}; 28^{D}; 8E; 9^{F}; 32^{G}; 59^{H}). All numerical variables are in means and standard deviation (SD). *MICA-/-:* not *MICA A5.1*; TX - kidney transplanted patients; CKD - chronic kidney disease patients never transplanted; DSA - donor-specific antibody; PRA - panel reactive antibody; sHLA-G - soluble HLA-G; sMICA - soluble MICA.

Table 2 - Immunosuppression protocol adopted for all patients.

Medication (type)	Quantity administered	Start of medication when:	
		Patient with living donors	Patient with deceased donors
Corticosteroid (Prednisone)	1mg/kg/day (progressively reducing to 5mg within 30 days after transplantation independent of weight)	Initiated 24 hours before transplantation	Immediately prior to surgery
Antiproliferative cell inhibitor (tacrolimus, sirolimus or azathioprine)	0.10 a 0.15 mg/kg/dose every 12 hours (dose adjustments depending on the blood level of toxicity)	Initiated 36 hours before transplantation	Immediately prior to surgery
Antimetabolite agent (mycophenolate mofetil or sodium)	360 mg every12 hours	Initiated 36 hours before transplantation	Immediately prior to surgery

Sampling

Blood samples of transplanted patients were obtained before transplantation and serially drawn up to 3 months after transplantation. Blood samples were drawn at the same time as the routine samples were collected for clinical tests, such as DSA detection and serum creatinine. After collection in ethylene-diamine-tetra-acetic acid (EDTA) - containing tubes, the samples were centrifuged 1000g for 15 minutes. The plasma obtained was stored at -80ºC and buffy coats at -20ºC until use. A total of 67 patients were genotyped for *MICA, NKG2D* and *HLA-G*. Blood samples from 32 chronic kidney disease patients that did not undergo transplantation and 79 healthy control subjects were used to obtain DNA to genotype *HLA-G*, *MICA* and *NKG2D* genes.

ELISA for sHLA-G and sMICA

Soluble HLA-G concentrations were evaluated by a specific sandwich ELISA in plasma using MEM-G/9 [59], anti-human β2-microglobulin as capture and detection antibodies, respectively [60, 61]. This reaction recognizes sHLA-G1 and HLA-G5 isoforms. In the same way, sMICA was measured using a commercial kit according to the manufacturer's protocol (DuoSet MICA ELISA, R & D Systems, Minneapolis, MN, USA). Plasma samples and calibrators were added to each well (100μL) in duplicates to perform sMICA and sHLA-G measurements. The final concentration was determined by optical density compared to standard curves. Measurements that fell at or below the minimum

detectable dose specific to each kit were considered not detectable (sHLA-G under 6.25 ng/mL; sMICA under 31.25 pg/mL).

HLA-G, *MICA* and *NKG2D* Genotyping

Peripheral blood DNA extraction was carried with a salting out technique [62]. DNA samples were genotyped by sequence-based typing (SBT) for the *HLA-G* gene, using the following primers amplified in a single reaction: i) exon 2 (5′ :GGGTCGGGCGGGTCTCAA, used to perform amplification and sequencing reactions, and 3′ :TCCGTGGGGCATGGAGGT, used just to perform amplification reaction; ii) exon 3 (5′ :CCCAGACCCTCTACCTGGGAGA, used just to perform amplification reaction, and 3′ :CTCTCCTTGTGCTAGGCCAGGCTG, used to perform amplification and sequencing reactions; and iii) exon 4 (5′ :CCATGAGAGATGCAAAGTGCT, used to perform amplification and sequencing reactions, and 3′ :TGCTTTCCCTAACAGACATGAT, used just to perform amplification reaction), as adapted from previous work [63]. All sequences were obtained using ABI Prism Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA) and analyzed through *ABI Prism SeqScape* (Applied Biosystems) software, based on the official 53 alleles listed in the International Immunogenetics Information System (IMGT). The resolution analysis was performed on typing results at the second field of resolution and genotypes were also separated according to their P group, which concerns IMGT genetic division that is based on exon 2 and 3 variations from *HLA-G* gene [64].

The polymerase chain reaction sequence specific oligonucleotide probes (PCR - SSOP) were used as first step to *MICA* genotyping, according to the manufacturer's recommendations, as outlined in the Kit LABType MICA (One Lambda Inc., Canoga Park, CA, USA). This reaction was analyzed through a flow fluorimeter (LABScanTM 100, Austin, TX, USA), which employs LUMINEX®technology. Analyses were performed with HLA Fusion Software, and *MICA* genotypes were discriminated according to *MICA-129 Val/Met* polymorphism and either as to the presence or absence of *MICA A5.1* allelic variation at exon 5.

The *NGK2D* gene was typed following the TaqMan® allelic

discrimination methodology and KLRC4-KLRK1 TaqMan® SNP Genotyping Assays (product number: C_9345347_10, Applied Biosystems). The SNP-based genotyping was specific to detect a G<C (dbSNP: rs1049174) replacement and discriminate allelic haplotype*s HNK1* and *LNK1*.

Statistical analysis

Analysis of binary logistic regression was the statistical tool chosen to predict the best model to estimate the behavior of the main clinical variables and genetic factors involved in the prognosis of kidney transplantation. The IBM SPSS (Statistical Package for Social Sciences) software, version 20 (https://www.ibm.com/analytics/ us/en/technology/spss/) was used to perform all the analyses.

The inclusion or exclusion of a variable in the model can change according to the problem under analysis. When the number of variables to be included in the model was minimized, a numerically more stable and more generalized model was obtained. Considering that, the creation of a risk score model was preceded by a pre-selection step for each independent variable *versus* the explanatory variable; i. e., detection (1) or not (0) of allograft dysfunction (as the development of acute rejection, acute pyelonephritis, humoral rejection or chronic graft nephropathy). In this step, also called null (empty) model, the univariate logistic regression was applied [65]. Then, the variables that present a *p* value of less than 0.25 were selected (**Table 3**) as candidates for the multivariate model for biological prediction. Description of all univariate logistic regression results are shown in the **Supplementary Table 1** and the set of analyzes and main results are summarized in **Figure 2**.

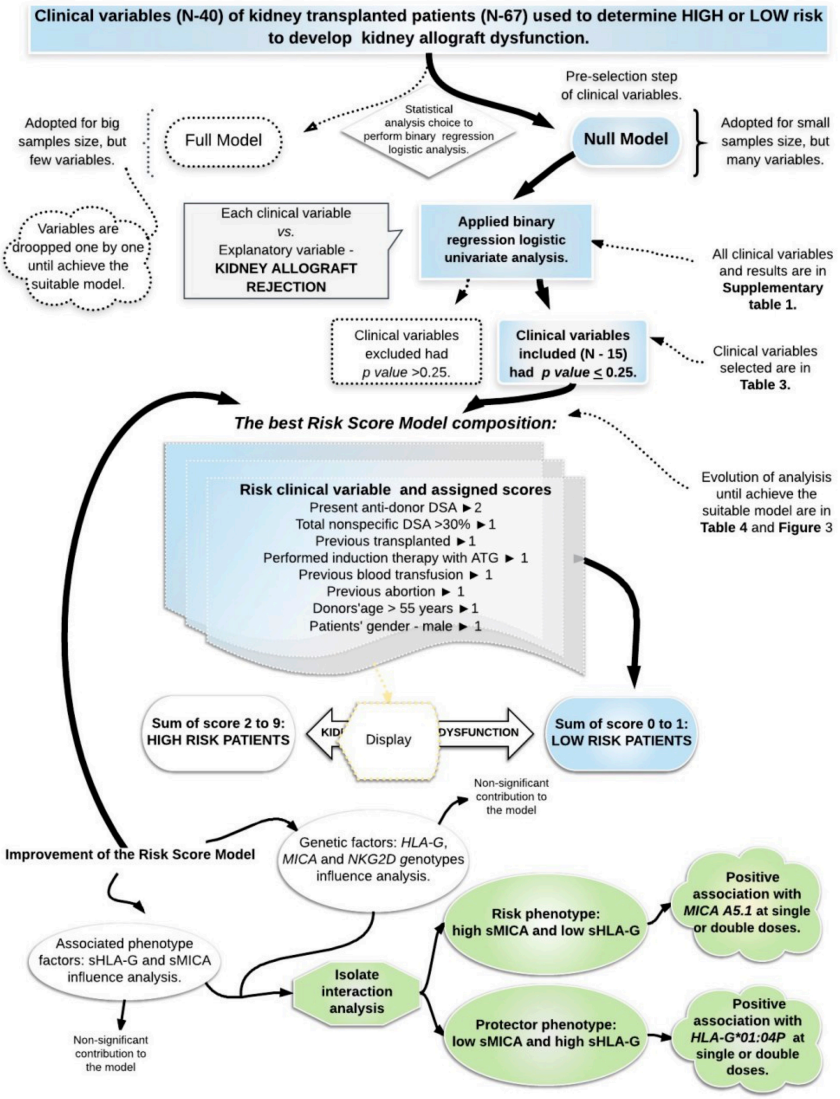


Figure 2 - The set of analyzes and main results are summarized from top to bottom: from Risk Score Model development to isolate interaction analysis of sHLA-G, sMICA, *HLA-G* and *MICA* genotypes. DSA - donor-specific antibody; sHLA-G - soluble HLA-G; sMICA - soluble MICA (Image by Vanessa Hauer & Matilde Risti, 2017).

Table 3 - Univariate logistic regression results for selected variables, considering as *p* value <0.25 and already related immunological importance.

Allograft dysfunction - yes (1) and no (0) - Main dependent variable.					
Variables passed through pre-selection step	β	S.E	Wald	Sig.	Exp(β)
Gender - man (1) or woman (0)	1.061	0.537	3.907	0.05	2.889
Transplanted - yes (1) and not (0)	-1.792	1.085	2.729	0.10	0.167
Previous blood transfusion - yes (0) or not (0)[b]	-0.012	0.101	0.013	0.91	0.989
Multiparous women - yes (1) or not (0)[a]	-0.875	0.776	1.272	0.25	0.417
Abortions - yes (1) or not (0)[ab]	-0.693	0.965	0.516	0.47	0.500
DSA against a population of possible donors detected as PRA higher than 30% in pre-transplant - yes (1) or not (0)	-1.114	0.564	3.899	0.05	0.328
Present DSA against specified donor in pre-transplant - yes (1) or not (0)	-1.353	1.103	1.504	0.22	0.259
Time on dialysis pre-transplant (numerical data in months)	0.018	0.009	4.169	0.04	1.019
Donors' type (nominal data)					
*Alive related 1 - (1)	-1.534	0.631	5.910	0.02	0.216
*Alive related 2 - (2)	-1.128	1.456	0.601	-1.13	1.456
*Alive related 3 - (3)	20.074	4.019E+04	0.000	1.00	5.23E+08
*Alive unrelated - (4)	-0.030	1.206	0.001	0.98	0.971
*Deceased donor - (5)			6.273	0.18	
Donors' age (numerical data in years)	0.054	0.023	5.493	0.02	1.056
Difference of height donor-receptor (numerical data in meters)	0.012	0.032	0.137	0.01	0.032
Induction - therapy with ATG- yes (1) and not (0)	-2.351	1.071	4.818	0.03	0.095
Base - Dyslipidemia - yes (1) and not (0)	1.564	0.910	2.951	0.09	4.778
Base - Pyelonephritis - yes (1) and not (0)	1.222	0.954	1.643	0.20	3.395
GFR CKD-EPI (numerical data in mL/min/1.73 m2)[c]	0.021	0.044	0.225	0.64	1.021

[a] Applied just for women. [b] Retained variable to compose Risk Score Model. [c] Retained variable because it is a common marker used for diagnostic kidney disease stage. DSA - Donor Specific Antibody; ATG - Anti-Thymocyte Globulin; GFR CKD-EPI - Glomerular filtration rate Chronic Kidney Disease Epidemiology Collaboration.

Before starting a multivariate logistic regression test for the explanatory variable (allograft dysfunction), selected variables with high influence on the production of DSAs and high immunological risk for developing rejection, such as patients who used induction therapy with ATG, were grouped in the Risk Score Models. Each DSA-related variable was associated with scores tested along eight models as described in **Table 4** and **Supplementary Figure 1**.

Table 4 - Variables aggregated to create Risk Score Model and its evolution

The scoring adopted for each Risk Score Model tested.								
Risk Score Model tested composition	1rst Model	2ndt Model	3rd Model	4th Model	5th Model	6th Model	**7th Model**	8th Model
Previous transplanted - yes (1) and not (0)	1	1	1	1	1	1	**1**	1
Previous blood transfusion - yes (1) or not (0)[b]	1	1	1	1	1	1	**1**	1
Multiparous women - yes (1) or not (0)[a]	1	1	ND	ND	ND	ND	**ND**	ND
Previous abortions - yes (1) or not (0)[ab]	ND	ND	1	1	1	1	**1**	1
DSA against a population of possible donors detected as PRA higher than 30% in pre-transplant - yes (1 or 2) or not (0)	2	1	1	1	1	1	**1**	1
Present DSA against specified donor in pre-transplant - yes (1 or 2) or not (0)	1	2	2	2	2	2	**2**	2
Induction therapy with ATG- yes (1) and not (0)	1	1	1	1	1	1	**1**	1
Gender - man (1) or woman (0)	ND	ND	ND	ND	1	1	**1**	ND
Base - Pyelonephritis - yes (1) and not (0)	ND	ND	ND	1	ND	1	**ND**	ND
Donors' age - Higher than 55 years (1) and lower age (0)	ND	ND	ND	ND	ND	ND	**1**	1
Minimum and maximum scoring for each model	0 to 7	0 to 7	0 to 7	0 to 8	0 to 8	0 to 9	**0 to 9**	0 to 8
Area from ROC curve	0.668	0.664	0.690	0.649	0.731	0.694	**0.779**	0.760
Standard error	0.070	0.070	0.069	0.072	0.069	0.074	**0.065**	0.066
Asymptotic significance	0.026	0.031	0.012	0.048	0.002	0.010	**<0.001**	0.001
Asymptotic 95% confidence Interval								
Lower bound	0.531	0.526	0.555	0.508	0.595	0.550	**0.651**	0.632
Upper bound	0.806	0.802	0.826	0.791	0.867	0.839	**0.908**	0.888

[a] Applied just for women. [b] Retained variable to compose Risk Score Model. ND - Not adopted; DSA - Donor Specific Antibody; ATG - Anti-Thymocyte Globulin; ROC - Receiver Operating Characteristic.

The multivariate logistic regression was applied as the main dependent variable, representing the risk of developing allograft dysfunction in relation to the first Risk Score Model as well as all the other pre-selected variables. All analyses were serially developed considering Risk Score Model from one to eight, and they were performed according to suggested main variables selected along the process of analysis (selection method - Forward Condition; selection p value ≤ 0.05; Omnibus Test of Model Coefficients - <0.05; Nagelkerke's R^2 - >0.300; Hosmer and Lemeshow test - >0.05 and the Prediction Power estimated in percentages). Changes in scoring adopted for each variable and their inclusion or

exclusion were considered, and the change in improvement was retained along the model evolution as suggested by the obtained results.

All proposed models were compared according to their performance (sensitivity and specificity) on the diagnosis of high and low risk patients through ROC (Receiver Operating Characteristic) curves. After identifying the best Risk Score Model (considered as the model with the highest area under curve), patients who reached higher scores (Score: 2 to 7) were placed into the higher risk group, while those who scored lower (Score: 0 to 1) were placed into the lower risk group. Models 4 to 8 included new main variables that were added to improve the predictive ability of the model and are discussed in the following sections.

BINARY LOGISTIC REGRESSION FOR DEPENDENT VARIABLES: HIGH AND LOW SOLUBLE VALUES

Levels of sMICA and sHLA-G were stratified as high and low production among analyzed individuals. The number of no detections observed in CKD patients (12.50%) and individuals from control group (57.63%) suggested a considerable distance between these groups and transplanted patients in relation to soluble molecule productions. Thus, the thresholds obtained for both molecules were evaluated in a specific condition (kidney transplanted patients), and were obtained considering TXs patients' medians (for sHLA-G a cutoff of 40ng/mL was adopted and for sMICA it was 80pg/mL).

Those two binary variables high (1) and low (0) sHLA-G and sMICA producers were applied in the analyses as independent variables with the aim to estimate their possible ability to discriminate the patients' risk to develop allograft dysfunction. The possible antagonistic effect of those molecules was also investigated, by setting sMICA or sHLA-G phenotypes as dependent variables and their related variables (genotypes and soluble measures).

ANALYSIS DISTRIBUTION OF SOLUBLE VALUES ALONG TIME

The skewed distribution of sHLA-G and sMICA values was surpassed through a logarithmic transformation. The quantifications of both molecules along five periods (pre-transplant, 1st week, 2nd week, 1st month and 2 to 3 months post-

transplant) of kidney-transplantated patients were analyzed in patients exhibiting or not allograft dysfunction. We compared CKD patients with control subjects. The one-way analysis of variance and Tukey test were used to compare the sHLA-G and sMICA levels along time and among the three groups (with significance set at $p \leq 0.05$; using the GraphPad Prism 5 software [66]).

Results

Risk Score Model determination

The improvement of Risk Score Model through adjustment with suggested variables added according to results of the logistic regression analysis achieved its peak in the seventh model (**Table 5**). This fit improvement in relation to the explanation of allograft dysfunction was confirmed in the related area under the ROC curve (0.779 - 95% of confidence interval [0.651; 0.908] - **Figure 3 A and B**).

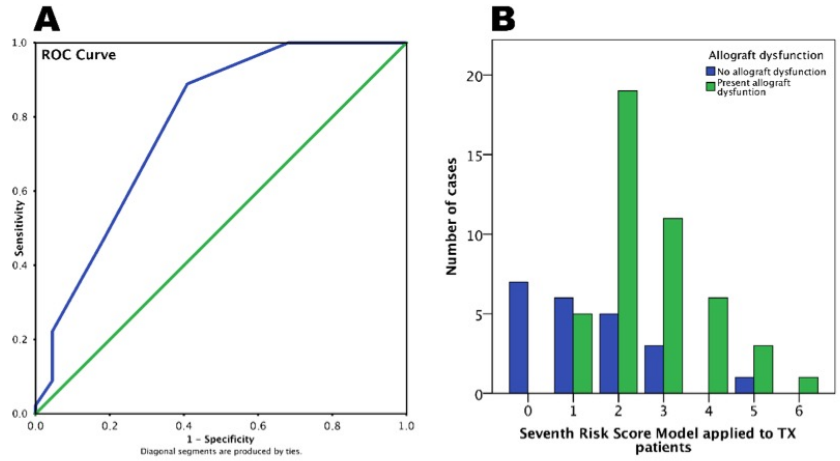


Figure 3 - A) ROC curve for the 7th allograft dysfunction model. **B)** Bar chart representing presence or not of allograft dysfunction; Risk Score Model scoring are shown on the X axis (scores from 7 to 9 were not found among patients).

Considering variables added or excluded to determine the model conformation, it was not possible to detect genetic or phenotypic variables associated to *HLA-G*, *MICA* and *NKG2D* genes. Donor's age was added at the end model by first transforming it from a numerical to a nominal variable; i. e., the

cutoff age estimated to discriminate between high and low risk was set to 55 years, since almost all transplanted patients with an allograft from a donor above that age developed allograft dysfunction (93.8%). The presence of pyelonephritis was another observed variable suggested along the fourth and sixth Risk Score Model tests. However, 60% of all patients who exhibited this complication in the pre-transplant period did not develop allograft dysfunction in the post-transplant time. Therefore, this underlying complication was not powerful enough to discriminate patients with allograft dysfunction, as evidenced through the ROC curve (**Table 4**) and the binary regression logistic results (**Supplementary Table 2**). At the end of the study, the male influence was added as well. This gender constitutes a risk factor, with 76.9% of men with detected allograft dysfunction.

Table 5 - 7th Risk Score Model Test with transplanted patients (N-63 and N(Variables)-11).

Nagelkerke R Square - 0.314	Prediction power – 79.40%		Dependent variable - Allograft dysfunction			
Variables that remained associated in the model	B	P value	Association	Exp(B)	95% CI for Exp(B) Lower bound	Upper bound
Seventh Risk Score Model	0.985	0.001	Positive	2.677	1.488	4.815
Constant	-1.247	0.042		0.287		

HLA-G and MICA interaction: genotype and soluble protein associations

Tests were performed to identify the ability of high and low sMICA and sHLA-G producers with the best Risk Score Model developed and pre-selected variables to help discriminate allograft dysfunction susceptibility. However, our results were not able to identify these molecules as good biomarkers in pre-transplant to discriminate patients at risk for allograft dysfunction.

The antagonistic effect of sMICA and sHLA-G phenotypes and their relation with genotypes were tested by placing these variables together, first as a protective phenotype - high production of sHLA-G plus low production of sMICA - and second as a risk phenotype - low production of sHLA-G plus high of sMICA. Tests were performed with dependent variables (protective or risk phenotype) and independent variables (*MICA*/*HLA-G* genotypes and haplotypes). Risk phenotype was positively associated to proposed dominance allelic interaction of MICA A5.1. Thus *MICA A5.1*- individuals were linked to the high

sMICA production plus low sHLA-G production (*p* value – 0.017; CI [0.030; 0.710]). The suggested dominance allelic interaction of *MICA A5.1* with transplanted and CKD patients was also confirmed through isolated analysis of high sMICA producers and *MICA* genotypes (*p* value – 0.013; CI [0.030; 0.504]) (**Table 6**).

Table 6 - Test with TX patients and inclusion of soluble analysis of high and low producers of sHLA-G and sMICA (N-112 and N(V)-4).

Nagelkerke R Square - 0.334	Prediction power - 82.60%		Dependent variable tested - Risk factor as high sMICA plus low sHLA-G production			
Variables that remained associated in the model	B	*P value*	Risk factor association	Exp(B)	95% CI for Exp(B)	
					Lower bound	Upper bound
TX and CKD patients	1.650	0.0013	Positive	5.209	1.656	118.857
Allele MICA -/- in MICA A5.1 a dominance allelic interaction test	-2.100	0.013	Negative	0.122	0.030	0.504
Constant	-2.785	0.007		0.620		

Analysis of protective phenotype related to high sHLA-G production and low sMICA evidenced *HLA-G* genotype association. In addition, isolated analysis of high producers of sHLA-G linked it to genotypes composed by allelic group *HLA-G*01:04P* (**Table 7**).

Table 7 - 12th Test with all group patients and inclusion of soluble analysis of high and low producers of sHLA-G and sMICA (N-129 and N(V)-6).

Nagelkerke R Square - 0.343	Prediction power – 82.80%			Dependent variable tested - High sHLA-G producers		
Variables that remained associated in the model	B	*P value*	High sHLA-G producers association	Exp(B)	95% CI for Exp(B)	
					Lower bound	Upper bound
CKD patients and control individuals	20.356	0.998	Positive	----	----	----
*HLA-G*01:04P*/- (in adominance allelic interaction test)	1.417	0.033	Positive	4.125	1.112	15.160
Constant	-21.773	0.997		<0.000		

Soluble HLA-G and MICA log-transformed quantifications analyzed in patients with or without allograft dysfunction from pre- to post-transplant had a significant variation when compared with those for CKD and control individuals (sMICA *p* value - < 0.001; sHLA-G *p* value - <0.003). We could observe a specific significant variation in sMICA plasma quantifications between control group and pre-transplant evaluation of patients that had an allograft dysfunction (**Figure 4**). A similar trend was detected for sHLA-G quantification. Significant differences were observed for controls *versus* first week and second to third

month after transplant in patients who developed an allograft dysfunction. Insertion of new post-transplant variables and also simultaneous analysis with soluble quantifications may clarify this fluctuation, which is still not discriminated by the proposed outcome (allograft dysfunction) and pre-transplant based Risk Score Model.

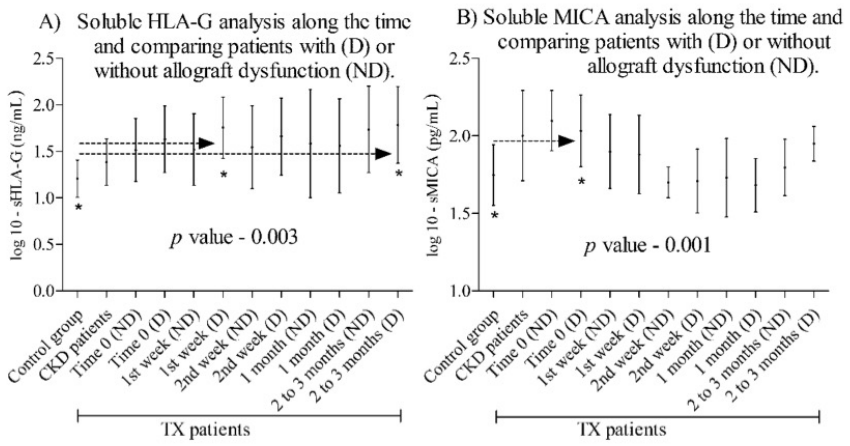


Figure 4 - Soluble levels performed in control group (N-59), CKD patients (chronic kidney disease patients, N-32) and for TX patients (transplanted patients, sMICA with N-30 and sHLA-G with N-37). All periods are represented separately among high and low risk groups - Time 0 (pre-transplant), 1st week, 2nd week, 1st month, 2nd month and 2 to 3 months post-transplant and no differences were observed along those periods in sHLA-G and in sMICA levels. **Graph A)** sHLA-G results (mean and standard errors) on the Y axis of the three groups. P value - <0.003 represents de difference between groups. **Graph B)** sMICA results (mean and standard errors) on the Y axis of the three groups. P value – 0.001 represents de difference between groups. Individuals with no soluble detection or zero were not illustrated in graphs and represent respectively for sHLA-G and sMICA: in control group 79.66% and 66.10%, in CKD patients 12.50% and 50.00% and in TX patients 2.70% and 40.00%. *Differences detected using the Tukey test.

Discussion

The Risk Score Model highlighted significant variables that help to discriminate between high and low risk for developing kidney allograft dysfunction in pre-transplant patients. Among the clinical variables included in the model, the presence of DSA had the greatest weight on the final score, increasing the allograft risk loss [67, 68]. The involvement of pre-existing specific donor antibodies, activating the immune response, frequently culminates in irreversible rejections; however, DSA detection does not detect non-HLA antibodies and its accuracy depends on the completeness of donor HLA typing [4]. Thus PRA, which detects total DSA relative to a population of donors continues to be the most joined analysis in pre-transplant waiting list banks, which is used to monitor and forward sensitized patients to induction therapies in pre-transplant procedures [69].

Still in our model, women with previous abortion appeared to be at a stronger risk factor than multiparous women. In fact, the immunological events that surround the fetus and allograft loss are comparable, mainly concerning the inflammation process, as other immune cells and cytokine balance deregulation [70]. Pregnancy *per se* has already been associated with the development of naturally acquired pregnancy-induced microchimerism. It is supposed to be developed from trafficking of T, B and NK cells that may persist decades later in women and in her progeny. This type of tolerance development mediated by pregnancy in case of donor-related transplant could even explain why it is not at all a risk factor associated to allograft dysfunction [71].

The finding of an association between the male gender and donor's age higher than 55 years has an important implication on donor selection considering the possible risk factors. In the recent years, a considerable change in donors' age distribution has been observed, especially in older populations. However, donors' age is known to negatively influence graft survival, as it is overlapped to pathologic lesions with age-related change [72], justifying its inclusion as a risk factor in the more relevant graft survival scores, such as the Kidney Donor Risk Index [73, 74]. Studies also suggest that for donor organs above 30 years, the risk of allograft failure begins to gradually increase [75], and donors' age above 55 years has already been evidenced as an important risk factor [76]. Donors' age has

received more importance than male's sex as risk factor, which has also been associated with graft loss after five years of transplantation [77, 78].

