HELOÍSA LIZOTTI CILIÃO

"**ESTUDOS EM TRANSPLANTADOS RENAIS:** AVALIAÇÃO DA INSTABILIDADE GENÔMICA, EXPRESSÃO GÊNICA E ANÁLISE DE VARIANTES POLIMÓRFICAS ASSOCIADAS AO RISCO DE REJEIÇÃO DO ENXERTO E AO DESENVOLVIMENTO DE CÂNCER"

Londrina
2017

Universidade Estadual de Londrina

Instituto Agronômico do Paraná

Empresa Brasileira de Pesquisa Agropecuária

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Tese apresentada ao Programa de Pós-Graduação em Genética e Biologia Molecular da Universidade Estadual de Londrina, como requisito parcial para a obtenção do título de doutor.

Orientadora: Profa. Dra. Ilce Mara de Syllos Cólus

Londrina
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Londrina, 24 de janeiro de 2017.

Dedico este trabalho:

Aos pacientes transplantados renais pela contínua lição de fé, determinação e coragem.

Ao meu querido marido Gilberto Araújo Brandina, amigo e companheiro de todos os momentos, que sempre esteve ao meu lado oferecendo seu ombro amigo e dedicando a mim, muito apoio, confiança e amor ilimitado. E à nossa filha Isabela.

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“Quando a gente acha que tem todas as respostas,
vem a vida e muda todas as perguntas.”
(Luís Fernando Veríssimo)

CILIÃO, Heloísa Lizotti. **Estudos em transplantados renais:** avaliação da instabilidade genômica, expressão gênica e análise de variantes polimórficas associadas ao risco de rejeição do enxerto e ao desenvolvimento de câncer. 158 f. Tese (Doutorado em Genética e Biologia Molecular) – Universidade Estadual de Londrina, Londrina, 2017.

RESUMO

O transplante renal é a melhor opção terapêutica para pacientes com doença renal crônica. Após a realização do transplante, é necessário o uso constante de drogas imunossupressoras para evitar a rejeição do enxerto. Porém, o uso prolongado destes medicamentos acarreta alguns efeitos adversos. Entre os pacientes transplantados é observada uma grande variabilidade da concentração plasmática dos imunossupressores, o que se deve à influência de diversos fatores, entre eles, a presença de variantes alélicas que pode levar a respostas distintas ao tratamento. Os objetivos do presente estudo foram estudar pacientes transplantados renais para: (i) avaliar se o uso prolongado de imunossupressores pode induzir efeitos mutagênicos; (ii) associar a presença de polimorfismos de nucleotídeo único (SNPs) em genes do metabolismo, transporte de drogas e sistema imunológico com a ocorrência de episódios de rejeição; (iii) associar SNPs com a concentração plasmática de tacrolimo; (iv) avaliar se a presença de SNPs em genes de reparo e sistema imunológico está associada com a ocorrência de câncer. Os resultados obtidos não demonstraram associação entre a frequência de micronúcleos em linfócitos com o tempo decorrido após o transplante. Por outro lado, os danos observados no ensaio do cometa aumentaram com o tempo de imunossupressão. Na análise de associação dos SNPs com episódios de rejeição em 246 pacientes foi observada associação de SNPs em genes do metabolismo rs7662029 (*UGT2B7*) com proteção (1,85 vezes) e rs6714486 (*UGT1A9*) com aumento de risco de rejeição de 1,6 vezes. Entre os genes de transportadores de drogas, apenas o rs2231142 (*ABCG2*) demonstrou diminuição em 1,92 vezes no risco de rejeição. O rs10889677 do gene do sistema imune *IL-23R* foi associado com o aumento de 1,9 vezes no risco de rejeição do enxerto. Entre os pacientes, 145 faziam uso do imunossupressor micofenolato de mofetil (MMF). Foram avaliados neste grupo SNPs em genes que influenciam a farmacocinética deste medicamento e observou-se associação do rs11706052 (*IMPDH2*) com proteção de 4,2 vezes, e do rs7438135 (*UGT2B7*) com proteção de 2,4 vezes contra episódios de rejeição. O rs2241409 (*CES2*) foi associado com o aumento de risco à rejeição de 7,2 vezes. Dos 246 pacientes avaliados 10,2 % desenvolveram câncer após 15±8,9 anos de transplante, sendo que destes, 68% eram constituídos por carcinoma de pele não melanoma. O rs1800872 (*IL-10*) foi associado com o risco de 3,5 vezes de desenvolver câncer. Foi avaliada a associação de 11 SNPs em genes do metabolismo e transporte de drogas com a concentração plasmática de tacrolimo em 55 pacientes transplantados renais que faziam uso, nos três primeiros meses de transplante, deste medicamento associado com MMF e corticóide, sendo observado que apenas os SNPs rs4646437 (*CYP3A4*) e rs776746 (*CYP3A5*) influenciaram a farmacocinética deste imunossupressor. Este estudo demonstrou que os pacientes tranplantados renais apresentam maior quantidade de dano no material genético em comparação com controles saudáveis e que o uso prolongado dos medicamentos imunossupressores pode induzir instabilidade genética. Os SNPs nos genes *UGT2B7, UGT1A9, ABCG2, IL-23R, IMPDH2* e *CES2* podem ser usados como marcadores candidatos para a triagem do risco de rejeição e o SNP no gene *IL-10* pode ser utilizado para a triagem do risco de desenvolvimento de câncer em pacientes transplantados renais. Também foi confirmado o impacto dos SNPs rs4646437 (*CYP3A4*), rs776746 (*CYP3A5*) na farmacocinética do imunossupressor tacrolimo.

Palavras-chave: Ensaio do cometa. Teste do micronúcleo. SNPs. Genes do metabolismo. Genes do sistema imune. Genes de reparo.

CILIÃO, Heloísa Lizotti. **Studies in kidney transplanted patients:** evaluation of genomic instability, gene expression and analysis of polymorphic variants associated with the risk of graft rejection and the development of cancer. 158 p. Thesis (Doctorate in Genetics and Molecular Biology) – Universidade Estadual de Londrina, Londrina, 2017.

ABSTRACT

Kidney transplant is the best option of treatment for patients with chronic kidney disease. After the transplant performing, it is need the use of immunosuppressive drugs to prevent the graft rejection, but prolonged use of these drugs leads to some adverse effects. Among transplanted patients a large variability of plasma concentration of immunosuppressives is observed, due to the influence of various factors, among them the presence of allelic variants that can lead to different responses to the treatment. The objectives of the present study were to study renal transplant patients to: (i) assess if prolonged use of immunosuppressants can induce mutagenic effects; (ii) associate the presence of single nucleotide polymorphisms (SNPs) in genes of metabolism, drug transport and immune system with the occurrence of rejection episodes; (iii) associate SNPs with tacrolimus plasma concentration; (iv) assess if the presence of SNPs in repair genes and immune system are associated with the occurrence of cancer. The results obtained showed no association between micronucleus frequency in lymphocytes and the time elapsed after transplantation. On the other hand, the damage observed in the comet assay increased with the time of immunosuppression. In the analysis of the association of SNPs with rejection episodes in 246 patients, was observed association of SNPs in metabolic genes rs6714486 (*UGT1A9*) with the protection (1.85 times) and of rs7662029 (*UGT2B7*) with risk of rejection of 1.6 times. Among the drug transporter genes, only rs2231142 (*ABCG2*) showed a 1.92-fold decrease in the risk of rejection. The rs10889677 of immune system gene IL-23R was associated with an increased risk of graft rejection of 1.9 fold. Among the patients, 145 used the immunosuppressive mycophenolate mofetil (MMF; in this group it were evaluated SNPs in genes that influence the pharmacokinetics of this drug and was observed association of rs11706052 (*IMPDH2*) with protection of 4.2 fold and rs7438135 (*UGT2B7*) with 2.4-fold protection against rejection episodes. The rs2241409 (*CES2*) was associated with an increased risk of rejection of 7.2 fold. Of the 246 patients evaluated, 10.2% developed cancer after 15±8.9 years of transplantation, of which 68% were nonmelanoma cutaneous carcinomas. The rs1800872 (IL-10) was associated with the 3.5-fold risk of developing cancer. It was done the evaluation of association of 11 SNPs in drug metabolism and transport genes with the plasma concentration of tacrolimus in 55 renal transplant patients who used of this drug associated with MMF and corticoid in the first three months of transplantation and it was observed that only SNPs rs4646437 (*CYP3A4*) and rs776746 (*CYP3A5*) influenced the pharmacokinetics of immunosuppressive tacrolimus. This study demonstrated that renal transplant recipients present greater amount of damage in genetic material compared to healthy controls and that prolonged use of immunosuppressive drugs may induce genetic instability. SNPs in the genes *UGT2B7, UGT1A9, ABCG2, IL-23R, IMPDH2* and *CES2* can be used as screening markers for risk of rejection and the SNP in the *IL-10* gene can be used for cancer risk screening in kidney transplant patients. This study also confirmed the impact of SNPs rs4646437 (*CYP3A4*) and rs776746 (*CYP3A5*) on the pharmacokinetics of tacrolimus.

Key words: Comet assay. Micronucleus test. SNP. Metabolism genes. Genes of the immune system. DNA repair genes.

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LISTA DE ABREVIATURAS E SIGLAS

ABCB1	*ATP-binding cassette, sub-family B (MDR/TAP), member 1*
ABCC2	*ATP-binding cassette, sub-family C (CFTR/MRP), member 2*
ABCG2	*ATP-binding cassette transporter BCRP/MXR1/ABCP*
AcMPAG	MPA-acil-glucuronído
APC	Células apresentadoras de antígenos
APE1	Apurínico/apirimidínico endonuclease I
AZA	Azatioprina
BER	Reparo por Excisão de Bases
CBMN-cyt	Ensaio do micronúcleo com bloqueio de citocinese
CCR5	Receptor de quimiocina
CDKs	Quinases dependentes de ciclinas
CES1	Carboxilesterase 1
CES2	Carboxilesterase 2
CNI	Inibidores de calcineurina
CsA	Ciclosporina
CYP	Citocromo P450
CYP3A4	Citocromo P450, família 3, subfamília A, polipeptideo 4
CYP3A5	Citocromo P450, família 3, subfamília A, polipeptideo 5
CYP2E1	Citocromo P450, família 2, subfamília E, polipeptídeo 1
DNA	Ácido desoxirribonucleico
eGFR	Taxa de filtração glomerular estimada
ERCC1	*Excision repair cross complementation, Group 1*
FK-506	Tacrolimo
HLA	Antígeno leucocitário humano
IC	Intervalo de confiança
IL-2	Interleucina 2
IL-2R	Receptor de interleucina 2
IL-10	Interleucina 10
IL-23R	Receptor de interleucina 23
IRF-5	Fator regulador de interferon 5
IMPDH	Inosina 5'-monofosfato desidrogenase
IFN-γ	Interferon gama

MDR-1	Proteína de resistência a múltiplas drogas
MGMT	O^6-metilguanina-DNA-metiltransferase
MHC	Complexo Principal de Histocompatibilidade
MN	Micronúcleo
MMF	Micofenolato de mofetil
MPA	Ácido micofenólico
MPAG	Ácido micofenólico glucuronídeo
mTOR	Alvo da rapamicina em mamíferos (mammalian target of rapamycin)
NFAT	Fator nuclear de células T ativadas
NFKBIA	Inibidor do fator de transcrição nuclear kappa β Alfa
OR	Razão de chance (*Odds Ratio*)
OKT3	Anticorpo monoclonal específico anti-CD3
PCR	Reação de polimerase em cadeia
POR	Citocromo P450 oxidorredutase
RFLP	Polimorfismo de comprimento dos fragmentos de restrição *(Restriction Fragment Length Polymorphism)*
SLCO1B1	Membro 1B1 da família de transportadores de ânions orgânicos portadores de solutos
SNP	Polimorfismo de nucleotídeo único
TCLE	Termo de Consentimento Livre e Esclarecido
TCR	Receptor de células T
TNF	Fator de necrose tumoral
TGFB1	Fator de crescimento transformante-Beta
Th1	Célula T auxiliar efetora tipo 1
Th2	Célula T auxiliar efetora tipo 2
UGTs	UDP-glucuronosiltransferases
UGT1A9	UDP glucuronosil transferase família 1, polipeptídio A9
UGT2B7	UDP glucuronosil transferase família 2, polipeptídio B7
XPA	Xeroderma pigmentoso, grupo A
XRCC1	*X-ray Cross Complementing Group1*
6-TGN	6- tioguanina

SUMÁRIO

1 INTRODUÇÃO GERAL

Após a realização de um transplante os pacientes necessitam fazer uso constante de medicamentos imunossupressores para prevenir episódios de rejeição. No entanto, o uso destes medicamentos após longos períodos pode acarretar o aparecimento de efeitos adversos, como nefrotoxicidade, hepatotoxicidade, neurotoxicidade, infecções, diabetes *mellitus*, câncer, entre outros. Estes efeitos adversos acometem os pacientes de maneira diferente devido vários fatores, entre eles a variabilidade interindividual na resposta aos medicamentos imunossupressores. Esta variação pode ser decorrente de polimorfismos de nucleotídeo único (SNPs) em genes do metabolismo e transporte de drogas que interferem na expressão e/ou atividades destes genes, modificando a concentração ou a atividade das proteínas responsáveis pelo metabolismo dos medicamentos imunossupressores, fazendo com que diferentes concentrações plasmáticas dos medicamentos sejam atingidas, mesmo após a administração de doses idênticas. SNPs em genes do sistema imune também podem influenciar na ocorrência de episódios de rejeição.

Estudos que colaboram para um maior conhecimento sobre a interferência de SNPs no resultado do transplante, bem como uma melhor compreensão da atuação de drogas imunossupressoras no organismo humano são importantes e necessários para proporcionar melhora na qualidade de vida dos pacientes transplantados.

Neste estudo foram avaliados e comparadas as frequências de danos no material genético em pacientes transplantados e em controles saudáveis; e em pacientes com diferentes tempos de transplante, com o intuito de verificar se os pacientes apresentam maior quantidade de dano que os controles e se o tempo prolongado de imunossupressão influencia na instabilidade genômica. Também foram avaliados SNPs de genes relacionados com o metabolismo, transporte de drogas e sistema imunológico, a fim de buscar associação entre variantes polimórficas com episódios de rejeição e com as concentrações plasmáticas do imunossupressor tacrolimo. SNPs em genes de reparo e sistema imune também foram avaliados em pacientes transplantados que desenvolveram câncer após tratamento com imunossupressores.

2 REVISÃO BIBLIOGRÁFICA

2.1 TRANSPLANTE RENAL: IMUNOSSUPRESSORES E PROCESSO DE REJEIÇÃO DO ENXERTO

Pacientes com doença renal crônica necessitam de diálise para substituir a função renal artificialmente (O'CALLAGHAN, 2008; GENSBURGER et al., 2010). Estes pacientes são selecionados de acordo com a idade e estado de saúde para entrarem ou não na fila de espera por um transplante, sendo este considerado a melhor opção terapêutica do ponto de vista médico, social e econômico, além de aumentar a expectativa de vida dos pacientes (WOLFE et al., 1999; TONELLI et al., 2011).

No Brasil, segundo senso realizado pela Associação Brasileira de Transplante de Órgãos, 19.440 adultos e 379 crianças aguardam na lista de espera por um transplante de rim, sendo realizados mais de 5000 transplantes de rim por ano (BRASIL, ABTO: RBT 2015). A taxa de rejeição no primeiro ano de transplante chega a 15%, sendo a maior barreira para o sucesso do transplante (OLIVEIRA; ZANKL; RATH, 2004).

Os episódios de rejeição apresentados pelo paciente são consequência da sua resposta imunológica contra células do órgão transplantado. Este reconhecimento é realizado por células do complexo principal de histocompatibilidade (MHC), conhecido em seres humanos como sistema antígeno leucocitário humano (HLA) (ABBAS; LICHTMAN, 2007). Didaticamente o complexo gênico MHC é dividido em três classes: I, II e III.

O sistema HLA está localizado no braço curto do cromossomo humano 6, na região 6p21.3 e contém mais de 220 genes com funções distintas. Muitos desses genes codificam proteínas para o sistema imunológico e são altamente polimórficos. As proteínas transcritas pelos genes do complexo MHC classe I são expressas nas superfícies de todas as células nucleadas, enquanto que as proteínas do complexo de classe II são expressas nas células dendríticas, macrófagos e linfócitos B. Estas duas classes participam do processo de apresentação do antígeno ao linfócito T, sendo que o MHC classe I faz a apresentação dos antígenos para os linfócitos T CD8+ e o MHC classe II, para os linfócitos CD4+ (MAGALHÃES; BÖHLKE; NEUBARTH, 2004).

A variação destes antígenos de histocompatibilidade faz com que o sistema imune distingua o próprio do não próprio, ou seja, as células do receptor das células do enxerto (ABBAS; LICHTMAN; POBER, 2003). Assim, quando células apresentadoras de antígeno (APC) são reconhecidas por células do complexo MHC, ocorre a indução da proliferação dos linfócitos através da ativação da calcineurina, uma proteína que leva à produção de várias citocinas, incluindo a interleucina 2 (IL-2). A estimulação autócrina por IL-2 resulta na

proliferação de células por uma via envolvendo o alvo da rapamicina em mamíferos (mTOR) e quinases dependentes de ciclinas (CDKs) (KOBASHIGAWA; PATEL, 2006) (Figura 1).

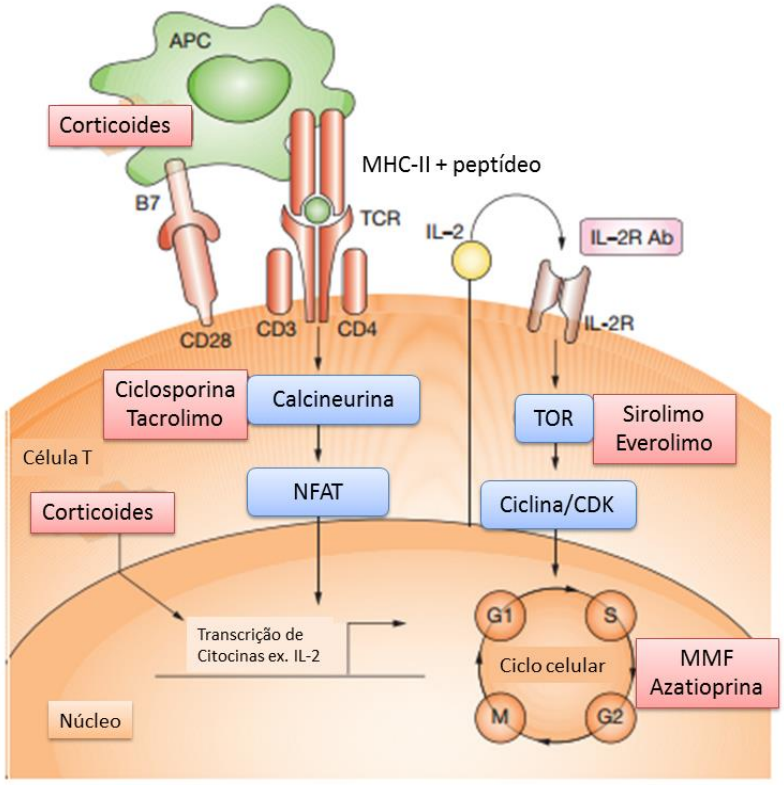

Figura 1: Indução de proliferação de linfócitos T e alvos dos imunossupressores. APC: células apresentadoras de antígenos, TCR: receptor de células T, MHC-II: complexo principal de histocompatibilidade – classe II, NFAT: fator nuclear de células T ativadas, IL-2R: receptor de IL-2, TOR: proteína alvo da rapamicina, MMF: micofenolato de mofetil. Fonte: Modificado de KOBASHIGAWA; PATEL, 2006.

Durante o processo de rejeição ocorre o recrutamento de linfócitos do sangue do transplantado para o endotélio e tecido do enxerto, desencadeando um processo inflamatório que pode resultar em lesão tubular renal, contribuindo para a perda da função do enxerto (ROBERTSON et al., 1998). O processo inflamatório é decorrente do aumento da expressão de quimiocinas pró-inflamatórias como RANTES, MIP-1α e MIP-1β, que se ligam ao receptor CCR5 e causam a infiltração de células mononucleares no enxerto, entre elas, linfócitos T de memória, monócitos e eosinófilos (PATTISON et al., 1994; ROBERTSON et al., 1998).

A rejeição aguda é um importante fator de risco para o desenvolvimento da rejeição crônica, pois após o primeiro episódio de rejeição, células T e B de memória são produzidas, propiciando respostas imunes mais rápidas e em maior magnitude em episódios de rejeição subsequentes (WOOD; GOTO, 2012).

Para evitar a ocorrência de rejeição e diminuir possíveis processos inflamatórios, tradicionalmente são administrados aos pacientes medicamentos imunossupressores de uso contínuo, além da realização de exames de rotina para avaliar a função renal (O'CALLAGHAN, 2008).

O Ministério da Saúde por meio da Portaria n^{o} 666 de 17 de julho de 2012, recomendou o esquema tríplice de imunossupressão (prednisona (corticoide), ciclosporina ou tacrolimo e azatioprina (AZA) ou micofenolato de mofetil (MMF)) (BRASIL, 2012), que é atualmente utilizado pela maioria dos centros de transplante.

Os medicamentos imunossupressores mais utilizados agem em diferentes alvos com o objetivo comum de diminuir a proliferação dos linfócitos, como demonstrado na Figura1.

2.1.1 Corticoides

Corticoides são essenciais na terapia de indução, realizada logo após a realização do transplante, pois exercem diferentes efeitos imunomoduladores e anti-inflamatórios (KOBASHIGAWA; PATEL, 2006). Os esteroides, ao se ligarem a receptores de glicocorticoides, são translocados para o núcleo da célula e diminuem a transcrição de genes de citocinas pró-inflamatórias (IL-1, IL-2, IL-6, TNF), causando a diminuição da quantidade de monócitos, macrófagos e células T CD4+ circulantes e, consequentemente, resultando na redução da resposta inflamatória (TAYLOR; WATSON; BRADLEY, 2005).

O tratamento com esteroides pode resultar em inúmeros efeitos adversos como diabetes *mellitus*, ganho de peso, miopatias, retenção de líquidos, hipertensão, hipercalemia (concentração baixa de potássio no sangue), hiperlipidemia, necrose avascular, atrofia da adrenal, osteoporose, desenvolvimento de doenças cardiovasculares, entre outros (BENFIELD et al., 2010).

2.1.2 Inibidores de Calcineurina (CNIs)

A ciclosporina foi introduzida na prática clínica em 1983, como um potente imunossupressor em pacientes transplantados renais, resultando em grandes melhoras na área do transplante, pois trouxe um aumento na taxa de sobrevivência do enxerto em 15% ao longo de dois anos. Em 1997 começou a ser utilizado o imunossupressor tacrolimo, com semelhante mecanismo de ação (CECKA, 1998). Estes medicamentos possuem ação específica sobre os linfócitos T, não tendo efeito sobre a síntese de anticorpos (KOBASHIGAWA; PATEL, 2006).

Os CNIs, ao entrarem no citoplasma da célula, formam um complexo que inibe a

atividade da calcineurina. A ciclosporina se liga à ciclofilina e o tacrolimo se liga à imunofilina FKBP-12, bloqueando a translocação de fator nuclear de células T ativadas (NFAT) e, consequentemente, a transcrição de genes de citocinas (BRAZELTON; MORRIS, 1996; TAYLOR; WATSON; BRADLEY, 2005).

Os efeitos adversos causados por estes medicamentos incluem hipertensão, nefrotoxicidade, hepatotoxicidade, efeitos neurológicos, hiperplasia gengival e aumento do risco de doenças malignas (MATTHEW, 2007). Certos efeitos adversos como a hipertrofia gengival, hirsutismo (pilosidade excessiva em locais normalmente desprovidos de pelos) e hiperlipidemia são mais frequentes como consequência do tratamento com ciclosporina enquanto tremores e intolerância à glicose são observados em pacientes que fazem uso de tacrolimo (NOVARTIS PHARMACEUTICALS CORP, 2004; ASTELLAS PHARMA US INC, 2005).

Em recente meta-análise realizada por Liu et al. (2014) foi comparada a utilização da ciclosporina e do tacrolimo após o transplante renal, demonstrando melhores resultados em pacientes que utilizaram tacrolimo, com redução da mortalidade, da perda do enxerto, da rejeição aguda e da hipercolesterolemia; porém, maior risco de desenvolvimento de diabetes. Dados semelhantes foram observados por Cheung et al. (2009), que compararam a eficácia da terapia imunossupressora à base de tacrolimo ou ciclosporina e observaram que pacientes que fizeram uso do tacrolimo apresentaram melhor função renal, menores taxas de rejeição aguda (18,4% vs 42,1% para tacrolimo e ciclosporina, respectivamente) e hipercolesterolemia; não sendo observadas diferenças entre os grupos quando parâmetros como diabetes *mellitus*, hipertensão, infecções oportunistas e neoplasias.

2.1.3 Inibidores de mTOR (Sirolimo e Everolimo)

O mecanismo de ação dos medicamentos sirolimo e everolimo, também conhecidos como rapamicina, ocorre pela ligação destes medicamentos à proteína imunofilina FKBP12, formando um complexo que inibe a proteína cinase mTOR, regulador chave do ciclo, crescimento e proliferação celular. O bloqueio da mTOR impede a progressão do ciclo celular da fase G1 para a fase S nos linfócitos ativados, inibindo a proliferação de linfócitos T e B (BARLOW; NICHOLSON; HERBERT, 2013).

