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ASPECTOS IMUNOGENÉTICOS DA IMUNOTOLERÂNCIA NA GESTAÇÃO E TRANSPLANTES

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Organização da tese

A presente tese está estruturada de forma análoga as etapas da gestação: pré-concepção, concepção/desenvolvimento e nascimento.

- Na fase de pré-concepção – ou seção I – é introduzida a temática de estudo, abordando os principais aspectos históricos e contemporâneos da relação entre a gestação e os transplantes. Ainda neste mesmo capítulo, descrevemos os principais aspectos da gestação humana e o papel central do complexo principal de histocompatibilidade (MHC) no sucesso ou não da gestação (Capítulo 1). Ao final da seção, são apresentados os objetivos gerais e específicos;
- A fase de desenvolvimento – ou seção II – expõe o conhecimento desenvolvido durante a elaboração desta tese, expresso na forma de artigos científicos. Nesta seção, serão apresentados quatro capítulos (capítulos 2, 3, 4 e 5). No capítulo 2, é introduzido um artigo de revisão sobre as bases genéticas da pré-eclâmpsia, as quais perfazem um dos tópicos centrais desta tese, e, complementando esta seção, são apresentados nos anexos (6.1.1 e 6.1.2) do capitulo 6 os dados originais e preliminares da presente tese.
- O nascimento – ou seção III – em analogia à gestação, é a finalização de uma etapa e início de novos desafios. Nesta seção, é discutido de forma crítica como os conhecimentos adquiridos podem contribuir para o entendimento da tolerância imunológica na gestação e transplante (capítulo 6), e de que forma essa abordagem traz novas perspectivas nesse campo em rápido desenvolvimento.
- A fase pós-natal – no fim da seção III (capítulo 7) - é apresentado os trabalhos que foram desenvolvidos paralelamente à presente tese e que auxiliaram na construção do conhecimento científico para elaboração desta tese.

Sumário

Lista de abreviaturas

AER	Abortamento espontâneo de repetição
APC	Células apresentadoras de antígenos
BCR	*B cell receptor*
CIITA	*Class II transactivator*
CLR	Receptores de lectina tipo C
DAMP	Padrões Moleculares Associados a Danos
DC	*Dendritic cell*
EVT	*Extravillous trophoblast*
FGF	*Fibroblast growth factor*
HLA	*Human leukocyte antigen*
IGF-1	*Insulin growth factor-1*
IL	Interleucina
KIR	*Killer cell immunoglobulin-like receptor*
LILRB1	*Leukocyte immunoglobulin-like receptor*
MHC	*Major histocompatibility complex*
MHC-Ia	*Major histocompatibility complex class-Ia*
MHC-Ib	*Major histocompatibility complex class-Ib*
MIC	*MHC class-I related sequence*
NK	Células natural killer
NKG2	*Natural Killer Cell lectin receptor*
NLR5	*NLR family, caspase recruitment domain-containing 5*
NOD	Receptores tipo NOD
PAMPS	Padrões moleculares associados aos patógenos
PBMC	*Peripheral blood mononuclear cells*
PD-1	*Programmed cell death 1*
PD-L1	*Programmed cell death ligand 1*
PE	Pré-eclâmpsia
PP	Parto prematuro
PRR	*Pattern recognition receptor*
sHLA-G	HLA-G solúvel
sMIC-A	MIC-A solúvel
SNP	*Single nucleotide polymorphism*

SPK	Transplante simultâneo de rins e pâncreas
STA5	*Signal transducer and activation of transcription 5*
TCR	*T cell receptor*
Th	*T helper*
TNF	Fator de necrose tumoral
TOL	Receptores tipo TOL
Treg	Células T regulatórias
uNK	Células natural *killer* uterinas
VEGF	*Vascular endothelium growth fator*

Lista de figuras e tabela

Resumo

O estudo da imunologia reprodutiva data da década de 1950. Seus estudos emergiram das observações pioneiras de Peter Medawar sobre a existência de antígenos de tecido, mais especificamente, em como antígenos expressos em enxertos de pele eram reconhecidos e rejeitados quando transplantados em indivíduos geneticamente distintos. Na gestação, a aceitação do feto é um evento único, e, demonstra como o sistema imunológico materno se adapta e tolera as células fetais semi-alogênicas sem rejeitá-las, mesmo considerando que o feto herda e expressa metade dos antígenos paternos e metade maternos. Diante disso, sugere-se que muitas desordens gestacionais, tais como, a pré-eclâmpsia e abortamentos, sejam causadas por desequilíbrios na tolerância materna. Tal tolerância é alcançada, entre outros mecanismos, através da elevada expressão das moléculas do MHC de classe I não clássico (HLA-E, -F e -G) e ausência de expressão de MHC de classe I clássico (exceto a baixa expressão de HLA-C) e de moléculas de MHC-II, as quais conferem baixa antigenicidade às células fetais.

No contexto dos transplantes (histocompatibilidade), a tolerância imunológica é estabelecida de maneira mais complexa, uma vez que deve se considerar dois genomas distintos. A rejeição do transplante é um fenômeno "criado pelo homem", uma vez que o transplante de órgãos não é evidenciado no mundo natural. Embora a rejeição seja altamente dependente da incompatibilidade entre os diferente alelos de HLA entre os pares de doador e receptor, a rejeição aguda ou crônica pode ocorrer mesmo em pares compatíveis, sugerindo a influência de outras moléculas não clássicas do MHC-I e moléculas não-HLA no desfecho dos transplantes.

Apesar de serem fenômenos biológicos distintos, as semelhanças entre a gravidez e o transplante não podem ser negligenciadas, especificamente, em relação ao papel imunorregulatório das moléculas do MHC-I não clássico e moléculas não HLA. Na presente tese, apresentaremos o papel das variantes genéticas em genes não clássicos do MHC-I (HLA-E, HLA-G, MIC-A) e receptores cognatos (NKG2C e NKG2D) no contexto da gravidez saudável ou patológica. Para complementar esta tese, avaliamos como os eixos imunológicos NKG2C/HLA-E e NKGD2/MIC-A influenciam na manutenção e sobrevivência do transplante.

No total, três artigos publicados em periódicos internacionais e, adicionalmente, três artigos em fase de preparação para publicação compõem está tese. Os resultados desses artigos (publicados) podem ser resumidos como seguem:

1. Michita *et al*. (2016) *A tug-of-war between tolerance and rejection - New evidence for 3'UTR HLAG haplotypes influence in recurrent pregnancy loss* (Hum Immunol. 77:892-897). Neste estudo observamos que as variantes genéticas +3010C/G, +3142G/C e +3187A/G localizados na região 3'UTR do *HLA-G* influenciam na suscetibilidade para o abortamento de repetição numa população brasileira. Além disso, haplótipos observados nessa região foram caracterizados na população estudada, e, a associação do haplótipo UTR-1 com o risco de abortamentos foi observada.
2. Michita *et al*. (2018) *A Valine Mismatch at Position 129 of MICA Is an Independent Predictor of Cytomegalovirus Infection and Acute Kidney Rejection in Simultaneous Pancreas–Kidney Transplantation Recipients* (Int J Mol Sci. 4;19). Nesse estudo avaliamos o impacto da variante *MICA* Val129Met no desfecho do primeiro ano após o transplante simultâneo de rim e pâncreas. Brevemente, observamos que incompatibilidade no MHC (HLA-A, -B, -DR) não influenciaram no desfecho (infecção pelo citomegalovírus, função do órgão e rejeição aguda). Porém, um acentuado efeito no desfecho foi influenciado pela incompatibilidade do alelo MICA 129Val (doador → receptor). Estas observações foram observadas pela primeira vez no contexto de transplantes de órgão sólidos e destacam a importância de outros genes não-HLA no desfecho dos transplantes.
3. Michita *et al*. (2018) *Genetic Variants in Preeclampsia: Lessons From Studies in Latin-American populations* (Front Physiol. 4;9:1771). Nesse estudo foi realizado um extensa revisão da literatura no que se refere aos estudos genéticos – variantes genéticas – realizados em populações latino-americanas investigando a patogênese da pré-eclâmpsia. Este artigo de revisão representa uma importante fonte de dados para estudos futuros devido a sua abrangência e profundidade. Além disso, o artigo destaca os principais grupos engajados na caracterização das bases genéticas da pré-eclâmpsia.

Os resultados da presente tese, destacam que apesar das diferenças entre os transplantes e a gestação, diversos mecanismos imunorregulatórios podem se sobrepor nestes dois fenômenos biológicos. Principalmente, no que se refere a investigação das moléculas não clássicas do MHC de classe-I.

Abstract

The field of reproductive immunology dates from the 1950s. It emerged from the pioneering observations of Peter Medawar on tissue antigens, more specifically, in how skin grafts were recognized and rejected when transplanted to a genetically different individual. In pregnancy, the acceptance of the fetus is a unique event, and shows how the maternal immune system should shape to tolerate and do not reject the semi-allogeneic fetal cells, even considering that the fetus inherited half of antigens from each parental - mother and father. Not surprisingly, many pregnancy disorders such as preeclampsia and miscarriages are thought to originate from a breakdown of tolerance. Such tolerance is achieved, amongst other mechanisms, by a high expression of non-classical MHC class I molecules such as HLA-E, -F and –G, and the lack of expression of classical MHC-I (or low expression of HLA-C) and MHC-II molecules. Both such mechanisms allow poor antigenicity to fetal cells.

In the context of allotransplantation, immunological tolerance is achieved in a more complex way (histocompatibility), as the attention must be directed to antigens derived from more than one genome. Graft rejection is a "man-made" phenomenon since tissue transplantation is no evidenced in the natural world. Although rejection is highly dependent on HLA mismatches between donor and recipient pairs, even fully matched patients can undergo acute or chronic rejection, suggesting the existence of other non-classical MHC-I and non-HLA molecules driving transplantation outcome.

Despite being different biological phenomena, the similarities between pregnancy and transplantation cannot be neglected, is essential to consider the overlapped functions towards a tolerogenic phenotype conferred by non-classical MHC-I and non-MHC molecules. In the current thesis, we will present the role of functional genetic variants in non-classical MHC-I (HLA-E, HLA-G, MIC-A) and cognate receptors (NKG2A and NKG2D) in the context of a healthy or a pathological pregnancy. In order to complement this thesis, we evaluated how the immunological axis NKG2C/HLA-E and NKGD2/MIC-A are involved in the maintenance of organ transplantation.

In total, three articles published in international journals and, additionally, three articles in preparation compose the current thesis. The results of these (published) articles can be summarized as follows:

1. Michita *et al*. (2016) A tug-of-war between tolerance and rejection - New evidence for 3'UTR HLAG haplotypes influence recurrent pregnancy loss (Hum Immunol 77: 892-897). In this study, we observed that the genetic variants +3010C/G, +3142G/C and +3187A/G located in the 3'UTR region of *HLA-G* influence the susceptibility to recurrent pregnancy loss in a Brazilian population. In addition, haplotypes observed in this region were characterized in the population, and an association of the UTR-1 haplotype with the risk of miscarriages was observed.

2. Michita *et al*. (2018) Valine Mismatch at Position 129 of *MICA* Is an Independent Predictor of Cytomegalovirus Infection and Acute Kidney Rejection in Simultaneous Pancreas-Kidney Transplantation Recipients (Int J Mol Sci. 4;19). In this study, we evaluated the impact of the *MICA* Val129Met variant on the first year outcome after simultaneous transplantation of the kidney and pancreas. Briefly, we found that MHC mismatch (HLA-A, -B, -DR) did not influence the outcome (cytomegalovirus infection, organ function, and acute rejection). However, a pronounced effect on the outcome was influenced by the incompatibility of the MICA 129Val allele (donor → recipient). These observations were first observed in the context of solid organ transplants and highlight the importance of other non-HLA genes in the outcome of transplants.

3. Michita et al. (2018) Genetic Variants in Preeclampsia: Lessons From Studies in Latin American Populations (Front Physiol. 4: 9: 1771). In this study, an extensive review of the literature was carried out regarding genetic studies - genetic variants - carried out in Latin American populations investigating the pathogenesis of preeclampsia. This review article represents an essential source of data for future studies because of its breadth and depth. In addition, the article highlights the main groups engaged in characterizing the genetic basis of preeclampsia in Latin America.

The results of this thesis point out that despite the differences between transplants and gestation, several immunoregulatory mechanisms can overlap in these two biological phenomena. Mainly for the investigation of non-classical MHC class I molecules.

Seção I

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Capítulo I
Introdução geral

1. Aspectos históricos e contemporâneos da Imunologia da Reprodução

O estudo das interações imunológicas envolvidas no processo gestacional – imunologia da reprodução – sempre foi muito atrelado às bases conceituais da aceitação ou rejeição de transplantes (imunologia dos transplantes). Isto, deve-se ao fato de que há cerca de 65 anos, em 1953, o então imunologista *Sir* Peter Medawar, pioneiro no estudo da imunologia dos transplantes, demonstrou como a tolerância imunológica é adquirida através de estudos envolvendo transplantes de alo-enxertos de pele em camundongos. Medawar ao realizar tais experimentos reconheceu a natureza paradoxal entre o sistema imune materno e o feto: "O problema imunológico da gestação pode ser formulado desta forma: como pode a gestante garantir a nutrição dentro de si mesma, por muitas semanas ou meses, de um feto que é um corpo antigenicamente estranho? " (Medawar, 1953, tradução nossa)[1].

Durante muito tempo acreditou se que os mecanismos envolvidos na transplantação poderiam ser análogos aos envolvidos na gestação, uma vez que o feto é considerado um semi-aloenxerto e expressa antígenos paternos. Diante disso, este seria eliminado pelo sistema imune materno, contanto que não houvesse uma constante imunossupressão. O paradigma (enxerto-hospedeiro) de que a gestação é um fenômeno análogo a transplantação por muito tempo direcionou os estudos na imunologia da reprodução, tal que os conceitos da imunologia dos transplantes foram aplicados à imunologia da gestação. Ainda que o ato do transplante seja criado pelo homem e sua ocorrência seja improvável no mundo natural, sendo a gestação o fenômeno mais próximo, o paradoxo imunológico da gestação proposto por Medawar, é por muitos considerado a contribuição mais influente para o desenvolvimento da Imunologia da reprodução moderna (Billington 2003; Colucci et al. 2014; Beaman et al. 2016).

Medawar, tendo identificado esse paradoxo propôs três hipóteses acerca das razões pelas quais o sistema imune materno não rejeita o feto, são elas: 1) uma separação anatômica entre as duas entidades, 2) uma imaturidade antigênica do feto e 3) um estado anérgico do sistema imunológico materno. Essas hipóteses foram por muito tempo a base conceitual de inúmeros estudos. No entanto, no decorrer do tempo e com os avanços tecnológicos

[1] Medawar, 1953: "*The immunological problem of pregnancy may be formulated thus: how does the pregnant mother contrive to nourish within itself, for many weeks or months, a foetus that is an antigenically foreign body?*"

alcançados nenhuma se mostrou satisfatória. A primeira provou ser falsa devido a identificação de um fluxo bidirecional de células imunes maternas e fetais na interface materno-placentária, sendo a mãe e o feto considerados quimeras, termo definido pela detecção de células ou material genético de um/ou mais indivíduo geneticamente estranho (Gammill and Harrington 2017).

Quanto à segunda hipótese, foi demonstrado que células dendríticas (DC) em fetos de camundongos expressam genes do complexo principal de histocompatibilidade (MHC) de classe Ia (MHC-Ia) e podem ativar a resposta inflamatória de linfócitos T (Elbe-Burger, et al. 2000; Zenclussen 2013). Além disso, em humanos as células do trofoblasto extraviloso (EVT) as quais estão em íntimo contato com as células imunes presente na decídua expressam moléculas MHC de classe Ib não clássico (MHC-Ib), sendo estas fundamentais na imunorregulação da gestação (Hackmon et al. 2017). A terceira hipótese mostrou-se infundada, pois as reações inflamatórias têm, de fato, um papel crítico na implantação do blastocisto (Chavan et al. 2017), na parturição e na resposta contra infecções durante a gestação. Desta forma, ao invés de um estado anérgico é atualmente sugerida a existência de um estado de tolerância imunológica adquirida (Zenclussen 2013).

Historicamente, alguns modelos imunológicos foram sugeridos na tentativa de explicar o paradoxo imunológico da gestação (Figura 1). Esses conceitos, baseados em observações empíricas da imunologia clássica exemplificam as diferentes estratégias utilizadas na vigilância imunológica contra antígenos estranhos e são brevemente descritos abaixo (Moffett and Loke 2004).

O reconhecimento do próprio e não próprio é fundamental no entendimento da imunologia (Figura 1a) (Burnet 1959). Este modelo propõe que os linfócitos (T e B) ao reconhecerem o não próprio (aloantígenos ou peptídeos de microrganismos) apresentado pelo MHC nas células alvo através de seus receptores específicos [receptor de célula T (TCR) e receptor de célula B (BCR)] iniciem a resposta imune no intuito de eliminação dos antígenos. Em contrapartida, o reconhecimento do próprio resulta numa resposta imune basal, geralmente associada a sinais de sobrevivência ou diferenciação. Uma versão alternativa deste modelo (Figura 1b), o modelo do não próprio infeccioso, propõe que o reconhecimento do não próprio possa ocorrer de forma independente de TCR e BCR (Janeway 1992). Em determinadas células apresentadoras de antígenos (APC) o reconhecimento ocorre através dos receptores de reconhecimento de padrão (PRR)

[receptores tipo NOD (NLR), receptores tipo TOL (TLR) e receptores de lectina tipo C (CLR)], os quais reconhecem padrões moleculares associados aos patógenos (PAMPS).

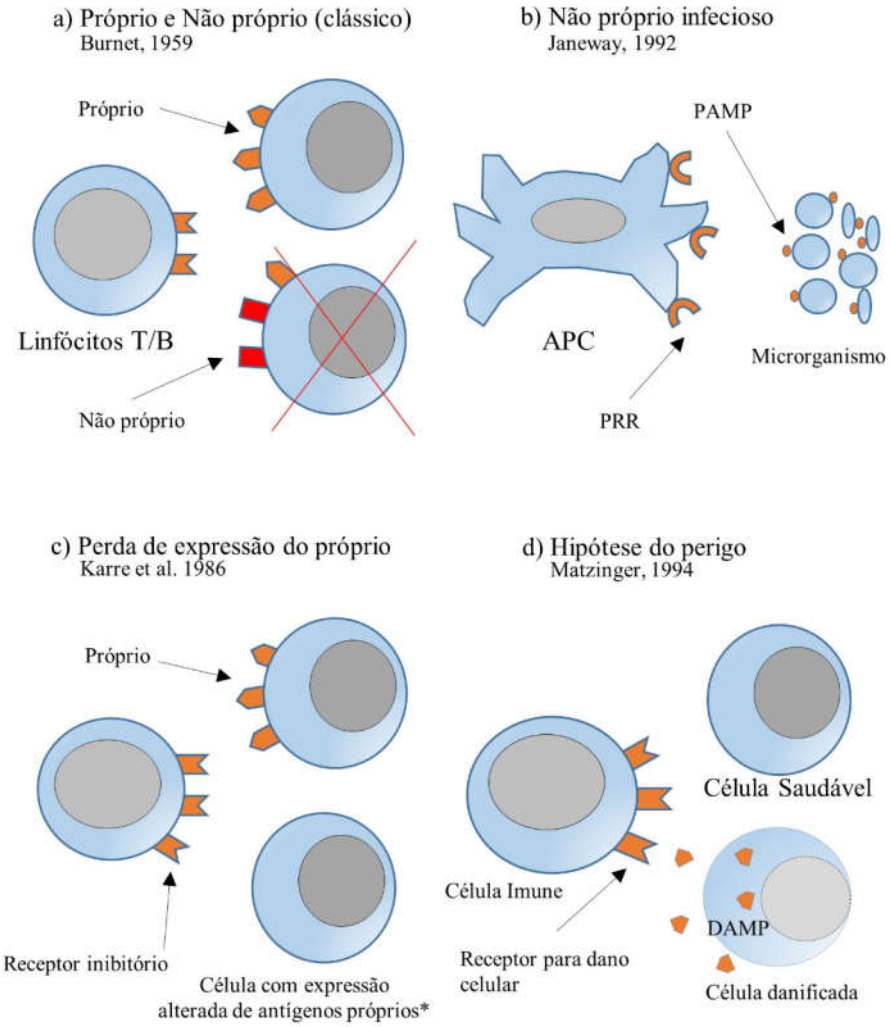

Figura 1. Modelos clássicos da imunologia. Na cronologia da imunologia da reprodução, diversos modelos da imunologia clássica, principalmente, do âmbito investigativo da imunologia dos transplantes foram explorados na tentativa de explicar o paradoxo imunológico da gestação. Atualmente, modelos mais complexos foram propostos. DAMP: Padrões Moleculares Associados a Danos. A versão original da figura se encontra em Moffett and Loke (2004).

O modelo da perda de expressão do próprio (Figura 1c) (Kärre et al. 1986), é relevante no contexto de infecções virais, malignidades e da gestação, uma vez que estas situações são caracterizadas pela baixa expressão de MHC-Ia. De acordo com este modelo, o não reconhecimento de antígenos próprios nas células por receptores inibitórios expressos nas células *Natural Killer* (NK) favorece o predomínio de sinais de ativação e consequente atividade citotóxica.

O modelo mais recente (Figura 1d), proposto por Matzinger (2002) é uma atualização do seu modelo prévio (Matzinger 1994). O modelo do perigo propõe que a resposta imune é desencadeada por sinais ou alarmes de perigo, os quais são liberados pelas células e sentidos pelas APCs. Em outras palavras, a resposta imune não é o resultado do reconhecimento do não próprio e sim do reconhecimento de perigo ou danos às próprias células. Desta forma, este modelo é visto como uma descrição a *posteriori* da resposta imune, pois ele não oferece uma explicação adequada na resposta imune a tumores, transplantes e bactérias comensais (Pradeu and Cooper 2012).

Em resumo, esses modelos exemplificam as diferentes maneiras em que a resposta imunológica atua na detecção de patógenos ou epitopos imunogênicos. No entanto, a aplicabilidade deles na imunologia da gestação é limitada, pois não consideram as características únicas da gestação humana – apresentadas no tópico 1.2 (Moffett and Loke 2004; Bonney 2017). É importante salientar, que esses modelos provêm da imunologia clássica e, portanto, a resposta imune aos patógenos e transplantes tem um papel predominante e não refletem a complexidade da gestação.

Atualmente, há uma transição de paradigmas dentro da imunologia da gestação. Isto, se deve à existência de diferentes escolas de pensamento (Beaman et al. 2016; Mor et al. 2017), há os que historicamente suportam a gestação como um fenômeno similar à imunologia dos transplantes e os que acreditam que a gestação pode ser vista como uma situação análoga à imunologia do câncer. A primeira escola de pensamento está muito atrelada ao paradoxo imunológico da gestação, e por afinidade ao modelo imunológico da discriminação do próprio e não próprio. Este modelo – paradigma clássico da gestação – desde a última década tem sido criticado, pois alguns autores o consideram obsoleto, e talvez o mais importante, é o fato de que ele tem prejudicado o entendimento da imunologia da gestação, devido a extrapolação equivocada de estudos em modelos não grávidos (Mor et al. 2017). Além de questões biológicas, o modelo traz importantes questões epistemológicas, por exemplo, qual a definição de próprio e não próprio? E como a gestação se enquadra neste contexto considerando que o sistema imunológico antecede a evolução da placenta? (Colucci et al. 2014; Bonney 2017). Bonney (2018) sugere que esse modelo limita a proposição de outros mecanismos biológicos para explicar a imunologia da gestação, além dos já teoricamente suportados pelo modelo de discriminação do próprio e não próprio proveniente dos transplantes.

A imunologia do câncer pode contribuir de certa forma para o entendimento da imunologia da gestação devido às similaridades entre o início da gestação e a formação de tumores (Figura 2). Neste contexto, os eventos de invasão, migração, proliferação e vasculogênese apresentam relativa semelhança (Holtan et al. 2009; Beaman et al. 2016).

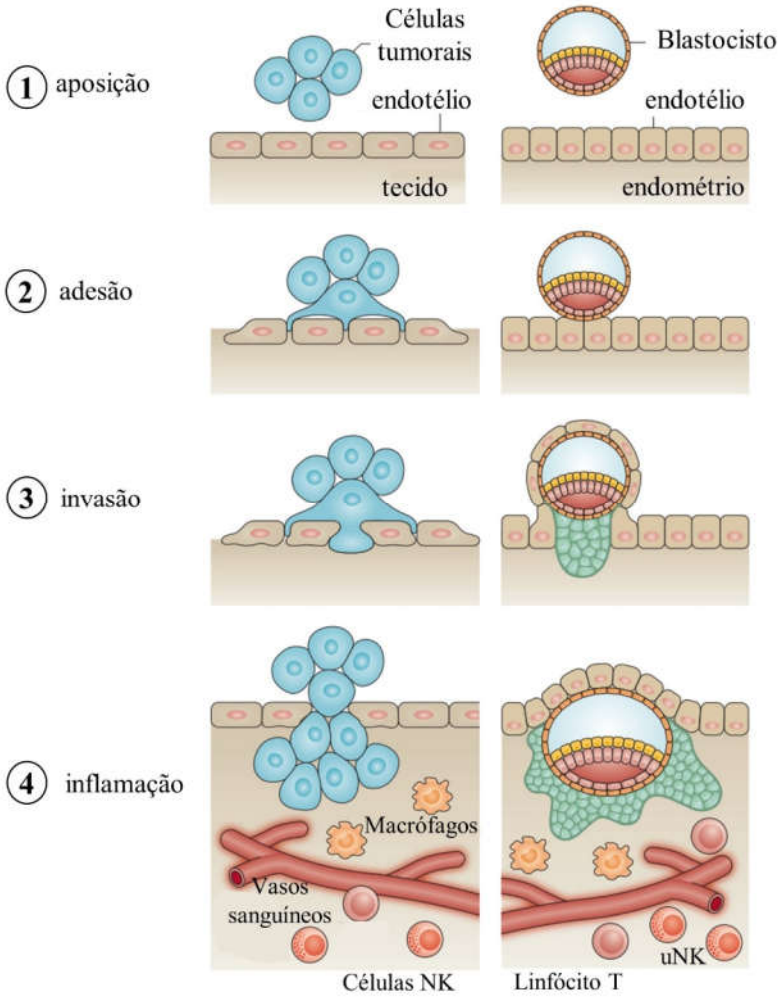

Figura 2. Similaridades entre os processos de metastização das células tumorais e implantação do blastocisto. Na primeira etapa, a aposição ocorre de forma similar nas duas entidades. Na etapa 2, moléculas de adesão auxiliam nas interações iniciais entre as células invasoras e o epitélio. Na etapa 3, os trofoblastos penetram o endotélio endometrial e invadem o estroma. Nesta etapa as células tumorais invadem o estroma antes de se diferenciarem em células epiteliais tumorais. Na etapa 4, o contínuo processo de invasão e proliferação das células promove um platô inflamatório, o qual é necessário para remodelação tecidual e recrutamento de macrófagos para a remoção de debris celulares. Adaptado de Mor et al. (2017).

Além disso, o processo de indução da tolerância imunológica na gestação é mais similar à micrometástase tumoral do que à transplantação (Mor et al. 2017). Nos dois contextos – gestacional e tumoral – as células do trofoblasto e células tumorais secretam moléculas e utilizam vias de sinalização em comum, por exemplo, a secreção de moléculas de resposta a estresse celular (hemeoxigenase-1) e proteases (colagenases e metaloproteinases de matrix), as quais são importantes para facilitar a invasão tecidual por atuarem na degradação do tecido (Mullen 1998; Beaman et al. 2016). Na fase proliferativa, a ativação de moléculas da via do IGF-1 (do inglês, *insulin growth factor-1*) favorece a proliferação e sobrevivência tanto do trofoblasto quanto das células cancerígenas (Mayama et al. 2013; Silva and Serakides 2016; Beaman et al. 2016). Tanto no câncer quanto na gestação o aporte sanguíneo é crucial para o desenvolvimento destas entidades. Neste contexto, a secreção dos mediadores vasculares: VEGF (do inglês, *vascular endothelium growth factor*) e FGF (do inglês, *fibroblast growth factor*) assumem importante função (Beaman et al. 2016; Presta et al. 2017; Viallard and Larrivée 2017). Não menos importantes, as células do trofoblasto assim como as células tumorais efetivamente modulam a atividade das células imunes adjacentes, essas células (macrófagos, DCs, NK e linfócitos T) estão presentes na interface materno-placentária e tumoral (Zhao et al. 2015). Apesar de diferirem na expressão de marcadores de superfície específicos, o que caracteriza as diferentes subpopulações, essas células geralmente possuem características imunorregulatórias e imunossupressivas (Mullen 1998; Zhao et al. 2015; Beaman et al. 2016; Mor et al. 2017; Viallard and Larrivée 2017).

Diante disso, propõe-se que as similaridades entre a imunologia da reprodução e do câncer podem auxiliar no delineamento de estudos futuros, através do direcionamento de novas abordagens conceituais, clínicas e terapêuticas. Um exemplo recente, é a utilização de bloqueadores da via de sinalização de PD-1 (do inglês, *programmed cell death 1*), a qual tem se mostrado importante para a imunorregulação da gestação e efetiva na resposta antitumoral (D'Addio et al. 2011; Zhang et al. 2015; Iwai et al. 2017; Xu-Monette et al. 2017). Por fim, é importante salientar que a gestação é única em sua natureza. Portanto, as extrapolações a partir de observações em modelos não-grávidos devem considerar tal limitação.

Na presente tese, é tomada uma posição distinta em relação aos diferentes paradigmas que circundam a imunologia da reprodução. Tal posicionamento considera que a gestação é

um estado imunológico dinâmico, adaptável e responsivo. Uma vez que a aceitação da gestação é algo natural, a investigação dos mecanismos que previnem seu acontecimento torna-se algo difícil, mas ao mesmo tempo intrigante e fascinante do ponto de vista imunológico. Desta forma, ao longo desta tese investigamos as bases moleculares da gestação saudável e patológica. Embora o foco da presente tese seja a gestação, apresentamos uma perspectiva de como os mesmos mecanismos envolvidos no sucesso gestacional podem contribuir para a manutenção de órgãos sólidos transplantados, a partir de estudos desenvolvidos em caráter colaborativo e dados originais incluídos na presente tese.

1.2. Aspectos da gestação humana

Em todos os mamíferos placentários (eutérios), o estabelecimento do contato íntimo entre o embrião e mãe depende de uma sucessão de etapas críticas que variam entre as espécies. A diversidade baseia-se principalmente na anatomia do útero e interações endócrinas e moleculares envolvendo a decídua materna e os tecidos embrionários (Chavatte-Palmer and Tarrade 2016). Particularmente, a estrutura histológica é geralmente considerada para descrever o tipo placentário e a funcionalidade (Furukawa et al. 2014). Em humanos, assim como roedores e lagomorfos, o tipo de placentação é uma forma bastante invasiva, denominada hemocorial. Apesar da similaridade entre os organismos acima citados, algumas características são únicas à espécie humana, como a anatomia uterina, duração da gestação, morfologia placentária e expressão de genes específicos (Moffett-King 2002; Chavatte-Palmer and Tarrade 2016). Desta forma, as divergências evolutivas somadas às considerações éticas representam um grande obstáculo para o estudo da placenta humana. Além disso, trofoblastos isolados a partir de placenta de primeiro trimestre rapidamente se diferenciam e não proliferam *in vitro* (Lee et al. 2016). No entanto, em estudo recente esses obstáculos foram parcialmente superados a partir de um modelo celular proposto, conhecido como organoide ou “mini placenta”, o qual utiliza isolados de trofoblasto da placenta expandidos *in vitro*. Estes organoides são geneticamente estáveis e se organizam de forma similar à sua origem no vilo coriônico sendo capazes de secretar proteínas e hormônios (Turco et al. 2018). Diante disso, esse modelo representa uma ferramenta promissora na investigação das interações iniciais entre o trofoblasto e o sistema imune materno, uma vez

que este é o modelo mais similar à placenta de primeiro trimestre e permite avaliar detalhadamente a formação e o desenvolvimento da placenta.

Após a fertilização, o zigoto, que é formado a partir do material genético paterno e materno sofre diversas divisões celulares (fase de segmentação/clivagem) resultando na formação do blastocisto (blástula). Este último, constituído por uma massa interna de células pluripotentes (embrioblasto), é circundado por uma camada externa de células, o trofectoderma, o qual sofre rápida expansão e diferenciação em subpopulações de trofoblasto, à medida que inicia a invasão da decídua (Gilbert and Barresi 2016).

Os diferentes tipos de trofoblasto podem ser identificados *in vivo* através da sua localização anatômica e expressão de marcadores de superfície. As principais subpopulações de trofoblasto são: citotrofoblasto, sinciciotrofoblasto e EVTs (Figura 3). Os citotrofoblastos são células não fusionadas que inicialmente cobriam a superfície do blastocisto. A segunda subpopulação, o sinciciotrofoblasto, consiste em células fusionadas que rapidamente se dividiram a partir do citotrofoblasto. Estas células formam uma barreira entre a circulação sanguínea materna e os vilos placentários, sendo responsáveis pela troca de nutrientes e produção de hormônios, como o hormônio da gonadotrofina coriônica e a progesterona (Chavatte-Palmer and Tarrade 2016). As células EVT são responsáveis por invadir a decídua, sendo essenciais para a ancoragem da placenta na decídua, e também são responsáveis por promover o remodelamento das artérias espirais e o consequente estabelecimento do aporte sanguíneo materno-placentário.

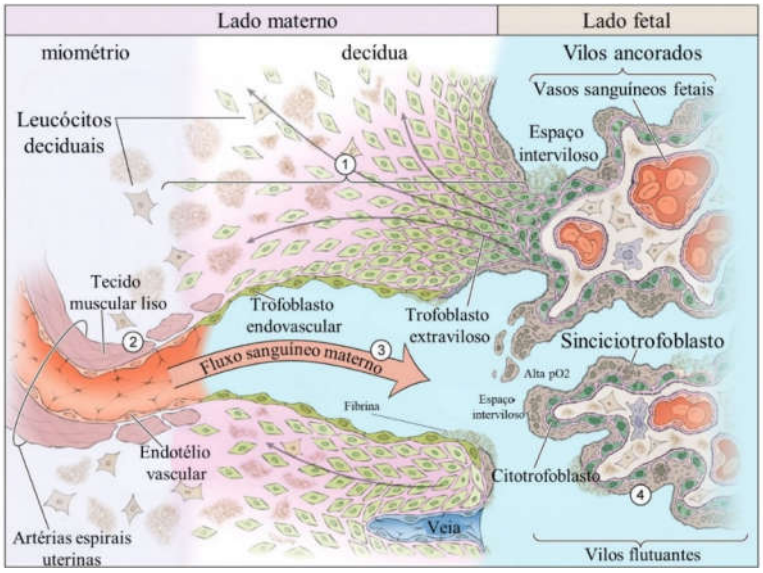

Figura 3. As diferentes subpopulações de trofoblasto nas etapas de invasão e placentação. As células do trofoblasto extravilosos proliferam ao passo que invadem a decídua materna (1) e as artérias espirais uterinas (2), ao promover a remodelação das artérias, as células extravilosas substituem as células endoteliais, diferenciando-se em trofoblastos endovasculares.
A partir desta etapa, o aporte sanguíneo materno é estabelecido para a unidade feto-placentária (3). O vilo placentário é coberto pelas células do sinciotrofoblasto no espaço interviloso e estão em direto contato com a circulação materna. Adaptado de (Iwai et al. 2017).

O crescimento e desenvolvimento fetal dependem de uma interação coordenada entre as células EVTs e a decídua materna receptiva. No lado fetal, diversos mecanismos celulares são regulados de maneira espacial e temporal, envolvendo de modificações epigenéticas à expressão seletiva de genes (Lee et al. 2016). No lado materno, a presença de diversos infiltrados imunológicos no entorno das EVTs paradoxalmente auxilia a implantação do blastocisto, embora durante a gestação anticorpos maternos específicos aos antígenos paternos possam ser detectados, geralmente sem prejuízos à gestação (Girardi et al. 2006). A falha de implantação geralmente é associada a perda gestacional (Sharkey and Smith 2003). De fato, estima-se que até 75% das perdas gestacionais ocorram devido a falhas na implantação do blastocisto (Norwitz et al. 2001). Além disso, a implantação ineficiente do blastocisto é associada a outras desordens gestacionais como restrição do crescimento fetal e pré-eclâmpsia (PE) (Norwitz 2006).