A combination of risk factors determines the immunological risk of each patient; however, there are general events commonly associated with higher risks. Previous transplants, abortion or blood transfusions are associated with a pre-transplant alloimmunization, as also inferred by our model. Besides being responsible for an increase in DSAs, these events are accompanied by other complications that alter the physiology and other components of the immune system, including the amount of MICA and HLA-G that may modulate the immune response. In this study, the levels of soluble MICA and HLA-G were not good biomarkers in pre-transplant analysis for discrimination of the kidney-transplanted patients' outcome. Soluble MICA and HLA-G levels fluctuated along the post-transplant period, and significant increased levels of sMICA and sHLA-G were observed in some post-transplant periods when compared to pre-transplant levels. Although mean soluble HLA-G levels exhibited a trend of increase along time while soluble MICA a trend of decrease along time, the overall opposite role of these molecules was not demonstrated in our risk score model.

To further understand the association between HLA-G and MICA, we studied the relationship between phenotypes and genotypes. The risk phenotype, i.e. high soluble MICA and low soluble HLA-G, was associated with the proposed dominance allelic interaction of *MICA A5.1* (*MICA A5.1/ A5.1* and *MICA A5.1*/-). Indeed, *MICA A5.1* is a common variant associated with higher levels of mRNA and protein expression in cell surface and in exosomes. This mutation was already reported by Tonnerre *et al*. (2013) as an immunodominant factor and a potential risk factor for transplant survival. The increased amount of MICA may increase the interaction with NKG2D, increasing NK cell activation, an effect that is certainly involved with the rejection mechanism of the kidney allograft [49].

Regarding the protective phenotype, i.e. high soluble HLA-G and low soluble MICA, we observed an association between the HLA-*G*01:01P/G*01:04P* genotype and sHLA-G high-producer individuals. This higher expression observed in patients with *HLA-G*01:04P* allelic group could be explained when the most frequent extended haplotype of allele *HLA-G*01:04* is analyzed. It includes variations present along promoter, coding and 3'UTR gene segments, described as *G0104a/G*01:04:01/UTR-3* and

*G0104a/G*01:04:04/UTR-3* extended haplotypes. The UTR-3 exhibits a relevant deletion of 14-bp polymorphism (dbSNP: rs371194629) [52], which has already been associated with high production of HLA-G [79]. However, in the presence of 14-bp, as observed in trophoblasts, it was associated with lower mRNA production for membrane and soluble isoforms of HLA-G [80, 81]. The allelic group *G*01:01P* includes a wide allelic diversity already associated with low or intermediate sHLA-G expression. Considering heterozygosis, the presence of *G*01:04P* possibly shows an influence of dominance linked to sHLA-G expression.

In conclusion, our Risk Score Model proposes a new way to predict the kidney-transplanted patients' prognostic still in pre-transplant, taking into account major variables involved in allograft dysfunction. The suggested risk phenotype (high producers of sMICA and low producers of sHLA-G) has shown an association with the allelic dominance of *MICA A5.1*. In contrast, the protective phenotype (low producers of sMICA and high producers of sHLA-G) was associated with the G**01:04P* allelic group in single or double doses. Individually, HLA-G and MICA genotypes and phenotypes were not associated with a pre-transplant prognostic of allograft dysfunction, but the carriage of specific genotypes of MICA or HLA-G differentially affects the risk factors in the post-transplant period.

Authors' contributions

Matilde Risti and Vanessa Hauer: planning and structure organization of the manuscript, research and analysis, writing of the manuscript, planning and creation of figures and tables. Geórgia F. Gelmini, Bruna L.M. Miranda, Dr. Carolina M. Pozzi, Dr. Ibrahim A. Sadissou and Dr. Eduardo A. Donadi: critical review of the manuscript. Dr. Maria da G. Bicalho: planning and structure organization of the manuscript and contributions to the sections writing / critical review of the manuscript.

Acknowledgments

We are most grateful to participants for generously agreeing to provide samples for this study. We also thank Hospital Universitário Evangélico de Curitiba, especially LIGH and the staff of the Clinical Immunology Division (Ribeirão Preto) for their technical support.

References

1. Ciszek M, Mucha K, Foroncewicz B, Zochowska D, Kosieradzki M, Grochowiecki T, Durlik M, Gorski A, Paczek L. Immune biomarkers and longterm graft survival: a prospective followup of 457 kidney transplant recipients. Pol Arch Intern Med 2017; **127**:178-83.

2. Mishra MN, Baliga KV. Significance of panel reactive antibodies in patients requiring kidney transplantation. Saudi J Kidney Dis Transpl 2013; **24**:495-9.

3. Konvalinka A, Tinckam K. Utility of HLA Antibody Testing in Kidney Transplantation. J Am Soc Nephrol 2015; **26**:1489-502.

4. Tait BD. Detection of HLA Antibodies in Organ Transplant Recipients - Triumphs and Challenges of the Solid Phase Bead Assay. Frontiers in immunology 2016; **7**:570.

5. Hayanga JA, Yang J, Aboagye J, Berko CA, Qiao CZ, Kaiser HE, Shigemura N, D'Cunha J. Risk factors associated with lung retransplantation: evaluation of a nationwide registry over a quarter century. The Annals of thoracic surgery 2014; **98**:1742-6; discussion 6-7.

6. Heaphy EL, Poggio ED, Flechner SM, Goldfarb DA, Askar M, Fatica R, Srinivas TR, Schold JD. Risk factors for retransplant kidney recipients: relisting and outcomes from patients' primary transplant. Am J Transplant 2014; **14**:1356-67.

7. Hönger G, Fornaro I, Granado C, Tiercy JM, Hösli I, Schaub S. Frequency and determinants of pregnancy-induced child-specific sensitization. Am J Transplant 2013; **13**:746-53.

8. Higgins R, Lowe D, Daga S, Hathaway M, Williams C, Lam FT, Kashi H, Tan LC, Imray C, Fletcher S, Krishnan N, Hart P, Zehnder D, Briggs D. Pregnancy-induced HLA antibodies respond more vigorously after renal transplantation than antibodies induced by prior transplantation. Hum Immunol 2015; **76**:546-52.

9. Pratschke J, Dragun D, Hauser IA, Horn S, Mueller TF, Schemmer P, Thaiss F. Immunological risk assessment: The key to individualized immunosuppression after kidney transplantation. Transplant Rev (Orlando) 2016; **30**:77-84.

10. Scornik JC, Meier-Kriesche HU. Blood transfusions in organ transplant patients: mechanisms of sensitization and implications for prevention. Am J Transplant 2011; **11**:1785-91.

11. Ming Y, Hu J, Luo Q, Ding X, Luo W, Zhuang Q, Zou Y. Acute Antibody-Mediated Rejection in Presence of MICA-DSA and Successful Renal Re-Transplant with Negative-MICA Virtual Crossmatch. PLoS One 2015; **10**:e0127861.

12. Gómez-Casado E, Martínez-Lasot J, Castro MJ, Morales P, Trápaga J, Berciano M, Lowy E, Arnaiz-Villena A. Detection of HLA-E and -G DNA alleles for population and disease studies. Cellular and molecular life sciences : CMLS 1999; **56**:356-62.

13. Racca AL, Veaute CM, Bailat AS, Gaite L, Arriola M, Hajos SE, Malan Borel IS. Expression of HLA-G and MICA mRNA in renal allograft. Transpl Immunol 2009; **21**:10-2.

14. Menier C, Riteau B, Carosella ED, Rouas-Freiss N. MICA triggering signal for NK cell tumor lysis is counteracted by HLA-G1-mediated inhibitory signal. Int J Cancer 2002; **100**:63-70.

15. Carosella ED, Moreau P, Lemaoult J, Rouas-Freiss N. HLA-G: from biology to clinical benefits. Trends in immunology 2008; **29**:125-32.

16. LeMaoult J, Le Discorde M, Rouas-Freiss N, Moreau P, Menier C, McCluskey J, Carosella ED. Biology and functions of human leukocyte antigen-G in health and sickness. Tissue Antigens 2003; **62**:273-84.

17. Demaria S, Schwab R, Gottesman SR, Bushkin Y. Soluble beta 2-microglobulin-free class I heavy chains are released from the surface of activated and leukemia cells by a metalloprotease. J Biol Chem 1994; **269**:6689-94.

18. Dong Y, Lieskovska J, Kedrin D, Porcelli S, Mandelboim O, Bushkin Y. Soluble nonclassical HLA generated by the metalloproteinase pathway. Hum Immunol 2003; **64**:802-10.

19. Zidi I, Guillard C, Marcou C, Krawice-Radanne I, Sangrouber D, Rouas-Freiss N, Carosella ED, Moreau P. Increase in HLA-G1 proteolytic shedding by tumor cells: a regulatory pathway controlled by NF-kappaB inducers. Cellular and molecular life sciences : CMLS 2006; **63**:2669-81.

20. Rizzo R, Trentini A, Bortolotti D, Manfrinato MC, Rotola A, Castellazzi M, Melchiorri L, Di Luca D, Dallocchio F, Fainardi E, Bellini T. Matrix metalloproteinase-2 (MMP-2) generates soluble HLA-G1 by cell surface proteolytic shedding. Mol Cell Biochem 2013; **381**:243-55.

21. Zingoni A, Cecere F, Vulpis E, Fionda C, Molfetta R, Soriani A, Petrucci MT, Ricciardi MR, Fuerst D, Amendola MG, Mytilineos J, Cerboni C, Paolini R, Cippitelli M, Santoni A. Genotoxic Stress Induces Senescence-Associated ADAM10-Dependent Release of NKG2D MIC Ligands in Multiple Myeloma Cells. J Immunol 2015; **195**:736-48.

22. Salih HR, Antropius H, Gieseke F, Lutz SZ, Kanz L, Rammensee HG, Steinle A. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood 2003; **102**:1389-96.

23. Camodeca C, Nuti E, Tepshi L, Boero S, Tuccinardi T, Stura EA, Poggi A, Zocchi MR, Rossello A. Discovery of a new selective inhibitor of A Disintegrin And Metalloprotease 10 (ADAM-10) able to reduce the shedding of NKG2D ligands in Hodgkin's lymphoma cell models. Eur J Med Chem 2016; **111**:193-201.

24. Huergo-Zapico L, Gonzalez-Rodriguez AP, Contesti J, Gonzalez E, López-Soto A, Fernandez-Guizan A, Acebes-Huerta A, de Los Toyos JR, Lopez-Larrea C, Groh V, Spies T, Gonzalez S. Expression of ERp5 and GRP78 on the membrane of chronic lymphocytic leukemia cells: association with soluble MICA shedding. Cancer Immunol Immunother 2012; **61**:1201-10.

25. Baranwal AK, Mehra NK. Major Histocompatibility Complex Class I Chain-Related A (MICA) Molecules: Relevance in Solid Organ Transplantation. Frontiers in immunology 2017; **8**:182.

26. Katsuyama Y, Ota M, Ando H, Saito S, Mizuki N, Kera J, Bahram S, Nose Y, Inoko H. Sequencing based typing for genetic polymorphisms in exons, 2, 3 and 4 of the MICA gene. Tissue antigens 1999; **54**:178-84.

27. Muro M, López-Hernández RL, S. , al. e. MICA Molecules in Disease and Transplantation, a Double-Edged Sword? *Current Immunology Reviews* 2012; **8**:307–25.

28. 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. Proc Natl Acad Sci U S A 1996; **93**:12445-50.

29. Bauer S, Groh V, Wu J, Steinle A, Phillips JH, Lanier LL, Spies T. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science 1999; **285**:727-9.

30. Romphruk AV, Naruse TK, Romphruk A, Kawata H, Puapairoj C, Kulski JK, Leelayuwat C, Inoko H. Diversity of MICA (PERB11.1) and HLA haplotypes in Northeastern Thais. Tissue antigens 2001; **58**:83-9.

31. Steinle A, Li P, Morris DL, Groh V, Lanier LL, Strong RK, Spies T. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics 2001; **53**:279-87.

32. Liang S, Horuzsko A. Mobilizing dendritic cells for tolerance by engagement of immune inhibitory receptors for HLA-G. Hum Immunol 2003; **64**:1025-32.

33. Sheshgiri R, Rao V, Tumiati LC, Xiao R, Prodger JL, Badiwala M, Librach C, Delgado DH. Progesterone induces human leukocyte antigen-g expression in vascular endothelial and smooth muscle cells. Circulation 2008; **118**:S58-64.

34. Créput C, Durrbach A, Menier C, Guettier C, Samuel D, Dausset J, Charpentier B, Carosella ED, Rouas-Freiss N. Human leukocyte antigen-G (HLA-G) expression in biliary epithelial cells is associated with allograft acceptance in liver-kidney transplantation. Journal of hepatology 2003; **39**:587-94.

35. Almasood A, Sheshgiri R, Joseph JM, Rao V, Kamali M, Tumiati L, Ross HJ, Delgado DH. Human leukocyte antigen-G is upregulated in heart failure patients: a potential novel biomarker. Hum Immunol 2011; **72**:1064-7.

36. Brugière O, Thabut G, Pretolani M, Krawice-Radanne I, Dill C, Herbreteau A, Poras I, Moreau P, Colombat M, Danel C, Dehoux M, Fournier M, Carosella ED, Rouas-Freiss N. Immunohistochemical study of HLA-G expression in lung transplant recipients. Am J Transplant 2009; **9**:1427-38.

37. Qiu J, Terasaki PI, Miller J, Mizutani K, Cai J, Carosella ED. Soluble HLA-G expression and renal graft acceptance. Am J Transplant 2006; **6**:2152-6.

38. Donadi EA, Castelli EC, Arnaiz-Villena A, Roger M, Rey D, Moreau P. Implications of the polymorphism of HLA-G on its function, regulation, evolution and disease association. Cellular and molecular life sciences : CMLS 2011; **68**:369-95.

39. Carosella ED, Rouas-Freiss N, Roux DT, Moreau P, LeMaoult J. HLA-G: An Immune Checkpoint Molecule. Adv Immunol 2015; **127**:33-144.

40. Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, Pan L, Solway J, Gern JE, Lemanske RF, Nicolae D, Ober C. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am J Hum Genet 2007; **81**:829-34.

41. Naji A, Menier C, Morandi F, Agaugué S, Maki G, Ferretti E, Bruel S, Pistoia V, Carosella ED, Rouas-Freiss N. Binding of HLA-G to ITIM-bearing Ig-like transcript 2 receptor suppresses B cell responses. J Immunol 2014; **192**:1536-46.

42. Manavalan JS, Kim-Schulze S, Scotto L, Naiyer AJ, Vlad G, Colombo PC, Marboe C, Mancini D, Cortesini R, Suciu-Foca N. Alloantigen specific CD8+CD28- FOXP3+ T suppressor cells induce ILT3+ ILT4+ tolerogenic endothelial cells, inhibiting alloreactivity. Int Immunol 2004; **16**:1055-68.

43. Contini P, Ghio M, Poggi A, Filaci G, Indiveri F, Ferrone S, Puppo F. Soluble HLA-A,-B,-C and -G molecules induce apoptosis in T and NK CD8+ cells and inhibit cytotoxic T cell activity through CD8 ligation. Eur J Immunol 2003; **33**:125-34.

44. Apps R, Gardner L, Moffett A. A critical look at HLA-G. Trends in immunology 2008; **29**:313-21.

45. Garrity D, Call ME, Feng J, Wucherpfennig KW. The activating NKG2D receptor assembles in the membrane with two signaling dimers into a hexameric structure. Proc Natl Acad Sci U S A 2005; **102**:7641-6.

46. Groh V, Smythe K, Dai Z, Spies T. Fas-ligand-mediated paracrine T cell regulation by the receptor NKG2D in tumor immunity. Nat Immunol 2006; **7**:755-62.

47. Espinoza JL, Takami A, Onizuka M, Sao H, Akiyama H, Miyamura K, Okamoto S, Inoue M, Kanda Y, Ohtake S, Fukuda T, Morishima Y, Kodera Y, Nakao S. NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignanciesHaematologica. Italy, 2009:1427-34.

48. Risti M, Bicalho MD. MICA and NKG2D: Is There an Impact on Kidney Transplant Outcome? Frontiers in immunology 2017; **8**.

49. Tonnerre P, Gérard N, Chatelais M, Poli C, Allard S, Cury S, Bressollette C, Cesbron-Gautier A, Charreau B. MICA variant promotes allosensitization after kidney transplantation. J Am Soc Nephrol 2013; **24**:954-66.

50. Borelli SD, Ferreira E, Oliveira AM, Krishnaswamy S, Hiraki DD, Grumet FC. Specific sHLA in healthy donors and donor-specific sHLA in renal transplant patients. Hum Immunol 1999; **60**:430-4.

51. Raache R, Belanteur K, Amroun H, Benyahia A, Heniche A, Azzouz M, Mimouni S, Gervais T, Latinne D, Boudiba A, Attal N, Abbadi MC. Association of major histocompatibility complex class 1 chain-related gene A dimorphism with type 1 diabetes and latent autoimmune diabetes in adults in the Algerian population. Clinical and Vaccine Immunology 2012; **19**:557-61.

52. Castelli EC, Ramalho J, Porto IO, Lima TH, Felício LP, Sabbagh A, Donadi EA, Mendes-Junior CT. Insights into HLA-G Genetics Provided by Worldwide Haplotype Diversity. Frontiers in immunology 2014; **5**:476.

53. Misra MK, Pandey SK, Kapoor R, Sharma RK, Kapoor R, Prakash S, Agrawal S. HLA-G gene expression influenced at allelic level in association with end stage renal disease and acute allograft rejection. Human immunology 2014; **75**:833-9.

54. Simoes RT, Goncalves MA, Castelli EC, Junior CM, Bettini JS, Discorde ML, Duarte G, Quintana SM, Simoes AL, Moreau P, Carosella ED, Soares EG, Donadi EA. HLA-G polymorphisms in women with squamous intraepithelial lesions harboring human papillomavirus. Mod Pathol 2009; **22**:1075-82.

55. Turk WJ, Kimani J, Bielawny T, Wachihi C, Ball TB, Plummer FA, Luo M. Associations of human leukocyte antigen-G with resistance and susceptibility to HIV-1 infection in the Pumwani sex worker cohort. AIDS 2013; **27**:7-15.

56. Luo L, Li Z, Wu W, Luo G, Mei H, Sun Z, Xu C. The effect of MICA antigens on kidney transplantation outcomes. Immunology letters 2013; **156**:54-8.

57. Lajoie J, Jeanneau A, Faucher MC, Moreau P, Roger M. Characterisation of five novel HLA-G alleles with coding DNA base changes. Tissue Antigens 2008; **72**:502-4.

58. Malyszko J, Malyszko J. Immune biomarkers: do they impact long-term graft survival? Pol Arch Intern Med 2017; **127**:149-51.

59. Rebmann V, Lemaoult J, Rouas-Freiss N, Carosella ED, Grosse-Wilde H. Report of the Wet Workshop for Quantification of Soluble HLA-G in Essen, 2004. Hum Immunol 2005; **66**:853-63.

60. Menier C, Saez B, Horejsi V, Martinozzi S, Krawice-Radanne I, Bruel S, Le Danff C, Reboul M, Hilgert I, Rabreau M, Larrad ML, Pla M, Carosella ED, Rouas-Freiss N. Characterization of monoclonal antibodies recognizing HLA-G or HLA-E: new tools to analyze the expression of nonclassical HLA class I molecules. Hum Immunol 2003; **64**:315-26.

61. Sadissou I, d'Almeida T, Cottrell G, Luty A, Krawice-Radanne I, Massougbodji A, Moreau P, Moutairou K, Garcia A, Favier B, Rouas-Freiss N, Courtin D. High plasma levels of HLA-G are associated with low birth weight and with an increased risk of malaria in infancy. Malar J 2014; **13**:312.

62. Lahiri DK, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991; **19**:5444.

63. Nardi FaS, Slowik R, Wowk PF, da Silva JS, Gelmini GF, Michelon TF, Neumann J, Bicalho MaG. Analysis of HLA-G polymorphisms in couples with implantation failure. Am J Reprod Immunol 2012; **68**:507-14.

64. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG. The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Res 2015; **43**:D423-31.

65. Sperandei S. Understanding logistic regression analysis. Biochem Med (Zagreb) 2014; **24**:12-8.

66. HJ M. Analyzing Data with GraphPad Prism, 1999.

67. Mengel M, Husain S, Hidalgo L, Sis B. Phenotypes of antibody-mediated rejection in organ transplants. Transpl Int 2012; **25**:611-22.

68. Susal C, Seidl C, Schonemann C, Heinemann FM, Kauke T, Gombos P, Kelsch R, Arns W, Bauerfeind U, Hallensleben M, Hauser IA, Einecke G, Blasczyk R. Determination of unacceptable HLA antigen mismatches in kidney transplant recipients: recommendations of the German Society for Immunogenetics. Tissue antigens 2015; **86**:317-23.

69. Castro CD, Luoma AM, Adams EJ. Coevolution of T-cell receptors with MHC and non-MHC ligands. Immunological reviews 2015; **267**:30-55.

70. Wilczynski JR. Immunological analogy between allograft rejection, recurrent abortion and pre-eclampsia - the same basic mechanism? Human immunology 2006; **67**:492-511.

71. Gammill HS, Nelson JL. Naturally acquired microchimerism. Int J Dev Biol 2010; **54**:531-43.

72. Halloran PF, Melk A, Barth C. Rethinking chronic allograft nephropathy: the concept of accelerated senescence. J Am Soc Nephrol 1999; **10**:167-81.

73. Rao PS, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM, Port FK, Sung RS. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. Transplantation 2009; **88**:231-6.

74. Rosenberger LH, Gillen JR, Hranjec T, Stokes JB, Brayman KL, Kumer SC, Schmitt TM, Sawyer RG. Donor risk index predicts graft failure reliably but not post-transplant infections. Surgical infections 2014; **15**:94-8.

75. Laging M, Kal-van Gestel JA, van de Wetering J, Ijzermans JN, Weimar W, Roodnat JI. The relative importance of donor age in deceased and living donor kidney transplantation. Transpl Int 2012; **25**:1150-7.

76. Matas AJ, Payne WD, Sutherland DE, Humar A, Gruessner RW, Kandaswamy R, Dunn DL, Gillingham KJ, Najarian JS. 2,500 living donor kidney transplants: a single-center experience. Annals of surgery 2001; **234**:149-64.

77. Aakhus S, Dahl K, Wideroe TE. Cardiovascular morbidity and risk factors in renal transplant patients. Nephrol Dial Transplant 1999; **14**:648-54.

78. Oien CM, Reisaeter AV, Leivestad T, Dekker FW, Line PD, Os I. Living donor kidney transplantation: the effects of donor age and gender on short- and long-term outcomes. Transplantation 2007; **83**:600-6.

79. Fernandes A, Sanches R, Matos F, Massaro J, Donadi E. HLA-G 14bp polymorphism on HIV-1 perinatal transmission (P3041). The Journal of Immunology 2013; **190**:55.21-55.21.

80. Harrison GA, Humphrey KE, Jakobsen IB, Cooper DW. A 14 bp deletion polymorphism in the HLA-G gene. Hum Mol Genet 1993; **2**:2200.

81. Hviid TV, Hylenius S, Rorbye C, Nielsen LG. HLA-G allelic variants are associated with differences in the HLA-G mRNA isoform profile and HLA-G mRNA levels. Immunogenetics 2003; **55**:63-79.

Supplementary table 1 - The univariate logistic regression results of all clinical variables, considering as selection p value < 0.25.

Allograft disfunction - yes (1) and no (0) - Main dependent variable					
Variables submitted through pre-selection step	β	S.E	Wald	Sig.	Exp(β)
Ethnic group (nominal data)					
*Mix race - (2)	-0.239	0.787	0.093	0.76	0.787
*Asian - (3)	-21.953	40192.97	0.000	1.00	0.000
*Black - (4)	-0.057	1.26	0.002	0.96	0.944
*Mulatto - (5)	20.453	2.84E+04	0.000	1.00	7.63E+08
*White - (7)			0.093	1.00	
Kidney replacement therapy (nominal data)					
* Hemodialysis (Hd) - (3)			1.529	0.68	
* numerical Ambulatory Peritoneal Dialysis (CAPD) - (2)	20.458	2.32E+04	0.000	1.00	7.67E+08
* Hd plus CAPD - (1)	-0.744	1.441	0.267	0.61	0.475
*No therapy before transplant or just one time in pre-transplant (0)	-1.438	1.256	1.310	0.25	0.238
Age (numerical data in years)	0.011	0.019	0.367	0.54	1.012
Gender - man (1) or woman (2)	1.061	0.537	3.907	0.05	2.889
Weight (numerical data in meters)	0.014	0.021	0.445	0.51	1.014
BMI (nominal data)					
*Underweight - (1)	1.099	1.528	0.517	0.47	3.000
*Normal weight - (2)	0.693	1.061	0.427	0.51	2.000
*Overweight - (3)	0.827	1.098	0.567	0.45	2.286
*Obesity - (4)			0.686	0.88	
Receptors' height (numerical data in kilograms)	0.020	0.029	0.476	0.49	1.020
Transplanted - yes (1) and not (0)	-1.792	1.085	2.729	0.10	0.167
Previous blood transfrusion - yes (0) or not (0)[b]	-0.012	0.101	0.013	0.91	0.989
Multiparous women - yes (1) or not (0)[a]	-0.875	0.776	1.272	0.25	0.417
Abortions - yes (1) or not (0)[ab]	-0.693	0.965	0.516	0.47	0.500
DSA against a poplation of possible donors detected as PRA higher than 30% in pre-transplant - yes (1) or not (0)	-1.114	0.564	3.899	0.05	0.328
Present DSA against specified donor in pre-transplant - yes (1) or not (0)	-1.353	1.103	1.504	0.22	0.259
Pre-transplant antibody anti-MHC class I - yes (1) or not (0)	0.263	0.551	0.228	0.63	1.301
Pre-transplant antibody anti-MHC class II - yes (1) or not (0)	-0.515	0.534	0.930	0.34	0.597
Time on dialysis pre-transplant (numerical data in months)	0.018	0.009	4.169	0.04	1.019
Dialysis post-transplant- yes (1) or not (0)	-0.131	0.524	0.063	0.80	0.877
Time of cold ischemia (numerical data in hour/minutes)	0.000	0.000	0.814	0.37	1.000
Number classical HLA mismatches between donors-receptors (nominal)					
*No mismatches - (0)	-1.099	1.528	0.517	0.47	0.333
*1 mismatch - (1)	20.510	4.019E+04	0.000	1.00	8.08E+08
*2 mismatches - (2)	0.000	1.414	0.000	1.00	1.000
*3 mismatches - (3)	-0.163	1.288	0.016	0.90	0.850
*4 mismatches - (4)	0.223	1.360	0.027	0.87	1.250

*5 mismatches - (5)	20.510	1.641E+04	0.000	1.00	8.08E+08
*6 mismatches - (6)			1.534	0.96	
Donors' type (nominal data)					
***Alive related 1 - (1)**	-1.534	0.631	5.910	0.02	0.216
*Alive related 2 - (2)	-1.128	1.456	0.601	-1.13	1.456
*Alive related 3 - (3)	20.074	4.019E+04	0.000	1.00	5.23E+08
*Alive unrelated - (4)	-0.030	1.206	0.001	0.98	0.971
***Deceased donor - (5)**			6.273	0.18	
Donors' gender - man (1) or woman (2)	0.049	0.522	0.009	0.93	1.050
Donors' age (numerical data in years)	0.054	0.023	5.493	0.02	1.056
Difference of age donor-receptor (numerical data in years)	-0.018	0.026	0.447	0.50	0.983
Difference of weight donor-receptor (numerical data in kilograms)	-0.001	0.024	0.003	0.96	0.999
Difference of height donor-receptor (numerical data in meters)	0.012	0.032	0.137	0.01	0.032
Induction - therapy with ATG- yes (1) and not (0)	-2.351	1.071	4.818	0.03	0.095
Base - Hypertension - yes (1) and not (0)	0.717	1.150	0.389	0.53	2.049
Base - Diabetes - yes (1) and not (0)	0.027	0.676	0.002	0.97	1.028
Base - Dyslipidemia - yes (1) and not (0)	1.564	0.910	2.951	0.09	4.778
Base - Glomerulonephritis - yes (1) and not (0)	-0.054	0.528	0.010	0.92	0.947
Base - Chronic kidey failure - yes (1) and not (0)	0.223	0.880	0.064	0.80	1.250
Base - Pyelonephritis - yes (1) and not (0)	1.222	0.954	1.643	0.20	3.395
Base - Polycystic kidney - yes (1) and not (0)	-1.173	1.114	1.109	0.29	0.310
Base - Hypertension from nephropathy - yes (1) and not (0)	-0.771	0.838	0.847	0.36	0.463
Base - disease - virus infection grouped - yes (1) and not (0)	-0.024	1.253	0.000	0.99	0.977
Base disease - parasitic infection - yes (1) and not (0)	-0.765	1.036	0.546	0.46	0.465
Base disease - other diseases grouped - yes (1) and not (0)	0.041	0.523	0.006	0.94	1.042
GFR Cockcroft-Gault (numerical data in mL/min/1.73 m2)	0.012	0.040	0.093	0.76	1.012
GFR MDRD (numerical data in mL/min/1.73 m2)	0.023	0.044	0.278	0.60	1.024
GFR CKD-EPI (numerical data in mL/min/1.73 m2)[c]	0.021	0.044	0.225	0.64	1.021

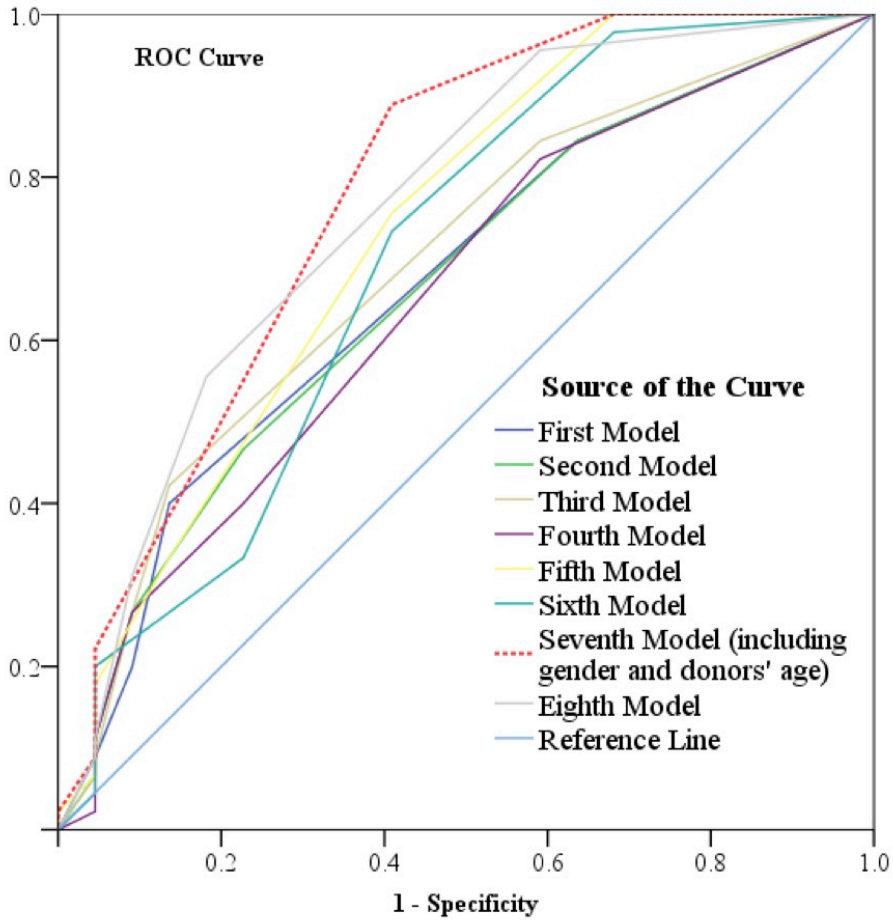
In bold are selected variable.[a]Applied just for women.[b]Retained variable to compose Risk Score Model. [c]Retained variable because it is a common marker used to diagnostic kidney disease stage. DSA - Donor Specific Antibody; GFR - Glomerular filtration rate; MDRD - Modification of Diet in Renal Disease (MDRD) Study Group; CKD-EPI - Chronic Kidney Disease Epidemiology Collaboration.