Os efeitos adversos mais comuns destes medicamentos são hipertensão, mielossupressão e resistência à insulina, e quando administrados em conjunto com a ciclosporina (CsA) podem causar nefrotoxicidade (AUGUSTINE; BODZIAK; HRICIK, 2007; BARLOW; NICHOLSON; HERBERT, 2013).

2.1.4 Agentes Antiproliferativos

A AZA é uma droga imunossupressora utilizada desde a década de 1950 e vem sendo substituída pelo MMF na maioria dos centros de transplantes, pois este tem se mostrado mais eficaz (STAATZ; TETT, 2014). Mathew (1998) observou que pacientes tratados com MMF apresentaram uma redução de aproximadamente 50% na taxa de rejeição aguda em relação aos tratados com AZA. No entanto, estudos mais recentes não observaram diferença na incidência de episódios de rejeição aguda e na sobrevida do enxerto entre os transplantados renais que receberam estes dois medicamentos (JOH et al., 2005; RAHEEM et al., 2011).

O efeito imunossupressor da AZA é resultante da incorporação do metabólito ativo 6-tioguanina (6-TGN) (estruturalmente análogo às bases purinas do DNA), que inibe a síntese de DNA e ocasiona a parada da replicação. Dentre os efeitos adversos causados pela AZA destaca-se a supressão da medula óssea e hepatotoxicidade (STAATZ; TETT, 2014).

O pró-fármaco MMF após ser metabolizado no fígado em ácido micofenólico (MPA), um fármaco ativo, inibe a inosina monofosfato desidrogenase (IMPDH), enzima que limita a taxa de síntese do nucleotídeo guanina pela via “de novo”. A inibição desta enzima resulta no bloqueio da síntese de DNA e, consequentemente, na diminuição da proliferação dos linfócitos (TAYLOR; WATSON; BRADLEY, 2005). O MMF possui atividade antiproliferativa específica para os linfócitos B e T, pois estes são desprovidos da via de recuperação de guanina, diferentemente dos outros tipos celulares que podem gerar nucleotídeos guanina por duas vias distintas, a via de síntese “de novo” e a via de recuperação, que permite a reciclagem das purinas (TAYLOR; WATSON; BRADLEY, 2005).

O MMF além de agir na proliferação dos linfócitos, também diminui a expressão de glicoproteínas e moléculas de adesão, responsáveis pelo recrutamento de monócitos e linfócitos para os locais de inflamação, como ocorre durante a rejeição do enxerto (STAATZ; TETT, 2014). Este medicamento causa desconfortos gastrointestinais (diarreia e vômitos) e hematotoxicidade (leucopenia (baixa quantidade de leucócitos), anemia ou trombocitopenia (baixa quantidade de plaquetas)) (STAATZ; TETT, 2014).

2.2 FARMACOGENÉTICA DOS IMUNOSSUPRESSORES E POLIMORFISMOS GENÉTICOS

O desenvolvimento da biotecnologia a partir das informações obtidas do sequenciamento do genoma humano permitiu identificar alguns genes que definem a resposta individual aos medicamentos, bem como a eficácia e segurança das drogas (BETONICO et

al., 2008).

Polimorfismos de nucleotídeo único (SNPs) em genes de bomba de efluxo de drogas (P-glicoproteína) e do metabolismo de drogas podem influenciar a resposta a medicamentos imunossupressores, resultando em uma ausência de correlação entre a dose, as concentrações plasmáticas e a resposta terapêutica aos medicamentos (HESSELINK et al., 2003; ZUNUNI VAHED et al., 2015). Assim, mesmo sendo administradas doses idênticas aos pacientes estes podem apresentar concentrações plasmáticas extremamente diferentes o que resulta em diferentes respostas ao tratamento.

Após a realização do transplante são recomendadas doses iniciais de imunossupressor; no entanto, alguns pacientes não alcançam as concentrações desejadas destes medicamentos. Os pacientes que metabolizam as drogas de maneira mais rápida eliminam rapidamente os medicamentos do organismo, o que resulta em baixas concentrações plasmáticas, podendo desencadear um processo de rejeição. Ao contrário, metabolizadores lentos podem apresentar concentrações supraterapêuticas (ELENS et al., 2011), resultando no aumento de efeitos adversos como hipertensão, nefrotoxicidade, neurotoxicidade, hipercolesterolemia, diabetes *mellitus* e câncer (PONTICELLI, 2005; FRAILE et al., 2009). Dentre os efeitos adversos observados nestes pacientes o mais preocupante é a alta incidência de neoplasias, que tem sido estimada em 20% após 10 anos de imunossupressão crônica (KAPOOR, 2008) e de 50% após 30 anos (WISGERHOF et al., 2011).

Como uma tentativa de minimizar estes efeitos adversos e obter maior sucesso no transplante, é realizado o monitoramento das concentrações plasmáticas dos medicamentos CNIs, que apresentam grande variabilidade farmacocinética e possuem estreita janela terapêutica, buscando a individualização da imunoterapia (ZHENG et al., 2003; ANTIGNAC et al., 2007). A biodisponibilidade destes medicamentos pode váriar entre os diferentes grupos étnicos já que estes podem apresentar diferentes frequências alélicas em SNPs presentes em genes importantes do metabolismo e transporte de drogas (RAMAMOORTHY et al., 2015). Segundo Neylan (1998), a média de tacrolimo administrada aos pacientes africanos chega a ser 37% maior que a administrada aos pacientes caucasianos para alcançarem as concentrações plasmáticas desejadas. Assim, devido à alta miscigenação da população brasileira, o monitoramento das concentrações plasmáticas nesta população é de extrema importância.

Avanços nos estudos moleculares permitirão a construção de um painel de marcadores para serem testados nos pacientes antes do início do tratamento imunossupressor, o que irá contribuir para a escolha de concentrações mais adequadas destes medicamentos

para cada paciente, diminuindo os efeitos adversos e possibilitando a predição do risco individual para o desenvolvimento de episódios de rejeição e, consequentemente, melhorando a qualidade de vida (FISCHEREDER et al., 2001; KAPOOR, 2008, WAVAMUNNO; CHAPMAN, 2008).

Entre tais estudos destacam-se os genômicos, que buscam novos biomarcadores candidatos em genes responsáveis pelo metabolismo de drogas, que têm papel na ativação e detoxificação, aumentando suas solubilidades e facilitando a excreção, e cujas variantes polimórficas influenciam na resposta farmacológica (BETONICO et al., 2008; GRINYÓ et al., 2008). Genes relacionados ao sistema imunológico também têm sido investigados pois estão diretamente relacionados com a ocorrência de episódios de rejeição, assim como genes de reparo que podem estar relacionados com a maior incidência de câncer observada após longos períodos de transplante.

2.2.1 Genes de resposta inflamatória

O papel da resposta inflamatória é combater infecções e lesões teciduais por meio das células da imunidade inata (fibroblastos, macrófagos, mastócitos, células dendríticas e leucócitos) que reconhecem a invasão do patógeno, desencadeando cascatas de sinalização que levam à liberação de fatores que promovem o recrutamento de leucócitos para a região (NEWTON; DIXIT, 2012). No entanto, algumas vezes o organismo do receptor reconhece o enxerto como um corpo estranho e desencadeia um processo de rejeição, durante o qual ocorre o recrutamento de células citotóxicas que podem causar a destruição do tecido (SPIVEY et al., 2011).

A interação entre os receptores de superfície celular e células do sistema imune (linfócitos ativados, fagócitos e leucócitos) é imunomodulada por citocinas, que participam da resposta imune inata e adaptativa (ALBERTS et al., 2010). A produção de citocinas pode ser alterada devido à presença de SNPs, principalmente na região promotora dos genes, afetando direta ou indiretamente a ligação de fatores de transcrição e aumentando ou diminuindo a produção de mRNA (SUTHANTHIRAN, 2000).

A resposta imune adaptativa é determinada pela subclasse de células T auxiliares efetoras tipo 1 (Th1) e tipo 2 (Th2), distinguidas pelas citocinas que secretam. As células Th1 secretam interferon gama (IFN-γ), fator de necrose tumoral (TNF-α) e interleucina-2 (IL-2), que são mediadores da resposta imune celular e pro-inflamatória, ativando macrófagos e células T citotóxicas, defendendo o organismo principalmente contra patógenos intracelulares (ALBERTS et al., 2010).

O TNF- α secretado pelas células Th1 participa do processo inflamatório por meio da ativação de células endoteliais, regulação de moléculas de adesão e recrutamento de leucócitos diferenciados (STOJANOVA; POUCHÉ; PICARD, 2016). A presença do SNP rs1800629 (-308G>A) na região promotora do gene *TNF-α* foi associada com alteração na produção desta proteína, sendo que a presença do alelo A aumenta a transcrição deste gene em comparação com o alelo G (WILSON et al., 1997). A presença desta variante polimórfica aumentou em 2,5 vezes o risco de rejeição aguda do enxerto renal (PAWLIK et al., 2005). Outros estudos também associaram este SNP com a maior incidência de rejeição (WILSON et al., 1993; SANKARAN et al., 1999). No entanto, estes dados são contraditórios na literatura, já que alguns estudos não confirmam esta associação (CARTWRIGHT et al., 2001; LEE et al., 2004).

A IL-2 também participa da resposta imune celular e pró-inflamatória, atuando como imunomoduladora em uma variedade de células imunes, sendo a mais importante estimuladora da expansão clonal dos linfócitos T efetores, participando também da eliminação de linfócitos T auto reativos (SEDER; PAUL, 1994). Sadlack et al. (1995) demonstraram a importância da IL-2 para o sistema imune, mostrando que camundongos deficientes para esta proteína desenvolveram síndrome linfoproliferativa letal após cinco semanas de vida, com lesões inflamatórias e presença de auto-anticorpos, indicando doença autoimune generalizada.

O SNP rs2069762 (-330G>T) do gene *IL-2* foi associado com mudanças na produção da citocina, resultando em um aumento de três vezes na produção desta proteína em comparação com indivíduos que possuem o genótipo T/T (HOFFMANN et al., 2001). Neste SNP, o alelo G tem sido relacionado com severidade das rejeições em transplante de células hematopoéticas. Estudo realizado por Harkensee et al. (2012) demonstrou que pacientes portadores do genótipo G/T possuem maior risco de desenvolverem rejeição crônica enquanto que o genotipo T/T foi associado com proteção contra rejeição.

Pawlik et al. (2005) não encontraram associação entre o rs2069762 na região promotora do gene *IL-2* com o risco de rejeição aguda do enxerto em um grupo de 129 pacientes transplantados renais, resultados estes confirmados por Grinyó et al. (2008) em estudo com 237 pacientes transplantados renais.

Outras interleucinas como IL-4, IL-5, IL-6, IL-10, são produzidas pelas células Th2 e atuam na resposta humoral, participam da defesa do organismo principalmente contra patógenos extracelulares estimulando a produção de anticorpos (ALBERTS et al., 2010). Dentre estas interleucinas, a IL-10 é frequentemente descrita como um regulador positivo durante a rejeição aguda do transplante de rim e fígado, produzindo efeitos pró-inflamatórios,

reforçando a liberação de IFN-g, TNF-α e IL-1, podendo ativar células natural killer (NK) (SPIVEY et al., 2011).

Trabalho realizado por Grinyó et al. (2008) avaliou a associação da rejeição aguda com 21 variantes polimórficas em 237 pacientes transplantados renais, encontrando associação entre os episódios de rejeição e o SNP rs1800872 do gene *IL-10*, onde pacientes homozigotos A/A apresentaram uma chance cinco vezes maior de desenvolver rejeição.

Além dos genes que transcrevem citocinas, genes responsáveis pela transcrição de fatores de crescimento, reguladores e fatores de transcrição também exercem importante papel na resposta imune, entre eles: *TGF-β* (fator de crescimento transformante-Beta), *IRF-5* (fator regulador de interfeton-5), *NFKB1A* (inibidor do fator de transcrição nuclear Kappa Beta 1 Alfa) e *NFAT* (fator nuclear de células T ativadas).

TGF-β age como regulador do desenvolvimento de células T no timo, assegurando a homeostase das células T periféricas e a tolerância para auto-antígenos; age também na diferenciação das células T durante a resposta imune (BLOBE; SCHIEMANN; LODISH, 2000; LI; FLAVELL, 2008). Os SNPs rs1800470 (29T>C) e rs1800471 (915G>C) influenciam a expressão deste gene e têm sido associados com episódios de rejeição entre os receptores de órgãos sólidos (GE et al., 2014). Benza et al. (2009) observaram que pacientes transplantados cardíacos que possuíam o genótipo G/G para o SNP rs1800471 do gene *TGF-β1* tiveram menor risco de rejeição em comparação com os pacientes com genótipo C/C ou G/C durante o primeiro ano após o transplante.

A proteína transcrita pelo gene *IRF-5* desempenha importante papel na defesa do hospedeiro, estimulando a resposta inflamatória através da transcrição de citocinas inflamatórias como IL-6 e TNF-α (CHEN; ROYER, 2010). Yu et al. (2014) observaram associação entre o SNP rs3757385 deste gene com episódios de rejeição aguda (OR = 2,34) em 289 pacientes transplantados de fígado durante os seis primeiros meses.

O gene *NFKB1A* codifica a proteína IKBα, que inibe o fator de transcrição nuclear kappa β (NF-KB), pois se liga a ele e o mantém inativo no citoplasma. Assim, *NFKB1A* desempenha um papel importante na imunidade inflamatória (YENMIS et al., 2015). Kramer et al. (2014) avaliaram em pacientes transplantados hepáticos os SNPs rs696 (126G/A), rs2233409 (-297C/T) e rs2233406 (-826C/T) do gene *NFKB1A*, sendo observada associação do genótipo A/A do SNP rs696 com a ocorrência de rejeição aguda. Segundo Yenmis et al. (2015) este SNP encontra-se na região não traduzida UTR 3’ e altera a regulação da proteína IKBα.

A proteína transcrita pelo gene *NFAT* participa da regulação da atividade

imunológica e da produção de insulina. Assim, os medicamentos imunossupressores inibidores de calcineurina que bloqueiam a atividade da NFAT, previnem episódios de rejeição e reduzem a capacidade de produção de insulina, contribuindo para o desenvolvimento precoce de diabetes *mellitus* (LAWRENCE et al., 2015). O diabetes *mellitus* é um dos efeitos adversos com alta incidência após o transplante renal em adultos, variando de 3-46% (AL-UZRI; STABLEIN; COHN, 2001).

Segundo Chen et al. (2012), estudos avaliando SNPs na família gênica *NFAT* são escassos. Estes autores avaliaram o SNP rs10141896 do gene *NFAT* em 319 pacientes transplantados renais (40,5% desenvolveram diabetes após um ano de transplante), e observaram uma associação entre a presença do alelo T com uma incidência 2,5% menor de desenvolver diabetes após o transplante.

As quimiocinas e seus receptores têm sido vistos como promissores alvos para drogas anti-inflamatórias, dirigidas contra a migração e ativação de populações selecionadas de células inflamatórias (SALLUSTO; BAGGIOLINI, 2008). Entre os receptores de quimiocinas encontram-se o receptor de interleucina-23 (IL-23R) e o receptor de quimiocina (CCR5), que participam do processo inflamatório mediado por células T auxiliares.

O IL-23R tem papel importante na inflamação auto-imune e na tumorigênese; este receptor pode diminuir a imunovigilância de CD8+, facilitando a evasão de células cancerosas do sistema imune (ZHOU et al., 2013). Foi identificado um SNP funcional rs10889677 na região não traduzida 3'-UTR deste gene. A presença do alelo A interrompe o sítio de ligação do microRNA let-7f, aumentando a transcrição de *IL-23R*; o alelo C, por sua vez, aumenta a afinidade de ligação do microRNA let-7f, regulando negativamente a expressão de *IL-23R*. Portadores do alelo C possuem menor risco de desenvolver câncer de mama, pulmão e nasofaringe em comparação com portadores do alelo A (ZHENG et al., 2012; ZHOU et al., 2013).

O receptor CCR5 é expresso principalmente na superfície de linfócitos Th1 ativados e de memória, em monócitos, macrófagos e células dendríticas. CCR5 pertence à superfamília de receptores de sinalização intracelular acoplados à proteínas G, com sete domínios transmembrana (ROSSI; ZLOTNIK, 2000). Este receptor, quando ativado, leva a um aumento na produção de interferon (IFN), amplificando os estímulos inflamatórios e a liberação de quimiocinas (SPIVEY et al., 2011).

A variante alélica mais conhecida do gene *CCR5*, a deleção *CCR5*Δ32 (rs333), gera uma proteína truncada, que não chega à superfície celular, alterando a resposta destas células durante a inflamação e diminuindo a taxa de rejeição. A análise desta deleção em 1227

pacientes transplantados em centros europeus mostrou que 1,7% dos pacientes eram homozigotos para a mutação *CCR5*Δ32 e 20,2% eram heterozigotos. Os pacientes homozigotos para a deleção demonstraram uma associação com sobrevida do enxerto 2,7 vezes maior, não apresentando nenhum efeito deletério aparente, o que sugere que *CCR5* pode ser um alvo terapêutico ideal para futuras investigações para prevenir a perda do transplante (FISCHEREDER et al., 2001).

Estes SNPs em genes das citocinas e em seus receptores são de grande importância para o resultado do transplante de órgãos, pois contribuem com a resposta imune, sendo provável que sutis diferenças na composição destas, principalmente no início da resposta imune, possam determinar o resultado do transplante (AWAD et al., 2001; SEYHUN et al., 2012). Além de influenciar na atividade do sistema imunológico, estes SNPs também podem modificar a suscetibilidade ao desenvolvimento de câncer (ZHENG et al., 2012), um dos efeitos adversos observados após longos períodos de imunossupressão.

2.2.2 Metabolismo dos fármacos

O metabolismo dos fármacos é classificado em reações de fase I e II. As reações de fase I resultam, na maioria das vezes, na perda da atividade farmacológica. No entanto, em alguns casos, pró-fármacos inicialmente inativos são convertidos em metabólitos biologicamente ativos. As reações de fase II resultam na formação de uma ligação covalente entre um grupo funcional do composto com o ácido glicurônico, sulfato, glutationa, aminoacidos ou acetato, formando um conjugado inativo altamente polar, que é rapidamente excretado pela urina ou bile (BUXTON; BENET, 2012) (Figura 2).

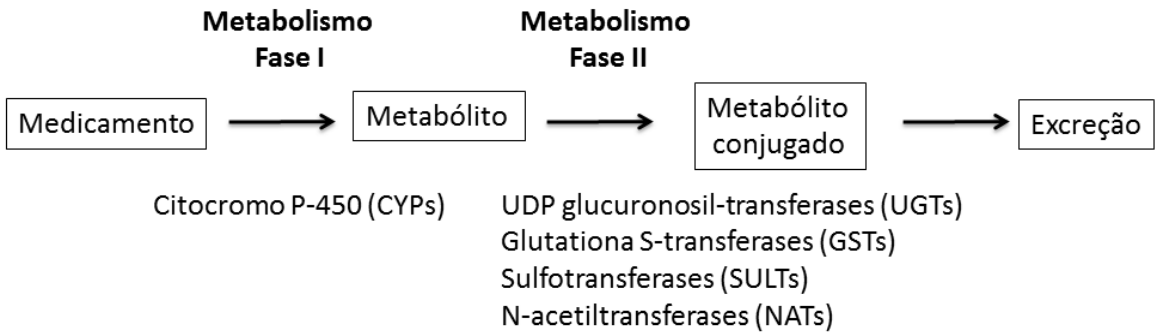

Figura 2: Metabolismos de Fase I e II e principais enzimas envolvidas.

A capacidade geneticamente determinada de metabolizar e eliminar os medicamentos do organismo de forma eficiente pode influenciar, pelo menos em parte, a chance do paciente

apresentar episódios de rejeição do enxerto. Pacientes com metabolismo mais rápido podem ser tratados com doses inadequadas e desencadearem episódios de rejeição, enquanto lentos metabolizadores podem acumular altas concentrações dos medicamentos desencadeando efeitos adversos (NUSSBAUM; MCINNES; WILLARD, 2007).

As enzimas da família do citocromo P450 (CYP) participam do metabolismo de fase I e parecem estar diretamente relacionadas ao processo de ativação de grande parte dos xenobióticos. A subfamília CYP3A é responsável pelo metabolismo hepático de mais de 50% das drogas, sendo as enzimas CYP3A4 e CYP3A5 as mais importantes e abundantes (KUEHL et al., 2001).

O gene *CYP3A5* possui vários SNPs que podem interferir em sua expressão resultando em diferentes respostas interindividuais na eliminação de drogas (KUEHL et al., 2001). Pacientes portadores do alelo prevalente *CYP3A5*1*, apresentam função normal do gene, eliminam o medicamento tacrolimo mais eficientemente e tendem a manter menores concentrações plasmáticas.

Quando comparados com o alelo prevalente *CYP3A5**1, o SNP *CYP3A5**7 (rs41303343) tem função nula enquanto os SNPs *CYP3A5**3 (rs776746 - 6986A>G), *5 (12952T>C), *6 (rs10264272 – 10264272G>A) e *10 (rs41279854 – 29748T>C) apresentam diminuição da função proteica (LEE; GOLDSTEIN, 2005), sendo o SNP rs776746 o mais conhecido (PARK et al., 2008).

A presença do genótipo *CYP3A5**3/*3 causa um defeito no *splicing*, que reduz significativamente a quantidade de mRNA, tendo como consequência o aumento das concentrações plasmáticas dos inibidores de calcineurina (XIE et al., 2004; TADA et al., 2005; STAATZ; GOODMAN; TETT, 2010) e, consequentemente, diminuição da rejeição e aumento da incidência de efeitos adversos. Estudos demonstraram que pacientes transplantados portadores deste genótipo podem atingir concentrações plasmáticas de tacrolimo cerca de duas vezes maiores que pacientes com o genótipo *CYP3A5*1/*1* ou **1/*3* (MACPHEE et al., 2005). A associação do SNP rs776746 deste gene com as concentrações plasmáticas do tacrolimo fornece suporte para a individualização da dosagem e consequente diminuição dos efeitos adversos (ZHENG et al., 2003; MACPHEE et al., 2005; TADA et al., 2005).

A expressão do gene *CYP3A4* também é influenciada pela presença de alguns SNPs. Wang et al. (2011) identificaram que o alelo *CYP3A4**22 (rs35599367) afeta a expressão e a atividade hepática da enzima CYP3A4, pois portadores dos genótipos CT e TT apresentaram uma redução de atividade enzimática de, respectivamente, 1,7 e 2,5 vezes em comparação ao

genótipo CC, sugerindo que este genótipo poderia servir como um biomarcador para prever respostas a drogas metabolizadas pela enzima CYP3A4.

A avaliação da variante alélica *CYP3A4**22 associada com *CYP3A5**3 (rs776746) em 185 pacientes transplantados, revelou que pacientes portadores das duas variações são pobres metabolizadores e requerem menores doses de tacrolimo para alcançarem a concentração plasmática desejada. Estes pacientes apresentaram concentrações plasmáticas de tacrolimo 179,3% maiores do que os pacientes portadores dos alelos prevalentes, apresentando concentrações supra terapêuticas (ELENS et al., 2011). Esta mesma variante foi avaliada em 113 pacientes transplantados, por Woillard et al. (2013), demonstrando que este SNP reduziu a taxa de metabolização do sirolimo em 20%.

Outro SNP do gene *CYP3A4* que pode afetar a variabilidade individual no metabolismo do tacrolimo entre pacientes transplantados renais é o rs4646437. Logo após o transplante, pacientes com o genótipo T/T mantêm menores concentrações plasmáticas de tacrolimo em relação aos pacientes com genótipo prevalente (LI et al., 2014).

A enzima CYP2E1 é expressa principalmente no fígado, participando do metabolismo de uma grande variedade de produtos químicos, em especial compostos de baixo peso molecular como alguns fármacos, etanol e acetona (TANAKA; TERADA; MISAWA, 2000).

Estudo realizado por Kim et al. (2014) encontraram associação entre o SNP rs2515641 do gene *CYP2E1* com rejeição aguda do enxerto em pacientes transplantados renais coreanos, sugerindo que este SNP pode estar relacionado com a ocorrência de rejeição nesta população.

Outro SNP rs3813867 do gene *CYP2E1* está localizado na região promotora, e pode afetar a atividade de transcrição deste gene, sendo responsável pelo aumento da atividade enzimática. A presença desta variante foi associada com a diminuição do risco de câncer de pulmão na população asiática (SHEN et al., 2015).

O gene *POR* (citocromo P450 oxidorredutase) transfere elétrons do NADPH para todas as enzimas do citocromo P450 microssomal (CYP). Este gene codifica uma flavoproteína com 78 kDa que se liga à membrana do retículo endoplasmático e possui dois domínios de ligação, um dinucleotídeo de flavina-adenina (FAD) e um mononucleotídeo de flavina (FMN) que lhe permitem doar elétrons, necessários para a atividade das CYPs (HUBBARD et al., 2001) (Figura 3).