Na decídua materna, células do sistema imune inato e adaptativo participam no desenvolvimento da unidade feto-placentária. Aproximadamente, 70% dos leucócitos deciduais são células uterinas NK (uNK). Em menor frequência, observam-se macrófagos (20-25%), linfócitos T (3-10%) e DCs (1,7%) (Mor et al. 2017). Linfócitos B também estão presentes, embora em baixa frequência (Muzzio et al. 2014). De forma interessante, a investigação dos perfis transcriptômicos de células individuais (*single-cell*) da placenta, da decídua e da circulação materna indicam a presença de três subpopulações de células uNK na decídua materna. A mais frequente destas populações de células uNK expressa receptores para HLA (do inglês, *human leukocyte antigen*) –G, como o LILRB1 (do inglês, *leukocyte immunoglobulin-like receptor B1*), receptores para HLA-E [NKG2A (do inglês, *natural killer cell lectin*) e NKG2C] e HLA–C [KIR2DL1 (do inglês, *killer cell immunoglobulin-like receptor*), KIR2DL2, KIR2DL3, KIR2DS1 e KIR2DS4] e possui alta atividade metabólica e grandes quantidades de conteúdo citoplasmático sugerindo que estas células interagem com as células EVTs. Além disso, uma alta expressão de PD-L1 (do inglês, *programmed cell death ligand 1*) e HLA-G é observada nas células EVTs, corroborando o papel destas moléculas na regulação da resposta inflamatória iniciada pela invasão e dano tecidual causada por essas células. Em contrapartida, as duas outras subpopulações de uNK provavelmente estão envolvidas na manutenção da homeostasia do microambiente uterino (Vento-Tormo et al. 2018).

Estas células, junto à decídua, contribuem para a receptibilidade do blastocisto e invasão do trofoblasto através da secreção de vários mediadores inflamatórios, como prostaglandinas e citocinas pró-inflamatórias, incluindo o fator de necrose tumoral (TNF) a interleucina (IL)-6, IL-8, IL-15 e quimiocinas (Vilella et al. 2013; Norwitz et al. 2015). A inflamação local contribui para a implantação do blastocisto. Além disso, é observado que a realização de biopsias endometriais aumenta o sucesso gestacional de mulheres submetidas à fertilização *in vitro*, possivelmente devido ao dano tecidual e inflamação local (Gnainsky et al. 2015). É observado que o microambiente inflamatório promove a expressão de moléculas de adesão na decídua, resultando no aumento da aderência e afinidade do blastocisto (Mor et al. 2017). Por outro lado, o uso de medicamentos anti-inflamatórios durante a janela de implantação é associado ao risco de abortamento (Li 2003), o que reforça a necessidade de uma inflamação basal para a correta implantação do blastocisto.

Apesar da inflamação ser necessária nas fases iniciais da gestação, ela é frequentemente associada a diversas desordens gestacionais como abortamento, nascimento prematuro e PE (Romero et al. 2007). No entanto, gestações saudáveis são compatíveis com o perfil inflamatório Th1 (do inglês, *T helper 1*) (Mor et al. 2017). Diante disso, sugere-se que uma gestação de sucesso seja um balanço entre estímulos pró-inflamatórios, anti-inflamatórios e imunorregulatórios caracterizado pelo modelo Th1/Th2/Th17 e Treg (do inglês, *T regulatory cell*) (Saito et al. 2010). Neste cenário, a habilidade do sistema imune materno em se adequar às diferentes fases do desenvolvimento fetal e estímulos imunológicos é crucial para o sucesso gestacional.

Recentemente, o "relógio imunológico da gestação humana" foi descrito a partir da técnica de citometria de massa (citometria de fluxo de nova geração) avaliando amostras de PBMC (do inglês, *peripheral blood mononuclear cells*) nos diferentes períodos gestacionais e no período pós-parto (Aghaeepour et al. 2017). Nesse estudo, os autores descrevem quais eventos imunológicos ocorrem do início ao término da gestação e propõem um referencial para o desenvolvimento normal do sistema imune durante a gestação. Nesse mesmo estudo é confirmado que as células NK e neutrófilos têm ação reforçada durante a gravidez. Pela primeira vez os pesquisadores demonstram uma atividade incomum da via de sinalização STAT5 (do inglês, *signal transducer and activation of transcription 5*) em células T CD4+. Esta atividade aumenta progressivamente apenas durante a gravidez e está positivamente correlacionada com o aumento dos níveis de IL-2, a qual é principalmente secretada por

células T ativadas (Crispín and Tsokos 2009). Além disso, STAT5 está envolvido na diferenciação de células Treg sugerindo um mecanismo adicional para a manutenção da imunorregulação na gestação.

Diante disso, é sugerido que desvios neste padrão podem predizer a ocorrência de nascimento prematuro, entre outras desordens gestacionais. No entanto, o valor preditivo deste modelo é limitado pois o processo de migração de células periféricas à decídua materna ainda permanece a ser investigado (Diemert and Arck 2018). Contudo, o "relógio imunológico da gestação" não representa toda a cronologia de fatos observados durante o período gestacional. Neste aspecto podem-se citar os "relógios": endócrino, miométrio, membrana fetal, decidual e fetal (Figura 4) (Menon et al. 2016; Diemert and Arck 2018).

Figura 4. A interdependência dos diferentes "relógios gestacionais" na cronologia do desenvolvimento gestacional. Adaptado de Diemert and Arck (2018).

Assim como no contexto gestacional saudável, muitas hipóteses acerca da patogênese das desordens gestacionais já foram propostas na tentativa de elucidar suas possíveis causas. Nesse sentido, o capítulo 2 que consiste em um artigo de revisão complementa a presente introdução, apresentando uma revisão sobre a PE e como a

investigação de SNPs (do inglês, *single nucleotide polymorphisms*) em genes candidatos de diferentes sistemas biológicos tem contribuído no entendimento da fisiopatologia da PE (Michita et al. 2018b).

1.3. A contribuição do MHC na gestação

O sistema MHC compreende um importante *locus* gênico localizado no braço curto do cromossomo 6 amplamente estudado devido a associação de múltiplas variantes genéticas com doenças autoimunes, doenças inflamatórias, transplantação e gestação (Fernando et al. 2008; Tsai and Santamaria 2013; Djurisic and Hviid 2014). Classicamente, o MHC pode ser dividido em duas grandes classes: MHC-I e MHC-II, embora MHC-III e MHC-IV também existam e influenciem a patogênese de doenças complexas (Figura 5) (Gruen 2001; Yau et al. 2016).

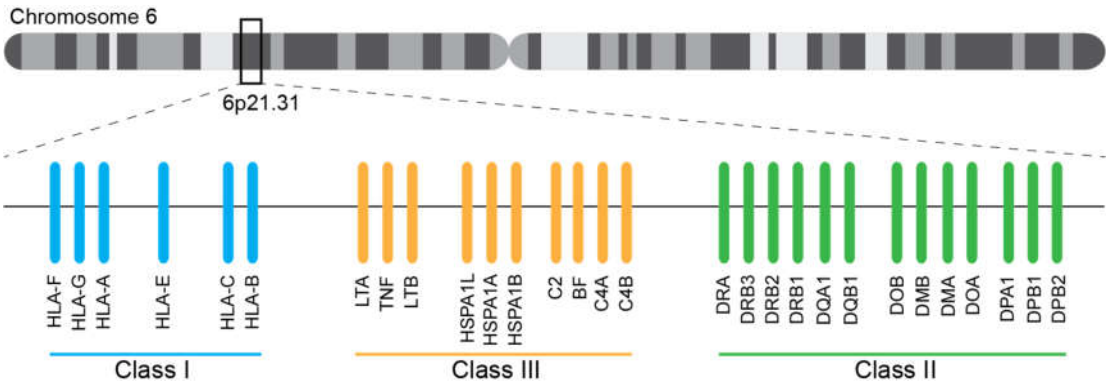

Figura 5. Organização do MHC no cromossomo 6. Genes pertencentes do MHC de classe IV estão localizados entre o MHC de classe I e III. Disponível em http://sciscogenetics.com/pages/technology.html data de acesso 08.01.2019 11:04.

Os genes do MHC-I se dividem em duas classes adicionais, conforme sua funcionalidade, diversidade genética e local de expressão, são eles: o MHC-Ia (*HLA-A*, *-B, -C*) e MHC-Ib (*HLA-E, -F, -G* e *HFE*). Adicionalmente, próximo aos *locus* do MHC-Ia encontram-se os genes *MIC (*do inglês, *MHC class-I related sequence*) (Baranwal and Mehra 2017). O MHC-Ia é expresso virtualmente em todas as células nucleadas (com algumas exceções), as proteínas por eles codificadas são altamente polimórficas, e sua função primária é a apresentação de peptídeos. Por outro lado, a expressão de MHC-Ib é restrita a tipos celulares e condições fisiológicas específicas, os genes possuem menor grau de

polimorfismo genético, as proteínas por eles codificadas não apresentam peptídeos como função primária, e são moléculas cruciais na sinalização imunológica, principalmente, na manutenção da gestação humana (Djurisic and Hviid 2014). A expressão do MHC de classe-II (*HLA-DR, -DQ*, e *-DP*) é restrita às APCs, tais como linfócitos B, macrófagos e DCs. MHC de classe-III e -IV são moléculas distintas que compõem os membros do sistema complemento e moléculas inflamatórias, respectivamente (Gruen 2001; Jongsma et al. 2017).

As moléculas do MHC diferem em diversos outros aspectos, ao passo que o MHC-II interage com linfócitos T CD4+, o MHC-I é principalmente reconhecido pelos linfócitos T CD8+. Apesar dos princípios/teoria envolvidos no carregamento de peptídeo e a apresentação do mesmo às células imunológicas serem similares, o modo e mecanismos associados à obtenção e processamento dos peptídeos diferem entre as distintas classes do MHC (Neefjes et al. 2011). Além disso, é sugerido que fatores de transcrição sejam específicos na regulação da expressão das diferentes moléculas, como o NLR5 (do inglês, *NLR family, caspase recruitment domain-containing 5*) para o MHC-I (Meissner et al. 2010; Jongsma et al. 2017) e CIITA (do inglês, c*lass II transactivator*) para o MHC-II (Muhlethaler-Mottet et al. 1998).

O sistema MHC é crucial para o estabelecimento de uma gestação saudável. Uma vez que o feto expressa metade dos antígenos paternos, a apresentação de antígenos pelas DCs deciduais representa um obstáculo para o desenvolvimento da gestação. Neste cenário, o alorreconhecimento de antígenos fetais ocorre de forma gradual e indireta (Figura 6a), além de ser caracterizado pela baixa quantidade de antígenos fetais apresentados (Collins et al. 2009). Em outras palavras, sugere-se que essa situação seja similar ao processo de indução de tolerância imunológica, uma vez que as células T efetoras reativas são destruídas e Tregs podem ser induzidas (figura 6b) (Mor et al. 2017). Alternativamente, a apresentação de antígenos paternos pelas APCs deciduais pode levar a produção de anticorpos anti-HLA paternos, porém, se a perda gestacional é diretamente causada por estes anticorpos contra antígenos fetais ainda deve ser investigado, visto que anticorpos anti-HLA paternos podem ser detectados em gestações saudáveis (Petroff 2011; Martínez-Varea et al. 2014).

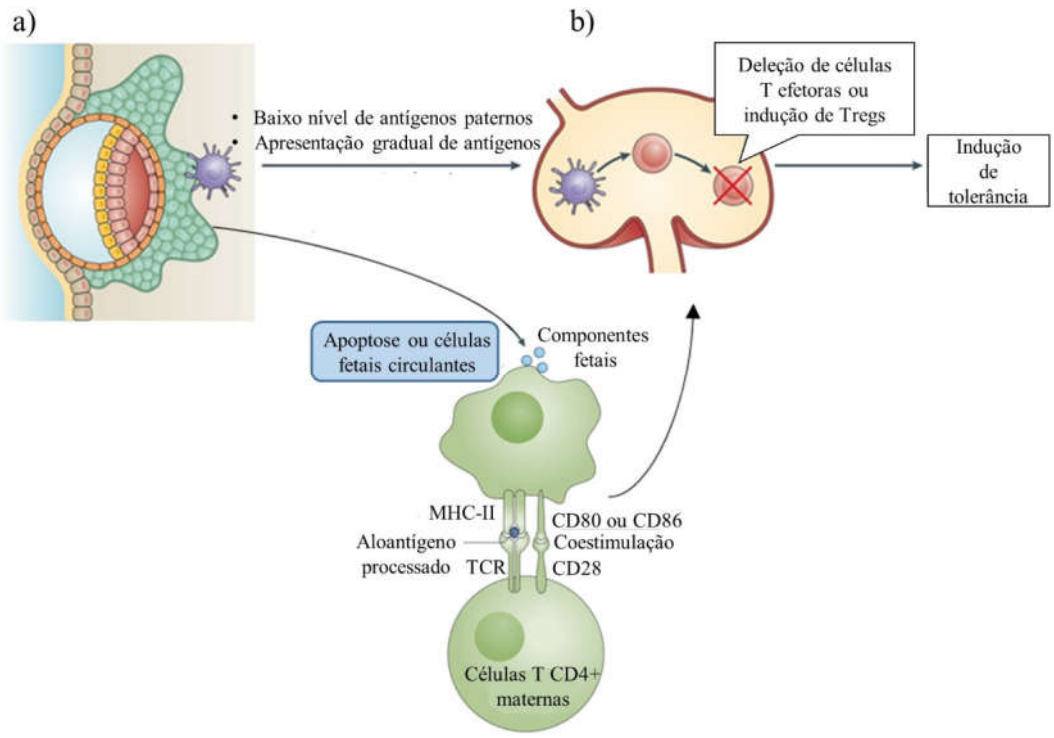

Figura 6. A apresentação e reconhecimento de antígenos fetais durante o período gestacional. Adaptado de Mor et al. 2017 e Yang et al. 2017.

Ainda, deve-se considerar que as células do trofoblasto (EVT e citotrofoblasto), principalmente as EVTs, não expressam os genes polimórficos *HLA-A*, *HLA-B* ou *MHC-II*, os quais são potente ativadores da resposta imunológica. No entanto, elas expressam uma combinação única composta pelo *HLA-C*, *HLA-E* e *HLA-G*, sendo o HLA-C o único a apresentar antígenos paternos (Tabela 1). Além disso, o sinciciotrofoblasto é nulo para a expressão de *MHC* (Blaschitz et al. 2001; Djurisic and Hviid 2014; Redman et al. 2015). Por um lado, sugere-se que a ausência de MHC evite respostas imunes de forma sistêmica, uma vez que este tipo celular está no espaço interviloso em contato com a circulação materna (Figura 3) (Redman et al. 2015). Por outro lado, a ausência de MHC no sinciciotrofoblasto pode ativar as funções efetoras imunes das células uNK, embora esta interação específica ainda precise ser investigada.

Tabela 1. Expressão de genes do MHC-I nas principais subpopulações do trofoblasto.

Tecido	MHC-Ia			MHC-Ib		
	HLA-A	HLA-B	HLA-C	HLA-E	HLA-G	HLA-F
Células adultas e fetais	Sim	Sim	Sim	Sim	Não	Sim*
EVTs, Citotrofoblasto	Não	Não	Sim	Sim	Sim	Sim*±
Sinciotrofoblasto	Não	Não	Não	Não	Não	N.D.

*transcritos de *HLA-F*. ± expressão proteica de HLA-F. N.D = não disponível.

Considerando que a implantação e placentação são eventos críticos para o sucesso gestacional, anormalidades nestes processos têm sido implicadas na fisiopatologia de diversas desordens gestacionais, incluindo abortamentos e PE (Norwitz 2006). Nesse sentido, o papel das moléculas do MHC-Ib na gestação tem sido o foco de intensa investigação desde a descoberta do HLA-G nas células do trofoblasto humano (Kovats et al. 1990; Michita et al. 2016; de Almeida et al. 2018). Além do HLA-G, as formas não clássicas do MHC-Ib também foram identificadas na interface materno-fetal: HLA-E, -F, –G e MIC-A (Mincheva-Nilsson et al. 2006; Hackmon et al. 2017). Interessantemente, a expressão seletiva dessas moléculas na interface materno-fetal contribui para a manutenção da gestação saudável, uma vez que elas auxiliam na manutenção da tolerância imunológica na interface materno-placentária, através do seu reconhecimento por receptores expressos nas células imunes maternas (Varla-Leftherioti et al. 2003; Szereday et al. 2003).

Nesta tese investigamos como aspectos imunogenéticos referentes aos genes do MHC-Ib: *HLA-G*, *HLA-E* e *MIC-A* influenciam tanto na gestação saudável, quanto na suscetibilidade à PE e o abortamento espontâneo de repetição (AER) idiopático. Desta forma, uma breve revisão sobre estes genes no contexto gestacional faz se necessária, sendo apresentada nos tópicos 1.4 (HLA-G), 1.5 (HLA-E) e 1.6 (MIC-A).

1.4. O papel imunorregulatório do HLA-G na gestação

O HLA-G expresso nas células do trofoblasto interage com diversas células do sistema imunológico presentes na decídua (por exemplo, células NK, macrófagos e linfócitos T CD8+), e pode tanto ativá-las ou inibi-las dependendo do estímulo e contexto celular (Hunt

et al. 2005). A afinidade do HLA-G por seus receptores cognatos, tais como: (ILT-2, ILT-4, KIR2DL4, CD8, CD160) varia conforme suas diferentes isoformas (HLA-G1 a -G7), formação de multímeros de HLA-G, e forma de expressão de membrana ou solúvel (Rajagopalan and Long 2012; Rebmann et al. 2014). Devido ao seu potencial imunorregulatório, o HLA-G tem sido investigado nos mais diferentes contextos biológicos (de Almeida et al. 2018). Interessantemente, monócitos podem adquirir porções da membrana celular de outras células e transferir as mesmas para leucócitos autólogos. Esse mecanismo, trogocitose, é responsável pela transferência de moléculas HLA-G temporariamente funcionais para monócitos, linfócitos T e célula NK (LeMaoult et al. 2007; Alegre et al. 2010; Carosella et al. 2015). A presença de HLA-G em microvesículas extracelulares secretadas durante a gestação reforça seu papel na imunorregulação da gestação (Kshirsagar et al. 2012).

A expressão do HLA-G geralmente é associada com o sucesso gestacional, enquanto a baixa expressão desta molécula é associada com desordens gestacionais (Persson et al. 2017). Em nível molecular, assim como os polimorfismos na região promotora do HLA-G (Castelli et al. 2011; Porto et al. 2015), algumas variantes genéticas na região 3'UTR (do inglês, *untranslated region*) do HLA-G têm papel preponderante na regulação gênica, visto que influenciam os níveis plasmáticos de HLA-G solúvel (sHLA-G) (Martelli-Palomino et al. 2013). Na circulação materna, o sHLA-G é predominantemente secretado pelas células do trofoblasto, e secundariamente pelas células Tregs e APCs (monócitos e DCs) (Feger et al. 2007; Alegre et al. 2007) (Feger et al. 2007; Alegre et al. 2007). Além disso, sHLA-G é detectado no fluido seminal (Larsen et al. 2011).

Na literatura, é descrito que polimorfismos na região 3'UTR do HLA-G influenciam na suscetibilidade a diversas desordens imunológicas, incluindo a PE e AER (Michita et al. 2016; de Almeida et al. 2018). No entanto, em diversas áreas os resultados ainda são conflitantes. É importante salientar que as regiões regulatórias (promotora ou 3'UTR) do HLA-G exibem alto desequilíbrio de ligação, e haplótipos específicos apresentam frequências similares entre diferentes populações (Castelli et al. 2014). Neste contexto, apenas um estudo caracterizou os haplótipos estendidos (promotor e 3'UTR) do HLA-G em díades (materna-fetal) saudáveis e com PE severa (Nilsson et al. 2016). Apesar dos autores não observarem uma associação com o risco de PE, o que permanece a ser respondido, é se determinados haplótipos ou a combinação dos mesmos pode influenciar na expressão de

sHLA-G e severidade da doença, visto que variantes na região promotora do gene são associadas a menor atividade transcricional do HLA-G em mulheres com dificuldades em manter a gestação (Agrawal et al. 2015). No capítulo 3 o papel da região 3'UTR do *HLA-G* na suscetibilidade ao abortamento de repetição idiopático foi avaliado. No capítulo 6 são retomados esses resultados em uma discussão geral.

1.5. HLA-E

Diferentemente das outras moléculas não clássicas, o HLA-E é expresso em diversos tipos celulares (linfócitos, células B e NK, monócitos, macrófagos e células endoteliais) (Rölle et al. 2018). No entanto, a expressão de membrana do HLA-E depende da estabilização do complexo MHC por um *pool* bastante restrito de peptídeos (Sullivan et al. 2015; Celik et al. 2016). Esses peptídeos são derivados principalmente da sequência líder/peptídeo sinal de moléculas do MHC-I (HLA-A, -B, -C, -G) e de proteínas virais [citomegalovírus (CMV)] (Celik et al. 2016). Portanto, HLA-E e os peptídeos que o estabilizam podem ser considerados um potencial aloantígeno no contexto dos transplantes, devido ao seu potencial para ativação da resposta imunológica (Guberina et al. 2017). Além disso, a expressão de certos alelos de HLA-C no trofoblasto pode fornecer peptídeos para a estabilização do complexo pMHC-HLA-E. As propriedades imunológicas do HLA-E devem-se majoritariamente à interação com os receptores CD94-NKG2A (inibitório) e CD94-NKG2C (ativação) expressos nas células uNK e linfócitos T CD8+, mas também via αβ-TCR (Braud et al. 1998; Ishitani et al. 2003; Vento-Tormo et al. 2018). Desta forma o HLA-E é relevante na resposta imunológica adaptativa e inata. É importante salientar que o eixo imunológico HLA-E/NKG2C é dinâmico, e devem-se considerar alguns aspectos: 1) em condições fisiológicas, HLA-E possui menor afinidade para com NKG2C, 2) esta afinidade pode ser dependente de alelos específicos do HLA-E (E*01:01, E*01:03), 3) a deleção do gene *NKG2C* é frequente (~4%) (Miyashita 2004) e, 4) infecções virais podem usurpar a maquinaria de expressão do HLA-E (van Hall et al. 2010).

O HLA-E é o menos polimórfico dos MHC-Ib (https://www.ebi.ac.uk/ipd/). Entre os alelos reconhecidos, o HLA-E*0101 e E*0103 apresentam frequências similares em diferentes populações, e juntos, representam até 98% dos alelos (Felício et al. 2014). Interessantemente, quando se considera as variantes regulatórias e codificadoras do gene,

uma alta variabilidade é observada para o alelo E*0103, o qual se separa em três grandes linhagens alélicas: E*01031, E*01032a e E*01032b (Felício et al. 2014).

Os alelos E*0101 e E*0103 diferem em uma única mutação não sinônima no códon 107, ocasionando a troca de um aminoácido arginina (E*0101) para uma glicina (E*0103). Esta modificação não altera a fenda peptídica, embora modifique a afinidade dos peptídeos com o HLA-E através de outras modificações estruturais. O alelo E*0103 possui maior estabilidade a diferentes peptídeos, sendo detectado em maiores quantidades na membrana celular (Rölle et al. 2018).

Considerando o potencial imunológico do HLA-E, somente alguns estudos avaliaram o seu papel na suscetibilidade às desordens gestacionais (Persson et al. 2017). Na PE nenhuma associação do HLA-E com o risco é observada, embora alguns estudos reportem a associação do genótipo E*0101/E*0103 e o alelo E*0101 com o risco de perdas gestacionais recorrentes. É importante salientar que dependendo do contexto, as diferentes formas alélicas assumem diferentes características, ora são protetoras ora são deletérias (van Hall et al. 2010; Guberina et al. 2017; Rölle et al. 2018). Além disso, esta molécula pode interagir com outros HLA não clássicos, como o HLA-G, potencializando o mecanismo de escape imunológico utilizado pelas células do trofoblasto (Marín et al. 2003; Morandi and Pistoia 2014). Por último, é provável que o HLA-E solúvel tenha um papel imunorregulatório na gestação, uma vez que altos níveis solúveis são detectados em pacientes com malignidades (Allard et al. 2011; Morandi et al. 2016). No anexo 6.1.1, são apresentados os dados preliminares sobre a influência do eixo imunológico HLA-E/NKG2C na suscetibilidade à PE. No capítulo 4, complementamos as lacunas do conhecimento acerca do eixo imunológico HLA-E/NKG2C, através da avaliação clínica do mesmo na transplantação. No capítulo 6 são retomados esses resultados em uma discussão geral.

1.6. MIC-A

MIC-A é uma proteína de membrana cuja expressão é induzida por estresse celular, e que atua como ligante para o receptor NKG2D expresso em células citotóxicas, como linfócitos T CD8+ e NK. A expressão de MIC-A é restrita à apenas alguns tipos celulares: especialmente células epiteliais do trato gastrointestinal, células endoteliais, fibroblastos, monócitos, queratinócitos e células dendríticas (Baranwal and Mehra 2017). Os transcritos

de MIC-A são detectados na decídua, na placenta e células do trofoblasto em gestações saudáveis, embora a expressão física de MIC-A seja dificilmente detectada em tecidos placentários (Mincheva-Nilsson et al. 2006). É proposto que as formas solúveis de MIC-A (sMIC-A) participam da imunotolerância materno-fetal (Mincheva-Nilsson et al. 2006; HUANG et al. 2011). No entanto, níveis elevados de sMIC-A estão associados a falha de implantação em mulheres submetidas à fertilização *in vitro* (Porcu-Buisson et al. 2007), o que pode ser explicado pois sMIC-A leva a internalização do receptor NKG2D e resulta num estado anérgico das células NK (Isernhagen et al. 2015; Isernhagen et al. 2016). Em contrapartida, a expressão de MIC-A na membrana celular geralmente é um sinal para destruição celular pelas células citotóxicas (Baranwal and Mehra 2017).

Entre as variantes genéticas estudadas, a mais importante é o polimorfismo MIC-A Val129Met (rs1051792), o qual é caracterizado por uma substituição não sinônima de uma valina (Val) para uma metionina (Met). Esta variante influencia tanto os níveis de sMIC-A quanto a afinidade para o receptor NKG2D. É descrito que as moléculas 129Met possuem de 10-50 vezes maior afinidade a NKG2D solúvel comparado às moléculas 129Val (Steinle et al.). Isto sugere que esta variante tenha um papel relevante em desordens inflamatórias (Isernhagen et al. 2016) e possivelmente influencie na severidade/desenvolvimento da PE. Outro polimorfismo também associado com condições patológicas e níveis de expressão de sMICA é o -2778G/A (rs2596538) localizado na região promotora do gene (Lo et al. 2013; Michita et al. 2018a).

Recentemente, a variante Val129Met foi avaliada em mulheres com parto prematuro (PP) (Von Linsingen et al. 2018). Ainda que a associação de MIC-A Val129Met com o risco de PP não tenha sido observada nesse estudo, possivelmente, devido ao baixo tamanho amostral do estudo, os autores reportam uma correlação positiva com os níveis de sMIC-A e o genótipo Val/Val, o que corrobora nosso estudo prévio (Michita et al. 2018a).

O papel desta variante na suscetibilidade à PE, assim como em outras desordens gestacionais ainda é desconhecido, embora altos níveis de sMIC-A tenham sido observados em mulheres pré-eclâmpticas, mas frequentemente ausente em gestantes saudáveis (Haumonte et al. 2014). Atualmente, a hipótese aceita é que altos níveis circulantes de sMICA resultem na super-estimulação das células NK através do NKG2D, resultando em um fenótipo anérgico incapaz de promover suas funções efetoras (secreção de mediadores angiogênicos e inflamatórios), desta forma prejudicando o remodelamento vascular na

interface materno-fetal (Haumonte et al., 2014). Diante disso, no anexo 6.1.2 é apresentado os dados preliminares sobre a influência do eixo imunológico MIC-A/NKG2D na suscetibilidade à PE.

Considerando que as variantes genéticas do *MIC-A* influenciam na severidade de condições inflamatórias, apresentamos no capítulo 5 o efeito das variantes genéticas do *MIC-A* no desfecho clínico do transplante simultâneo de pâncreas e rim. No capítulo 6 as diferentes abordagens investigativas na avaliação do potencial imunorregulatório das moléculas do MHC-I são retomadas em uma discussão geral.

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1.8 Objetivos

1.8.1. Objetivo Geral

Avaliar o papel de variantes genéticas em genes envolvidos na imunorregulação da resposta imunológica na gestação saudável e patológica e avaliar o potencial imunorregulatório dos mesmos na manutenção de transplantes de órgãos sólidos.

1.8.2. Objetivos específicos

- Avaliar o efeito das variantes genéticas e haplótipos da região 3'UTR do *HLA*-G na susceptibilidade ao abortamento espontâneo de repetição idiopático: inserção/deleção de 14-pares de bases (pb) na posição +2960 (rs371194629), +3003 T/C (rs1707), +3027 C/A (rs17179101), +3035 C/T (rs17179108), +3142 G/C (rs1063320), +3187 A/G (rs9380142) e +3196 C/G (rs1610696) (**Capítulo 3**);
- Avaliar o eixo imunológico NKG2C/HLA-E na susceptibilidade à pré-eclâmpsia, através da determinação dos alelos do *HLA-E* e a deleção completa do gene *NKG2C* (**Anexo 6.1.1**);
- Avaliar o eixo imunológico NKG2C/HLA-E no desfecho do transplante simultâneo de pâncreas e rim durante um período de acompanhamento de um ano pós-transplante, através da determinação dos alelos do *HLA-E* e a deleção completa do gene *NKG2C* (**Capítulo 4**);
- Avaliar o eixo imunológico NKG2D/MIC-A na susceptibilidade à pré-eclâmpsia, através da genotipagem das variantes genéticas do gene *MIC-A* [-2778GA (rs2596538GA) na região promotora e Val129Met (rs1051792) na região codificadora] e NKG2D (rs1049174GC; região 3'UTR) (**Anexo 6.1.2**);
- Avaliar o eixo imunológico NKG2D/MIC-A no desfecho do transplante simultâneo de pâncreas e rim durante um período de acompanhamento de um ano pós-transplante, através da quantificação dos níveis solúveis de MIC-A e genotipagem das variantes genéticas do gene *MIC-A* [-2778GA (rs2596538GA) na região promotora e Val129Met (rs1051792) na região codificadora] e NKG2D (rs1049174GC; região 3'UTR) (**Capítulo 5**);

- Avaliar a contribuição das variantes genéticas do gene *MIC-A* [-2778GA (rs2596538GA) na região promotora e Val129Met (rs1051792)] nos níveis plasmáticos solúveis de MIC-A em 50 indivíduos controle saudáveis (**Capítulo 5)**.

Seção II

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Capítulo 2

Genetic Variants in Preeclampsia: Lessons From Studies in Latin-American Populations

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REVIEW
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Genetic Variants in Preeclampsia: Lessons From Studies in Latin-American Populations

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Placental vascularization is a tightly regulated physiological process in which the maternal immune system plays a fundamental role. Vascularization of the maternal-placental interface involves a wide range of mechanisms primarily orchestrated by the fetal extravillous trophoblast and maternal immune cells. In a healthy pregnancy, an immune cross-talk between the mother and fetal cells results in the secretion of immunomodulatory mediators, apoptosis of specific cells, cellular differentiation/proliferation, angiogenesis, and vasculogenesis, altogether favoring a suitable microenvironment for the developing embryo. In the context of vasculopathy underlying common pregnancy disorders, it is believed that inefficient invasion of extravillous trophoblast cells in the endometrium leads to a poor placental blood supply, which, in turn, leads to decreased secretion of angiogenic factors, hypoxia, and inflammation commonly associated with preterm delivery, intrauterine growth restriction, and preeclampsia. In this review, we will focus on studies published by Latin American research groups, providing an extensive review of the role of genetic variants from candidate genes involved in a broad spectrum of biological processes underlying the pathophysiology of preeclampsia. In addition, we will discuss how these studies contribute to fill gaps in the current understanding of preeclampsia. Finally, we discuss some trending topics from important fields associated with pregnancy vascular disorders (e.g., epigenetics, transplantation biology, and non-coding RNAs) and underscore their possible implications in the pathophysiology of preeclampsia. As a result, these efforts are expected to give an overview of the extent of scientific research produced in Latin America and encourage multicentric collaborations by highlighted regional research groups involved in preeclampsia investigation.

Keywords: preeclampsia, vasculopathy, endothelial damage, inflammation, SNPs, Latin America, polymorphism

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INTRODUCTION

In all pregnancies that can potentially lead to living birth, a major concern is the high prevalence of disorders that can affect healthy pregnancies. Maternal mortality is a global health issue. One of the eight goals of the United Nations Millennium Development Goals (MDG) was to reduce maternal mortality by three quarters from 1990 to 2015. As of 2013, the worldwide maternal mortality ratio has dropped 45%, yet maternal deaths are still the primary cause of death. For the same period, an

estimated 289,000 maternal deaths due to pregnancy- or childbirth-related complications occurred, particularly in developing countries, since mortality rates vary according to geographical area and different social and ethnic characteristics. These estimates expose the alarming healthcare situation in developing countries where the maternal mortality ratio is ∼14 times higher than in developed countries. Actual numbers might be even higher because only 51% of the countries evaluated in the MDG had data on maternal causes of death (United Nations, 2015). In Latin America, pregnancy vascular disorders are the leading cause of maternal mortality and morbidity (Khan et al., 2006). These disorders cover a wide range of clinically characterized phenotypes with a common underlying dysfunction in the endothelial and vascular systems, including preeclampsia (PE), and will be appropriately discussed in this review.

Owing to a lack of robust experimental animal models and ethical issues related to early pregnancy tissue usage, elucidation of the underlying mechanisms involved in the pathophysiology of pregnancy disorders remains the "holy grail" of reproductive biology. Considering that fetal cells inherit half paternal genetic material, this "non-self" status (compared to the mother) represents a challenge to the maternal immunological system. In this sense, a question naturally arises: How does the fetus avoid rejection by the maternal immune system? Since rejection occurs at different levels, it is reasonable to consider that genetic disparity, or the genetic background of the parents may account for an increased risk of pregnancy disorders (Goldenberg et al., 2009; Gardosi et al., 2013; Lisonkova and Joseph, 2013). Human pregnancy is a phenomenon that relies on immunological adaptations (Aghaeepour et al., 2017). Since maternal immune tolerance is essential to the maintenance of pregnancy, breakage of such tolerance is an accepted hypothesis for the occurrence of pregnancy-related disorders, including PE (Christiansen, 2013; Redman et al., 2014), which is briefly reviewed in sections Placental Vasculogenesis and Angiogenesis: Immune System and Vascular Remodeling During Pregnancy and Preeclampsia.

Pregnancy is a highly coordinated process that requires the involvement of a well-regulated network of biological mechanisms. Briefly, pregnancy establishment initiates through blastocyst implantation and endometrial invasion. Blastocyst invasion requires the expression of a wide range of factors by both maternal and fetal cells, including adhesion molecules, pregnancy hormones, and inflammatory mediators (Norwitz et al., 2001). In this context, inefficient blastocyst implantation is related to impaired endometrial vascular remodeling and immunological tolerance, which are commonly observed in a broad spectrum of pregnancy disorders. The extent of maternal physiological responses driven by the foreign developing embryo involves both maternal/paternal and fetal aspects. The response for such stimuli varies between healthy and pathological pregnancies, or even among individuals of the same group. This implies that the genetic variability is a critical component and accounts in the susceptibility for (but not limited to) pregnancy vascular disorders by influencing both local and systemic responses. In Latin America, the genetic and molecular basis of PE is a rapidly developing field of investigation, and many studies approaching basic science or even extending to cutting-edge technologies have been published and will be reviewed in the sections Genetic Studies in Latin-American Populations, Genetic Variation in Histocompatibility-Related Genes in PE, Gene Variants Involved in Metabolic Processes, and Variants in Detoxification, DNA-Repair, and Apoptosis-Related Genes.

Latin America contains a highly diverse human population. This admixed population is also under the influence of environmental factors, such as climate, lifestyle, and pathogen exposure. As pregnancy disorders are affected by both genetic and environmental factors, it is difficult to extrapolate data obtained in specific human populations to other ones. Therefore, we provide an extensive review of studies developed in Latin America (**Figure 1**) as a contribution to the understanding of pregnancy disorders, mainly focusing on PE. Since Brazil and Mexico are at the forefront of PE investigation in Latin America, we call attention to the lack of investigative studies in countries not represented here. Also, we highlight the urgent need for collaborative studies and extensive efforts to fill gaps in the current scenario of hypertensive pregnancy disorder epidemiology in Latin America. Here, we will discuss current knowledge about the role of the maternal immune system in pregnancy vasculogenesis and PE. Also, we will review the literature concerning genetic studies evaluating the contribution of single nucleotide polymorphisms (SNPs) in candidate genes from distinct biological systems and discuss their involvement in PE pathogenesis by analyzing data from Latin America as well as from other human populations when appropriate. For the sake of clarity, reference SNP cluster (rs#) will be cited as it appears in the text and the SNP nomenclature will be maintained according to the original cited article.