Supplementary Table 2 - 4th Risk Score Model Test with transplanted patients (N-64 and N(Variables)-17).

Nagelkerke R Square - 0.345	Prediction power - 75%		Dependent variable tested - Allograft dysfunction			
Variables that remained associated in the model	B	*P value*	Risk association to allograft dysfunction	Exp(B)	95% CI for Exp(B)	
					Lower bound	Upper bound
Patients' gender (Female)	1.414	0.0275	Negative	0.243	0.069	0.855
Donors' age	0.080	0.006	Positive	1.083	1.023	1.148
Fourth Model (risk to develop allograft dysfunction)*	0.679	0.008	Positive	1.972	1.197	3.252
Constant	-2.920	0.035		0.053		

* Included variables: Base disease - pyelonephritis, previous transplant, previous blood transfusion, previous abortion, induction therapy with ATG, PRA higher than 30% and positive donor DAS adopted with 2 points.

Supplementary Figure 1



5.3.0 SOLVING MICA AMBIGUITIES

To solve MICA's ambiguities derived by the use of the PCR-SSOP technique, we looked at its linkage disequilibrium with HLA-B (APPENDIX 5).

This approach allowed us to gather the allelic frequencies of the three analyzed populations: the control group, the chronic and the transplanted subjects (FIGURE 11).

In transplanted subjects (TX), the allele frequency for *MICA*001* is 2%, *MICA*002* is 23%, *MICA*004* is 7%, *MICA*007* is 1%, *MICA*008* is 25%, *MICA*009* is 10%, *MICA*010* is 7%, *MICA*011* is 6%, *MICA*012* is 4%, *MICA*016* is 4%, *MICA*018* is 4%, *MICA*019* is 2%, *MICA*027* is 2%, *MICA*045* is 1% and *MICA*056* is 1%.

In chronic patients (NTXC), the allele frequency for *MICA*002* is 21%, *MICA*004* is 6%, *MICA*006* is 2%, *MICA*008* is 29%, *MICA*009* is 15%, *MICA*010* is 13%, *MICA*011* is 2%, *MICA*017* is 2%, *MICA*018* is 3%, *MICA*027* is 6% and *MICA*080* is 2%.

Healthy subjects' (NTXS) allele frequency for *MICA*001* is 2%, *MICA*002* is 15%, *MICA*004* is 17%, *MICA*007* is 6%, *MICA*008* is 28%, *MICA*009* is 6%, *MICA*010* is 3%, *MICA*011* is 7%, *MICA*012* is 1%, *MICA*016* is 2%, *MICA*017* is 3%, *MICA*018* is 3%, *MICA*019* is 1% and *MICA*027* is 3%.

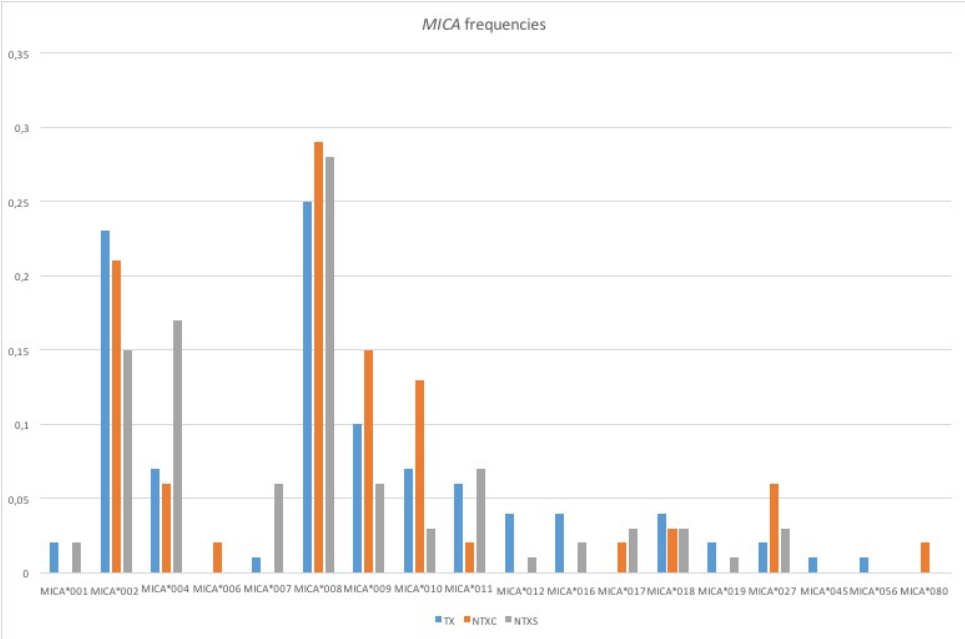


FIGURE 11 – *MICA* FREQUENCIES.
SOURCE: Matilde Risti (2017)

For further study, we also drew six primers that pear in exons 2, 3, 4 and 5 of the MICA gene. Specific primers were selected in order to solve the specific ambiguity of each sample,

optimizing the procedure (APPENDIX 6). This work will be carried forward by a student of scientific initiation. At the end of this study we will be able to confront these two methodologies.

5.4.0 RESUME OF RESULTS

1. We completed a detailed review of the MICA and NKG2D genes and their relevance in kidney transplantation, publishing it successfully on “Frontiers in Immunology”.
2. The multivariate logistic regression and ROC curve allowed us to identify the best Risk Score Model.
3. Risk phenotype was positively associated to proposed dominance allelic interaction of MICA A5.1. Thus, MICA A5.1- individuals were linked to the high sMICA and low sHLA-G production (p value – 0.017; CI [0.030; 0.710]).
4. Soluble HLA-G and MICA log-transformed quantifications analysed in patients with or without allograft dysfunction from pre- to post-transplant had a significant variation when compared to those for CKD and control individuals (sMICA p value - < 0.001; sHLA-G p value - <0.003).
5. MICA's ambiguities derived by the use of the PCR-SSOP were resolved by using its linkage disequilibrium with HLA-B, and the same ambiguities will be resolved with a different methodology (SBT) in a new project for an "iniciação científica" by a student who is working under prof. Maria da Graça Bicalho’s orientation and my co-orientation.

6.0.0 CONCLUSIONS

The MICA molecule is involved in: transplant rejection, inflammation, epithelia homeostasis, immune response against viruses and intracellular bacteria, and immune response against tumors. MICA's receptor is the NKG2D molecule. This activating receptor and its ligands are deeply involved in the outcome of transplanted grafts. The MICA molecule is also considered a minor histocompatibility antigens (MiHAs), present in the transplanted organ and that could trigger rejection and graft loss. For all of these reasons it is important to perform further studies and reviews to comprehend MICA's immunobiology and its role in the innate and adaptive immune response.

Meanwhile HLA-G molecule has a high potential to modulate immune response towards the improvement of graft survival after transplantation. Considering these two molecules, and their possible opposite effects, we performed a study on renal transplant subjects.

Considering the numerous variables that we could collect from clinical data of each transplant patients, a very important step was to select what the most important variables would be for us researchers as well as for the medical staff involved in this research. The Risk Score Model that we propose is a new way to predict the kidney-transplanted patients' prognostic in pre-transplant, considering the major non-invasive variables involved in allograft dysfunction. The suggested risk phenotype (high producers of sMICA and low producers of sHLA-G) has shown an association with the allelic dominance of MICA A5.1. In contrast, the protective phenotype (low producers of sMICA and high producers of sHLA-G) was associated with the G*01:04P allelic group in single or double doses. Individually, HLA-G and MICA genotypes and phenotypes were not associated with a pre-transplant prognostic of allograft dysfunction, but the carriage of specific genotypes of MICA or HLA-G differentially affects the risk factors in the post-transplant period. As far as we know this is the first study that considered sMICA and sHLA-G levels simultaneously on plasma but further studies will be needed to better understand the role of MICA and HLA-G.

6.1 Future prospective

It remains to be further investigated in other studies, whether the presence of MICA would be the result of alloreaction or if its presence would be indicative of an alert condition, considering that its expression occurs only under stress conditions. We will evaluate the best methodology to resolve *MICA* ambiguity: on one hand, looking at allelic frequencies (considering *MICA's* linkage disequilibrium with *HLA-B*); on the other hand, by analysing MICA with the SBT technique. We will also elucidate the influence of MICA and HLA-G in the post-transplant period, improving the

risk score model, according to the evolution in the renal transplantation. We will analyse sHLA-G and sMICA through time using the linear mixed model (LLM), a statistic methodology specific for this type of data. And finally we will evaluate the influence of *MICB* on kidney transplant, comparing it to *MICA's*.

7.0.0 APPENDIX:

7.1.0 APPENDIX 1: TCLE TRANSPLANTED PATIENTS

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE GENÉTICA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós do Laboratório de Immunogenética e Histocompatibilidade, pesquisadores da Universidade Federal do Paraná, estamos convidando o(a) Sr(a), transplantado( ) ou doador( ), com idade igual ou superior a 18 anos, a participar do estudo intitulado "INVESTIGAÇÃO DE POTENCIAIS BIOMARCADORES PARA O PROGNÓSTICO DA ACEITAÇÃO DE ALOENXERTO RENAL", que visa analisar a "OCORRÊNCIA DE VARIAÇÃO EM GENES e o MONITORAMENTO DE MOLÉCULAS PRODUZIDAS PARTIR DESTES GENES EM PACIENTES TRANSPLANTADOS RENAIS". A tipagem destes genes tem se mostrado uma importante etapa da pesquisa genética, avaliando o impacto da resposta imune na aceitação ou rejeição do rim transplantado. A avaliação por meio da analise de uma amostra de sangue, informará características relevantes que diferenciem pacientes transplantados renais de indivíduos saudáveis auxiliando no diagnostico de eventos de rejeição.

a) O objetivo desta pesquisa é investigar a possível relação entre a tolerância ao transplante e o gene HLA-G, monitorando também, via quantificação, as proteínas produzidas a partir deste gene em pacientes trai1splantados renais.
b) Com relação a possíveis benefícios:
   1. É possível que para o(a) Sr(a) não exista nenhum benefício direto a curto prazo.
   2. No entanto, a médio prazo, caso haja a necessidade de um novo transplante, ou mesmo para outras pessoas que venham a necessitar de um novo transplante. Os resultados obtidos com este estudo poderão auxiliar na busca de duplas compatíveis (doador e receptor) e no monitoramento da evolução do transplante.
c) Optando por participar desta pesquisa, será necessário o fornecimento de uma amostra de sangue, a qual será colhida junto com a coleta para o exame de rotina solicitado pelo médico. Também será necessário a coleta de alguns dados presentes em seu prontuário. Os dados a serem coletados seja o Sr(a) paciente receptor ou doador serão: nome, sexo, data de nascimento, estatura, peso, etnia, tipo sanguíneo ABO, fator Rh, tipagem HLA-A, HLA-B e HLA-DRBI (realizada na rotina do pré-trai1splante), existência de doenças basais e realização de transfusão(s) sanguínea(s) prévia. Ainda, apenas de pacientes receptores serão coletados os seguintes dados: data do último transplante, data de alta após a cirurgia deste último transplante, grau de parentesco com o doador do rim transplantado, painel reativo de anticorpos (PRA) pré-transplante, existência de transplantes anteriores, ocorrido de urna ou mais rejeições e imunossupressão pré- e pós-transplante adotada.

Rubricas:
Sujeito da Pesquisa e/ou responsável legal _____
Pesquisadores Responsáveis _____
Orientador _____ Orientados _____

Comitê de Ética em Peqsuisa do Setor de Ciências da Saúde da UFPR
Telefone: (41) 3360-7259 I e-mail: cometica.saude@ufpr.br

Os dados serão coletados após o último transplante realizado pelo paciente receptor, e seu monitoramento ocorrerá até um ano após o transplante.

d) Cada paciente (doador e receptor) receberá um código, o qual manterá o anonimato e privacidade das informações dos pacientes estudados.

e) O recrutamento e coleta de amostras para este estudo serão realizados quando do seu comparecimento no Hospital Universitário Evangélico de Curitiba, na oportunidade de suas consultas rotineiras, solicitadas pelo médico responsável, sem alterar a rotina que existiria mesmo na ausência deste estudo. Os procedimentos serão realizados por enfermeiras aptas do setor de transplante renal, sem a necessidade da realização de consultas extras para a participação neste estudo. As amostras de sangue coletadas serão armazenadas conforme o disposto na resolução do CNS No 441, de 12 de maio de 2011, para o caso de existir a necessidade da confirmação dos resultados obtidos por meio desta pesquisa, ou para a realização de novos estudos relacionados a mesma linha de pesquisa também por meio do Laboratório de Imunogenética e Histocompatibilidade - Universidade Federal do Paraná. Considerando nova pesquisa a ser realizada com este material armazenado, esta será realizada apenas mediante aprovação pelo Comitê de Ética em Pesquisa (CEP) institucional, e quando for o caso, pela Comissão Nacional de Ética em Pesquisa (CONEP).

f) Esta pesquisa não representará nenhum risco adicional para o(a) Sr(a)., a ém do desconforto da coleta de sangue, que é rotina de seus exames.

g) Embora a quebra de confidencialidade seja um risco inerente a qualquer pesquisa, serão tornadas todas as medidas cabíveis e disponíveis para a proteção da identidade e dos dados obtidos, seja de prontuários ou das amostras de sangue coletadas, os quais serão codificados de modo a proteger sua identidade. É importante ressaltar que a equipe de enfermagem disposta para recrutamento e coleta está apta para contornar o desconforto pela punção para a coleta do sangue.

h) Os pesquisadores (Orientadora deste estudo: Prof'. Dr". Maria da Graça Bicalho, Co-orientador deste estudo: Prof. Dr. Pablo Sandro Carvalho Santos, Mestranda responsável por este estudo: Vanessa Hauer - <vanessa.hauer@yahoo.com>), responsáveis por este estudo poderão ser contatados no Laboratório de imunogenética e Histocompatibilidade - Universidade Federal do Paraná (<ligh@ufpr.br>), Centro Politécnico, Rua Coronel Francisco Heráclito dos Santos, 210,Jard m das Américas, (41)3361- 1729, os quais estão à disposição para quaisquer esclarecimentos que possam surgir, antes, durante ou depois de encerrado o estudo.

i) A participação neste estudo é voluntária e o(a) Sr(a). poderá desistir a qualquer momento, solicitando que lhe seja devolvido o presente termo de consentimento assinado. A sua desistência não trará prejuízo para o(a) Sr(a). e nem para o seguimento de nosso estudo.

j) As informações relacionadas ao estudo poderão ser conhecidas por pessoas autorizadas (Médica responsável pelo setor de Transplante Renal do Hospital Universitário Evangélico de Curitiba:

Rubricas:
Sujeito da Pesquisa e/ou responsável legal _____
Pesquisadores Responsáveis _____
Orientador _____ Orientados _____

Dr' Fabiana L. C. Contieri, Orientadora deste estudo: Prof". Dr'. Maria da Graça Bicalho, Co-

orientador deste estudo: Prof. Dr. Pablo Sandro Carvalho Santos, Mestranda responsável por este estudo: Vanessa Hauer, Pesquisadora e Técnica: Renata Slowik, Pesquisador e Técnico: José Samuel Silva). No entanto, se qualquer informação for divulgada em relatório ou publicação, isto será feito sob forma codificada. para que a sua identidade seja preservada e seja mantida a confidencialidade.

k) As despesas necessárias para a realização da pesquisa (exames, medicamentos etc.) não são de sua responsabilidade e pela sua participação no estudo você não receberá qualquer valor em dinheiro.

l) Na hipótese de publicação dos resultados, não será divulgado sua identidade, e sim um código.

Eu, _____ li esse termo de consentimento e compreendi a natureza e objetivo do estudo com o qual concordo em participar. A explicação que recebi menciona os riscos e benefícios. Eu entendi que sou livre para interromper minha participação a qualquer momento sem justificativa prévia.

Eu concordo voluntariamente em participar deste estudo.

_____

(Assinatura do sujeito de pesquisa ou responsável legal – CPF n.°)

Curitiba, _____ de _____ 20____

_____

(Assinatura do Pesquisador)

Comitê de Ética em Peqsuisa do Setor de Ciências da Saúde da UFPR
Telefone: (41) 3360-7259 | e-mail: cometica.saude@ufpr.br

7.2.0 APPENDIX 2: TCLE CHRONIC PATIENTS

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE GENÉTICA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós, do Laboratório de Imunogenética e Histocompatibilidade, pesquisadores da Universidade Federal do Paraná, estamos convidando o(a) Sr(a) com idade igual ou superior a 18 anos, a participar do estudo intitulado "INVESTIGAÇÃO DE POTENCIAIS BIOMARCADORES PARA O PROGNÓSTICO DA ACEITAÇÃO DE ALOENXERTO RENAL", que visa analisar a "OCORRÊNCIA DE VARIAÇÃO EM GENES e o MONITORAMENTO DE MOLÉCULAS PRODUZIDAS PARTIR DESTES GENES EM PACIENTES TRANSPLANTADOS RENAIS". A tipagem destes genes tem se mostrado uma importante etapa da pesquisa genética, avaliando o impacto da resposta imune na aceitação ou rejeição do rim transplantado. A avaliação por meio da análise de uma amostra de sangue, informará características relevantes que diferenciem pacientes transplantados renais de pacientes não transplantados auxiliando no diagnóstico de eventos de rejeição.

.

a) O objetivo desta pesquisa é investigar a possível relação entre a tolerância ou não ao transplante e genes associados a resposta imune, monitorando também, via quantificação, moléculas produzidas a partir destes genes em pacientes transplantados renais.

b) Com relação a possíveis benefícios:

1. É possível que para o(a) Sr(a) não exista nenhum benefício direto a curto prazo.

2. No entanto, a médio prazo, caso haja a necessidade de um transplante, ou mesmo para outras pessoas que venham a necessitar de transplante, os resultados obtidos com este estudo poderão auxiliar no diagnóstico de eventos de rejeição.

c) Optando por participar desta pesquisa, será necessário o fornecimento de uma amostra de sangue, a qual será colhida junto com a coleta para o exame de rotina solicitado pelo médico. Também será necessário obter dados de seu prontuário. Os dados a serem coletados seja o Sr(a) paciente receptor ou doador serão: nome, sexo, data de nascimento, estatura, peso, etnia, tipo sanguíneo ABO, fator Rh, tipagem HLA-A, HLA-B e HLA-DRB1 (realizada na rotina do pré-transplante), existência de doenças basais e realização de transfusão(s) sanguínea(s) prévia.

Rubricas:
Sujeito da Pesquisa e /ou responsável legal_____
Pesquisadores Responsáveis _____
Orientador_____ Orientados_____

d) Cada paciente receberá um código, o qual manterá o anonimato e privacidade das informações dos pacientes estudados.

e) O recrutamento e coleta de amostras para este estudo serão realizados quando do seu comparecimento no Hospital Universitário Evangélico de Curitiba, na oportunidade de suas consultas rotineiras, solicitadas pelo médico responsável, sem alterar a rotina que existiria mesmo na ausência deste estudo. Os procedimentos serão realizados por enfermeiras aptas do setor de transplante renal, sem a necessidade da realização de consultas extras para a participação neste estudo. As amostras de sangue coletadas serão armazenadas conforme o disposto na resolução do CNS Nº 441, de 12 de maio de 2011, para o caso de existir a necessidade da confirmação dos resultados obtidos por meio desta pesquisa, ou para a realização de novos estudos relacionados a mesma linha de pesquisa também por meio do Laboratório de Imunogenética e Histocompatibilidade – Universidade Federal do Paraná. Considerando nova pesquisa a ser realizada com este material armazenado, esta será realizada apenas mediante aprovação pelo Comitê de Ética em Pesquisa (CEP) institucional e, quando for o caso, pela Comissão Nacional de Ética em Pesquisa (CONEP).

f) Esta pesquisa não representará nenhum risco adicional para o(a) Sr(a)., além do desconforto da coleta de sangue, que é rotina de seus exames.

g) Embora a quebra de confidencialidade seja um risco inerente a qualquer pesquisa, serão tomadas todas as medidas cabíveis e disponíveis para a proteção da identidade e dos dados obtidos, seja de prontuários ou das amostras de sangue coletadas, os quais serão codificados de modo a proteger sua identidade. É importante ressaltar que a equipe de enfermagem disposta para recrutamento e coleta está apta para contornar o desconforto pela punção para a coleta do sangue.

h) Os pesquisadores (Orientadora deste estudo: Profª. Drª. Maria da Graça Bicalho, Doutoranda responsável por este estudo: Vanessa Hauer - <vanessa.hauer@yahoo.com>, Doutoranda: Matilde Risti - <matilderisti@gmail.com>), responsáveis por este estudo poderão ser contatados no Laboratório de Imunogenética e Histocompatibilidade – Universidade Federal do Paraná (<ligh@ufpr.br>), Centro Politécnico, Rua Coronel Francisco Heráclito dos Santos, 210, Jardim das Américas, (41)3361-1729, os quais estão à disposição para quaisquer esclarecimentos que possam surgir, antes, durante ou depois de encerrado o estudo.

Rubricas:
Sujeito da Pesquisa e /ou responsável legal_____
Pesquisadores Responsáveis _____
Orientador_____ Orientados_____

i) A participação neste estudo é voluntária e o(a) Sr(a). poderá desistir a qualquer momento, solicitando que lhe seja devolvido o presente termo de consentimento assinado. A sua desistência não trará prejuízo para o(a) Sr(a). e nem para o seguimento de nosso estudo.

j) As informações relacionadas ao estudo poderão ser conhecidas por pessoas autorizadas (Médica responsável pelo setor de Transplante Renal do Hospital Universitário Evangélico de Curitiba: Drª Fabiana L. C. Contieri, Orientadora deste estudo: Profª. Drª. Maria da Graça Bicalho, Doutoranda responsável por este estudo: Vanessa Hauer, Doutoranda: Matilde Risti, Pesquisadora e Técnica: Renata Slowik, Pesquisador e Técnico: José Samuel Silva). No entanto, se qualquer informação for divulgada em relatório ou publicação, isto será feito sob forma codificada, para que a **sua identidade seja preservada e seja mantida a confidencialidade**.

k) As despesas necessárias para a realização da pesquisa (exames, medicamentos etc.) não são de sua responsabilidade e pela sua participação no estudo você não receberá qualquer valor em dinheiro.

l) Na hipótese de publicação dos resultados, não será divulgado sua identidade, e sim um código.

Eu,_____ li esse termo de consentimento e compreendi a natureza e objetivo do estudo com o qual concordo em participar . A explicação que recebi menciona os riscos e benefícios. Eu entendi que sou livre para interromper minha participação a qualquer momento sem justificativa prévia.

Eu concordo voluntariamente em participar deste estudo e autorizo o acesso ao meu prontuário.

_____
(Assinatura do sujeito de pesquisa ou responsável legal – CPF n.º)

Curitiba,___ de _____20__

_____
Assinatura dos Pesquisadores Responsáveis

Comitê de Ética em Pesquisa do Setor de Ciências da Saúde da UFPR
Telefone: (41) 3360-7259 e-mail: cometica.saude@ufpr.br

7.3.0 APPENDIX 3: TCLE CONTROL GROUP

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS BIOLÓGICAS
DEPARTAMENTO DE GENÉTICA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós do Laboratório de Imunogenética e Histocompatibilidade, pesquisadores da Universidade Federal do Paraná, estamos convidando o(a) Sr(a) com idade igual ou superior a 18 anos, saudável, não gestante, não portador de doença renal crônica e não transplantado, a participar do estudo intitulado "INVESTIGAÇÃO DE POTENCIAIS BIOMARCADORES PARA O PROGNÓSTICO DA ACEITAÇÃO DE ALOENXERTO RENAL", que visa analisar a "OCORRÊNCIA DE VARIAÇÃO EM GENES e o MONITORAMENTO DE MOLÉCULAS PRODUZIDAS PARTIR DESTES GENES EM PACIENTES TRANSPLANTADOS RENAIS". A tipagem destes genes tem se mostrado uma importante etapa da pesquisa genética, avaliando o impacto da resposta imune na aceitação ou rejeição do rim transplantado. A avaliação por meio da análise de uma amostra de sangue, informará características relevantes que diferenciem pacientes transplantados renais de indivíduos saudáveis auxiliando no diagnóstico de eventos de rejeição.

a) O objetivo desta pesquisa é investigar a possível relação entre a tolerância ou não ao transplante e genes associados a resposta imune, monitorando também, via quantificação, moléculas produzidas a partir destes genes em pacientes transplantados renais.

b) Com relação a possíveis benefícios:

1. É possível que para o(a) Sr(a) não exista nenhum benefício direto a curto prazo.