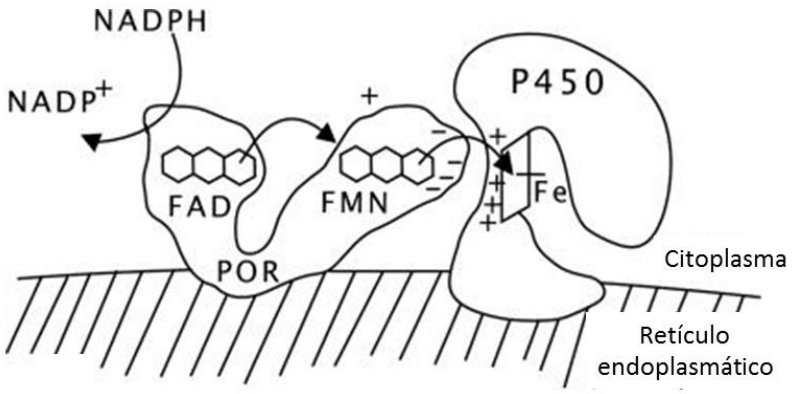

Figura 3: Relação da proteína POR com a enzima citocromo P450. POR contém duas flavinas em dois lóbulos distintos, uma dinucleotídeo adenina flavina (FAD) e uma mononucleotídeo flavina (FMN). Eletróns reduzidos de nicotinamida adenina dinucleotídeo fosfato (NADPH) são ligados à porção FAD e transferidos para a porção FMN, em seguida o eletrón passa para o citocromo P450 por interações eletrostáticas da ligação, permitindo a catálise. Fonte: Modificado de HUANG et al., 2008.

Estudo com camundongos nocautes para o gene *Por* demonstrou que este gene é essencial para as funções celulares normais e/ou embriogênese, pois os animais morreram durante o desenvolvimento embrionário (SHEN; O'LEARY; KASPER, 2002). Camundongos nocautes específicos para a enzima Por presente no fígado possuem o metabolismo hepático severamente prejudicado (HENDERSON et al., 2003).

Já foram identificados 43 SNPs neste gene, sendo o SNP rs1057868 (*POR*28*) o único que parece exercer efeito sobre a atividade das isoformas CYP, sendo encontrado em alta frequência alélica em diferentes populações: 19,1% nos afro-americanos, 26,4% nos caucasianos, 36,7% nos americanos chineses e 31% nos americanos mexicanos. Este SNP induz a substituição de um aminoácido alanina por valina, o que influencia a porção de ligação do elétron, reduzindo assim, a atividade da proteína (HUANG et al., 2008). O genótipo T/T deste SNP foi associado com o aumento de 1,6 vezes na atividade da enzima codificada pelo gene *CYP3A* em comparação com indivíduos portadores do genótipo C/C (ONEDA et al., 2009).

Zhang et al. (2013) demonstraram que o gene *POR**28 pode influenciar a variabilidade da resposta interindividual ao tacrolimo, pois indivíduos portadores do alelo *POR**28 T apresentaram menores concentrações de tacrolimo em comparação com os portadores do alelo *POR**28 C, sugerindo que este alelo T possa aumentar a atividade das enzimas codificadas pelos genes *CYPs*.

A avaliação conjunta dos SNPs *CYP3A5* e *POR**28 em 43 crianças transplantadas renais 14 dias após o transplante, demonstrou que indivíduos que expressam *CYP3A5* com pelo menos um alelo *POR**28 apresentaram concentrações 20,2% mais baixas de tacrolimo

quando comparadas com indivíduos que expressam *CYP3A5* portadores do genótipo *POR**1/*1. Em indivíduos que não expressam *CYP3A5* não foram observadas diferenças na concentração de tacrolimo em relação ao genótipo do gene *POR* (GIJSEN et al., 2014).

O pro-fármaco MMF, quando administrado via oral, é rapidamente ativado por esterases, sendo as carboxilesterases 1 e 2 (CES1 e CES2) as principais enzimas que catalisam a hidrólise deste medicamento, transformando o MMF em MPA. Estas enzimas são encontradas no fígado, e apenas a CES2 é encontrada no intestino (FUJIYAMA et al., 2010). Segundo Fujiyama et al. (2010), a atividade destas enzimas pode ajudar a explicar a variabilidade interindividual observada pelo uso do MMF, uma vez que já foram descritos diferentes SNPs nestes genes (PICARD; MARQUET, 2012).

A ativação deste pró-fármaco depende da enzima CES1 para produzir um grupo terapêutico funcional, assim, variações neste gene poderiam dificultar a ativação da pró-droga, resultando em alterações no efeito terapêutico. O SNP rs71647871, conduz à substituição não conservativa (Gly143Glu), reduzindo a atividade da enzima. Esta variação foi observada em uma frequência alélica de 3,7 % em brancos, 4,3 % em negros e 2,0 % em população hispânica (ZHU et al., 2008; WALTER SORIA et al., 2010). No entanto, Lewis et al. (2013) identificaram uma frequência alélica de 0,6% deste SNP em população caucasiana, frequência menor do que as observadas pelos autores acimas citados.

Fujiyama et al. (2009), após avaliarem a relação entre os SNPs rs2303218 (4595A>G), rs2241409 (8721C>T) e rs3890213 (-1548A>G) do gene *CES2* e a farmacocinética do ácido micofenólico em 80 pacientes transplantados renais após 28 dias de transplante, concluíram que estas variantes polimórficas não afetaram as concentrações plasmáticas do MMF, sugerindo que o gene *CES2* tem uma pequena contribuição nas diferenças interindividuais quanto à farmacocinética do MPA.

A atividade imunossupressora do MPA é atribuída à inibição das isoformas IMPDH1 e IMPDH2, enzimas limitantes da síntese de purinas “de novo”, levando à redução do crescimento celular, principalmente dos linfócitos que são desprovidos da via de salvamento de purina, não ocorrendo, assim, a reciclagem de nucleotídeos guanina. Portanto, a proliferação dos linfócitos depende da atividade da proteína IMPDH (ALLISON; EUGUI, 2000; PICARD; MARQUET, 2012).

Gensburger et al. (2010) analisaram a associação entre quatro SNPs do gene *IMPDH2* e os SNPs rs2278293 e rs2278294 do gene *IMPDH1* com a rejeição aguda em 546 pacientes transplantados renais e encontraram associação apenas do SNP rs2278294 com o menor risco de rejeição aguda. Estudo realizado por Wang et al. (2008) encontrou associação

entre os SNPs rs2278294 e rs2278293 com baixa incidência de rejeição aguda em 191 pacientes transplantados renais.

Kagaya et al. (2010) observaram que pacientes transplantados que possuíam o genótipo G/G do SNP rs2278293 do gene *IMPDH1* eram mais propensos a desenvolver rejeição aguda e os pacientes que apresentavam o genótipo A/A possuíam um risco menor devido à maior eficácia do MMF.

Picard e Marquet (2012) avaliaram o SNP rs11706052 do gene *IMPDH2* em 237 pacientes transplantados renais e observaram que os pacientes portadores de um ou dois alelos C apresentaram probabilidade três vezes maior de sofrer rejeição quando comparados com pacientes homozigotos para o alelo T.

Dentre as enzimas de conjugação do metabolismo de fase II destacam-se as UDP-glucuronosiltransferases (UGTs) que fazem parte da superfamília de genes dividida entre *UGT1A*, *UGT2A* e *UGT2B*. Essas enzimas realizam a biotransformação de compostos lipofílicos em metabólitos solúveis em água, o que facilita a subsequente eliminação dos xenobióticos do organismo por meio da bile ou urina. O processo ocorre por meio da ligação do ácido glicurônico ao xenobiótico, reação esta catalisada pelas enzimas UGTs (TUKEY; STRASSBURG, 2000; INNOCENTI et al., 2008).

Os membros da subfamília *UGT1A* são codificados por um único locus gênico com aproximadamente 160 kb que codifica nove proteínas funcionais: UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9 e UGT1A10. Estas proteínas são sintetizadas a partir da transcrição de cinco éxons, sendo um éxon variável, uma vez que apenas um dos 13 diferentes éxons localizados na região N-terminal é associado com quatro exons na região C-terminal, comum para todas as isoformas de *UGT1A* (Figura 4) (TUKEY; STRASSBURG, 2000).

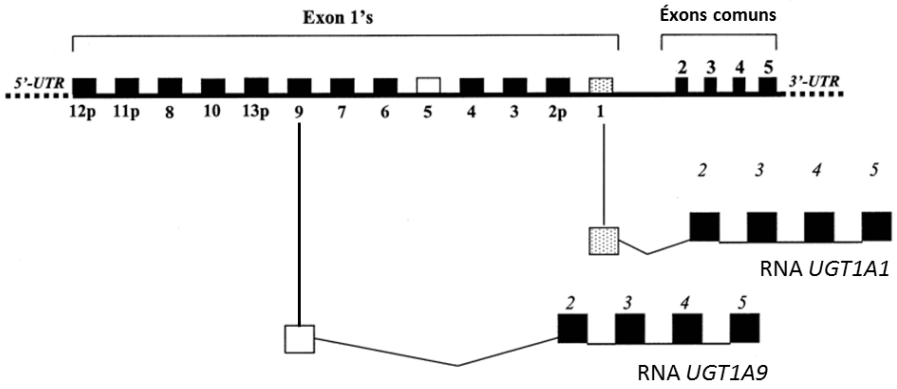

Figura 4: Estrutura da família *UGT1A*. Organização genômica dos trezes genes UGTs e processamento dos genes *UGT1A1* e *UGT1A9*. Fonte: modificado de INNOCENT; RATAIN, 2004.

O gene *UGT1A9* tem importante papel na detoxificação de vários xenobióticos, sendo responsável pela inativação do metabólito MPA, que é transformado em ácido micofenólico glucuronídeo (MPAG) pelo fígado, rim, e na mucosa intestinal em uma taxa de 55%, 75%, e 50%, respectivamente. Parte do MPAG é excretado pela bile e contribui para a recirculação entero-hepática do MPA (PICARD et al., 2004).

A expressão e atividade do produto do gene *UGT1A9* depende da presença de SNPs localizados na região promotora. Girard et al. (2004) avaliaram a relação entre genótipo e fenótipo em 48 frações microssomais de fígado humano. Estes autores descobriram 10 SNPs na região promotora do gene *UGT1A9*, sendo que 5 deles (-275, -331/-440, -665 e -2152) foram associados com maior concentração da proteína UGT1A9. O SNP rs6714486 (-275T>A), além de ser associado ao aumento de 1,4 vezes no nível hepático da proteína, também aumentou em 1,9 vezes a glucuronidação do MPA. O aumento da expressão e atividade da enzima UGT1A9, uma das principais enzimas envolvidas no metabolismo do MPA, reduz a concentração de MPA e, consequentemente, reduz a imunossupressão induzida pelo mesmo (PICARD et al., 2004), facilitando a ocorrência de episódios de rejeição.

Pazik et al. (2011) observaram que pacientes portadores das variantes alélicas -275A (rs6714486) e -2152T (rs17868320) tendem a desenvolver rejeição aguda durante os 3 primeiros meses de transplante. Pacientes portadores da variante polimórfica rs6714486 apresentam fator de risco de 4,4 vezes, enquanto que os portadores do SNP rs17868320 apresentaram fator de risco de 3,62 vezes. Van Schaik et al. (2009) demonstraram que pacientes transplantados que possuem as duas variantes alélicas (-275A e -2152T) foram associados com reduzida exposição ao MPA e um aumento de risco de 13 vezes de desenvolver rejeição aguda.

Enquanto os SNPs rs17868320 (-2152C>T) e rs6714486 (-275T>A) induzem a expressão do gene *UGT1A9*, o polimorfismo rs72551330 foi associado com a diminuição da atividade da enzima (GIRARD et al., 2004). Pazik et al. (2011) não encontraram associação deste polimorfismo com episódios de rejeição, no entanto, este polimorfismo foi associado com a diminuição da taxa de filtração glomerular estimada (eGFR) durante o primeiro ano após o transplante. Este efeito na função renal pode ter sido consequência da baixa taxa de detoxificação do MPA, levando a uma exposição tóxica ao medicamento.

Dentre os genes da família *UGT2B*, o gene *UGT2B7* (UDP glucuronosil transferase família 2, polipeptídeo B7) é altamente expresso no fígado (RADOMINSKA-PANDYA et al., 2002) codificando uma enzima que participa do metabolismo de diversos medicamentos e

xenobióticos, dentre eles alguns medicamentos imunossupressores, como a ciclosporina e o tacrolimo (STRASSBURG et al., 2001). A proteína também metaboliza o MMF, sendo a responsável por transformar o metabólico ativo, o ácido micofenólico (MPA), no metabótilo tóxico MPA-acil-glucuronido (AcMPAG) sem função de imunossupressão (PICARD et al., 2004).

São conhecidos três haplótipos do gene *UGT2B7* (Figura 5), sendo que o polimorfismo rs7438135 (-842A/G / -900A/G) encontra-se em desequilíbrio de ligação completo com a maioria dos polimorfismos únicos conhecidos na região promotora, fazendo parte dos haplótipos B e C (Figura 5) (HOLTHE et al., 2003). Este polimorfismo também encontra-se em equilíbrio de ligação inverso com o polimorfismos no exon 2 *UGT2B7**2 (802C/T) (DJEBLI et al., 2007).

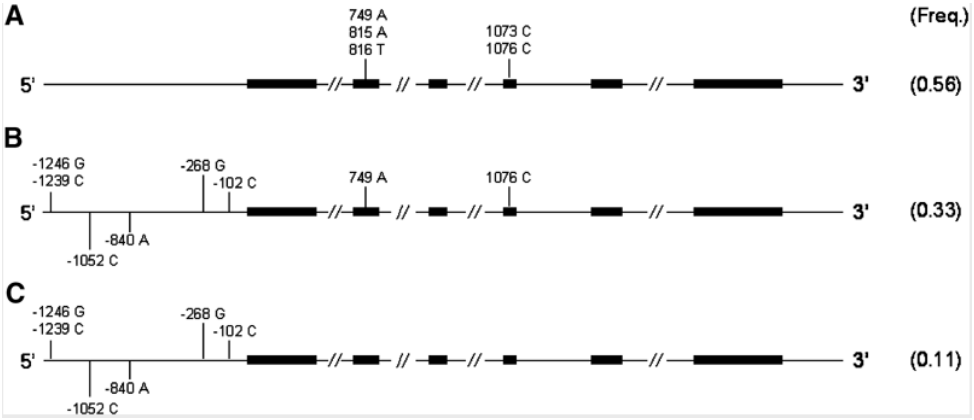

Figura 5: Três principais haplótipos do gene *UGT2B7*, preditos a partir da genotipagem de 239 indivíduos por sequenciamento do DNA e análise com sondas de hibridização. Fonte: HOLTHE et al., 2003.

Pacientes transplantados renais homozigotos prevalentes do haplótipo B, que fazem uso concomitante de MMF, sirolimus e corticoide são mais propensos a ter concentrações elevadas de AcMPAG, estando predispostos a efeitos adversos relacionados ao MPA (DJEBLI et al., 2007).

Trabalho realizado por Duguay et al. (2004), demonstrou forte desequilíbrio de ligação entre seis variantes polimórficas na região promotora do gene *UGT2B7* (-1248, -1241, -1054, -842, -268, -102). Estes autores observaram que a presença das variantes aumentou em 2 vezes a expressão de *UGT2B7* em células modelo de fígado e intestino quando comparado com o promotor do tipo prevalente, e sugeriram que o aumento da atividade transcricional é resultante do polimorfismo -842.

Os metabólitos AcMPAG e MPAG são excretados pela urina e pela bile. A excreção pela bile é realizada por proteínas de resistência a multidrogas 2 (MDR2) presentes na superfície dos hepatócitos, sendo que parte do MPAG sofre desconjugação por bactérias intestinais, volta para sua forma ativa e contribui para a recirculação entero-hepática do MPA (Figura 6) (PICARD et al., 2004; PICARD; MARQUET, 2012).

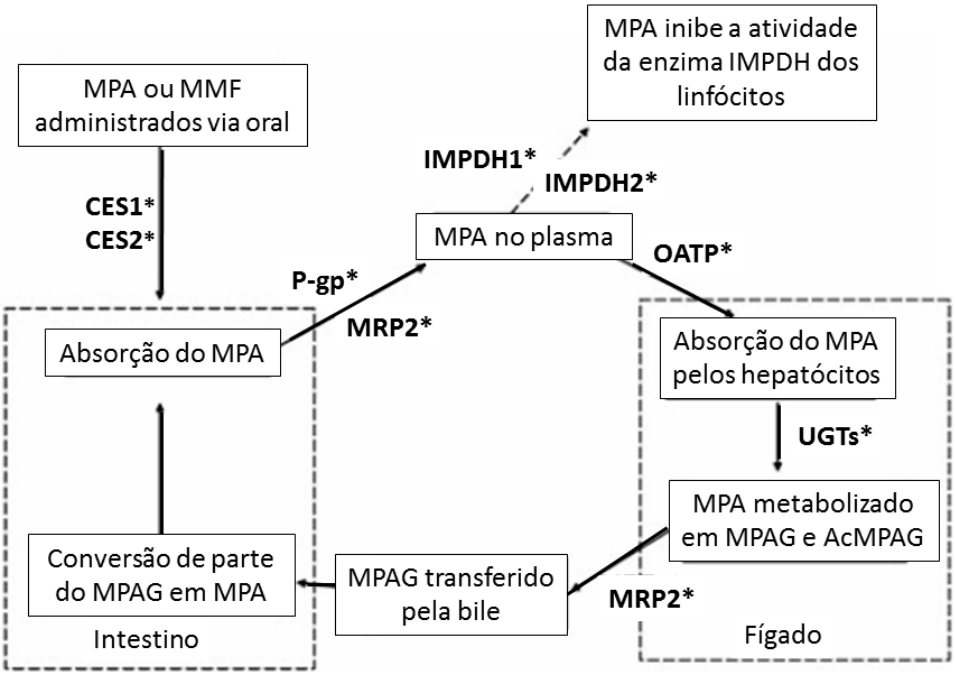

Figura 6: Metabolismo e recirculação entero-hepática do micofenolato de mofetil – MMF. Fonte: modificado de WANG et al., 2008. *Principais proteínas que atuam no processo.

2.2.3 Genes de Transportadores de Drogas

Em seres humanos já foram descritos 48 transportadores de membrana ABC divididos em 7 superfamílias. Estes transportadores podem translocar substrato através das membranas extra e intracelulares, atuando como bombas de efluxo de xenobióticos e metabólicos. Dentre estes transportadores estão: ABCB1 (P-glicoproteína, MDR-1), ABCC2 (MRP2) e ABCG2, que utilizam ATP para mover substratos através das membranas (HAUFROID, 2011). Estas proteínas são essenciais para a absorção de drogas, biodisponibilidade, eficácia, toxicidade e depuração (SISSUNG et al., 2014), e polimorfismos nestes genes podem ter impacto na farmacocinética de drogas, podendo ter importantes implicações clínicas principalmente em drogas com estreita janela terapêutica (HAUFROID, 2011).

Os membros desta família de transportadores estão envolvidos na distribuição e eliminação de algumas drogas imunossupressoras. Assim, polimorfismos nestes genes podem

contribuir para a variabilidade interindividual na farmacocinética e resposta do medicamento imunossupressor MMF, podendo desencadear o processo de rejeição (SHUKER et al., 2012).

O gene *ABCB1(ATP-binding cassette, sub-family B (MDR/TAP), member 1)* codifica uma P-glicoproteína que atua como transportador transmembrana presente no fígado, rim e intestino. Este transportador foi descrito pela primeira vez em células tumorais resistentes a várias drogas antineoplásicas, por isso ficou conhecido como proteína de resistência a múltiplas drogas (MDR-1) (HAUFROID, 2011).

O nível de expressão e função destas P-glicoproteínas afeta diretamente a concentração plasmática de drogas e assim determina a resposta terapêutica (KROETZ et al., 2003). Os imunossupressores CNIs são substratos do transportador ABCB1 e a variabilidade na absorção e eliminação destes imunossupressores podem ser atribuídas à quantidade e atividade deste transportador no intestino (GRINYÓ et al., 2008).

São conhecidas 48 variantes polimórficas neste gene, sendo as variantes rs1128503 (C1236T), rs1045642 (C3435T) e rs2032582 (G2677T/A) as mais estudadas e as únicas que possuem alta frequência alélica e estão presentes em exóns. As duas primeiras resultam de mutações sinônimas enquanto a última é uma mutação "*missense*" devido à substituição do aminoácido 899 Ala>Ser/Thr (KROETZ et al., 2003). Estas três variantes encontram-se em forte desequilíbrio de ligação e foi proposto que prejudicam a expressão do gene resultando na menor atividade da bomba de efluxo ABCB1, diminuindo a capacidade de expulsar a droga dos enterócitos e elevam a concentração plasmática dos CNI (SÁNCHEZ-LÁZARO et al., 2015).

Análise da expressão gênica em amostra de fígado humano revelou que a presença do polimorfismo rs1045642 (3435T) resulta em significativa diminuição da expressão do gene *ABCB1*. Isso ocorre porque este SNP afeta a estabilidade do mRNA que é degradado mais rapidamente após sua síntese (WANG et al., 2005).

Grinyó et al. (2008) encontraram associação entre a incidência de rejeição em pacientes transplantados renais e os polimorfismos rs1128503 (T/T OR = 3,9 p = 0,009), rs2032582 (T/T OR = 4,19 p = 0,003) e rs1045642 (T/T OR = 4,19 p = 0,0099). Porém, quando o modelo foi ajustado com a interação do tratamento, apenas o polimorfismo rs2032582 continuou associado com os episódios de rejeição, sendo o alelo T associado a uma chance três vezes maior de desenvolvimento de rejeição aguda (OR = 3,16 p = 0,003).

Garcia et al. (2013) não encontraram associação entre os polimorfismos rs1128503, rs2032582 e rs1045642 e episódios de rejeição ou a farmacocinética da ciclosporina em 68 pacientes transplantados renais. Porém, quando os dados foram reanalisados em pacientes que

não expressam o gene *CYP3A5* (CYP3A5*3/*3) estes autores observaram que pacientes homozigotos T/T para o rs1045642 tinham menor concentração plasmática de CsA que os carregadores do genótipo CC.

O gene *ABCC2 (ATP-binding cassette, sub-family C (CFTR/MRP), member 2)* também codifica um transportador de membrana MRP2 que pertence à família de proteínas de resistência a múltiplos fármacos e está envolvida no transporte de substâncias para fora da célula. Este transportador é encontrado principalmente no fígado e em quantidades menores no rim e intestino (KEPPLER, 2014).

Este transportador participa da metabolização da forma ativa do imunossupressor MMF, o MPA, que é glucuronidado pelas UGTs para a formação do metabólito farmacologicamente inativo, MPAG. Este é excretado pela bile através da proteína de resistência a multidroga 2 (ABCC2) (NAESENS et al., 2006), mas parte deste MPAG participa da recirculação entero-hepática, sendo novamente convertido em MPA no trato gastrointestinal (Figura 6) (BULLINGHAM; NICHOLLS; KAMM, 1998).

Trabalho realizado por Naesens et al. (2006) avaliou sete polimorfismos do gene *ABCC2* em 95 pacientes transplantados renais que faziam uso dos medicamentos MMF, tacrolimo e corticoides. Estes autores observaram que os polimorfismos em desequilíbrio de ligação rs3740066 (3972C/T) e rs717620 (-24C/T) foram associados ao aumento da concentração plasmática do MPA, pois contribuem para a recirculação entero-hepática. Sendo os genótipos T/C e T/T do polimorfismo rs717620 associados com melhor prognóstico em pacientes transplantados cardíacos (BURCKART et al., 2014).

Ogasawara et al. (2013) demonstraram que o polimorfismo rs2273697 (1249G/A) do gene *ABCC2* também influenciou na farmacocinética do tacrolimo. Estes autores observaram que pacientes transplantados renais que possuem a variante alélica 1249 G>A, em homozigose ou heterozigose, atingiram concentrações plasmáticas de tacrolimo 1,4 vezes menor no tempo 0 (pré-dose) e concentração de 1,59 vezes menor duas horas após a administração da dose.

O gene *ABCG2 (ATP-binding cassette transporter BCRP/MXR1/ABCP)* também confere resistência a drogas anticâncer, e codifica uma proteína com função de transportador de efluxo expressa no epitélio do intestino delgado e no fígado, desempenhando importante papel na regulação da absorção de xenobióticos (TAMURA et al., 2007).

Dentre os mais de 80 polimorfismos já identificados neste gene, o polimorfismo de relevância clínica rs2231142 (421C>A), resulta na mudança de aminoácido lisina por glutamina no códon 141, exon 5, expressando metade da quantidade de proteína produzida em

relação ao genótipo prevalente (TAMURA et al., 2007). Além disso, este polimorfismo afeta a estabilidade da proteína ABCG2 no retículo endoplasmático aumentando a suscetibilidade à degradação mediada por ubiquitina proteossômica (FURUKAWA et al., 2009).

Outro gene que expressa um importante transportador é o *SLCO1B1* (membro 1B1 da família de transportadores de ânions orgânicos portadores de solutos) que codifica o transportador OATP1B1 expresso na membrana basolateral dos hepatócitos, que facilita a absorção de vários medicamentos, sendo importante para a depuração hepática. O polimorfismo rs4149056 (521T>C), também chamado de *SLCO1B1**5, resulta em uma mutação missense (Val174Ala) que prejudica a atividade deste transportador, aumentando as concentrações plasmáticas destes medicamentos devido à sua eliminação prejudicada (NIEMI, 2007).