PLACENTAL VASCULOGENESIS AND ANGIOGENESIS: IMMUNE SYSTEM AND VASCULAR REMODELING DURING PREGNANCY

Tissue remodeling and angiogenesis are the results of a tightly regulated interaction between the immune system and the vascular system (Ribatti and Crivellato, 2009). In pregnancy, an adequate placental vascularization depends on the proliferation and differentiation of the trophoblast cells in the placental villi (Herr et al., 2010). Adaptation and changes in maternal anatomy and physiology are fundamental for the establishment of an adequate blood supply for the developing fetus (Boeldt and Bird, 2017). After implantation, the invasion of the endometrium by the cytotrophoblast drives the first steps of human placentation. Initially, myometrial spiral arteries are remodeled in the second trimester, changing from a high-resistance state of coiled vessels to dilated low-resistance vessels (Boeldt and Bird, 2017). In low-resistance vessels, the exchange of gas and nutrients is highly facilitated, since there is a decrease in blood flow to the intervillous spaces of the placenta (Boeldt and Bird, 2017). According to the immunological aspects of pregnancy, it is accepted that a mild pro-inflammatory stimulus is essential for local tissue remodeling, neovascularization, and

FIGURE 1 | The contribution from Latin American countries to the understanding of genetic predisposition in pregnancy vascular disorders. In the map, areas in red represent the number of published articles covered by our review in Latin America. Areas in weak red represent low numbers of publications, and areas in strong red represent countries/populations with a production of 50 articles or more.

the establishment of successful embryo attachment enabling fetal development (Chaouat, 2002). Decidual immune cells, invading trophoblasts and endothelial cells interact and orchestrate placental vascularization. Leukocytes represent 15–30% of all cells in human early pregnant decidua (Mincheva-Nilsson et al., 1994). The organization of these immune cells is unique and includes lymphoid cell clusters, and randomly distributed immune cells, such as uterine natural killer (uNK) cells, αβ-T, and γδ-T cells, dendritic cells (DCs), and macrophages. B cells and regulatory B cells are less represented in number, and their emerging roles in pregnancy are discussed elsewhere (Muzzio et al., 2013; Fettke et al., 2014; Mor et al., 2017; Esteve-Solé et al., 2018). uNK cells represent ∼70% of leukocytes in the decidua (Moffett-King, 2002), and are essential to the angiogenesis and maintenance of vascular stability by secreting specific sets of cytokines: the vascular endothelial growth factor C (VEGFC), the placental growth factor (PIGF), and angiopoietin 2 (ANG2) (Li et al., 2001).

PREECLAMPSIA

Worldwide, PE affects 2–8% of pregnant women. In addition, it accounts for ∼40% of preterm births (<35 weeks of gestation) (Khan et al., 2006; Duley, 2009). PE incidence differs mainly between low- and high-income countries. In Latin American countries, ∼26% of maternal deaths are attributed to PE. However, the actual impact of PE in developing countries is underestimated due to differences in PE diagnostic criteria and the fact that reporting the maternal cause of death is not compulsory in several countries (Giachini et al., 2017).

PE usually manifests in the second trimester. Although new definitions for PE include organ dysfunction (Tranquilli et al., 2014) and no longer require proteinuria if other severe PE features are present (ACOG, 2013), traditionally PE is defined by onset of hypertension after 20 weeks of gestation (systolic ≥140 mmHg; diastolic ≥90 mmHg), proteinuria (≥300 mg/24 h or protein/creatinine ratio ≥0.5 in random sample) and edema. While untreated PE can be lethal, the clinical complications vary and include seizures, liver rupture, pulmonary edema, and renal insufficiency (Adu-Bonsaffoh et al., 2013). Despite advances in the clinical management of PE (symptomatic treatment), the only effective treatment remains clinical intervention and delivery, resulting in low birth weight and premature birth. In fact, ∼23% of low birth weight and ∼20% of preterm birth occurrences in Latin America are attributed to PE (Bilano et al., 2014). In clinical practice, therapies involving antiplatelet agents such as low aspirin doses (Duley et al., 2007; Roberge et al., 2013; Xu et al., 2015; ACOG, 2018) and calcium supplementation in women with low calcium diets (Hofmeyr et al., 2014) have proven to bring small to moderate benefit to women with high risk pregnancies. Symptomatic treatments include different strategies targeting gestational hypertension (antihypertensive therapy), eclamptic seizures (anticonvulsive therapy), and other symptoms as reviewed elsewhere (Ramos et al., 2017).

The impact of PE on both maternal and fetal health goes beyond pregnancy, and represents a significant burden on public health services, especially, in low-income countries where the incidence rates can reach up to 6% in Latin America, 2.3% in Africa, and 3.2% in Asia (Bilano et al., 2014). Preeclamptic women have an increased risk of post-partum depression, cardiovascular disorders, metabolic diseases and hypertension later in life (Ramsay et al., 2003; Hoedjes et al., 2011; Behrens et al., 2017; Neiger, 2017; Timpka et al., 2017; Zoet et al., 2018), while newborns are at higher risk to develop autistic spectrum disorders, cerebral palsy, and bronchopulmonary dysplasia due to low birth weight and preterm birth (Hansen et al., 2010; Mann et al., 2010; Strand et al., 2013).

Despite extensive efforts in the last two decades, the etiopathology of PE is still unclear, although some environmental and genetic risk factors have been reported (Fong et al., 2014; Ye et al., 2017). The variety of candidate genes evaluated by Latin American research groups and the critical events of each stage of PE development are summarized in **Figure 2** (for more details see Redman, 2014; Redman et al., 2014). Classically, PE development follows a two-stage model including a pre-clinical and a clinical period (Redman, 1991). This model was recently updated into a sequential four- and six-stage model to accommodate all immune aspects of PE: In the first stage of PE, environmental and genetic factors represent a critical component. The latter element involves several genes from different signaling pathways, revealing the polygenic nature of PE (for example, it is suggested that limited exposure to paternal antigens likely increases PE risk, being clinically relevant in primiparous women). In the next stage, inefficient trophoblast invasion in the decidua may result

in poor placentation and abnormal uteroplacental perfusion. In the third stage, placental ischemia and hypoxia result in local oxidative stress and inflammatory response. Secondary to placental damage, in the fourth stage, impaired secretion of placental and maternal factors lead to the manifestation of the clinical symptoms of PE. In the fifth stage, diagnosis of PE is clear. At this stage, the vascular damage is augmented in response to systemic inflammation (i.e., Th1/Th17 cytokines). The last stage characterizes a more severe form of the disorder (observed in up to 40% of placentas) and involves atherosis, a focal lesion in the spiral arterial wall associated with placental infarction and arterial thrombosis (Harsem et al., 2007).

Placental hypoxia and impaired perfusion lead to the release of reactive oxygen species (ROS) and endothelial damage. Thus, the release of fetal cell debris and syncytiotrophoblast microparticles into maternal circulation prompts an intense pro-inflammatory response by maternal immune cells (Redman and Sargent, 2000; Sibai et al., 2005). Also worth mentioning is the pregnancy stress test hypothesis, which postulates that pregnancy is a maternal stress test for the vascular, metabolic or immunological systems (Williams, 2003; Roberts and Hubel, 2010; Myatt and Roberts, 2015). Following this idea, women with pre-existing vascular dysfunction would present a lower threshold for the stress test, and a higher predisposition to develop PE and chronic disorders later in life.

PE might also be the manifestation of two extreme situations converging in a common phenotype. Sometimes, in maternal PE, normal placentation occurs in women with the pre-existing chronic disease. Conversely, in placental PE, abnormal placentation results in poor placental perfusion (Valenzuela et al., 2012). This concept highlights a not exclusive dependency of PE in placentation failure and explains the variability of clinical phenotypes and timing of PE development.

Familial history and hypertensive disorders increase the risk of PE, implying that the genetic components are also risk-modifying factors (Bezerra et al., 2010). PE is a polygenic disorder, and although no single genetic variant is believed to be responsible for all cases of PE, individual *loci*, environmental factors, and epistasis are components that should not be neglected (Staines-Urias et al., 2012; Williams, 2016). In this sense, the evaluation of genetic variants in PE risk could partially explain disorder susceptibility and would be of great importance to identify candidate targets for gene-gene interaction analyses, as well as to better follow-up/management of women at higher risk.

GENETIC STUDIES IN LATIN-AMERICAN POPULATIONS

Pro- and Anti-inflammatory Mediators in PE

In Latin America, several immune-related genes have been evaluated, and most of the studies are summarized in **Table 1.** For example, costimulatory molecules play a role in immune cell differentiation and activation, SNPs in the *CTLA4* (rs231775), *CD28* (rs3116496), and *ICOS* (rs4675378) were evaluated in Brazilian women with PE (Pendeloski et al., 2011). An association between the *ICOS* (−1564 T/C) SNP and PE was suggested based on a lower frequency of the *ICOS* "T" allele and the "TT" genotype in PE cases compared to controls. A systemic inflammatory response mediated by cytokines can cause endothelial damage, and thus it plays a central role in PE severity. In this scenario, six SNPs of pro-inflammatory genes were studied: *IL1R1* (rs2234650), *IL12* (rs3212227), *IL18* (rs187238), *IL18* (rs1946519), *TLR2* (rs5743708), and *TLR4* (rs4986790). However, no differences in genotypic and allelic frequencies between PE and controls were observed (Franchim et al., 2011). In a Northern Mexico population study, the association between PE risk and the *TGFB1* SNPs: −800G/A (rs1800468), −509C/T (rs1800469), and +869T/C (rs1800470) and their haplotypes were evaluated. No association between PE development and the SNPs or haplotypes was observed, although the +869TT genotype was suggested as a protective factor against severe PE (Aguilar-Duran et al., 2014).

Since different cytokine profiles have been associated with PE development (Saito and Sakai, 2003), de Lima et al. (2009) investigated SNPs of cytokine genes in eclampsia and PE in northwestern Brazilian individuals. They evaluated the SNPs *TNFA* (−308 G>A), *IL6* (−174 G>C), *IFNG* (+874 A>T), *IL10* (−1082 A>G, −819 C>T, −592 C>A), and *TGFB1* (+869 T>C, +915 G>C). No differences in genotypes and allelic frequencies were observed (de Lima et al., 2009). However, in a previous study by the same group, individuals were stratified according to ethnic origin in Caucasian and non-Caucasian, and the association of PE with *TNFA* (−308), *TGFB1* (+10;25), *IL10* (−1082), *IL6* (−174), and *IFNG* (+874) SNPs was evaluated. Intriguingly, the *IL10* −1082G/G SNP was associated with PE in Caucasian women, which is the most frequent allelic variant in people of African ancestry (Daher et al., 2006). A possible association between SNPs in *IL1B* was investigated in Brazilian women with severe PE. In this study, the "rs1143630 T" allele was associated with PE (Leme Galvão et al., 2016). In a Maya-Mestizo population sample, no association between *TNFA* (−308G/A, −850C/T) SNPs and PE was observed (Canto-Cetina et al., 2007). Another study reported no association of *IL10* (rs1800896), *IL6* (rs1800795), and *IL1RA* variable number of tandem repeats (VNTR) in intron 2 with PE susceptibility in Mexican-Mestizo women and Maya-Amerindian women from Mexico (Valencia Villalvazo et al., 2012).

The influence of *TNFA*, *IL6*, *IFNG,* and *IL10* gene SNPs and their relationship with cytokine plasma levels in severe PE, normotensive pregnancy, and in non-pregnant women from Brazil was investigated by Pinheiro et al. (2015). The SNPs evaluated in the study were *TNFA* (−308), *TGFB1* (+10;25), *IL10* (−1082), *IL6* (−174), and *IFNG* (+874). Interesting, they observed higher IL-10 levels in normotensive pregnant women compared to preeclamptic women. Conversely, higher plasma levels of IL-6 and IFN-γ were detected in PE in comparison to non-pregnant and normotensive pregnant women. Also, a positive correlation between IFN-γ plasma levels and the *IFNG* +874T allele was observed, and when the three groups were evaluated separately, a positive correlation between IL-6 levels

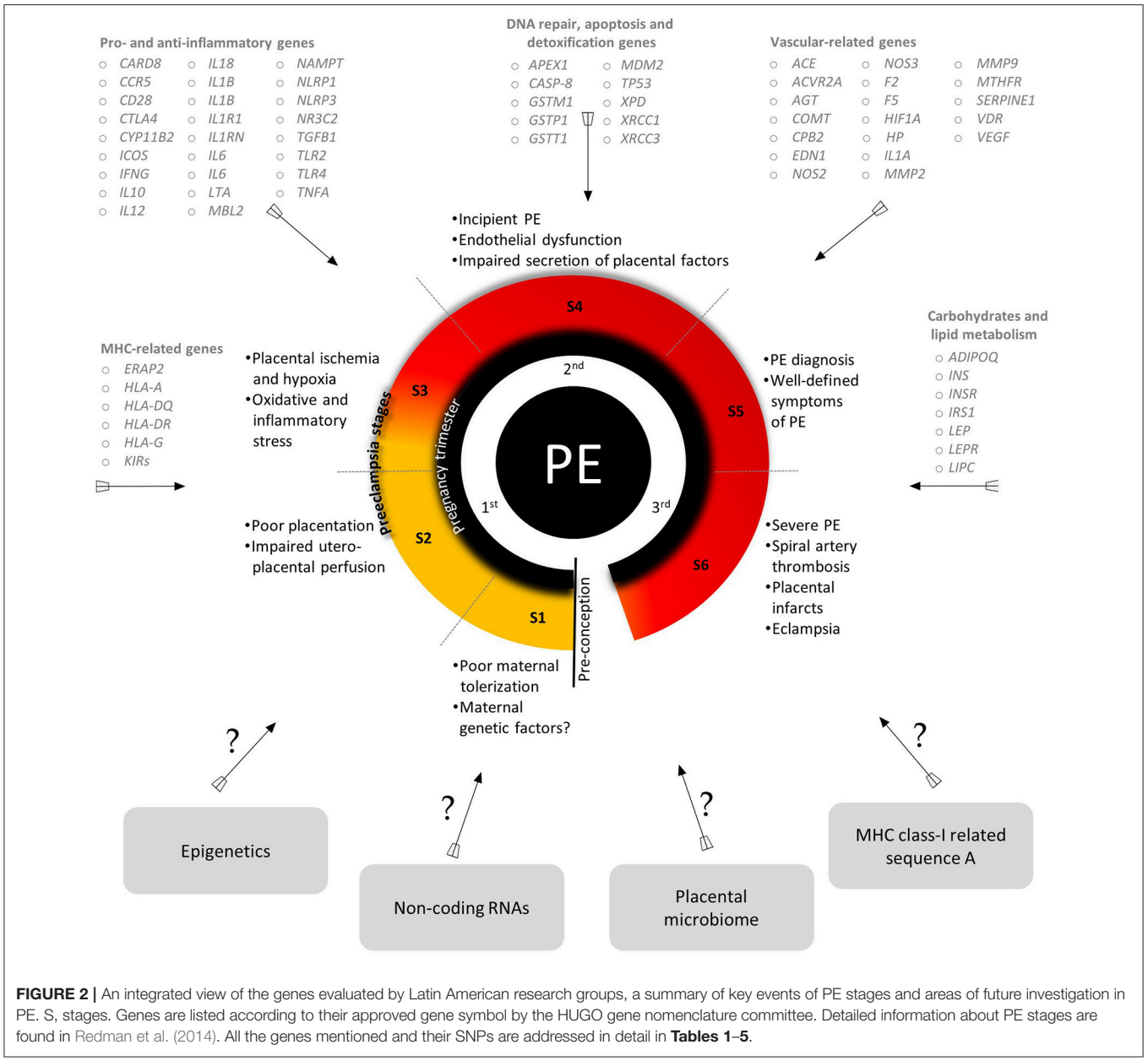

FIGURE 2 | An integrated view of the genes evaluated by Latin American research groups, a summary of key events of PE stages and areas of future investigation in PE. S, stages. Genes are listed according to their approved gene symbol by the HUGO gene nomenclature committee. Detailed information about PE stages are found in Redman et al. (2014). All the genes mentioned and their SNPs are addressed in detail in **Tables 1**–**5**.

and the presence of the IL6 −174C allele in normotensive pregnant women was observed (Pinheiro et al., 2015).

Mannose-binding lectin (MBL) is a pro-inflammatory protein that modulates inflammation and ultimately induces apoptosis (Turner, 2003). Polymorphisms in the *MBL2* gene located at exon 1: at codons 54 (allele B, rs1800450), 57 (allele C, rs1800451), and 52 (allele D, rs5030737) were evaluated in women with PE and in healthy pregnant controls from Brazil. The absence of all the variants characterize the wild-type allele "A." In this study, an association between genotypes coding for low MBL levels and a severe PE was evidenced. In the AD genotype, the C and D alleles were more frequent in PE compared to controls. Moreover, in relation to MBL levels, three groups of haplotypes were observed: group 1 (H/L)YA/(H/L)YA and (H/L)YA/LXA genotypes were related to high MBL serum levels; group 2 encompasses LXA/LXA and (H/L)YA/O genotypes, which were related to intermediate MBL serum levels; and group 3 was defined by low MBL serum levels, resulting in MBL deficiency, corresponding to LXA/O and O/O genotypes (Vianna et al., 2010). Cysteine-cysteine chemokine receptor type 5 (CCR5) is an essential receptor for inflammatory reactions expressed in leukocytes and other cell types (Barmania and Pepper, 2013). CCR5Δ32 is a 32-base pair deletion in the *CCR5* that, in homozygosis, results in a lack of expression of the functional CCR5 on the cell surface. Heterozygous carriers express lower levels of functional CCR5 compared to wild-type homozygous individuals (Venkatesan et al., 2002). Considering the intense inflammatory response in PE, and based on the high frequency

of the deletion allele in healthy pregnant women, Telini et al. (2014) suggested a protective role of the *CCR5Δ32* allele against PE development in a Brazilian study.

Adipocytokines are involved in trophoblast invasion and successful placentation. Visfatin is an adipocytokine also known as nicotinamide phosphoribosyltransferase (NAMPT), which has a potential role in the pathophysiology of metabolic disorders such as hypertension and obesity (Dahl et al., 2012). A study by Luizon et al. (2015) evaluated visfatin/NAMPT plasma levels in healthy pregnant women and in patients with gestational hypertension or PE, in the context of the *NAMPT* SNPs −423T<C (rs1319501) and rs3801266A<G in intron 1. No effects were observed concerning rs1319501. Nevertheless, gestational hypertensive patients carrying the rs3801266 "AG" and "GG" genotypes had higher visfatin/NAMPT levels compared to gestational hypertensive patients carrying the "AA" genotype (Luizon et al., 2015). Moreover, Luizon et al. (2017) evaluated whether *NAMPT* SNPs (rs1319501T>C, rs3801266A>G), haplotypes and gene-gene interactions in the NAMPT pathway could affect plasma visfatin/NAMPT levels, and the response to antihypertensive therapy in PE and hypertensive pregnant women. Low circulating visfatin/NAMPT levels were seen in non-responsive PE patients with the rs1319501 TC+CC genotypes. Conversely, high circulating visfatin/NAMPT levels were detected in non-responsive PE patients with the rs3801266 AG+GG genotypes. Haplotype analysis revealed an association of the 'C, A' haplotype with response to antihypertensive treatment and with low visfatin/NAMPT levels in PE (Luizon et al., 2017). Since lymphotoxin alpha (LTα) is an inflammatory mediator, this molecule was evaluated in the context of PE development, but no association of LTA +252 (rs909253) with PE risk was reported in a Brazilian study (Pissetti et al., 2015).

In order to evaluate the contribution of the inflammasome in PE development, SNPs in the genes coding for nod-like receptors with a pyrin domain 1 (*NLRP1*), *NLRP3*, caspase recruitment domain 8 (*CARD8*), and *IL1B* were studied in a Brazilian population. The *NLRP1* rs12150220A/T SNP was associated with PE. The minor "T" allele was more frequent in PE compared to healthy pregnancy controls, indicating that this allele might be relevant in PE susceptibility. A strong association with *NLRP1* (rs12150550) was also observed in this study, suggesting a role for this molecule in the pathogenesis of PE. Besides, *NLRP1* SNPs produce six main haplotypes, and the rs11651270/C-rs12150220/A-rs2670660/A combination was less frequent amongst PE women compared to controls, suggesting a protective effect against PE (Pontillo et al., 2015).

In the context of gestational hypertension, the relationship between aldosterone levels and SNPs of the aldosterone synthase (*CYP11B2*) gene (−344T/C) and the mineralocorticoid receptor gene (S810L) was investigated in a Mexican population. No differences in genotype distributions or in aldosterone levels were found (Ramírez-Salazar et al., 2011). Similar results were obtained for a Brazilian population (de Vasconcelos et al., 2009).

In summary, several studies covered in this review (**Table 1**), and other approaches have reinforced that PE is a polygenic disorder and manifests as complex phenotypes, resulting from both maternal and fetal genetic features (Triche et al., 2014). In Latin American populations, conflicting results regarding genetic variants and PE risk were observed, implying that genetic variability does account for this complex phenomenon. Therefore, the search for potential genetic components involved in PE, or its severity, is of paramount importance for a better understanding of the genetic basis of PE pathophysiology (**Figure 2**). Importantly, we observed a worrying lack of family-based studies evaluating the genetic components of both the fetus/placenta and its biological parents. Thus, such an approach would provide a more actual picture of the genetic risk factors involved in PE and possibly a more accurate disease monitoring and clinical management.

Vascular and Angiogenic Mediators

Studies also examined gene variants involved in vasculogenesis and angiogenesis, given the importance of establishing an adequate and efficient placental vascular system for a favorable gestational outcome (Herr et al., 2010). Studies from Latin America are summarized in **Table 2**. Nitric Oxide (NO) has a primary role in the circulatory system. Also, NO is a critical regulatory molecule in ovulation, embryo implantation, pregnancy maintenance, labor, and delivery. Imbalances in NO levels during gestation were suggested as a cause of the development of pregnancy-induced hypertension and PE (Maul et al., 2003). Several studies have evaluated SNPs in both endothelial and inducible nitric oxide synthase genes (*eNOS* and *iNOS*, respectively). In a multicenter study in Colombia, Serrano et al. (2004) evaluated the role of *eNOS* SNPs: Glu298Asp, −786T→ C, and VNTR b/a (27 bp-tandem repeat, where "a" and "b" refer to PCR product size, comprising 420 bp for "b" and 393 bp for "a" alleles) as potential risk factors for PE. Young Colombian women homozygous for the Asp298 allele were reported to have an increased risk for PE. The authors suggested that homozygous women for the Asp298 allele are more susceptible to endothelial dysfunction and at increased risk for PE development, since the homozygous state is likely to generate low NO levels. In addition, the presence of the "Asp298-786C-4b" haplotype was associated with an increased PE risk (Serrano et al., 2004). Similarly, Sandrim et al. (2008) compared the same *eNOS* SNPs in women with and without PE from Brazil. Interestingly, the study observed that two *eNOS* haplotypes ("T Glu a" and "C Glu a") were associated with PE and gestational hypertension. The same SNPs were also evaluated in a Maya-Mestizo Mexican population. The Asp298 allele was associated with PE in a recessive model. In addition, the "−786C-4b-Asp298" haplotype was more frequent in PE than in controls, whereas the "−786T-4b-Asp298" and "−786C-4b-Glu298" haplotypes had lower frequencies or were absent in patients (Díaz-Olguín et al., 2011). In another study, Alpoim et al. (2014) evaluated these same *eNOS* SNPs in early and late severe preeclamptic Brazilian women, and in a group of normotensive/healthy pregnant controls. The frequency of the 894T allele was higher in late severe PE compared to early severe PE. Also, the overall 894T frequency was higher in PE when compared to controls. Regarding the VNTR b/a SNP, higher "aa" genotype and "a"

TABLE 1 | Summary of studies developed in Latin America evaluating the role of genetic variation in pro- and anti-inflammatory mediators in PE.

Factors	Sample size[†]	Key findings	Country	References
ICOS (T-1564C) *CTLA4* (A49G) *CD28* (T17C)	130/260	Association with protection for PE: ICOS−1564T allele and−1564TT genotype.	Brazil	Pendeloski et al., 2011
TGFB1 (G800A, C509T, T869C)	175/253	Association with protection for severe PE: TGFB1 869TT genotype.	Mexico	Aguilar-Duran et al., 2014
IL1R1 (rs2234650) *IL12* (rs3212227) *IL18* (rs187238, rs1946519) *TLR2* (rs5743708) *TLR4* (rs4986790)	109/174	No association with PE.	Brazil	Franchim et al., 2011
TNFA (G308A) *IL6* (G174C) *IFNG* (A874T) *IL10* (A1082G, C819T, C592A) *TGFB1* (T869C, G915C)	165/101[a]	No association with PE.	Brazil	de Lima et al., 2009
TNFA (G308A) *TGFB1* (T10C, C25G) *IL10* (G1082A) *IL6* (G174C) *IFNG* (A874T)	151/189[b]	Association with PE risk: IL10−1082GG genotype in white women.	Brazil	Daher et al., 2006
IL1B (rs1143630)	169/287	Association with PE risk: IL1B rs1143630 'T' allele.	Brazil	Leme Galvão et al., 2016
TNFA (G308A,C850T)	105/200	No association with PE.	Mexico	Canto-Cetina et al., 2007
IL10 (G1082A) *IL6* (G174C) *IL1RA* (86bp-VNTR)	411/613	No association with PE.	Mexico	Valencia Villalvazo et al., 2012
TNFA (G308A) *IL6* (G-174C) *IFNG* (A874T) *IL10* (A1082G, C819T, C592A) *TGFB1* (T869C,G915C)	116/165[c]	Association with protection for PE: IL6−174C allele.	Brazil	Pinheiro et al., 2015
MBL2 allele B (rs1800450), allele C (rs1800451), allele D (rs5030737)	157/162	Association with PE severity: "AD" genotype, "C" and "D" alleles.	Brazil	Vianna et al., 2010
CCR5 (CCR5Δ32)	155/144	Association with protection for PE: CCR5Δ32 allele.	Brazil	Telini et al., 2014
NAMPT (rs3801266)	389/212[d]	Association with GH: rs3801266 "AG" and "GG" genotypes.	Brazil	Luizon et al., 2015
NAMPT (rs1319501; rs3801266)	379/207[e]	Association with PE risk: rs1319501 "TC+CC" and rs3801266 "AG+GG" genotypes.	Brazil	Luizon et al., 2017
LTA (+252A>G)	30/115	No association with PE.	Brazil	Pissetti et al., 2015
NLRP1 (rs11651270, rs12150550, rs2670660) *NLRP3* (rs35829419, rs10754558) *CARD8* (rs2043211, rs6509365) *IL1B* (rs1143634)	286/309	Association with risk for PE: rs12150220 (L155H) and the "rs11651270/C-rs12150220/A-rs2670660/A" haplotype.	Brazil	Pontillo et al., 2015
CYP11B2 (T344C) *MR* (S810L)	100/100	No association with PE.	Mexico	Ramírez-Salazar et al., 2011
CYP11B2 (T344C)	185/118[f]	No association with PE.	Brazil	de Vasconcelos et al., 2009

[†] *Pooled cases/controls.*

[a] *Cases were grouped according severity: PE (n = 92) and eclampsia (n = 73).*

[b] *Studied population was grouped according to skin color (white and non-white); white: PE (n = 56) and control (n = 92); non-white: PE (n = 95) and control (n = 97).*

[c] *Cases were compared to healthy pregnant (n =107) and non-pregnant women (n = 58).*

[d] *Cases correspond to PE (n = 208) and gestational hypertension (GH) cases (n=181).*

[e] *Cases were grouped according to disorder severity and response to anti-hypertensive therapy: PE responsive (n = 60) and non-responsive (n = 145); GH responsive (n = 120) and non-responsive (n= 54).*

[f] *Cases were grouped in PE (n = 70) and GH (n = 115).*

allele frequencies were observed in early severe PE compared to late severe PE and controls. Also, the "T-b-C" haplotype was more frequent in late severe PE compared to early severe PE and controls.

Although anti-hypertensive treatment has never been demonstrated to reverse PE outcome, its usage could prevent cardiovascular and cerebrovascular adverse consequences, due to severe and rapid elevations of the blood pressure, being a critical tool for clinical PE management. In this sense, it was proposed that anti-hypertensive therapy can enable maintenance of gestation and increase the gestational age of delivery, thus decreasing adverse fetal and maternal outcomes (Podymow and August, 2008). In this context, an elegant study compared the distribution of *eNOS* variants in gestational hypertensive and PE cases who were responsive to anti-hypertensive therapy versus cases who did not respond to treatment. Interestingly, a difference in the overall distribution of *eNOS* haplotypes was observed when PE responsive to treatment groups and PE nonresponsive to treatment groups were compared. The "C Glu a" haplotype was more frequent in the responsive PE group than in the nonresponsive PE group, and the "T Asp a" haplotype was less frequent in the active PE group than in the nonresponsive PE group. This was a pioneer study approaching the genetic background in the context of gestational hypertension treatment (Sandrim et al., 2010b).

The distribution of two *eNOS* Tag SNPs, rs743506 and rs7830, as well as the SNPs T-768C, Intron-4, and G894T, among healthy pregnant controls, gestational hypertensive subjects, and PE subjects was assessed by Muniz et al. (2012). No differences were detected among genotype frequencies in the three groups studied. However, the haplotype H5 "CbGGC" ("C" of rs2070744, "b" of intron 4, "G" of rs1799983, "G" of rs743506, and "C" of rs7830) was more frequent in the control group compared to gestational hypertensive and PE individuals, suggesting a potential protective effect against hypertensive disorders development in pregnancy.

Two *iNOS* SNPs, C-1026A (rs2779249) and G2087A (rs2297518), were evaluated in Brazilian healthy pregnant/control, gestational hypertension, and PE groups. The "GA" genotype and the "A" allele for the G2087A were more commonly found amongst PE subjects. No differences were observed concerning the other variants evaluated (Amaral et al., 2012).

Considering that increased levels of hemoglobin (Hb) and haptoglobin (Hp) complexes contribute to impaired NO bioavailability in PE (Sandrim et al., 2010a), the role of a haptoglobin SNP (duplication of exons 3 and 4 of *HP* gene) was evaluated in PE and non-pregnant women, in the context of NO bioavailability. Higher NO consumption was detected in association with increased cell-free Hb in plasma from PE patients carrying the allele *HP2* (duplicated exons 3 and 4 of the *HP1*), suggesting a functional association between *HP* SNPs and the hemodynamic imbalances observed in PE (Sertório et al., 2013).

Thrombin-activated fibrinolysis inhibitor (*TAFI*) gene has also attracted attention in the context of SNPs and their possible association with vascular disorders in pregnancy. In this scenario, a case-control study investigated the possible association between PE and *TAFI* SNPs (G505A, C1040T, and G-438A), together with TAFIa plasma levels in a Mexican-Mestizo population. No associations with increased PE risk were observed. However, due to higher plasma TAFIa levels and the presence of the G505A mutant genotype, together with wild-type forms of C1040C and G-438G, it was suggested that *TAFI* SNPs in the coding region or in nearby regulatory elements could contribute to variations in TAFIa plasma concentrations (Acosta-Tejeda et al., 2011).

The methylenetetrahydrofolate reductase (MTHFR) enzyme is critical for homocysteine (HCY) metabolism, where it catalyzes the NADPH-linked reduction of 5,10-MTHF to 5-MTHF, and subsequently the methylation of HCY to methionine in a vitamin B12-dependent manner (Barbosa et al., 2008). In a Tunisian study, low MTHFR activity levels were associated with mild to moderate increases in plasma HCY levels in placental vascular complications (Klai et al., 2011). In the same study, the *MTHFR* A1298C variant was associated with recurrent pregnancy loss, intrauterine growth restriction, and placental abruption. In the context of PE, a differential distribution of the *MTHFR* C677T alleles was associated with thrombosis markers and endothelial activation in a study with Mexican women (Rojas et al., 2010). Moreover, a possible association between C677T SNP of *MTHFR* gene and PE was investigated in pregnant women from the Yucatan Peninsula in southeastern Mexico, although no differences between cases and controls were observed (Pérez-Mutul et al., 2004).

In another study evaluating *MTHFR* (C677T) in Maya-Mestizo PE women, it was observed that *MTHFR* "T" allele and the "TT" genotype were more frequent in controls, suggesting a decreased risk of PE in women carrying this variant (Canto et al., 2008). Amongst a Mestizo-Ecuadorian population, the prevalence of C677T and A1298C *MTHFR* SNPs was also investigated in the context of PE, with the "CC" genotype of A1298C occurring in higher prevalence in PE women than controls (Chedraui et al., 2014). Nevertheless, contradictory results regarding PE, the placental genotype, and allele frequencies of the *MTHFR* C677T were observed (Chedraui et al., 2015). Interestingly, for the C677T SNP, the mutant "TT" genotype was threefold more frequent in preeclamptic placentas than controls. In a Chilean population, epistatic interactions between *MTHFR* and catechol-O-methyltransferase (COMT) gene were evaluated in maternal-fetus dyads. The increased PE risk was observed exclusively when the fetus harbored both the *COMT* "ATCA" haplotype (respectively composed by the SNPs rs6269, rs4633, rs4818, rs4680) and the *MTHFR* 677T allele (Hill et al., 2011a).

SNPs in the vascular endothelial growth factor (*VEGF*) gene are also largely studied in PE. Importantly, the low production of VEGF by peripheral blood mononuclear cells is associated with PE (Cardenas-Mondragon et al., 2014). The possible role of SNPs at the promoter region of *VEGF* was addressed by Sandrim et al. (2009). The study reported an association between PE development and the SNPs −2578C/A (rs699947), −1154G/A (rs1570360), and −634G/C (rs2010963). Importantly, inter-ethnic differences account for differential allelic and haplotype distributions, and this is particularly relevant for Latin American populations. In this context, the authors observed that *VEGF*

TABLE 2 | Summary of studies developed in Latin America evaluating the role of genetic variation in vascular- and angiogenesis-related genes in PE.