2. No entanto, a médio prazo, caso haja a necessidade de um transplante, ou mesmo para outras pessoas que venham a necessitar de transplante, os resultados obtidos com este estudo poderão auxiliar no diagnóstico de eventos de rejeição.

c) Optando por participar desta pesquisa, será necessário o fornecimento de uma amostra de sangue, a qual será colhida mediante campanha de recrutamento voluntário. Também será necessária a coleta de alguns dados através de questionário que será aplicado no momento do recrutamento. Os dados a serem coletados serão: nome completo, informações para re-contato (endereço, telefone e e-mail), sexo, data de nascimento, estatura, peso, etnia, tipo sanguíneo ABO, fator Rh, existência de doenças de base não renal, tipo de medicação de uso contínuo adotada e realização de transfusão(s) sanguínea(s) prévia. Indivíduos do sexo feminino recrutadas terão os seguintes dados reprodutivos coletados: número de gestações, número de abortos, fase do período reprodutivo em que se encontram e anticonceptivos adotados.

Rubricas:
Sujeito da Pesquisa e /ou responsável legal_____
Pesquisadores Responsáveis_____
Orientador_____ Orientados_____

Comitê de Ética em Pesquisa do Setor de Ciências da Saúde da UFPR
Telefone: (41) 3360-7259 e-mail: cometica.saude@ufpr.br

d) Cada paciente receberá um código, o qual manterá o anonimato e privacidade das informações dos pacientes estudados.

e) O recrutamento e coleta de amostras para este estudo serão realizados mediante campanha de recrutamento voluntário no Laboratório de Imunogenética e Histocompatibilidade – Universidade Federal do Paraná (<ligh@ufpr.br>), Centro Politécnico, Rua Coronel Francisco Heráclito dos Santos, 210, Jardim das Américas, (41)3361-1729 e nos locais de sensibilização disponibilizados para campanha. Os procedimentos serão realizados pela farmacêutica-bioquímica apta Suelen Camargo Zeck (CRF: 22400, <suelenzeck@gmail.com>). As amostras de sangue coletadas serão armazenadas conforme o disposto na resolução do CNS Nº 441, de 12 de maio de 2011, para o caso de existir a necessidade da confirmação dos resultados obtidos por meio desta pesquisa, ou para a realização de novos estudos relacionados a mesma linha de pesquisa também por meio do Laboratório de Imunogenética e Histocompatibilidade – Universidade Federal do Paraná. Considerando nova pesquisa a ser realizada com este material armazenado, esta será realizada apenas mediante aprovação pelo Comitê de Ética em Pesquisa (CEP) institucional e, quando for o caso, pela Comissão Nacional de Ética em Pesquisa (CONEP).

f) Esta pesquisa não representará nenhum risco adicional para o(a) Sr(a)., além do desconforto da coleta de sangue, que será voluntária.

g) Embora a quebra de confidencialidade seja um risco inerente a qualquer pesquisa, serão tomadas todas as medidas cabíveis e disponíveis para a proteção da identidade e dos dados obtidos, seja de prontuários ou das amostras de sangue coletadas, os quais serão codificados de modo a proteger sua identidade. É importante ressaltar que a equipe de enfermagem disposta para recrutamento e coleta está apta para contornar o desconforto pela punção para a coleta do sangue.

h) Os pesquisadores (Orientadora deste estudo: Profª. Drª. Maria da Graça Bicalho, Doutoranda responsável por este estudo: Vanessa Hauer - <vanessa.hauer@yahoo.com>, Doutoranda: Matilde Risti - <matilderisti@gmail.com>), responsáveis por este estudo poderão ser contatados no Laboratório de Imunogenética e Histocompatibilidade – Universidade Federal do Paraná (<ligh@ufpr.br>), Centro Politécnico, Rua Coronel Francisco Heráclito dos Santos, 210, Jardim das Américas, (41)3361-1729, os quais estão à disposição para quaisquer esclarecimentos que possam surgir, antes, durante ou depois de encerrado o estudo.

Rubricas:
Sujeito da Pesquisa e /ou responsável legal_____
Pesquisadores Responsáveis_____
Orientador_____ Orientados_____

i) A participação neste estudo é voluntária e o(a) Sr(a). poderá desistir a qualquer momento, solicitando que lhe seja devolvido o presente termo de consentimento assinado. A sua desistência não trará prejuízo para o(a) Sr(a).
j) As informações relacionadas ao estudo poderão ser conhecidas por pessoas autorizadas (Médica responsável pelo setor de Transplante Renal do Hospital Universitário Evangélico de Curitiba: Drª Fabiana L. C. Contieri, Orientadora deste estudo: Profª. Drª. Maria da Graça Bicalho, Doutoranda responsável por este estudo: Vanessa Hauer, Doutoranda: Matilde Risti, Pesquisadora e Técnica: Renata Slowik, Pesquisador e Técnico: José Samuel Silva). No entanto, se qualquer informação for divulgada em relatório ou publicação, isto será feito de forma codificada, para que a **sua identidade seja preservada e seja mantida a confidencialidade**.

k) As despesas necessárias para a realização da pesquisa (exames, medicamentos etc.) não são de sua responsabilidade e pela sua participação no estudo você não receberá qualquer valor em dinheiro.

l) Na hipótese de publicação dos resultados, não será divulgada sua identidade, e sim um código.

Eu,_____ li esse termo de consentimento e compreendi a natureza e objetivo do estudo com o qual concordo em participar. A explicação que recebi menciona os riscos e benefícios. Eu entendi que sou livre para interromper minha participação a qualquer momento sem justificativa prévia.

Eu concordo voluntariamente em participar deste estudo.

_____
(Assinatura do sujeito de pesquisa ou responsável legal – CPF n.º)

Curitiba,___ de _____20__

_____
Assinatura do Pesquisador Responsável

Comitê de Ética em Pesquisa do Setor de Ciências da Saúde da UFPR
Telefone: (41) 3360-7259 e-mail: cometica.saude@ufpr.br

7.4.0 APPENDIX 4: QUESTIONNAIRE CONTROL GROUP

INSTRUMENTO DE AVALIAÇÃO

Nome completo: _____

Sexo: F ( ) / M ( ) Idade: _____ RG ou CPF: _____

INFORMAÇÕES PARA RECONTATO

Telefone celular:

Telefone residencial: _____ _____

Endereço residencial: _____ Cidade/Estado: _____

Endereço de e-mail: _____

INFORMAÇÕES A SEREM UTILIZADAS NA PESQUISA

Data de nascimento: _____ Peso: _____ Altura: _____

Tipo sanguíneo: A ( ) / B ( ) / AB ( ) / O ( ) Fator Rh: Rh + ( ) / Rh - ( )

Etnia: Amarelo ( ) / Branco ( ) / Indígena ( ) / Negróide ( ) / Miscigenado ( )

DOENÇAS /CONDIÇÕES CRÔNICAS:

( ) hipertensão arterial	( ) pedras no rim (cálculo)	( ) hipercolesterolemia
( ) diabetes *mellitus* congênita	( ) tabagista	( ) hipertireoidismo
( ) diabetes *mellitus* adquirida	( ) ex-tabagista	( ) hipotireoidismo
( ) histórico - doença de chagas	( ) histórico - toxplasmose	( ) histórico - hepatite
( ) herpes - EBV (Vírus Epstein-Barr)	( ) HIV	( ) câncer

( ) outras doenças/condições ( ) doença auto-imune diversa

Qual(s)? _____ Qual(s)? _____

Por favor, liste seus medicamentos e doses atuais, incluindo VITAMINAS:

a)	d)	g)
b)	e)	h)
c)	f)	i)

QUESTÕES APENAS A SEREM RESPONDIDAS PELAS MULHERES

Em qual período reprodutivo se encontra? ( ) período fértil ( ) perimenopausa ( ) menopausa

Número de gestações: ( ) 0 ( ) 1 ( ) 2 ( ) 3 ou mais

Número de abortos: ( ) 0 ( ) 1 ( ) 2 ( ) 3 ou mais

Estando no período fértil, qual é a fase do seu ciclo atual? ( ) fase da menstruação (1º ao 4º dia) ( ) fase proliferativa (5º ao 13º dia) ( ) ovulação (13º ao 16º dia) ( ) fase luteínica/secretora (16º ao 28º dia)

No momento está grávida? Sim ( ) / Não ( )

Método(s) contraceptivo(s) adotado(s)? ( ) pílula ( ) DIU ou SIU ( ) anel vaginal ( ) preservativo ( ) injeção anticoncepcional ( ) nenhum

7.5.0 APPENDIX 5: SOLVING MICA AMBIGUITIES THROUGH ITS LINKAGE DISEQUILIBRIUM WITH HLA-B

Amostras	MICA resultados	HLA-B	ALLELE FRENQUENCIES
TXR1R01	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	HLA-B*44:02 HLA-B*49:01	MICA*004 MICA*008
TXR1R02	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	HLA-B*44:03 HLA-B*39:01	MICA*002 MICA*004
TXR1R03	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03	HLA-B*51:01 HLA-B*35:05	MICA*002:01 MICA*009:01
TXR1R04	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*35:01/07 HLA-B*39:01/05	MICA*002:AC MICA*002:AC
TXR1R05	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*53:01 HLA-B*58:01	MICA*002:AC MICA*002:AC
TXR1R06	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*07:02 HLA-B*40:01	MICA*008:AD MICA*008:AD
TXR1R07	MICA*011 MICA*018:01	HLA-B*14:02 HLA-B*18:01	MICA*011 MICA*018:01
TXR1R08	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*08:01 HLA-B*38:01	MICA*002:AC MICA*008:AD
TXR1R09	MICA*009:01 MICA*012:01 MICA*012:01 MICA*049	HLA-B*52:01 HLA-B*55:01	MICA*009:01 MICA*012:01
TXR1R10	MICA*010:01 MICA*027 MICA*010:01 MICA*048 MICA*027 MICA*069 MICA*048 MICA*069	HLA-B*44 HLA-B*52	MICA*008 MICA*010:01
TXR1R11	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/0	HLA-B*08:01 HLA-B*35:01/07/05	MICA*002:AC MICA*008:AD
TXR1R12	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	HLA-B*50:01 HLA-B*51:01	MICA*009:01 MICA*010:01
TXR1R13	MICA*009:01 MICA*045 MICA*045 MICA*049	HLA-B*13:01/02 HLA-B*51:01	MICA*009:01 MICA*045
TXR1R14*	MICA*002:AC MICA*010:01 MICA*002:AC MICA*069 MICA*010:01 MICA*020 MICA*010:01 MICA*055 MICA*010:01 MICA*068 MICA*010:01 MICA*072 MICA*010:01 MICA*075 MICA*020 MICA*069 MICA*055 MICA*069 MICA*068 MICA*069 MICA*069 MICA*072 MICA*069 MICA*075 AC:=:01/03	HLA-B*39:05 HLA-B*15:14	MICA*002:AC MICA*010:01

TXR1R15	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03	HLA-B*35:01/07/04 HLA-B*51:01/02	MICA*002:AC MICA*009:01
TXR1R16	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	HLA-B*07:02 HLA-B*15:01/07	MICA*008:AD MICA*010:01
TXR1R17	MICA*008:AD MICA*016 MICA*016 MICA*080 MICA*016 MICA*082 MICA*016 MICA*085 AD:=:01/04	HLA-B*44:02 HLA-B*51:05	MICA*008:AD MICA*016
TXR1R18	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*40	MICA*008:AD MICA*008:AD
TXR1R19	MICA*009:01 MICA*016 MICA*016 MICA*049	HLA-B*35:02 HLA-B*52:01	MICA*009:01 MICA*016
TXR1R20	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*08:01 HLA-B*38:01	MICA*002:AC MICA*008:AD
TXR1R21	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	HLA-B*50:01 HLA-B*51:01	MICA*009:01 MICA*009:01
TXR1R22	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	HLA-B*39:05 HLA-B*44:03	MICA*002:AC MICA*004

TXR1R23	MICA*018:01 MICA*019:AB AB:=:01/02	HLA-B*14:01 HLA-B*18:04	MICA*018:01 MICA*019:AB
TXR1R24	MICA*011 MICA*011 MICA*011 MICA*030	HLA-B*14:02 HLA-B*58:01/02	MICA*011 MICA*011
TXR1R25	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*35:01/07 HLA-B*44:02	MICA*002:AC MICA*008:AD
TXR1R26	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	HLA-B*15 HLA-B*44	MICA*008:AD MICA*010:01
TXR1R27	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	HLA-B*27:05 HLA-B*07	MICA*007:01 MICA*008:AD
TXR1R28	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*08:01 HLA-B*53:01	MICA*002:AC MICA*008:AD

TXR1R29	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	HLA-B*15:04 HLA-B*44:02	MICA*008:AD MICA*010:01
TXR1R30	MICA*008:MV MICA*008:MV MICA*008:MV MICA*070 MICA*008:MV MICA*073 MICA*008:MV MICA*080 MICA*008:MV MICA*082 MICA*070 MICA*073 MICA*070 MICA*080 MICA*070 MICA*082 MICA*073 MICA*080 MICA*073 MICA*082 MV:=:01/04/05	HLA-B*08 HLA-B*44	MICA*008:MV MICA*008:MV
TXR1R31	MICA*001 MICA*004	HLA-B*18 HLA-B*44	MICA*001 MICA*004
TXR1R32	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/0	HLA-B*07 HLA-B*49	MICA*004 MICA*008:AD
TXR1R33	MICA*002:AC MICA*027 MICA*002:AC MICA*048 MICA*020 MICA*027 MICA*020 MICA*048 MICA*027 MICA*055 MICA*027 MICA*081 MICA*048 MICA*055 MICA*048 MICA*081 AC:=:01/03	HLA-B*35 HLA-B*40	MICA*002:AC MICA*027
TXR1R34	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	HLA-B*14:02 HLA-B*35:01/07	MICA*002:AC MICA*011
TXR1R35	MICA*010:01 MICA*012:01 MICA*012:01 MICA*069	HLA-B*15 HLA-B*55	MICA*010:01 MICA*012:01
TXR1R36	MICA*012:01 MICA*019:AB AB:=:01/02	HLA-B*51 HLA-B*55	MICA*012:01 MICA*019:AB
TXR1R37	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*15:01/07	MICA*008:AD MICA*010:01
TXR1R38	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*07:02 HLA-B*38:01	MICA*002:AC MICA*008:AD
TXR1R39	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*08:01 HLA-B*39:01/05	MICA*002:AC MICA*008:AD
TXR1R40	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	HLA-B*44 HLA-B*50	MICA*008:AD MICA*009:01
TXR1R41	MICA*004 MICA*004	HLA-B*48 HLA-B*49	CA*004 MICA*004 no match
TXR1R42	MICA*010:01 MICA*011 MICA*011 MICA*069	HLA-B*14:02 HLA-B*15:01	MICA*010:01 MICA*011
TXR1R43	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	HLA-B*07 HLA-B*35	MICA*002:AC MICA*008:AD

TXR1R44	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*07 HLA-B*35	MICA*002:AC MICA*008:AD
TXR1R45	MICA*007:02 MICA*012:01	HLA-B*18:01 HLA-B*82:02	MICA*007:02 MICA*012:01
TXR1R46	repetir	HLA-B*14:02 HLA-B*58:01/02	¿ 002-011 ?
TXR1R47	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*35:01/07 HLA-B*39:03	MICA*002:AC MICA*002:AC
TXR1R48	MICA*004 MICA*016	HLA-B*35 HLA-B*44	MICA*004 MICA*016
TXR1R49	MICA*001 MICA*027 MICA*001 MICA*048 MICA*001 MICA*080 MICA*001 MICA*082	HLA-B*15 HLA-B*44	MICA*001 MICA*027
TXR1R50	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	HLA-B*35 HLA-B*40	MICA*002:AC MICA*008:AD
TXR1R51	MICA*001 MICA*010:01 MICA*001 MICA*069	HLA-B*15:01 HLA-B*18:01/02/03	MICA*001 MICA*010:01
TXR1R52	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	HLA-B*08:091 HLA-B*44:02	MICA*008:AD MICA*009:01
TXR1R53	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	HLA-B*07 HLA-B*18	MICA*002:AC MICA*008:AD
TXR1R54*	MICA*008:AD MICA*016 MICA*016 MICA*070 MICA*016 MICA*073 AD:=:01/04	HLA-B*08:01 HLA-B*35:08	MICA*008:AD MICA*016
TXR1R55	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*008:AD MICA*074 MICA*009:01 MICA*070 MICA*009:01 MICA*073 MICA*049 MICA*070 MICA*049 MICA*073 MICA*070 MICA*074 MICA*073 MICA*074 AD:=:01/04	HLA-B*37 HLA-B*51	MICA*008:AD MICA*009:01
TXR1R56	MICA*008:AD MICA*018:01 MICA*018:01 MICA*080 MICA*018:01 MICA*082 MICA*018:01 MICA*085 AD:=:01/04	HLA-B*44:02 HLA-B*57:03	MICA*008:AD MICA*018:01
TXR1R57	MICA*002:AC MICA*018:01 MICA*018:01 MICA*020 MICA*018:01 MICA*055 MICA*018:01 MICA*081 AC:=:01/03	HLA-B*18:02 HLA-B*58:01	MICA*002:AC MICA*018:01
TXR1R58	MICA*002:AC MICA*016 MICA*016 MICA*020 MICA*016 MICA*055 MICA*016 MICA*081 AC:=:01/03	HLA-B*35:02 HLA-B*53:01	MICA*002:AC MICA*016
TXR1R59	MICA*009:01 MICA*018:01 MICA*018:01 MICA*049 MICA*018:01 MICA*07	HLA-B*45:01 HLA-B*51:01	MICA*009:01 MICA*018:01
TXR1R60	MICA*008:AD MICA*011 AD:=:01/04	HLA-B*07 HLA-B*14	MICA*008:AD MICA*011
TXR1R61	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*068 MICA*011 MICA*072 MICA*011 MICA*075 MICA*011 MICA*081 AC:=:01/03	HLA-B*11 HLA-B*39	MICA*002:AC MICA*011
TXR1R62	MICA*019 MICA*056 MICA*056 MICA*056	HLA-B*14:01 HLA-B*15:16	MICA*019 MICA*056
TXR1R63	MICA*002:AC MICA*012:01 MICA*012:01 MICA*020 MICA*012:01 MICA*055 MICA*012:01 MICA*081 AC:=:01/03	HLA-B*55 HLA-B*80	MICA*002:AC MICA*012:01 there's no B*80 on allelefrequencies
TXR1R64	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*44	MICA*004 MICA*008:AD

TXR1R65	MICA*009:01 MICA*012:03 MICA*012:03 MICA*049 MICA*012:03 MICA*074	HLA-B*35:03 HLA-B*40:01	MICA*009:01 MICA*012:03
TXR1R66	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*35 HLA-B*35	MICA*002:AC MICA*002:AC
TXR1R67	MICA*009:01 MICA*027 MICA*009:01 MICA*048 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*027 MICA*049 MICA*027 MICA*074 MICA*048 MICA*049 MICA*048 MICA*074 MICA*049 MICA*080 MICA*049 MICA*082 MICA*074 MICA*080 MICA*074 MICA*082	HLA-B*40 HLA-B*51	MICA*009:01 MICA*027 MICA*027 MICA*049
Amostras	MICA resultodos	HLA-B	ALLELE FRENQUENCIES
NTXC01	MICA*004 MICA*017	HLA-B*44:04 HLA-B*57:01	MICA*004 MICA*017
NTXC02	MICA*010:01 MICA*018:01 MICA*018:01 MICA*069	HLA-B* 15HLA-B*18	MICA*010:01 MICA*018:01
NTXC03	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*35:01/07/17 HLA-B*35:01/07/17	MICA*002:AC MICA*002:AC
NTXC05	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	HLA-B*44:02 HLA-B*51:01	MICA*008:AD MICA*009:01
NTXC06	MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*13:02 HLA-B*15:03	MICA*008:AD MICA*080
NTXC07	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	HLA-B*15:01/30 HLA-B*51:01/02	MICA*009:01 MICA*010:01
NTXC09	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*08:01 HLA-B*38:01	MICA*002:AC MICA*008:AD
NTXC12	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*27:02/05 HLA-B*08:01	MICA*008:AD MICA*008:AD
NTXC14	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	HLA-B*15:15 HLA-B*51:01	MICA*009:01 MICA*010:01
NTXC15	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*08:01 HLA-B*15:10	MICA*008:AD MICA*008:AD
NTXC16	MICA*027 MICA*027 MICA*027 MICA*048 MICA*048 MICA*048	HLA-B*40:02 HLA-B*40:02/04/09	MICA*027 MICA*027

NTXC19	MICA*010:01 MICA*010:01 MICA*010:01 MICA*019:AB MICA*010:01 MICA*069 MICA*019:AB MICA*069 MICA*069 MICA*069 AB:=:01/02	HLA-B*15:01 HLA-B*48:02	MICA*010:01 MICA*010:01
NTXC22	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*07:02 HLA-B*15:17	MICA*008:AD MICA*008:AD
NTXC24	MICA*004 MICA*010:01 MICA*004 MICA*069	HLA-B*15:01 HLA-B*44:03	MICA*004 MICA*010:01
NTXC25	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*08:01 HLA-B*44:02	MICA*008:AD MICA*008:AD
NTXC26	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	HLA-B*50:01 HLA-B*52:01	MICA*009:01 MICA*009:01
NTXC30	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*07 HLA-B*39	MICA*002:AC MICA*008:AD
NTXC31	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	HLA-B*44:03 HLA-B*47:01	MICA*004 MICA*008:AD
NTXC32	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*35:01/07 HLA-B*48:02	MICA*002:AC MICA*002:AC
NTXC37	MICA*002:AC MICA*027 MICA*002:AC MICA*048 MICA*020 MICA*027 MICA*020 MICA*048 MICA*027 MICA*055 MICA*027 MICA*081 MICA*048 MICA*055 MICA*048 MICA*081 AC:=:01/03	HLA-B*40:06 HLA-B*53:01	MICA*002:AC MICA*027
NTXC38	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	HLA-B*51:01 HLA-B*51:01	MICA*009:01 MICA*009:01
NTXC39	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*35:01/07 HLA-B*44:02/03	MICA*002:AC MICA*008:AD
NTXC40	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	HLA-B*50:01 HLA-B*15:01	MICA*009:01 MICA*010:01
NTXC41	MICA*004 MICA*009:01 MICA*004 MICA*049	HLA-B*13:02 HLA-B*49:01	MICA*004 MICA*009:01
NTXC43	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	HLA-B*15:15 HLA-B*44:02	MICA*008:AD MICA*010:01
NTXC44	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	HLA-B*14:02 HLA-B*35:05	MICA*002:AC MICA*011

NTXC45	MICA*008:AD MICA*027 MICA*008:AD MICA*048 MICA*027 MICA*080 MICA*027 MICA*082 MICA*027 MICA*085 MICA*048 MICA*080 MICA*048 MICA*082 MICA*048 MICA*085 AD:=:01/04	HLA-B*08:01 HLA-B*40:02/04/09	MICA*008:AD MICA*027
NTXC46	MICA*002:AC MICA*018:01 MICA*018:01 MICA*020 MICA*018:01 MICA*055 MICA*018:01 MICA*081 AC:=:01/03	HLA-B*18:01 HLA-B*39:07	MICA*002:AC MICA*018:01
NTXC47	MICA*006 MICA*008:AD MICA*006 MICA*080 MICA*006 MICA*082 MICA*006 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*51	MICA*006 MICA*008:AD
NTXC48	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*07 HLA-B*39	MICA*002:AC MICA*008:AD
NTXC49	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*39:03 HLA-B*48:02	MICA*002:AC MICA*002:AC
Amostras	MICA resultodos	HLA-B	ALLELE FRENQUENCIES
NTXS01	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	HLA-B*08 HLA-B*15	MICA*008:AD MICA*010:01
NTXS02	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04		
NTXS03	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*37 HLA-B*44	MICA*008:AD MICA*008:AD
NTXS04	MICA*016 MICA*027 MICA*016 MICA*048	HLA-B*35 HLA-B*40	MICA*016 MICA*027
NTXS05	MICA*004 MICA*004	HLA-B*41 HLA-B*49	MICA*004 MICA*004
NTXS06	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*44	MICA*004 MICA*008:AD
NTXS07	MICA*010:01 MICA*017 MICA*017 MICA*069		
NTXS08	MICA*004 MICA*007:01 MICA*004 MICA*026 MICA*004 MICA*079	HLA-B*44 HLA-B*52	MICA*004 MICA*007:01
NTXS09	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*57	MICA*008:AD MICA*017
NTXS10	MICA*009:01 MICA*018:01 MICA*018:01 MICA*049	HLA-B*18 HLA-B*50	MICA*009:01 MICA*018:01
NTXS11	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*07 HLA-B*35	MICA*002:AC MICA*008:AD
NTXS12	MICA*007:AB MICA*018:AB MICA*018:01 MICA*079 AB:=:01/02		

NTXS13	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04		
NTXS14	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	HA-B*14 HLA-B*39	MICA*002:AC MICA*011
NTXS15	MICA*008:AD MICA*016 MICA*016 MICA*080 MICA*016 MICA*082 MICA*016 MICA*085 AD:=:01/04	HLA-B*15 HLA-B*35	MICA*008:AD MICA*016
NTXS16	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04	HLA-B*40 HLA-B*57	MICA*008:AD MICA*017
NTXS17	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03		
NTXS18	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04		
NTXS19	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049		
NTXS20	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04		
NTXS21	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03		
NTXS22	MICA*004 MICA*004		MICA*004 MICA*004
NTXS23	MICA*002:AC MICA*019:AB MICA*019:AB MICA*020 MICA*019:AB MICA*055 MICA*019:AB MICA*081 AC:=:01/03 AB:=:01/02	HLA-B*35 HLA-B*51	
NTXS24	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*08 HLA-B*14	
NTXS25	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*27	
NTXS26	MICA*001 MICA*011		MICA*001 MICA*011
NTXS27	MICA*002:AC MICA*007:01 MICA*002:AC MICA*026 MICA*002:AC MICA*079 MICA*007:01 MICA*020 MICA*007:01 MICA*055 MICA*007:01 MICA*081 MICA*020 MICA*026 MICA*020 MICA*079 MICA*026 MICA*055 MICA*026 MICA*081 MICA*055 MICA*079 MICA*079 MICA*081 AC:=:01/03		
NTXS28	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	HLA-B*15 HLA-B*35	MICA*007:01 MICA*008:AD

NTXS29	MICA*002:AC MICA*010:01 MICA*002:AC MICA*069 MICA*010:01 MICA*020 MICA*010:01 MICA*055 MICA*010:01 MICA*081 MICA*020 MICA*069 MICA*055 MICA*069 MICA*069 MICA*081 AC:=:01/03	HLA-B*35 HLA-B*38	
NTXS30	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04		
NTXS31	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	HLA-B*39 HLA-B*44	MICA*002:AC MICA*004
NTXS32	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*27 HLA-B*49	MICA*008:AD MICA*008:AD
NTXS33	MICA*002:AC MICA*017 MICA*017 MICA*020 MICA*017 MICA*055 MICA*017 MICA*068 MICA*017 MICA*081 AC:=:01/03		
NTXS34	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03		
NTXS35	MICA*004 MICA*011	-B*14:01 HLA-B*49	MICA*004 MICA*011
NTXS36	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03		
NTXS37	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04		
NTXS38	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04		
NTXS39	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04		
NTXS40	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04		
NTXS41	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04		
NTXS42	MICA*008:AD MICA*018:01 MICA*018:01 MICA*080 MICA*018:01 MICA*082 MICA*018:01 MICA*085 AD:=:01/04		
NTXS43	MICA*001 MICA*009:01 MICA*001 MICA*049		
NTXS44	MICA*009:01 MICA*011 MICA*011 MICA*049		
NTXS45	MICA*007:01 MICA*009:01 MICA*007:01 MICA*049 MICA*009:01 MICA*026 MICA*009:01 MICA*079 MICA*026 MICA*049 MICA*049 MICA*079		
NTXS46	MICA*009:01 MICA*018:01 MICA*018:01 MICA*049		