Estudo realizado por Michelon et al. (2010) avaliou a influência de polimorfismos de genes do metabolismo sobre a resposta ao MMF em 218 pacientes transplantados renais, quanto à incidência de efeitos adversos e episódios de rejeição no primeiro ano de transplante. A variante *SLCO1B1**5 foi a única que demonstrou associação com os efeitos adversos; pacientes que possuíam o alelo prevalente (521T ou *1a) apresentaram mais efeitos adversos em comparação com os portadores da variante (571C ou *5). Experimentos *in vitro* realizados por estes mesmos autores indicaram que o MPA não é um substrato de OATP1B1, sendo que os portadores do alelo *SLCO1B1**5 têm maior absorção hepática de MPAG e AcMPAG, levando a uma reduzida reciclagem de MPA.

2.2.4 Genes de Reparo

O sistema de reparo auxilia na manutenção da integridade e estabilidade do genoma, removendo lesões do material genético causadas por fatores externos (exposição ambiental) e fatores internos (atividade metabólica). Esses sistemas eventualmente falham e algumas lesões podem causar mutações, que podem desencadear o desenvolvimento de câncer (GOODE; ULRICH; POTTER, 2002; AGNEZ-LIMA et al., 2003). Entre os mecanismos de reparo por excisão encontram-se os reparos por excisão de bases (BER) e por excisão de nucleotídeos (NER) (Figura 7).

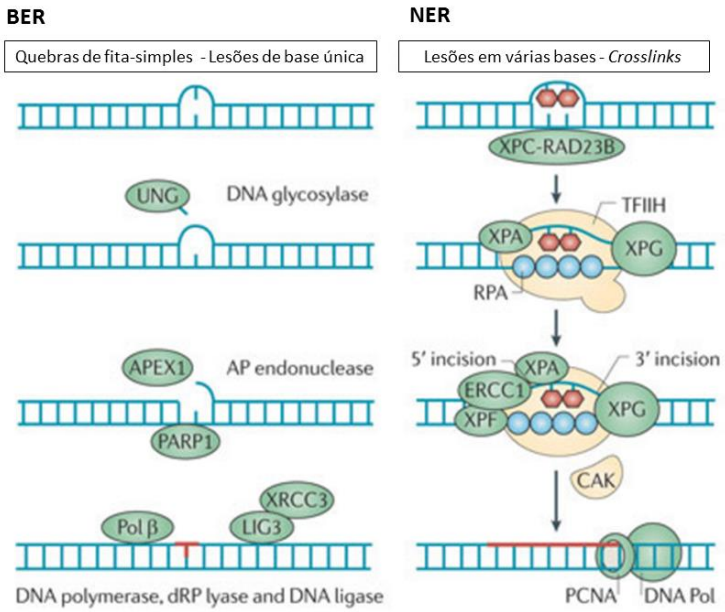

Figura 7: Vias de reparo por excisão de bases (BER) e de nucleotídeo (NER). Fonte: Modificado de LANGE; TAKATA; WOOD, 2011.

BER é o tipo de reparo mais frequente no material genético, caracterizado pela excisão de uma única base lesada. A lesão é reconhecida pelas enzimas DNA glicosilases que promovem a hidrólise da ligação N-glicosil que liga a base ao esqueleto de fosfato-açúcar do DNA, resultando em um sítio abásico (sítio AP) reconhecido pela endonuclease APE1. Esta enzima cliva regiões adjacentes ao sítio AP pela quebra da ligação fosfodiester, e a lacuna formada é preenchida pela ação das enzimas DNA polimerase e ligase (COOPER; HAUSMAN, 2007). Defeitos no mecanismo de reparo por BER estão relacionados com risco de câncer em idades precoses (KARAHALIL; BOHR; WILSON, 2012).

O SNP rs1130409 presente no gene *APE1* (apurínico/apirimidínico endonuclease I – também conhecido como HAP1 e REF-1) resulta na substituição do aminoácido ácido aspártico por ácido glutâmico (Asp148Glu) no exón 5 e pode estar associada com hipersensibilidade à radiação ionizante e risco de câncer (KARAHALIL; BOHR; WILSON, 2012).

Outra importante proteína que atua nesta via de reparo é a XRCC1 (*X-ray Cross Complementing Group1*), que age como arcabouço para alocar diversas enzimas como DNA-polimerase β que remove os resíduos de fosfato 5'-desoxirribose deixados pela enzima APE1 e DNA-ligase III que fecha a fita de DNA recém reparada. Uma variante genética comum do gene *XRCC1* é o SNP rs25487 (1196A>G) que resulta na substituição de uma arginina por uma glutamina (Arg399Gln), variante esta que pode alterar a função da proteína e,

consequentemente, a capacidade de reparo, resultando na instabilidade genética (KARAHALIL; BOHR; WILSON, 2012).

O reparo por NER identifica uma maior quantidade de bases lesadas que causam a distorção da molécula de DNA. O dano ao DNA é identificado pelo complexo XPC/hHR23B, que desencadeia o recrutamento de proteínas helicases XPA e XPD para o local da lesão. Estas proteínas desenrolam o DNA em aproximadamente 30 pb em torno do sítio com o dano e então o complexo XPF/ERCC1 de endonucleases é recrutado para o local, removendo a lesão. A lacuna resultante é preenchida por DNA polimerase, empregando a fita intacta como molde, e selada pela proteína ligase (COOPER; HAUSMAN, 2007).

Pacientes que apresentam deficiência nesta via de reparo são portadores de síndrome xeroderma pigmentoso (XP), e as células destes indivíduos apresentam altas taxas de mutação quando irradiadas com luz UV. Assim, os portadores de XP apresentam alta frequência de tumores de pele em regiões expostas a luz solar, degeneração neurológica progressiva e envelhecimento precose (AGNEZ-LIMA et al., 2003).

O SNP rs1800975 do gene *XPA* (xeroderma pigmentoso, grupo A) está localizado próximo do códon de iniciação e a presença deste SNP afeta a capacidade de NER, aumentando o risco de câncer de pulmão, carcinoma de células escamosas (LOU et al., 2014) e câncer de mama (HAN et al., 2012).

O gene *ERCC1* (*excision repair cross complementation Group 1),* por desempenhar papel essencial no reparo NER, quando produz a proteína alterada pode ter um papel importante na carcinogênese. O SNP rs3212986 (8092 C>A) neste gene tem sido amplamente estudado. Chen et al. (2000) observaram associação entre este SNP com o risco de tumores cerebrais, sugerindo que este SNP pode alterar a estabilidade transcricional deste gene. Xue et al. (2015) demonstraram que pacientes com câncer gástrico (n=410) portadores do genótipo raro A/A para o polimorfismo rs3212986 apresentaram taxa de sobrevivência 6 vezes maior do que os pacientes portadores do genótipo C/C. Estes autores sugeriram que este SNP pode estar influenciando a resposta à quimioterapia e a evolução clínica do câncer gástrico.

Outra proteína de reparo que tem importante papel na manutenção da integridade do genoma é a enzima O^6-metilguanina-DNA-metiltransferase (MGMT), que participa da via direta de remoção de dano, removendo lesões causadas pelos agentes alquilantes (KAINA et al., 2007). O reparo das lesões O^6-alquilguanina (adição do grupo alquila ao oxigênio na posição 6 da guanina no DNA) é fundamental para a célula, pois esta lesão leva ao pareamento incorreto das bases durante a replicação do DNA, resultando em mutação do tipo transição G:C para A:T podendo afetar genes importantes para a manutenção da estabilidade

do DNA (JACINTO; ESTELLER, 2007).

A reparação destas lesões resulta de uma reação que transfere o grupo alquil do oxigênio da guanina para um resíduo de cisteína na região catalítica da proteína MGMT, restaurando o DNA e inativando MGMT, que sofre ubiquitinização. Como a proteína MGMT pode reparar apenas uma lesão, a capacidade da célula em remover estas lesões depende do número total de moléculas MGMT na célula e da taxa de síntese de novas proteínas (KAINA et al., 2007). Assim, se a quantidade de proteína presente na célula for diminuída, as lesões O^6-alquilguanina podem acumular-se, originarem mutações e levarem ao surgimento de câncer.

A quantidade da proteína MGMT encontrada em alguns tumores humanos é menor do que a quantidade presente em tecidos normais. Esta diminuição da expressão está relacionada com o silenciamento do promotor deste gene por hipermetilação, resultando na falta de expressão do mRNA. A inativação do MGMT foi associada com gliomas, linfomas, câncer de cólon, pulmão, cabeça e pescoço (ESTELLER et al., 1999).

Polimorfismos presentes neste gene podem afetar a capacidade de reparo do DNA, e assim influenciar na suscetibilidade individual ao câncer. Kaur et al. (2000) observaram associação entre o SNP rs2308321 e o aumento no risco de câncer de pulmão (OR=2,1). Este SNP rs2308321 resulta na substituição de uma isoleucina por uma valina na posição 143, nas proximidades do sítio catalítico da proteína MGMT e pode inativar ou diminuir a atividade da proteína (CRONE; PEGG, 1993).

2.3 TESTES DE GENOTOXICIDADE E MUTAGENICIDADE

Um dos passos cruciais para o desenvolvimento do câncer e outras doenças crônicas é a indução e acúmulo de danos genéticos, resultando na instabilidade genômica (STRATTON; CAMPBLELL; FUTREAL, 2009). Na literatura são encontrados poucos estudos que avaliaram a instabilidade em pacientes transplantados submetidos a diferentes terapias imunossupressoras (OLIVEIRA; ZANKL; RATH, 2004; OZTÜRK et al., 2008; RATH; OLIVEIRA-FRICK, 2009; LA MANNA et al., 2011), mesmo sabendo que as neoplasias malignas estão entre as complicações mais frequentes após longos períodos de exposição a medicamentos imunossupressores e representam uma das principais causas de morte de pacientes transplantados (CHAPMAN; WEBSTER; WONG, 2013). Entre os fatores de risco para o câncer destacam-se o tempo após o transplante, infecções por vírus e aumento da idade do paciente (STALLONE; INFANTE; GRANDALIANO, 2015).

Para a avaliação do risco de câncer, danos genotóxicos e mutagênicos podem ser

avaliados, respectivamente, pelos ensaios do cometa e micronúcleo e estes podem ser realizados concomitantemente a fim de avaliar *in vivo* dois parâmetros distintos da genotoxicidade individual (KANG et al., 2013). Estes ensaios são amplamente utilizados no biomonitoramento humano, por serem rápidos e sensíveis, sendo muito adequados para a avaliação de danos ao DNA em populações expostas a agentes químicos (FENECH, 2007; COLLINS et al., 2014).

O ensaio do cometa pode contribuir para a determinação de "dosagem e efeito biológico", sendo um indicador precoce do risco à saúde (COLLINS et al., 2014). La Manna et al. (2011) utilizaram o ensaio do cometa para avaliar linfócitos de 30 pacientes transplantados renais em diferentes tempos após o transplante (2 dias, 1 mês e 6 meses) e observaram uma significativa redução na fragmentação do DNA após 6 meses de transplante. Aykanat et al. (2011) utilizaram o mesmo ensaio para comparar 3 grupos de crianças com doença renal crônica, pré-diálise (N = 17), hemodiálise (N = 15) e transplantadas (N = 17), não sendo observada diferença significativa na fragmentação do DNA entre os grupos avaliados.

O ensaio do micronúcleo com bloqueio de citocinese (CBMN-cyt) está bem estabelecido e detecta perdas de fragmentos cromossômicos ou cromossomos inteiros que resultam em instabilidade cromossômica, tendo sido proposto como um marcador promissor para o estudo do câncer (KANG et al., 2013).

Utilizando o ensaio CBMN-cyt em linfócitos periféricos de pacientes transplantados renais, Oliveira, Zankl e Rath (2004) observaram maior frequência de células binucleadas micronucleadas e menor índice de divisão celular nos pacientes transplantados (n = 14) em comparação com controles saudáveis. Rath e Oliveira-Frick (2009) também observaram redução na capacidade de proliferação dos linfócitos, sendo que dos 79 pacientes avaliados após 2-3 semanas de imunossupressão, foi possível realizar a análise de apenas 36 pacientes devido a baixa quantidade de células binucleadas. Estes autores não observaram diferença na quantidade de danos mutagênicos entre os pacientes que faziam uso de diferentes medicamentos imunossupressores ou entre os que apresentaram episódios de rejeição e os que não apresentaram.

Trabalho realizado por Speit (2013) questiona a realização do ensaio do micronúcleo com bloqueio da citocinese utilizando citocalasina B em cultura de linfócitos humanos como uma ferramenta sensível para detectar efeito mutagênico em populações ocupacionalmente expostas, pelo fato dos micronúcleos (MNs) avaliados nas células binucleadas serem formados em sua maioria *in vitro* durante a cultura. Assim, os MNs produzidos como

resultado de dano ao DNA *in vivo*, durante a divisão dos linfócitos na médula óssea ou nos linfonodos, não contribuem substancialmente para a frequência de MNs observada em células binucleadas. Essa falta de sensibilidade neste ensaio, segundo Speit (2013), decorre do fato da citocalasina B ser adicionada à cultura relativamente tarde, fazendo com que nem todas as células binucleadas analisadas representem células que sofreram apenas uma divisão celular. Outro agravante de se analisar células binucleadas após cultivo *ex vivo* por tempos maiores do que um ciclo celular é que as células danificadas podem ser eliminadas durante a cultura por apoptose ou reparadas antes da divisão, na presença da citocalasina.

Para a avaliação do MN já presente nos linfócitos *in vivo*, a cultura não pode ser realizada por longos períodos de tempo, garantindo que as células não se dividam *in vitro.* Para isso é indicada a estimulação das células por 24 horas com fitohemaglutinina (FENECH, 2007; SPEIT, 2013).

A prevenção das neoplasias após o transplante é realizada por meio do rastreio do câncer, mas não existem estudos sobre a abordagem específica para o rastreio da população transplantada. Assim, devem ser adotadas com maior frequência triagens individualizadas com base no risco individual, sendo sugerida a realização de exames de rastreio como mamografia, endoscopia, exames de próstata, ultrassonografias, entre outros, além de consultas periódicas com dermatologista (STALLONE; INFANTE; GRANDALIANO, 2015). No entanto, na prática clínica não são realizados ensaios para a detecção de possível indução de danos no material genético e consequente aumento da suscetibilidade ao desenvolvimento de câncer.

3 OBJETIVOS

3.1 OBJETIVO GERAL:

Avaliar, em pacientes transplantados renais, variantes polimórficas em genes do biometabolismo, resposta imune e reparo, a fim de estabelecer marcadores moleculares para os processos de rejeição e desenvolvimento de câncer e analisar a possível indução de instabilidade genética promovida pelo uso prolongado de imunossupressores.

3.2 OBJETIVOS ESPECÍFICOS:

ARTIGO I

a) Avaliar e comparar a incidência de danos genotóxicos e mutagênicos em linfócitos do sangue periférico de pacientes transplantados renais e de controles saudáveis;

b) Verificar a associação entre os danos genotóxicos e mutagênicos observados nos pacientes com o tempo após transplante e com a função renal.

ARTIGO II

c) Determinar as frequências genotípicas dos genes envolvidos na metabolização de drogas imunossupressoras (*CYP3A4*, *CYP3A5*, *CYP2E1*, *POR*, *UGT2B7*, *UGT1A9*, *IMPDH1*, *IMPDH2*, *CES1* e *CES2*), de bomba de efluxo de drogas (*ABCC2*, *ABCB1*, *ABCG2* e *SLCO1B1*) e na resposta imune (*CCR5*, *TNF-α, IRF-5, TGFβ, NFAT, NFKB1A, IL-23R*, *IL-10* e *IL-2*), em pacientes transplantados renais que apresentaram ou não episódios de rejeição;

d) Selecionar os genes que na genotipagem dos pacientes apresentarem associação com o processo de rejeição e analisá-los quanto a expressão dos respectivos mRNAs.

ARTIGO III

e) Associar SNPs relacionados com a farmacocinética do MMF em pacientes que fazem uso deste medicamento com episódio de rejeição.

ARTIGO VI

f) Analisar se os SNPs em genes do metabolismo e transporte de drogas interferem na farmacocinética do imunossupressor tacrolimo durante os três primeiros meses do transplante.

ARTIGO V

g) Verificar a possível associação entre os SNPs em genes de reparo (*ERCC1*, *APE1*, *XPA, XRCC1* e *MGMT)* e em genes do sistema imune (*IL-23R, IL-2, IL-10* e *TGF-β)* com a incidência de câncer em pacientes transplantados renais comparados com controles livre de neoplasias.

4 ARTIGO I

Long-term genotoxic effects of immunosuppressive drugs on lymphocytes of kidney transplant recipients

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Long-term genotoxic effects of immunosuppressive drugs on lymphocytes of kidney transplant recipients

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ABSTRACT

Immunosuppressive therapy can prevent rejection after organ transplantation. However, increased cancer risk is a serious complication among patients undergoing such therapy. We have evaluated whether prolonged use of immunosuppressive drugs is genotoxic. DNA instability was assessed, using the comet and micronucleus assays, in blood lymphocytes of 76 kidney transplant patients. DNA damage detected by the comet assay increased with time after transplantation. The estimated glomerular filtration rate of the patients did not influence the incidence of DNA damage. No association between micronucleated mononucleated cells and time elapsed after transplantation was observed. Our results suggest that prolonged use of immunosuppressive drugs in kidney transplant patients can induce genetic instability.

1. Introduction

End Stage Renal Disease (ESRD) patients may be treated by dialysis or undergo transplantation. Kidney transplantation is considered the best therapeutic option for those ESRD patients who are able to receive it, as it results in higher survival rates and better quality of life [1]. After kidney transplantation, patients need to take immunosuppressive medications indefinitely, to prevent immune rejection of the graft. Usually, these drugs have high pharmacokinetic ranges but narrow therapeutic indices [2]. In general, two or three immunosuppressive medications are used in combination, to minimize the risk of organ rejection [3].

Long-term use of immunosuppressive drugs is associated with severe adverse effects, such as nephrotoxicity, neurotoxicity, gastrointestinal disturbances, increased cholesterol and triglyceride levels, insulin resistance, and diabetes mellitus [4]. Among adverse effects, the development of cancer is a major cause of morbidity and mortality [5,6]. Apel et al. [7], examining the incidence of cancer in a group of 1882 German kidney transplant recipients with post-transplant times ranging 0.4–25.5 y, observed an overall incidence of 13.7%, and a risk of malignant non-skin tumors 12.1 times higher than that expected in the general population.

The long-term care of kidney transplant patients, therefore, involves a delicate balance: avoiding graft rejection, without causing excessive immunosuppression or increasing the incidence of nephrotoxicity by calcineurin inhibitors. The estimated glomerular filtration rate (eGFR), from serum creatinine, provides an adequate tool to evaluate the function of the graft, and is used routinely in clinical transplantation [8]. Monitoring the plasma concentrations of some immunosuppressive drugs is also commonly performed, in an attempt to minimize their adverse effects by adjusting the doses. However, there are no routine tests to evaluate DNA damage in transplant patients.

Some studies have assessed the mutagenic effects of immunosuppressive drugs in lymphocytes *in vitro*, using the sister-chromatid exchange [9,10], micronucleus (MN), nuclear division index (NDI) [10–12], and comet assays [12]. In general, these studies have demonstrated that high concentrations of immunosuppressive drugs can induce DNA damage. Moreover, *in vivo* studies in patients after short post-transplant periods revealed that

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the lymphocytes showed an increased incidence of sister chromatid exchanges in patients that received cyclosporine [13] and an increase in MN frequencies and a reduction in NDI after transplantation [14].

The advantage of using assays that allow monitoring of DNA stability is the prediction of the risk of cancer development and other adverse health effects induced by exposure to these immunosuppressants. Among the assays widely used for human biomonitoring, the comet assay has high sensitivity, requires small numbers of cells, and is inexpensive and quick [15]. The test can be performed concurrently with the MN assay, which evaluates mutagenic damage resulting from loss of acentric fragments or whole chromosomes [16].

According to Speit [17], the 24-h time-point after the start of the culture is ideal for analyzing MN, produced *in vivo* during division of lymphocytes in the bone marrow, thymus and lymph nodes. Therefore, mononucleated lymphocytes present *in vivo* prior to starting the culture can be analyzed for the presence of DNA damage [18], complementing the data obtained by evaluation of binucleated cells.

The aim of this study was to evaluate whether kidney transplant recipients exhibit DNA damage caused by immunosuppressive treatment and whether the degree of damage is associated with time following transplantation.

2. Materials and methods

2.1. Patients

The experiments were approved by the Ethics Committee on Human Research of the State University of Londrina, (CEP/UEL 153/2013 CAAE: 18263413.4.0000.5231). Seventy-six kidney transplant patients who regularly underwent medical monitoring at the Kidney Institute of Londrina (Paraná State, Brazil) participated in this study. Written informed consent and a questionnaire about their lifestyle were obtained from all study participants. Participants who stated that they consume alcoholic beverages were considered "drinkers" and those who stated that they smoke were classified as "smokers". Additional data, such as post-transplantation time, rejection episodes, degree of histocompatibility with the donor, and occurrence of cancer were obtained from medical records. Venous blood samples (2 mL) were collected using EDTA (6%)-coated vacuum tubes (Labor Import, Brazil) and the coded blood samples were brought to the laboratory (stored on ice) within 2 h and processed immediately, under identical conditions. Baseline DNA damage was also estimated by MN and comet assays in 17 healthy individuals (control-group) and whole blood of three individuals was treated with methyl methanesulfonate solution (Sigma-Aldrich, CAS: 6627-3, St. Louis, MO, USA) at a final concentration of 5×10^{-5} M and incubation for 1 h at 37 °C and used as positive control in the comet assay.

2.2. eGFR and plasma concentration of tacrolimus

The patients' eGFR values were calculated from the abridged Modification of Diet in Renal Disease formula (MDRD-4), modified by Levey et al. [19]. The transplant patients were clustered according to eGFR levels; the cutoff value used was 60 mL min^{-1} per 1.73 m^2, because GFR <60 mL min^{-1} per 1.73 m^2 for at least 3 months is evidence of kidney damage or chronic kidney disease [8,20]. Patients with eGFR >60 mL min^{-1} per 1.73 m^2 were defined as "better graft function" (BGF) and patients who showed eGFR <60 mL min^{-1} per 1.73 m^2 were defined as "worse graft function" (WGF).

For all patients studied, determination of plasma creatinine levels was obtained from the patient's record on the same day of blood collection for mutagenicity assays. Concurrently, assessment of plasma tacrolimus levels by radioimmunoassay was also obtained from the 44 patients who were using this drug as part of their immunosuppressive regimen.

2.3. Comet assay

The alkaline comet assay was performed as described by Singh et al. [21]. Peripheral blood (20 μL) was mixed with low-melting-point agarose (Gibco, CAS: 9012-36-6, Grand Island, NY; 0.6% in phosphate-buffered saline – PBS), 120 μL. This mixture was applied to microscope slides pre-coated with normal-melting-point agarose (Life Technologies, Paisley, UK) (1.5% in PBS). Two slides were made for each patient and respective controls. The slides were immediately covered with coverslips and the agarose was allowed to solidify for 30 min at 4 °C. After removal of the coverslips, these slides were immersed in ice-cold alkaline lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM TRIS, 10% DMSO, 1% Triton-X) pH 10, for 24 h at 4 °C. After lysis, the slides were incubated for 20 min in alkaline buffer (200 mM EDTA, 10 N NaOH) pH 13 at 4 °C, followed by electrophoresis (25 V; 300 mA; 1 V/cm) for 20 min in the same buffer. Then, the slides were neutralized with Tris (0.4 M, pH 7.5) for 15 min, dried, and fixed with absolute alcohol for another 15 min and stored at 4 °C until analysis.

Staining was performed with GelRed Nucleic Acid Stain 10000X (Biotium, Hayward, CA), 45 μL, diluted in 0.1 M NaCl solution to 30X. A total of 300 nucleoids per patient were scored using a fluorescence microscope (Nikon Eclipse, Tokyo, Japan) with excitation filter 515–560 nm and 590 nm emission (barrier) filter, under a magnification of 400×.

The criteria used for quantification of DNA damage [22] was visual scoring of the size of the comet tail in comparison to the nucleoid. The cells were classified into four categories of DNA damage, ranging from no visible migration (class 0, undamaged cells) to the maximum length comet cells (class 3). The comet score was calculated according to the formula proposed by Manoharan and Banerjee [23], multiplying the number of nucleoids observed in each class (n0, n1, n2 and n3) by the value of the corresponding class (0, 1, 2, 3) divided by the total number of cells analyzed (N).