Factors	Sample size[†]	Key findings	Country	References
eNOS (−786T>C, intron-4 b/a, Glu298Asp)	322/522	Association with PE risk: 298Asp/Asp genotype and *eNOS* C-b-Asp haplotype.	Colombia	Serrano et al., 2004
eNOS (−786T>C, intron-4 b/a, Glu298Asp)	216/110[a]	Association with PE and GH risk: *eNOS* C-a-Glu haplotype. Association with protection for PE and GH: *eNOS* T-a-Glu haplotype	Brazil	Sandrim et al., 2008
e*NOS* (−786T>C, intron-4 b/a, Glu298Asp)	127/263	Association with PE risk: 298Asp/Asp genotype and *eNOS* C-b-Asp.	Mexico	Díaz-Olguín et al., 2011
eNOS (−786T>C, intron-4 b/a, Glu298Asp) MMP2 (C-1306T) MMP9 (C-1562T)	77/266	Association with PE risk and severity: −786CC genotype and −786C allele, respectively.	Brazil	Leonardo et al., 2015
eNOS (−786T>C, intron-4 b/a, Glu298Asp)	98/103[b]	Association with late-onset PE risk: 298Asp/Asp genotype and 298Asp allele; intron-4 aa genotype and a allele; *eNOS* C-b-Asp.	Brazil	Alpoim et al., 2014
eNOS (−786T>C), intron-4 b/a, Glu298Asp	152/152[c]	Association with anti-hypertensive therapy in PE, *eNOS* haplotypes: C-a-Glu responsive and T-a-Asp non-responsive.	Brazil	Sandrim et al., 2010a
eNOS (−786T>C, intron-4 b/a, Glu298Asp, rs743506, rs7830)	295/122[d]	Association with protection for PE and GH: eNOS C-b-Glu-G-C haplotype.	Brazil	Muniz et al., 2012
eNOS (C-1026A, G2087A)	353/212[e]	Association with PE risk: 2087GA genotype and the 2087A allele.	Brazil	Amaral et al., 2012
HP (Hp1-1, Hp2-1, Hp2-2)	92/105	No association with PE risk. Nitric Oxide byproducts in PE associated with Hp2-1 and Hp2-2 genotypes.	Brazil	Sertório et al., 2013
TAFI (G505A, C1040T, G-438A)	87/87	No association with PE.	Mexico	Acosta-Tejeda et al., 2011
MTHFR (C677T) *FV LEIDEN* (G1691A) PROTHROMBIN (G20210A)	28/41	No association with PE.	Mexico	Rojas et al., 2010
MTHFR (C677T)	148/490[f]	No association with PE	Mexico	Pérez-Mutul et al., 2004
MTHFR (C677T, A1298C)	150/150	Association with PE risk: 1298CC genotype.	Ecuador	Chedraui et al., 2014
MTHFR (C677T)	125/274	Association with protection for PE: 677TT genotype and 677T allele.	Mexico	Canto et al., 2008
VEGF (C-2578A, G-1154A, G-634C)	195/108[g]	Association with protection for PE: *VEGF*−2578C/-1154G/-634C haplotype. Low proportion of-2578AA and−634GG genotypes in white PE women.	Brazil	Sandrim et al., 2009
VEGF (C936T, C-2578A)	52/28	Association with protection for PE: *VEGF*−2578A allele.	Brazil	Cunha et al., 2011
VEGF (C2578A, G634C)	113[h]	No association with PE.	Brazil	Sandrim et al., 2015
VEGF (G634C) *IL1A* (rs3783550)	79/210	Association with PE risk: *IL1A* rs3783550 "A" allele.	Brazil	Silva et al., 2015
VEGF (A2578C, C1498T, A1154G, C634G,C936T)	31/31[i]	No association with PE.	Ecuador	Chedraui et al., 2013
eNOS (T786C, VNTR, G894T) *MTHFR* (C677T) *AGT* (C704T)	230/350	No association with PE.	Mexico	Coral-Vázquez et al., 2013
MMP9 (C1562T, (CA)n repeats)	300/176[j]	Association with risk for GH: *MMP9* C1562 T allele. No association with PE.	Brazil	Palei et al., 2010
eNOS (T786C, VNTR, G894T) *MMP9* (C1562T, (CA)n repeats) *VEGF* (C2578A, G634C)	229/102[k]	Association with protection for PE: combination of MMP9-1562CC with VEGF-634GG genotypes. Association with PE risk: combination of MMP9-1562CC with VEGF-634CC or MMP9-1562CT with VEGF-634CC or-634GG genotypes.	Brazil	Luizon et al., 2012
MMP2 (C1306T, C735T)	263/130[l]	No association with PE.	Brazil	Palei et al., 2012a
MMP9 (C1562T, (CA)n repeats)	399/214[m]	Association with GH: combination of the "T" allele for the C1562T and "H" allele of 90(CA)13–25.	Brazil	Palei et al., 2012b

(Continued)

TABLE 2 | Continued

Factors	Sample size[†]	Key findings	Country	References
MTHFR (C677T) *Factor II* (G20210A) *FV LEIDEN* (G1691A) *PAI1* (4G/5G I/D)	75/145	No association with PE.	Brazil	Dalmáz et al., 2006
MTHFR (C677T) *FV LEIDEN* (G1691A)	33/62	No association with PE.	Mexico	Dávalos et al., 2005
ACVR2A (rs1424954, rs1014064, rs1424941, rs2161983, rs3768687)	613/693[n]	Association with severe early-onset PE risk: SNPs rs1014064 "G," rs1424954 "A," and rs2161983 "A."	Brazil	Ferreira et al., 2015
ACE (287 bp I/D in intron 16)	51/71	No association with PE.	Brazil	Galão et al., 2004
FV LEIDEN (G1691A) *Factor II* (G20210A) *MTHFR* (C677T)	30/83	No association with PE.	Brazil	Dusse et al., 2007
ACE (287 bp I/D in intron 16)	66/37	Association with risk for PE: *ACE* "DD" genotype.	Mexico	González-Garrido et al., 2017
EDN1 (G5665T)	61/49[o]	Association with protection for PE: paternal *EDN1* "GG" and "GT" genotypes.	Mexico	Galaviz-Hernandez et al., 2016
MTHFR (C677T, A1298C)	50/50[p]	Association with risk for PE: *MTHFR* 677TT genotype.	Ecuador	Chedraui et al, 2015
ACE (287 bp I/D in intron 16)	665/1,046	No association with PE.	Colombia	Serrano et al., 2006
HIF1A (C1772T, G1790A)	150/105	No association with PE.	Mexico	Nava-Salazar et al., 2011
VDR (*FokI, ApaI, BsmI*)	316/213[q]	No association with PE.	Brazil	Rezende et al., 2012
COMT (rs6269, rs4633, rs4680, and rs4818), *MTHFR* (C677T)	528/575[r]	Association with PE risk: "ATCA" haplotype of *COMT* (SNPs rs6269, rs4633, rs4818, rs4680, and MTHFR 677T)	Chile	Hill et al., 2011a

[†] *Pooled cases/controls.*
[a] *Cases were stratified in PE (n = 113) and gestational hypertension (GH, n = 103).*
[b] *Cases were stratified in early severe PE (n = 53) and late severe PE (n = 45).*
[c] *Cases were stratified in PE (n = 152) and GH (n = 152).*
[d] *Cases were stratified in PE (n = 157) and GH (n = 138).*
[e] *Cases were stratified in PE (n = 187) and GH (n = 166).*
[f] *Sample size composed by PE cases (n = 148), health pregnant woman (N = 177), and health non-pregnant volunteers (313).*
[g] *Cases were stratified in PE (n = 94) and GH (n = 101).*
[h] *Sample size was composed by 113 PE white women who were responsive (n = 46) and non-responsive (n = 67) to anti-hypertensive treatment.*
[i] *Sample size was composed by 62 cord vessels of singleton gestations with severe PE (n = 31) and controls (n = 31).*
[j] *Cases were stratified in PE (n = 154) and GH (n = 146).*
[k] *Cases were stratified in PE (n = 122) and GH (n = 107).*
[l] *Cases were stratified in PE (n = 133) and GH (n = 130).*
[m] *Cases were stratified in PE (n = 214) and GH (n = 185).*
[n] *Cases were stratified in PE (n = 443), eclampsia (n = 138), and HELLP syndrome (n = 693).*
[o] *Sample size composed by PE cases (n = 61) and their partners (n = 61), and the control group was health pregnant woman (N = 49) and their partners (n = 49).*
[p] *Sample size composed by 100 placental tissues of PE cases (n = 50) and controls (n = 50).*
[q] *Cases were stratified in PE (n = 162) and GH (n = 154).*
[r] *Sample size was composed by maternal-fetus dyads from PE cases (n = 528) and controls (n = 575).*

−2578A and −1154A alleles were more frequent in European-descendants subjects compared to Afro-descendants, while no inter-ethnic differences were observed regarding the G-634C SNP. Ethnic origin is also correlated with differences in *VEGF* haplotypic frequencies (Muniz et al., 2009). Cunha et al. (2011) evaluated *VEGF* variants +936C/T and −2578C/A in PE cases and controls. The *VEGF* −2578A allele showed a higher frequency in the control group, suggesting a possible protective effect against PE, while no association of *VEGF* +936C/T was observed in PE or controls (Cunha et al., 2011).

In the context of antihypertensive therapy, *VEGF* SNPs (C-2578A and G-634C) were evaluated in European-derived Brazilian women with PE classified according to response to antihypertensive therapy. No associations were observed, suggesting that these *VEGF* SNPs does not influence the antihypertensive therapy responsiveness in PE (Sandrim et al., 2015). The *VEGF* G-634C and *IL1A* (rs3783550) SNPs were evaluated in Brazilian women with PE and in controls. An association between *IL1A* (rs3783550) SNP and PE development was observed in this population sample. However, no differences were observed regarding the *VEGF* G-634C variant (Silva et al., 2015).

An elegant study investigated *VEGF* SNPs (−2578 A/C, −1498 C/T, −1154 A/G, −634 C/G, and +936 C/T) in samples

from cord vessels of singleton gestations with severe PE. Additionally, they investigated NO plasmatic levels, asymmetric dimethyllarginine and VEGF levels in fetal circulation. The SNPs showed similar distributions in cases and controls. Significantly higher NO plasma levels in umbilical vessels were seen in PE. Arterial VEGF levels were significantly lower in PE cases, and a positive correlation was found between NO and asymmetric dimethylarginine levels amongst PE cases (Chedraui et al., 2013).

The influence of SNPs in *eNOS*, *MTHFR*, *GSTP1*, and angiotensinogen (*AGT*) genes on PE was evaluated by Coral-Vázquez et al. (2013). The *eNOS* variants covered in the study were: −786T→C (rs2070744), VNTR (27 bp) in intron 4, and G-894T→Glu298Asp (rs1799983). The C-704T→Met235Thr (rs699) was the variant studied in *MTHFR*, the C-704T→Met235Thr (rs699) in *AGT*, and the A-313G→Ile105Val (rs1695) in *GSTP1*. No differences in the distribution of the genotypes or haplotypes between controls and PE cases were observed.

Matrix metalloproteinases (MMPs) are enzymes responsible for the degradation of various extracellular matrix molecules. In pregnancy, a disturbance in MMP activity could indicate abnormal trophoblast invasion. Moreover, detection of MMP up-regulation could reflect an interaction between oxidative stress and inflammatory mediators, which could result in the delivery of cell debris in maternal circulation and accumulation in various maternal organs (Chen and Khalil, 2017). The involvement of MMPs in vascular disorders of pregnancy may worsen the response to antihypertensive therapy (Palei et al., 2012a). In this context, a study investigated two matrix metalloproteinase 9 (*MMP9)* SNPs, the g.−1562C>T (rs3918242) and microsatellite g.−90(CA)13-25 (rs3222264). They report an association of the *MMP9* SNP with gestational hypertension, but not with PE (Palei et al., 2010).

The association of PE and SNPs of nitric oxide synthase 3, *NOS3* (G894T, T-786C, and a VNTR with intron 4), *MMP2* (C-1306T), and *MMP9* (C-1562T) genes was investigated through a prospective case-control study in a southeastern Brazilian population. No association with PE development was found regarding *MMP2* and *MMP9* variants. Considering the *NOS3* gene, the SNP T-786C showed association with PE development (Leonardo et al., 2015). Luizon et al. (2012) evaluated whether epistatic interactions among seven clinically relevant SNPs of *eNOS* (T-786C, rs2070744, a VNTR in intron 4, and Glu298Asp, rs1799983), *MMP-9* [C-1562T, rs3918242 and −90(CA)13-25, rs2234681] and *VEGF* (C-2578A, rs699947, and G-634C, rs2010963) could be associated with PE or gestational hypertension. Significant interactions between the *MMP9* and *VEGF* genes were seen in PE samples (Luizon et al., 2012). The *MMP2* SNPs: g-1306 C>T (rs243865) and g-735 C>T (rs2285053) were analyzed in the context of both PE and gestational hypertension together with circulating MMP-2 and tissue inhibitor of metalloproteinase (TIMP)-2 levels. High MMP-2/TIMP-2 ratios were observed in gestational hypertensive patients, but no differences in the genotype and allelic frequencies were found (Palei et al., 2012a). The same above-mentioned approach was used regarding *MMP9* SNPs: g.−90(CA)13-25 (rs3222264) and g.−1562C>T (rs3918242) and circulating levels of MMP-9 and TIMP-1. Higher plasma concentrations of MMP-9 and TIMP-1 were detected in gestational hypertensive patients compared to controls. TIMP-1 levels were higher in PE cases, but MMP-9 and MMP-9/TIMP-1 ratios were similar between PE and gestational hypertensive subjects. Haplotype analyses suggested that the presence of the H4 haplotype increases susceptibility to gestational hypertension (Palei et al., 2012b).

Dalmáz et al. (2006) assessed the prevalence of four thrombophilic genes in women with mild or severe PE in Southern Brazil. Variants studied include the *MTHFR* C677T, prothrombin gene (*F II*) G20210A, *Factor V* (*FV Leiden*) G1691A, and insertion/deletion (4G/5G) in the plasminogen activator inhibitor type 1 (*PAI1*) gene promoter region. No association between PE and the SNPs was observed (Dalmáz et al., 2006). *MTHFR* C677T and *Factor V Leiden* SNPs were also investigated as potential genetic risk factors for eclampsia and PE in a group of women from western Mexico, without statistically significant results (Dávalos et al., 2005). Furthermore, *Factor V Leiden* (G1691A), *Factor II* (G20210A), and *MTHFR* (C677T) variants were investigated in the context of inherited thrombophilia in Brazilian PE women and controls. Again, no differences were observed (Dusse et al., 2007).

The gene *ACVR2A* encodes the activin A type II receptor (ActRIIA), an essential factor for pregnancy establishment during decidualization, trophoblast invasion, and placentation. Concerning the regulation of trophoblast invasion, abnormal decidual *ACVR2A* expression may affect placentation and lead to PE development (Yong et al., 2018a). In this context, five *ACVR2A* SNPs (rs1424954, rs1014064, rs1424941, rs2161983, and rs3768687) were investigated in a northwestern Brazilian population approaching PE cases and controls. These five SNPs showed no association with PE. Nevertheless, haplotype analysis revealed a strong association among SNPs rs1014064, rs1424954, and rs2161983 and severe early-onset PE (Ferreira et al., 2015).

Considering that the cardiovascular system of a pregnant woman adapts to allow and support increased blood flow toward the placenta, angiotensin-converting enzyme gene (*ACE*) SNPs were investigated in vascular disorders of pregnancy. A common 287-bp insertion/deletion SNP within *ACE* (*ACE*-I/D) was investigated as a possible risk factor for PE development in a south Brazilian population. The allele frequencies of this *ACE* variant were not associated with PE development (Galão et al., 2004). Subsequently, a case-control study and meta-analysis were also unable to show the association between the *ACE*-I/D variant and PE (Serrano et al., 2006). In a Mexican population, González-Garrido et al. (2017) evaluated the *ACE*-I/D SNP in relation to ACE activity and oxidative damage in PE. Higher ACE activity was found in PE cases compared to controls. Also, higher "DD" genotype and "D" allelic frequencies were found in PE compared to the control group. In summary, the results suggested that *ACE*-I/D SNP, high ACE activity, body mass index and oxidative damage are important factors in the pathogenesis of PE (González-Garrido et al., 2017).

The Endothelin 1 protein is a potent vasoconstrictor molecule, and its encoding gene, *END1*, is also a candidate gene for PE. A case-control study investigated women affected with PE and their partners in comparison to healthy pregnant women and their

partners regarding the *EDN1* rs5370 SNP. A negative association between the rs5370 SNP and PE in the male sub-group was observed, while no association was observed between cases and controls in the female sub-group (Galaviz-Hernandez et al., 2016). This study reminds us of the importance of including the paternal genetic background and the effect of the male genetic contribution in pregnancy outcomes.

Hypoxia-inducible factor (HIF) is a highly conserved transcription factor that coordinates an adaptive response in physiological and pathophysiological situations. Several cell types up-regulate *HIF* in response to low oxygen levels. In human pregnancy, HIF signaling in the gravid uterus is critical for fetal and placental development (Macklin et al., 2017). The Influence of *HIF1A* C1772T and G1790A SNPs was evaluated in PE patients and controls in a Mexican population, although no association of these variants with PE risk was observed (Nava-Salazar et al., 2011).

Finally, vitamin D is an essential molecule during pregnancy. The levels of its active form increase throughout healthy gestation and are critical for an adequate calcium supply for fetal growth (Urrutia and Thorp, 2012). Given its importance and relevance in gestational outcome, Vitamin D Receptor (*VDR*) SNPs have been studied in PE and gestational hypertension. Rezende et al. (2012) evaluated three *VDR* SNPs (*FokI, ApaI,* and *BsmI*) in a Brazilian population, and also investigated the potential association of hypertensive pregnancy disorders with *VDR* haplotypes. No differences in genotype, allele, or haplotype frequencies were observed between PE or pregnant hypertensive women and controls, these findings suggested that the investigated SNPs do not influence pregnancy outcome (Rezende et al., 2012).

Studies in vascular and angiogenic gene polymorphisms have shown conflicting results in Latin American populations (**Table 2**). Besides genetic variants alone, PE-associated haplotypes and the interaction among SNPs of distinct genes further support the importance of exploratory studies in this rapidly developing field. The conflicting results evidenced in this review are partially explained by the differences in the genetic background of distinct Latin American populations, which result from high admixture (Salzano and Sans, 2014). Besides, the different genotypic and allelic frequencies of the studied SNPs corroborate the PE classification as a complex disease. For a better understanding of the whole scenario involving this disease, robust studies and several exploratory studies still need to be put into practice. Also, the publication of negative results is important, mainly for the correct performance of meta-analyses encompassing preexisting data that would better reflect the actual frequency of genetic variants in Latin American populations.

GENETIC VARIATION IN HISTOCOMPATIBILITY-RELATED GENES IN PE

The major histocompatibility complex (MHC) is fundamental to the immunological system allowing the development of immune responses against foreign antigens or immunogenic epitopes through recognition of self- and non-self. Traditionally, the MHC complex is defined by two well-known genetic *loci*: MHC class-I and MHC class-II, although MHC class-III and -IV also exist and are relevant to complex diseases (Gruen and Weissman, 2001; Yau et al., 2016). MHC class-I members split in "classical" [human leukocyte antigen (*HLA*)-*A*, -*B,* and -*C*] and "non-classical" (*HLA-E*, -*F*, -*G* and -*H*: or MHC-Ib) molecules. Classical genes are ubiquitously expressed on virtually all nucleated cells (with a few exceptions), are highly polymorphic, and their primary function is as peptide presenting molecules. On the contrary, expression of non-classical molecules are restricted to some cellular types (for example, EVT), have a limited degree of polymorphism, and do not present peptides as a major function but rather act as signaling molecules to immune cells. Classical MHC class-II (*HLA-DR*, -*DQ,* and -*DP*) expression is restricted to antigen presenting cells, such as B cells, macrophages, and DCs. MHC class-III and IV are otherwise very distinct molecules comprising members of the complement system and induced-stress/inflammatory proteins, respectively (Gruen and Weissman, 2001).

The role of the MHC-Ib molecules in pregnancy has been a focus since the discovery of HLA-G expression in human trophoblast cells (Kovats et al., 1990). In the maternal-placental interface, an exciting aspect is the expression of HLA-G, -E, -F, and -C antigens on EVT cells (Hackmon et al., 2017).

Among the non-classical MHC-I molecules, HLA-G is a most enigmatic member. It interacts with several maternal immune cells, including those in the decidua (i.e., dNK, decidual macrophages, dCD4+, dCD8+), and has the potential to inhibit or activate their immunologic functions. Recently, it was reported that soluble HLA-G (sHLA-G) affinity for its cognate receptors [i.e., dimers binding to LILRB1 (leukocyte Ig-like receptor 1) with increased affinity] is likely impacted by sHLA-G heterodimerization in inflamed patients, which is likely to occur in PE and explains the variable findings reported so far (Veit et al., 2015). Also, LILRB1 receptors bind to β2-microglobulin(m)-associated HLA-G, whereas the LILRB2 receptors bind to non-β2m-associated HLA-G molecules. Alternative splicing of the gene results in seven isoforms: four membrane-bound HLA-G isoforms (HLA-G1 to -G4) and three soluble isoforms (HLA-G5 to G7). HLA-G1 undergoes proteolytic cleavage by metalloproteinase-2 (MMP-2) giving rise to sHLA-G1 (Rizzo et al., 2013).

In Latin America, several groups have evaluated the role of candidate genes belonging to the MHC loci and PE susceptibility (**Table 3**). *HLA-G* is the most studied *MHC* gene due to its immunotolerogenic properties, and several aspects of *HLA-G* have been explored. An SNP located in the 3′ untranslated region (UTR) of the gene, namely 14-bp insertion(ins)/deletion(del) (rs66554220), is well-known due to its influence on mRNA stability which affects the expression patterns of the gene (Rousseau et al., 2003; Porto et al., 2015). We have recently reported that specific haplotypes and variants in the 3′UTR increase the risk for recurrent pregnancy loss in Brazilian women (Michita et al., 2016). Also, we suggested that a maternal 14 bp del/del homozygous status might predispose primiparous women to PE (Vianna et al., 2007). An increased risk for

PE was also observed in neonates who preferentially inherit the maternal *HLA-G**0104 allele (Carreiras et al., 2002), which has been associated with the 14 bp del allele present in the UTR-3 haplotype (Castelli et al., 2014). In another study, a concomitantly low frequency of CD8+CD28- T cells (CD8+T memory cells), low monocyte (CD14+HLA-G+), and low T cell (CD3+HLA-G+) counts in PE women were associated with a pro-inflammatory status, which was confirmed by pro-inflammatory cytokine measurements (Vianna et al., 2016); however, no differences in 14 bp ins/del and +3142C/G (rs1063320) SNP frequencies between PE and non-PE women were observed. Similarly, in a Mexican PE cohort, although HLA-G expression was not evaluated, a reduced frequency of CD3+ T cells was observed in third trimester decidual tissue, and most importantly, dNK cells (CD3-CD56+CD16-CD9+) persisted throughout pregnancy and shared the same phenotype as the ones detected in early pregnancy (Sánchez-Rodríguez et al., 2011). This implies that long-term persistence of dNK cells could play important physiological roles in labor by the secretion of inflammatory mediators and fighting against infectious agents. Still considering HLA-G, Ferreira et al. (2017) reported that the 14 bp variant had no influence on PE predisposition, although the specific contribution of this SNP for PE in primiparous women was not evaluated. Hitherto, the role of the 14 bp variant in PE has been a matter of debate (Vianna et al., 2007; Pabalan et al., 2015; Ferreira et al., 2017). However, in a recent meta-analysis, the ethnicity (European-derived) and the 14 bp ins/ins genotype status in neonates were pointed as likely involved in PE risk in primiparous women (Pabalan et al., 2015).

PE development probably involves the interaction of maternal and fetal features. Also, a contribution of paternal origin has been suggested (Dahl et al., 2014; Saftlas et al., 2014). Functional variants within the endoplasmic reticulum aminopeptidase gene (*ERAP2*) have been associated with PE in non-Latin American populations (Johnson et al., 2009). In a study by Hill et al. (2011a), the *ERAP* variants rs2549782 and rs17408150 were evaluated in Chilean dyads (mother-neonate) and African American subjects (78% were dyads) with PE. In this study, no influence of *ERAP* SNPs in PE predisposition was reported. The lack of association with PE risk could be partially explained by differences in population structure and linkage disequilibrium patterns (Hill et al., 2011b). A study evaluating Venezuelan dyads reported an increased risk for PE in both mothers and neonates carrying the *HLA-DRB1**07 *DQA1**0201 *DQB1**0201 haplotype. In addition, mothers carrying the *HLA-DRB1**06/07 allele were more likely to be infected by the human cytomegalovirus (HCMV) (Carreiras et al., 2002). Since the recent Zika virus epidemics in Brazil (Schuler-Faccini et al., 2016), the relevance of viral infections during pregnancy is once more in the spotlight. Of note, recent evidence suggests that some viral infections modify the threshold of placental cell immunologic response to bacterial lipopolysaccharides (LPS) resulting in an exacerbated inflammatory response, and thus contributing to the development of pregnancy disorders including PE (Cross et al., 2017; Nourollahpour Shiadeh et al., 2017).

Other polymorphic loci immunologically relevant in PE comprise the *KIR* (Killer-cell immunoglobulin-like receptors) family. This gene family encompasses both activating (S) and inhibitory (L) receptors and can be functionally characterized in two additional groups: A (inhibitory) or B (activating) group. There is evidence of maternal *KIR* contribution in PE development. Indeed, it was suggested that the predominance of inhibitory receptors in PE women conferred an increased risk for PE in Mexican women (Sánchez-Rodríguez et al., 2011). Interestingly, a higher frequency of CMV-positivity was observed in third trimester Mexican women carrying the inhibitory *KIR* bB03|tA01 haplotype (*KIR* A) (Alvarado-Hernández et al., 2016), reinforcing the theory that imbalances between activating and inhibitory receptors expressed on cytotoxic cells influence viral infection predisposition and are possibly a risk-modifying factor for pregnancy disorder development.

GENE VARIANTS INVOLVED IN METABOLIC PROCESSES

Changes in maternal metabolism occur during gestation, allowing adaptation to the energetic and nutritional needs of the developing fetus and ensuring its healthy development. Some changes involve the metabolism of carbohydrates and lipids. Such metabolic changes occur in a spatial and temporal manner as pregnancy develops. Early in gestation, glucose and insulin levels are comparable to those of non-pregnant women, with a slight increase in insulin sensitivity (Butte, 2000). A decrease in insulin sensitivity occurs naturally, becoming evident in the second trimester, however, a noticeable loss of insulin sensitivity can lead to systemic resistance, hyperglycemia, and gestational diabetes mellitus (DM). The effects of hyperglycemia in pregnancy are associated with several adverse clinical outcomes for both mother and newborn, the latter associated with overweight and cardiometabolic risk later in life (Thaware et al., 2015; Zhu et al., 2016; Tam et al., 2017). It was suggested that gestational hyperglycemia or pre-pregnancy DM are risk factors for gestational disorders, including PE (Wendland et al., 2008). Interestingly, the expression of cytokines (i.e., IL-10 and TNF-alpha) relevant to the pathophysiology of PE (Daher et al., 2006; Pinheiro et al., 2015) is associated with maternal glycemia (Moreli et al., 2012), implying that maternal glycemia not only affects the metabolic status but also the immunological profile of pregnant women.

Some studies have evaluated the role of critical mediators in metabolic processes and their influence on PE development (**Table 4**). Adiponectin (ADIPOQ) is an adipokine, a term referring to adipose tissue-derived signaling molecules with broad biological functions (Ruan and Dong, 2016). ADIPOQ enhances cellular insulin sensitivity and thus is involved in adipose tissue expansion. Besides metabolic signaling, ADIPOQ has anti-inflammatory, anti-atherogenic and anti-proliferative functions, but paradoxically it is associated with coronary diseases (Sattar and Nelson, 2008). In addition, it enhances human EVT cell invasion *in vitro* by means of MMP-9 and -2 expression and TIMP-2 repression (Benaitreau et al., 2010). Expression of both MMPs in EVT cells may

TABLE 3 | Summary of studies in Latin America evaluating the role of genetic variants in histocompatibility-related genes in PE.

Factors	Sample size[†]	Key findings	Country	References
HLA-A, *-G*, *-DRB1*, *-DQA1*, *-DQB1* alleles	27/29[a]	Association with PE risk: *HLA-G**0104 allele, *DRB1**07 *DQA1**0201 *DQB1**0201 haplotype and *DRB1**07 and/or *DRB1**06 alleles in presence of HCMV detection.	Venezuela	Carreiras et al., 2002
HLA-G (14 bp ins/del)	157/162	No association with PE.	Brazil	Vianna et al., 2007
KIR inhibitory(*2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3*); activating (*2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1*); pseudogenes (*2PQ1, 3DP1*)	90/86	No association with PE.	Mexico	Sánchez-Rodríguez et al., 2011
HLA-G (14 bp ins/del, +3142C>G).	26/32[b]	No association with PE.	Brazil	Vianna et al., 2016
HLA-G (14 bp ins/del)	409/332[c]	No association with PE.	Brazil	Ferreira et al., 2017
ERAP2 (rs2549782, rs17408150)	528/575[d]	No association with PE.	Chile	Hill et al., 2011b

[†] *Pooled cases/controls.*
[a] *Samples were mother-neonate dyads.*
[b] *Controls were grouped in non-PE (n = 25) and healthy group (n = 7).*
[c] *Cases were grouped in PE (n = 246), eclampsia (n = 57), and HELLP (n = 106). PE, preeclampsia; HLA, human leukocyte antigen; HCMV, human cytomegalovirus; ins, insertion; del, deletion; KIR, killer cell immunoglobulin-like receptor; ERAP2, endoplasmic reticulum aminopeptidase-2*
[d] *Only Chilean mother-neonate dyads.*

increase membrane cleavage of the immunomodulatory molecules MIC-A and HLA-G (Sun et al., 2011; Rizzo et al., 2013). SNPs in *ADIPOQ* influence basal expression of the gene and predispose occurrences of metabolic disorders in French and Japanese populations (Hara et al., 2002; Fumeron et al., 2004). In a cohort of Brazilian PE women *ADIPOQ* variants −11391G/A (rs17300539), −11377C/G (rs266729), 45T/G (rs2241766), and 276G/T (rs1501299) were evaluated. The rs266729 GG genotype presented a higher frequency in PE (Machado et al., 2014). The −11377G allele is suggested to decrease the affinity of nuclear proteins in the *ADIPOQ* promoter and putatively the transcriptional activity (Bouatia-Naji et al., 2006; Wang et al., 2009; Zhang et al., 2009). Therefore, preeclamptic -11377GG genotype carriers are likely to express low levels of adiponectin, resulting in impaired control of glycemia. Also, −11377G allele carriers have been associated with chronic hypertension (Ong et al., 2010), recurrent pregnancy loss (Dendana et al., 2018), and gestational diabetes (Pawlik et al., 2017) in non-Latin American populations.

Lipid metabolism and plasmatic concentration are regulated by an enzyme encoded in lipase hepatic gene *LIPC*. *LIPC* −514C/T (rs1800588) is a promoter SNP which influences hepatic lipase levels. In fact, the −514TT genotype is associated with the lowest enzyme activity, although the variant effect is variable among non-Latin American populations (Tahvanainen et al., 1998; Ordovas et al., 2002; Isaacs et al., 2004). This variant was evaluated in a cohort of PE Peruvian women (Enquobahrie et al., 2005). Although no direct association with PE risk was observed, overweight status during pregnancy was a modifying risk factor for PE in *LIPC* −514TT genotype.

Changes in insulin responsiveness are essential in pregnancy and affect both mother and fetus. As pregnancy develops, maternal insulin resistance increases, which in turn facilitates glucose transport across the placenta and stimulates fetal insulin production, favoring normal fetal growth and development (Farrar, 2016). Hyperinsulinemia is harmful and resembles the endothelial dysfunction observed in PE pathophysiology (Muniyappa and Sowers, 2014). An interesting Mexican study evaluating the role of genetic variants of genes involved in insulin responsiveness in PE development focused on: insulin [(*INS*); *Pst*I (rs3842752) and *Mae*III (rs689)], insulin receptor [(*INSR*); *Nsi*I (rs2059806)], and insulin receptor substrate [(*IRS1*); Ala513Pro (rs1801276) and Gly972Arg (rs1801278)] (Machorro-Lazo et al., 2009). Although no statistical difference in SNPs frequencies was observed, a previous study evaluating different ethnic groups in Mexico observed differences in the *Mae*II, *Pst*I, and *Nsi*I genotype distribution when stratified by fasting insulin and serum triglyceride levels (Flores-Martínez et al., 2004; Sánchez-Corona et al., 2004). Also, the *IRS1* 972Arg allele was associated with gestational diabetes in a meta-analysis (Zhang et al., 2013) and the *INSR Nsi*I SNP (rs2059806AA genotype) was associated with PE in an Australian cohort and also in PE newborns small for the gestational age in a Sinhalese cohort (Andraweera et al., 2017). The lack of association with PE is possibly due to the stringent inclusion/exclusion criteria of the study since pregnant women with undiagnosed insulin resistance before pregnancy were excluded.

As insulin signaling involves an intricate network of molecules, it is unlikely that a single gene or SNP results in an insulin-resistant phenotype. Nevertheless, SNPs in leptin (*LEP*) and leptin receptor (*LEPR*) genes seem to have the potential to

TABLE 4 | Summary of studies in Latin America evaluating the role of genetic variants within genes involved in metabolic changes during pregnancy.

Factors	Sample size[†]	Key findings	Country	References
ADIPOQ (-11391G>A,−11377C>G, 45T>G, 276G>T)	240/161[a]	Association with PE risk: −11377GG genotype.	Brazil	Machado et al., 2014
INS (*Pst*I, *Mae*III) *INSR* (*Nsi*I) *IRS1* (Ala513Pro, Gly972Arg)	43/46	No association with PE.	Mexico	Machorro-Lazo et al., 2009
LEP (G2548A) LEPR (Gln223Arg, Lys109Arg)	146[b]	Association with GH clinical findings: LEP 2548AA genotype with BMI and 2548G allele with systemic BP; LEP 109 Lys/Lys genotype with BMI and Insulin resistance.	Brazil	Farias et al., 2017
LIPC (-514C>T)	157/180	Association with PE risk: *LIPC* −514TT genotype in overweight pregnant women.	Peru	Enquobahrie et al., 2005

[†] *Pooled cases/controls.*

[a] *Cases were grouped in PE (n = 127) and gestational hypertension (n = 113).*

[b] *Prospective cohort of pregnant women. PE, preeclampsia; ADIPOQ, adiponectin; INS, insulin; INSR, insulin receptor; IRS1, insulin receptor substrate-1; LEP, leptin; LEPR, leptin receptor; GH, gestational hypertension; BP, blood pressure; BMI, body mass index; LIPC, hepatic lipase.*

influence blood pressure during pregnancy as an indirect effect on insulin sensitivity and BMI, and therefore are relevant in PE pathophysiology (Fan and Say, 2014; Taylor et al., 2015). In a Brazilian study, *LEP* G2548A (rs7799039), *LEPR* Q223R (rs1137101), and K(Lys)109R(Arg) (rs1137100) variants were evaluated regarding their influence on maternal blood pressure during pregnancy and the postpartum period (Farias et al., 2017). Although no association with leptin levels and SNPs were observed, homozygous individuals for 2548AA genotype had lower BMI in early pregnancy, and the effect of BMI on blood pressure levels was higher in 2548AA homozygous carriers compared to G allele carriers (GA+GG). On the contrary, 2548GG+GA showed a positive increase in systemic blood pressure in early pregnancy. In a more recent study, the 2548A allele was associated with an increased risk for gestational weight gain (Martins et al., 2017). The influence of G2548A SNP in leptin levels during pregnancy is still not evident (Sugathadasa et al., 2010; Yang et al., 2016; Farias et al., 2017). Nevertheless, in non-pregnant Brazilian women, associations with obesity risk and increased leptin levels for 2548GG genotype and 2548G allele were reported (Hinuy et al., 2008). In PE, plasma levels of leptin are higher than in normotensive pregnant women (Sugathadasa et al., 2010). Also, women with impaired fasting glucose have higher levels of both insulin and leptin compared to euglycemic pregnant women (Yang et al., 2016). These observations are relevant since leptin-induced obesity is associated with hyperglycemia, hypertension, and endothelial damage.

VARIANTS IN DETOXIFICATION, DNA-REPAIR, AND APOPTOSIS-RELATED GENES

Vascular dysfunction is one hallmark of PE that is intensified by positive feedback involving altered maternal immune tolerance and placental hypoxia. In addition, endothelial damage observed in PE is the *prima facie* of impaired clearance of oxidative stress byproduct by endogenous detoxifying agents. Oxidative stress causes membrane lipid peroxidation, DNA damage and is possibly implicated in the pathogenesis of essential hypertension (González, 2014). Functional SNPs in candidate genes of the detoxification system, DNA repair, and apoptosis genes have been suggested to play roles in PE development (**Table 5**). Glutathione-S-transferase (GST) is an endogenous detoxifying enzyme superfamily that protects against oxidative stress and exogenous toxins or xenobiotics. The functional variant *GSTP1* 313A/G (rs1695) lies within the active site of the GSTP1 enzyme, and the 313G allele (valine) is associated with low catalytic activity (Ali-Osman et al., 1997). Studies evaluating this variant in different continental cohorts of PE have reported conflicting results (Zusterzeel et al., 2000; Gerhardt et al., 2004; Canto et al., 2008; Coral-Vázquez et al., 2013; Gao et al., 2016). On the one hand, it was observed that *GSTP1* 313G allele and 313GG/AG genotypes are protective factors for PE development in Maya-Mestizo women (Canto et al., 2008), a finding inconsistent with a Dutch study (Zusterzeel et al., 2000). This same variant had no influence on severe PE development in Mexican-Mestizo women (Coral-Vázquez et al., 2013), highlighting differences in results according to ethnic origin. Studies evaluating the role of *GSTP1* 313A/G in PE risk reported conflicting results, probably due to the high inter-variation and intra-variation (i.e., admixture) of the *GSTP1* 313G allele frequency (Zerbino et al., 2018). Another interesting *GST* variant is the complete deletion of *GSTM1* and *GSTT1* (Anvar et al., 2011). It is reported that Mexican-Mestizo women homozygous for *GSTT1* null genotype have a higher risk for PE, and those double homozygous for both *GSTM1* and *GSTT1* null genotypes have a 5-fold increased risk for PE (Sandoval-Carrillo et al., 2014a). These findings contribute to the conflicting body of evidence as pointed out by a meta-analysis (Anvar et al., 2011; Ge et al., 2015). Although the frequency of single deletions varies (Palma-Cano et al., 2017), we hypothesized that populations showing a high frequency of both *GSTs* deletions could have a high frequency of individuals carrying both deletion

TABLE 5 | Summary of the studies in Latin America evaluating the role of genetic variants in genes involved in detoxification, DNA repair and apoptosis in PE.

Factors	Sample size†	Key findings	Country	References
GSTP1 (313A>G)	125/274	Association with protection for PE: 313GG and AG genotypes.	Mexico	Canto et al., 2008
GSTP1 (313A>G)	230/352	No association with PE.	Mexico	Coral-Vázquez et al., 2013
GSTM1, *GSTT1*	112/233	Association with PE risk: *GSTT1* deletion, and combined *GSTM1/GSTT1* deletion (highest risk).	Mexico	Sandoval-Carrillo et al., 2014a
APEX1 (Asp148Glu) *XPD* (Lys751Gln) *XRCC* (Arg399Gln) *XRCC3* (Thr241Met)	202/350	Association with PE risk and disorder severity: *APEX1* 148Glu allele.	Mexico	Sandoval-Carrillo et al., 2014b
TP53 (Arg72Pro) *MDM2* (309T>G)	119/99	No association with PE.	Brazil	Busatto et al., 2015
CASP-8 (*rs13416436, rs2037815*)	55/162	No association with PE.	Brazil	Orlando et al., 2018

† *Pooled cases/controls. PE, preeclampsia; GSTP, glutathione s-transferase Pi-1; GTSM1, glutathione s-transferase Mu-1; GSTT1, glutathione s-transferase Theta-1; APEX1, Apex nuclease 1; XPD, Xeroderma pigmentosum complementation group D; XRCC, x-ray repair cross-complementing protein; XRCC3, x-ray repair cross-complementing protein 3; TP53, tumor protein p53; MDM2, mouse double minute-2 homolog; CASP8, caspase-8.*

alleles, implying an increased risk to oxidative stress-related disorders such as PE or vasculopathies.