NTXS47	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049		
NTXS48	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03		
NTXS49	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04		
NTXS50	MICA*027 MICA*047 MICA*047 MICA*048		
NTXS51	MICA*008:AD MICA*011 MICA*011 MICA*080 MICA*011 MICA*082 MICA*011 MICA*085 AD:=:01/04		
NTXS52	MICA*001 MICA*010:01 MICA*001 MICA*069		
NTXS53	MICA*002:AC MICA*018:01 MICA*018:01 MICA*020 MICA*018:01 MICA*055 MICA*018:01 MICA*081 AC:=:01/03		
NTXS54	MICA*011 MICA*012:01	HLA-B*14 HLA-B*56	MICA*011 MICA*012:01
NTXS55	MICA*002:AC MICA*027 MICA*002:AC MICA*048 MICA*020 MICA*027 MICA*020 MICA*048 MICA*027 MICA*055 MICA*027 MICA*081 MICA*048 MICA*055 MICA*048 MICA*081 AC:=:01/03	HLA-B*01 HLA-B*40	MICA*002:AC MICA*027
NTXS56	MICA*004 MICA*009:01 MICA*004 MICA*049	HLA-B*41 HLA-B*51	MICA*004 MICA*009:01
NTXS57	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*44	MICA*004 MICA*008:AD
NTXS58	MICA*007:01 MICA*009:01 MICA*007:01 MICA*049 MICA*009:01 MICA*026 MICA*009:01 MICA*079 MICA*026 MICA*049 MICA*049 MICA*079	HLA-B*27 HLA-B*51	MICA*007:01 MICA*009:01
NTXS59	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	HLA-B*15 HLA-B*38	MICA*002:AC MICA*008:AD
NTXS60	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*44	MICA*008:AD MICA*008:AD
NTXS61	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*08	MICA*008:AD MICA*008:AD
NTXS62	MICA*008:AD MICA*011 MICA*011 MICA*080 MICA*011 MICA*082 MICA*011 MICA*085 AD:=:01/04	HLA-B*07 HLA-B*14	MICA*008:AD MICA*011
NTXS63	MICA*018:01 MICA*027 MICA*018:01 MICA*048	HLA-B*18 HLA-B*40	MICA*018:01 MICA*027
NTXS64	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	HLA-B*40 HLA-B*57	MICA*008:AD MICA*008:AD
NTXS65	MICA*004 MICA*018:01		MICA*004 MICA*018:01

NTXS66	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03		
NTXS67	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	HLA-B*14 HLA-B*35	MICA*002:AC MICA*011
NTXS68	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	HLA-B*39 HLA-B*44	MICA*002:AC MICA*004
NTXS69	MICA*008:AD MICA*008:02 MICA*008:02 MICA*080 MICA*008:02 MICA*082 MICA*008:02 MICA*085 AD:=:01/04		
NTXS70	MICA*009:01 MICA*017 MICA*017 MICA*049	HLA-B*50 HLA-B*03	MICA*009:01 MICA*017
NTXS71	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04		
NTXS72	MICA*002:AC MICA*019:AB MICA*019:AB MICA*020 MICA*019:AB MICA*055 MICA*019:AB MICA*081 AC:=:01/03 AB:=:01/02		
NTXS73	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	HLA-B*HLA-B*	MICA*002:AC MICA*002:AC
NTXS74	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	HLA-B*13 HLA-B*44	MICA*008:AD MICA*009:01
NTXS75	MICA*004 MICA*004	-B*48:03 HLA-B*49	MICA*004 MICA*004
NTXS79	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04		
NTXS80	MICA*001 MICA*010:01 MICA*001 MICA*069	HLA-B*15 HLA-B*18	MICA*001 MICA*010:01
NTXS81	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	HLA-B*39 HLA-B*67	MICA*001 MICA*010:01
NTXS82	MICA*002:AC MICA*007:01 MICA*002:AC MICA*026 MICA*002:AC MICA*079 MICA*007:01 MICA*020 MICA*007:01 MICA*055 MICA*007:01 MICA*081 MICA*020 MICA*026 MICA*020 MICA*079 MICA*026 MICA*055 MICA*026 MICA*081 MICA*055 MICA*079 MICA*079 MICA*081 AC:=:01/03	HLA-B*35 HLA-B*44	MICA*002:AC MICA*007:01

7.6.0 APPENDIX 6: SOLVING MICA AMBIGUITIES USING SBT

Amostras	MICA resultados	Primers a ser utilizados
trasplantados		
TXR1R01	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
TXR1R02	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R03	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R04	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R05	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R06	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
TXR1R07	MICA*011 MICA*018:01	OK
TXR1R08	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R09	MICA*009:01 MICA*012:01 MICA*012:01 MICA*049	DIFERENçAS NO EXON 6
TXR1R10	MICA*010:01 MICA*027 MICA*010:01 MICA*048 MICA*027 MICA*069 MICA*048 MICA*069	MICA-2R305 MICA-3F557 MICA-5F915
TXR1R11	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/0	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R12	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R13	MICA*009:01 MICA*045 MICA*045 MICA*049	DIFERENçAS NO EXON 6
TXR1R14*	MICA*002:AC MICA*010:01 MICA*002:AC MICA*069 MICA*010:01 MICA*020 MICA*010:01 MICA*055 MICA*010:01 MICA*068 MICA*010:01 MICA*072 MICA*010:01 MICA*075 MICA*020 MICA*069 MICA*055 MICA*069 MICA*068 MICA*069 MICA*069 MICA*072 MICA*069 MICA*075 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915

TXR1R15	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R16	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R17	MICA*008:AD MICA*016 MICA*016 MICA*080 MICA*016 MICA*082 MICA*016 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
TXR1R18	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
TXR1R19	MICA*009:01 MICA*016 MICA*016 MICA*049	DIFERENçAS NO EXON 6
TXR1R20	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R21	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	DIFERENçAS NO EXON 6
TXR1R22	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R23	MICA*018:01 MICA*019:AB AB:=:01/02	ok
TXR1R24	MICA*011 MICA*011 MICA*011 MICA*030	MICA-3R553

TXR1R25	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R26	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R27	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
TXR1R28	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R29	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915

TXR1R30	MICA*008:MV MICA*008:MV MICA*008:MV MICA*070 MICA*008:MV MICA*073 MICA*008:MV MICA*080 MICA*008:MV MICA*082 MICA*070 MICA*073 MICA*070 MICA*080 MICA*070 MICA*082 MICA*073 MICA*080 MICA*073 MICA*082 MV:=:01/04/05	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779
TXR1R31	MICA*001 MICA*004	ok
TXR1R32	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/0	MICA-3F557 MICA-4R759 MICA-4F779
TXR1R33	MICA*002:AC MICA*027 MICA*002:AC MICA*048 MICA*020 MICA*027 MICA*020 MICA*048 MICA*027 MICA*055 MICA*027 MICA*081 MICA*048 MICA*055 MICA*048 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R34	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R35	MICA*010:01 MICA*012:01 MICA*012:01 MICA*069	DIFERENçAS NO EXON 6
TXR1R36	MICA*012:01 MICA*019:AB AB:=:01/02	ok
TXR1R37	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R38	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915

TXR1R39	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R40	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R41	MICA*004 MICA*004	ok
TXR1R42	MICA*010:01 MICA*011 MICA*011 MICA*069	DIFERENçAS NO EXON 6
TXR1R43	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R44	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R45	MICA*007:02 MICA*012:01	ok
TXR1R46	repetir	purificar o DNA

TXR1R47	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R48	MICA*004 MICA*016	ok
TXR1R49	MICA*001 MICA*027 MICA*001 MICA*048 MICA*001 MICA*080 MICA*001 MICA*082	MICA-3F557 MICA-4F779 MICA-5F915
TXR1R50	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R51	MICA*001 MICA*010:01 MICA*001 MICA*069	DIFERENçAS NO EXON 6
TXR1R52	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R53	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R54*	MICA*008:AD MICA*016 MICA*016 MICA*070 MICA*016 MICA*073 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779
TXR1R55	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*008:AD MICA*074 MICA*009:01 MICA*070 MICA*009:01 MICA*073 MICA*049 MICA*070 MICA*049 MICA*073 MICA*070 MICA*074 MICA*073 MICA*074 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R56	MICA*008:AD MICA*018:01 MICA*018:01 MICA*080 MICA*018:01 MICA*082 MICA*018:01 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779

TXR1R57	MICA*002:AC MICA*018:01 MICA*018:01 MICA*020 MICA*018:01 MICA*055 MICA*018:01 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R58	MICA*002:AC MICA*016 MICA*016 MICA*020 MICA*016 MICA*055 MICA*016 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R59	MICA*009:01 MICA*018:01 MICA*018:01 MICA*049 MICA*018:01 MICA*07	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R60	MICA*008:AD MICA*011 AD:=:01/04	ok
TXR1R61	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*068 MICA*011 MICA*072 MICA*011 MICA*075 MICA*011 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R62	MICA*019 MICA*056 MICA*056 MICA*056	MICA-2R305
TXR1R63	MICA*002:AC MICA*012:01 MICA*012:01 MICA*020 MICA*012:01 MICA*055 MICA*012:01 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R64	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
TXR1R65	MICA*009:01 MICA*012:03 MICA*012:03 MICA*049 MICA*012:03 MICA*074	MICA-3R553
TXR1R66	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXR1R67	MICA*009:01 MICA*027 MICA*009:01 MICA*048 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*027 MICA*049 MICA*027 MICA*074 MICA*048 MICA*049 MICA*048 MICA*074 MICA*049 MICA*080 MICA*049 MICA*082 MICA*074 MICA*080 MICA*074 MICA*082	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
doadores		

TXD01	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*070 MICA*010:01 MICA*073 MICA*069 MICA*070 MICA*069 MICA*073 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXD03	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*009:01 MICA*074 MICA*049 MICA*049 MICA*049 MICA*074	MICA-3R553
TXD04	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*008:AD MICA*074 MICA*009:01 MICA*070 MICA*009:01 MICA*073 MICA*049 MICA*070 MICA*049 MICA*073 MICA*070 MICA*074 MICA*073 MICA*074 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXD07	MICA*008:AD MICA*018:01 MICA*018:01 MICA*073 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779
TXD08	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXD15	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*002:AC MICA*074 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*068 MICA*009:01 MICA*072 MICA*009:01 MICA*075 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*020 MICA*074 MICA*049 MICA*055 MICA*049 MICA*068 MICA*049 MICA*072 MICA*049 MICA*075 MICA*049 MICA*081 MICA*055 MICA*074 MICA*068 MICA*074 MICA*072 MICA*074 MICA*074 MICA*075 MICA*074 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXD17	MICA*008:AD MICA*016 MICA*016 MICA*070 MICA*016 MICA*073 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779
TXD19	MICA*011 MICA*016	ok
TXD21	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*009:01 MICA*074 MICA*049 MICA*049 MICA*049 MICA*07	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXD23	MICA*008:AD MICA*019 MICA*019 MICA*070 MICA*019 MICA*073 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779
TXD24	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*068 MICA*004 MICA*072 MICA*004 MICA*075 MICA*004 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
TXRD28	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXRD29	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*070 MICA*010:01 MICA*073 MICA*069 MICA*070 MICA*069 MICA*073 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXRD34	MICA*004 MICA*011	ok
TXRD39	MICA*002:AC MICA*008:AD MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*068 MICA*008:AD MICA*072 MICA*008:AD MICA*075 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXD44	MICA*008:AD MICA*019 MICA*019 MICA*070 MICA*019 MICA*073 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779

TXRD47	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*068 MICA*002:AC MICA*072 MICA*002:AC MICA*075 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*068 MICA*020 MICA*072 MICA*020 MICA*075 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*068 MICA*055 MICA*072 MICA*055 MICA*075 MICA*055 MICA*081 MICA*068 MICA*068 MICA*068 MICA*072 MICA*068 MICA*075 MICA*068 MICA*081 MICA*072 MICA*072 MICA*072 MICA*075 MICA*072 MICA*081 MICA*075 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
TXRD51	MICA*001 MICA*001	ok
TXD53	MICA*008:AD MICA*011 AD:=:01/04	ok
TXD55	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*008:AD MICA*074 MICA*009:01 MICA*070 MICA*009:01 MICA*073 MICA*049 MICA*070 MICA*049 MICA*073 MICA*070 MICA*074 MICA*073 MICA*074 AD:=:01/0	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
cronicos		
NTXC01	MICA*004 MICA*017	ok
NTXC02	MICA*010:01 MICA*018:01 MICA*018:01 MICA*069	DIFERENçAS NO EXON 6
NTXC03	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXC04 *	PURIFICAR o DNA	DNA inviavel
NTXC05	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC06	MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXC07	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXC09	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC12	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXC14	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXC15	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXC16	MICA*027 MICA*027 MICA*027 MICA*048 MICA*048 MICA*048	MICA-5F915

NTXC19	MICA*010:01 MICA*010:01 MICA*010:01 MICA*019:AB MICA*010:01 MICA*069 MICA*019:AB MICA*069 MICA*069 MICA*069 AB:=:01/02	MICA-2R305
NTXC22	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXC24	MICA*004 MICA*010:01 MICA*004 MICA*069	DIFERENçAS NO EXON 6
NTXC25	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXC26	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	DIFERENçAS NO EXON 6
NTXC30	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC31	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXC32	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXC37	MICA*002:AC MICA*027 MICA*002:AC MICA*048 MICA*020 MICA*027 MICA*020 MICA*048 MICA*027 MICA*055 MICA*027 MICA*081 MICA*048 MICA*055 MICA*048 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC38	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	DIFERENçAS NO EXON 6
NTXC39	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC40	MICA*009:01 MICA*010:01 MICA*009:01 MICA*069 MICA*010:01 MICA*049 MICA*049 MICA*069	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXC41	MICA*004 MICA*009:01 MICA*004 MICA*049	DIFERENçAS NO EXON 6
NTXC43	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC44	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915

NTXC45	MICA*008:AD MICA*027 MICA*008:AD MICA*048 MICA*027 MICA*080 MICA*027 MICA*082 MICA*027 MICA*085 MICA*048 MICA*080 MICA*048 MICA*082 MICA*048 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC46	MICA*002:AC MICA*018:01 MICA*018:01 MICA*020 MICA*018:01 MICA*055 MICA*018:01 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXC47	MICA*006 MICA*008:AD MICA*006 MICA*080 MICA*006 MICA*082 MICA*006 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXC48	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXC49	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
saudaveis		
NTXS01	MICA*008:AD MICA*010:01 MICA*008:AD MICA*069 MICA*010:01 MICA*080 MICA*010:01 MICA*082 MICA*010:01 MICA*085 MICA*069 MICA*080 MICA*069 MICA*082 MICA*069 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS02	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779

NTXS03	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS04	MICA*016 MICA*027 MICA*016 MICA*048	MICA-5F915
NTXS05	MICA*004 MICA*004	ok
NTXS06	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS07	MICA*010:01 MICA*017 MICA*017 MICA*069	DIFERENçAS NO EXON 6
NTXS08	MICA*004 MICA*007:01 MICA*004 MICA*026 MICA*004 MICA*079	MICA-4F779 MICA-5F915
NTXS09	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS10	MICA*009:01 MICA*018:01 MICA*018:01 MICA*049	DIFERENçAS NO EXON 6
NTXS11	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS12	MICA*007:AB MICA*018:AB MICA*018:01 MICA*079 AB:=:01/02	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779
NTXS13	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS14	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915

NTXS15	MICA*008:AD MICA*016 MICA*016 MICA*080 MICA*016 MICA*082 MICA*016 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS16	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS17	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS18	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS19	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	DIFERENçAS NO EXON 6
NTXS20	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS21	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS22	MICA*004 MICA*004	ok
NTXS23	MICA*002:AC MICA*019:AB MICA*019:AB MICA*020 MICA*019:AB MICA*055 MICA*019:AB MICA*081 AC:=:01/03 AB:=:01/02	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915

NTXS24	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS25	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS26	MICA*001 MICA*011	ok
NTXS27	MICA*002:AC MICA*007:01 MICA*002:AC MICA*026 MICA*002:AC MICA*079 MICA*007:01 MICA*020 MICA*007:01 MICA*055 MICA*007:01 MICA*081 MICA*020 MICA*026 MICA*020 MICA*079 MICA*026 MICA*055 MICA*026 MICA*081 MICA*055 MICA*079 MICA*079 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS28	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS29	MICA*002:AC MICA*010:01 MICA*002:AC MICA*069 MICA*010:01 MICA*020 MICA*010:01 MICA*055 MICA*010:01 MICA*081 MICA*020 MICA*069 MICA*055 MICA*069 MICA*069 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS30	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS31	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915

NTXS32	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS33	MICA*002:AC MICA*017 MICA*017 MICA*020 MICA*017 MICA*055 MICA*017 MICA*068 MICA*017 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS34	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS35	MICA*004 MICA*011	ok
NTXS36	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS37	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS38	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS39	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS40	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS41	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS42	MICA*008:AD MICA*018:01 MICA*018:01 MICA*080 MICA*018:01 MICA*082 MICA*018:01 MICA*085 AD:=:01/04	MICA-2R305 MICA-3F557 MICA-4R759 MICA-4F779
NTXS43	MICA*001 MICA*009:01 MICA*001 MICA*049	DIFERENçAS NO EXON 6
NTXS44	MICA*009:01 MICA*011 MICA*011 MICA*049	DIFERENçAS NO EXON 6
NTXS45	MICA*007:01 MICA*009:01 MICA*007:01 MICA*049 MICA*009:01 MICA*026 MICA*009:01 MICA*079 MICA*026 MICA*049 MICA*049 MICA*079	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS46	MICA*009:01 MICA*018:01 MICA*018:01 MICA*049	DIFERENçAS NO EXON 6
NTXS47	MICA*009:01 MICA*009:01 MICA*009:01 MICA*049 MICA*049 MICA*049	DIFERENçAS NO EXON 6
NTXS48	MICA*002:AC MICA*009:01 MICA*002:AC MICA*049 MICA*009:01 MICA*020 MICA*009:01 MICA*055 MICA*009:01 MICA*081 MICA*020 MICA*049 MICA*049 MICA*055 MICA*049 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS49	MICA*007:01 MICA*008:AD MICA*007:01 MICA*080 MICA*007:01 MICA*082 MICA*007:01 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS50	MICA*027 MICA*047 MICA*047 MICA*048	MICA-5F915
NTXS51	MICA*008:AD MICA*011 MICA*011 MICA*080 MICA*011 MICA*082 MICA*011 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS52	MICA*001 MICA*010:01 MICA*001 MICA*069	DIFERENçAS NO EXON 6

NTXS53	MICA*002:AC MICA*018:01 MICA*018:01 MICA*020 MICA*018:01 MICA*055 MICA*018:01 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS54	MICA*011 MICA*012:01	ok
NTXS55	MICA*002:AC MICA*027 MICA*002:AC MICA*048 MICA*020 MICA*027 MICA*020 MICA*048 MICA*027 MICA*055 MICA*027 MICA*081 MICA*048 MICA*055 MICA*048 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS56	MICA*004 MICA*009:01 MICA*004 MICA*049	DIFERENçAS NO EXON 6
NTXS57	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS58	MICA*007:01 MICA*009:01 MICA*007:01 MICA*049 MICA*009:01 MICA*026 MICA*009:01 MICA*079 MICA*026 MICA*049 MICA*049 MICA*079	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS59	MICA*002:AC MICA*008:AD MICA*002:AC MICA*080 MICA*002:AC MICA*082 MICA*002:AC MICA*085 MICA*008:AD MICA*020 MICA*008:AD MICA*055 MICA*008:AD MICA*081 MICA*020 MICA*080 MICA*020 MICA*082 MICA*020 MICA*085 MICA*055 MICA*080 MICA*055 MICA*082 MICA*055 MICA*085 MICA*080 MICA*081 MICA*081 MICA*082 MICA*081 MICA*085 AC:=:01/03 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS60	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779

NTXS61	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS62	MICA*008:AD MICA*011 MICA*011 MICA*080 MICA*011 MICA*082 MICA*011 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS63	MICA*018:01 MICA*027 MICA*018:01 MICA*048	MICA-5F915
NTXS64	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS65	MICA*004 MICA*018:01	ok
NTXS66	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS67	MICA*002:AC MICA*011 MICA*011 MICA*020 MICA*011 MICA*055 MICA*011 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS68	MICA*002:AC MICA*004 MICA*004 MICA*020 MICA*004 MICA*055 MICA*004 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS69	MICA*008:AD MICA*008:02 MICA*008:02 MICA*080 MICA*008:02 MICA*082 MICA*008:02 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS70	MICA*009:01 MICA*017 MICA*017 MICA*049	DIFERENçAS NO EXON 6
NTXS71	MICA*008:AD MICA*017 MICA*008:AD MICA*083 MICA*017 MICA*080 MICA*017 MICA*082 MICA*017 MICA*085 MICA*080 MICA*083 MICA*082 MICA*083 MICA*083 MICA*085 AD:=:01/04	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915

NTXS72	MICA*002:AC MICA*019:AB MICA*019:AB MICA*020 MICA*019:AB MICA*055 MICA*019:AB MICA*081 AC:=:01/03 AB:=:01/02	MICA-2R305 MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS73	MICA*002:AC MICA*002:AC MICA*002:AC MICA*020 MICA*002:AC MICA*055 MICA*002:AC MICA*081 MICA*020 MICA*020 MICA*020 MICA*055 MICA*020 MICA*081 MICA*055 MICA*055 MICA*055 MICA*081 MICA*081 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915
NTXS74	MICA*008:AD MICA*009:01 MICA*008:AD MICA*049 MICA*009:01 MICA*080 MICA*009:01 MICA*082 MICA*009:01 MICA*085 MICA*049 MICA*080 MICA*049 MICA*082 MICA*049 MICA*085 AD:=:01/04	MICA-3R553 MICA-3F557 MICA-4R759 MICA-4F779 MICA-5F915
NTXS75	MICA*004 MICA*004	ok
NTXS79	MICA*008:AD MICA*008:AD MICA*008:AD MICA*080 MICA*008:AD MICA*082 MICA*008:AD MICA*085 MICA*080 MICA*080 MICA*080 MICA*082 MICA*080 MICA*085 MICA*082 MICA*082 MICA*082 MICA*085 MICA*085 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS80	MICA*001 MICA*010:01 MICA*001 MICA*069	DIFERENçAS NO EXON 6
NTXS81	MICA*004 MICA*008:AD MICA*004 MICA*080 MICA*004 MICA*082 MICA*004 MICA*085 AD:=:01/04	MICA-3F557 MICA-4R759 MICA-4F779
NTXS82	MICA*002:AC MICA*007:01 MICA*002:AC MICA*026 MICA*002:AC MICA*079 MICA*007:01 MICA*020 MICA*007:01 MICA*055 MICA*007:01 MICA*081 MICA*020 MICA*026 MICA*020 MICA*079 MICA*026 MICA*055 MICA*026 MICA*081 MICA*055 MICA*079 MICA*079 MICA*081 AC:=:01/03	MICA-2R305 MICA-3R553 MICA-4R759 MICA-4F779 MICA-5F915

7.7.0 APPENDIX 7: CONGRESSES

Risk Score Model defines a new phenotype for kidney transplant genetic association

Vanessa Hauer*†, Matilde Risti*†, Geórgia F. Gelmini*, Bruna L.M. Miranda*, Fabiana C. Contieri ‡, Carolina M. Pozzi‡, Maria da G. Bicalho*

* Laboratory of Immunogenetics and Histocompatibility-LIGH, Department of Genetics, Federal University of Paraná, Curitiba, Brazil

† Authors contributed equally to the work.

‡ Hospital Universitário Evangélico de Curitiba, Alameda Augusto Stellfeld, 1908 Curitiba, 80.730-150, Brazil

Increasing the success rate of transplants avoiding rejections is still a challenge and many factors influence patients' prognostic. The exploration of MICA and HLA-G, two non-classical MHC-I molecule, as biomarkers can be useful in predicting the transplant outcome. Their expression exhibits a tissue and cell specific regulation that may interplays a relevant role in immunoregulation. Generally HLA-G has been associated to a immunosupression response, while MICA with a stress imune response. Thus, we analyzed *MICA* and *HLA-G* genotypes and quantified their soluble isoforms (sMICA and sHLA-G). The sample was composed by a total of 67 patients undergoing kidney transplantation at the Kidney Transplant Sector of the Hospital Universitário Evangélico do Paraná (Brazil), 32 kidney chronic disease patients and 73 control subjects. A total of 48 variables were obtained for transplanted patients from pre-transplant to up to three months after transplants. sHLA-G and sMICA were measured by ELISA, while *MICA* and *HLA-G* genotyping was performed by PCR–SSOP and SBT (sequence base typing), respectively. We identified a few main variables in order to create a Risk Score Model to discriminate patients with higher and lower risk of developing rejection: total DSA, DSA against selected donor, previous transplant, previous transfusion, being a multiparous woman, ATG induction therapy. We found a strong association between the Risk Model and patients with presumed rejection. Therefore, this model shows a potential to discriminate patients with greater or lesser chance of developing episodes of rejection (immunological risk). The *MICA-129 Met/Met* genotype was associated with a better prognosis in graft acceptance. The quantitative data of sMICA shows it as possible biomarker. The levels of sMICA was increased in high risk patients while the opposite was true in low risk patients. Regarding HLA-G, we confirmed a strong association between the *HLA-G*01:01P/G*01:04P* genotype and individuals producing higher sHLA-G, this suggest a probable dominance of *HLA-G*01:04* in *HLA-G *01:01P*. Low risk patients may have this sHLA-G phenotype and genotype association, however more analysis are necessary to prove our findings.

References:

.

ABBAS A.K, B.L., LICHTMAN A.H., SHIV PILLAI. Cell and Molecular Immunology (7th ed.). Philadelphia: Elsevier Saunders. 2012.

ADAMS, E.J., CHIEN, Y.H. & GARCIA, K.C. Structure of a gammadelta T cell receptor in complex with the nonclassical MHC T22. Science, 308(5719), 227-231. 2005.

ADRIAN CABESTRE, F., MOREAU, P., RITEAU, B., IBRAHIM, E.C., LE DANFF, C., DAUSSET, J., ROUAS-FREISS, N., CAROSELLA, E.D. & PAUL, P. HLA-G expression in human melanoma cells: protection from NK cytolysis. J Reprod Immunol, 43(2), 183-193. 1999.

AMROUN, H., DJOUDI, H., BUSSON, M., ALLAT, R., EL SHERBINI, S.M., SLOMA, I., RAMASAWMY, R., BRUN, M., DULPHY, N., KRISHNAMOORTHY, R., TOUBERT, A., CHARRON, D., ABBADI, M.C. & TAMOUZA, R. Early-onset ankylosing spondylitis is associated with a functional MICA polymorphism. Hum Immunol, 66, 1057-1061. 2005.

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. Hum Immunol, 71(6), 560-565. 2010.

ANTOUN, A., JOBSON, S., COOK, M., O'CALLAGHAN, C.A., MOSS, P. & BRIGGS, D.C. Single nucleotide polymorphism analysis of the NKG2D ligand cluster on the long arm of chromosome 6: Extensive polymorphisms and evidence of diversity between human populations. Hum Immunol, 71(6), 610-620. 2010.

AQUINO-GALVEZ, A., PEREZ-RODRIGUEZ, M., CAMARENA, A., FALFAN-VALENCIA, R., RUIZ, V., MONTANO, M., BARRERA, L., SADA-OVALLE, I., RAMIREZ, R., GRANADOS, J., PARDO, A. & SELMAN, M. MICA polymorphisms and decreased expression of the MICA receptor NKG2D contribute to idiopathic pulmonary fibrosis susceptibility. Hum Genet, 125(5-6), 639-648. 2009.