2.4. Micronucleus assay in mononucleated cells

The MN assay in mononucleated cells was performed according to Speit [17] by using whole blood, 600 μL, in 6 mL RPMI 1640 medium (Gibco, CAS: 31800-014, Grand Island, NY, USA), 2.0 g L^{-1} $NaHCO_3$ (Merck SA Industrias Químicas, Rio de Janeiro, RJ, Brazil), HEPES 25 mM (Sigma-Aldrich, CAS: 7365-45-9, St. Louis, MO, USA), 0.06 g L^{-1} penicillin G (Sigma-Aldrich, CAS: 113-98-4), 0.10 g L^{-1} streptomycin sulfate (Sigma-Aldrich, CAS: 3810-74-0), supplemented with 20% fetal bovine serum (Gibco, CAS: 12657-029) and 2% phytohemaglutinin A (Gibco, CAS: 10576-015) to stimulate the division of lymphocytes. Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. Cells were collected by centrifugation and processed further by hypotonic treatment for 15 min in 0.075 M KCl at 4 °C. Cells were fixed three times in methanol/acetic acid (3:1). After fixation, the slides were prepared and stained with 5% Giemsa (CAS: 1.09204.0500, Merck SA Industrias Químicas) solution in phosphate buffer (0.06 M $NaHPO_4$ and 0.06 M KH_2PO_4, pH 6.8) for 10 min, washed with water and dried. Analysis under a light microscope (Nikon Eclipse E200, Tokyo, Japan) with 400× magnification was carried out to determine the frequency of micronucleated cells per 1000 mononucleated cells analyzed per patient.

Table 1
Demographic characteristics of kidney transplant patients (n = 76), parameters analyzed and comparison between BGF and WGF patients.

Characteristics of patients	General		BGF[g]		WGF[h]		*p-value*[i]
	N (%)	Mean ± SD[f]	N (%)	Mean ± SD	N (%)	Mean ± SD	
Age (y)	76 (100)	46± 13	34 (100)	46 ± 13	42 (100)	45 ± 12	0.80
Time after transplantation (y)	76 (100)	6.8 ± 5.8	34 (100)	5.9 ± 4.6	42 (100)	7.6 ± 6.5	0.20
Serum creatinine (mg dL^{-1})	76 (100)	1.5 ± 0.70	34 (100)	1.1 ± 0.18	42 (100)	1.8 ± 0.77	<0.0010
eGFR[a] (mL min^{-1} per 1.73 m^2)	76 (100)	58 ± 22	34 (100)	77 ± 14	42 (100)	42 ± 12	<0.0010
Comet Score[b]	76 (100)	0.21 ± 0.094	34 (100)	0.20 ± 0.080	42 (100)	0.22 ± 0.11	0.26
MN[c]	76 (100)	3.9 ± 2.5	34 (100)	3.7 ± 2.1	42 (100)	4.0 ± 2.9	0.56
Tacrolimus[d] (ng mL^{-1})	44 (58)	6.6 ± 3.0	19 (56)	6.0 ± 2.8	25 (60)	7.0 ± 3.2	0.31
Gender							0.20
Men	43 (57)	–	22 (65)		21 (50)		
Women	33 (43)	–	12 (35)	–	21 (50)	–	
Degree of HLA compatibility[e]							0.30
Deceased unrelated donor	34 (45)	–	14 (41)	–	20 (48)	–	
Live HLA-haploidentical	23 (30)	–	9 (27)	–	14 (33)	–	
Live HLA-identical	19 (25)	–	11 (32)	–	8 (19)	–	
Rejection episode(s) (yes)	20 (26)	–	3 (8.8)	–	17 (40)	–	0.0010**
Smoker (yes)	4 (5.3)	–	2 (5.9)	–	2 (4.8)	–	0.83
Drinker (yes)	10 (13)	–	1 (2.9)	–	9 (21)	–	0.011*
Cancer (yes)	5 (6.6)	–	3 (8.8)	–	2 (4.8)	–	0.48

[a] Glomerular filtration rate; estimated by MDRD.
[b] Arbitrary units.
[c] Micronucleated cells per 1000.
[d] Concentration of tacrolimus.
[e] Human leukocyte antigen.
[f] Standard deviation.
[g] BGF – better graft function.
[h] WGF – worse graft function.
[i] *p*-values calculated by *t*-test for independent samples comparing patients BGF and WGF.
* $p < 0.050$.
** $p < 0.010$.

Table 2
Pearson's correlations (ρ) between gender, age, time of transplantation, comet score, MN and other covariates.

	Gender	Age	Time after transplant	Rejection episode(s)	Degree of HLA compatibility	Drinker	Smoker	Comet Score	MN	Cancer	eGFR
Gender[a]	–	**−0.25***	−0.074	0.041	−0.016	0.18	0.21	−0.10	0.078	0.018	−0.10
Age[b]	**−0.25***	–	0.15	**0.23***	0.034	−0.072	0.049	0.042	−0.021	−0.16	0.025
Time after transplant[c]	−0.074	0.15	–	−0.17	0.14	**−0.25***	0.050	0.20	−0.037	**−0.26***	**−0.29***
Rejection episode(s)[d]	0.041	**0.23***	−0.17	–	0.11	0.12	−0.0070	−0.031	0.028	0.082	**0.48****
Degree of HLA compatibility[e]	−0.016	0.034	0.14	0.11	–	0.0010	0.015	−0.047	−0.12	**−0.33****	0.13
Drinker[f]	0.18	−0.072	**−0.25***	0.12	0.0010	–	**0.26***	−0.15	−0.036	0.054	**0.23***
Smoker[g]	0.21	0.049	0.050	−0.0070	0.015	**0.26***	–	−0.16	−0.036	−0.063	−0.040
Comet Score[h]	−0.10	0.042	0.20	−0.031	−0.047	−0.15	−0.16	–	0.028	0.13	−0.058
MN[i]	0.078	−0.021	−0.037	0.028	−0.12	−0.036	−0.036	0.028	–	0.0070	−0.13
Cancer[j]	0.018	−0.16	**−0.26***	0.082	**−0.33****	0.054	−0.063	0.13	0.0070	–	−0.081
eGFR[k]	−0.10	0.025	**−0.29***	**0.48****	0.13	**0.23***	−0.040	−0.058	−0.13	−0.081	–

[a] Female was taken as reference.
[b] Years.
[c] Years.
[d] Non-rejection episodes were taken as reference.
[e] Human leukocyte antigen compatibility (live HLA-identical donor was taken as reference).
[f] Non-drinkers were taken as reference.
[g] Non-smokers were taken as reference.
[h] Arbitrary units.
[i] micronucleated cells per 1000.
[j] Absence of cancer was taken as reference.
[k] Estimated glomerular filtration rate (mL min^{-1} per 1.73 m^2).
* $p < 0.050$.
** $p < 0.010$.

2.5. Statistical analysis

Age (y), time since transplantation, serum creatinine (mg dL^{-1}), eGFR (mL min^{-1} per 1.73 m^2), plasma concentration of tacrolimus (ng mL^{-1}), micronuclei (micronucleated cells per 1000), and comet score (arbitrary units) were analyzed as continuous variables; gender (female or male), alcohol consumption (yes or no), smoking (yes or no), donors (group 1: live HLA identical; group 2: live HLA-haploidentical and group 3: deceased donor), rejection episodes (yes or no), and cancer (yes or no) were analyzed as categorical variables. Time after transplantation was ln-transformed due to the skewed distribution.

Independent-samples Student's *t*-test was performed to compare the comet score means and MN frequencies between the patients with BGF and WGF and between patients with 0–5 y of transplant and patients with >5 y. Comparison of the group of

Table 3
Comet score and micronucleus frequency in patients with 0–5 y post-transplant or >5 y and controls.

Group	Number of individuals	Comet score	MN
Negative controls	17	0.11 ± 0.050^{a}	1.76 ± 1.25^{a}
Positive control (MMS 5×10^{-5} M)	3	1.28 ± 0.26^{b}	–
Transplant patients			
0–5 y	39	0.19 ± 0.088^{c}	$4.28 \pm 2.96^{b,c}$
>5 y	37	0.23 ± 0.097^{c}	3.43 ± 1.92^{ac}
Total	76	0.21 ± 0.090^{c}	3.87 ± 2.53^{c}

Values followed by the same letter do not differ significantly from each other at 5% significance by ANOVA followed by Tukey's test.

patients with different duration of transplant and the control group was performed using ANOVA followed by Tukey's test.

Parametric correlations (Pearson) were performed in order to examine the associations between age, time after transplantation, eGFR, micronuclei frequencies, comet score, gender, alcohol consumption, smoking, donor categories, rejection episodes, and cancer.

Univariate and multivariable linear regression models were used to evaluate the association between micronuclei frequency, comet score and time after transplantation. The multivariable models were calculated with all variables that showed $p \leq 0.20$ (i.e., age, gender, alcohol consumption, smoking and cancer) obtained from previous univariate models.

Results were assumed as statistically significant for a value of $p \leq 0.050$. Analyses were performed using SPSS® 20 Statistics software (IBM; Armonk, NY, USA) and models for multivariable analysis are detailed as footnotes of the respective tables. All analyses were corrected by the number of each variable.

3. Results

3.1. General characteristics

Table 1 summarizes the characteristics of all individuals enrolled in the current study. The mean age of patients was 46 ± 13 y, and there were more men than women (43 men vs. 33 women). Time after transplantation ranged from 1 month-28 y and eGFR ranged from 12.4–110 mL min^{-1} per 1.73 m^2 (58 ± 22) (Table 1). Among the donors, there were 19 individuals who were live HLA-identical, 23 live HLA-haploidentical and 34 deceased. Only 5.3% and 13% of individuals declared that they consumed alcoholic beverages and had smoking habits, respectively. At the time of data collection, 5 patients had cancer. Among them, 3 patients presented with non-melanoma skin cancer and 2 non-skin cancers (utero cervix and colon cancer). Time elapsed until documentation of the first post-transplant case ranged from 5 to 13 y (8.4 ± 3.1). Comet score ranged from 0.060-0.57 and micronucleated cells ranged from 0 to 13 per 1000 analyzed cells (not presented in Table 1). The control group was composed of 17 healthy individuals (9 women and 8 men), having a mean age of 48 ± 11 y.

3.2. Influence of eGFR on DNA damage

To evaluate the influence of eGFR on DNA damage, intra-group comparisons were performed according to renal function of patients, divided into BGF and WGF groups. Independent-samples *t*-test of comet score and MN between the BGF and WGF groups did not show any significant difference ($p = 0.26$ and $p = 0.56$, respectively) (Table 1).

3.3. Correlation analyses

Results from Pearson's correlations are presented in Table 2. Positive correlations were observed between age and rejection episodes ($\rho = 0.23$; $p < 0.050$), alcohol consumption and smoking habits ($\rho = 0.26$; $p < 0.050$), and rejection episodes and eGFR ($\rho = 0.48$; $p < 0.010$); negative associations were seen between time after transplantation and eGFR ($\rho = -0.29$; $p < 0.050$) and between time after transplantation and cancer ($\rho = -0.26$; $p < 0.050$). No correlations were found between plasma concentration of tacrolimus and comet score, MN or cancer incidence (data not shown).

3.4. Impact of time after transplantation on comet formation and MN

Both the MN and comet assays indicated that kidney transplant patients exhibited more DNA damage than healthy individuals. No statistically significant difference in the MN frequency was observed by Student's *t*-test between the group of patients with 0–5 y of transplant and the group with >5 y. However, a tendency of increasing score in the comet assay ($p = 0.081$) was observed.

Table 4 summarizes the estimates of time after transplantation obtained from univariate and multivariable regression analyses on comet score and MN. It can be seen that comet formation is associated with time after transplant, i.e., longer the time after transplant, the higher the DNA damage observed (either in univariate ($\beta = 0.024$; $p = 0.031$) or multivariable models ($\beta = 0.025$; $p = 0.042$)). Although an association between MN and time after transplantation was observed, these results were not statistically significant and, therefore, further conclusions cannot be drawn.

4. Discussion

In our previous study, high concentrations of tacrolimus and cyclosporine showed genotoxic (comet assay) and mutagenic (MN assay) effects on MRC-5 cells *in vitro* [12]. *In vitro* studies using normal human lymphocytes also have shown mutagenic effects of these drugs. Tacrolimus, mycophenolate mofetil, and cyclosporine increased the MN frequency [10,11], while sirolimus had lower mutagenic effects, inducing MN only at higher concentrations [11].

Table 4
Multivariable linear regression parameters for the associations between score and time after transplantation.

	Comet score				MN			
	Univariate		Multivariable		Univariate		Multivariable	
	β	*p*	β	*p*	β	*p*	β	*p*
Time after transplantation[a]	0.024	0.031	0.025	0.042	−0.38	0.20	−0.40	0.19

Unstandardized beta (β) coefficients for the $\beta 1 \times$ time after transplantation (continuous) adjusted. Adjusted models as follow: Score = $\alpha + \beta 1 \times$ time after transplantation + $\beta 2 \times$ age + $\beta 3 \times$ gender + $\beta 4 \times$ drinker + $\beta 5 \times$ smoker + $\beta 6 \times$ cancer. MN = $\alpha + \beta 1 \times$ time after transplantation + $\beta 2 \times$ age + $\beta 3 \times$ gender.

[a] ln-transformed.

In the present study, we evaluated the impact of long-term immunosuppressive therapy on DNA of lymphocytes in kidney transplant patients through the MN assay in mononucleated cells and the comet assay. The cytokinesis-block micronucleus cytome assay (CBMN-Cyt) was performed as described by Fenech and Morley [24]. However, in most of the samples, the lymphocytes did not proliferate in culture, so it was not possible to obtain binucleated cells in order to perform the cytological analyses. Rath and Oliveira-Frick [14] also observed a reduction in the ability of lymphocytes to proliferate *in vitro* after three weeks of immunosuppressive therapy, demonstrating that this inhibition of proliferation occurs after short-term immunosuppressive therapy. In the present study, the average time of transplant was 6.8 y; thus, the absence of proliferation of lymphocytes observed in culture may be explained by the prolonged use of immunosuppressive drugs.

Due to the lack of *in vitro* lymphocyte proliferation of transplant patients, we evaluated the MN in mononucleated cells, performing the assay 24 h after culture initiation. Therefore, all MN present in mononucleated lymphocytes were formed *in vivo* and were already present in the blood when the sample was taken. Our results did not show increased frequency of micronucleated mononucleated cells in relation to the transplantation time, but it should be noted that these patients were under chronic treatment, where a relatively constant rate of MN formation is expected. However, the transplant patients presented a significant increase in MN frequency in mononucleated cells compared to the control group. The loss of acentric fragments or whole chromosome, as detected by the MN assay, can potentially lead to a variety of DNA/chromosome contents into the cell. The accumulation of genetic changes may lead to genetic instability, which may result in cancer [25]. Although the MN frequency of transplant patients was not correlated with the cancer episodes, the significant increase in MN frequency observed in this group when compared with the control group suggest that the transplant patients have higher probability of developing cancer than the general population.

Cakmak-Demircigil et al. [26] assessed MN frequencies in mononucleated and binucleated cells using the same culture and observed that the frequency of micronucleated binucleated cells in hemodialysis and pre-dialysis patients was significantly higher than in the transplant patients ($p < 0.050$). The authors also showed that there was no difference in MN frequencies in mononucleated cells among this subgroup of patients. The fact that these authors had examined both cell types in the same slides of CBMN-Cyt, i.e., 72 h after the start of culture, is contentious. According to Speit [17], at this time-point of culture it is not possible to distinguish between MN produced *in vivo* or *in vitro*.

This is the first time that DNA damage has been assessed using the comet assay in peripheral blood of patients living long-term with a transplant kidney. To minimize the effect of DNA repair processes on reversal of DNA damage, the comet assay was performed in unstimulated cells of whole blood that, according to Bausinger and Speit [27], have low repair activities. The cell samples were maintained at approx. 8 °C between collection and processing and the experiments were always performed within 2 h of blood collection. Therefore, the DNA damage observed in the present study was already present in the lymphocytes and not due to mis-repair or mis-replication.

There was no significant difference in MN frequency and comet score by Student's *t*-test between the group of patients with 0–5 y duration post-transplantation and >5 y (Table 3), although the comet score exhibited an upward trend. However, univariate and multivariable regression analyses showed an association between the time of transplantation and the genotoxic damage in lymphocytes of kidney transplant patients (Table 4). The DNA fragmentation observed in the patient group could reflect reduced cell viability, less effective repair capacity and a stronger possibility of development of cancer and graft rejection in these patients.

Two other studies [28,29] have evaluated DNA fragmentation by the comet assay in transplant patients, although with shorter post-transplant follow-up and smaller numbers of patients. Aykanat et al. [28] evaluated basal damage, through the amount of DNA (%) in the tail of comet in children at different stages of treatment of chronic kidney disease (CKD), including 17 patients on pre-dialysis, 15 patients on regular hemodialysis, 17 kidney transplant patients with 22.35 ± 19.43 months after transplantation and 20 healthy children. They observed that there was no difference in the basal DNA damage between the subgroups of CKD, but the basal damage was significantly increased in the CKD group vs. healthy group. As expected, the results of the present study also showed a higher amount of DNA damage in the transplant patient group compared with healthy individuals.

In the present study, no differences in DNA damage were found between BGF and WGF patients ($p = 0.26$). These results corroborate the findings of La Manna et al. [29] who used the comet assay in 30 patients and found that kidney transplant patients categorized as BGF and WGF did not differ in the levels of DNA fragmentation, 6 months after transplant. However, contrary to our findings, they observed a significant time-dependent decline in the DNA fragmentation post-transplantation. This difference is probably a consequence of different times of evaluation, post-transplantation; the present study evaluated patients between 1 month and 28 y post-transplantation, while La Manna et al. [29] evaluated patients in the early follow-up period (up to 6 months) after transplantation.In the present study, time after transplant was correlated with decreased eGFR and increased cancer episodes ($p < 0.050$) (Table 2). These data corroborate some studies that have shown an increased incidence of cancer after long periods of transplantation, probably due to immunosuppressive treatment [30,31]. Nevertheless, there was no correlation between the cancer incidence and eGFR, contrary to a previous study by Wong et al. [32] in CKD patients, which found an association between increased cancer incidence and lower eGFR. The lack of correlation between cancer and eGFR may be due to the low number of cancer cases in this study.

There was no correlation between the levels of tacrolimus and the occurrence of cancer ($p = 0.95$). This result is not surprising, as we were referring only to the tacrolimus levels at the time of genotoxicity analysis. Importantly, physicians often adjust the doses of tacrolimus after transplantation, so that the patients are within the recommended therapeutic range for each stage of the transplant.

In conclusion, our study shows that with longer time after transplant, eGFR levels were lower and DNA damage was higher, possibly due to prolonged therapy with immunosuppressive drugs. The MN and comet results did not correlate with cancer episodes, but further investigations are needed to test the link between immunosuppression and cancer risk.

Despite the association between DNA damage and time post-transplantation, the sample size of this study does not justify using the DNA damage score as a marker to identify patients at risk of cancer in routine clinical practice, over long duration after kidney transplantation and immunosuppressive therapy.

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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.mrgentox.2016.07.001.

References

[1] P. Roderick, C. Byrne, A. Casula, R. Steenkamp, D. Ansell, R. Burden, D. Nitsch, T. Feest, Survival of patients from South Asian and black populations starting renal replacement therapy in England and Wales, Nephrol. Dial. Transplant. 24 (12) (2009) 3774–3782, http://dx.doi.org/10.1093/ndt/gfp348.

[2] A. Albring, L. Wendt, N. Harz, H. Engler, B. Wilde, A. Kribben, M. Lindemann, M. Schedlowski, O. Witzke, Relationship between pharmacokinetics and pharmacodynamics of calcineurin inhibitors in renal transplant patients, Clin. Transplant. 29 (4) (2015) 294–300, http://dx.doi.org/10.1111/ctr.12504.

[3] B. Hutton, L. Joseph, F. Yazdi, J. Tetzlaff, M. Hersi, M. Kokolo, N. Fergusson, A. Bennett, C. Buenaventura, D. Fergusson, A. Tricco, S. Strauss, D. Moher, G. Knoll, Checking whether there is an increased risk of post-transplant lymphoproliferative disorder and other cancers with specific modern immunosuppression regimens in renal transplantation: protocol for a network meta-analysis of randomized and observational studies, Syst. Rev. 3 (2014) 16, http://dx.doi.org/10.1186/2046-4053-3-16.

[4] M. Naesens, D.R. Kuypers, M. Sarwal, Calcineurin inhibitor nephrotoxicity, Clin. J. Am. Soc. Nephrol. 4 (2) (2009) 481–508, http://dx.doi.org/10.2215/CJN.04800908.

[5] T. Vial, J. Descotes, Immunosuppressive drugs and cancer, Toxicology 185 (3) (2003) 229–240, http://dx.doi.org/10.1016/S0300-483X(02)00612-1.

[6] P. Fraile, P. García-Cosmes, P. Martín, V. García-Bernalt, J.M. Tabernero, Non-skin solid tumors as a cause of morbidity and mortality after liver transplantation, Transplant. Proc. 41 (6) (2009) 2433–2434, http://dx.doi.org/10.1016/j.transproceed.2009.06.022.

[7] H. Apel, K. Walschburger-Zorn, L. Häberle, S. Wach, D.G. Engehausen, B. Wullich, De novo malignancies in renal transplant recipients: experience at a single center with 1882 transplant patients over 39 yr, Clin. Transplant. 27 (1) (2013) E30–E36, http://dx.doi.org/10.1111/ctr.12050, 2013.

[8] W.L. Eppenga, C. Kramers, H.J. Derijks, M. Wensing, J.F. Wetzels, P.A. De Smet, Individualizing pharmacotherapy in patients with renal impairment: the validity of the modification of diet in renal disease formula in specific patient populations with a glomerular filtration rate below 60 ml/min. A systematic review, PLoS One 10 (3) (2015) e0116403, http://dx.doi.org/10.1371/journal.pone.0116403.

[9] K. Yuzawa, I. Kondo, K. Fukao, Y. Iwasaki, H. Hamaguchi, Mutagenicity of cyclosporine Induction of sister chromatid exchange in human cells, Transplantation 42 (1) (1986) 61–63.

[10] E.L. Kurtoglu, S. Yuksel, Genotoxic effects of tacrolimus on human lymphocyte cells, Genetika 48 (6) (2012) 762–767, http://dx.doi.org/10.1134/S1022795412050134.

[11] V.D. Oliveira, H. Zankl, T. Rath, Mutagenic and cytotoxic effects of immunosuppressive drugs on human lymphocyte cultures, Exp. Clin. Transplant. 2 (2) (2004) 273–279, http://dx.doi.org/10.1159/000244646.

[12] H.L. Cilião, D.L. Ribeiro, R.B. Camargo-Godoy, A.F. Specian, J.M. Serpeloni, I.M. Cólus, Cytotoxic and genotoxic effects of high concentrations of the immunosuppressive drugs cyclosporine and tacrolimus in MRC-5 cells, Exp. Toxicol. Pathol. 67 (2) (2015) 179–187, http://dx.doi.org/10.1016/j.etp.2014.11.008.

[13] S. Oztürk, T.K. Ayna, K. Cefle, S. Palanduz, H.S. Ciftçi, S.A. Kaya, A.S. Diler, A. Türkmen, M. Gürtekin, M.S. Sever, M. Carin, Effect of cyclosporin A and tacrolimus on sister chromatid exchange frequency in renal transplant patients, Genet. Test. 12 (3) (2008) 427–430, http://dx.doi.org/10.1089/gte.2008.0006.

[14] T. Rath, V. Oliveira-Frick, Mutagenicity of immunosuppressive medications among renal transplant recipients, Am. J. Nephrol. 30 (6) (2009) 514–520, http://dx.doi.org/10.1159/000244646.

[15] R.R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J.C. Ryu, Y.F. Sasaki, Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35 (3) (2000) 206–221, http://dx.doi.org/10.1002/(SICI)1098-2280(2000)35:3<206::AID-EM8>3.0.CO;2-J.

[16] M. Fenech, N. Holland, W.P. Chang, E. Zeiger, S. Bonassi, The HUman MicroNucleus project–an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans, Mutat. Res. (1–2) (1999) 271–283, http://dx.doi.org/10.1016/S1383-5742(99)00053-8.

[17] G. Speit, Does the recommended lymphocyte cytokinesis-block micronucleus assay for human biomonitoring actually detect DNA damage induced by occupational and environmental exposure to genotoxic chemicals? Mutagenesis 28 (4) (2013) 375–380, http://dx.doi.org/10.1093/mutage/get026.

[18] M. Kirsch-Volders, M. Fenech, Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes, Mutagenesis 16 (1) (2001) 51–58, http://dx.doi.org/10.1093/mutage/16.1.51.

[19] A.S. Levey, L.A. Stevens, C.H. Schmid, Y.L. Zhang, A.F. Castro 3rd, H.I. Feldman, J.W. Kusek, P. Eggers, F. Van Lente, T. Greene, J. Coresh, CKD-EPI (chronic kidney disease epidemiology collaboration) a new equation to estimate glomerular filtration rate, Ann. Intern. Med. 150 (9) (2009) 604–612, http://dx.doi.org/10.7326/0003-4819-150-9-200905050-00006.

[20] I.H. Bleskestad, H. Bergrem, T. Leivestad, A. Hartmann, L.G. Gøransson, Parathyroid hormone and clinical outcome in kidney transplant patients with optimal transplant function, Clin. Transplant. 28 (4) (2014) 479–486, http://dx.doi.org/10.1111/ctr.12341.

[21] N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, Exp. Cell Res. 175 (1) (1988) 184–191, http://dx.doi.org/10.1016/0014-4827(88)90265-0.

[22] H. Kobayashi, C. Sugiyama, Y. Morikawa, M. Hayashi, T. Sofuny, A comparison between manual microscopic analysis and computerized image analysis in the single cell gel electrophoresis assay, MMS Commun. 3 (1995) 103–115.