Most DNA damage caused by endogenous ROS generated from oxidative stress is corrected by the DNA repairing machinery through diverse pathways (see Chatterjee and Walker, 2017). It is not clear whether DNA damage is an effect or cause of PE pathophysiology, although impaired DNA repair is observed in placental tissue from PE women (Tadesse et al., 2014). Also, accumulation of DNA errors results in cell death, and DNA repair efficiency is impacted by genetic variation in DNA repair genes. Hitherto, few studies have investigated such variants in PE development (Vural et al., 2009; Saadat et al., 2012; Sandoval-Carrillo et al., 2014b). In a study enrolling Mexican women with PE, SNPs in DNA repair genes from nucleotide and base excision pathways, homologous recombination and single-strand break repair mechanisms were evaluated. Among the variants evaluated, a possible role for the functional variant T1349G (Asp148Glu; rs1130409) in the apurinic/apyrimidinic (AP) endonuclease (*APEX1*) gene in PE development was observed. Although no difference in overall genotype distribution between PE and normotensive pregnant women was observed, consistent with a previous study (Vural et al., 2009), the 1349G (148Glu) allele frequency was higher in PE subjects compared to normotensive women. Also, the G allele frequency was higher in severe PE compared to mild-PE (Sandoval-Carrillo et al., 2014b). Although a functional study reported no difference in endonuclease activity between APEX1-148Glu and APEX1-148Asp molecules (Hadi et al., 2000), the role of this variant in PE is supported by impaired enzyme functionality (impaired DNA-binding and endonuclease activity) associated with the 1349G (148Glu) allele (Almutairi et al., 2015), and also by the fact that APEX-1 is essential for the base excision repair pathway, apoptosis, response to oxidative stress, and cell cycle control.

Essential for genomic stability and cell cycle control, the tumor suppressor protein p53 is also implicated in human reproduction (Kang and Rosenwaks, 2018). Our research group has investigated the role of the *TP53* Arg72Pro (rs1042522) and *MDM2* 309T/G (rs2279744) variants in PE development (Busatto et al., 2015). Despite a lack of association with PE risk in our study, it is reported that *MDM2* 309GG genotype confers an increased risk for PE in an Iranian population (Salimi et al., 2017). Interestingly, MDM2 309G allele frequency in normotensive and PE women was similar in both studies, although genotypic frequencies differed. These findings highlight that interaction among SNPs from the regulatory *TP53* network are likely to account for observed differences and should be addressed in further studies (Jacovas et al., 2015). Genetic variants in apoptosis-related genes, such as *CASPASE-8* (rs13416436T/A and rs2037815G/A) were evaluated in PE in a small cohort of Brazilian women, although no association with disorder risk was observed (Orlando et al., 2018).

FUTURE DIRECTIONS: CHALLENGES AND PERSPECTIVES

Over the past decade, our understanding of the molecular basis of many disorders has increased in an unprecedented manner. Despite improvements in understanding the contribution of paternal, maternal, and placental factors in PE pathophysiology, the identification of reliable predictive biomarkers for PE remains elusive. We do not wish to distract from the importance and biological implications of the many other advances in PE understanding, however, based on our knowledge we suggest future directions/studies and challenges in PE research by highlighting and discussing some emerging trends from distinct but related biological fields (**Figure 2**).

MHC Class-I Related Sequence A

It is well known that some biological aspects inherent to host immunologic tolerance to solid organ allograft transplantation (tx) could overlap to some extent with those directly related to human pregnancy (sometimes considered as a naturally

occurring grafting event). Relevant in human pregnancy, the MHC Class-Ib molecules are becoming a target of studies in human transplantation, since the rejection of allografts fully matched for HLA antigens still occur. In this context, the non-classical MHC class-I related sequence A (MIC-A or MICA), a stress-induced protein has attracted attention due to its immunomodulatory properties (Baranwal and Mehra, 2017; Risti and Bicalho, 2017). MICA has restricted tissue expression in normal physiological conditions (i.e., gastrointestinal tract and endothelial cells) (Baranwal and Mehra, 2017). *MICA mRNA* transcripts are detected in decidual, placental, and trophoblast cells from healthy pregnancies, although the MICA molecule is barely detected on placental tissues (Mincheva-Nilsson et al., 2006; Apps et al., 2008). It has been proposed that soluble MICA (sMICA) in pregnant women may participate in fetal immune escape (Mincheva-Nilsson et al., 2006; Huang et al., 2011), although high levels of sMICA were considered a predictive biomarker for *in vitro* fertilization failure (Porcu-Buisson et al., 2007). Indeed, in pathological situations, *MICA* expression patterns might change. A dimorphism known as MICA-129Val/Met (rs1051792), is reported to influence both sMICA levels and affinity to the NKG2D receptor expressed on cytotoxic cells, including uNK cells. It was observed that soluble NKG2D has a higher affinity to 129Met molecules (range 10- to 50-fold) compared to 129Val MICA (Steinle et al., 2001). Thus, this variant seems relevant in inflammatory disorders (Isernhagen et al., 2016), but its influence on pregnancy disorders is yet to be addressed. Besides, high sMICA levels are observed in PE and other vascular pregnancy disorders, often being absent in healthy pregnancies. Further, sMICA maternal plasma from PE women downregulates NKG2D expression on CD3-CD56+NK cells from healthy donors (Haumonte et al., 2014), suggesting that sMICA impairs vascular remodeling through downregulation of NK effector functions by means of interferon-gamma secretion and cytotoxicity (Haumonte et al., 2014; Zhou et al., 2014). Additionally, microvesicles derived from early placenta harbor MICA which has potential to downregulate NKG2D (Hedlund et al., 2009).

Non-coding RNAs and Epigenetics

In the era of genomics, next-generation DNA sequencing is becoming a technique accessible to most laboratories. The possibility of massively interrogating millions of DNA strands at the same time has fostered research in the search of causal genetic variation involved in PE pathophysiology (see Yong et al., 2018b). The profile of non-coding RNA (ncRNA) in distinct tissues, body fluids and disorders has revealed a universe of RNAs, which is currently under extensive investigation. Traditionally, ncRNAs are divided into two classes based on size: small ncRNA (<200 nt) and long ncRNA (>200 nt). The small ncRNA includes microRNA (miRNA), small interfering RNA, small nuclear RNA, small nucleolar RNA, ribosomal RNA, transfer RNA, and P-element-induced-wimpy testis (piwi)-interacting RNA. Their regulatory activity extends to different levels of transcription and post-transcriptional control (Anfossi et al., 2018). Although little is known about long non-coding RNA (lncRNA) functions, they participate in several biological processes such as epigenetic regulation, transcriptional and post-transcriptional control, regulation of miRNAs (by acting as sequence decoys) and acting as scaffolds for protein complex (Li et al., 2014), suggesting a more extensive biological versatility compared to small ncRNAs.

Currently, ncRNAs are considered promising diagnostic tools and disease progression biomarkers in the clinical setting, because their level of presence is expected to correlate with repressive activity (Bounds et al., 2017). In PE, an increasing number of studies have identified potential regulatory ncRNAs, most of them miRNAs such as miR-155 and miR-210 (Bounds et al., 2017; Wei et al., 2017; Winger et al., 2018; Yoffe et al., 2018). miRNAs are of particular interest due to their high stability in body fluids (Brase et al., 2010) and their potential to be released inside microvesicles (Salomon et al., 2017). An emerging role of genetic variants within miRNAs (even virally encoded miRNAs) highlights their influence in the susceptibility to viral infections (Ellwanger et al., 2018).

Despite the promising applications of ncRNAs as biomarkers in distinct pathologies, the increasing complexity for use due to ncRNA heterogeneity as well as the diversity of methodologies implemented for their isolation (Anfossi et al., 2018) highlight some challenges that should be addressed, including the need for sample collection, processing, and analysis standardization in order to increase the feasibility and replicability of studies.

It is clear that epigenetics is a mechanism involved in the development of a healthy pregnancy. Partially methylated domains (PMDs) are regions showing reduced average methylation levels which cover up to 40% of the genome. PMDs are observed in only a few cell types: cultured cells, malignant cells and placental cells (Hansen et al., 2011; Schroeder and LaSalle, 2013). Interestingly, PMD covers 37% of the placental genome, and most of the genes in PMD are repressed, suggesting that repression of specific genes within PMD during pregnancy is needed for healthy development (Schroeder et al., 2013). Following this reasoning, disruption in the epigenetic program could lead to placental dysfunction and associated disorders (Robinson and Price, 2015). Some studies have evaluated the methylation status, or methylome, of the placenta in pregnancy disorders such as preterm birth (Hong et al., 2017), intrauterine growth restriction (Hillman et al., 2015), and PE (Blair et al., 2013; Anton et al., 2014; Chu et al., 2014; Liu et al., 2014). Owing to the fact that different methodologies exist, the comparison between studies is not always possible. Nonetheless, two genes (*DAPK3* and *PAPPA2*) were observed to share methylation patterns in preeclamptic placenta (Blair et al., 2013; Chu et al., 2014; Bianco-Miotto et al., 2016). Apart from pregnancy disorders, the methylation patterns in placental PMD suggest a causal link to autism spectrum disorders because behavioral genes are overrepresented in placental PMD (Schroeder et al., 2016). There is also a suggestion of an interaction between environmental factors and DNA, altering epigenetic features and therefore susceptibility to many disorders including PE (Chelbi and Vaiman, 2008).

Analysis of DNA methylation in cord blood cells may improve our knowledge of epigenetic signatures in pregnancy (and PE) and improve understanding of their implications

for adult life. For example, hypomethylation of the 11β-hydroxysteroid dehydrogenase type-2 (*HSD11B2*) gene promoter is suggested to increase fetal glucocorticoid levels identified as risk factors for metabolic diseases (Hu et al., 2014). In a study evaluating cord blood in early preeclamptic women, different sets of genes from lipid metabolism, cellular proliferation and inflammation showed variable levels of methylation in their promoter regions, suggesting that early epigenetic signatures are detected in newborns and could be associated with predisposition to cardiovascular diseases in adulthood (Ching et al., 2015). Nevertheless, whether early risk epigenetic modifications remain constant and act as disease triggers or risk-modifying factors is still an open question.

Genomic imprinting is closely associated with parental origin, which highlights that epigenetic disruption can result in abnormal expression of imprinted genes in the placenta and contributes to PE development. The distal-less homeobox-5 (*DLX5*) gene is paternally imprinted (maternally expressed gene) in normal healthy placenta, but its status is upregulated in PE as a result of the loss of paternal imprinting. *DLX5* was upregulated in up to 70% of PE placentas correlating positively with classical PE markers (i.e., PlGF:sFLT). Of note, overexpression of DLX5 *in vitro* led to reduced proliferation and endoplasmic reticulum stress of trophoblast cells (Zadora et al., 2017). GATA-binding protein 3 (*GATA3*), a gene relevant to trophoblast invasion, was also identified as a candidate for future research concerning dysregulated imprint and pregnancy disorders (Chiu and Chen, 2016; Zadora et al., 2017).

Overall, the methylome opens new perspectives for comprehension of the phenomenon of inherited traits unrelated to classical nucleotide sequence changes in the genome (SNPs or mutations) and how they affect phenotype. The future is promising, but some important issues should be addressed. For example, PMD is overrepresented in the placental methylome, but most of the studies published so far have ignored them, raising the question of whether PMD occurs in specific trophoblast cell lineages or at specific stages of development (Schroeder et al., 2013; Bianco-Miotto et al., 2016). Interestingly, methylation patterns in early extraembryonic tissues resemble those commonly observed in cancer (Smith et al., 2017), implying that comprehension of the epigenomic landscape of these two phenomena would provide some clues to the inherent process of cellular invasion, proliferation, and vasculogenesis. Also, the paradox of high methylation of CpG islands in genes within placental PMD is yet to be addressed. Lastly, future studies should differentiate hypomethylation patterns occurring in PMD regions from those occurring in other genomic regions (Schroeder et al., 2013).

Placental Microbiome—Friend or Foe

The fact that microorganisms are detected in the placenta, the womb, and the fetus, once thought of as sterile entities, has attracted much attention. The detection of bacterial DNA in the placenta (Aagaard et al., 2014; Collado et al., 2016) and in the amniotic fluid (Collado et al., 2016) has brought the "placental microbiome" into the spotlight. This concept challenges the traditional belief that newborns acquire their first bacteria only

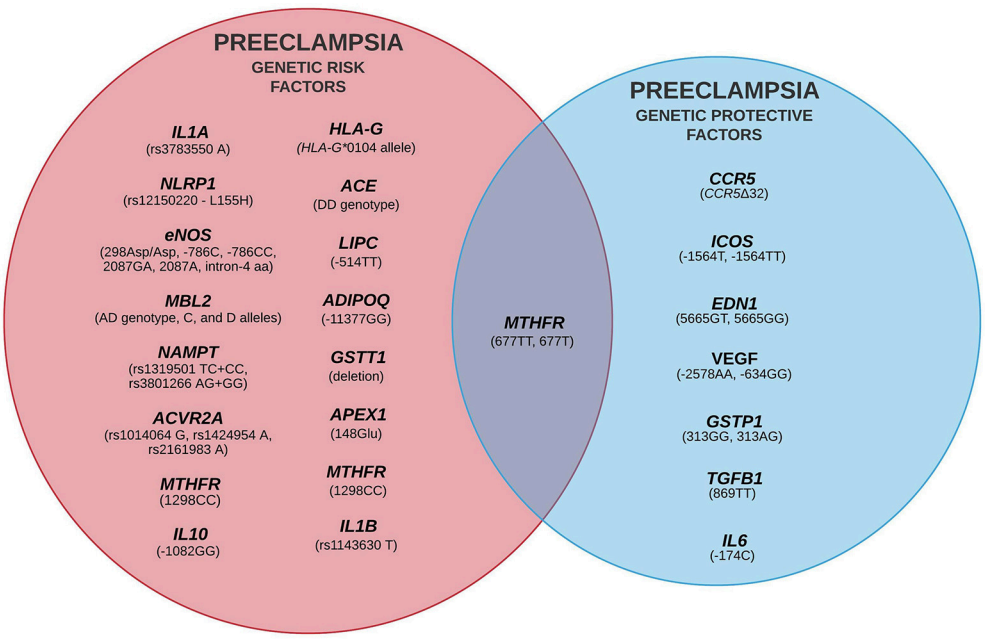

FIGURE 3 | Summary of preeclampsia-associated genetic variants addressed in Latin American studies. Only single locus polymorphisms are shown. Risk and protection associated variants are shown where the intersection represents variants found in both protective and risk factors in different studies. Additional information and haplotypes are detailed in **Tables 1**–**5**.

as they pass through the birth canal. The observation that *Enterococcus faecium* from human breast milk orally inoculated in pregnant mice can be detected in the amniotic fluid, and the pup's meconium (Jiménez et al., 2005, 2008; Aagaard et al., 2014) further supports the concept of the placental microbiome. In this same line, it seems that the newborn gut microbiome shares similarities to the maternal oral microbiome (Aagaard et al., 2014). The nature of symbiosis between extraembryonic tissues and the local community of microorganisms is still unknown. Although studies support the existence of fetal microbiomes, there is currently skepticism surround the concept, as discussed in other studies (Lauder et al., 2016; Perez-Muñoz et al., 2017).

The presence of placental microbiota in normal pregnancy (Aagaard et al., 2014; Parnell et al., 2017) intuitively implies that an altered microbiome would underlie pregnancy disorders such as chorioamnionitis and preterm birth (Antony et al., 2015; Prince et al., 2016). In this sense, a novel mechanism by which viruses may alter immunologic tolerance to intrauterine bacteria was suggested (Cross et al., 2017). It demonstrated that polymicrobial exposure of human fetal membranes (FM; amnion and chorion) explanted to bacterial LPS and virus [Herpes simplex virus type 2 (HSV2)] samples result in the aberrant expression of IL-1β, which is commonly observed in chorioamnionitis and preterm birth (Gomez-Lopez et al., 2017). The mechanism is not fully understood, however, it involves downregulation of the MER tyrosine kinase proto-oncogene (MERKT) receptor, allowing the activation of Nod-like receptor protein-3 (NLRP3) also known as the NLRP3 inflammasome through a synergistic signaling by LPS/TLR4 (TLR: toll-like receptor-4) and viral double strand dsRNA/TLR3 (dsRNA: double strand RNA) (Cross et al., 2017). It is worth mentioning that some viruses exploit TAM receptors for cell attachment and entry, but whether they are surrogates capable of suppressing TLR signaling is unclear (Best, 2013; Bhattacharyya et al., 2013). This observation is relevant since NLRP3 expression seems to be higher in the placental villi of preeclamptic women compared to normotensive women (Weel et al., 2017). However, if polymicrobial exposure underlies NLRP3 expression in preeclamptic placentas, and if different herpesviruses (i.e., congenital Cytomegalovirus infection) besides the ones evaluated are also able to reduce LPS threshold response are still open questions.

GENERAL CONCLUDING REMARKS

In Latin America, several studies approached the molecular basis of PE pathogenesis, documented by the increasing amount of scientific study and its impact on local and international scientific communities. In this endeavor, Brazil and Mexico are at the forefront of scientific production. However, we call attention to the need for studies in other Latin American countries, since these regions are characterized by a highly genetically diverse human population. Additionally, PE and gestational hypertensive disorders are a heavy burden in Latin America, strongly affecting maternal and fetal health.

Several genetic variants influencing PE predisposition were reported (**Figure 3**), some consistently associated with PE across different populations, despite disparities in the genetic/ethnic background inherent in Latin American populations. Genetic intra- and inter-variation have a great influence on genetic predisposition to PE. Although a comprehensive literature review was performed in this study, it may not be representative of the genetic variability present in Latin America since human population studies focus on small samples and therefore may not represent the genetic variability of entire local populations. Additionally, in developing countries, medical specialties (i.e., high-risk pregnancy care) are often centralized in the biggest cities. Therefore, replication of studies in different populations and multicentric collaborative studies are encouraged and would provide a better evaluation of the maternal genetic components of PE development in Latin America. Finally, PE and other hypertensive pregnancy disorders are the primary cause of maternal-fetal morbidity and mortality in low- and middle-income countries, representing a significant burden on public healthcare services. Therefore, it is imperative that public health policies assure prenatal care, perinatal monitoring, and health education in order to reduce the risk of pregnancy-related complications.

AUTHOR CONTRIBUTIONS

RM designed the review, planned the topics, wrote the review, reviewed the literature, and designed the figures and tables. VK wrote the review, reviewed the literature, designed the figures, and proofread the review. JC designed the review, contributed to writing the topics and critically reviewed the manuscript.

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Capítulo 3

A tug-of-war between tolerance and rejection - New evidence for 3'UTR HLA-G haplotypes influence in recurrent pregnancy loss

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A tug-of-war between tolerance and rejection – New evidence for 3′UTR HLA-G haplotypes influence in recurrent pregnancy loss

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ABSTRACT

HLA-G is a molecule essential to the maintenance of the maternal-fetal interface tolerance, thus contributing to a healthy pregnancy. Here we investigate the role of HLA-G single nucleotide polymorphisms (SNPs) and whether a specific HLA-G haplotype influence or not recurrent pregnancy loss (RPL) risk. A total of 296 DNA samples from RPL (N = 140) and controls (N = 156) were evaluated. The HLA-G 3′UTR region was sequenced and eight major SNPs were evaluated (14pb insertion/deletion, +3003T/C, +3010C/G, +3027C/A, +3035C/T, +3142G/C, +3187A/G, +3196C/G). A high linkage disequilibrium (LD) among all pairs and a perfect LD between +3010C/G and +3142G/A (D′ = 1.0, r^2 = 1.0) were observed. Our data showed an increased risk to +3010CC genotype carriers in comparison with control [odds ratio (OR) 2.05 95% confidence interval (CI) 1.05–4.00, p = 0.035] and to a decreased risk of RPL in +3142CC genotype carriers (OR = 0.49 95%CI 0.25–0.95, p = 0.035) and +3187AG genotype carriers (OR = 0.58 95%CI 0.35–0.94, p = 0.029). A total of eight haplotypes were observed in the sample, being UTR-1 and UTR-2 the most represented. An association between UTR-1 haplotype carriers with a reduced risk of both RPL and secondary RPL was observed. Our results indicate that the HLA-G 3′UTR plays important roles in RPL and might be an important marker of susceptibility to this, and possible to other, pregnancy disorders.

1. Introduction

Recurrent pregnancy loss (RPL) is historically defined as three consecutive pregnancy losses prior to 20 weeks. Nevertheless, due to a high risk of miscarriage in subsequent pregnancies after two consecutive pregnancy losses, some guidelines consider two consecutive pregnancy losses as a relevant point, that deserves evaluation in patients with no prior live birth (reviewed in Christiansen [1]). When defined by the presence of at least two consecutive miscarriages, RPL affects 2–5% of couples [1,2]. This disorder is classified in different clinical subgroups according to the woman reproductive history. Primary RPL is characterized by consecutive losses and no prior successfully pregnancy, while at least one successful pregnancy, regardless of the number of miscarriages, characterizes the secondary RPL [3]. Notably, after the identification of possible etiologic factors, a specific cause of RPL is clearly identified in only ~50% of all cases [3].

As the fetus is not genetically identical to its mother, it is reasonable to think that mechanisms must exist to allow the mother to carry the fetus throughout gestation without a rejection. Identified in the maternal-placental interface mainly on extravillous trophoblast cells (EVT), Human leukocyte antigen (HLA)-G has a relevant role in reproduction, it contributes to trophoblast invasiveness, vascular remodeling, decidual cell differentiation and establishment of a immuno tolerogenic environment [4–7].

Among the non-classical class I molecules, *HLA-G* is the best characterized and when compared to its classical class I counterparts, HLA-G is a low polymorphic molecule. At present, 51 alleles

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have been recognized by the international ImMunoGeneTics project (IMGT) [8]. Seven different isoforms of HLA-G are reported as the result of the alternative splicing of messenger RNA three of which are soluble (sHLAG5-G7) and four are membrane bound (G1-G4) [9]. In addition, a soluble G1 (sHLA-G1) form can be generated by cell surface proteolytic shedding [10].

In pregnancy, sHLA-G5, HLA-G1 and its shed form seem to be molecules of great importance [11]. Although functionality of the different isoforms is poorly understood, they might be involved in the modulation of immune activity. Through interaction with inhibitory receptors immunoglobulin-like transcript (ILT)2, ILT-4 and the killer immunoglobulin-like (KIR)2DL4, HLA-G promotes tolerance by regulating innate and adaptive responses (reviewed in Rebmann et al. [12]). Therefore, specific interactions contribute to: (a) the immune suppression of the cytotoxic activity of the decidual NK cells (dNK); (b) the inhibition of proliferation of CD4 + T lymphocytes, CD8+ T and B lymphocytes and induction of apoptosis of CD8+ T cells via FAS-FAS-L pathway and; (c) the placental stimulation and development through the secretion of angiogenic factors by dNK cells and macrophages [4,13,14].

The 3′ untranslated region (UTR) of *HLA-G* is of particular interest as it impacts the expression patterns of the gene due to the presence of regulatory elements such as AU-rich motifs, polyadenylation signals and miRNA binding sites [15]. Several *HLA-G* SNPs have been associated with an increased maternal risk to pregnancy disorders such as preeclampsia, infertility and recurrent miscarriage [5,16–18]. Three polymorphic sites have been reported to modify HLA-G expression: (1) the 14-bases pair (bp) insertion/deletion (ins/del) which is associated to mRNA stability [19]; (2) the +3142G which has been described as influencing micro RNA (miRNA) binding thus interfering on mRNA availability [20,21] and; (3) the +3187A described to affect mRNA stability due to a proximal AU-rich motif [22]. In despite of being a quite short segment, the 3′UTR presents at least eight polymorphic sites that are more frequently evaluated in worldwide populations. Recently, several other single nucleotide polymorphisms and 3′UTR haplotypes have been characterized [15,23].

A study evaluating the influence of 3′ UTR haplotypes and the expression patterns of sHLA-G in healthy non-pregnant individuals of a Brazilian cohort and French showed that some haplotypes were associated with high (UTR-1) and low (UTR-5, UTR-7) HLA-G levels in blood plasma [24]. Although conflicting results exist due to methodological issues, HLA-G UTR-1 haplotype seems to be one of the most important determinants on sHLA-G expression in pregnant and non-pregnant individuals [11,25]. Genetic variability in this region has been approached in a wide range of association studies, but in most cases, a limited set of putative functional variants was evaluated, whereas only few studies have evaluated the full sequence variation in unrelated healthy, non-healthy individuals as well as in the pregnancy outcome [16,17,26–28]. Therefore, and since the individual genetic variability in the 3′UTR might impact the pattern of HLA-G expression, we performed a comprehensive analysis of the *HLA-G* 3′ UTR in recurrent pregnancy loss and healthy multiparous women, in order to evaluate the maternal influence of this genetic *locus* in pregnancy outcome.

2. Materials and methods

2.1. Subjects

The present study enrolled a total of 296 women (156 multiparous control and 140 RPL idiopathic women). The subjects were recruited between 2000 and 2011 from the Prenatal Diagnosis Clinic of the Medical Genetics Service of the *Hospital de Clínicas de Porto Alegre* (HCPA), located in Southern Brazil. All women reporting at least two pregnancy losses before 24 weeks of gestation with the same partner and with no report of full-term pregnancy were invited to participate in the study [29,30]. Clinical and demographic data was obtained through a structured interview (obstetric history, family history of malformations, weight, height, occupation, use of tobacco, alcohol consumption, drug use). All women were subjected to a preliminary standard diagnostic protocol evaluating known causes of pregnancy losses [hysteroscopy, laparoscopy, ultrasound, karyotypic examination, immunological risk factors (anticardiolipin, lupus anticoagulant, antinuclear antibodies) and hormonal status (gonadotrophins, FSH, LH, prolactin, thyroid hormones, thyroperoxidase] before inclusion in the study. Exclusion criterion was considered by the presence of any clinical condition that could prevent full-term pregnancies.

The control group consisted of 156 healthy multiparous women with at least two successful pregnancies reported and no history of pregnancy loss and/or infertility, randomly selected to participate in the study during blood collection for routine laboratory analyses at the HCPA. This study was approved by the Research Ethics Committee of the Research and Postgraduate Studies Group of the HCPA, under the protocol number #11-242. Written informed consent in accordance with the Declaration of Helsinki was obtained from every participant before their inclusion in the study.

2.2. DNA extraction

Genomic DNA was obtained from the blood samples and saliva. The DNA extraction from blood was performed according to Lahiri and Nurnberger [31] and the DNA from saliva samples was extracted using the Oragene® DNA collection kit (DNA Genotek Inc., Canada), in accordance with the manufacturer's protocol.

2.3. HLA-G 3′ UTR analysis

The 3′ UTR of *HLA-G* gene (hg38 assembly chr6:29830768-29831270) was amplified by polymerase chain reaction (PCR) as previously described [32]. The PCR products were directly sequenced using the reverse primer GmiRNA in an ABI 3730 XL DNA sequencer (Applied Biosystems, Foster City, CA, USA). Finally, *HLA-G* polymorphic variants were assessed by interpretation of chromatogram peaks using the FinchTV software version 1.4.0 (available on http://www.geospiza.com/Products/finchtv.shtml). In the total, 133 RPL and 152 control samples were efficiently sequenced while seven and four samples, respectively, were excluded from genetic analysis due to low reactions quality.

2.4. Haplotype analysis

Linkage disequilibrium (LD) between pairs of the genetic variants evaluated in the study was assessed by r2 and D′ coefficient using the Haploview 4.1 software [33]. Haplotype inference was performed using a Bayesian method implemented in the PHASE software version 2.1 using default parameters [34]. Ten independent runs with different seed values for the random number generator were used to check the consistency across the results. The haplotype overall frequencies generated from PHASE showed highly consistent results across different runs. One sample of each group was excluded from the haplotype analysis due to a low confidence in the estimate. The haplotype Hardy-Weinberg equilibrium expectation was tested by exact test implemented in the Arlequin software version 3.5.1.3 [35]. All haplotype were named accordingly with previous studies [15,23].

Table 1
Clinical and demographic characteristics of RPL and control women.

	RPL	Control	*p*-Value
Age at first pregnancy [median (25–75%)]	22 (18.0–28.2)	22 (20.0–25.0)	0.764[a]
Number of pregnancies [median (25–75%)]	3.0 (2.0–4.0)	2.0 (2.0–3.0)	<0.001[a]
Body Mass Index [median (25–75%)]	26.0 (23.0–29.0)	24.0 (21.0–28.0)	0.097[a]
European-derived [n(%)]	88 (62.9)	121 (77.6)	0.006[b]
Alcohol consumption [n(%)]	60 (42.9)	32 (20.5)	<0.001[b]
Smoking [n(%)]	21 (15.0)	34 (21.8)	0.133[b]
Alcohol and Smoking habit [n(%)]	15 (10.7)	9 (5.7)	0.113[b]
Primary RPL [n(%)]	101 (72.1)	–	
Secondary RPL [n(%)]	39 (27.9)	–	
Miscarriages [mean (±SD)]	3.3 (±1.6)	–	
2 miscarriages [n(%)]	50 (35.7)	–	
3 miscarriages [n(%)]	50 (35.7)	–	
>4 miscarriages [n(%)]	40 (28.6)	–	

[a] Mann-Whitney *U*-test.
[b] Pearson Chi-square test. RPL n = 140 and Control n = 156. SD = standard deviation.

2.5. Statistical analysis

Categorical and quantitative variables were analyzed, respectively, by Chi-square and Mann-Whitney *U* tests. Asymmetric continuous distributions are described by the median and the 25th and 75th percentiles. The Hardy-Weinberg equilibrium was calculated for the RPL and control groups, for each polymorphism and haplotype evaluated in the study. The analyses were conducted in overall RPL women and its clinical subgroups (primary and secondary RPL) in comparison to the control group. Adjusted Logistic regression was performed to evaluate the strength of association between genetic markers and outcomes using odds ratio (OR) and respective 95% confidence interval (CI). Potential confounders evaluated were smoking, alcohol consumption and ethnicity. Covariates were entered in the logistic regression models only if they were associated with the study factor and with the outcome at $p < 0.20$. All statistical analyses were performed with standard software SPSS v.18.0 for Windows (SPSS Inc., Chicago, Illinois, USA). For all instances, p-value < 0.05 were considered to be significant.

3. Results

In the current study we assessed clinical and demographic characteristics of 140 RPL and 156 healthy multiparous women (Table 1). We observed a similar median age at the first pregnancy between RPL women and the control group ($p = 0.764$). Likewise, similar results were observed for body mass index ($p = 0.097$), smoking habit ($p = 0.133$) and combined alcohol and smoking habit ($p = 0.113$) between groups. Our analysis showed a higher frequency of European-derived women among controls (78%) as compared to RPL group (63%, $p = 0.006$), although stratifying individuals in European-derived and African-derived revealed similar frequencies for all polymorphisms evaluated independently of the ethnic/geographic origin (Table S1). The obstetric history of the subjects enrolled in the study was evaluated. The median number of pregnancies was higher among RPL women as compared to the control group ($p < 0.001$), and the proportion of RPL women who had two, three or four/plus miscarriage was of 35.7, 35.7 and 28.6%, respectively. 72.1% of RPL patients were classified as primary RPL and 27.9% as secondary RPL. Among the known risk factors to pregnancy complications evaluated, the frequency of alcohol consumption was twice as high in RPL women (42.9%) compared with the control group (20.5%, $p < 0.001$).

A comprehensive analysis of the 3′UTR region allowed the evaluation of the frequencies of eight main variants (Table 2) although ten other variant sites (rs567747015, rs146339774, rs569057854,

Table 2
Genotypic and allelic frequencies of polymorphic sites observed in the *HLA-G* 3′ UTR in RPL and control women.

Genotypes	RPLn(%)	Control n(%)	OR	95% C.I	*p*-Value
14 bp Del/Del	41 (30.8)	58 (38.2)	1		
Ins/Del	62 (46.6)	71 (46.7)	1.24	0.73–2.08	0.430
Ins/Ins	30 (22.6)	23 (15.1)	1.84	0.94–3.62	0.075
Del	144 (54.1)	187 (61.5)			0.082[a]
Ins	122 (45.9)	117 (38.5)			
+3003T/C					
TT	103 (77.4)	118 (77.6)	1		
TC	28 (21.1)	32 (21.1)	1.00	0.57–1.78	0.993
CC	2 (1.5)	2 (1.3)	1.14	0.16–8.28	0.893
T	234 (88.0)	268 (88.2)			0.949[a]
C	32 (12.0)	36 (11.8)			
+3010C/G					
GG	23 (17.3)	40 (26.4)	1		
CG	64 (48.1)	73 (48.0)	1.53	0.82–2.81	0.177
CC	46 (34.6)	39 (25.6)	2.05	1.05–4.00	0.035
G	110 (41.3)	153 (50.3)			0.032[a]
C	156 (58.7)	151 (49.7)			
+3027C/A					
CC	118 (88.7)	139 (91.4)	1		
CA	15 (11.3)	13 (8.6)	1.36	0.62–2.97	0.442
C	251 (94.4)	291 (95.7)			0.503[a]
A	15 (5.6)	13 (4.3)			
+3035C/T					
CC	94 (70.7)	115 (75.7)	1		
CT	37 (27.8)	36 (23.7)	1.26	0.74–2.14	0.400
TT	2 (1.5)	1 (0.6)	2.45	0.22–27.4	0.468
C	225 (84.6)	266 (87.5)			0.304[a]
T	41 (15.4)	38 (12.5)			
+3142G/C					
GG	46 (34.6)	39 (25.7)	1		
GC	64 (48.1)	73 (48.0)	0.74	0.43–1.28	0.284
CC	23 (17.3)	40 (26.3)	0.49	0.25–0.95	0.035
G	156 (58.6)	151 (49.7)			0.032[a]
C	110 (41.4)	153 (50.3)			
+3187A/G					
AA	82 (61.6)	72 (47.4)	1		
AG	44 (33.1)	69 (45.4)	0.58	0.35–0.94	0.029
GG	7 (5.3)	11 (7.2)	0.57	0.21–1.56	0.272
A	208 (78.2)	213 (70.0)			0.025[a]
G	58 (21.8)	91 (30.0)			
+3196C/G					
CC	65 (48.9)	79 (52.0)	1		
CG	56 (42.1)	66 (43.4)	1.03	0.64–1.67	0.901
GG	12 (9.0)	7 (4.6)	2.08	0.78–5.60	0.145
C	186 (70.0)	224 (73.7)			0.328[a]
G	80 (30.0)	80 (26.3)			

OR, Odds. 95% C.I, Confidence interval.
[a] *p*-Values for alleles were obtained by Pearson Chi-square.

rs180827037, rs554784083, rs138249160, rs554076817, rs187320344, rs1233331, rs541542414) were monomorphic in our sample. A logistic regression was performed to assess the impact of genotype variants in the risk of RPL. Our results indicated differences on +3010C/G allele frequencies between patients and controls ($p = 0.032$) and association of the +3010CC genotype to a higher risk of RPL [OR = 2.05 (95% CI 1.05–4.00), $p = 0.035$] compared to the GG genotype. A statistical difference was observed for the +3142G/C allele frequencies ($p = 0.032$) and a reduced risk of RPL was associated with the +3142CC genotype [OR = 0.49 (95% CI 0.25–0.95), $p = 0.035$] compared to GG genotype. Also, an association of the +3187AG genotype with a reduced risk of RPL was observed [OR = 0.58 (95% CI 0.35–0.94), $p = 0.029$]. Additionally, a statistical difference was observed in the +3187A/G allele frequencies between groups ($p = 0.025$). The +3187G allele behaved according to a dominant model [OR = 0.60 (95% CI 0.37-0.97), $p = 0.039$] rather than to a recessive one ($p = 0.569$). Multivariate models including the eight single nucleotide polymorphisms and haplotypes were carried out to evaluate their role in the prediction of RPL risk. No statistically significant results were observed even when covariates were included in the model (data not shown).