ARNAIZ-VILLENA, A., MARTINEZ-LASO, J., CORELL, A., ALLENDE, L., ROSAL, M., GOMEZ-REINO, J.J. & VICARIO, J.L. Frequencies of HLA-A24 and HLA-DR4-DQ8 are increased and that of HLA-B blank is decreased in chronic toxic oil syndrome. Eur J Immunogenet, 23(3), 211-219. 1996.

ASHIRU, O., BOUTET, P., FERNANDEZ-MESSINA, L., AGUERA-GONZALEZ, S., SKEPPER, J.N., VALES-GOMEZ, M. & REYBURN, H.T. Natural killer cell cytotoxicity is suppressed by exposure to the human NKG2D ligand MICA*008 that is shed by tumor cells in exosomes. Cancer Res, 70(2), 481-489. 2010.

ASHIRU, O., LOPEZ-COBO, S., FERNANDEZ-MESSINA, L., PONTES-QUERO, S., PANDOLFI, R., REYBURN, H.T. & VALES-GOMEZ, M. A GPI anchor explains the unique biological features of the common NKG2D-ligand allele MICA*008. Biochem J, 454(2), 295-302. 2013.

AUCHINCLOSS, H., JR. & SULTAN, H. Antigen processing and presentation in transplantation. Curr Opin Immunol, 8(5), 681-687. 1996.

BACON, L., EAGLE, R.A., MEYER, M., EASOM, N., YOUNG, N.T. & TROWSDALE, J. Two human ULBP/RAET1 molecules with transmembrane regions are ligands for NKG2D. J Immunol, 173(2), 1078-1084. 2004.

BAEK, I.C., JANG, J.P., CHOI, H.B., CHOI, E.J., KO, W.Y. & KIM, T.G. Microarrays for high-throughput genotyping of MICA alleles using allele-specific primer extension. Tissue Antigens, 82(4), 259-268. 2013.

BAHRAM, S. MIC genes: from genetics to biology. Adv Immunol, 76, 1-60. 2000.

BAHRAM, S., BRESNAHAN, M., GERAGHTY, D.E. & SPIES, T. A second lineage of mammalian major histocompatibility complex class I genes. Proc Natl Acad Sci U S A, 91(14), 6259-6263. 1994.

BAHRAM, S., MIZUKI, N., INOKO, H. & SPIES, T. Nucleotide sequence of the human MHC class I MICA gene. Immunogenetics, 44(1), 80-81. 1996.

BARGOSTAVAN, M.H., ESLAMI, G., ESFANDIARI, N. & SHAMS SHAHEMABADI, A. MMP9 Promoter Polymorphism (-1562 C/T) Does not Affect the Serum Levels of Soluble MICB and MICA in Breast Cancer. Iran J Immunol, 13(1), 45-53. 2016.

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, 285(5428), 727-729. 1999.

BILBAO, J.R., MARTIN-PAGOLA, A., VITORIA, J.C., ZUBILLAGA, P., ORTIZ, L. & CASTANO, L. HLA-DRB1 and MHC class 1 chain-related A haplotypes in Basque families with celiac disease. Tissue Antigens, 60(1), 71-76. 2002.

BILLINGHAM, R.E., BRENT, L. & MEDAWAR, P.B. Actively acquired tolerance of foreign cells. Nature, 172(4379), 603-606. 1953.

BJORKMAN, P.J., SAPER, M.A., SAMRAOUI, B., BENNETT, W.S., STROMINGER, J.L. & WILEY, D.C. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature, 329(6139), 512-518. 1987.

BLOOM, D., CHANG, Z., PAULY, K., KWUN, J., FECHNER, J., HAYES, C., SAMANIEGO, M. & KNECHTLE, S. BAFF is increased in renal transplant patients following treatment with alemtuzumab. Am J Transplant, 9(8), 1835-1845. 2009.

BOUKOUACI, W., AL-DACCAK, R., DULPHY, N., LAUDEN, L., AMOKRANE, K., FORTIER, C., MARZAIS, F., BENNABI, M., PEFFAULT DE LATOUR, R., SOCIE, G., TOUBERT, A., CHARRON, D., KRISHNAMOORTHY, R. & TAMOUZA, R. Soluble MICA-NKG2D interaction upregulates IFN-gamma production by activated CD3-CD56+ NK cells: potential impact on chronic graft versus host disease. Hum Immunol, 74(12), 1536-1541. 2013.

BOUKOUACI, W., BUSSON, M., PEFFAULT DE LATOUR, R., ROCHA, V., SUBERBIELLE, C., BENGOUFA, D., DULPHY, N., HAAS, P., SCIEUX, C., AMROUN, H., GLUCKMAN, E., KRISHNAMOORTHY, R., TOUBERT, A., CHARRON, D., SOCIE, G. & TAMOUZA, R. MICA-129 genotype, soluble MICA, and anti-MICA antibodies as biomarkers of chronic graft-versus-host disease. Blood, 114(25), 5216-5224. 2009.

BURGESS, S.J., MARUSINA, A.I., PATHMANATHAN, I., BORREGO, F. & COLIGAN, J.E. IL-21 down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells. J Immunol, 176(3), 1490-1497. 2006.

CAMODECA, C., NUTI, E., TEPSHI, L., BOERO, S., TUCCINARDI, T., STURA, E.A., POGGI, A., ZOCCHI, M.R. & ROSSELLO, A. Discovery of a new selective inhibitor of A Disintegrin And Metalloprotease 10 (ADAM-10) able to reduce the shedding of NKG2D ligands in Hodgkin's lymphoma cell models. Eur J Med Chem, 111, 193-201. 2016.

CAROSELLA, E.D., DAUSSET, J. & ROUAS-FREISS, N. Immunotolerant functions of HLA-G. Cell Mol Life Sci, 55(3), 327-333. 1999.

CAROSELLA, E.D., GREGORI, S. & LEMAOULT, J. The tolerogenic interplay(s) among HLA-G, myeloid APCs, and regulatory cells. Blood, 118(25), 6499-6505. 2011.

CAROSELLA, E.D., ROUAS-FREISS, N., TRONIK-LE ROUX, D., MOREAU, P. & LEMAOULT, J. HLA-G: An Immune Checkpoint Molecule. Adv Immunol, 127, 33-144. 2015.

CARREL, A. The Operative Technique of Vascular Anastomoses and the Transplantation of Viscera. Clinical Orthopaedics and Related Research, 29(98), 859. 1963.

CASTELLI, E.C., RAMALHO, J., PORTO, I.O., LIMA, T.H., FELICIO, L.P., SABBAGH, A., DONADI, E.A. & MENDES-JUNIOR, C.T. Insights into HLA-G Genetics Provided by Worldwide Haplotype Diversity. Front Immunol, 5, 476. 2014.

CHANG, Y.T., TSAI, S.F., LEE, D.D., SHIAO, Y.M., HUANG, C.Y., LIU, H.N., WANG, W.J. & WONG, C.K. A study of candidate genes for psoriasis near HLA-C in Chinese patients with psoriasis. Br J Dermatol, 148(3), 418-423. 2003.

CHAUVEAU, A., TONNERRE, P., PABOIS, A., GAVLOVSKY, P.J., CHATELAIS, M., COUPEL, S. & CHARREAU, B. Endothelial cell activation and proliferation modulate NKG2D activity by regulating MICA expression and shedding. J Innate Immun, 6(1), 89-104. 2014.

CHEN, A., SHEN, Y., XIA, M., XU, L., PAN, N., YIN, Y., MIAO, F., SHEN, C., XIE, W. & ZHANG, J. Expression of the nonclassical HLA class Iand MICA/B molecules in human hepatocellular carcinoma. Neoplasma, 58(5), 371-376. 2011.

CHEN, D., JUKO-PECIREP, I., HAMMER, J., IVANSSON, E., ENROTH, S., GUSTAVSSON, I., FEUK, L., MAGNUSSON, P.K., MCKAY, J.D., WILANDER, E. & GYLLENSTEN, U. Genome-wide association study of susceptibility loci for cervical cancer. J Natl Cancer Inst, 105(9), 624-633. 2013.

CHEN, H., ZHIDAN, W., XIA, R., ZHAOXIA, W., QING, J., QIANG, G., HAIPENG, Y. & HENGXIAO, W. Scorpion venom activates natural killer cells in hepatocellular carcinoma via the NKG2D-MICA pathway. Int Immunopharmacol, 35, 307-314. 2016.

CHEN, Y., LIN, W.S., ZHU, W.F., LIN, J., ZHOU, Z.F., HUANG, C.Z., CHEN, G., SHI, Y., GUO, Z.Q. & YE, Y.B. Tumor MICA status predicts the efficacy of immunotherapy with cytokine-induced killer cells for patients with gastric cancer. Immunol Res, 64(1), 251-259. 2016.

CHICK, L.R. Brief history and biology of skin grafting. Ann Plast Surg, 21(4), 358-365. 1988.

CHOI, H.B., HAN, H., YOUN, J.I., KIM, T.Y. & KIM, T.G. MICA 5.1 allele is a susceptibility marker for psoriasis in the Korean population. Tissue Antigens, 56, 548-550. 2000.

CHOY, M.K. & PHIPPS, M.E. MICA polymorphism: biology and importance in immunity and disease. Trends Mol Med, 16(3), 97-106. 2010.

CLEMENTS, C.S., KJER-NIELSEN, L., KOSTENKO, L., HOARE, H.L., DUNSTONE, M.A., MOSES, E., FREED, K., BROOKS, A.G., ROSSJOHN, J. & MCCLUSKEY, J. Crystal structure of HLA-G: a nonclassical MHC class I molecule expressed at the fetal-maternal interface. Proc Natl Acad Sci U S A, 102(9), 3360-3365. 2005.

CODO, P., WELLER, M., MEISTER, G., SZABO, E., STEINLE, A., WOLTER, M., REIFENBERGER, G. & ROTH, P. MicroRNA-mediated down-regulation of NKG2D ligands contributes to glioma immune escape. Oncotarget, 5(17), 7651-7662. 2014.

COLLINS, R.W. Human MHC class I chain related (MIC) genes: their biological function and relevance to disease and transplantation. Eur J Immunogenet, 31(3), 105-114. 2004.

COUPEL, S., MOREAU, A., HAMIDOU, M., HOREJSI, V., SOULILLOU, J.P. & CHARREAU, B. Expression and release of soluble HLA-E is an immunoregulatory feature of endothelial cell activation. Blood, 109(7), 2806-2814. 2007.

COX, S.T., MADRIGAL, J.A. & SAUDEMONT, A. Diversity and characterization of polymorphic 5' promoter haplotypes of MICA and MICB genes. Tissue Antigens, 84(3), 293-303. 2014.

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. Hum Immunol, 72(10), 827-834. 2011.

CRANE, C.A., HAN, S.J., BARRY, J.J., AHN, B.J., LANIER, L.L. & PARSA, A.T. TGF-beta downregulates the activating receptor NKG2D on NK cells and CD8+ T cells in glioma patients. Neuro Oncol, 12(1), 7-13. 2010.

CURRY, M.P. Hepatitis B and hepatitis C viruses in liver transplantation. Transplantation, 78(7), 955-963. 2004.
DE VRIES, R.R., MEERA KHAN, P., BERNINI, L.F., VAN LOGHEM, E. & VAN ROOD, J.J. Genetic control of survival in epidemics. J Immunogenet, 6(4), 271-287. 1979.
DELVES, P.J. & ROITT, I.M. The immune system. Second of two parts. N Engl J Med, 343(2), 108-117. 2000.
DIEFENBACH, A., TOMASELLO, E., LUCAS, M., JAMIESON, A.M., HSIA, J.K., VIVIER, E. & RAULET, D.H. Selective associations with signaling proteins determine stimulatory versus costimulatory activity of NKG2D. Nat Immunol, 3(12), 1142-1149. 2002.
DING, Y., XIA, B., LU, M., ZHANG, Y., LI, J., YE, M., LUO, H., YU, J., ZHANG, X. & TAN, J. MHC class I chain-related gene A-A5.1 allele is associated with ulcerative colitis in Chinese population. Clin Exp Immunol, 142(1), 193-198. 2005.
DONADI, E.A., CASTELLI, E.C., ARNAIZ-VILLENA, A., ROGER, M., REY, D. & MOREAU, P. Implications of the polymorphism of HLA-G on its function, regulation, evolution and disease association. Cell Mol Life Sci, 68(3), 369-395. 2011.
DOUIK, H., BEN CHAABEN, A., ATTIA ROMDHANE, N., ROMDHANE, H.B., MAMOGHLI, T., FORTIER, C., BOUKOUACI, W., HARZALLAH, L., GHANEM, A., GRITLI, S., MAKNI, M., CHARRON, D., KRISHNAMOORTHY, R., GUEMIRA, F. & TAMOUZA, R. Association of MICA-129 polymorphism with nasopharyngeal cancer risk in a Tunisian population. Hum Immunol, 70(1), 45-48. 2009.
ELEME, K., TANER, S.B., ONFELT, B., COLLINSON, L.M., MCCANN, F.E., CHALUPNY, N.J., COSMAN, D., HOPKINS, C., MAGEE, A.I. & DAVIS, D.M. Cell surface organization of stress-inducible proteins ULBP and MICA that stimulate human NK cells and T cells via NKG2D. J Exp Med, 199(7), 1005-1010. 2004.
ESPINOZA, J.L., TAKAMI, A., ONIZUKA, M., SAO, H., AKIYAMA, H., MIYAMURA, K., OKAMOTO, S., INOUE, M., KANDA, Y., OHTAKE, S., FUKUDA, T., MORISHIMA, Y., KODERA, Y. & NAKAO, S. NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies Haematologica (Vol. 94, pp. 1427-1434). Italy. 2009.
ESPINOZA, J.L., TAKAMI, A., ONIZUKA, M., SAO, H., AKIYAMA, H., MIYAMURA, K., OKAMOTO, S., INOUE, M., KANDA, Y., OHTAKE, S., FUKUDA, T., MORISHIMA, Y., KODERA, Y., NAKAO, S. & PROGRAM, J.M.D. NKG2D gene polymorphism has a significant impact on transplant outcomes after HLA-fully-matched unrelated bone marrow transplantation for standard risk hematologic malignancies. Haematologica, 94(10), 1427-1434. 2009.
ESTESO, G., LUZON, E., SARMIENTO, E., GOMEZ-CARO, R., STEINLE, A., MURPHY, G., CARBONE, J., VALES-GOMEZ, M. & REYBURN, H.T. Altered microRNA expression after infection with human cytomegalovirus leads to TIMP3 downregulation and increased shedding of metalloprotease substrates, including MICA. J Immunol, 193(3), 1344-1352. 2014.
FDEZ-MORERA, J.L., RODRIGO, L., LOPEZ-VAZQUEZ, A., RODERO, S.R., MARTINEZ-BORRA, J., NINO, P., GONZALEZ, S. & LOPEZ-LARREA, C. MHC class I chain-related gene A transmembrane polymorphism modulates the extension of ulcerative colitis. Hum Immunol, 64(8), 816-822. 2003.
FERNANDEZ-SANCHEZ, A., BARAGANO RANEROS, A., CARVAJAL PALAO, R., SANZ, A.B., ORTIZ, A., ORTEGA, F., SUAREZ-ALVAREZ, B. & LOPEZ-LARREA, C. DNA demethylation and histone H3K9 acetylation determine the active transcription of the NKG2D gene in human CD8+ T and NK cells. Epigenetics, 8(1), 66-78. 2013.

FERRARINI, M., FERRERO, E., DAGNA, L., POGGI, A. & ZOCCHI, M.R. Human gammadelta T cells: a nonredundant system in the immune-surveillance against cancer. Trends Immunol, 23(1), 14-18. 2002.

FODIL, N., LALOUX, L., WANNER, V., PELLET, P., HAUPTMANN, G., MIZUKI, N., INOKO, H., SPIES, T., THEODOROU, I. & BAHRAM, S. Allelic repertoire of the human MHC class I MICA gene. Immunogenetics, 44(5), 351-357. 1996.

FOJTIKOVA, M., NOVOTA, P., CEJKOVA, P., PESICKOVA, S., TEGZOVA, D. & CERNA, M. HLA class II, MICA and PRL gene polymorphisms: the common contribution to the systemic lupus erythematosus development in Czech population. Rheumatol Int, 31(9), 1195-1201. 2011.

FURUE, H., KUMIMOTO, H., MATSUO, K., SUZUKI, T., HASEGAWA, Y., SHINODA, M., SUGIMURA, T., MITSUDO, K., TOHNAI, I., UEDA, M., TAJIMA, K. & ISHIZAKI, K. Opposite impact of NKG2D genotype by lifestyle exposure to risk of aerodigestive tract cancer among Japanese. Int J Cancer, 123(1), 181-186. 2008.

FURUE, H., MATSUO, K., KUMIMOTO, H., HIRAKI, A., SUZUKI, T., YATABE, Y., KOMORI, K., KANEMITSU, Y., HIRAI, T., KATO, T., UEDA, M., ISHIZAKI, K. & TAJIMA, K. Decreased risk of colorectal cancer with the high natural killer cell activity NKG2D genotype in Japanese. Carcinogenesis, 29(2), 316-320. 2008.

GABER, L.W., GABER, A.O., VERA, S.R., BRAXTON, F. & HATHAWAY, D. Successful reversal of hyperacute renal allograft rejection with the anti-CD3 monoclonal OKT3. Transplantation, 54(5), 930-932. 1992.

GAMBELUNGHE, G., BROZZETTI, A., GHADERI, M., CANDELORO, P., TORTOIOLI, C. & FALORNI, A. MICA gene polymorphism in the pathogenesis of type 1 diabetes. Annals of the New York Academy of Sciences, 1110, 92-98. 2007.

GAMBELUNGHE, G., FALORNI, A., GHADERI, M., LAURETI, S., TORTOIOLI, C., SANTEUSANIO, F., BRUNETTI, P. & SANJEEVI, C.B. Microsatellite polymorphism of the MHC class I chain-related (MIC-A and MIC-B) genes marks the risk for autoimmune Addison's disease. Journal of Clinical Endocrinology and Metabolism, 84, 3701-3707. 1999.

GAMBELUNGHE, G., GERLI, R., BOCCI, E.B., DEL SINDACO, P., GHADERI, M., SANJEEVI, C.B., BISTONI, O., BINI, V. & FALORNI, A. Contribution of MHC class I chain-related A (MICA) gene polymorphism to genetic susceptibility for systemic lupus erythematosus. Rheumatology (Oxford), 44(3), 287-292. 2005.

GAO, X., SINGLE, R.M., KARACKI, P., MARTI, D., O'BRIEN, S.J. & CARRINGTON, M. Diversity of MICA and linkage disequilibrium with HLA-B in two North American populations. Hum Immunol, 67(3), 152-158. 2006.

GARRITY, D., CALL, M.E., FENG, J. & WUCHERPFENNIG, K.W. The activating NKG2D receptor assembles in the membrane with two signaling dimers into a hexameric structure. Proc Natl Acad Sci U S A, 102(21), 7641-7646. 2005.

GHADERI, M., HJELMSTRÖM, P., HALLMANS, G., WIKLUND, F., LENNER, P., DILLNER, J. & SANJEEVI, C.B. MICA gene polymorphism and the risk to develop cervical intraepithelial neoplasia. Him, 60, 970-973. 1999.

GILFILLAN, S., HO, E.L., CELLA, M., YOKOYAMA, W.M. & COLONNA, M. NKG2D recruits two distinct adapters to trigger NK cell activation and costimulation. Nat Immunol, 3(12), 1150-1155. 2002.

GLIENKE, J., SOBANOV, Y., BROSTJAN, C., STEFFENS, C., NGUYEN, C., LEHRACH, H., HOFER, E. & FRANCIS, F. The genomic organization of NKG2C, E, F, and D receptor genes in the human natural killer gene complex. Immunogenetics, 48(3), 163-173. 1998.

GOBIN, S.J. & VAN DEN ELSEN, P.J. Transcriptional regulation of the MHC class Ib genes HLA-E, HLA-F, and HLA-G. Hum Immunol, 61(11), 1102-1107. 2000.

GONEN-GROSS, T., GOLDMAN-WOHL, D., HUPPERTZ, B., LANKRY, D., GREENFIELD, C., NATANSON-YARON, S., HAMANI, Y., GILAD, R., YAGEL, S. & MANDELBOIM,

O. Inhibitory NK receptor recognition of HLA-G: regulation by contact residues and by cell specific expression at the fetal-maternal interface. PLoS One, 5(1), e8941. 2010.
GONZALEZ, S., BRAUTBAR, C., MARTINEZ-BORRA, J., LOPEZ-VAZQUEZ, A., SEGAL, R., BLANCO-GELAZ, M.A., ENK, C.D., SAFRIMAN, C. & LOPEZ-LARREA, C. Polymorphism in MICA rather than HLA-B/C genes is associated with psoriatic arthritis in the Jewish population. Hum Immunol, 62(6), 632-638. 2001.
GONZALEZ, S., LOPEZ-SOTO, A., SUAREZ-ALVAREZ, B., LOPEZ-VAZQUEZ, A. & LOPEZ-LARREA, C. NKG2D ligands: key targets of the immune response. Trends Immunol, 29(8), 397-403. 2008.
GONZALEZ, S., MARTINEZ-BORRA, J., TORRE-ALONSO, J.C., GONZALEZ-ROCES, S., SANCHEZ DEL RÍO, J., RODRIGUEZ PÉREZ, A., BRAUTBAR, C. & LÓPEZ-LARREA, C. The MICA-A9 triplet repeat polymorphism in the transmembrane region confers additional susceptibility to the development of psoriatic arthritis and is independent of the association of Cw*0602 in psoriasis. Arthritis Rheum, 42, 1010-1016. 1999.
GONZÁLEZ-ESCRIBANO, M.F., RODRÍGUEZ, M.R., AGUILAR, F., ALVAREZ, A., SANCHEZ-ROMAN, J. & NÚÑEZ-ROLDÁN, A. Lack of association of MICA transmembrane region polymorphism and Behçet's disease in Spain. Tissue Antigens, 54, 278-281. 1999.
GOODWIN, W.E., KAUFMAN, J.J., MIMS, M.M., TURNER, R.D., GLASSOCK, R., GOLDMAN, R. & MAXWELL, M.M. Human renal transplantation. I. Clinical experiences with six cases of renal homotransplantation. J Urol, 89, 13-24. 1963.
GOTO, K., OTA, M., OHNO, S., MIZUKI, N., ANDO, H., KATSUYAMA, Y., MAKSYMOWYCH, W.P., KIMURA, M., BAHRAM, S. & INOKO, H. MICA gene and ankylosing spondylitis: linkage analysis via a transmembrane-encoded triplet repeat polymorphism. Tissue Antigens, 49, 503-507. 1997.
GRIMSLEY, C., KAWASAKI, A., GASSNER, C., SAGESHIMA, N., NOSE, Y., HATAKE, K., GERAGHTY, D.E. & ISHITANI, A. Definitive high resolution typing of HLA-E allelic polymorphisms: Identifying potential errors in existing allele data. Tissue Antigens, 60(3), 206-212. 2002.
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. Proc Natl Acad Sci U S A, 93(22), 12445-12450. 1996.
GROH, V., BRUHL, A., EL-GABALAWY, H., NELSON, J.L. & SPIES, T. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. Proc Natl Acad Sci U S A, 100(16), 9452-9457. 2003.
GROH, V., RHINEHART, R., RANDOLPH-HABECKER, J., TOPP, M.S., RIDDELL, S.R. & SPIES, T. Costimulation of CD8alphabeta T cells by NKG2D via engagement by MIC induced on virus-infected cells. Nat Immunol, 2(3), 255-260. 2001.
GROH, V., RHINEHART, R., SECRIST, H., BAUER, S., GRABSTEIN, K.H. & SPIES, T. Broad tumor-associated expression and recognition by tumor-derived gamma delta T cells of MICA and MICB. Proc Natl Acad Sci U S A, 96(12), 6879-6884. 1999.
GROH, V., SMYTHE, K., DAI, Z. & SPIES, T. Fas-ligand-mediated paracrine T cell regulation by the receptor NKG2D in tumor immunity. Nat Immunol, 7(7), 755-762. 2006.
GUERRA, N., PESTAL, K., JUAREZ, T., BECK, J., TKACH, K., WANG, L. & RAULET, D.H. A selective role of NKG2D in inflammatory and autoimmune diseases. Clin Immunol, 149(3), 432-439. 2013.
HALL, B.M. Cells mediating allograft rejection. Transplantation, 51(6), 1141-1151. 1991.
HAYASHI, T., IMAI, K., MORISHITA, Y., HAYASHI, I., KUSUNOKI, Y. & NAKACHI, K. Identification of the NKG2D haplotypes associated with natural cytotoxic activity of peripheral blood lymphocytes and cancer immunosurveillance. Cancer Res, 66(1), 563-570. 2006.