[23] K. Manoharan, M.R. Banerjee, beta-Carotene reduces sister chromatid exchanges induced by chemical carcinogens in mouse mammary cells in organ culture, Cell Biol. Int. Rep. 9 (9) (1985) 783–789, ISSN 0309-1651.

[24] M. Fenech, A.A. Morley, Measurement of micronuclei in lymphocytes, Mutat. Res. 147 (1–2) (1985) 29–36, http://dx.doi.org/10.1016/0165-1161(85)90015-9.

[25] M. Kirsch-Volders, G. Plas, A. Elhajouji, M. Lukamowicz, L. Gonzalez, K. Vande Loock, I. Decordier, The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance, Arch. Toxicol. 85 (8) (2011) 873–899, http://dx.doi.org/10.1007/s00204-011-0691-4.

[26] G. Cakmak Demircigil, B. Aykanat, K. Fidan, K. Gulleroglu, U.S. Bayrakci, A. Sepici, B. Buyukkaragoz, H. Karakayali, M. Haberal, E. Baskin, N. Buyan, S. Burgaz, Micronucleus frequencies in peripheral blood lymphocytes of children with chronic kidney disease, Mutagenesis 26 (5) (2011) 643–650, http://dx.doi.org/10.1093/mutage/ger027.

[27] J. Bausinger, G. Speit, The impact of lymphocyte isolation on induced DNA damage in human blood samples measured by the comet assay, Mutagenesis (2016), http://dx.doi.org/10.1093/mutage/gew021, pii: gew021. [Epub ahead of print].

[28] B. Aykanat, G.C. Demircigil, K. Fidan, N. Buyan, K. Gulleroglu, E. Baskin, U.S. Bayrakci, A. Sepici, B. Buyukkaragoz, H. Karakayali, M. Haberal, S. Burgaz, Basal damage and oxidative DNA damage in children with chronic kidney disease measured by use of the comet assay, Mutat. Res. 725 (1–2) (2011) 22–28, http://dx.doi.org/10.1016/j.mrgentox.2011.07.005.

[29] G. La Manna, N. Lanci, E. Della Bella, G. Comai, M.L. Cappuccilli, K. Nisi, P. Todeschini, E. Carretta, M.P. Scolari, S. Stefoni, Reduction of oxidative damage reflects a better kidney transplantation outcome, Am. J. Nephrol. 34 (6) (2011) 496–504, http://dx.doi.org/10.1159/000329320.

[30] M.L. Agraharkar, R.D. Cinclair, Y.F. Kuo, J.A. Daller, V.B. Shahinian, Risk of malignancy with long-term immunosuppression in renal transplant recipients, Kidney Int. 66 (1) (2004) 383–389, http://dx.doi.org/10.1111/j.1523-1755.2004.00741.x.

[31] H.C. Wisgerhof, L.G. van der Geest, J.W. de Fijter, G.W. Haasnoot, F.H. Claas, S. le Cessie, R. Willemze, J.N. Bouwes Bavinck, Incidence of cancer in kidney-transplant recipients: a long-term cohort study in a single center, Cancer Epidemiol. 35 (2) (2011) 105–111, http://dx.doi.org/10.1016/j.canep.2010.07.002.

[32] G. Wong, A. Hayen, J.R. Chapman, A.C. Webster, J.J. Wang, P. Mitchell, J.C. Craig, Association of CKD and cancer risk in older people, J. Am. Soc. Nephrol. 20 (6) (2009) 1341–1350, http://dx.doi.org/10.1681/ASN.2008090998.

5 ARTIGO II – Aceito para publicação

Association of *UGT2B7*, *UGT1A9*, *ABCG2*, and *IL23R* polymorphisms with rejection risk in kidney transplant patients

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Association of *UGT2B7*, *UGT1A9*, *ABCG2*, and *IL23R* polymorphisms with rejection risk in kidney transplant patients

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Running title: Polymorphism and rejection risk in kidney transplant

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Abstract

Despite advances in testing compatibility between donor and recipient, graft rejection remains a current concern. Single nucleotide polymorphisms (SNP) that codify altered enzymes of metabolism, drug transport, and the immune system may contribute to graft rejection in transplant patients. This study examined the association between SNP present in genes of these processes and occurrence of rejection episodes in 246 kidney transplant patients, 35% of which were diagnosed with rejection. Genotype-gene expression associations were also assessed. Peripheral blood samples were used for genotyping of 24 SNP on the following genes: *CYP3A4, CYP3A5, CYP2E1, POR, UGT2B7, UGT1A9, ABCB1, ABCC2, ABCG2, SLCO1B1, TNF, IL2, IRF5, TGFB1, NFKBIA, IL10, IL23R, NFAT,* and *CCR5* by real-time PCR. The analysis of gene expression was performed by RT-qPCR. The association between rejection episodes and polymorphic variants was assessed using odds ratios. Polymorphisms rs7662029 (*UGT2B7*) and rs6714486 (*UGT1A9*) were associated with occurrence of rejection episodes, rs7662029 (*UGT2B7*) exhibited a protective effect (1.85 fold) and rs6714486 (*UGT1A9*) an increased 1.6 fold increased risk of rejection. Among drug transporter genes, only rs2231142 (*ABCG2*) demonstrated an association with a 1.92-fold decrease in the risk of rejection. The immunological SNP rs10889677 (*IL23R*) was associated with a 1.9-fold enhanced risk of graft rejection. Association between genotypes and gene expression was not detected. Therefore, SNP of *UGT2B7, UGT1A9, ABCG2,* and *IL23R* genes may be useful as candidate markers for screening of risk graft rejection in renal transplant patients. These markers may improve medical decisions, avoiding adverse effects.

Introduction

In the absence of contraindications, kidney transplantation is considered the best option for restoring renal function in patients with advanced chronic kidney disease, not only for medical, social, and economic perspective, but also to increase survival of these patients (Wolfe et al., 1999). Following transplantation procedures, patients use immunosuppressive drugs to reduce the risk of organ rejection such as the triple immunosuppressive regimen consisting of corticosteroids, tacrolimus, and mycophenolate mofetil (MMF). However, prolonged use of these drugs triggers adverse effects (Chapman et al., 2005; Burckart and Amur, 2010), which limit the long-term benefits of transplantation. The adverse effects influence patients differently owing to inter-individual variability in the response to immunosuppressive drugs, a consequence that may arise, among other factors, from the presence of single nucleotide polymorphisms (SNP) in genes involved in the pharmacokinetics/ pharmacodynamics of these drugs. These SNP may be associated with rapid drug clearance, resulting in low plasma chemical concentrations, which initiate rejection episodes (Elens et al., 2011). In contrast, high concentrations of immunosuppressive drugs over a long period after transplantation might lead to (i) decline in renal function and/or graft loss due to nephrotoxicity (Fadili et al., 2013); (ii) loss of renal function due to immunological damage as evidenced by inflammation and antibody-mediated injuries (Ong and Gaston, 2015), and (iii) development of neoplasia and inflammation (Apel et al., 2013).

Phase I metabolism genes – namely cytochrome P450 (CYPs) and drug-transporting P-glycoprotein genes (ABCB1) have been reported as the most important genes involved in the pharmacokinetics/pharmacodynamics of calcineurin inhibitors (Hebert, 1997; MacPhee et al., 2005) and the immunosuppressant sirolimus (Sattler et al., 1992). Proteins encoded by *UGT2B7* and *UGT1A9* phase II metabolism genes are involved in mycophenolate mofetil (MMF) metabolism (Picard et al., 2004; Picard and Marquet, 2012), and genes coding for efflux pumps, such as multidrug resistance protein 2 (*ABCC2*), breast cancer resistance protein (BCRP, *ABCG2* gene), and organic anion-transporting polypeptide (*SLCO1B1*), are responsible for the elimination of the major metabolites of MMF (Miura et al., 2008). Therefore, the presence of polymorphisms in these genes might affect the availability of the immunosuppressive drugs and consequently result in a poor correlation between dose, plasma concentration, and therapeutic response (Hesselink et al., 2003; Zununi-Vahed et al., 2015).

Polymorphisms in genes of the immune system may also influence the occurrence of graft rejection episodes (Karimi et al., 2014) including cytokines and/or their receptors (such

as *IL2*, *IL10*, *TNF*, *IL23R*, and *CCR5*), growth factors (*TGFB1*) and regulatory factors (*IRF5*). Therefore, SNP may prove to be of therapeutic use as biomarkers to identify appropriate doses, predict patient tolerance to treatment and risk of graft rejection (Burckart and Amur, 2010), and consequently have potential to be utilized for prognosis and/or diagnosis of graft rejection episodes.

Taking this into consideration, the aim of this study was to examine the association between incidence of graft rejection and presence of SNP in phase I (*CYP3A4*, *CYP3A5*, *CYP2E1*, and *POR*) and phase II (*UGT2B7*, *UGT1A9*) metabolism, drug-transporting (*ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1*), and immune system genes (*TNF*, *IL2*, *IRF5*, *TGFB1*, *NFKBIA*, *IL10*, *IL23R*, *NFAT*, and *CCR5*).

Methods

Population studied

A total of 246 renal transplant patients undergoing post-transplant treatment at the Kidney Institute of Londrina (Londrina, PR, Brazil) were selected. These patients were classified into two groups, those who had graft rejection episodes (N = 86) and those with none (N = 160). Graft rejection episodes were confirmed by histological examination of the graft material obtained through biopsy.

The research protocol was approved by the Ethics Committee for Research in Human Beings at the State University of Londrina (CEPE/UEL 153/2013 CAAE: 18263413.4.0000.5231). Patients signed a Free and Informed Consent Form, filled a questionnaire regarding lifestyle and history of environmental exposure, and each individual received a code. Patients were considered smokers and/or alcoholics when they in an interview responded to consuming any amount of cigarettes or alcohol. Patients were divided into two groups: descendant of Caucasian and descendant of Afro/Asian (African (N = 47) and Asian (N = 2) descendant). Information regarding the transplantation process, such as immunosuppressive therapy, graft rejection history, and diseases frequently developed after transplantation, were obtained from medical records of the patients. Peripheral blood samples (4 ml) were collected intravenously from each patient in vacuum blood collection tubes (EDTA 6%) (Labor Import, Osasco, Brazil).

Analysis of polymorphic allelic variants

Genomic DNA was extracted from 200 µl blood, using the mini spin extraction kit (KASVI, Curitiba, Brazil; code K9-0250), following the manufacturer's recommendations. DNA samples were quantified using a NanoDrop 2000 spectrophotometer (ThermoScientific, Waltman, MA, USA).

The genes were selected based upon criteria previously associated with pharmacokinetics of immunosuppressive drugs (metabolism and drug transport genes) or with the immune response. Genotyping was performed by real-time PCR in a Quantica thermocycler (TECHNE, Staffordshire, UK), using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA), TaqMan Genotyping Master Mix (Applied Biosystems) (Table 1). Genotyping of the polymorphism rs333 of the *CCR5* gene was performed by conventional Polymerase Chain Reaction (PCR) in a Veriti 96-well thermocycler (Life Technologies of Brazil Ltda., São Paulo, Brazil), using 1.5 mM deoxynucleotide (dNTP), 30 mM $MgCl_2$, 2.5 µM of each primer, 0.5 U Taq DNA polymerase in 10X PCR buffer (Invitrogen-Life Technologies, São Paulo, Brazil), and 20 ng genomic DNA, in a final volume of 15 µl. The primers used were: sense 5' - ACC AGA TCT CAA AAA GAA - 3' and antisense 5' - CAT GAT GGT GAA GAT AAG CCT CA - 3'; the PCR conditions were: 94^{o}C for 5 min, 30 cycles consisting of 94^{o}C for 30 sec, 57^{o}C for 30 sec, and 72^{o}C for 30 sec, followed by a final annealing step of 10 min at 72^{o}C. The PCR product had a different size owing to a 32-bp deletion. The fragment was 225-bp long when the prevalent allele was present and 193 bp in the presence of the allele with the deletion. Genotypes were determined by electrophoresis in 10% polyacrylamide gels stained with silver nitrate (Quimex, Brazil).

Reverse Transcription Quantitative PCR (RT-qPCR)

Total RNA from 58 patients was extracted using TRIzol® Reagent (Ambion, Carlsbad, CA, USA) and PureLink® Total RNA Blood Kit (Ambion, Carlsbad, CA, USA); RNA samples were quantified in a Qubit 2.0 Fluorometer using the Qubit RNA HS Assay (Life Technologies, ref Q32855, Eugene, OR, USA) and its integrity was assessed using the Agilent 2100 Bioanalyzer RNA 6000 LabChip Kit (Agilent Technologies, Inc., Wilmington, DE, USA). For each sample, cDNA was synthesized using 500 ng total RNA and SuperScript® III (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations. Expression levels of *UGT2B7*, *UGT1A9*, *ABCG2*, and *IL23R* were evaluated by RT-qPCR. Predesigned oligonucleotide primers were purchased from Sigma-Aldrich (KiCqStart® SYBR® Green primers) and real-time thermocycler model

7900 (Applied Biosystems, USA) was employed. *GUSB* (fwd: CCTGCGTCCCACCTAGAATC, rev: ATACGGAGCCCCCTTGTCTG) and *PUM1* (fwd: CACAGACACCACCTCCTTCC, rev: CCATTCGTGAGTCCTCCCAG) genes were selected as a reference based on geNorm software analysis (http://medgen.ugent.be/~jvdesomp /genorm/).

Statistical analysis

The continuous variables age (years) and graft survival (years), and the categorical variables (gender, degree of HLA compatibility, tobacco and alcohol consumption, ancestry, use of immunosuppressive drugs, development of cancer, diabetes and cardiovascular disease after transplantation) were compared in groups of patients according to graft rejection episodes, using the Student's t-test. Univariate logistic regression analysis was performed associating rejection episodes with each of the variables. Hardy-Weinberg equilibrium and linkage disequilibrium analysis were performed using HAPLOVIEW version 4.1 (Barrett et al., 2005). The haplotypes and their frequencies were determined using the Program PHASE version 2.1 (Stephens and Donnelly, 2003).

Associations between graft rejection episodes, and genotypes and haplotypes were performed using multivariate logistic regression analysis with SPSS version 20 (IBM, Armonk, NY, USA), and results presented as odds ratios (OR) with a 95% confidence interval (CI). The degree of HLA compatibility and ancestry ($p < 0.2$ in the univariate logistic regression analysis) were included to adjust the multivariate model. The relative expression of each gene was performed using the ΔΔCt method (Pfaffl, 2001). Association between transcripts expression levels and different genotypes was performed using the Student's t-test. For all statistical tests used the criterion for significance was set at $p<0.05$.

Results

The main characteristics of the 246 patients included in this study (145 were men and 101 women) are showed in Table 2. Transplant patients displayed a mean age of 48.6 ± 12.6 years and duration of transplant ranging from six months to 34 years (10.6 ± 8.3 years). The comparison of these variables showed no significant differences in cases with or without graft rejection episodes.

Most grafts were obtained from living donors, 22% from identical living donors, 44.3% from haploidentical living donors, and 33.7% from deceased donors. Most patients were Caucasian (80.1%), followed by 19.9% Afro or Asian descendant. Eighteen patients (7.3%) reported being smokers and thirty-five (14.2%) consumed alcohol.

Triple immunosuppressive regimen (corticosteroids, tacrolimus, and MMF) was reported in 87 patients (35.4%); only two subjects (0.80%) did not use steroids and MMF was the second immunosuppressant more frequently utilized by individuals (58.5%). Among the most prevalent diseases presented after transplantation, the development of diabetes (21.5%), cardiovascular diseases (11.8%), and cancer (10.2%) were predominant (Table 2).

A total of 86 patients (34.9%) displayed graft rejection episodes. Multivariate regression analysis demonstrated that the degree of human leukocyte antigen (HLA) compatibility and ancestry are risk factors for graft rejection and therefore data were adjusted for these variables. Receiving the graft from an HLA-haploidentical living donor or from a deceased donor resulted in a marked greater than 2-fold increase in graft rejection risk. Afro or Asian descendant patients also were found to show a significant 2-fold rise in organ rejection risk.

All examined SNP were in Hardy-Weinberg equilibrium. The multivariate logistic regression analysis revealed four SNP *UGT2B7* (rs7662029), *UGT1A9* (rs6714486), *ABCG2* (rs2231142), and *IL23R* (rs10889677) with a significant association with rejection episodes (Table 3). Protection against organ rejection episodes was observed among carriers of the rare alleles of either the rs7662029 (UGT2B7) and rs2231142 (ABCG2) SNP. The association of the genotypes A/A and A/G in the polymorphism rs7662029 represented a significant 1.85-fold higher protective factor, while genotypes C/A and A/A at SNP rs2231142 resulted in a 1.92-fold marked decrease in rejection risk.

The presence of the polymorphisms rs6714486 (*UGT1A9*) and rs10889677 (*IL23R*) resulted in significant elevated risk of organ rejection, with the association of genotypes T/A and A/A at SNP rs6714486 showing a 1.6-fold significant rise in risk of rejection, while subjects with genotypes A/A and A/C at SNP rs10889677 of the *IL23R* gene displayed a 1.9-fold higher risk of developing rejection.

Two *UGT2B7* polymorphisms were in linkage disequilibrium ($D' = 0.99$; $r^2 = 0.97$). Linkage disequilibrium was also observed between three other SNP: rs4646450 (*CYP3A5*) and rs776746 (*CYP3A5*) ($D' = 0.92$; $r^2 = 0.51$); rs4646450 (*CYP3A5*) and rs4646437 (*CYP3A4*) ($D' = 0.90$; $r^2 = 0.45$), and rs4646437 (*CYP3A4*) and rs776746 (*CYP3A5*) ($D' =$

0.86; r^2 = 0.68). Using multivariate logistic regression analysis, the presence of these haplotypes did not show marked association with rejection episodes (data not shown).

ABCG2 and *IL23R* gene expression analysis demonstrated no marked association with the SNP mapped in these genes (rs2231142 and rs10889677, respectively) (Figure 1). Due to the low number of patients with genotype AA (rs10889677), analysis of association between gene expression and genotype was not performed. Furthermore, no detectable transcripts levels were found in peripheral blood samples for *UGT1A9* and *UGT2B7* genes.

Discussion

In our cohort of cases, 34.9% of the patients presented rejection episodes and the average duration of transplants was 10.6 ± 8.3 years. This rejection frequency is higher than described by Ro et al. (2012) in subjects where mean duration of kidney transplant of about 4.2 years had an organ rejection rate of 20.5% and Karimi et al. (2014) reported 28% rejection in patients after 3 months of transplant. One of the main risk factors for graft dysfunction and rejection is the compatibility between donor and recipient in the genes involved in human leukocyte antigen (HLA). Lack of compatibility reduces the long-term survival of the graft; therefore, molecular typing of HLA improves clinical outcomes (Tiercy, 2002). In this study patients who did not receive a graft from a live donor with identical HLA showed a 2-fold increased risk of graft rejection indicating that HLA compatibility enhancing organ graft survival.

Data also showed that Afro or Asian descendants patients displayed a higher than 2-fold risk of developing rejection than Caucasian descendants patients. This corroborates the findings of Palanisamy et al. (2015), which demonstrated that African-American (AA) patients displayed an 8% rise in graft loss after 5 years of transplantation compared with non-AA patients. Palanisamy et al. (2015) also noted that AA patients demonstrated a higher prevalence of hypertension, diabetes mellitus, acute rejection, delayed graft function, and elevated incidence of cardiovascular diseases, when compared with non-AA patients. In contrast, in the present study, a significant difference between incidence of these diseases and the two ethnic groups examined was not detected (data not shown).

SNP in genes involved in the phase I metabolism analyzed in this study were not markedly associated with graft rejection episodes. However, SNP in phase II metabolism genes (*UGT1A9* and *UGT2B7*) were associated with organ rejection episodes. After oral

administration, the MMF prodrug is usually hydrolyzed to mycophenolic acid (MPA), its active metabolite. The immunosuppressive effect of MPA is inactivated by UGT1A9 enzyme through glucuronidation producing its major metabolite, the mycophenolic acid glucuronide (MPAG) (Picard et al., 2004). However, MPA is also inactivated by the UGT2B7 enzyme, generating mycophenolic acyl-glucuronide acid (AcMPAG) (Shipkova et al., 1999). This metabolite, although produced in lower amounts, may induce pro-inflammatory responses that subsequently lead to various adverse effects (Wieland et al., 2000).

In this study, it was observed that the association between genotypes (T/A and A/A) of SNP rs6714486 (*UGT1A9)* enhanced the risk of graft rejection 1.6-fold. Our results corroborate those of Van Schaik et al. (2009), which also found a higher risk of rejection in transplant patients with the same allelic variants. The increased risk of rejection and its association with the *UGT1A9* gene was explained by Girard et al. (2004), who demonstrated that the polymorphism rs6714486 was associated with a 1.4-fold elevation in hepatic levels of the protein and a 1.9-fold increase in the glucuronidation of MPA. The enhanced expression and activity of UGT1A9 enzyme reduced the concentration of MPA and therefore diminished its immunosuppressive activity (Picard et al., 2004), promoting the initiation of organ rejection episodes.

Analysis of SNP rs7662029 (*UGT2B7* gene) showed that association of A/G and A/A genotypes produced a 1.85-fold greater protection against graft rejection. The effect of this polymorphism on gene expression has not been elucidated. This polymorphism is in strong linkage disequilibrium (LD) with the polymorphism rs7438135. According to Hu et al. (2014), 23 SNP mapped in the *UGT2B7* promoter region are in LD. These authors also observed LD between SNP rs7662029 and rs7438135, and reported that the haplotype carrying the G allele for the rs7438135 showed a 50% decrease in promoter activity of the gene and enzyme compared with the haplotype carrying the A allele (wild-type), thus identifying this polymorphism as functional. In the present study, the association of genotypes A/G and G/G demonstrated a non-significant trend toward protection, with an elevated 1.69-fold rise in protection against organ rejection episodes. This finding suggests that the presence of the G allele may contribute to a lower activity of the enzyme and consequently reduced inactivation of MPA into AcMPAG, resulting in a lower risk of rejection.

As the rs7662029 polymorphism (genotype AA) is in LD with rs7438135 (genotype GG), it is possible that patients with a combination of these genotypes possess lower levels of enzymatic activity, eliminating immunosuppressive drugs more slowly. Univariate logistic regression analysis of the haplotypes formed between these two genotypes showed marked

association with protection against rejection of almost 2-fold. However, multivariate analysis, which considered the degree of HLA compatibility and ancestry, found no significant association (data not shown).

MPA metabolites (MPAG and AcMPAG) are eliminated through the bile by drug efflux transporter proteins ABCC2 and ABCG2 (Kobayashi et al., 2004; Miura et al., 2008). Subsequently, some metabolites undergo deconjugation by bacteria and are resorbed in the gastrointestinal tract through enterohepatic recirculation. This occurs in 10–60% of all MPAG, producing a second peak in MPA drug concentration (Bullingham et al., 1988). In this study, the association of C/A and A/A genotypes of SNP rs2231142 of the *ABCG2* gene was associated with a 1.92-fold increase in protection against organ rejection episodes. The presence of this SNP leads to an amino acid change, from lysine to glutamine, at codon 141, which decreases expression of the protein by 50% compared with the prevalent genotype (Tamura et al., 2007). Functionally, this SNP affects stability of the ABCG2 protein in the endoplasmic reticulum and raises susceptibility to ubiquitin-mediated degradation by the proteasome (Furukawa et al., 2009). Patients with the C/A and A/A genotypes, compared with those with C/C genotype, demonstrated an elevation in the concentration of plasmatic MPAG (Miura et al., 2008), possibly as a result of a reduced efflux of MPAG into the bile ducts. This may account for protective effect in carriers of the rs2231142 minor allele, because reduced expression of the ABCG2 transporter for rs2231142 is in agreement with findings of Miura et al., (2008). This study demonstrated for the first time the association between SNP rs2231142 and diminished risk of graft rejection. However, additional studies are required to clarify the effect of this SNP on pharmacokinetics of immunosuppressive MMF.

Of the 10 immune system genes analyzed in this study, only IL23 receptor (*IL23R*) showed association with graft rejection episodes. This gene is involved in the inflammatory process mediated by T helper 17 cells and plays an important role in autoimmune inflammation (Zhou et al., 2013). A study by Tsai et al. (2011) with 422 Chinese renal transplant patients showed that the C allele of the rs10889677 polymorphism leads to a 1.79-fold increase in the risk of developing interstitial fibrosis and tubular atrophy in the graft. In the present study, the association of A/A and A/C genotypes of this SNP resulted in a 1.83-fold rise in graft rejection risk. Karini et al. (2014) also observed an increase in the incidence of acute rejection in renal transplant male patients with the A/A genotype.

According Zheng et al. (2012) and Zhou et al. (2013) the presence of the polymorphic variant A of rs10889677 in the 3'-UTR of the mRNA prevents binding of the microRNA let-7f and consequently increases transcription of *IL23R*. On the other hand, the C

allele enhances the binding affinity of the microRNA let-7f and thus negatively regulates *IL23R* expression (Zheng et al, 2012; Zhou et al, 2013). Vanden-Eijnden et al., (2005) found elevated expression of *IL23R* and amount of receptors present on the cell surface might assist in activation of IL23 interleukins, production of interferon-γ, and differentiation of T helper 1 cells, leading to increased inflammation and stimulation of pro-inflammatory cytokines levels. The findings cited above might account for the observed increased risk of rejection shown in our study.