Linkage disequilibrium (LD) analysis indicated a strong LD among the *loci* studied. A perfect LD was observed between +3010C/G and +3142G/A ($D' = 1.0$, r2 = 1.0, Table S2). The whole haplotype distributions adhere to the Hardy-Weinberg equilibrium expectations. Table 3 presents the haplotype distribution in RPL and controls. HLA-G UTR-1 and UTR-2 were the most frequent haplotypes in both RPL and control groups, although in RPL group UTR-2 was the most frequent (30.3%) and in the control group UTR-1 was the most frequent (29.8%). However, no statistical differences in haplotype frequencies were observed between RPL and controls ($p = 0.549$). HLA-G UTR-1 carriers in RPL and control group were compared. An association with reduced risk of RPL in UTR-1 carriers was observed [OR = 0.59 (95% CI 0.37–0.95; $p = 0.032$), Table 4]. Furthermore, an association with reduced risk of secondary RPL [OR = 0.47 (95% CI 0.22–0.99); $p = 0.049$, Table 5] and the number of miscarriages (4 or plus) were observed [OR = 0.46 (95% CI 0.21–0.98); $p = 0.045$].

A total of 25 and 23 diplotypes were observed in RPL women and control group, respectively (Table S3). The overall diplotype frequencies were similar between groups ($p = 0.357$). The UTR-1/UTR-2 diplotype was the most frequent accounting for 19% and 10.6% [respectively, control and RPL group ($p = 0.039$)]. Interestingly, a statistical significance was observed in the frequency of diplotypes carrying at least one copy of the UTR-1, being the frequency of the UTR-1 higher in the control group (45%) than in RPL women (33%, $p = 0.020$). Also, the frequency of diplotypes carrying at least one copy of UTR-2 haplotype was higher in the RPL woman (41%) as compared to controls (29%, $p = 0.038$).

Table 3
HLA-G 3′UTR haplotype frequencies.

Haplotypes[a]	RPL N (%)	Control N (%)
UTR-1 (DelTGCCCGC)	58 (22.0)	90 (29.8)
UTR-2 (InsTCCCGAG)	80 (30.3)	80 (26.5)
UTR-3 (DelTCCCGAC)	34 (12.9)	33 (10.9)
UTR-4 (DelCGCCCAC)	31 (11.7)	35 (11.6)
UTR-5 (InsTCCTGAC)	25 (9.5)	23 (7.7)
UTR-6 (DelTGCCCAC)	20 (7.5)	27 (8.9)
UTR-7 (InsTCATGAC)	15 (5.7)	13 (4.3)
UTR-13 (DelTCCTGAC)	1 (0.4)	1 (0.3)

Sample size: Control 2n = 302 and RPL 2n = 264.
[a] Haplotype consensus sequences are represented by 14 bp Ins/Del +3003T/C +3010C/G +3027C/A +3035C/T +3142G/C +3187A/G +3196C/G.

Table 4
Logistic regression analysis to assess the influence of *HLA-G* UTR-1 carriers in RPL and healthy multiparous control women.

	RPL n(%)	Control n(%)	OR	95% C.I	*P*-Value
Non-carriers	81 (61.4)	72 (47.7)	1		
UTR-1 Carriers	51 (38.6)	79 (52.3)	0.593	0.37–0.95	0.032[a]

[a] *P*-Value, multiple logistic regression test; Hosmer and Lemeshow test: $P = 0.998$; Omnibus test: $P = 0.034$. Sample size: RPL n = 132 and Control n = 151.

Table 5
HLA-G aggregated UTR-1 haplotype overall distribution and frequencies accordingly to clinical features of recurrent pregnancy loss.

Variables	UTR-1 Carriers		OR	95% C.I	*P*-Value
	RPL n(%)	Control n(%)			
Primary RPL	38 (40.4)		0.64	0.38–1.09	0.103
Secondary RPL	13 (34.2)		0.47	0.22–0.99	0.049
2 pregnancy losses	19 (39.6)	79 (52.3)	0.60	0.31–1.16	0.126
3 pregnancy losses	20 (43.5)		0.75	0.38–1.48	0.414
>4 pregnancy losses	26 (68.4)		0.46	0.21–0.98	0.045

Sample size is Control n = 151, Primary RPL n = 94, Secondary RPL n = 38, Pregnancy loss categories: 2 (n = 48), 3 (n = 46) and > 4 (n = 38). OR, Odds Ratio. 95% C.I, Confidence Interval.

4. Discussion

In the present study it was possible to put in evidence associations of the +3010CC, +3142GG and +3187AG HLA-G 3′UTR genotypes with RPL predisposition. Nevertheless, when correction for multiple comparisons was applied, these associations lack their statistical significance. Of note, Bonferroni adjustment is very conservative, and an unfortunate byproduct of such adjustment is the probability of false negatives where there is a real effect [36]. The following discussion will take this situation into consideration.

The associations of +3010CC and +3142GG with RPL risk might reflect the high linkage disequilibrium between these markers. Both variants were previously evaluated *in silico* as putative binding sites for miRNAs [21]. Further, JEG-3 cells transfected with pre-miR-152 shown reduced HLA-G expression and increased NK cytolysis activity, clearly suggesting a role for miRNAs in HLA-G gene expression [37]. As previously stated, the +3142G/C polymorphism has no influence on HLA-G expression on its own and such variants should not be interpreted as individual determinants of miRNA binding [11,38]. That is, the cellular microenvironment and the 3′UTR haplotype/region may account for a final balancing in respect to HLA-G expression. Furthermore, a high linkage disequilibrium among variation sites in both the upstream regulatory region and 3′UTR may contribute to differential expression of HLA-G [12,39].

Additionally, a reduced risk of RPL in +3187AG carriers was reported. To the best of our knowledge, this is the first study evaluating this polymorphism in RPL. The +3187A confers a decreased *HLA-G* expression due to its proximity to an AU-rich element related to mRNA degradation [15]. This allele was associated with lower mRNA stability *in vitro* and has been associated with preeclampsia development in a Canadian population. However, a recent finding of the same research group contradicts its own findings, highlighting the need for further studies [17,22].

The 14 bp ins/del is the most studied due to its impact on mRNA stability and expression. The insertion allele is related to a higher stability of HLA-G transcripts in comparison to the deletion allele [19,40]. Recently, an allelic imbalance in the 14 bp ins/del polymorphism was shown to modulates the HLA-G surface expression on primary trophoblast cells, highlighting potential implications of this variant on pregnancy [41]. A statistically difference regarding

14 bp ins/del was not observed, although a remarkably tendency to a higher proportion of the 14 bp ins allele and homozygous carriers in the miscarriage group was observed, corroborating previous data from the literature [42–44]. Interestingly, higher rates of implantation failure in couples who underwent ART were associated with both the presence of 14 bp ins allele and the 5′URR PROMO-G010102a haplotype [45], although other studies evaluated the role of this variant in RPL and the results are still conflicting [46,47].

The polymorphic sites assessed in this study are encompassed in a short region in the gene and given a higher LD observed between them we observed that UTR-1 haplotype, which has all variation sites related to a high sHLA-G production [24], was protective against RPL development. Here we were not able to measure sHLA-G levels, although other studies have evaluated it on in non-pregnant healthy individuals taking into account the 3′UTR haplotypes [24,25]. Regardless of methodological differences in the studies aforementioned, UTR-1 haplotype seems to be one of the most important determinants of sHLA-G1 and -G5 blood levels. Circulating sHLA-G levels from non-pregnant healthy individuals is associated with the *HLA-G* genotypes/haplotypes, although in pregnant women one would take into account both the maternal and fetal contributions. Recently, a study evaluating the contribution of mother-child 3′UTR SNPs for sHLA-G levels have shown that heterozygous women for 14 bp del/+3142C genotype carrying a fetus with 14 bp ins/+3142G homozygous genotype had higher sHLA-G peripheral levels at term than those carrying a fetus with other genotypes. These data was interpreted by the authors as an advantage of heterozygous, reducing the risk of pregnancy disorders [11]. Such findings, and the dual role of the HLA-G molecule, could partially explain the higher frequency of heterozygous (UTR-1/UTR-2) in healthy multiparous women and need to be further evaluated. Indeed, the *Janus face* of HLA-G is quite established, and if its expression is advantageous in some circumstances (maintenance of maternal-fetal tolerance, decreased risk of miscarriage, protection against high inflammatory conditions) it is clearly detrimental in others (immunologic surveillance to tumors and invading pathogens) suggesting that the maintenance of high-expressing and low-expressing haplotypes may be individually beneficial [11,12,23,27,28,48]. Given that, individuals carrying low and high-expressing haplotypes may be at fine balance between optimal levels of expression allowing for both fetal tolerance and an appropriate defense against pathogens [23].

We observed an association of the UTR-1 haplotype with a decreased risk of secondary RPL. Of note, few studies evaluated the influence of 3′UTR polymorphisms in secondary RPL probably because it represents a poorly understood group in which a distinction from primary RPL is not usually made.

In conclusion, this is the first study to perform a comprehensive analysis of the 3′UTR of *HLA-G* in RPL. Our results a possible role of +3010CC, +3142CC and +3187AG genotypes in RPL susceptibility. Also, carriers of the *HLA-G* 3′ UTR-1 haplotype were associated with a decreased risk of both RPL and secondary RPL development in a Southern Brazilian population. In this sense, the overall picture on the differential genotype and haplotype distribution of *HLA-G* in pregnancy disorders is emerging and new studies will help in the establishment of the role of all the actors of this play.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.humimm.2016.07.004.

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Table S1. Minor Allele frequency comparisons according to European-derived and African-derived individuals.

SNP ID	Associated Allele	Frequencies in European-derived		Frequencies in African-derived		*p*-value[a]
		RPL	Control	RPL	Control	
rs371194629	Insertion	0.488	0.374	0.415	0.435	0.934
rs1707	C	0.135	0.122	0.096	0.133	0.390
rs1710	G	0.406	0.513	0.436	0.468	0.688
rs17179101	A	0.053	0.050	0.064	0.016	0.724
rs17179108	T	0.141	0.134	0.181	0.081	0.951
rs1063320	C	0.406	0.513	0.436	0.468	0.687
rs9380142	G	0.224	0.319	0.213	0.226	0.161
rs1910696	G	0.353	0.244	0.213	0.355	0.579

[a]*p*-values obtained by Pearson Chi-Square test. SNP= Single Nucleotide polymorphism, ID= Identification. RPL= RecurrentPregnancyLoss.

Table S2. Analysis of linkage disequilibrium patterns for all pairs of polymorphic sites observed in the 3' UTR of the *HLA-G*.

	+2960 Ins/del[a]	+3003 T/C	+3010 C/G	+3027 C/A	+3035 C/T	+3142 G/C	+3187 A/G	+3196 C/G
+2960 Ins/del	—	0.086	0.606	0.072	0.197	0.606	0.256	0.540
+3003 T/C	0.939	—	0.158	0.007	0.022	0.158	0.048	0.053
+3010 C/G	0.999	1.000	—	0.044	0.138	1.000	0.413	0.334
+3027 C/A	1.000	1.000	1.000	—	0.321	0.044	0.003	0.020
+3035 C/T	0.941	1.000	1.000	1.000	—	0.138	0.057	0.063
+3142 G/C	0.999	1.000	1.000	1.000	1.000	—	0.413	0.334
+3187 A/G	1.000	1.000	1.000	0.396	1.000	1.000	—	0.138
+3196 C/G	1.000	1.000	1.000	1.000	1.000	1.000	1.000	—

D′ Lewontin Normalized Coefficient (below the line) and r^2 values between pairs (above the line).[a]+2960 Ins/del= 14bp Insertion/deletion.

Table S3. Diplotype frequencies observed in RPL and control women.

Diplotypes	Control n(%)	RPL n(%)
UTR-1/UTR-1	11 (7.3)	7 (5.3)
UTR-1/UTR-2	29 (19.2)	14 (10.6)
UTR-1/UTR-3	8 (5.3)	5 (3.8)
UTR-1/UTR-4	11 (7.3)	7 (5.3)
UTR-1/UTR-5	3 (2.0)	5 (3.8)
UTR-1/UTR-6	11 (7.3)	6 (4.5)
UTR-1/UTR-7	6 (4.0)	7 (5.3)
UTR-2/UTR-2	7 (4.6)	12 (9.1)
UTR-2/UTR-3	9 (6.0)	12 (9.1)
UTR-2/UTR-4	7 (4.6)	10 (7.6)
UTR-2/UTR-5	9 (6.0)	10 (7.6)
UTR-2/UTR-6	6 (4.0)	5 (3.8)
UTR-2/UTR-7	6 (4.0)	5 (3.8)
UTR-3/UTR-3	1 (0.7)	3 (2.3)
UTR-3/UTR-4	4 (2.6)	3 (2.3)
UTR-3/UTR-5	5 (3.3)	2 (1.5)
UTR-3/UTR-6	1 (0.7)	6 (4.5)
UTR-4/UTR-13	1 (0.7)	1 (0.8)
UTR-4/UTR-4	2 (1.3)	2 (1.5)
UTR-4/UTR-5	3 (2.0)	3 (2.3)
UTR-4/UTR-6	5 (3.3)	1 (0.8)
UTR-4/UTR-7	1 (0.7)	2 (1.5)
UTR-5/UTR-5	5 (3.3)	1 (0.8)
UTR-5/UTR-6	–	2 (1.5)
UTR-5/UTR-7	–	1 (0.8)

RPL= Recurrent Pregnancy Loss. Sample size Controls=151 and RPL =132.

Capítulo 4

*The HLA-E*0103 and +3777G alleles on grafts increase the risk of kidney acute rejection after simultaneous pancreas and kidney transplantation – A report from a one-year follow-up study*

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The *HLA-E0103 and +3777G alleles on grafts increase the risk of kidney acute rejection after simultaneous pancreas and kidney transplantation – A report from a one-year follow-up study**

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Conflicts of Interest

The authors have declared no conflicting interests.

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Abstract

Human Leukocyte Antigen (HLA)-E is a potential immunomodulatory and antigen-presenting molecule. While specific *HLA-E* alleles (E*0101 and 01:03) have been reported to impact transplantation outcomes (i.e., graft rejection, CMV infection, graft versus host disease), no investigation of *HLA-E* was reported in simultaneous pancreas and kidney transplantation (SPKT). We report that donor's *HLA-E* genotype has a more significant role in graft acute rejection (AR) than the recipient's *HLA-E* or allelic mismatch. Also, with the inclusion of the *HLA-E* +3777G/A genotyping we observed that most of the donor's E*01:03 alleles were E*01:03:02:01, while most of E*01:01 alleles were E*01:01:01. Notably, we observed that grafts carrying E*01:03:02:01 or +3777G alleles have a shorter kidney AR-free-survival (log-rank test, $P < 0.05$). Also, the donor's +3777G status was an independent predictor for kidney AR in the first-year post-SPKT ($P = 0.031$). These preliminary findings highlight that the *HLA-E* status of the graft is an important factor for the occurrence of acute rejection episodes in the first-year post-SPKT. Moreover, it also calls our attention to potential functional differences between the most common HLA-E alleles, which should be taken into account in the context of transplantation.

The role of classical MHC-class Ia (A,B,C) molecules in graft allorecognition and graft rejection is well established. However, the impact of non-classical MHC-class Ib (E,F,G) molecules is little explored in solid organ transplantation (Guberina et al., 2018; Guberina et al., 2017; Cristofaro et al., 2016). SPKT is of particular interest as it differs from other solid organ transplantations making it not easily comparable (for example, induction therapy and maintenance of immunosuppression are much stronger in SPKT patients). However, immunological mechanisms involved in the acceptance or rejection of these SPK grafts should be similar to those accounting to the same phenomena in other grafts.

Contrasting with other non-classical molecules, *HLA-E* is a critical alloantigen, and its expression correlates with mouse skin allograft rejection and human kidney allograft rejection (Pecasova et al. 1999; Guberina et al. 2017). The HLA-E immunological properties are mainly transduced through the binding with inhibitory (NKG2A) or activating (NKG2C) receptors expressed on NK and CD8+ T cells, but can also be effective via the αβ-TCR complex (Garcia et al. 2002). Therefore, HLA-E bridges innate and adaptive responses. HLA-E presents a restricted set of peptides (leader sequence/peptide) derived from specific class-I HLA molecules. Also, HLA-E can present cellular stress-related proteins and pathogen-derived peptides. Thus, HLA-E expression is suggested as a qualitative measure of the integrity of the intracellular antigen processing machinery (Braud et al., 1999; Hall et al., 2010). In transplantation settings, expression of HLA-E on grafts has several implications: (i) even in the absence of allelic mismatch at *HLA-E* between donor and recipient alloreactivity may occur due to (but not limited to) the pool of peptides derived from donor's MHC-I or *HLA-E* expression (Lauterbach et al., 2015; Guberina et al. 2017). (ii) Viruses (i.e., CMV, HIV, EBV) can usurp the HLA-E machinery favoring their pathogenesis and negatively impacting in allograft survival (Joosten et al. 2016; Guberina et al. 2017), and (iii) cellular stress signals provided by surgical trauma, viral infection and ischemia-reperfusion injury might increase HLA-E/HSP60 expression modifying cytotoxic cells response (Michaelsson et al., 2002). Therefore, it is important to evaluate how *HLA-E* mismatches or specific alleles affect graft acceptance, maintenance, and survival.

According to the Immuno Polymorphism Database, *HLA-E* has the lowest allelic diversity amongst the MHC-Ib, only 27 alleles and 8 proteins being described to date (https://www.ebi.ac.uk/ipd/) (Release 3.34.0, Robinson et al. 2013). Of note, even in highly admixed populations, together, the E*0101 and the E*0103 alleles can be found in frequencies as

high as ~97.5% (Felicio et al. 2014). These alleles differ by an arginine (E*01:01) to glycine (E*01:03) exchange at codon 107 [+756A/G (rs115492845)] (Ulbrecht 1999). Worldwide, E*0101 is the most frequent allele, followed by E*0103 (Felicio et al. 2014). Remarkably, sequencing of exons 1-4 and the 3'unstranslated region (UTR) of *HLA-E* revealed a high allelic variability for E*0103 allele as it splits amongst E*01:03:01, E*01:03:02a, and E*01:03:02b lineages, each one with distinct haplotypes (Felicio et al. 2014). However, only few alleles/haplotypes have a frequency above 1% in worldwide populations: E*01:01:01 (51.4%), E*01:03:02:01 (25.3%), E*01:03:02:01^{424T}(3.33%), E*01:03:01 (12.7%), E*01:06 (1.54%), E*01:03:05 (1.46%). Noteworthy, together these alleles represent up to 96% of *HLA-E* alleles segregating in worldwide populations. Contrary to previous observations, it is important to note that some more recent data do not favor the hypothesis of balancing selection acting on +756A/G (Grimsley and Ober, 1997; Felicio et al., 2014).

In this report, we present a one-year follow-up of 50 diabetes mellitus type 1 (DM1) patients after SPK transplantation. Patients were recruited at *Universitätsklinikum Knappschaftskrankenhaus* (Bochum, Germany, from 2012-2016). Transplantation outcomes included a graft function evaluation, occurrence of acute rejection episodes and CMV infection. All recipients received thymoglobulin induction therapy, immunosuppression maintenance, and CMV prophylaxis. Kidney AR was evaluated through biopsy and was graded according to the BANFF classification (Racusen et al. 1999). Pancreas biopsy was only performed when the isolated occurrence of AR was suspected. Treatment of AR consisted in methylprednisolone for three consecutive days, and, if resistant, with thymoglobulin. Antibody-mediated rejections were treated with plasmapheresis and/or thymoglobulin and intravenous immune globulin injection.

Clinical and demographic characteristics according to the overall occurrence of AR episodes are detailed in a previous study by our group (Michita et al. 2018). In the aforementioned study, the incidence of rejection episodes of any kind in the first-year post-transplantation was (19/50; 38%), being kidney AR (15/19; 79.0%) the most frequent, followed by pancreas AR (3/19; 15.8%) and both kidney and pancreas AR (1/19; 5.2%). In the present study, we evaluate whether *HLA-E* allelic mismatches influence SPKT outcome. In all transplant situations (kidney AR, pancreas AR, no rejection group) most of the recipient-donor pairs were matched for *HLA-E* and no association of *HLA-E* mismatches with SPKT outcome was observed. Since the *HLA-E**01:03 allele has a great potential to present peptides, and elicit immune responses, recipients were

evaluated in terms of graft genotype (Table 1). No influence of *HLA-E* genotype on graft function and CMV infection was observed. Also, since *NKG2C* allele deletion genotyping was not performed before transplantation, as expected, no differences in the *NKG2C* allele deletion frequency in recipients receiving an E*01:03 positive or an E*01:03 negative graft was evidenced (P = 0.450). Genotyping frequencies are detailed in Supplementary Table 1.

Table 1. Clinical characteristics of SPK recipients according to the presence of HLA-E*0103 on donor grafts

Variables	HLA-E*0103 positive (*n* = 28)	HLA-E*0103 negative (*n* = 17)	*p*
Age (±SD)	44.9 (11.9)	43.8 (11.0)	0.760
Recipient gender (male/female)	18/10	8/9	0.257
Gender mismatch (%)	7 (25.0)	4 (23.5)	0.911
BMI (±SD)	24.4 (3.6)	24.2 (3.4)	0.839
Urea, mg/dL (±SD)	21.9 (8.8)	17.7 (7.0)	0.135
Creatinine, mg/dL (median: 25–75%)	1.50 (1.02-1.69)	1.30 (1.13-1.48)	0.526
Glucose, mg/dL (median: 25–75%)	103 (91-115)	94 (88-116)	0.559
HbA1C, % (median: 25–75%)	5.70 (5.33-6.50)	5.69 (5.10-6.40)	0.521
Kidney graft cold ischemia time, min (±SD)	807.5 (166.1)	761.1 (168.5)	0.306
Pancreas graft cold ischemia time, min (±SD)	680.2 (131.0)	657.9 (142.3)	0.415
HLA-A, *-B*, *-DR* mismatches	5 (4-5.5)	5 (4-5)	0.243
HLA-A and *-B* mismatches (median: 25%–75%)	3 (2.5-4)	3 (2-3)	0.208
HLA–DR mismatch (median: 25–75%)	2 (1-2)	2 (1-2)	0.685
Anti–MICA pretransplantation (%)	4 (14.3)	1 (6.3)	0.638
Anti–MICA post-transplantation (%)	3 (11.5)	2 (13.3)	1.000
Anti–MHC I post-transplantation (%)	7 (26.9)	1 (6.7)	0.220
Anti–MHC II post-transplantation (%)	7 (26.9)	1 (6.7)	0.220
Cytomegalovirus D+ (%)	13 (46.4)	7 (41.2)	0.731
Cytomegalovirus R+ (%)	14 (50.0)	8 (47.1)	0.848
Cytomegalovirus, R−/D+ (%)	7 (25.0)	5 (29.4)	0.743
Cytomegalovirus infection, first-year post-transplantation (%)	3 (10.7)	3 (17.6)	0.658
Pancreas or kidney graft AR (%)	13 (46.4)	5 (29.4)	0.259
Pancreas graft AR (%)	1 (3.6)	3 (17.6)	0.144
Kidney graft AR (%)	13 (46.4)	2 (11.8)	**0.017**
NKG2C allele deletion, frequency	0.19	0.26	0.450

Bold p values indicate statistically different parameters. AR = acute rejection; ATG = antithymocyte globulin; BMI = body-mass index; D = donor; HLA = human leukocyte antigen; MICA = MHC class I-related sequence A; R = recipient; SD = standard deviation; SPKT = simultaneous pancreas and kidney transplantation. ND = no data available.

A higher incidence of kidney AR (46.4%) was observed in patients receiving E*01:03 grafts (P = 0.017). Also, the E*01:03 status on graft contributed to a shorter kidney AR-free-survival compared to E*0101 homozygous grafts in survival analysis for a period of one-year post-transplantation (log-rank test, P = 0.036, Figure 1a). Survival analysis for E*01:03 overlap the data for +3777G carriers, showing similar survival probabilities (log-rank test, P = 0.009, Figure 1b).

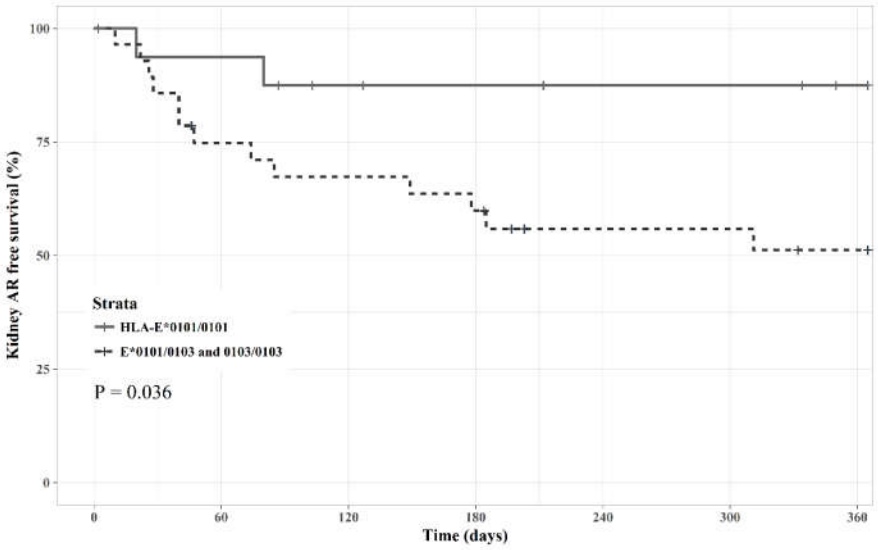

Figure 1a.

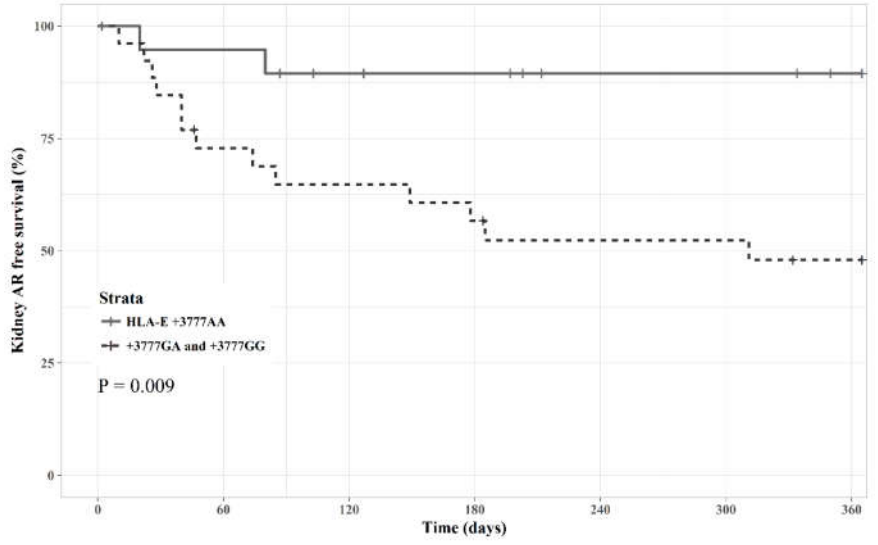

Figure 1b.

Figure 1. Survival analysis according to the occurrence of kidney acute rejection (AR) episodes and *HLA-E* status on the donor. **A.** Influence of E*0103 allele on graft in kidney AR in the first-year post-SPKT. **B.** Shorter kidney AR free survival for +3777G allele carriers in the first-year post-SPKT.

The inclusion of *HLA-E* +3777G/A genotyping (Supplementary Table 2) allowed us to evaluate whether the previously identified *HLA-E* alleles could be classified as E*01:03:02:01, E*01:03:02:01^{424T}, E*01:01:01, E*01:03:01, E*01:06 or E*01:03:05. We observed that most of E*01:01 alleles were E*01:01:01 as this allele has a +3777A at 3'UTR. In contrast, the presence of +3777G is unlikely since this combination (haplotype 27: 1.3%) has a low frequency in worldwide populations (Felicio et al. 2014).

In the case of E*01:03 alleles, the presence of +3777A at 3'UTR is shared by E*01:03:01, E*01:06, E*01:03:05 and E*01:03:02:01^{424T}. Thus, differentiation was not possible, although it is likely that most of E*01:03/+3777A are E*01:03:01 based on its high frequency (haplotype 1: 12.7%) and the low frequency (haplotypes 3,5 and 28;range 1.3-3.3%) of the others (Felicio et al. 2014). Lastly, the presence of +3777G allele was only found in E*01:03:02:01.

The overall frequency of *HLA-E* alleles and +3777G/A genotypes are presented in Supplementary Table 1. *HLA-E**01:01 allelic frequency in donors was in agreement with the expected for European populations, being this the most frequent allele in our sample (Felicio et al., 2014). In contrast, a higher frequency of E*01:03 alleles (~54%) was observed amongst the recipients, and might be related to the pathogenesis of DM1 (Hodgkinson et al. 2000). Noteworthy, in multivariate Cox-regression analyses (Table 2), the *HLA-E**01:03 status on grafts was not associated with kidney AR risk [Hazard Ratio (HR): 3.53, 95% Confidence Interval (CI): 0.77-16.1, P = 0.104]. However, when grafts were designated as E*01:03:02:01 carriers an independent association with kidney AR risk was observed (HR: 4.65, 95% CI: 1.04-20.9, P = 0.044). Similarly, the donor's *HLA-E* +3777G status increased the risk for kidney AR (HR: 5.22, 95% CI: 1.16-23.4, P = 0.031).

Table 2. Univariate and multivariate Cox-regression analysis of covariates and their impact on Kidney AR in the first-year post-SPKT

Risk factors	*Univariate analysis*		*Multivariate analysis*			
			Model 1		**Model 2**	
	HR (95% C.I.)	***P***	**HR (95% C.I.)**	***P***	**HR (95% C.I.)**	***P***
Anti–MICA pre-Tx	2.79 (0.89-8.68)	0.077	1.78 (0.48-6.59)	0.384	1.90 (0.53-6.94)	0.326
CMV R+ pre-Tx	2.40 (0.83-6.93)	0.105	2.22 (0.75-6.61)		2.43 (0.83-7.18)	0.107
*HLA-E**0103	4.30 (0.97-19.11)	0.055	3.53 (0.77-16.1)	0.104		
HLA-E +3777G	5.70 (1.28-25.3)	**0.022**			5.22 (1.16-23.4)	**0.031**

In bold statistically significant results. HR = hazard ratio. C.I. = confidence interval. MICA = MHC-class I related-sequence A. Tx = transplantation. CMV = Cytomegalovirus. R = recipient. Covariates were entered in the multivariate analysis based on a statistical threshold of $P \leq 0.15$).

The data suggest that *HLA-E* allele mismatch *per se* does not account for the risk of kidney AR in the first-year post-SPKT, but that it is important to evaluate the donor *HLA-E* status. It should be noted that SPKT differs in several clinical aspects from other solid organ transplantation, meaning that comparisons are limited. Thus, we encourage additional replicative studies to confirm our findings. Nevertheless, our data is in accordance with a previous study, showing that *HLA-E* expression on renal allografts and concomitant infiltrating NKG2C+ cytotoxic cells are critical for graft acute cellular rejection after kidney transplantation (Guberina et al. 2017).

Also worth to mention, data from the literature already suggested that the E*0103 allele on recipients increases the risk for chronic allograft dysfunction in lung transplantation (Cristofaro et al., 2016), and CMV infection after kidney transplantation (Guberina et al., 2018). Actually, CMV infection has been implicated in the expansion of HLA-$E^{VMAPRTLIL}$ -restricted CD8+ T cells (Sullivan et al., 2015), and in the differentiation and expansion of adaptive NKG2C+ NK cells (Hammer et al., 2018). Also, the VMAPRTLIL sequence present in CMV-UL40 protein is similar to a sequence present on HLA-C*03 allele (Tomasec et al., 2000; Pietra et al., 2003). This is of foremost relevance in transplantation, since recipients with permissive HLA genetics (i.e., HLA-C*02 or C*07 which does not contain a VMAPRTLIL sequence) may have a better immunologic surveillance for CMV infection/reactivation (Sullivan et al., 2015). Further, in this same direction, the HLA-E*01:03 allele has been shown to more efficiently binds to VMAPRTLIL than E*01:01 allele (Celik et al., 2016).

In summary, we observed that the presence of E*01:03:02:01 allele on graft was an independent predictor for kidney AR in the first year post-SPKT.A similar association was observed for grafts carrying +3777G allele. In addition, we report that *HLA-E* +3777G/A genotyping provide insights into the allelic composition of the most frequent *HLA-E* alleles. Also, since this SNP is located at the *HLA-E* 3'UTR, it is likely involved in the post-transcriptional control of *HLA-E.* Nevertheless, whether this SNP contributes to the variable expression of *HLA-E* alleles is yet to be addressed.

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Supplementary material

Supplementary table 1. Genotype and allelic frequencies of SPKT recipient-donor pairs.

Supplementary table 2. HLA-E +3777G/A (rs1059655) genotyping method.

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Supplementary Table 1. Genotype and allelic frequencies of SPKT recipient-donor pairs.

Variable	Recipients	Donors
HLA-E allele typing [1]		
E*01:01/01:01	12 (24.0)	17 (37.8)
E*01:01/01:03:01	3 (6.0)	2 (4.4)
E*01:01/01:03:02	19 (38.0)	19 (42.2)
E*01:03:01/E*01:03:02	7 (14.0)	2 (4.5)
E*01:03:02/ E*01:03:02	9 (18.0)	5 (11.1)
Allele		
E*01:01	46 (46.0)	55 (61.1)
E*01:03:01	10 (10.0)	4 (4.4)
E*01:03:02	44 (44.0)	31 (34.5)
HLA-E +3777A/G [2]		
AA	17 (34.0)	20 (43.5)
AG	23 (46.0)	21 (45.7)
GG	10 (20.0)	5 (10.9)
Allele		
A	57 (57.0)	61 (66.3)
G	43 (43.0)	31 (33.7)
NKG2C deletion [3]		
Wt/Wt	33 (66.0)	31 (67.4)
Wt/Del	13 (26.0)	13 (28.3)
Del/Del	4 (8.0)	2 (4.3)
Allele		
Wt	79 (79.0)	75 (81.5)
Del	21 (21.0)	17 (18.5)

SPKT = simultaneous pancreas and kidney transplantation. Wt = wild-type. Del = deletion. [1] *HLA-E* allelic typing was performed as previously described by Grimsley et al., 2002. [2] HLA-E +3777G/A genotyping method is described in Supplementary Table 2. [3] *NKG2C* typing was performed as previously described in Miyashita et al., 2004.

Supplementary Table 2. HLA-E +3777G/A (rs1059655) genotyping method

Primers [1]	**Fragment size**	**PCR conditions** [2]			***AgsI* digestion** [3]	
		Temperature	Time	Cycles	Genotype	Fragment size
Forward 5′ CAAGGGCCTCTGAATCTGTC 3′	564pb [1]	94°C	5 min			
		94°C	30 sec		AA	564pb
		59°C	30 sec	30	AG	564pb, 443pb,121pb
Reverse 5′ TTTGCTAGAGATGTGCTGTGG 3′		72°C	30 sec		GG	443pb, 121pb
		72°C	5 min			

[1] Primers were designed according to the *HLA-E* sequence retrieved on NCBI-Gene repository (Gene ID: 3133) under the number NC_000006.12. [2] The PCR conditions were as follows: final volume of 25 μL containing the genomic DNA (30–50 ng), 0.4 pmol of each primer, 0.2 mM of each dNTP (Thermo Fisher Scientific, USA), 2.0 mM of MgCl2 (Invitrogen, USA), PCR buffer 1X (Invitrogen, USA) and 1U of Platinum Taq DNA polymerase (Invitrogen, USA). [3] Samples were digested according to the manufacturers' instructions (*Ags I*; Sibenzyme, RUS) – positive controls for all genotypes were included in all genotyping reaction batches, with 100% reproducibility. min = minutes. sec = seconds. Genotypes were determined according to digestion patterns visualized in Agarose gels at 2.5% concentration for 30 min.

Capítulo 5

A Valine Mismatch at Position 129 of MICA Is an Independent Predictor of Cytomegalovirus Infection and Acute Kidney Rejection in Simultaneous Pancreas–Kidney Transplantation Recipients

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A Valine Mismatch at Position 129 of *MICA* Is an Independent Predictor of Cytomegalovirus Infection and Acute Kidney Rejection in Simultaneous Pancreas–Kidney Transplantation Recipients

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Abstract: The polymorphic major histocompatibility complex class I chain-related molecule A (MICA) and its soluble form (sMICA) interact with activating receptor natural-killer group 2 member D (NKG2D) on natural-killer (NK) and T cells, thereby modifying immune responses to transplantation and infectious agents (e.g., cytomegalovirus). Two single-nucleotide polymorphisms (SNPs), rs2596538GA in the *MICA* promoter and rs1051792AG in the coding region (*MICA*-129Val/Met), influence MICA expression or binding to NKG2D, with MICA-129Met molecules showing higher receptor affinity. To investigate the impact of these SNPs on the occurrence of cytomegalovirus infection or acute rejection (AR) in individuals who underwent simultaneous pancreas–kidney transplantation (SPKT), 50 recipient-donor pairs were genotyped, and sMICA levels were measured during the first year post-transplantation. Recipients with a Val-mismatch (recipient Met/Met and donor Val/Met or Val/Val) showed shorter cytomegalovirus infection-free and shorter kidney AR-free survival. Additionally, Val mismatch was an independent predictor of cytomegalovirus infection and kidney AR in the first year post-transplantation. Interestingly, sMICA levels were lower in rs2596538AA and MICA129Met/Met-homozygous recipients. These results provide further evidence that genetic variants of *MICA* influence sMICA levels, and that Val mismatch at position 129 increases cytomegalovirus infection and kidney AR risk during the first year post-SPKT.