HEEGER, P.S. & DINAVAHI, R. Transplant immunology for non-immunologist. Mt Sinai J Med, 79(3), 376-387. 2012.
HILL, R.B., DAHRLING, B.E., STARZL, T.E. & RIFKIND, D. Death after transplantation. The American Journal of Medicine, 42(3), 327-334. 1967.
HO, E.L., HEUSEL, J.W., BROWN, M.G., MATSUMOTO, K., SCALZO, A.A. & YOKOYAMA, W.M. Murine Nkg2d and Cd94 are clustered within the natural killer complex and are expressed independently in natural killer cells. Proc Natl Acad Sci U S A, 95(11), 6320-6325. 1998.
HORNG, T., BEZBRADICA, J.S. & MEDZHITOV, R. NKG2D signaling is coupled to the interleukin 15 receptor signaling pathway. Nat Immunol, 8(12), 1345-1352. 2007.
HOV, J.R., LLEO, A., SELMI, C., WOLDSETH, B., FABRIS, L., STRAZZABOSCO, M., KARLSEN, T.H. & INVERNIZZI, P. Genetic associations in Italian primary sclerosing cholangitis: Heterogeneity across Europe defines a critical role for HLA-C. Journal of Hepatology, 52, 712-717. 2010.
HOWANGYIN, K.Y., LOUSTAU, M., WU, J., ALEGRE, E., DAOUYA, M., CAUMARTIN, J., SOUSA, S., HORUZSKO, A., CAROSELLA, E.D. & LEMAOULT, J. Multimeric structures of HLA-G isoforms function through differential binding to LILRB receptors. Cell Mol Life Sci. 2012.
HUANG, Y., LEE, Y.J., CHEN, M.R., HSU, C.H., LIN, S.P., SUNG, T.C., CHANG, S.C. & CHANG, J.G. Polymorphism of transmembrane region of MICA gene and Kawasaki disease. Experimental and clinical immunogenetics, 17, 130-137. 2000.
HUERGO-ZAPICO, L., GONZALEZ-RODRIGUEZ, A.P., CONTESTI, J., GONZALEZ, E., LOPEZ-SOTO, A., FERNANDEZ-GUIZAN, A., ACEBES-HUERTA, A., DE LOS TOYOS, J.R., LOPEZ-LARREA, C., GROH, V., SPIES, T. & GONZALEZ, S. Expression of ERp5 and GRP78 on the membrane of chronic lymphocytic leukemia cells: association with soluble MICA shedding. Cancer Immunol Immunother, 61(8), 1201-1210. 2012.
HUGHES, E.H., COLLINS, R.W., KONDEATIS, E., WALLACE, G.R., GRAHAM, E.M., VAUGHAN, R.W. & STANFORD, M.R. Associations of major histocompatibility complex class I chain-related molecule polymorphisms with Behcet's disease in Caucasian patients. Tissue Antigens, 66(3), 195-199. 2005.
HVIID, T.V. HLA-G in human reproduction: aspects of genetics, function and pregnancy complications. Hum Reprod Update, 12(3), 209-232. 2006.
IMAI, K., HAYASHI, T., YAMAOKA, M., KAJIMURA, J., YOSHIDA, K., KUSUNOKI, Y. & NAKACHI, K. Effects of NKG2D haplotypes on the cell-surface expression of NKG2D protein on natural killer and CD8 T cells of peripheral blood among atomic-bomb survivors. Hum Immunol, 73(6), 686-691. 2012.
ISERNHAGEN, A., MALZAHN, D., BICKEBOLLER, H. & DRESSEL, R. Impact of the MICA-129Met/Val Dimorphism on NKG2D-Mediated Biological Functions and Disease Risks. Front Immunol, 7, 588. 2016.
ISERNHAGEN, A., MALZAHN, D., VIKTOROVA, E., ELSNER, L., MONECKE, S., VON BONIN, F., KILISCH, M., WERMUTH, J.M., WALTHER, N., BALAVARCA, Y., STAHL-HENNIG, C., ENGELKE, M., WALTER, L., BICKEBÖLLER, H., KUBE, D., WULF, G. & DRESSEL, R. The MICA-129 dimorphism affects NKG2D signaling and outcome of hematopoietic stem cell transplantation. EMBO Mol Med, 7(11), 1480-1502. 2015.
ISERNHAGEN, A., SCHILLING, D., MONECKE, S., SHAH, P., ELSNER, L., WALTER, L., MULTHOFF, G. & DRESSEL, R. The MICA-129Met/Val dimorphism affects plasma membrane expression and shedding of the NKG2D ligand MICA. Immunogenetics, 68(2), 109-123. 2016.
ITO, A., SHIMURA, H., NITAHARA, A., TOMIYAMA, K., ITO, M., KANEKURA, T., OKUMURA, K., YAGITA, H. & KAWAI, K. NK cells contribute to the skin graft rejection

promoted by CD4+ T cells activated through the indirect allorecognition pathway. Int Immunol, 20(10), 1343-1349. 2008.
JAMIESON, A.M., DIEFENBACH, A., MCMAHON, C.W., XIONG, N., CARLYLE, J.R. & RAULET, D.H. The role of the NKG2D immunoreceptor in immune cell activation and natural killing. Immunity, 17(1), 19-29. 2002.
JENSEN, H., FOLKERSEN, L. & SKOV, S. Regulation and gene expression profiling of NKG2D positive human cytomegalovirus-primed CD4+ T-cells. PLoS One, 7(8), e41577. 2012.
KABALAK, G., THOMAS, R.M., MARTIN, J., ORTEGO-CENTENO, N., JIMENEZ-ALONSO, J., DE RAMON, E., BUYNY, S., HAMSEN, S., GROSS, W.L., SCHNARR, S., ZEIDLER, H., GROMNICA-IHLE, E., SCHMIDT, R.E. & WITTE, T. Association of an NKG2D gene variant with systemic lupus erythematosus in two populations. Hum Immunol, 71(1), 74-78. 2010.
KAISER, B.K., YIM, D., CHOW, I.T., GONZALEZ, S., DAI, Z., MANN, H.H., STRONG, R.K., GROH, V. & SPIES, T. Disulphide-isomerase-enabled shedding of tumour-associated NKG2D ligands. Nature, 447(7143), 482-486. 2007.
KAMOUN, A., BOUZID, D., MAHFOUDH, N., AMOURI, A., GADDOUR, L., HAKIM, F., TAHRI, N., MASMOUDI, H. & MAKNI, H. Association study of MICA-TM polymorphism with inflammatory bowel disease in the South Tunisian population. Genet Test Mol Biomarkers, 17(8), 615-619. 2013.
KARIMI, M.A., AGUILAR, O.A., ZOU, B., BACHMANN, M.H., CARLYLE, J.R., BALDWIN, C.L. & KAMBAYASHI, T. A truncated human NKG2D splice isoform negatively regulates NKG2D-mediated function. J Immunol, 193(6), 2764-2771. 2014.
KATSUYAMA, Y., OTA, M., ANDO, H., SAITO, S., MIZUKI, N., KERA, J., BAHRAM, S., NOSE, Y. & INOKO, H. Sequencing based typing for genetic polymorphisms in exons, 2, 3 and 4 of the MICA gene. Tissue Antigens, 54(2), 178-184. 1999.
KAUFMANN, R.A., OBERHOLZER, P.A., CAZZANIGA, S. & HUNGER, R.E. Epithelial skin cancers after kidney transplantation: a retrospective single-centre study of 376 recipients. Eur J Dermatol. 2016.
KAWABATA, Y., IKEGAMI, H., KAWAGUCHI, Y., FUJISAWA, T., HOTTA, M., UEDA, H., SHINTANI, M., NOJIMA, K., ONO, M., NISHINO, M., TANIGUCHI, H., NOSO, S., YAMADA, K., BABAYA, N. & OGIHARA, T. Age-related association of MHC class I chain-related gene A (MICA) with type 1 (insulin-dependent) diabetes mellitus. Hum Immunol, 61, 624-629. 2000.
KIM, R., EMI, M. & TANABE, K. Cancer immunoediting from immune surveillance to immune escape. Immunology, 121(1), 1-14. 2007.
KIRSTEN, H., PETIT-TEIXEIRA, E., SCHOLZ, M., HASENCLEVER, D., HANTMANN, H., HEIDER, D., WAGNER, U., SACK, U., HUGO TEIXEIRA, V., PRUM, B., BURKHARDT, J., PIERLOT, C., EMMRICH, F., CORNELIS, F. & AHNERT, P. Association of MICA with rheumatoid arthritis independent of known HLA-DRB1 risk alleles in a family-based and a case control study. Arthritis Res Ther, 11(3), R60. 2009.
KISHIKAWA, T., OTSUKA, M., OHNO, M., YOSHIKAWA, T., SATO, M. & KOIKE, K. Development of a screening method to identify regulators of MICA shedding. Biochem Biophys Res Commun, 465(4), 764-768. 2015.
KLOESS, S., HUENECKE, S., PIECHULEK, D., ESSER, R., KOCH, J., BREHM, C., SOERENSEN, J., GARDLOWSKI, T., BRINKMANN, A., BADER, P., PASSWEG, J., KLINGEBIEL, T., SCHWABE, D. & KOEHL, U. IL-2-activated haploidentical NK cells restore NKG2D-mediated NK-cell cytotoxicity in neuroblastoma patients by scavenging of plasma MICA. Eur J Immunol, 40(11), 3255-3267. 2010.
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, 49(7-8), 620-628. 1999.
KOOPMANN, J.O., ALBRING, J., HÜTER, E., BULBUC, N., SPEE, P., NEEFJES, J., HÄMMERLING, G.J. & MOMBURG, F. Export of antigenic peptides from the endoplasmic reticulum intersects with retrograde protein translocation through the Sec61p channel. Immunity, 13(1), 117-127. 2000.
KUMAR, V., YI LO, P.H., SAWAI, H., KATO, N., TAKAHASHI, A., DENG, Z., URABE, Y., MBAREK, H., TOKUNAGA, K., TANAKA, Y., SUGIYAMA, M., MIZOKAMI, M., MUROYAMA, R., TATEISHI, R., OMATA, M., KOIKE, K., TANIKAWA, C., KAMATANI, N., KUBO, M., NAKAMURA, Y. & MATSUDA, K. Soluble MICA and a MICA variation as possible prognostic biomarkers for HBV-induced hepatocellular carcinoma. PLoS One, 7(9), e44743. 2012.
LAHIRI, D.K. & NURNBERGER, J.I., JR. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res, 19(19), 5444. 1991.
LAND, W. Innate alloimmunity: history and current knowledge. Exp Clin Transplant, 5(1), 575-584. 2007.
LE BOUTEILLER, P. & LENFANT, F. Antigen-presenting function(s) of the non-classical HLA-E, -F and -G class I molecules: the beginning of a story. Res Immunol, 147(5), 301-313. 1996.
LE CLERC, S., DELANEAU, O., COULONGES, C., SPADONI, J.L., LABIB, T., LAVILLE, V., ULVELING, D., NOIREL, J., MONTES, M., SCHACHTER, F., CAILLAT-ZUCMAN, S. & ZAGURY, J.F. Evidence after imputation for a role of MICA variants in nonprogression and elite control of HIV type 1 infection. J Infect Dis, 210(12), 1946-1950. 2014.
LEE, Y.J., HUANG, F.Y., WANG, C.H., LO, F.S., TSAN, K.W., HSU, C.H., HUANG, C.Y., CHANG, S.C. & CHANG, J.G. Polymorphism in the transmembrane region of the MICA gene and type 1 diabetes. J Pediatr Endocrinol Metab, 13(5), 489-496. 2000.
LEELAYUWAT, C., HOLLINGSWORTH, P., PUMMER, S., LERTMEMONGKOLCHAI, G., THOM, G., MULLBERG, J., WITT, C., KAUFMAN, J., DEGLI-ESPOSTI, M.A., COSMAN, D. & DAWKINS, R. Antibody reactivity profiles following immunization with diverse peptides of the PERB11 (MIC) family. Clin Exp Immunol, 106(3), 568-576. 1996.
LI, H., LIU, F., ZHU, H., ZHOU, X., LU, J., CHANG, H. & HU, J. Interaction Between Polymorphisms of IFN-gamma and MICA Correlated with Hepatocellular Carcinoma. Med Sci Monit, 22, 549-553. 2016.
LI, H., LIU, F., ZHU, H., ZHOU, X., LU, J., CHANG, H. & HU, J. Interaction Between Polymorphisms of IFN-γ and MICA Correlated with Hepatocellular Carcinoma. Med Sci Monit, 22, 549-553. 2016.
LI, J., CUI, L. & HE, W. Distinct pattern of human Vdelta1 gammadelta T cells recognizing MICA. Cell Mol Immunol, 2(4), 253-258. 2005.
LI, J., GOLDSTEIN, I., GLICKMAN-NIR, E., JIANG, H. & CHESS, L. Induction of TCR Vbeta-specific CD8+ CTLs by TCR Vbeta-derived peptides bound to HLA-E. J Immunol, 167(7), 3800-3808. 2001.
LI, P., MORRIS, D.L., WILLCOX, B.E., STEINLE, A., SPIES, T. & STRONG, R.K. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. Nat Immunol, 2(5), 443-451. 2001.
LI, P., WILLIE, S.T., BAUER, S., MORRIS, D.L., SPIES, T. & STRONG, R.K. Crystal structure of the MHC class I homolog MIC-A, a gammadelta T cell ligand. Immunity, 10(5), 577-584. 1999.
LIU, W. & LI, X.C. An overview on non-T cell pathways in transplant rejection and tolerance. Curr Opin Organ Transplant, 15(4), 422-426. 2010.
LOPEZ-HERNANDEZ, R., VALDES, M., LUCAS, D., CAMPILLO, J.A., MARTINEZ-GARCIA, P., SALAMA, H., LOPEZ, M., SALGADO, G., BOTELLA, C., MINGUELA, A., MIRAS,

M., ALVAREZ-LOPEZ, M.R., CARBALLO, F. & MURO, M. Association analysis of MICA gene polymorphism and MICA-129 dimorphism with inflammatory bowel disease susceptibility in a Spanish population. Hum Immunol, 71(5), 512-514. 2010.

LU, M., XIA, B., GE, L., LI, Y., ZHAO, J., CHEN, F., ZHOU, F., ZHANG, X. & TAN, J. Role of major histocompatibility complex class I-related molecules A*A5.1 allele in ulcerative colitis in Chinese patients. Immunology, 128(1 Suppl), e230-236. 2009.

LU, S.M., XIAO, P., XUE, L., CHE, L.H., YANG, P., LI, Y. & QIAO, H. Prevalent expression of MHC class I chain-related molecule A in human osteosarcoma. Neoplasma, 55(3), 266-272. 2008.

LUO, J., TIAN, W., PAN, F., LIU, X. & LI, L. Allelic and haplotypic diversity of 5'promoter region of the MICA gene. Hum Immunol, 75(4), 383-388. 2014.

LUO, L., LI, Z., WU, W., LUO, G., MEI, H., SUN, Z. & XU, C. The effect of MICA antigens on kidney transplantation outcomes. Immunol Lett, 156(1-2), 54-58. 2013.

LUO, L., LU, J., WEI, L., LONG, D., GUO, J.Y., SHAN, J., LI, F.S., LU, P.Y., LI, P.Y. & FENG, L. The role of HIF-1 in up-regulating MICA expression on human renal proximal tubular epithelial cells during hypoxia/reoxygenation. BMC Cell Biol, 11, 91. 2010.

MA, J., GUO, X., WU, X., LI, J., ZHU, X., LI, Z., LI, J., PAN, L., LI, T., LI, H. & LIU, Y. Association of NKG2D genetic polymorphism with susceptibility to chronic hepatitis B in a Han Chinese population. J Med Virol, 82(9), 1501-1507. 2010.

MALYSZKO, J. & MALYSZKO, J. Immune biomarkers: do they impact long-term graft survival? Pol Arch Intern Med, 127(3), 149-151. 2017.

MARIN, M.L., SAVIOLI, C.R., YAMAMOTO, J.H., KALIL, J. & GOLDBERG, A.C. MICA polymorphism in a sample of the São Paulo population, Brazil. Eur J Immunogenet, 31(2), 63-71. 2004.

MARTELLI-PALOMINO, G., PANCOTTO, J.A., MUNIZ, Y.C., MENDES-JUNIOR, C.T., CASTELLI, E.C., MASSARO, J.D., KRAWICE-RADANNE, I., PORAS, I., REBMANN, V., CAROSELLA, E.D., ROUAS-FREISS, N., MOREAU, P. & DONADI, E.A. Polymorphic Sites at the 3' Untranslated Region of the HLA-G Gene Are Associated with Differential hla-g Soluble Levels in the Brazilian and French Population. PLoS One, 8(10), e71742. 2013.

MARTINEZ, A., FERNANDEZ-ARQUERO, M., BALSA, A., RUBIO, A., ALVES, H., PASCUAL-SALCEDO, D., MARTIN-MOLA, E. & DE LA CONCHA, E.G. Primary association of a MICA allele with protection against rheumatoid arthritis. Arthritis Rheum, 44(6), 1261-1265. 2001.

MARTINEZ-CHAMORRO, A., MORENO, A., GÓMEZ-GARCÍA, M., CABELLO, M.J., MARTIN, J. & LOPEZ-NEVOT, M.Á. MICA*A4 protects against Ulcerative Colitis whereas MICA*A5.1 is associated with abscesses formation and the age on onset. Clinical and experimental immunology, 1-9. 2016.

MEHRA, N.K. Histocompatibility Antigens. In Wiley-Blackwell (Ed.), eLS: John Wiley & Sons, Ltd. 2001.

MEHRA, N.K., JAINI, R., RAJALINGAM, R., BALAMURUGAN, A. & KAUR, G. Molecular diversity of HLA-A*02 in Asian Indians: predominance of A*0211. Tissue Antigens, 57(6), 502-507. 2001.

MEI, B., LUO, Q., DU, K., HUO, Z., WANG, F. & YU, P. Association of MICA gene polymorphisms with Chlamydia trachomatis infection and related tubal pathology in infertile women. Hum Reprod, 24(12), 3090-3095. 2009.

MELLERGAARD, M., SKOVBAKKE, S.L., SCHNEIDER, C.L., LAURIDSEN, F., ANDRESEN, L., JENSEN, H. & SKOV, S. N-glycosylation of asparagine 8 regulates surface expression of major histocompatibility complex class I chain-related protein A (MICA) alleles dependent on threonine 24. J Biol Chem, 289(29), 20078-20091. 2014.

MELUM, E., KARLSEN, T.H., SCHRUMPF, E., BERGQUIST, A., THORSBY, E., BOBERG, K.M. & LIE, B.A. Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms. Hepatology, 47(1), 90-96. 2008.

MENIER, C., RITEAU, B., CAROSELLA, E.D. & ROUAS-FREISS, N. MICA triggering signal for NK cell tumor lysis is counteracted by HLA-G1-mediated inhibitory signal. Int J Cancer, 100(1), 63-70. 2002.

MERESSE, B., CHEN, Z., CISZEWSKI, C., TRETIAKOVA, M., BHAGAT, G., KRAUSZ, T.N., RAULET, D.H., LANIER, L.L., GROH, V., SPIES, T., EBERT, E.C., GREEN, P.H. & JABRI, B. Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity, 21(3), 357-366. 2004.

MERRIL, J.P. The Artificial Kidney. Archives of Internal Medicine, 106(1), 143-148. 1960.

MERRILL, J.P., MURRAY, J.E., HARRISON, J.H. & GUILD, W.R. Successful homotransplantation of the human kidney between identical twins. Journal of the American Medical Association, 160(4), 277-282. 1956.

MIZUKI, N., MEGURO, A., TOHNAI, I., GUL, A., OHNO, S. & MIZUKI, N. Association of Major Histocompatibility Complex Class I Chain-Related Gene A and HLA-B Alleles with Behcet's Disease in Turkey. Jpn J Ophthalmol, 51(6), 431-436. 2007.

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 Rheum, 42(9), 1961-1966. 1999.

MIZUKI, N., OTA, M., KIMURA, M., OHNO, S., ANDO, H., KATSUYAMA, Y., YAMAZAKI, M., WATANABE, K., GOTO, K., NAKAMURA, S., BAHRAM, S. & INOKO, H. Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behcet disease. Proc Natl Acad Sci U S A, 94(4), 1298-1303. 1997.

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, 41, 3702-3708. 2000.

MIZUKI, N., YABUKI, K., OTA, M., KATSUYAMA, Y., ANDO, H., NOMURA, E., FUNAKOSHI, K., DAVATCHI, F., CHAMS, H., NIKBIN, B., GHADERI, A.A., OHNO, S. & INOKO, H. Analysis of microsatellite polymorphism around the HLA-B locus in Iranian patients with Behcet's disease. Tissue Antigens, 60(5), 396-399. 2002.

MOLINERO, L.L., FUERTES, M.B., RABINOVICH, G.A., FAINBOIM, L. & ZWIRNER, N.W. Activation-induced expression of MICA on T lymphocytes involves engagement of CD3 and CD28. J Leukoc Biol, 71(5), 791-797. 2002.

MORADI, S., BERRY, R., PYMM, P., HITCHEN, C., BECKHAM, S.A., WILCE, M.C., WALPOLE, N.G., CLEMENTS, C.S., REID, H.H., PERUGINI, M.A., BROOKS, A.G., ROSSJOHN, J. & VIVIAN, J.P. The structure of the atypical killer cell immunoglobulin-like receptor, KIR2DL4. J Biol Chem, 290(16), 10460-10471. 2015.

MORANDI, F., RIZZO, R., FAINARDI, E., ROUAS-FREISS, N. & PISTOIA, V. Recent Advances in Our Understanding of HLA-G Biology: Lessons from a Wide Spectrum of Human Diseases. J Immunol Res, 2016, 4326495. 2016.

MUNOZ-SAA, I., CAMBRA, A., PALLARES, L., ESPINOSA, G., JUAN, A., PUJALTE, F., MATAMOROS, N., MILA, J. & JULIA, M.R. Allelic diversity and affinity variants of MICA are imbalanced in Spanish patients with Behcet's disease. Scand J Immunol, 64(1), 77-82. 2006.

MUNTASELL, A., MAGRI, G., PENDE, D., ANGULO, A. & LOPEZ-BOTET, M. Inhibition of NKG2D expression in NK cells by cytokines secreted in response to human cytomegalovirus infection. Blood, 115(25), 5170-5179. 2010.

MURO, M., LÓPEZ-HERNÁNDEZ, R.L., S. & AL., E. MICA Molecules in Disease and Transplantation, a Double-Edged Sword? Current Immunology Reviews, 8(4), 307–325. 2012.

MURRAY, J.E., MERRILL, J.P., DAMMIN, G.J., DEALY, J.B., JR., ALEXANDRE, G.W. & HARRISON, J.H. Kidney transplantation in modified recipients. Ann Surg, 156, 337-355. 1962.

NARAYAN, S., TSAI, E.W., ZHANG, Q., WALLACE, W.D., REED, E.F. & ETTENGER, R.B. Acute rejection associated with donor-specific anti-MICA antibody in a highly sensitized pediatric renal transplant recipient. Pediatr Transplant, 15(1), E1-7. 2011.

NARDI, F.A.S., SLOWIK, R., WOWK, P.F., DA SILVA, J.S., GELMINI, G.F., MICHELON, T.F., NEUMANN, J. & BICALHO, M.A.G. Analysis of HLA-G polymorphisms in couples with implantation failure. Am J Reprod Immunol, 68(6), 507-514. 2012.

NORRIS, S., KONDEATIS, E., COLLINS, R., SATSANGI, J., CLARE, M., CHAPMAN, R., STEPHENS, H., HARRISON, P., VAUGHAN, R. & DONALDSON, P. Mapping MHC-encoded susceptibility and resistance in primary sclerosing cholangitis: the role of MICA polymorphism. Gastroenterology, 120(6), 1475-1482. 2001.

NUTI, E., CASALINI, F., AVRAMOVA, S.I., SANTAMARIA, S., FABBI, M., FERRINI, S., MARINELLI, L., LA PIETRA, V., LIMONGELLI, V., NOVELLINO, E., CERCIGNANI, G., ORLANDINI, E., NENCETTI, S. & ROSSELLO, A. Potent arylsulfonamide inhibitors of tumor necrosis factor-alpha converting enzyme able to reduce activated leukocyte cell adhesion molecule shedding in cancer cell models. J Med Chem, 53(6), 2622-2635. 2010.

OGASAWARA, K., BENJAMIN, J., TAKAKI, R., PHILLIPS, J.H. & LANIER, L.L. Function of NKG2D in natural killer cell-mediated rejection of mouse bone marrow grafts. Nat Immunol, 6(9), 938-945. 2005.

OKADA, Y., HAN, B., TSOI, L.C., STUART, P.E., ELLINGHAUS, E., TEJASVI, T., CHANDRAN, V., PELLETT, F., POLLOCK, R., BOWCOCK, A.M., KRUEGER, G.G., WEICHENTHAL, M., VOORHEES, J.J., RAHMAN, P., GREGERSEN, P.K., FRANKE, A., NAIR, R.P., ABECASIS, G.R., GLADMAN, D.D., ELDER, J.T., DE BAKKER, P.I. & RAYCHAUDHURI, S. Fine mapping major histocompatibility complex associations in psoriasis and its clinical subtypes. Am J Hum Genet, 95(2), 162-172. 2014.

OLIVEIRA, L.A., RIBAS, F., BICALHO, M.G., TSUNETO, L.T. & PETZL-ERLER, M.L. High frequencies of alleles MICA*020 and MICA*027 in Amerindians and evidence of positive selection on exon 3. Genes Immun, 9(8), 697-705. 2008.

ONO, S.J. The birth of transplantation immunology: the Billingham-Medawar experiments at Birmingham University and University College London. 1951. J Exp Biol, 207(Pt 23), 4013-4014. 2004.

OPELZ, G. Non-HLA transplantation immunity revealed by lymphocytotoxic antibodies. The Lancet, 365(9470), 1570-1576. 2005.

ORCHARD, T.R., DHAR, A., SIMMONS, J.D., VAUGHAN, R., WELSH, K.I. & JEWELL, D.P. MHC class I chain-like gene A (MICA) and its associations with inflammatory bowel disease and peripheral arthropathy. Clin Exp Immunol, 126(3), 437-440. 2001.

PARK, S.H., PARK, K.S., SEO, Y.I., MIN, D.J., KIM, W.U., KIM, T.G., CHO, C.S., MOK, J.W., PARK, K.S. & KIM, H.Y. Association of MICA polymorphism with HLA-B51 and disease severity in Korean patients with Behcet's disease. J Korean Med Sci, 17(3), 366-370. 2002.

PARK, Y., CHEONG, J.W., PARK, M.H., KIM, M.S., KIM, J.S. & KIM, H.S. Effect of major histocompatibility complex haplotype matching by C4 and MICA genotyping on acute graft versus host disease in unrelated hematopoietic stem cell transplantation. Hum Immunol, 77(2), 176-183. 2016.