In conclusion, this study demonstrated an association between SNP rs7662029 (*UGT2B7*), rs6714486 (*UGT1A9*), rs2231142 (*ABCG2*), and rs10889677 (*IL23R*), and the incidence of graft rejection episodes in 246 Brazilian kidney transplant patients. Although transcription studies in cell lines to determine the functionality of SNP were not conducted, these findings may contribute in the future to be used as candidate markers for screening kidney transplant patients with higher or lower rejection risk and thus help in medical management.

Conflict of interest

The authors declare no conflicts of interest.

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References

Apel, H., Walschburger-Zorn, K., Häberle, L., Wach, S., Engehausen, D. G., and Wullich, B. 2013. De novo malignancies in renal transplant recipients: experience at a single center with 1882 transplant patients over 39 yr. *Clin Transplant.* 27: E30-36.

Barrett, J. C., Fry, B., Maller, J., and Daly, M. J. 2005. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics*. 21: 263-265.

Bullingham, R. E., Nicholls, A. J., and Kamm, B. R. 1998. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet*. 34: 429-455.

Burckart, G. J. and Amur, S. 2010. Update on the clinical pharmacogenomics of organ transplantation. *Pharmacogenomics*. 11: 227-236.

Chapman, J. R., O'Connell, P. J., and Nankivell, B. J. 2005. Chronic renal allograft dysfunction. *J Am Soc Nephrol.*16: 3015-3026.

Elens, L., Bouamar, R., Hesselink, D. A., Haufroid, V., van der Heiden, I. P., van Gelder, T., and van Schaik, R. H. 2011. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. *Clin Chem*. 57: 1574-1583.

Fadili, W., Habib Allah, M., and Laouad, I. 2013. Chronic renal allograft dysfunction: risk factors, immunology and prevention. *Arab J Nephrol Transplant*. 6:45-50.

Furukawa, T., Wakabayashi, K., Tamura, A., Nakagawa, H., Morishima, Y., Osawa, Y., and Ishikawa, T. 2009. Major SNP (Q141K) variant of human ABC transporter ABCG2 undergoes lysosomal and proteasomal degradations. *Pharm Res*. 26: 469-479.

Girard, H., Court, M. H., Bernard, O., Fortier, L. C., Villeneuve, L., Hao, Q., Greenblatt, D. J., von Moltke, L. L., Perussed, L., and Guillemette, C. 2004. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. *Pharmacogenetics*. 14: 501-515.

Grinyó, J., Vanrenterghem, Y., Nashan, B., Vincenti, F., Ekberg, H., Lindpaintner, K., Rashford, M., Nasmyth-Miller, C., Voulgari, A., Spleiss, O., Truman, M., and Essioux, L. 2008. Association of four DNA polymorphisms with acute rejection after kidney transplantation. *Transplant Int*. 21: 879-891.

Hebert, M. F. 1997. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. *Adv Drug Deliv Rev*. 27: 201-214.

Hesselink, D. A., van Schaik, R. H., van der Heiden, I. P., van der Werf, M., Gregoor, P. J., Lindemans, J., Weimar, W., and van Gelder, T. 2003. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clin Pharmacol Ther*. 74: 245-254.

Hu, D. G., Meech, R., Lu, L., McKinnon, R. A. and Mackenzie, P. I. 2014. Polymorphisms, and haplotypes of the UDP-glucuronosyltransferase 2B7 gene promoter. *Drug Metab Dispos*. 42: 854-862.

Karimi, M. H., Hejr, S., Geramizadeh, B., Yaghobi, R., Sagheb, M. M., and Kamali-Sarvestani, E. 2014. Combined analysis of cytokine gene polymorphism and the level of expression with allograft function in kidney transplant recipients. *Transplant Immunol*. 30: 46-51.

Kobayashi, M., Saitoh, H., Kobayashi, M., Tadano, K., Takahashi, Y., and Hirano, T. 2004. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. *J Pharmacol Exp Ther*. 309: 1029-1035.

Miura, M., Kagaya, H., Satoh, S., Inoue, K., Saito, M., Habuchi, T., and Suzuki, T. 2008. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. *Ther Drug Monit*. 30: 559-564.

MacPhee, I. A. M., Fredericks, S., Mohamed, M., Moreton, M., Carter, N. D., Johnston, A., Goldberg, L., and Holt, D. W. 2005. Tacrolimus pharmacogenetics: The CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in Whites and South Asians. *Transplantation*. 79: 499-502.

Ong, S. C. and Gaston, R. S. 2015. Medical management of chronic kidney disease in the renal transplant recipient. *Curr Opin Nephrol Hypertens*. 24: 587-593.

Palanisamy, A. P., Schiltz, C. E., Pilch, N. A., Hunt, K. J., Nadig, S. N., Dowden, J. E., McGillicuddy, J. W., Baliga, P. K., Chavin, K. D., and Taber, D. J. 2015. Cardiovascular risk factors contribute to disparities in graft outcomes in African American renal transplant recipients: a retrospective analysis. *Blood Press*. 24: 14-22.

Picard, N. and Marquet, P. 2012. Pharmacogenomics of immunosuppressants. In: Dasgupta LJLAA. (Ed.). Pharmacogenomics in Clinical Therapeutics. 1. Wiley-Blackwell, cap. 6. 1: 70-97.

Picard, N., Ratanasavanh, D., Prémaud, A., Le Meur, Y., and Marquet, P. 2004. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. *Drug Metab Dispos*. 33: 139-146, 2004.

Pfaffl, M. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucl Acids Res.* 29: e45.

Ro, H., Min, S. I., Yang, J., Moon, K. C., Kim, Y. S., Kim, S. J., Ahn, C., and Ha, J. 2012. Impact of tacrolimus intraindividual variability and CYP3A5 genetic polymorphism on acute rejection in kidney transplantation. *Ther Drug Monit*. 34: 680-685.

Sattler, M., Guengerich, F.P., Yun, C.H., Christians, U., and Sewing, K.F. 1992. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. *Drug Metab Dispos*. 20: 753-761.

Shipkova, M., Armstrong, V.W., Wieland, E., Niedmann, P.D., Schütz, E., Brenner-Weiss, G., Voihsel, M., Braun, F., and Oellerich, M. 1999. Identification of glucoside and carboxyl-linked glucuronide conjugates of mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. *Br J Pharmacol*. 126: 1075-1082.

Stephens, M. and Donnelly, P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Human Genet*. 73:1162-1169.

Tamura, A., Wakabayashi, K., Onishi, Y., Takeda, M., Ikegami, Y., Sawada, S., Tsuji, M., Matsuda, Y., and Ishikawa, T. 2007. Re-evaluation and functional classification of non-synonymous single nucleotide polymorphisms of the human ATP-binding cassette transporter ABCG2. *Cancer Sci*. 98: 231-239.

Tiercy, J. M. 2002. Molecular basis of HLA polymorphism: Implications in clinical transplantation. *Transpl Immunol*. 9: 173-180.

Tsai, J. P., Yang, S. F., Wu, S. W., Hugn, T. W., Tsai, H C., Lian, J. D. and Chang, H. D. 2011. Association between interleukin 23 receptor polymorphism and kidney transplant outcomes: A 10-year Taiwan cohort study. *Clin Chim Acta*. 412: 958-962.

van Schaik, R. H., van Agteren, M., de Fijter, J. W., Hartmann, A., Schmidt, J., Budde, K., Kuypers, D., Le Meur, Y., van der Werf, M., Mamelok, R., and van Gelder, T. 2009. UGT1A9 -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. *Clin Pharmacol Ther*. 86: 319-327.

Vanden Eijnden, S., Goriely, S., De Wit, D., Willems, F., and Goldman, M. 2005. IL-23 up-regulates IL-10 and induces IL-17 synthesis by polyclonally activated naive T cells in human. *Eur J Immunol*. 35: 469-475.

Wieland, E., Shipkova, M., Schellhaas, U., Schütz, E., Niedmann, P. D., Armstrong, V. W., and Oellerich, M. 2000. Induction of cytokine release by the acyl glucuronide of mycophenolic acid: A link to side effects? *Clin Biochem*. 33: 107-113.

Wolfe, R. A., Ashby, V. B., Milford, E. L., Ojo, A. O., Ettenger, R. E., Agodoa, L. Y., Held, P. J., and Port, F. K. 1999. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med*. 341: 1725-1730.

Zheng, J., Jiang, L., Zhang, L., Yang, L., Deng, J., You, Y., Li, N., Wu, H., Li, W., Lu, J., and Zhou, Y. 2012. Functional genetic variations in the IL-23 receptor gene are associated with risk of breast, lung and nasopharyngeal cancer in Chinese populations. *Carcinogenesis*. 33: 2409-2016.

Zhou, S., Ruan, Y., Yu, H., Chen, Y., Yao, Y., Ma, Y., and Gao, Y. 2013. Functional IL-23R rs10889677 genetic polymorphism and risk of multiple solid tumors: a meta-analysis. *PLoS One.* 8: e80627.

Zununi Vahed, S., Ardalan, M., Samadi, N., and Omidi, Y. 2015. Pharmacogenetics and drug-induced nephrotoxicity in renal transplant recipients. *Bioimpacts*. 5: 45-54.

Table 1: Genes evaluated in renal transplant patients, their polymorphisms and type of mutation that originated them.

Gene	SNP_ID	Alternative nomenclature	Change	Chromosome location
CYP3A4	rs35599367	15389C>T (CYP3A4*22)	Intron	7q22.1
	rs4646437	-	Intron	
CYP3A5	rs776746	6986A>G (CYP3A5*3)	Intron, splice acceptor variant	7q21.1
	rs4646450	-	Intron	
CYP2E1	rs3813867	-1295G>C *(CYP2E1*5B)*	Promoter	10q24.3
POR	rs1057868	(POR*28)	Exon (Ala503Val)	7q11.2
UGT1A9	rs6714486	-275T>A	Promoter	2q37
UGT2B7	rs7662029	-327G>A	Promoter	4q13
	rs7438135	-900A>G / -842A>G	Promoter	
ABCB1	rs1045642	3435C>T	Exon (Ile1145Ile)	7q21.12
ABCC2/MRP2	rs717620	-24C>T	Promoter (5'UTR)	10q24
	rs2273697	1249G>A	Exon (Ile417Val)	
ABCG2/BCRP	rs2231142	421C>A	Exon (Lys141Gln)	4q22
SLCO1B1	rs4149056	521T>C (SLCO1B1*5)	Exon (Val174Ala)	12p
TNF	rs1800629	-308G>A	Promoter	6p21.3
TGFB1	rs1800470	29T>C	Exon (Leu10Pro)	19q13.1-13.3
	rs1800471	915G>C	Exon (Arg25Pro)	
IL2	rs2069762	-330G>T	Promoter	4q26-q27
IL10	rs1800872	-592C>A	Promoter (5'UTR)	1q31-q32
IL23R	rs10889677	-	3'UTR	1p31.3
IRF5	rs3757385	-	Promoter	7q32
NFAT	rs10141896	*NFATC4*	Intron	14q11.2
NFKBIA	rs696	2758A>G	3'UTR	14q13
CCR5	rs333	CCR5Δ32	Intron deletion	3p21

Table 2: General and clinical characteristics of kidney transplant patients (246) who developed and who did not develop rejection episodes.

Characteristics of patients	General (n = 246)	With rejection episode(s) (n = 86)	Without rejection episode(s) (n = 160)
	N (%)	N (%)	N (%)
Gender			
Male	145 (58.9)	53 (61.6)	92 (57.5)
Female	101 (41.1)	33 (38.4)	68 (42.5)
Degree of HLA[a] compatibility			
Live HLA-identical	54 (22.0)	11 (12.8)	43 (26.9)*
Live HLA-haploidentical	109 (44.3)	39 (45.3)	70 (43.8)
Deceased unrelated donor	83 (33.7)	36 (41.9)	47 (29.4)
Smoker			
Yes	18 (7.30)	5 (5.80)	13 (8.10)
No	228 (92.7)	81 (94.2)	147 (91.9)
Alcoholic			
Yes	35 (14.2)	13 (15.1)	22 (13.8)
No	211 (85.8)	73 (84.9)	138 (86.2)
Ancestry			
Caucasian	197 (80.1)	62 (72.1)	135 (84.4)*
Afro or Asian descendant	49 (19.9)	24 (27.9)	25 (15.6)
Use of immunosuppressive			
Cyclosporine	47 (19.1)	20 (23.3)	27 (16.9)
Tacrolimus	112 (45.5)	35 (40.7)	77 (48.1)
Sirolimus	16 (6.50)	6 (7.00)	10 (6.30)
AZA[b]	61 (24.8)	20 (23.3)	41 (25.6)
MMF[c]	144 (58.5)	49 (57.0)	95 (59.4)
Steroids	244 (99.2)	86 (100)	158 (98.8)
MMF+Tacrolimus +Steroids	87 (35.4)	24 (27.9)	63 (39.4)
Development of disease			
Cancer	25 (10.2)	7 (8.10)	18 (11.3)
Diabetes	53 (21.5)	19 (22.1)	34 (21.3)
Cardiovascular	29 (11.8)	18 (20.9)	11 (6.90)*
Warts	91 (37.0)	35 (40.7)	56 (35.0)

[a]HLA – Human Leukocyte Antigen, [b]AZA – Azathioprine, [c]MMF – mycophenolate mofetil; *$p<0.05$ (Student's t-test comparing patients with rejection and without rejection).

Table 3: Genotypic frequencies of genes *CYP3A4*, *CYP3A5*, *CYP2E1*, *POR*, *UGT1A9*, *UGT2B7*, *ABCB1*, *ABCC2*, *ABCG2*, *SLCO1B1*, *TNF*, *TGFB1*, *IL2*, *IL10*, *IL23R*, *IRF5*, *NFAT*, *NFKBIA* and *CCR5* in 246 kidney transplant patients, and the association between gene polymorphisms and rejection episodes.

Genes (SNP_ID)	**Genotypes**	**With rejection episode(s) n (%)**	**Without rejection episode(s) n (%)**	***Odds Ratio*[a] (CI95%)**
CYP3A4 (rs35599367)	C/C	81 (94.2)	153 (95.6)	Ref.[b]
	C/T	5 (5.80)	7 (4.40)	1.5 (0.58-4.0)
CYP3A4 (rs4646437)	G/G	54 (62.8)	106 (66.3)	Ref.
	G/A	25 (29.1)	45 (28.1)	0.95 (0.56-1.6)
	A/A	7 (8.10)	9 (5.60)	1.1 (0.41-3.0)
	A/G and A/A	32 (37.2)	54 (33.7)	0.98 (0.66-1.4)
CYP3A5 (rs776746)	G/G	53 (61.6)	102 (63.8)	Ref.
	A/G	25 (29.1)	49 (30.6)	0.87 (0.47-1.6)
	A/A	8 (9.30)	9 (5.60)	1.3 (0.41-4.0)
	A/A and A/G	33 (38.4)	58 (36.2)	0.92 (0.57-1.5)
CYP3A5 (rs4646450)	C/C	33 (38.4)	85 (53.1)	Ref.
	C/T	41 (47.7)	58 (36.2)	1.5 (0.78-3.1)
	T/T	12 (13.9)	17 (10.6)	1.6 (0.54-4.7)
	C/T and T/T	53 (61.6)	75 (46.8)	1.6 (0.77-3.2)
CYP2E1 (rs3813867)	G/G	77 (89.5)	136 (85.0)	Ref.
	G/C and C/C	9 (10.5)	24 (15.0)	0.69 (0.24-2.0)
POR (rs1057868)	C/C	42 (48.8)	91 (56.9)	Ref.
	C/T	36 (41.9)	59 (36.9)	1.4 (0.71-2.7)
	T/T	8 (9.30)	10 (6.20)	2.4 (0.72-8.4)
	C/T and T/T	44 (51.2)	69 (43.1)	1.5 (0.78-3.0)

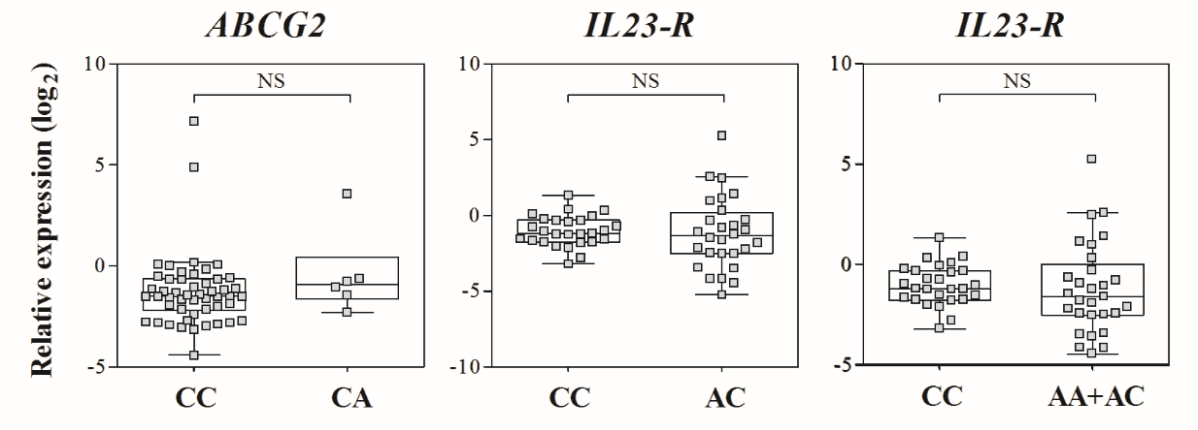
***UGT1A9* (rs6714486)**	T/T	69 (80.2)	142 (88.7)	Ref.
	T/A and A/A	17 (19.8)	18 (11.3)	1.6 (1.0-2.5)*
UGT2B7 (rs7662029)	G/G	40 (46.5)	48 (30.0)	Ref.
	A/G	39 (45.4)	89 (55.6)	0.57 (0.32-1.0)
	A/A	7 (8.10)	23 (14.4)	0.42 (0.16-1.1)
	A/A and A/G	46 (53.5)	112 (70.0)	0.54 (0.3-1.0)*
UGT2B7 (rs7438135)	A/A	39 (45.3)	49 (30.6)	Ref.
	A/G	39 (45.3)	87 (54.4)	0.61 (0.35-1.1)
	G/G	8 (9.30)	24 (15.0)	0.48 (0.19-1.2)
	A/G and G/G	47 (54.6)	111 (69.4)	0.59 (0.33-1.0)
ABCB1 (rs1045642)	C/C	34 (39.5)	56 (35.0)	Ref.
	T/C	39 (45.4)	72 (45.0)	0.96 (0.61-1.5)
	T/T	13 (15.1)	32 (20.0)	0.70 (0.38-1.3)
	T/C and T/T	52 (60.5)	104 (65.0)	0.88 (0.60-1.3)
ABCC2 (rs717620)	C/C	61 (71.0)	104 (65.0)	Ref.
	C/T	23 (26.7)	51 (31.9)	0.82 (0.43-1.6)
	T/T	2 (2.30)	5 (3.10)	0.65 (0.10-4.1)
	C/T and T/T	25 (29.0)	56 (35.0)	0.81 (0.50-1.3)
ABCC2 (rs2273697)	G/G	54 (62.8)	103 (64.4)	Ref.
	A/G	30 (34.9)	50 (31.2)	1.1 (0.74-1.7)
	A/A	2 (2.30)	7 (4.40)	0.52 (1.6-1.7)
	A/G and A/A	32 (37.2)	57 (35.6)	1.0 (0.75-1.5)
***ABCG2* (rs2231142)**	C/C	76 (88.4)	129 (80.6)	Ref.
	C/A and A/A	10 (11.6)	31 (19.4)	0.52 (0.28-0.96)*

SLCO1B1 (rs4149056)	T/T	63 (73.2)	107 (66.9)	Ref.
	T/C	20 (23.3)	48 (30.0)	0.75 (0.31-1.8)
	C/C	3 (3.50)	5 (3.10)	1.0 (0.13-8.2)
	T/C and C/C	23 (26.8)	53 (33.1)	0.77 (0.40-1.5)
TNF (rs1800629)	G/G	63 (73.3)	116 (72.5)	Ref.
	G/A and A/A	23 (26.7)	44 (27.7)	0.94 (0.41-2.1)
TGFB1 (rs1800470)	C/C	26 (30.2)	39 (24.4)	Ref.
	C/T	34 (39.6)	83 (51.9)	0.72 (0.35-1.5)
	T/T	26 (30.2)	38 (23.7)	1.1 (0.50-2.5)
	C/T and T/T	60 (69.8)	121 (75.6)	0.85 (0.56-1.3)
TGFB1 (rs1800471)	G/G	73 (84.9)	137 (85.6)	Ref.
	G/C and C/C	13 (15.1)	23 (14.4)	1.1 (0.46-2.5)
IL2 (rs2069762)	T/T	48 (55.8)	91 (56.9)	Ref.
	T/G	32 (37.2)	58 (36.2)	1.2 (0.64-2.1)
	G/G	6 (7.00)	11 (6.90)	1.0 (0.34-3.2)
	T/G and G/G	38 (44.2)	69 (43.1)	1.1 (0.65-2.0)
IL10 (rs1800872)	C/C	32 (37.2)	63 (39.4)	Ref.
	C/A	44 (51.2)	77 (48.1)	1.1 (0.76-1.6)
	A/A	10 (11.6)	20 (12.5)	0.91 (0.51-1.6)
	C/A and A/A	54 (62.8)	97 (60.6)	1.1 (0.81-1.4)
***IL23R* (rs10889677)**	C/C	33 (38.4)	85 (53.1)	Ref.
	A/C	43 (50.0)	66 (41.3)	1.7 (0.98-3.1)
	A/A	10 (11.6)	9 (5.60)	3.0 (1.1-8.5)*
	A/A and A/C	53 (61.6)	75 (46.9)	1.9 (1.3-2.7)*

IRF5 (rs3757385)	T/T	17 (19.8)	29 (18.1)	Ref.
	T/G	43 (50.0)	79 (49.4)	1.1 (0.48-2.6)
	G/G	26 (30.2)	52 (32.5)	0.94 (0.38-2.3)
	T/G and G/G	69 (80.2)	131 (81.9)	1.0 (0.62-1.8)
NFAT (rs10141894)	G/G	81 (94.2)	150 (93.7)	Ref.
	G/T and T/T	5 (5.80)	10 (6.30)	0.92 (0.22-3.8)
NFKBIA (rs696)	G/G	30 (34.9)	59 (36.9)	Ref.
	G/A	42 (48.8)	73 (45.6)	0.97 (0.41-2.3)
	A/A	14 (16.3)	28 (17.5)	0.81 (0.26-2.6)
	G/A and A/A	56 (65.1)	101 (63.1)	0.92 (0.63-1.4)
CCR5 (n=244)	wt/wt	77 (91.7)	150 (93.7)	Ref.
n=244	wt/Δ32	7 (8.30)	10 (6.30)	1.2 (0.48-3.0)

[a]Odds Ratio (calculated by logistic regression using degree of HLA compatibility and ancestry as covariates); [b]Ref – genotype used as reference; *$p < 0.05$.

Figure 1: Relative expression of *ABCG2* and *IL23R* detected by RT-qPCR in different genotypes of each gene. The transcript expression values are shown in log scale.*: *P* value < 0.05.



6 ARTIGO III

Polymorphisms in *IMPDH2*, *UGT2B7*, and *CES2* genes influence the risk of graft rejection in kidney transplant recipients taking mycophenolate mofetil

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Polymorphisms in *IMPDH2*, *UGT2B7*, and *CES2* genes influence the risk of graft rejection in kidney transplant recipients taking mycophenolate mofetil

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Abstract

The immunosuppressant mycophenolic acid (MPA), derived from the prodrug mycophenolate mofetil (MMF), is a drug used widely by kidney transplant recipients. This drug selectively inhibits inosine monophosphate dehydrogenase that controls the proliferation of lymphocytes, aiding in the prevention of rejection of the transplanted organ. Polymorphisms in key genes involved in MMF metabolism may alter the function of the enzymes encoded by them and contribute to interindividual variability in the response to the drug and its efficacy. The aim of this study was to investigate the association of nine polymorphic variants of eight genes involved in MMF pharmacokinetics, with rejection and adverse effects exhibited by kidney transplant recipients who use this drug. Our sample comprised 145 patients of the average age 46.9 ± 12.5 years, who underwent transplantation 7.00 ± 5.71 years ago. The combination of the T/C and C/C genotypes of the polymorphism rs11706052 (*IMPDH2*) was associated with a 4.2-fold protection, and the combination of the genotypes A/G and G/G of the polymorphism rs7438135 (*UGT2B7*) showed a 2.4-fold protection, against rejection. The T/T genotype of the polymorphism rs2241409 (*CES2*) was associated with a 7.2-fold increased risk of rejection. In the analysis using only patients receiving tacrolimus associated with MMF and corticoids, the association of T/C and C/C genotypes in the SNP rs11706052 (IMPDH2) demonstrated association with protection against rejection of 15.6-fold. Therefore, these polymorphisms that showed a strong association with rejection episodes should be considered in future studies as new prognostic markers for rejection in patients treated with MMF.

Key-words: Renal transplantation, acid mycophenolic, allelic variants, metabolism gene, drug transporter gene, rejection episodes.

Introduction

Mycophenolate mofetil (MMF) is a drug widely used as an immunomodulator by transplant patients [1,2] and patients with autoimmune diseases [3]. MMF is commonly combined with calcineurin inhibitors and corticoids in kidney transplant recipients, and they act at different stages of the lymphocyte proliferation pathway, inhibiting lymphocyte proliferation and preventing organ rejection [4].