Keywords: simultaneous pancreas–kidney transplantation; Val129Met; MICA; cytomegalovirus; acute and latent cytomegalovirus infection; sMICA; dimorphism; genetic predisposition

1. Introduction

Simultaneous pancreas and kidney transplantation (SPKT) is the most effective treatment for diabetes mellitus type 1 patients with end-stage renal disease. Graft survival following SPKT has improved over the previous decade due to advances in immunosuppressive regimens: antithymocyte globulin and basiliximab induction therapies are used by the majority of transplant centers, with the former associated with lower rejection rates during the first year post-transplantation [1].

Despite the overall improvement in SPKT, acute rejection (AR) remains the major challenge in solid-organ transplantation, as it has a negative effect on the allograft function and may trigger chronic rejection. Notably, the occurrence of AR in SPKT is partially attributed to donor type, as the majority of pancreas and kidney donors are deceased (cardiac-dead and brain-dead donors). Consequently, allografts from such donors are more strongly affected by ischemia-reperfusion injury capable of causing delayed graft function and/or ultimate rejection.

Another complication influencing graft survival is human cytomegalovirus infection, which frequently occurs after transplantation and leads to severe complications. In solid-organ transplantation, the main risk factors associated with cytomegalovirus infection are serological mismatch between recipient and donor (recipient is cytomegalovirus-negative and donor is cytomegalovirus-positive; cytomegalovirus R−/D+), high doses of methylprednisolone, and T cell-depletion therapy [2]. In immunocompetent hosts, cytomegalovirus infection is usually asymptomatic; however, in immunosuppressed transplantation recipients, the infection manifests as a systemic syndrome that affects multiple organs. Importantly, cytomegalovirus infection increases the risk of opportunistic infections, allograft dysfunction, and the overall cost of transplantation.

The major histocompatibility complex (MHC) class I chain-related molecule A (*MICA*) encodes a stress-induced protein located within MHC loci and is close to the human leukocyte antigen (*HLA*)-*B* gene [3]. *MICA* is a highly polymorphic non-MHC gene that includes 107 alleles encoding 82 proteins, according to Immuno Polymorphism Database [4]. MICA proteins exist as membrane-bound and soluble molecules (sMICA), each with distinct biological properties. Unlike classic MHC class I molecules, MICA is not involved in antigen presentation, but rather acts as a ligand for natural-killer (NK) group 2-member D (NKG2D), an activating C-type lectin-like receptor. The constitutive expression of MICA is restricted to few cell types, including endothelial cells, dendritic cells, fibroblasts, and epithelial cells (reviewed in Reference [5]). MICA upregulation in tissues indicates virus-induced stress or injury, malign transformation, or ischemia-reperfusion injury of allografts in the transplantation setting [6]. Because MICA expression functions as a costimulatory signal for CD8+ T cells and triggers cytotoxic and cytokine immune responses by NK effectors, its expression and potential for stress-induced upregulation represent an additional boundary between tolerance and rejection in allogeneic situations such as transplantation.

MICA single-nucleotide polymorphisms (SNPs) influence expression patterns of the gene. Specifically, rs2596538G/A located in the promoter region at position –2778 from exon 1 influences sMICA levels by modifying the affinity to transcription factor specificity protein-1. The variant rs2596538G is associated with higher sMICA expression levels, especially in patients with hepatitis C virus-induced hepatocellular carcinoma [7]. SNP rs1051792, also known as *MICA*-129Val/Met, is characterized by a substitution of guanine with adenine at position 454 in exon 3, which results in a nonsynonymous mutation of valine to methionine at codon 129. *MICA*-129Val/Met not only affects sMICA levels, but also its affinity to the NKG2D receptor expressed on CD8+ T cells, γδ T cells, and NK cells. MICA-129Met proteins bind soluble NKG2D with a 10- to 50-fold higher affinity than MICA-129Val proteins [5,8]. Additionally, MICA-129Met proteins are expressed at low levels in the cell membrane due to intracellular retention, and are highly susceptible to membrane shedding [9].

The clinical relevance of *MICA*-129Val/Met has been highlighted by diverse association studies, including those in patients with autoimmune disorders, malignancies, and infections [3,10]. Recent studies on hematopoietic stem-cell transplantation have produced conflicting results, with different MICA-129 genotypes being associated with different outcomes [11–15]. The effects of both functionally relevant SNPs (rs2596538 and *MICA*-129Val/Met) on the incidence of cytomegalovirus infection and AR after SPKT remain unknown. Therefore, we investigated the impact of these SNPs on the occurrence of cytomegalovirus infection or AR in SPKT by genotyping 50 recipient-donor pairs and defining their sMICA levels during the first year post-transplantation.

2. Results

Clinical and demographic characteristics of SPKT recipients with or without at least one AR event are provided in Table 1. AR was observed in 19 (38%) individuals with SPKT and at different frequencies in kidney (84.2%) and pancreas (21.1%) allografts. Only one patient exhibited graft rejection of both. The median age, biochemical parameters, cold ischemia time of the graft, and the number of human leukocyte antigen (*HLA)-A*, *-B*, and *-DR* mismatches between AR and functional/stable graft recipients were similar. Before transplantation, anti-MICA antibodies were more frequently detected in patients in the AR group (26.3% vs. 3.4%; $p = 0.030$) as compared with those without AR. After transplantation, the frequencies of anti-MICA antibody detection were similar in both groups.

Table 1. Clinical and demographic characteristics of SPKT patients stratified by the presence of AR in the first year post–transplantation.

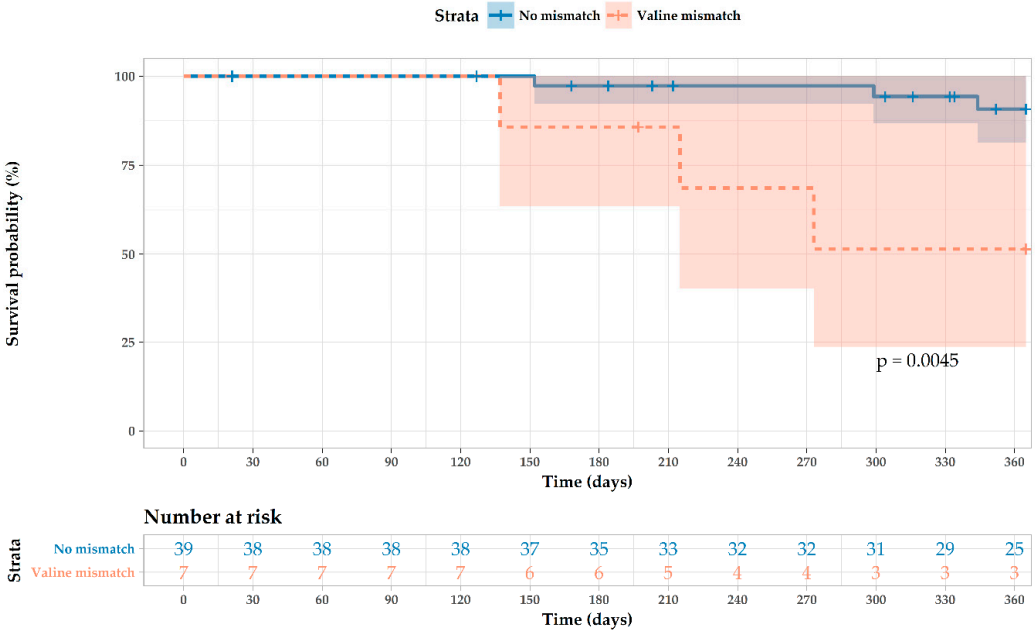
Variables	AR (*n* = 19)	No AR (*n* = 31)	*p*
Age (median: 25–75%)	50 (35.0–56.5)	43.0 (34.5–53.0)	0.289
Recipient gender (male/female)	10/9	19/12	0.547
Donor gender (male/female)	11/8	14/17	0.382
BMI (median: 25–75%)	24.2 (21.8–26.43)	25.0 (20.8–27.8)	0.818
Urea, mg/dL (median: 25–75%)	18.6 (16.1–27.2)	18.5 (15.4–20.5)	0.431
Creatinine, mg/dL (median: 25–75%)	1.36 (1.09–1.61)	1.30 (1.02–1.55)	0.516
Glucose, mg/dL (median: 25–75%)	104 (90.0–119.0)	97.5 (88.0–116.0)	0.382
HbA1C, % (median: 25–75%)	5.85 (5.33–6.70)	5.70 (5.3–6.4)	0.693
Kidney graft cold ischemia time, min (±SD)	816.7 (158.4)	798.4 (173.9)	0.772
Pancreas graft cold ischemia time, min (±SD)	677.4 (133.4)	682.6 (137.2)	0.697
HLA-A and *-B* mismatches (median: 25%–75%)	3.0 (3.0–4.0)	3.0 (2.0–3.0)	0.267
HLA–DR mismatch (median: 25–75%)	2.0 (1.5–2.0)	2.0 (1.0–2.0)	0.225
Anti–MICA pretransplantation	5 (26.3)	1 (3.4)	**0.030**
Anti–MICA post-transplantation	2 (11.1)	4 (14.8)	1.000
Anti–MHC I post-transplantation	5 (27.8)	5 (18.5)	0.489
Anti–MHC II post-transplantation	4 (22.3)	6 (22.2)	1.000
Cytomegalovirus D+	11 (57.9)	11 (35.5)	0.121
Cytomegalovirus R+	5 (26.3)	19 (61.3)	**0.016**
Cytomegalovirus, R−/D+	8 (42.1)	5 (16.1)	**0.042**
Cytomegalovirus infection, first-year post-transplantation	3 (15.8)	4 (12.9)	1.000
Pancreas and kidney graft AR	1 (5.2)	ND	
Pancreas graft AR	4 (21.1)	ND	
Kidney graft AR	16 (84.2)	ND	
Immunosuppressive drugs (*n* (%))			
ATG	19 (100)	31 (100)	
Steroids	19 (100)	31 (100)	
Tacrolimus	18 (94.7)	30 (96.8)	
Mycophenolic acid	19 (100)	30 (96.8)	
Cyclosporine A	1 (5.3)	1 (3.2)	
Azathioprine	ND	1 (3.2)	
Simulect	ND	1 (3.2)	
Rituximab	ND	1 (3.2)	

Bold *p* values indicate statistically different parameters. AR = acute rejection; ATG = antithymocyte globulin; BMI = body-mass index; D = donor; *HLA* = human leukocyte antigen; *MICA* = MHC class I-related sequence A; R = recipient; SD = standard deviation; SPKT = simultaneous pancreas and kidney transplantation. ND = no data available.

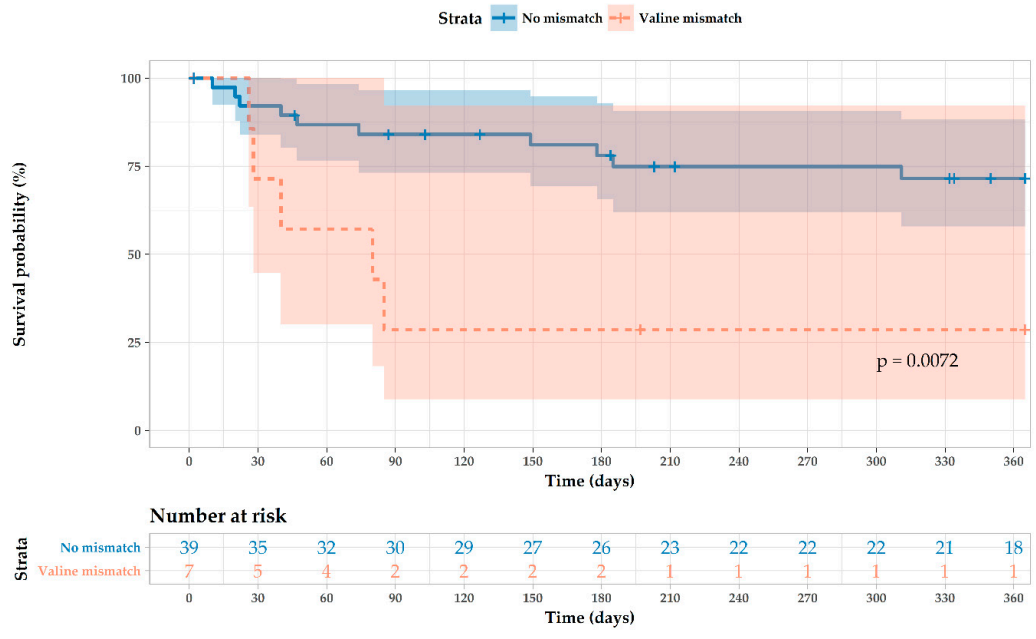
Cytomegalovirus-positive recipients were more prevalent among those with stable grafts than with AR allografts (61.3 vs. 26.3; $p = 0.016$), and the frequency of patients receiving cytomegalovirus-positive grafts was higher in the AR group (42.1 vs. 16.1, $p = 0.042$). After a one-year follow-up, active infection was observed with similar frequencies in both groups (AR: 15.8% vs. stable grafts: 12.9%; $p > 0.05$).

We then evaluated the impact of *MICA*-129Val/Met mismatches between recipient and donor pairs. Kaplan–Meier curves indicated that *MICA*-129 mismatch status (recipient Met/Met and donor Met/Val or Val/Val) conferred shorter cytomegalovirus-infection-free survival ($p = 0.004$;

Figure 1A) and kidney-rejection-free survival rates ($p = 0.007$; Figure 1B) during the first year post-SPKT. Multivariate Cox regression analysis indicated that *MICA*-129 mismatch represented an independent prognostic risk factor for cytomegalovirus infection ($p = 0.049$; hazard ratio (HR): 5.32; 95% confidence interval (CI): 1.00–28.1; Table 2) and kidney AR ($p = 0.006$; HR: 6.04; 95% CI: 1.68–21.7, Table 3) during the first year post-transplantation.



(**A**)



(**B**)

Figure 1. Impact of MICA-129Val/Met on survival following simultaneous pancreas and kidney transplantation. (**A**) Effect of MICA-129-Val mismatch (recipient Met/Met and donor Met/Val or Val/Val) in patients with cytomegalovirus infection at one year post-transplantation. (**B**) Effect of MICA-129-Val mismatch in patients with kidney acute rejection in one year post-transplantation.

Table 2. Univariate and multivariate Cox regression analysis of individual covariates and their impact on the risk of cytomegalovirus infection.

Variables	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Univariate analysis (n = 7)				
Valine mismatch [1]	7.37 (1.47–36.9)	**0.015**	5.32 (1.0–28.1)	**0.049**
MICA antibodies pretransplantation [2]	0.04 (0.00–1381.6)	0.546		
HLA mismatch [3]				
4/6	1.39 (0.12–15.3)	0.790		
5/6	0.80 (0.07–8.76)	0.851		
6/6	1.74 (0.16–19.2)	0.651		
Cytomegalovirus R−/D+ [4]	4.29 (0.96–19.6)	0.057	4.89 (0.86–27.9)	0.074
Kidney graft ischemia time	1.00 (1.00–1.00)	0.860		
Kidney graft AR	1.61 (0.36–7.20)	0.532		

Reference categories: [1] No mismatch. [2] No donor-specific antibodies. [3] 3/6 mismatch number (*HLA-A*, *-B*, and *-DR*). [4] Recipient cytomegalovirus+. CI = confidence interval; HR = hazard ratio. Bold values indicate statistically significant effects.

Table 3. Univariate and multivariate Cox regression analyses of predictor variables in SPKT patients with acute kidney rejection.

Variables	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Univariate analysis (n = 16)				
Valine mismatch [1]	4.00 (1.4–12.0)	**0.012**	6.04 (1.68–21.7)	**0.006**
MICA antibodies pretransplantation [2]	2.80 (0.89–8.7)	0.077	3.40 (0.84–13.8)	0.086
HLA mismatch [3]				
4/6	2.91 (0.32–26.0)	0.340	3.65 (0.39–34.0)	0.256
5/6	4.02 (0.49–32.8)	0.193	5.34 (0.64–44.7)	0.122
6/6	4.03 (0.45–36.1)	0.212	3.11 (0.32–30.6)	0.330
HLA-DR mismatch [4]	1.82 (0.58–5.64)	0.300		
Gender mismatch [5]	1.47 (0.51–4.20)	0.480		
Cytomegalovirus infection [6]	1.40 (0.40–4.90)	0.590		
Kidney graft ischemia time	1.00 (1.00–1.00)	0.860		

Acute kidney rejection (n = 16). Reference categories: [1] No mismatch. [2] No donor-specific antibodies. [3] 3/6 mismatch number (*HLA-A*, *-B*, and *-DR*). [4] *HLA-DR* mismatch number (n = 0 or 1). [5] Female recipient and male donor. [6] No infection in the first year post-transplantation. Bold values indicate statistically significant effects.

Additionally, in the first year post-transplantation: SPK patient, pancreas-, and kidney-graft survival rates were 96%, 84%, and 88%, respectively. In recipients without valine mismatch: SPK patient, pancreas-, and kidney-graft survival rates were 97.5%, 90%, and 92.5%. In the valine mismatch group: SPK patient, pancreas-, and kidney-graft survival rates were slightly worse, but this difference did not reach statistical significance (90%, 70%, and 60%, respectively).

Evaluation of *MICA*-129Val/Met genotypes based on plasma sMICA levels from SPKT recipients showed that in all measurements, Met/Met homozygotes had the lowest plasma sMICA levels (below the detection limit) relative to those in patients with Val/Val and Val/Met genotypes (p = 0.005, Table 4). Evaluation of SNP rs2596538G/A in the *MICA*-promoter region revealed that for all measurements, rs2596538AA homozygotes had the lowest median sMICA levels, with these differences being more pronounced when AA homozygotes were compared with rs2596538G-allele carriers [GG + GA = 0.73 (range: 0.54–1.05) vs. AA < 0.02; p = 0.025]. Furthermore, additional sMICA measurements from voluntary blood donors confirmed that *MICA*-129Met/Met and rs2596538AA genotypes were associated with the lowest sMICA levels (p < 0.001).

Table 4. Effect of Val129Met substitution and rs2596538GA on sMICA levels in SPKT recipients.

Measurements, Days after Transplantation (± SD)	Val/Val	Val/Met	Met/Met	*p* *
Val129Met				
1st, 0.58 (0.90) [1]	0.87 (0.75–1.44)	1.22 (0.60–1.75)	<0.02	0.265
2nd, 98.5 (46.0) [2]	0.42 (0.35–0.90)	0.55 (0.53–0.88)	<0.02	**0.007**
3rd, 238.9 (72.5) [3]	0.66 (0.40–0.75)	0.50 (0.45–1.08)	<0.02	**0.035**
Median values at one year post-transplantation [4]	0.69 (0.49–0.87)	1.04 (0.71–1.19)	<0.02	**0.005**
Healthy individuals **	1.16 (0.88–1.54)	0.55 (0.44–0.62)	0.00 (0.00–0.04)	**<0.001**
rs2596538GA	**GG**	**GA**	**AA**	
First, 0.58 (0.9) [1]	0.87 (0.65–1.59)	0.87 (0.81–1.64)	<0.02	0.241
Second, 98.5 (46.0) [2]	0.49 (0.42–0.76)	0.55 (0.45–0.72)	0.00 (0.00–0.45)	0.103
Third, 238.9 (72.5) [3]	0.58 (0.32–1.17)	0.63 (0.47–0.89)	<0.02	**0.036**
Median values at one year post-transplantation [4]	0.76 (0.60–1.17)	0.71 (0.47–0.97)	<0.02	0.075
Healthy individuals **	1.37 (0.91–1.55)	0.58 (0.43–0.83)	0.00 (0.00–0.06)	**<0.001**

sMICA values are presented as medians (25%–75%). Bold values indicate statistically significant effects. [1] First measurement: Val129Met Val/Val (n = 6), Val/Met (n = 6), and Met/Met (n = 1); rs2596528 GG (n = 7), GA (n = 5), and AA (n = 1). [2] Second measurement: Val129Met Val/Val (n = 8), Val/Met (n = 7), and Met/Met (n = 4); rs2596538 GG (n = 7), GA (n = 7), and AA (n = 5). [3] Third measurement: Val129Met Val/Val (n = 6), Val/Met (n = 5), and Met/Met (n = 3); rs2596538 GG (n = 4), GA (n = 7), and AA (n = 3). [4] Median values at one year post-transplantation: Val129Met Val/Val (n = 10), Val/Met (n = 7), and Met/Met (n = 4); rs2596538 GG (n = 8), GA (n = 8), and AA (n = 5). sMICA-detection limit: >0.02 pg/mL. * Kruskal–Wallis test. ** Healthy voluntary individuals: Val/Val (n = 20), Val/Met (n = 11), and Met/Met (n = 7); rs2596358, GG (n = 16), GA (n = 23), and AA (n = 5). sMICA = soluble MICA.

Analysis of linkage disequilibrium (LD) between *MICA* variants in recipients (D′ = 0.61, r^2 = 0.37) and recipients plus donors (D′ = 0.64, r^2 = 0.40) suggested only recombination and low incidence of LD. Interestingly, 89.9% (8/9) of *MICA*-129Met/Met homozygotes carried at least one rs2596538A allele, with 55.6% (5/8) being homozygous for this allele (rs2596538AA). Furthermore, 96.3% (26/27) of *MICA*-129Val/Val homozygotes carried at least one rs2596538G allele, with 77.8% (21/27) being homozygous for this allele (rs2596538GG). Similar to SPKT recipients, all Met/Met homozygotes carried at least one rs2596538A allele, and 57.1% of them (4/7) were also rs2596538AA homozygous, whereas all Val/Val homozygotes harbored at least one rs2596538G allele, and 70.0% (14/20) were also GG homozygous.

3. Discussion

Studies evaluating *MICA*-129 mismatch in solid-organ transplantation are scarce, as *MICA* typing is seldom reported, except for the detection of anti-MICA antibodies, which affect allograft survival [16,17]. In the present study, we report that the direction and type of *MICA* mismatches, rather than their number, influence SPKT outcomes. Specifically, we observed for the first time that *MICA*-129Val mismatch (donor *MICA*-129Val carrier and recipient *MICA*-129Met homozygote) is an independent predictor of cytomegalovirus infection and kidney AR in the first year post-SPKT. The functional implications of variability of MICA-129 molecules that bind to NKG2D with low (Val) or high (Met) affinity have been reported [12]. In hematopoietic stem-cell transplantation studies, *MICA* has gained special attention due to the functional impact of MICA-129Val/Met dimorphism. However, despite extensive investigations, the question of whether *MICA* mismatches increase or decrease the risk of graft-versus-host disease or overall patient survival remains a matter of debate [11–15].

The increased risk of AR in recipients harboring *MICA*-129Met/Met can be explained by the low level of sMICA present in AR patients, which could cause insufficient host immune suppression due to the downregulation of surface NKG2D expression on NK and CD8+ T cells. We also suggest that the expression of membrane MICA-129 valine molecules on the allograft tissue has potential for stimulating an immune response in *MICA*-129Met/Met recipients and leading to worse clinical outcomes.

Consistent with our observations, decreased expression of membrane-bound and sMICA molecules is associated with the *MICA*-129Met allele [12]. However, it should be noted that in our study, Met/Met homozygotes were likely to be rs2596538AA homozygous, and the latter genotype is associated with lower sMICA levels. Moreover, the rs2596538G allele influences specificity protein-1

transcription-factor binding, and thus is associated with higher sMICA levels than the rs2596538A allele [7].

In heart and kidney transplantation patients, consistent sMICA expression is associated with improved graft tolerance [18,19]. In our SPKT study, *MICA*-129Val/rs2596538G carriers exhibited the highest sMICA levels, whereas *MICA*-129Met/Met and rs2596538AA homozygotes had the lowest sMICA levels. Concomitant with low sMICA levels, *MICA*-129Met/Met carriers are exposed to MICA-129Val proteins expressed on donor allograft cells (Val mismatches), with the expression levels of these proteins correlating with NKG2D-mediated downstream signaling in NK and CD8+ T cells that promotes secretion of tissue-injury related mediators. Additionally, overexpression of *MICA*-129Met proteins leads to NKG2D internalization, resulting in impaired functions of both NK and CD8+ T cells [10,12]. Therefore, the evaluation of recipient-donor *MICA*-129Val/Met genotypes might provide reliable markers for patients that are at higher AR risk based on the mismatch type.

Currently, few studies evaluated the impact of MICA genetic variation on the clinical outcome of immunologic disorders and transplantation (reviewed in Reference [10]). An exception includes a study evaluating the variation in regulatory regions of the gene, such as the rs2596542 and rs2596538 SNPs [7]. The investigation and impact of *MICA* alleles in distinct outcomes are even more limited since *MICA* gene is highly polymorphic (>100 alleles). Consequently, a comprehensive evaluation requires high-throughput methodologies. Noteworthy, MICA Val129Met genotyping allows differentiation of the most frequent alleles in two groups.

It has been shown that genotyping of *MICA*-129Val/Met allows classification of *MICA* alleles by low (Val) or high (Met) affinity of the protein product to NKG2D [5]. Therefore, depending on the population under investigation, this might provide a rough estimate of segregated *MICA* alleles. We were unable to confirm donor–recipient *MICA*–allele typing in SPKT patients. However, *MICA*-129Val is present in the *MICA**008 allele, which is most frequently identified in Caucasians, and is currently under intense investigation [5].

Interestingly, the product of *MICA**008 lacks the cytoplasmic domain due to a microsatellite SNP (A5.1). However, this allele still expresses a protein on the membrane through a dynamic mechanism involving glycosylphosphatidylinositol anchoring. Because the latter is a slow process, unbound MICA proteins can be released as nanovesicles and soluble proteins, indicating that mechanisms other than those currently explored (i.e., SNPs) impact *MICA* expression patterns [10,20]. Additionally, it is possible that *MICA**008- and *MICA*-129Val-expression patterns overlap.

In the specific context of cytomegalovirus infection, cytomegalovirus eludes host immune system by targeting and downregulating NKG2D ligands at multiple checkpoints, including those involved in protein sequestration, mRNA degradation, translational repression, protein degradation, in the allele-specific manner [21]. The *MICA**008 allele illustrates this specificity as it represents an "escape variant" resistant to most cytomegalovirus viral glycoproteins, except for the recently identified US9 protein. Therefore, *MICA**008 carriers are less prone to cytomegalovirus infection than individuals with full-length *MICA* alleles [22,23]. A higher risk of cytomegalovirus reactivation is associated with A5.1 homozygosity (which includes the *MICA**008 allele) in HIV-immunodeficient patients [24]. It should be noted that HIV-1 Nef protein downregulates *MICA* expression, and this circumstance needs to be taken into account in coinfected and immunosuppressed patients [25].

Consequently, we suggest that susceptibility to cytomegalovirus infection in SPKT represents a cumulative effect of recipient–donor *MICA*-129Val/Met genotypes, with equivalent effects of serological mismatches, immunosuppressive regimens, and lymphocyte-depletion therapy. In this study, we observed that Val-mismatch recipients were at higher risk of cytomegalovirus infection post-SPKT. The fact that Met/Met homozygotes lack the "escape variant" and likely carry a 2596538A allele makes them susceptible to cytomegalovirus-specific immune evasion because *MICA* expression becomes downregulated in favor of viral replication [21,26]. However, additional studies are required to clarify the mechanisms associated with the susceptibility of Val-mismatch recipients to cytomegalovirus-infection risk, as well as the impact of donor-allograft genotypes and

different cytomegalovirus strains. Although we focused our attention on cytomegalovirus infection, our observations are also relevant for assessing the risk of other opportunistic infections, especially those caused by other herpesviruses that adopt multiple means to evade immune response, including *MICA* downregulation [27]. It is noteworthy that *MICA* Val129Met SNP influence on cytomegalovirus infection and AR could be specific for SPKT since the current cohort under investigation differs in the clinical practice/management and characteristics of other types of solid organ transplantation. Consequently, it makes SPKT and kidney only transplantation not comparable, although the functional relevance of *MICA* Val129Met SNP may overlap.

In summary, for the first time, we evaluated *MICA*-129Val/Met and rs2596538G/A genetic variations in SPKT recipients. We demonstrated that *MICA*-129Val mismatch is an effective predictor of AR risk and cytomegalovirus infection in the first year post-SPKT. Additionally, *MICA*-129Val/Met genotypes affect overall sMICA levels in individuals with SPKT, and, more importantly, rs2596538G/A SNP in the *MICA*-promoter region also affects sMICA levels. These findings indicated that evaluation of *MICA*-129-mismatch in individuals with SPKT might help to stratify them by the probable risks of developing AR and contracting cytomegalovirus infection. Furthermore, a simultaneous determination of rs2596538G/A genotype in the *MICA*-promoter region might provide a better understanding of the mechanisms associated with sMICA expression, which will be beneficial in the context of allotransplantation.

4. Materials and Methods

4.1. Study Population and Outcome Parameters

A total of 50 diabetes mellitus type 1 patients undergoing SPKT were recruited from 2012 to 2016 at Universitätsklinikum Knappschaftskrankenhaus (Bochum, Germany). Induction immunosuppression was employed in all patients. Most patients received thymoglobulin (Sanofi, Frankfurt, Germany) induction in daily doses of 1.5 mg/kg body weight for 3 days. Maintenance immunosuppression consisted of tacrolimus, mycophenolic acid, and prednisolone in most cases.

Serum creatinine, blood-glucose values, and serum-lipase levels were used to detect allograft dysfunction. When rejection was suspected, a biopsy of the kidney graft was performed primarily and graded according to the BANFF classification [28]. A biopsy of the pancreas graft was performed only when isolated pancreas graft rejection was suspected. AR episodes were treated with methylprednisolone initially for 3 consecutive days, and, if resistant, with thymoglobulin. Antibody-mediated rejections were treated with thymoglobulin and/or plasmapheresis and intravenous immune globulin.

All patients received cytomegalovirus prophylaxis with ganciclovir, followed by valganciclovir for at least 3 months postoperatively. In individuals with presumed high-risk sensitivity to cytomegalovirus (D+/R−; D+/R+), cytomegalovirus prophylaxis was prolonged to 6 months. Surveillance for cytomegalovirus infection was performed by PCR (Cobas 4800; Roche Diagnostics, Mannheim, Germany) weekly during the first month following SPKT and monthly thereafter until the end of the first year or in cases of clinical suspicion. Any level of detection was considered positive. Cytomegalovirus disease was defined as cytomegalovirus infection with at least one of the symptoms: newly occurred malaise, fever > 38 °C, leukopenia and thrombocytopenia, elevation of hepatic transaminases to more than twice the standard values, or graft dysfunction. Treatment of cytomegalovirus infection was performed using intravenous ganciclovir adjusted to the calculated glomerular-filtration rate, followed by 3 additional months of prophylactic valganciclovir treatment.

Recipients and deceased-donor grafts were typed for *HLA-A*, *-B*, and *-DR*. The recipients were followed-up with over a 1-year period after SPKT and evaluated for the occurrence of graft failure, cytomegalovirus infection, and AR. Demographic and clinical data were collected before and after SPKT and summarized according to cytomegalovirus status and AR outcomes (Table 1). Voluntary blood donors (n = 44) were included in the study in order to evaluate the effect of *MICA*-129Val/Met

and rs2596538G/A genotypes on sMICA levels. The protocols used in this study were approved by the local ethics board of the Faculty of Medicine, Ruhr-University of Bochum (No. 12-4380; date: 20 September 2012) and were in agreement with the Declaration of Helsinki. Before inclusion in the study, all participants provided written informed consent.

4.2. MIC-A 454G/A and rs2596538A/G Genotyping

Genomic DNA was obtained using a QIAmp DNA mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions from whole-blood samples collected in ethylenediaminetetraacetic acid. Genotypes associated with SNP rs1051792 involving an A > G substitution at position 454 in exon 3 of the *MICA* gene were determined by nested PCR, followed by RsaI (New England Biolabs, Ipswich, MA, USA) restriction digestion (PCR conditions are described in Table S1). Electrophoresis was performed on a 2.5% agarose gel at 140 V for 20 min in order to visualize digestion patterns and determine the presence of MIC-A 454G/A genotypes associated with the following nonsynonymous substitutions: 454GG = Val/Val (106 and 21 bp (base pair)); 454GA = Val/Met (127, 106, and 21 bp), and 454AA = Met/Met (127 bp).

Genotyping of rs2596538A/G was performed using a PCR-restriction fragment length polymorphism technique described in Table S2. All PCR amplifications were evaluated by 1% agarose gel electrophoresis and AluI (New England Biolabs) restriction digest reactions according to manufacturer instructions. The amplified sequences had a total of 339 bp and contained 4 AluI-recognition sites, yielding the following fragments after digestion: 139, 92, 78, 23, and 7 bp. The presence of the A allele led to the digestion of the 170 bp fragment resulting in the segments of 78 and 92 bp in length. Electrophoresis on a 3.5% agarose gel was used to visualize the digestion patterns and determine the following *MICA* rs2596538A/G genotypes: AA (139, 92, 78, 23, and 7 bp), AG (170, 139, 92, 78, 23, and 7 bp), and GG (170, 139, 23, and 7 bp). Samples were processed in batches, including all known genotypes as additional positive controls. At the end, 10% of samples were randomly selected and retested; 100% reproducibility was obtained for both methods.

4.3. sMICA Measurement

Determination of sMICA levels was performed as previously described [29]. Briefly, sMICA molecules were captured using an AMOI antibody (BAMOMAB, Munich, Germany) at a final concentration of 25 ng/mL. Detection of bound sMICA molecules was performed using a biotin-labeled polyclonal goat anti-human MICA antibody (R and D Systems GmbH, Wiesbaden-Nordenstadt, Germany) diluted to 400 ng/mL in phosphate-buffered saline containing 1% bovine serum albumin and 2% inactivated goat serum. Detection of bound antibodies was performed by streptavidin conjugated with horseradish peroxidase (R and D Systems GmbH) diluted 1:200 in phosphate-buffered saline containing 1% bovine serum albumin. 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich Chemie GmbH, Munich, Germany) served as substrate solution, and absorbance was measured at 450 nm (Biotek Instruments, Winooski, VT, USA). Recombinant MICA protein fused with the Fc portion of human IgG (R and D Systems GmbH) was used as standard. The detection limit of sMICA was 20.4 pg/mL or 0.024 ng/mL, with detection limits calculated according to DIN 32645 standard. Intra-assay coefficients of variation were 4.1%, and interassay coefficients of variation were 14.3%.

4.4. Statistical Analysis

Asymmetric distributions were described by the median (25–75 percentiles). Continuous and categorical variables were compared using the Mann–Whitney U or Kruskal–Wallis test and chi-squared tests, as appropriate. The SPKT outcomes evaluated included AR, graft function, and cytomegalovirus infection in the first year post-SPKT. Survival in groups with allograft AR and cytomegalovirus infection was estimated by Kaplan–Meier curves and evaluated through the log-rank test implemented in the R package survminer (v0.4.0; https://www.r-project.org/). Multivariate Cox regression according to proportional hazards assumption was used to assess the risk of allograft AR and

cytomegalovirus infection. Covariates were entered in the multivariate analysis based on conceptual evaluation of the literature or/and by means of a statistical threshold (association of the covariate with the outcome and with the study factor at $p \leq 0.20$). LD was tested by D′ and r^2 statistics was calculated by using Haploview software (https://www.broadinstitute.org/haploview/haploview). All remaining analyses were performed in SPSS for Windows (v18.0; SPSS Inc., Chicago, IL, USA). Effects were considered statistically if $p < 0.05$.

Supplementary Materials: The following are available online at http://www.mdpi.com/1422-0067/19/9/2618/s1.

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Abbreviations

AR	Acute rejection
bp	base pair
D	Donor
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
LD	Linkage disequilibrium
Met	Methionine
MHC	Major histocompatibility complex
MICA	Major histocompatibility complex class I chain-related molecule A
NK	Natural killer
NKG2D	Natural-killer group 2 member D
PBS	Phosphate buffered saline
R	Recipient
SD	Standard deviation
sMICA	soluble MICA
SNP	Single nucleotide polymorphism
SPKT	Simultaneous pancreas-kidney transplantation
Val	Valine

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Table S1. Primer sequences and PCR condition used for MICA rs1051792 genotyping.