PARK, Y., LEE, H., SANJEEVI, C.B. & EISENBARTH, G.S. MICA polymorphism is associated with type 1 diabetes in the Korean population. Diabetes Care, 24(1), 33-38. 2001.
PARK, Y.P., CHOI, S.C., KIESLER, P., GIL-KRZEWSKA, A., BORREGO, F., WECK, J., KRZEWSKI, K. & COLIGAN, J.E. Complex regulation of human NKG2D-DAP10 cell surface expression: opposing roles of the gammac cytokines and TGF-beta1. Blood, 118(11), 3019-3027. 2011.
PARK, Y.S., SANJEEVI, C.B., ROBLES, D., YU, L., REWERS, M., GOTTLIEB, P.A., FAIN, P. & EISENBARTH, G.S. Additional association of intra-MHC genes, MICA and D6S273, with Addison's disease. Tissue Antigens, 60, 155-163. 2002.
PEGO-FERNANDES, P.M. & GARCIA, V.D. Current status of transplantation in Brazil. Sao Paulo Med J, 128(1), 3-4. 2010.
PERALDI, M.N., BERROU, J., DULPHY, N., SEIDOWSKY, A., HAAS, P., BOISSEL, N., METIVIER, F., RANDOUX, C., KOSSARI, N., GUERIN, A., GEFFROY, S., DELAVAUD, G., MARIN-ESTEBAN, V., GLOTZ, D., CHARRON, D. & TOUBERT, A. Oxidative stress mediates a reduced expression of the activating receptor NKG2D in NK cells from end-stage renal disease patients. J Immunol, 182(3), 1696-1705. 2009.
PETERSDORF, E.W., SHULER, K.B., LONGTON, G.M., SPIES, T. & HANSEN, J.A. Population study of allelic diversity in the human MHC class I-related MIC-A gene. Immunogenetics, 49(7-8), 605-612. 1999.
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, 66(8), 931-936. 2005.
PICCO, P., PORFIRIO, B., GATTORNO, M., BUONCOMPAGNI, A., FALCINI, F., CUSANO, R., BORDO, D., PISTOIA, V., RAVAZZOLO, R. & SERI, M. MICA gene polymorphisms in an Italian paediatric series of juvenile Behcet disease. Int J Mol Med, 10(5), 575-578. 2002.
PISELLI, P., SERRAINO, D., SEGOLONI, G.P., SANDRINI, S., PIREDDA, G.B., SCOLARI, M.P., RIGOTTI, P., BUSNACH, G., MESSA, P., DONATI, D., SCHENA, F.P., MARESCA, M.C., TISONE, G., VEROUX, M., SPARACINO, V., PISANI, F., CITTERIO, F., IMMUNOSUPPRESSION & CANCER STUDY, G. Risk of de novo cancers after transplantation: results from a cohort of 7217 kidney transplant recipients, Italy 1997-2009. Eur J Cancer, 49(2), 336-344. 2013.
POLLOCK, R.A., CHANDRAN, V., PELLETT, F.J., THAVANESWARAN, A., EDER, L., BARRETT, J., RAHMAN, P., FAREWELL, V. & GLADMAN, D.D. The functional MICA-129 polymorphism is associated with skin but not joint manifestations of psoriatic disease independently of HLA-B and HLA-C. Tissue Antigens, 82, 43-47. 2013.
PYO, C.W., HUR, S.S., KIM, Y.K., CHOI, H.B., KIM, T.Y. & KIM, T.G. Distribution of MICA alleles and haplotypes associated with HLA in the Korean population. Hum Immunol, 64(3), 378-384. 2003.
QIN, H., LEE, I.F., PANAGIOTOPOULOS, C., WANG, X., CHU, A.D., UTZ, P.J., PRIATEL, J.J. & TAN, R. Natural killer cells from children with type 1 diabetes have defects in NKG2D-dependent function and signaling. Diabetes, 60(3), 857-866. 2011.
QIU, J., TERASAKI, P.I., MILLER, J., MIZUTANI, K., CAI, J. & CAROSELLA, E.D. Soluble HLA-G expression and renal graft acceptance. Am J Transplant, 6(9), 2152-2156. 2006.
QUEIRO, R., ALPERI, M., ALONSO-CASTRO, S., BALLINA, J., HUERGO-ZAPICO, L., FERNANDEZ-GUIZAN, A., ACEBES-HUERTA, A., MARTINEZ-BORRA, J., SARASQUETA, C., LOPEZ-LARREA, C. & GONZALEZ, S. Patients with psoriatic arthritis may show differences in their clinical and genetic profiles depending on their age at psoriasis onset. Clin Exp Rheumatol, 30(4), 476-480. 2012.
RAACHE, R., BELANTEUR, K., AMROUN, H., BENYAHIA, A., HENICHE, A., AZZOUZ, M., MIMOUNI, S., GERVAIS, T., LATINNE, D., BOUDIBA, A., ATTAL, N. & ABBADI,

M.C. Association of major histocompatibility complex class 1 chain-related gene A dimorphism with type 1 diabetes and latent autoimmune diabetes in adults in the Algerian population. Clinical and Vaccine Immunology, 19, 557-561. 2012.
RACCA, A.L., VEAUTE, C.M., BAILAT, A.S., GAITE, L., ARRIOLA, M., HAJOS, S.E. & MALAN BOREL, I.S. Expression of HLA-G and MICA mRNA in renal allograft. Transpl Immunol, 21(1), 10-12. 2009.
RAJAGOPALAN, S. Endosomal signaling and a novel pathway defined by the natural killer receptor KIR2DL4 (CD158d). Traffic, 11(11), 1381-1390. 2010.
REBMANN, V., BARTSCH, D., WUNSCH, A., MOLLENBECK, P., GOLDA, T., VIEBAHN, R. & GROSSE-WILDE, H. Soluble total human leukocyte antigen class I and human leukocyte antigen-G molecules in kidney and kidney/pancreas transplantation. Hum Immunol, 70(12), 995-999. 2009.
REBMANN, V., DA SILVA NARDI, F., WAGNER, B. & HORN, P.A. HLA-G as a tolerogenic molecule in transplantation and pregnancy. J Immunol Res, 2014, 297073. 2014.
RIBAS, F., OLIVEIRA, L.A., PETZL-ERLER, M.L. & BICALHO, M.G. Major histocompatibility complex class I chain-related gene A polymorphism and linkage disequilibrium with HLA-B alleles in Euro-Brazilians. Tissue Antigens, 72(6), 532-538. 2008.
RIBEIRO, C.H., KRAMM, K., GALVEZ-JIRON, F., POLA, V., BUSTAMANTE, M., CONTRERAS, H.R., SABAG, A., GARRIDO-TAPIA, M., HERNANDEZ, C.J., ZUNIGA, R., COLLAZO, N., SOTELO, P.H., MORALES, C., MERCADO, L., CATALAN, D., AGUILLON, J.C. & MOLINA, M.C. Clinical significance of tumor expression of major histocompatibility complex class I-related chains A and B (MICA/B) in gastric cancer patients. Oncol Rep, 35(3), 1309-1317. 2016.
RICCI-VITIANI, L., VACCA, A., POTOLICCHIO, I., SCARPA, R., BITTI, P., SEBASTIANI, G., PASSIU, G., MATHIEU, A. & SORRENTINO, R. MICA gene triplet repeat polymorphism in patients with HLA-B27 positive and negative ankylosing spondylitis from Sardinia. J Rheumatol, 27(9), 2193-2197. 2000.
RISTI, M. & BICALHO, M.D. MICA and NKG2D: Is There an Impact on Kidney Transplant Outcome? Front Immunol, 8(179). 2017.
ROBINSON, J., HALLIWELL, J.A., HAYHURST, J.D., FLICEK, P., PARHAM, P. & MARSH, S.G. The IPD and IMGT/HLA database: allele variant databases. Nucleic Acids Res, 43(Database issue), D423-431. 2015.
ROMPHRUK, A.V., NARUSE, T.K., ROMPHRUK, A., KAWATA, H., PUAPAIROJ, C., KULSKI, J.K., LEELAYUWAT, C. & INOKO, H. Diversity of MICA (PERB11.1) and HLA haplotypes in Northeastern Thais. Tissue Antigens, 58(2), 83-89. 2001.
ROSHANI, R., BOROUJERDNIA, M.G., TALAIEZADEH, A.H. & KHODADADI, A. Assessment of changes in expression and presentation of NKG2D under influence of MICA serum factor in different stages of breast cancer. Tumour Biol. 2015.
RZYMSKI, T., PETRY, A., KRACUN, D., RIESS, F., PIKE, L., HARRIS, A.L. & GORLACH, A. The unfolded protein response controls induction and activation of ADAM17/TACE by severe hypoxia and ER stress. Oncogene, 31(31), 3621-3634. 2012.
SACRAMENTO, W., MAZINI, P.S., FRANCESCHI, D.A.S., DE MELO, F.C., BRAGA, M.A., SELL, A.M., TSUNETO, L.T. & LAGUILA VISENTAINER, J.E. Frequencies of MICA alleles in patients from southern Brazil with multibacillary and paucibacillary leprosy. International Journal of Immunogenetics, 39(3), 210-215. 2012.
SAEZ-BORDERIAS, A., GUMA, M., ANGULO, A., BELLOSILLO, B., PENDE, D. & LOPEZ-BOTET, M. Expression and function of NKG2D in CD4+ T cells specific for human cytomegalovirus. Eur J Immunol, 36(12), 3198-3206. 2006.
SALIH, H.R., ANTROPIUS, H., GIESEKE, F., LUTZ, S.Z., KANZ, L., RAMMENSEE, H.G. & STEINLE, A. Functional expression and release of ligands for the activating immunoreceptor NKG2D in leukemia. Blood, 102(4), 1389-1396. 2003.

SALVARANI, C., BOIARDI, L., MANTOVANI, V., OLIVIERI, I., CIANCIO, G., CANTINI, F., SALVI, F., MALATESTA, R., MOLINOTTI, C., GOVONI, M., TROTTA, F., FILIPPINI, D., PAOLAZZI, G. & VIGGIANI, M. Association of MICA alleles and HLA-B51 in Italian patients with Behcet's disease. J Rheumatol, 28(8), 1867-1870. 2001.

SAMUELS, S., FERNS, D.M., MEIJER, D., VAN STRAALEN, J.P., BUIST, M.R., ZIJLMANS, H.J., KENTER, G.G. & JORDANOVA, E.S. High levels of soluble MICA are significantly related to increased disease-free and disease-specific survival in patients with cervical adenocarcinoma. Tissue Antigens, 85(6), 476-483. 2015.

SAYEGH, M.H. & CARPENTER, C.B. Transplantation 50 years later--progress, challenges, and promises. N Engl J Med, 351(26), 2761-2766. 2004.

SEILER, M., BRABCOVA, I., VIKLICKY, O., HRIBOVA, P., ROSENBERGER, C., PRATSCHKE, J., LODERÉROVA, A., MATZ, M., SCHONEMANN, C., REINKE, P., VOLK, H.D. & KOTSCH, K. Heightened expression of the cytotoxicity receptor NKG2D correlates with acute and chronic nephropathy after kidney transplantation. Am J Transplant, 7(2), 423-433. 2007.

SEKI, S.S., SUGIMURA, K., OTA, M., MATSUZAWA, J., KATSUYAMA, Y., ISHIZUKA, K., MOCHIZUKI, T., SUZUKI, K., YONEYAMA, O., MIZUKI, N., HONMA, T., INOKO, H. & ASAKURA, H. Stratification analysis of MICA triplet repeat polymorphisms and HLA antigens associated with ulcerative colitis in Japanese. Tissue Antigens, 58(2), 71-76. 2001.

SHIN, S., EL-DIWANY, R., SCHAFFERT, S., ADAMS, E.J., GARCIA, K.C., PEREIRA, P. & CHIEN, Y.H. Antigen recognition determinants of gammadelta T cell receptors. Science, 308(5719), 252-255. 2005.

SHIRAISHI, K., MIMURA, K., KUA, L.F., KOH, V., SIANG, L.K., NAKAJIMA, S., FUJII, H., SHABBIR, A., YONG, W.P., SO, J., TAKENOSHITA, S. & KONO, K. Inhibition of MMP activity can restore NKG2D ligand expression in gastric cancer, leading to improved NK cell susceptibility. J Gastroenterol. 2016.

SHIROISHI, M., KUROKI, K., OSE, T., RASUBALA, L., SHIRATORI, I., ARASE, H., TSUMOTO, K., KUMAGAI, I., KOHDA, D. & MAENAKA, K. Efficient leukocyte Ig-like receptor signaling and crystal structure of disulfide-linked HLA-G dimer. J Biol Chem, 281(15), 10439-10447. 2006.

SHUM, B.P., FLODIN, L.R., MUIR, D.G., RAJALINGAM, R., KHAKOO, S.I., CLELAND, S., GUETHLEIN, L.A., UHRBERG, M. & PARHAM, P. Conservation and variation in human and common chimpanzee CD94 and NKG2 genes. J Immunol, 168(1), 240-252. 2002.

SILVA, H.T., JR., FELIPE, C.R., ABBUD-FILHO, M., GARCIA, V. & MEDINA-PESTANA, J.O. The emerging role of Brazil in clinical trial conduct for transplantation. Am J Transplant, 11(7), 1368-1375. 2011.

SINGAL, D.P., LI, J. & ZHANG, G. Microsatellite polymorphism of the MICA gene and susceptibility to rheumatoid arthritis. Clin Exp Rheumatol, 19(4), 451-452. 2001.

SINNAKIROUCHENAN, R. & HOLLEY, J.L. Peritoneal dialysis versus hemodialysis: risks, benefits, and access issues. Adv Chronic Kidney Dis, 18(6), 428-432. 2011.

SONG, G.G., KIM, J.H. & LEE, Y.H. Associations between the major histocompatibility complex class i chain-related gene A transmembrane (MICA-TM) polymorphism and susceptibility to psoriasis and psoriatic arthritis: A meta-analysis. Rheumatology International, 34, 117-123. 2014.

SORIANI, A., ZINGONI, A., CERBONI, C., IANNITTO, M.L., RICCIARDI, M.R., DI GIALLEONARDO, V., CIPPITELLI, M., FIONDA, C., PETRUCCI, M.T., GUARINI, A., FOA, R. & SANTONI, A. ATM-ATR-dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. Blood, 113(15), 3503-3511. 2009.

STARZL, T.E. History of clinical transplantation World J Surg (Vol. 24, pp. 759-782). United States. 2000.
STARZL, T.E., MARCHIORO, T.L. & WADDELL, W.R. The reversal of rejection in human renal homografts with subsequent development of homograft tolerance. Surg Gynecol Obstet, 117, 385-395. 1963.
STEGALL, M.D., CHEDID, M.F. & CORNELL, L.D. The role of complement in antibody-mediated rejection in kidney transplantation. Nature Reviews Nephrology, 8(11), 670-678. 2012.
STEINLE, A., LI, P., MORRIS, D.L., GROH, V., LANIER, L.L., STRONG, R.K. & SPIES, T. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics, 53(4), 279-287. 2001.
STERN-GINOSSAR, N., GUR, C., BITON, M., HORWITZ, E., ELBOIM, M., STANIETSKY, N., MANDELBOIM, M. & MANDELBOIM, O. Human microRNAs regulate stress-induced immune responses mediated by the receptor NKG2D. Nat Immunol, 9(9), 1065-1073. 2008.
SUAREZ-ALVAREZ, B., LOPEZ-VAZQUEZ, A., BALTAR, J.M., ORTEGA, F. & LOPEZ-LARREA, C. Potential role of NKG2D and its ligands in organ transplantation: new target for immunointervention Am J Transplant (Vol. 9, pp. 251-257). Denmark. 2009.
SUEMIZU, H., RADOSAVLJEVIC, M., KIMURA, M., SADAHIRO, S., YOSHIMURA, S., BAHRAM, S. & INOKO, H. A basolateral sorting motif in the MICA cytoplasmic tail. Proc Natl Acad Sci U S A, 99(5), 2971-2976. 2002.
SUGIMURA, K., OTA, M., MATSUZAWA, J., KATSUYAMA, Y., ISHIZUKA, K., MOCHIZUKI, T., MIZUKI, N., SEKI, S.S., HONMA, T., INOKO, H. & ASAKURA, H. A close relationship of triplet repeat polymorphism in MHC class I chain-related gene A (MICA) to the disease susceptibility and behavior in ulcerative colitis. Tissue Antigens, 57, 9-14. 2001.
SUN, D., WANG, X., ZHANG, H., DENG, L. & ZHANG, Y. MMP9 mediates MICA shedding in human osteosarcomas. Cell Biol Int, 35(6), 569-574. 2011.
SUTHANTHIRAN, M. & STROM, T.B. Renal transplantation. N Engl J Med, 331(6), 365-376. 1994.
SUTHERLAND, C.L., CHALUPNY, N.J., SCHOOLEY, K., VANDENBOS, T., KUBIN, M. & COSMAN, D. UL16-binding proteins, novel MHC class I-related proteins, bind to NKG2D and activate multiple signaling pathways in primary NK cells. J Immunol, 168(2), 671-679. 2002.
TAY, G.K., HUI, J., GAUDIERI, S., SCHMITT-EGENOLF, M., MARTINEZ, O.P., LEELAYUWAT, C., WILLIAMSON, J.F., EIERMANN, T.H. & DAWKINS, R.L. PERB11 (MIC): a polymorphic MHC gene is expressed in skin and single nucleotide polymorphisms are associated with psoriasis. Clinical and experimental immunology, 119, 553-558. 2000.
TERASAKI, P.I. Humoral theory of transplantation. Am J Transplant, 3(6), 665-673. 2003.
TIAN, W., BOGGS, D.A., DING, W.Z., CHEN, D.F. & FRASER, P.A. MICA genetic polymorphism and linkage disequilibrium with HLA-B in 29 African-American families. Immunogenetics, 53(9), 724-728. 2001.
TIAN, W., BOGGS, D.A., UKO, G., ESSIET, A., INYAMA, M., BANJOKO, B., ADEWOLE, T., DING, W.Z., MOHSENI, M., FRITZ, R., CHEN, D.F., PALMER, L.J. & FRASER, P.A. MICA, HLA-B haplotypic variation in five population groups of sub-Saharan African ancestry. Genes Immun, 4(7), 500-505. 2003.
TONG, H.V., TOAN, N.L., SONG, L.H., BOCK, C.T., KREMSNER, P.G. & VELAVAN, T.P. Hepatitis B virus-induced hepatocellular carcinoma: functional roles of MICA variants. J Viral Hepat, 20(10), 687-698. 2013.
TONNERRE, P., GERARD, N., CHATELAIS, M., POLI, C., ALLARD, S., CURY, S., BRESSOLLETTE, C., CESBRON-GAUTIER, A. & CHARREAU, B. MICA variant

promotes allosensitization after kidney transplantation. J Am Soc Nephrol, 24(6), 954-966. 2013.
TONNERRE, P., GERARD, N., GAVLOVSKY, P.J., MAZALREY, S., HOURMANT, M., CHENEAU, M.L., CESBRON GAUTIER, A., RENAUDIN, K., BRESSOLLETTE-BODIN, C. & CHARREAU, B. MICA mutant A5.1 influences BK Polyomavirus Reactivation and Associated Nephropathy after Kidney Transplantation. J Infect Dis. 2016.
TONNERRE, P., GERARD, N., GAVLOVSKY, P.J., MAZALREY, S., HOURMANT, M., CHENEAU, M.L., CESBRON-GAUTIER, A., RENAUDIN, K., BRESSOLLETTE-BODIN, C. & CHARREAU, B. MICA Mutant A5.1 Influences BK Polyomavirus Reactivation and Associated Nephropathy After Kidney Transplantation. J Infect Dis, 214(5), 807-816. 2016.
TOSH, K., RAVIKUMAR, M., BELL, J.T., MEISNER, S., HILL, A.V.S. & PITCHAPPAN, R. Variation in MICA and MICB genes and enhanced susceptibility to paucibacillary leprosy in South India. Human Molecular Genetics, 15, 2880-2887. 2006.
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 Rheum, 41, 68-73. 1998.
TSUKAGOSHI, M., WADA, S., YOKOBORI, T., ALTAN, B., ISHII, N., WATANABE, A., KUBO, N., SAITO, F., ARAKI, K., SUZUKI, H., HOSOUCHI, Y. & KUWANO, H. Overexpression of natural killer group 2 member D ligands predicts favorable prognosis in cholangiocarcinoma. Cancer Sci, 107(2), 116-122. 2016.
UCISIK-AKKAYA, E. & DORAK, M.T. A study of natural killer cell lectin-like receptor K1 gene (KLRK1/NKG2D) region polymorphisms in a European population sample. Tissue Antigens, 73(2), 177-183. 2009.
UHRBERG, M., VALIANTE, N.M., SHUM, B.P., SHILLING, H.G., LIENERT-WEIDENBACH, K., CORLISS, B., TYAN, D., LANIER, L.L. & PARHAM, P. Human diversity in killer cell inhibitory receptor genes. Immunity, 7(6), 753-763. 1997.
ULBAR, F., NICOLINI, B., CHIRUMBOLO, G., TOLOMELLI, G., STEINLE, A., RONDELLI, D. & ARPINATI, M. Human hematopoietic CD34+ progenitor cells induce natural killer cell alloresponses via NKG2D activation. Exp Hematol, 44(1), 14-23 e11. 2016.
UPSHAW, J.L., ARNESON, L.N., SCHOON, R.A., DICK, C.J., BILLADEAU, D.D. & LEIBSON, P.J. NKG2D-mediated signaling requires a DAP10-bound Grb2-Vav1 intermediate and phosphatidylinositol-3-kinase in human natural killer cells. Nat Immunol, 7(5), 524-532. 2006.
VANCE, R.E., TANAMACHI, D.M., HANKE, T. & RAULET, D.H. Cloning of a mouse homolog of CD94 extends the family of C-type lectins on murine natural killer cells. Eur J Immunol, 27(12), 3236-3241. 1997.
VINY, A.D., CLEMENTE, M.J., JASEK, M., ASKAR, M., ISHWARAN, H., NOWACKI, A., ZHANG, A. & MACIEJEWSKI, J.P. MICA polymorphism identified by whole genome array associated with NKG2D-mediated cytotoxicity in T-cell large granular lymphocyte leukemia. Haematologica, 95(10), 1713-1721. 2010.
WADA, H., MATSUMOTO, N., MAENAKA, K., SUZUKI, K. & YAMAMOTO, K. The inhibitory NK cell receptor CD94/NKG2A and the activating receptor CD94/NKG2C bind the top of HLA-E through mostly shared but partly distinct sets of HLA-E residues. Eur J Immunol, 34(1), 81-90. 2004.
WALDHAUER, I., GOEHLSDORF, D., GIESEKE, F., WEINSCHENK, T., WITTENBRINK, M., LUDWIG, A., STEVANOVIC, S., RAMMENSEE, H.G. & STEINLE, A. Tumor-associated MICA is shed by ADAM proteases. Cancer Res, 68(15), 6368-6376. 2008.

WALLACE, G.R., VERITY, D.H., DELAMAINE, L.J., OHNO, S., INOKO, H., OTA, M., MIZUKI, N., YABUKI, K., KONDIATIS, E., STEPHENS, H.A., MADANAT, W., KANAWATI, C.A., STANFORD, M.R. & VAUGHAN, R.W. MIC-A allele profiles and HLA class I associations in Behcet's disease. Immunogenetics, 49(7-8), 613-617. 1999.

WANG, L.P., NIU, H., XIA, Y.F., HAN, Y.L., NIU, P., WANG, H.Y. & ZHOU, Q.L. Prognostic significance of serum sMICA levels in non-small cell lung cancer. Eur Rev Med Pharmacol Sci, 19(12), 2226-2230. 2015.

WANG, W., TIAN, W., ZHU, F., LIU, X., LI, L. & WANG, F. MICA, MICB polymorphisms and linkage disequilibrium with HLA-B in a Chinese Mongolian population. Scand J Immunol. 2016.

WANG, X., LUNDGREN, A.D., SINGH, P., GOODLETT, D.R., PLYMATE, S.R. & WU, J.D. An six-amino acid motif in the alpha3 domain of MICA is the cancer therapeutic target to inhibit shedding. Biochem Biophys Res Commun, 387(3), 476-481. 2009.

WANG, Y.J., ZHANG, N.J., CHEN, E., CHEN, C.J., BU, Y.H. & YU, P. Allele polymorphism and haplotype diversity of MICA/B in Tujia nationality of Zhangjiajie, Hunan Province, China. Hum Immunol. 2016.

WANG, Y.J., ZHANG, N.J., CHEN, E., CHEN, C.J., BU, Y.H. & YU, P. MICA/B genotyping of Tujias from Zhangjiajie, Hunan Province, China. Hum Immunol, 77(4), 340-341. 2016.

WIENCKE, K., SPURKLAND, A., SCHRUMPF, E. & BOBERG, K.M. Primary sclerosing cholangitis is associated to an extended B8-DR3 haplotype including particular MICA and MICB alleles. Hepatology, 34, 625-630. 2001.

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 gamma delta T cells. J Immunol, 169(3), 1236-1240. 2002.

WU, X., TAO, Y., HOU, J., MENG, X. & SHI, J. Valproic acid upregulates NKG2D ligand expression through an ERK-dependent mechanism and potentially enhances NK cell-mediated lysis of myeloma. Neoplasia, 14(12), 1178-1189. 2012.

XIAO, L., ZHOU, W.Q., SHI, B.Y., FENG, K., HE, X.Y., WEI, Y.X., GAO, Y., MENG, X.Y. & XIAO, W. HLA-G expression in the peripheral blood of live kidney transplant recipients. Chin Med J (Engl), 126(14), 2652-2655. 2013.

XU, B., PIZARRO, J.C., HOLMES, M.A., MCBETH, C., GROH, V., SPIES, T. & STRONG, R.K. Crystal structure of a gammadelta T-cell receptor specific for the human MHC class I homolog MICA. Proc Natl Acad Sci U S A, 108(6), 2414-2419. 2011.

YABUKI, K., MIZUKI, N., OTA, M., KATSUYAMA, Y., PALIMERIS, G., STAVROPOULOS, C., KOUMANTAKI, Y., SPYROPOULOU, M., GIZIAKI, E., KAKLAMANI, V., KAKLAMANI, E., INOKO, H. & OHNO, S. Association of MICA gene and HLA-B*5101 with Behcet's disease in Greece. Invest Ophthalmol Vis Sci, 40(9), 1921-1926. 1999.

YABUKI, K., OTA, M., GOTO, K., KIMURA, T., NOMURA, E., OHNO, S., MIZUKI, N., KATSUYAMA, Y., MAKYSYMOWYCH, W.P., BAHRAM, S., KIMURA, M. & INOKO, H. Triplet Repeat Polymorphism in the MICA Gene in HLA-B27 Positive and Ankylosing Spondylitis. Hum Immunol, 60, 83-86. 1999.

YOSHIDA, K., KOMAI, K., SHIOZAWA, K., MASHIDA, A., HORIUCHI, T., TANAKA, Y., NOSE, M., HASHIRAMOTO, A. & SHIOZAWA, S. Role of the MICA polymorphism in systemic lupus erythematosus. Arthritis Rheum, 63, 3058-3066. 2011.

YU, R., XU, S., WANG, Y., CAI, H. & XU, P. Role of MICA expression, anti-MICA antibodies and serum MICA during acute rejection in a rat-to-mouse cardiac transplantation model. Int J Clin Exp Pathol, 8(11), 14514-14520. 2015.

ZHANG, A., REVILLE, P., NOWACKI, A., THOMAS, D., KAWCZAK, P., MCMICHAEL, J., SOBECKS, R. & ASKAR, M. Racial diversity in MICA129 genotypes and linkage genotypes and linkage disequilibrium with HLA-B. Hum Immunol, 74, 104. 2013.

ZHANG, Y., HAN, M., VORHABEN, R., GIANG, C., LAVINGIA, B. & STASTNY, P. Study of MICA alleles in 201 African Americans by multiplexed single nucleotide extension (MSNE) typing. Hum Immunol, 64(1), 130-136. 2003.

ZHANG, Y., LAZARO, A.M., ZOU, Y., LAVINGIA, B., MORAES, E.M., MORAES, R.J. & STASTNY, P. MICA polymorphism in South American Indians. Immunogenetics, 53(10-11), 900-906. 2002.

ZHANG, Z.Q., REN, S.C., TAN, Y., LI, Z.Z., TANG, X., WANG, T.T., HAO, D.L., ZHAO, X., CHEN, H.Z. & LIU, D.P. Epigenetic regulation of NKG2D ligands is involved in exacerbated atherosclerosis development in Sirt6 heterozygous mice. Sci Rep, 6, 23912. 2016.

ZHAO, J., JIANG, Y., LEI, Y., ZOU, K., WANG, C., HUANG, S., YI, F. & XIA, B. Functional MICA-129 polymorphism and serum levels of soluble MICA are correlated with ulcerative colitis in Chinese patients. J Gastroenterol Hepatol, 26(3), 593-598. 2011.

ZHAO, Y.K., JIA, C.M., YUAN, G.J., LIU, W., QIU, Y. & ZHU, Q.G. Expression and clinical value of the soluble major histocompatibility complex class I-related chain A molecule in the serum of patients with renal tumors. Genet Mol Res, 14(2), 7233-7240. 2015.

ZHOU, X., WANG, J., ZOU, H., WARD, M.M., WEISMAN, M.H., ESPITIA, M.G., XIAO, X., PETERSDORF, E., MIGNOT, E., MARTIN, J., GENSLER, L.S., SCHEET, P. & REVEILLE, J.D. MICA, a gene contributing strong susceptibility to ankylosing spondylitis. Ann Rheum Dis, 73(8), 1552-1557. 2014.

ZHU, F., ZHAO, H., HE, Y., ZHANG, W., HE, J., XU, X. & YAN, L. Distribution of MICA diversity in the Chinese Han population by polymerase chain reaction sequence-based typing for exons 2-6. Tissue Antigens, 73(4), 358-363. 2009.

ZINGONI, A., CECERE, F., VULPIS, E., FIONDA, C., MOLFETTA, R., SORIANI, A., PETRUCCI, M.T., RICCIARDI, M.R., FUERST, D., AMENDOLA, M.G., MYTILINEOS, J., CERBONI, C., PAOLINI, R., CIPPITELLI, M. & SANTONI, A. Genotoxic Stress Induces Senescence-Associated ADAM10-Dependent Release of NKG2D MIC Ligands in Multiple Myeloma Cells. J Immunol, 195(2), 736-748. 2015.

ZIRM, M.E. Eduard Konrad Zirm and the "wondrously beautiful little window". Refract Corneal Surg, 5(4), 256-257. 1989.

ZOCCHI, M.R., CATELLANI, S., CANEVALI, P., TAVELLA, S., GARUTI, A., VILLAGGIO, B., ZUNINO, A., GOBBI, M., FRATERNALI-ORCIONI, G., KUNKL, A., RAVETTI, J.L., BOERO, S., MUSSO, A. & POGGI, A. High ERp5/ADAM10 expression in lymph node microenvironment and impaired NKG2D ligands recognition in Hodgkin lymphomas. Blood, 119(6), 1479-1489. 2012.

ZOU, Y., MIRBAHA, F., LAZARO, A., ZHANG, Y., LAVINGIA, B. & STASTNY, P. MICA is a target for complement-dependent cytotoxicity with mouse monoclonal antibodies and human alloantibodies. Hum Immunol, 63(1), 30-39. 2002.

ZOU, Y. & STASTNY, P. Alternatively spliced forms of MICA and MICB lacking exon 3 in a human cell line and evidence of presence of similar RNA in human peripheral blood mononuclear cells. Immunogenetics, 54(9), 671-674. 2002.

ZWIRNER, N.W., DOLE, K. & STASTNY, P. Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes, and monocytes. Hum Immunol, 60(4), 323-330. 1999.

ZWIRNER, N.W., FERNÁNDEZ-VIÑA, M.A. & STASTNY, P. MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes. Immunogenetics, 47(2), 139-148. 1998.

ZWIRNER, N.W., FUERTES, M.B., GIRART, M.A.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. Immunologìa, 25(1), 25-38. 2006.