PCR primers and conditions	Sequence (5′->3′)	Product size
MICA1-F	CAGGGAGGCATACCCCCTG	864 bp
MICA1-R	TCCGGGACCCCTGACCTG	
MICA2-F*	GGGTCTGTGAGATCCATGA	127 bp
MICA2-R*	TGAGCTCTGGAGGACTGGGGTA	
PCR Steps	Temperature (°C)-Duration	Cycles
Initial denaturation	95°C–2 min	1
Denaturation	95°C–30 sec	
Primer annealing	62.5°C–40 sec	40
Extension	72°C–1 min, 15 sec*	
Final step	72°C–5 min	1

The first PCR was prepared in a final volume of 30 µL: 1X Master mix (Ampliqon), 0.33µM of each MICA-1 primer and 1µL [50ng/µL] DNA. *Second PCR was prepared in final volume of 39.5 µL: 1X master mix, 0.33 µM of each MICA-2 primer and 0.5µL from first PCR product. Bp=base pairs. Min=minutes, Sec=seconds.

Table S2. Primer sequence and PCR condition used for MICA rs2596538A/G genotyping.

PCR primers and conditions	Sequence (5′->3′)	Product size
MICA538F	GTGAGTGCATGGGGTATAAGGC	339 bp
MICA538R	GTGCCAGCTCCAGCA AAGGAT	
PCR Steps	Temperature (°C)-Duration	Cycles
Initial denaturation	94°C–5 min	1
Denaturation	94°C–30 sec	
Primer annealing	56°C–30sec	32
Extension	72°C–1 min	
Final step	72°C–5 min	1

PCR was performed in a final volume of 25µL, 1X PCR Buffer–MgCl2 (Invitrogen), 2mM MgCl2 (Invitrogen), 0.4 µM of each primer, 0.5 mM dNTPmix (Thermo Fisher Scientific), 1Unit of Taq Platinum DNA (Invitrogen) and 1 µL DNA [50ng/µL]. Bp=base pairs. Min=minutes, Sec=seconds.

Seção III

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Capítulo 6
Discussão geral

6. Discussão geral

A imunologia da gestação é uma área do conhecimento biológico em rápido desenvolvimento. Para sua compreensão é necessária uma abordagem multidisciplinar, visto que redes complexas de sistemas biológicos estão atuando simultaneamente em prol do desenvolvimento saudável do feto. Uma vez que a aceitação materna da gestação é algo natural, a investigação dos mecanismos que permitem seu acontecimento torna-se algo fascinante do ponto de vista imunológico, além de permitir o entendimento das bases moleculares da gestação patológica.

Apesar do grande número de estudos já realizados, as causas precisas do AER e pré-eclâmpsia ainda permanecem desconhecidas. Na patogênese dessas desordens gestacionais é determinante a interação entre fatores genéticos e imunológicos, além do fator ambiente (Rai and Regan 2006; Fong et al. 2014). O primeiro artigo desta tese, através de uma extensa revisão é discutido a contribuição do fator genético materno na suscetibilidade à PE em populações latino-americanas. Na revisão realizada, genes envolvidos nos processos de vascularização e inflamação estão entre os mais investigados, devido ao reconhecido papel destes processos na patogênese da PE. Ainda, deve-se destacar a multiplicidade de genes candidatos já investigados. Neste cenário investigativo, limitações como o tamanho amostral desses estudos de associação, reprodutibilidade dos resultados, diversidade genética das populações latino-americanas, e a escassez de dados epidemiológicas básicos, como mortalidade materna e incidência da PE são questões importantes que devem ser direcionadas no futuro. Não menos importante, reforça-se o fato de que a epidemiologia da PE na América Latina é alarmante, uma vez que em muitos países se desconhece a incidência desta desordem, a qual é a principal causa de mortalidade materna e infantil em países em desenvolvimento (Michita et al 2018b).

Para superar os obstáculos previamente citados, salienta-se a necessidade de estudos em caráter colaborativo, os quais além de impulsionar os trabalhos científicos a um patamar de excelência, permitirão ao mesmo tempo avaliar a influência de diferentes variantes genéticas em distintas populações miscigenadas. Por fim, é importante salientar que esta revisão foi realizada para destacar a contribuição da América Latina em estudos genéticos da PE, os quais são principalmente representados por grupos de pesquisa situados no México e no Brasil.

Atualmente, uma das causas que pode levar ao desenvolvimento de PE assim como ao AER é a implantação inadequada do blastocisto no endométrio (Norwitz 2006). Sabe-se que a implantação é dependente tanto de fatores fetais quanto de fatores maternos (Norwitz 2006). No segundo artigo (capítulo 3) desta tese, avaliamos o papel do HLA-G na suscetibilidade materna ao AER. O HLA-G, assim como os outros genes não clássicos do MHC-I (HLA-E e HLA-F), é expresso em grande quantidade nas células EVTs e é importante na imunorregulação da interface materno-placentária (Hackmon et al. 2017). É reconhecido que variações genéticas nas regiões regulatórias afetam os padrões de expressão dos genes. Deste modo, a avaliação das variantes polimórficas e haplótipos da região 3'UTR do *HLA-G* em mulheres com AER idiopático faz-se justificável. De fato, nosso estudo foi primeiro a inicialmente avaliar o papel da região 3'UTR na suscetibilidade ao AER. Nós observamos em menor frequência os alelos +3010G, +3142C e +3187G em mulheres com AER. Ainda que não tenha sido possível avaliar se essas variantes e os haplótipos que elas compõem influenciam os níveis solúveis de HLA-G em mulheres com AER, sabe-se que esses alelos estão presentes no haplótipos UTR-1, o qual em homozigose é geralmente associado a altos níveis de HLA-G solúvel em indivíduos saudáveis (Martelli-Palomino et al. 2013). Recentemente, baixos níveis de HLA-G solúvel foram observados em mulheres com múltiplos abortos (Zidi et al. 2016), sugerindo um efeito de haplótipos de baixa expressão nessas mulheres. De fato, em outro estudo recente, foi reportado em uma população holandesa que o UTR-2 é um fator de risco para AER secundário, enquanto o UTR-4 confere proteção ao AER (Meuleman et al. 2018). Nesse contexto, o UTR-2 é um dos haplótipos composto por alelos (Inserção de 14pb, +3142G e +3187A) associados a menor expressão do HLA-G (Martelli-Palomino et al. 2013). É importante salientar que o tamanho amostral deste estudo pode ter influenciado as observações reportadas, visto que a frequência do UTR-4 (19,8%) no grupo controle é superior à média observada em populações europeias (~16%) (Sabbagh et al. 2014). Ademais, o efeito do UTR-4 na expressão do HLA-G ainda não está totalmente compreendido. No entanto, os autores sugerem que este haplótipo esteja associado a níveis consideráveis de expressão do HLA-G, visto que o alelo +3003C - exclusivo do UTR-4 - está em desequilíbrio de ligação com o alelo -725G (Castelli et al. 2014), previamente associado a maior expressão de HLA-G (Ober et al. 2006). Outra observação que corrobora a hipótese do UTR-4 influenciar a expressão

de HLA-G é a associação do mesmo ao risco de malignidades, por exemplo, câncer de próstata e carcinoma colorretal (Zambra et al. 2016; Garziera et al. 2016).

Na pré-eclâmpsia, o papel da região 3'UTR ainda permanece desconhecido (Quach et al. 2014, Lee et al. 2018). Visto que a maioria dos estudos conduzidos até o momento avaliou apenas algumas variantes nesta região (principalmente a variante referente a ausência ou presença de 14 pares de base) (de Almeida et al. 2018), o que dificulta uma visão geral, uma vez que a caracterização dos haplótipos nesta região pode fornecer informações relevantes acerca da sua influência na patogênese da PE.

O potencial imunorregulatório do HLA-G é investigado em diferentes contextos biológicos (de Almeida et al. 2018). Numa segunda abordagem desta tese, tal potencial foi paralelamente avaliado na manutenção dos transplantes. No transplante renal, por exemplo, uma maior quantidade de HLA-G solúvel é associada ao genótipo +3142CC nos receptores (tópico 7.4). É reconhecido que este SNP influencia a afinidade a micro RNAs, sendo que o alelo G apresenta maior afinidade (Tan et al. 2007). Apesar de não ser evidenciada uma associação do SNP com a aceitação ou rejeição aguda do órgão no primeiro ano após a transplantação, tais níveis elevados somados a terapias imunossupressoras já foram associadas ao risco de infecção por citomegalovírus, o qual é um reconhecido fator de risco para a rejeição e função do transplante (Guberina et al. 2018). De forma interessante, estes estudos contextualizam as situações em que a expressão do HLA-G pode ser benéfica (gestação) e deletéria (câncer, infecções virais e parasitárias) (Garcia et al. 2013; da Silva et al. 2014; Rebmann et al. 2014; Dahl et al. 2015). Nesse contexto, especulamos que heterozigotos para os haplótipos (HLA-G 3'UTR) associados a maior e menor expressão de HLA-G possam ter vantagem em relação a homozigotos devido a maior capacidade de resposta a diferentes estímulos. De fato, essa hipótese é corroborada pelo nosso estudo, visto que heterozigotos UTR-1/UTR-2 são estatisticamente mais frequentes em mulheres multíparas saudáveis (Michita et al. 2016).

No terceiro artigo desta tese, avaliamos a influência do eixo imunológico HLA-E/NKG2C no transplante simultâneo de pâncreas e rim (SPK). Como principal resultado deste estudo, destaca-se a influência do doador no risco de rejeição aguda renal, diferente de observações prévias, nas quais unicamente o perfil genético do receptor foi associado a rejeição do transplante (Di Cristofaro et al. 2016).

Na presente tese, receptores transplantados com órgãos portando o alelo HLA-E*01:03 apresentaram um maior risco para rejeição aguda renal no primeiro ano após a transplantação. Talvez, o mais interessante nesse estudo seja o fato de que a inclusão da genotipagem de um SNP (+3777GA) na região 3'UTR do *HLA-E* nos permitiu inferir, a partir da genotipagem prévia do HLA-E pelo método PCR-SSP o alelo E*01:03:02:01, visto que o alelo +3177G é frequentemente observado neste último, embora esta observação precise ser confirmada, através de metodologias mais robustas, como o sequenciamento de nova geração. Apesar do efeito da variante +3777G/A ainda ser desconhecido, devido a sua localização, é plausível seu envolvimento no controle pós-transcricional do gene (Bartel 2004). No entanto, em estudo recente é descrito que a posição da variante +3777G/A coincide com a presença de um elemento *Alu*, sugerindo a ausência de ligação de micro RNAs nesta região (Ramalho et al. 2017).

Paralelamente a este estudo, foi reportado que o HLA-E influencia no risco de rejeição aguda (Guberina et al. 2017; Guberina et al. 2018) e infecção pelo citomegalovírus em outras coortes de transplante renal (tópico 7.6). Apesar de não observarmos a associação da deleção do *NKG2C* com o desfecho pós-transplante, bem como o efeito do eixo HLA-E/NKG2C, a importância desse eixo imunológico na transplantação é evidente, visto ser observada a expressão de HLA-E em biópsias renais, além de infiltrados de células citotóxicas positivas para NKG2C+ na rejeição aguda do transplante renal (Guberina et al. 2017). Diante disso, sugerimos que o envolvimento do HLA-E nos transplantes depende de diversos aspectos, tais como: (1) mesmo na ausência de incompatibilidade entre os alelos de HLA-E entre doador e receptor, a ativação de células imunes pode ocorrer devido (mas não limitado) ao *pool* de peptídeos derivados da expressão de MHC-I ou HLA-E do doador (Lauterbach et al.,2015; Guberina et al. 2017). (2) os vírus (CMV, HIV, EBV) podem usurpar a maquinaria de expressão do HLA-E favorecendo a sua patogênese e impactando negativamente na sobrevida do órgão (Joosten et Al. 2016; Guberina et al. 2017) e (3) sinais de estresse celular resultantes do trauma cirúrgico, bem como a lesão da isquemia e reperfusão podem induzir a expressão de HLA-E/HSP60, o qual possui menor afinidade à NKG2A, favorecendo o predomínio de sinais de ativação em células citotóxicas (Michaelsson et al., 2002).

Na gestação, um número limitado de estudos avaliou as variantes alélicas do HLA-E, bem como a deleção do NKG2C. Na literatura são reportadas associações do genótipo

E*01:01/E*01:03 (Fotoohi et al. 2016) e o alelo E*01:01 (Tripathi et al. 2006) com risco à AER, embora alguns outros estudos não corroborem estes resultados (Kanai et al. 2001; Pfeiffer et al. 2001; Steffensen et al. 1998). Similar aos outros artigos desta tese, abordamos de forma original o efeito do eixo HLA-E/NKG2C na suscetibilidade à PE. Ainda que os dados sejam preliminares, há uma tendência a não associação dos diferentes alelos do HLA-E e da deleção do NKG2C com o risco à PE. Nossos dados estão de acordo com observações prévias de Nilson et al. (2016). No entanto, nesse estudo os alelos E*01:01 e E*01:03 foram inferidos através da genotipagem única da variante A107G (rs1264457), o que limita as suas conclusões.

Apesar da não associação do HLA-E/NKG2C com o risco de PE na presente tese, deve-se salientar que o genótipo fetal pode contribuir para a patogênese da PE, visto que as células EVTs expressam as principais moléculas que compõem o *pool* de peptídeos que o HLA-E é capaz de apresentar. Desta forma, além do HLA-C, o HLA-E influencia no potencial de ativação das células inatas e adaptativas na interface materno-fetal, através da apresentação de antígenos paternos. No entanto, esta hipótese deve ser direcionada em estudos futuros.

Por fim, o quarto artigo da presente tese avaliou a influência do gene *MIC-A* no desfecho do transplante SPK no primeiro ano pós-transplante. Nesse artigo, foi observado que a variante funcional MIC-A Val129Met influencia significativamente o risco da rejeição renal aguda e a infecção pelo citomegalovírus no primeiro ano pós-transplante. Além disso, os níveis de sMIC-A foram associados tanto a variante Val129Met quanto a variante rs2596538 localizada na região promotora do gene. Assim como o HLA-E, a associação da variante MIC-A Val129Met depende da interação entre doador e receptor. Nesse sentido, um maior risco à rejeição aguda renal em receptores homozigotos para MIC-A 129Met/Met pode ser explicado devido a menores níveis de sMIC-A, o que resulta numa menor interação e internalização do receptor NKG2D (ativação) expresso em células NK e linfócitos T CD8+. Além disso, sugere-se que as moléculas MIC-A 129Val expressas no transplante renal possuem potencial diferencial para estimular resposta imunes nos pacientes MIC-A 129Met/Met, como demonstrado previamente (Isernhagen et al. 2016).

Na gestação, apenas um estudo avaliou a variante Val129Met. Nesse estudo é reportada a associação do MIC-A Val129Met com os níveis de sMIC-A, no entanto, os mesmos não foram associados ao risco de parto prematuro (Von Linsingen et al. 2018). É

proposto que as formas de sMIC-A participem da imunotolerância materno-fetal (Mincheva-Nilsson et al. 2006; Huang et al. 2011). Níveis elevados de sMIC-A estão associados a falha de implantação em mulheres submetidas à implantação *in vitro* (Porcu-Buisson et al. 2007), o que poderia ser explicado pelo fato de sMIC-A levar a internalização do receptor NKG2D provocando um estado anérgico das células NK (Isernhagen et al. 2015; Isernhagen et al. 2016). Diante disso, nesta tese a variante MIC-A Val129Met, bem como a variante rs2596538 do MIC-A e rs1049174 do NKG2D foram avaliadas em uma grande coorte de mulheres com PE e gestantes normotensas saudáveis (anexo 6.1.2).

A variante rs1049174G/C localizado na região 3'UTR do *NKG2D* foi descrita como um *tag* SNP para a determinação de haplótipos de alta atividade (alelo G) e baixa atividade (alelo C) citotóxica em células NK (Risti and Bicalho 2017). Nossos dados preliminares sugerem que as frequências alélicas e genotípicas de NKG2D rs1049174 diferem entre os grupos. Análises mais robustas devem ser conduzidas afim de controlar para fatores de confusão previamente identificados. Independente do potencial resultado (associação ou não associação) após estas correções, acreditamos que os desdobramentos a partir de nossos achados irão impulsionar novos estudos funcionais no intuito de avaliar a interação e contribuição materno-fetal neste eixo imunológico.

Tendo em vista os aspectos imunogenéticos apresentados nesta tese, nossos dados reforçam o fato de que variantes genéticas em genes do MHC-Ib desempenham papéis cruciais na imunorregulação da resposta imunológica, sendo estas variantes decisivas para a manutenção da gestação e transplantes. Apesar dos fenômenos previamente citados, possuírem características únicas, é evidente que o conhecimento gerado a partir da imunologia da gestação pode ser uma ferramenta útil no entendimento dos mecanismos que governam a aceitação dos transplantes. Deste modo, o entendimento destes mecanismos poderá contribuir, futuramente, para o desenvolvimento de imunoterapias promissoras, como o bloqueio da interação entre o HLA-G e ILT-2, assim como imunoterapias direcionadas ao bloqueio das vias de sinalização MIC-A/NKG2D e HLA-E/NKG2C. Apesar de propormos a investigação de importantes componentes imunorregulatórios na gestação, deve-se salientar que investigamos apenas uma pequena parcela da vasta rede de interações complexas que atuam simultaneamente em prol da manutenção da gestação. Além disso, em estudos genéticos devemos considerar a heterogeneidade genética das populações, o que limita a extrapolação dos nossos dados a outras populações, mas implica diretamente na

necessidade de esforços colaborativos no âmbito investigativo das desordens gestacionais. Esforços, os quais serão pauta na continuidade deste trabalho pelo nosso grupo. O futuro é promissor!

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6.1. Anexos

Os anexos desta tese constituem os dados preliminares da genotipagem das variantes genéticas dos genes HLA-E, NKG2C, MIC-A e NKG2D.

6.1.1. The role of NKG2C-HLA-E axis in preeclampsia susceptibility.

Os dados preliminares irão compor um artigo para posterior apreciamento em revista indexada de circulação internacional. Revista almejada para publicação: HLA (editora: Wiley) Fator de impacto: 2.558 (*Journal Citation Reports*., 2017). Autores: Rafael Tomoya Michita, Vera Rebmann, Priscila Vianna, Citânia Tedoldi e José Artur Bogo Chies.

- Este estudo está avaliando mulheres com pré-eclâmpsia e gestantes saudáveis em uma população da região metropolitana de Porto Alegre – Rio Grande do Sul – Brazil. As instituições envolvidas são: Universidade Federal do Rio Grande do Sul, Hospital Nossa Senhora da Conceição e Universisätsklinikum Essen – Alemanha.

Amostra do estudo:

- 104 gestantes diagnosticas com PE;
- 165 gestantes normotensas saudáveis.

Tabela 1. Características clínicas e demográficas de mulheres diagnosticadas com PE e gestantes normotensas saudáveis.

	PE	Controles	Valor de *P*[1]
Idade maternal, mediana (IIQ 25-75)	28,0 (22,0-35,0)	29,0 (21,5-34,0)	0,702
Primíparas, n (%)	33 (32,7)	36 (22,1)	0,057
Gestações, mediana (25-75)	2 (1-3)	2 (2-3)	0,462
Histórico de abortamento, n(%)	19 (19,4)	42 (26,4)	0,198
Parceiro novo, n (%)	15 (26,3)	32 (41,6)	0,068
IMC. Média (±DP)	32,3 (5,35)	28,4 (4,79)	<0,001*
Caucasoide, n(%)	67 (65,7)	126 (77,3)	0,039*
Diabetes gestacional, n(%)	18 (17,5)	8 (5,1)	0,002*
Etilismo, n(%)	0 (0,0)	4 (2,6)	0,149
Tabagismo, n(%)	19 (19,4)	37 (23,1)	0,480
Pré-natal, n (%)	86 (93,5)	146 (96,7)	0,243
Idade do recém-nascido, semanas (IIQ 25-75)	37 (35,0-38,0)	39 (38,0-41,0)	<0,001*
Peso do recém-nascido, gramas (IIQ 25-75)	3010 (2125-3495)	3280 (2835-3540)	<0,001*

Histórico familiar de hipertensão, n (%)	27 (35,1)	19 (17,6)	**0,007***
Restrição do crescimento fetal, n (%)	13 (13,0)	N.A	N.A
PE leve	50 (48,1)	N.A	N.A
PE severa	54 (51,9)	N.A	N.A

PE= pré-eclâmpsia. IMC = Índice de massa corporal, DP = desvio padrão. [1]*Student T-test*, *Mann-Whitney U-test*, *Fisher's exact test* e *Pearson Chi-square test* foram utilizados quando necessário. NA = não se aplica. IIQ = intervalo interquartil, 25-75%. *valores de *P* estatísticamente significativos.

Tabela 2. Frequências alélicas e genotípicas do HLA-E em gestantes pré-eclâmpticas e grupo controle.

Genótipo	PE n (%)	Controles n (%)	Valor de *P*
E*01:01/E*01:01	4 (4,8)	1 (0,9)	
E*01:01/E*01:03	65 (77,3)	92 (81,4)	0,272[1]
E*01:03/E*01:03	15 (17,9)	20 (17,7)	
E*01:01	73 (43,5)	94 (41,6)	0,719[2]
E*01:03	95 (56,5)	132 (58,4)	

PE = Pré-eclâmpsia, n = 84. Controles, n = 113. [1] *Fisher's exact test*. [2] *Pearson Chi-square test*.

Tabela 3 Frequências alélicas e genotípicas da deleção do gene NKG2C em gestantes pré-eclâmpticas e grupo controle.

Genótipo	PE n(%)	Controles n(%)	Valor de *p*
NKG2C/NKG2C	49 (63,6)	71 (65,1)	
NKG2C/Del	25 (32,5)	37 (33,9)	0,475[1]
Del/Del	3 (3,9)	1 (1,0)	
NKG2C	123 (79,9)	179 (82,1)	0,547[2]
Del	31 (20,1)	39 (17,9)	

PE = Pré-eclâmpsia, n = 77. Controle, n = 109. [1] *Fisher's exact test*. [2] *Pearson Chi-square test*.

6.1.2. The effect of single nucleotide polymorphisms of the NKG2D-MIC-A immunological axis in preeclampsia susceptibility.

Os dados preliminares irão compor um artigo para posterior apreciamento em revista indexada de circulação internacional. Revista almejada para publicação: *American Journal of Repdroductive Immunology* (editora: Wiley) Fato de Impacto: 2.745 (*Journal Citation Reports.* 2017). Autores: Rafael Tomoya Michita, Vera Rebmann, Priscila Vianna, Citânia Tedoldi, Alessandra Pontillo, Valeria Sandim e José Artur Bogo Chies.

- Este estudo está avaliando mulheres com pré-eclâmpsia e gestantes saudáveis em duas populações localizadas nas regiões metropolitanas de Porto Alegre/RS e Belo Horizonte/MG – Brasil. As instituições envolvidas são: Universidade Federal do Rio Grande do Sul, Universidade de São Paulo, Universidade Estadual Paulista, Hospital Nossa Senhora da Conceição – Porto Alegre, Hospital Sofia Feldman-Belo Horizonte e Universisätsklinikum Essen – Alemanha.

Amostra do estudo:

- 257 gestantes diagnosticas com PE (Porto Alegre, n = 104; Belo Horizonte, n = 153);
- 385 gestantes normotensas saudáveis (Porto Alegre, n = 165; Belo Horizonte, n = 220).
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Tabela 1. Características clínicas e demográficas de mulheres diagnosticadas com PE e gestantes normotensas saudáveis.

	PE	Controles	Valor de *P*[1]
Idade maternal[2]	26,0 (21,0-32,0)	28,5 (21,5-34,0)	0,291
IMC[2]	27,1 (24,0-31,0)	28,4 (25,2-31,2)	0,877
Caucasoide, n(%)	99 (40,2)	126 (77,3)	<0,001
Parturição, idade gestacional[2]	36,0 (33,5-37,5)	39,0 (38,0-41,0)	<0,001
Peso do recém-nascido, gramas[2]	2315 (1925-3000)	3270 (2825-3540)	<0,001

PE= pré-eclâmpsia. IMC = índice de massa corporal. PAS = pressão arterial sistêmica. PAD = pressão artertial diastólica. [2] As variáveis estão representadas em mediana (variação interquartil: 25-75%); [1] *Mann-Whitney U-test.* *valores de *P* estatísticamente significativos.

Tabela 2. Frequências alélicas e genotípicas das variantes genéticas do eixo imunológicos NKG2D-MIC-A em gestantes pré-eclâmpticas e grupo controle.

Genótipo	PE n (%)	Controles n (%)	Valor de *P*
***MIC-A* Val129Met**			
Val/Val	99 (39,4)	73 (46,5)	
Val/Met	106 (42,4)	60 (38,2)	0,360
Met/Met	46 (18,3)	24 (15,3)	
Val	304 (60,5)	206 (65,6)	0,148
Met	198 (39,5)	108 (34,4)	
***MIC-A* rs2596538G/A**			
GG	24 (24,0)	53 (33,5)	
GA	54 (54,0)	79 (50,0)	0,215
AA	22 (22,0)	26 (16,5)	
G	102 (51,0)	185 (58,5)	0,093
A	98 (49,0)	131 (41,5)	
***NKG2D* rs1049174G/A**			
GG	70 (32,9)	67 (42,7)	
GC	98 (46,0)	74 (47,1)	0,013*
CC	45 (21,1)	16 (10,2)	
G	238 (55,9)	208 (66,2)	0,004*
C	188 (44,1)	106 (33,8)	

PE = Pré-eclâmpsia. Val = valina. Met = metionina. [1] *Pearson Chi-square test*. * valores de P estatísticamente significativos.

Capítulo 7
Outras produções científicas

7.1 Evaluation of cell death pathway genes FAS, FAS-L, BAX and BCL-2 polymorphisms with susceptibility to unexplained recurrent pregnancy loss.

Artigo em segundo *round* de revisão. Autores: **Rafael Tomoya Michita**, Francis Maria Báo Zambra, Lucas Rosa Fraga, Maria Teresa Sanseverino, Lavínia Schuler-Faccini, José Artur Bogo Chies e Priscila Vianna. *Journal of Assisted Reproduction and Genetics*. Fator de Impacto: 2,788 (*Journal Citation Reports*, 2017).

Abstract

Purpose: Idiopathic recurrent pregnancy loss (RPL) is a multifactorial reproductive disorder that impaired control of the apoptosis is likely involved. Triggering of cell-death mechanism occurs in spatiotemporal manner and are strongly related to healthy pregnancy. Single nucleotide polymorphisms (SNPs) in the genes at regulatory regions are known to influence expression patterns of apoptosis-related molecules. Therefore, evaluation of SNPs in critical genes for apoptosis signaling could represent an informative susceptibility factor, allowing identify and improve clinical management of women at increased risk for RPL. **Methods:** A total of 296 Brazilian RPL unrelated patients were evaluated for clinical-demographic variables and genetic factors: 140 unexplained RPL (with at least 2 consecutive abortions) and 156 healthy multiparous women. In all patients, 6 SNPs in genes of the death-receptors pathway: FAS (rs2234767, rs1800682), FAS-L (rs763110, rs5030772) and mitochondrial pathway: BAX (rs4645878) and BCL-2 (rs2279115) were evaluated by PCR-RFLP. **Results:** Our results suggest that BAX -248GA genotype is independently associated with idiopathic RPL [adjusted OR = 0.30, 95% CI 0.13-0.70, P = 0.005] susceptibility. Also, in the same multivariate model the variables: ethnicity, smoking, and alcohol consumption statistically associated with RPL susceptibility ($P < 0.05$). No association was reported for the remaining SNPs with RPL susceptibility. **Conclusions:** Our study is the first to evaluate the role of main SNPs from both extrinsic and intrinsic apoptosis pathway in RPL susceptibility. The association of BAX -248G/A with RPL susceptibility in our population suggests that maternal predisposition for RPL has essential contribution of genes involved in the delicate balance of endometrium cellular turnover (cell death/proliferation). Therefore, apoptosis genes may represent promising targets for future studies approaching healthy pregnancy and the spectrum of pregnancy disorders, as well as potential molecular biomarkers/target to identifying women with fertility issues.

7.2 The donor MICA allele rs2596538 G predicts Cytomegalovirus viremia in kidney transplant recipients.

Publicado como: Rohn H, **Tomoya Michita R**, Schwich E, Dolff S, Gäckler A, Trilling M, Le-Trilling VTK, Wilde B, Korth J, Heinemann FM, Horn PA, Kribben A, Witzke O, Rebmann V. The Donor Major Histocompatibility Complex Class I Chain-Related Molecule A Allele rs2596538 G Predicts Cytomegalovirus Viremia in Kidney Transplant Recipients. Front Immunol. 2018 May 8;9:917. doi: 10.3389/fimmu.2018.00917. Fator de Impacto: 5,511 (*Journal citation reports*, 2017).

Abstract

The interaction of major histocompatibility complex class I chain-related protein A (MICA) and its cognate activating receptor natural killer (NK) group 2 member D (NKG2D) receptor plays a significant role in viral immune control. In the context of kidney transplantation (KTx), cytomegalovirus (CMV) frequently causes severe complications. Hypothesizing that functional polymorphisms of the MICA/NKG2D axis might affect antiviral NK and T cell responses to CMV, we explored the association of the MICA-129 Met/Val single nucleotide polymorphism (SNP) (affecting the binding affinity of MICA with the NKG2D receptor), the MICA rs2596538 G/A SNP (influencing MICA transcription), and the NKG2D rs1049174 G/C SNP (determining the cytotoxic potential of effector cells) with the clinical outcome of CMV during the first year after KTx in a cohort of 181 kidney donor-recipients pairs. Univariate analyses identified the donor MICA rs2596538 G allele status as a protective prognostic determinant for CMV disease. In addition to the well-known prognostic factors CMV high-risk sero-status of patients and the application of lymphocyte-depleting drugs, the donor MICA rs2596538 G allele carrier status was confirmed by multivariate analyses as novel-independent factor predicting the development of CMV infection/disease during the first year after KTx. The results of our study emphasize the clinical importance of the MICA/NKG2D axis in CMV control in KTx and point out that the potential MICA transcription in the donor allograft is of clinically relevant importance for CMV immune control in this allogeneic situation. Furthermore, they provide substantial evidence that the donor MICA rs2596538 G allele carrier status is a promising genetic marker predicting CMV viremia after KTx. Thus, in the kidney transplant setting, donor MICA rs2596538 G may help to allow the future development of personal CMV approaches within a genetically predisposed patient cohort.

7.3 Helicobacter pylori eradication: influence of interleukin-1beta -31 C/T polymorphism.

Publicado como: Rech TF, Mazzoleni LE, Mazzoleni F, Francesconi CFM, Sander GB, **Michita RT**, Nabinger DD, Milbradt TC, Torresini RJS, Simon D. Helicobacter pylori eradication: influence of interleukin-1beta -31 C/T polymorphism. Braz J Infect Dis. 2018 Jul - Aug;22(4):311-316. doi: 10.1016/j.bjid.2018.06.005. Fator de Impacto: 2,082 (Journal citation reports, 2017).

Abstract

AIM: To analyze the influence of the -31 C/T polymorphism of the interleukin-1β gene on Helicobacter pylori eradication therapy success in patients with functional dyspepsia. **METHODS:** Functional dyspepsia was diagnosed according to the Rome III criteria. All patients underwent upper gastrointestinal endoscopy, and gastric biopsies were obtained at screening and 12 months after randomization (last follow-up visit). Urease test and histological examination were performed to define the H. pylori status. Patients received twice-daily amoxicillin, clarithromycin and omeprazole for 10 days. Genotyping of the interleukin-1beta -31 C/T polymorphism (rs1143627) was performed using polymerase chain reaction-restriction fragment length polymorphism. **RESULTS:** One hundred forty-nine patients received treatment with triple therapy for H. pylori eradication. Only one patient was lost to follow-up, and adherence to study medication was 94.6%. A total of 148 patients (mean age 46.08 ± 12.24 years; 81.8% women) were evaluated for the influence of the interleukin-1beta -31 C/T polymorphism on the outcome of H. pylori eradication therapy. After treatment, bacteria were eradicated in 87% of patients (129/148). Genotype frequencies of the polymorphism were as follows: CC, 38/148 (25.7%); CT, 71/148 (47.9%); and TT, 39/148 (26.4%). Successful eradication rate was 78.9%, 94.4% and 82.1% for the CC, CT and TT genotypes, respectively. The CT genotype was significantly associated with successful H. pylori eradication ($p = 0.039$). **CONCLUSION:** This study suggests that the CT genotype of the interleukin-1beta -31 C/T polymorphism plays a role in the successful eradication of H. pylori among patients with functional dyspepsia.

7.4 Recipient HLA-G +3142 CC Genotype and Concentrations of Soluble HLA-G Impact on Occurrence of CMV Infection after Living-Donor Kidney Transplantation.

Publicado como: Guberina H, **Tomoya Michita R**, Dolff S, Bienholz A, Trilling M, Heinemann FM, Horn PA, Kribben A, Witzke O, Rebmann V. Recipient HLA-G +3142 CC Genotype and Concentrations of Soluble HLA-G Impact on Occurrence of CMV Infection after Living-Donor Kidney Transplantation. Int J Mol Sci. 2017 Nov 5;18(11). pii: E2338. doi: 10.3390/ijms18112338. Fator de Impacto: 3,687 (Journal citation reports, 2017).

Abstract

The expression modulation of the immunosuppressive non-classical Human leukocyte antigen-G (HLA-G) molecule and its soluble isoforms is an immune evasion strategy being deployed by cytomegalovirus (CMV). The +3142 C>G single nucleotide polymorphism (SNP) located within the 3' untranslated region (3'UTR) is of crucial importance for the regulation of HLA-G expression. Therefore, we analyzed the influence of the +3142 C>G HLA-G SNP on the occurrence of CMV infection in a cohort of 178 living-donor kidney recipients and their 178 corresponding donors. In addition, soluble HLA-G (sHLA-G) levels were quantified before and after transplantation. The presence of the HLA-G +3142 CC genotype in recipients, but not donors of our cohort as along with elevated sHLA-G levels (≥ 6.1 ng/mL) were associated with higher susceptibility to CMV infection after transplantation. Our results provided evidence that i) HLA-G is implicated in the establishment of CMV after living-donor kidney transplantation and ii) recipient HLA-G +3142 CC genotype and sHLA-G concentration levels could represent important predictive risk markers for CMV infection.

7.5 CYP2B6 516 G> T polymorphism and side effects of the central nervous system in HIV-positive individuals under Efavirenz treatment: Study of a sample from southern Brazil.

Publicado como: Müller TE, Ellwanger JH, **Michita RT**, Matte MCC, Renner JDP. CYP2B6 516 G>T polymorphism and side effects of the central nervous system in HIV-positive individuals under Efavirenz treatment: Study of a sample from southern Brazil. An Acad Bras Cienc. 2017 May;89(1Suppl 0):497-504. doi: 10.1590/0001-3765201720160355. Fator de Impacto: 0,956 (Journal citation reports, 2017).

Abstract

This study aimed to identify the 516 G>T polymorphism of the CYP2B6 gene and evaluate its influence on central nervous system (CNS) side effect development in HIV-positive individuals undergoing Efavirenz (EFV) treatment in a population from southern Brazil. Additionally, we performed a survey on the clinical and epidemiological characteristics of our sample. In addition to medical records evaluation, whole blood of 89 individuals was analyzed for viral load, T lymphocyte count (CD4+ and CD8+), and the polymorphism. Considering the side effects of the CNS reported by individuals but without considering the genetic variables, no statistically significant association was noted between the adverse effects and the antiretroviral treatment (including or not EFV). In addition, no statistically significant difference was noted for the influence of genotype on the viral load or the number of T lymphocytes (CD4+ and CD8+) among individuals undergoing EFV treatment. This is the first study that investigated the impact of the 516 G>T polymorphism of the CYP2B6 gene among HIV-positive individuals from southern Brazil. Its clinical significance indicates the need for prospective studies in this population.

7.6 Susceptibility of HLA-E*01:03 Allele Carriers to Develop Cytomegalovirus Replication After Living-Donor Kidney Transplantation.

Publicado como: Guberina H, da Silva Nardi F, **Michita RT**, Dolff S, Bienholz A, Heinemann FM, Wilde B, Trilling M, Horn PA, Kribben A, Witzke O, Rebmann V. Susceptibility of HLA-E*01:03 Allele Carriers to Develop Cytomegalovirus Replication After Living-Donor Kidney Transplantation. J Infect Dis. 2018 May 25;217(12):1918-1922. doi: 10.1093/infdis/jix638.. Fator de Impacto: 5,186 (Journal citation reports, 2017).

Abstract

Cytomegalovirus (CMV) causes serious complications among solid organ transplant recipients. We report the positive correlation between the presence of the HLA-E*01:03 allele in living-donor kidney recipients and CMV reactivation during the first year after transplantation. Thus, HLA-E genotyping may help identify CMV replication-prone patients who require individualized patient-based CMV management.