MMF is a prodrug, which is hydrolyzed to mycophenolic acid (MPA) after oral administration (Figure 1). Though this activation of the drug occurs mainly in the liver catalyzed by the carboxylesterase enzymes, CES1 and CES2, it may also occur in the intestine and blood through the action of CES2 and acetylcholinesterase, respectively [5,6]. Hence, polymorphisms in genes responsible for MMF activation may contribute to interindividual variations and affect the efficacy of this drug.

The immunosuppressive activity of MPA occurs through the inhibition of inosine 5'-monophosphate dehydrogenase (IMPDH) [6], which leads to a reduction in guanine nucleotide synthesis that is essential for DNA synthesis. Selective inhibition of T and B lymphocyte proliferation occurs because lymphocytes, unlike other cell types, are exclusively dependent on the pathway catalyzed by IMPDH, for guanine nucleotide synthesis [7].

In addition to causing depletion of guanine nucleotides, MPA also induces apoptosis in activated T lymphocytes, inhibits the formation of antibodies, and the expression of glycoproteins and adhesion molecules responsible for the recruitment of monocytes and lymphocytes to sites of inflammation, which occurs during graft rejection [1, 8-9].

IMPDH has two isoforms, *IMPDH1* and *IMPDH2*, which are located on chromosomes 7q31.3 and 3p21.2, respectively, and show 84% homology [10]. *IMPDH1* is expressed in all tissues to maintain the basal level of guanine nucleotides, while *IMPDH2* is expressed in activated lymphocytes thus increasing their proliferation [1]. Variations in the activity of these proteins are observed among patients and can be explained by the presence of genetic polymorphisms. However, knowledge of the consequences of polymorphisms and their association with the response to MPA treatment is still scarce [2].

The inactivation of MPA is catalyzed by uridine 5'-diphospho-glucuronosyltransferases (UGTs). The UGT2B7 enzyme transforms MPA into MPA acyl glucuronide (AcMPAG) and the enzyme UGT1A9 transforms MPA into MPA glucuronide (MPAG). These two metabolites are excreted in the urine and bile. Biliary excretion is performed by multidrug resistance protein 2 (MRP2/*ABCC2*) and organic anion-transporting

polypeptides (*SLCO1B1*) present on the surface of hepatocytes, and a part of the excreted MPAG undergoes intestinal bacterial deconjugation, returns to its active form, and contributes to the enterohepatic recirculation of MPA [8, 11-12].

Polymorphisms in genes related to MMF activation (*CES1* and *CES2*), the mechanism of action (*IMPDH1* and *IMPDH2*), drug transporters (*ABCC2* and *SLCO1B1*), and MPA inactivation (*UGT1A9* and *UGT2B7*), may affect drug availability and cause interindividual variations in the efficacy of treatment with MPA. In this study, we selected nine single nucleotide polymorphisms (SNPs) in genes that are involved in the pharmacokinetics/pharmacodynamics of MMF to assess whether they contribute to the incidence of rejection in kidney transplant recipients.

Methods

Population studied

A total of 145 kidney transplant recipients undergoing post-transplant treatment at the Kidney Institute of Londrina (Londrina, PR, Brazil) and that immunosuppressive therapy consisted of MMF and corticosteroid combined or not with a calcineurin inhibitor or mTOR inhibitor were selected. These patients were classified into two groups, those who had graft rejection episodes (N = 49) and those with none (N = 96). Graft rejection episodes were confirmed by histopathological examination of the graft material obtained through biopsy.

The research protocol was approved by the Ethics Committee for Research in Human Beings at the State University of Londrina (CEP/UEL 153/2013 CAAE: 18263413.4.0000.5231). Patients signed a Free and Informed Consent Form, filled a questionnaire regarding lifestyle and history of environmental exposure. Patients were considered smokers and/or alcoholics when they in an interview responded to consuming any amount of cigarettes or alcohol. Peripheral blood samples (1 mL) were collected intravenously from each patient in vacuum blood collection tubes (EDTA 6%) (Labor Import, Osasco, Brazil).

Information regarding the transplantation process, such as immunosuppressive therapy, graft rejection history, and diseases frequently developed after transplantation, were obtained from medical records of the patients.

Analysis of polymorphic allelic variants

Genomic DNA was extracted from 200 µL of blood, using the mini spin extraction kit (KASVI, Curitiba, Brazil; code K9-0250), following the manufacturer's recommendations. DNA samples were quantified using a NanoDrop 2000 spectrophotometer (ThermoScientific, Waltman, MA, USA). Genotyping was performed by real-time PCR in a Quantica thermocycler (TECHNE, Staffordshire, UK), using 0.5 µL TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA), 5 µL TaqMan Genotyping Master Mix (Applied Biosystems) and 5 ng of genomic DNA. Table 1 shows the genes and their respective SNPs evaluated.

Statistical analysis

The continuous variables age (years) and time after transplantation (years), and the categorical variables (gender, degree of HLA compatibility, tobacco and alcohol consumption, use of immunosuppressive drugs, development of cancer and diabetes after transplantation) were compared in groups of patients according to graft rejection episodes, using the Student's t-test.

Associations between graft rejection episodes and genotypes were performed using multivariate logistic regression analysis with SPSS version 20 (IBM, Armonk, NY, USA), and results presented as Odds Ratios (OR) with a 95% confidence interval (CI). The time after transplantation, alcohol consumption and use of the immunosuppressive tacrolimus and ciclosporine ($p < 0.2$ in the univariate logistic regression analysis) were included to adjust the multivariate model.

Associations between graft rejection episodes and genotypes were performed in patients receiving ciclosporin (N = 20) or tacrolimus (N = 89) associated with MMF and corticoids as immunosuppressants. The time after transplantation and alcohol consumption were included to adjust the multivariate model.

Results

Table 2 shows the general and clinical characteristics of patients who exhibited (33.8%) and those who did not exhibit (66.2%) rejection. Differences were observed between the averages of two variables—time after transplantation and alcohol consumption (Student's t test, $p < 0.050$).

Of the nine SNPs evaluated, rs11706052 (*IMPDH2*) and rs7438135 (*UGT2B7*) showed a significant association with protection against rejection episodes and rs2241409 (*CES2*) was associated with an increased risk of rejection (Table 3).

The frequency of the C/C genotype of rs11706052 (*IMPDH2*) was 1.4%. Since only two patients were carriers of this genotype, we performed the analysis using the sum of T/C and C/C genotypes. In the multivariate logistic regression analysis, patients with T/C and C/C genotypes showed a 4.2-fold increased protection against rejection.

Patients with the G/G and A/G genotypes in rs7438135 (*UGT2B7*) showed a 2.4-fold increased protection against rejection. The T/T genotype of rs2241409 (*CES2*) was associated with a 7.2-fold increased risk of rejection.

When the analysis was performed among patients receiving cyclosporine or tacrolimus associated with MMF and corticoids, it was observed association with rejection only between the association of T/C and C/C genotypes in the SNP rs11706052 (*IMPDH2*) with a protection against rejection 15.6-fold (OR = 0.064 (0.009-0.463) p = 0.006), in patients who take tacrolimus.

The SNPs rs62028647 (*CES1*), rs2278293 (*IMPDH1*), rs6714486 (*UGT1A9*), rs717620 (*ABCC2*), rs2273607 (*ABCC2*), and rs4149056 (*SLCO1B1*) were not associated with the risk of graft rejection.

Discussion

In the present study it was observed that renal transplant patients who used any amount of alcohol had an increase of rejection episodes. According to a review performed by Parker et al. [13], the alcohol after transplantation is associated with poor medication compliance and this may increase risk of graft loss. Others authors showed that the intake of alcohol may induce complications like hypertension [14], that contribute to kidney injure and consequently with for occurrence of rejection. Patients with more time after transplant also present increasing in rejection episodes (Table 2); this can be explained by the appearance of complications such as diabetes, nephrotoxicity caused by immunosuppressive drugs, chronic allograft dysfunction, infection and cancer; these side effects has significant negative long-term consequences that may well be worse with the increase of ages [15].

Hydrolysis of MMF is the first step in the pharmacokinetics of this drug, which is performed primarily by the genes, *CES1* and *CES2*. In this study, no association was observed

between the SNP rs62028647 (*CES1*) and episodes of rejection. However, our results demonstrate that patients with the T/T genotype of the SNP rs2241409 (*CES2)* had a 7.2-fold increased risk of graft rejection. Fujiyama et al. [5] found no association between this SNP and episodes of acute rejection in 80 Japanese renal transplant patients, 28 days after transplantation. They assessed the association of rs2241409 with interindividual variation in MPA pharmacokinetics, and found no significant differences in dose-adjusted C_{max}, $AUC_{0–6}$ and t_{max} values for MPA between different *CES2* genotype groups. They also found that the dose-adjusted $AUC_{0–12}$ of MPAG was significantly greater in recipients with the *CES2* T allele than in those with the C allele; however, this observation was not explored further.

There is scarce data on the contribution of rs2241409 to *CES2* function. Therefore, future studies with larger samples may shed more light on the function of this SNP and its influence on the prognosis of graft rejection in kidney transplant recipients.

The immunosuppressive activity of MPA occurs through the selective inhibition of IMPDHs, enzymes responsible for the synthesis of purines that are essential for the proliferation of T and B-lymphocytes [16]. Thus, quantitative or qualitative changes in these enzymes interfere with immune responses and may alter the efficacy of immunosuppressive therapy.

In this study, the presence of at least one C allele (T/C and C/C) in the SNP rs11706052 (*IMPDH2)* resulted in a 4.2-fold increased protection against rejection. When this SNP was associated with the occurrence of rejection episodes only among patients who used the immunosuppressive drugs MMF, tacrolimus and corticoid, the protection observed increased to more than 15-fold. This result warrants future studies, since conflicting data have been reported previously. Several studies have found no association between this SNP and rejection episodes in renal transplant recipients [2, 17-19]. However, Sombogaard et al. [17] demonstrated its association with the lowest inhibition of IMPDH2 in 101 MMF-treated kidney transplants, six days after transplantation, observing that patients with this polymorphic variant showed higher IMPDH2 enzyme activity and reduced the anti-proliferative effect of MPA on lymphocytes. Pazik et al. [18] reported that the C allele of rs11706052 is associated with reduced incidence of leukopenia in renal transplant patients, whereas Grinyó et al. [20] reported an association between this allele and a 3.39-fold increased risk of rejection, in the first year after transplantation.

The lack of replicability of the effects of rs11706052 on rejection episodes can be attributed to differences in the post-transplant time points chosen for evaluation. Here, we evaluated patients who developed both acute and chronic rejection, with post-transplant times

ranging from six months to 27 years (average 7.00 ± 5.71 years). Grinyó et al. [20] and Shah et al. [19] studied the association of rs11706052 with rejection only during the first year post-transplantation.

In the present study, it was not find a significant association between rs2278293 (*IMPDH1*) and rejection episodes in kidney transplant recipients, consistent with other studies [2, 19, 21]. However, it has been reported to be associated with a lower incidence of rejection in kidney transplant recipients during the first year post-transplantation (OR = 0.34, p = 0.008) [22].

We found that the G (G/G and G/A) allele of the SNP rs7438135 (*UGT2B7)* is associated with a 2.4-fold protection against rejection. Some studies suggest that the presence of the G variant in this SNP is related to lower UGT2B7 protein activity, compared to the A allele [23-24].

Duguay et al. [23] evaluated the function of the haplotype -1248G, -1241C, -1054C, -842A, -268G, and -102C in the proximal promoter region of *UGT2B7*, using plasmids containing different associations of these polymorphisms. They observed a 2-fold increase in gene activity, suggesting that higher transcriptional activity is caused by the A-allele of the -842 polymorphism (rs7438135). It was also suggested that this variant should have a minimal impact on the binding of transcription factors, and that it is unlikely that it alters the rate of glucuronidation of drugs. Matic et al. [24] studied the effect of this polymorphism in newborns who received morphine and suggested that the G allele led to reduced *UGT2B7* gene activity, compared to the A allele, because patients with the GG genotype reached higher plasma concentrations of morphine.

Protection against rejection in patients with at least one G allele may be attributed to the decreased activity of UGT2B7, which is one of the factors responsible for MPA inactivation by transforming MPA into MPA-acyl-glucuronide. Lower UGT2B7 activity thus results in MPA being active for a longer time, which in turn lowers the risk of rejection.

Based on previous reports and our findings, we suggest that the SNPs rs2241409 (*CES2*), rs11706052 (*IMPDH2*), and rs7438135 (*UGT2B7*) be studied in further studies to validate them as prognostic markers of graft rejection in renal transplant patients.

Conclusion

The results observed in the present study regarding the SNP rs11706052 (*IMPDH2*) contradict previously reported data. The lack of replicability of these results precludes validation of this gene as a marker. Therefore, we suggest that this SNP be analyzed with caution.

The SNPs rs11706052 (*IMPDH2)* and rs7438135 (*UGT2B7),* are associated with a lower risk of rejection, whereas the SNP rs2241409 (*CES2)* is associated with an increased risk of rejection.

Conflict of interest

The authors declare no conflicts of interest.

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References

[1] A.C. Allison, E.M. Eugui. Mycophenolate mofetil and its mechanisms of action. Immunopharmacology. 47 (2000) 85-118. doi: 10.1016/S0162-3109(00)00188-0.

[2] O. Gensburger, R.H. Van Schaik, N. Picard, Y. Le Meur, A. Rousseau, J.B. Woillard, T. Van Gelder, P. Marquet. Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil. Pharmacogenet Genomics. 20 (2010) 537-43. doi: 10.1097/FPC.0b013e32833d8cf5.

[3] M. Walsh, N. Solomons, L. Lisk, D.R. Jayne. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis with poor kidney function: a subgroup analysis of the Aspreva Lupus Management Study. Am J Kidney Dis. 61 (2013) 710-5. doi: 10.1053/j.ajkd.2012.11.042.

[4] J.A. Kobashigawa, J.K. Patel. Immunosuppression for heart transplantation: where are we now? Nat Clin Cardiovasc Med. 3 (2006) 203-12. doi:10.1038/ncpcardio0510.

[5] N. Fujiyama, M. Miura, S. Satoh, K. Inoue, H, Kagaya, M. Saito, T. Habuchi, T. Suzuki. Influence of carboxylesterase 2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. Xenobiotica. 39 (2009) 407-14. doi: 10.1080/00498250902807338.

[6] N. Fujiyama, M. Miura, S. Kato, T. Sone, M. Isobe, S. Satoh. Involvement of carboxylesterase 1 and 2 in the hydrolysis of mycophenolate mofetil. Drug Metab Dispos. 38 (2010) 2210-7. doi: 10.1124/dmd.110.034249.

[7] A.L. Taylor, C.J. Watson, J.A. Bradley. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol. 56 (2005) 23-46. . doi: 10.1016/j.critrevonc.2005.03.012.

[8] N. Picard, P. Marquet. Pharmacogenomics of Immunosuppressants. In: Langman LJ, Dasgupta A, editors. Pharmacogenomics in Clinical Therapeutics. Wiley-Blackwell. (2012) 70-97.

[9] C.E. Staatz, S.E. Tett. Pharmacology and toxicology of mycophenolate in organ transplant recipients: an update. Arch Toxicol. 88 (2014) 1351-89. doi 10.1007/s00204-014-1247-1.

[10] A.G. Zimmermann, J. Spychala, B.S. Mitchell. Characterization of the human inosine-5'-monophosphate dehydrogenase type II gene. J Biol Chem. 270 (1995) 6808-14.

[11] N. Picard, D. Ratanasavanh, A. Prémaud, Y. Le Meur, P. Marquet. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. Drug Metab Dispos. 33 (2004) 139-46. doi: https://doi.org/10.1124/dmd.104.001651.

[12] D.R. Kuypers, G. Verleden, M. Naesens, Y. Vanrenterghem. Drug interaction between mycophenolate mofetil and rifampin: possible induction of uridine diphosphate-glucuronosyltransferase. Clin Pharmacol Ther. 1 (2005) 81-8. doi: 10.1016/j.clpt.2005.03.004

[13] R. Parker, M.J. Armstrong, C. Corbett, E.J. Day, J.M. Neuberger. Alcohol and Substance Abuse in Solid-Organ Transplant Recipients. Transplantation. 96 (2013) 1015-24. doi: 10.1097/TP.0b013e31829f7579.

[14] J. Rehm, R. Room, K. Graham, M. Monteiro, G. Gmel, C.T. Sempos. The relationship of average volume of alcohol consumption and patterns of drinking to burden of disease: an overview. Addiction. 98 (2003) 1209-28. doi: 10.1046/j.1360-0443.2003.00467.x

[15] C. Legendre, G. Canaud, F. Martinez. Factors influencing long-term outcome after kidney transplantation. Transplant International. 27 (2014) 19-27. doi:10.1111/tri.12217.

[16] P. Glander, K.P. Braun, P. Hambach, S. Bauer, I. Mai, I. Roots, J. Waiser, L. Fritsche, H.H. Neumayer, K. Budde. Non-radioactive determination of inosine 5'-monophosphate dehydro-genase (IMPDH) in peripheral mononuclear cells. Clin Biochem. 34 (2001) 543-9. doi:10.1016/S0009-9120(01)00267-3.

[17] F. Sombogaard, R.H. van Schaik, R.A. Mathot, K. Budde, M. van der Werf, A.G. Vulto, W. Weimar, P. Glander, L. Essioux, T. van Gelder. Interpatient variability in IMPDH activity in MMF-treated renal transplant patients is correlated with IMPDH type II 3757T > C polymorphism. Pharmacogenet Genomics. 19 (2009) 626-34. doi: 10.1097/FPC.0b013e32832f5f1b.

[18] J. Pazik, M. Ołdak, M. Podgórska, Z. Lewandowski, E. Sitarek, R. Płoski, J. Szmidt, A. Chmura, M. Durlik, J. Malejczyk. Lymphocyte counts in kidney allograft recipients are associated with IMPDH2 3757T>C gene polymorphism. Transplant Proc. 43 (2011) 2943-5. doi: 10.1016/j.transproceed.2011.08.037.

[19] S. Shah, S.M. Harwood, B. Döhler, G. Opelz, M.M. Yaqoob. Inosine monophosphate dehydrogenase polymorphisms and renal allograft outcome. Transplantation. 94 (2012) 486-91. doi: 10.1097/TP.0b013e31825b7654.

[20] J. Grinyó, Y. Vanrenterghem, B. Nashan, F. Vincenti, H. Ekberg, K. Lindpaintner, M. Rashford, C. Nasmyth-Miller, A. Voulgari, O. Spleiss, M. Truman, L. Essioux. Association of four DNA polymorphisms with acute rejection after kidney transplantation. Transpl Int. 21 (2008) 879-91. doi: 10.1111/j.1432-2277.2008.00679.x.

[21] H. Kagaya, M. Miura, M. Saito, T. Habuchi, S. Satoh. Correlation of IMPDH1 Gene Polymorphisms with Subclinical Acute Rejection and Mycophenolic Acid Exposure Parameters on Day 28 after Renal Transplantation. Basic Clin Pharmacol Toxicol. 107 (2010) 631–6. doi: 10.1111/j.1742-7843.2010.00542.x.

[22] J. Wang, J.W. Yang, A. Zeevi, S.A. Webber, D.M. Girnita, R. Selby, J. Fu, T. Shah, V. Pravica, I.V. Hutchinson, G.J. Burckart. IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. Clin Pharmacol Ther. 83 (2008) 711-7. doi:10.1038/sj.clpt.6100347.

[23] Y. Duguay, C. Báár, F. Skorpen, C. Guillemette. A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. Clin Pharmacol Ther. 75 (2004) 223-33. doi:10.1016/j.clpt.2003.10.006.

[24] M. Matic, E. Norman, A. Rane, O. Beck, M. Andersson, L. Elens, D. Tibboel, V. Fellman, R.H. van Schaik. Effect of UGT2B7 -900G>A (-842G>A; rs7438135) on morphine glucuronidation in preterm newborns: results from a pilot cohort. Pharmacogenomics. 15 (2014) 1589-97. doi: 10.2217/pgs.14.115.

[25] H. Girard, M.H. Court, O. Bernard, L.C. Fortier, L. Villeneuve, Q. Hao, D.J. Greenblatt, L.L. von Moltke, L. Perussed, C. Guillemette. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. Pharmacogenetics. 14 (2004) 501-15. doi: 10.1097/01.fpc.0000114754.08559.27.

[26] J.N. Tian, I.K. Ho, H.H. Tsou, C.P. Fang, C.F. Hsiao, C.H. Chen, H.K.Tan, L. Lin, C.S. Wu, L.W. Su, C.L. Huang, Y.H. Yang, M.L. Liu, Y.T. Chen, S.C. Liu, Y.T. Hsu, H.W. Kuo, C.T. Liu, Y.T. Yang, A.C.H. Chen, Y.H. Shih, Y.L. Liu. UGT2B7 genetic polymorphisms are associated with the withdrawal symptoms in methadone maintenance patients. Pharmacogenomics. 13 (2012) 879-88. doi:10.2217/pgs.12.69.

[27] M. Naesens, D.R. Kuypers, K. Verbeke, Y. Vanrenterghem. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. Transplantation. 82 (2006) 1074-84. doi: 10.1097/01.tp.0000235533.29300.e7.

[28] M. Hirouchi, H. Suzuki, M. Itoda, S. Ozawa, J. Sawada, I. Ieiri, R. Ohtsubo, Y. Sugiyama. Characterization of the cellular localization, expression level, and function of SNP

variants of MRP2/ABCC2. Pharm Res. 21 (2004) 742-8.
doi:10.1023/B:PHAM.0000026422.06207.33

[29] M. Niemi. Role of OATP transporters in the disposition of drugs. Pharmacogenomics. 8 (2007) 787-802. doi: 10.2217/14622416.8.7.787.

Table 1: Genes evaluated in renal transplant patients, their polymorphisms and type of mutation that originated them and function.

Gene	SNP_ID	Nomenclatura alternativa	Change	Chromosome location	Function	Reference
CES1	rs62028647	S82L (356C>T)	Exon (Ser83Leu)	16q22.2	Function unknown	-
CES2	rs2241409	IVS10-108C>T (8721C>T)	Intron	16q22.1	No significant differences in MPA pharmacokinetics were observed between genotype groups.	Fujiyama et al. [5]
IMPDH1	rs2278293	IVS7+119G>A	Intron	7q31.3	Function unknown	-
IMPDH2	rs11706052	IVS7+10T (3757T>C)	Intron	3p21.2	Increased IMPDH activity in MMF-treated renal transplant patients	Sombogaard et al. [17]
UGT1A9	rs6714486	-275T>A	Promoter	2q37	Increased glucuronidating activity in liver using MMF substrates	Girard et al. [25]
UGT2B7	rs7438135	-842G>A/ -900G>A	Promoter	4q13	tSNP; Function unknown Increased UGT2B7 activity in patients treated with morphine	Tian et al. [26] Matic et al. [24]
ABCC2	rs717620	-24C>T	Promoter (5'UTR)	10q24	Associated with mild liver dysfunction and significantly higher dose MPA levels in renal recipients.	Naesens et al. [27]
	rs2273697	1249G>A	Exon (Val417Ile)		Not affect protein expression levels, but may result in different substrate specificities	Hirouchi et al. [28]
SLCO1B1	rs4149056	521T>C (SLCO1B1*5)	Exon (Val174Ala)	12p	Reduced transporter activity	Niemi, [29]

Table 2: General and clinical characteristics of kidney transplant patients (145) who developed and who did not develop rejection episodes.

Characteristics of patients	General		With rejection episode(s)		Without rejection episode(s)		*p-value*[d]
	N (%)	Média ± SD[c]	N (%)	Média ± SD	N (%)	Média ± SD	
Age (years)	145 (100)	46.9 ± 12.5	49 (100)	45.9 ± 11.9	96 (100)	47.4 ± 12.8	0.498
Time after transplantation (years)	145 (100)	7.00 ±5.71	49 (100)	8.56 ± 7.07	96 (100)	6.21 ± 4.72	0.018*
Gender							0.199
Men	81 (55.9)	-	31 (63.3)	-	50 (52.1)	-	
Women	64 (44.1)	-	18 (36.7)	-	46 (47.9)	-	
Degree of HLA[a] compatibility							0.208
Live HLA-identical	20 (13.8)	-	5 (10.2)	-	15 (15.6)	-	
Live HLA-haploidentical	72 (49.7)	-	23 (46.9)	-	49 (51.0)	-	
Deceased unrelated donor	53 (36.6)	-	21 (42.9)	-	32 (33.3)	-	
Smoker (yes)	8 (5.50)	-	3 (6.10)	-	5 (5.20)	-	0.826
Drinker (yes)	18 (12.4)	-	11 (22.4)	-	7 (7.30)	-	0.024*
Use of immunosuppressive							
Cyclosporine	20 (13.8)	-	10 (20.4)	-	10 (10.4)	-	0.135
Tacrolimus	89 (61.4)	-	25 (51.0)	-	64 (66.7)	-	0.068
Sirolimus	10 (6.90)	-	4 (8.20)	-	6 (6.30)	-	0.683
MMF[b]	145 (100)	-	49 (100)	-	96 (100)	-	-
Steroids	145 (100)	-	49 (100)	-	96 (100)	-	-
Development of disease							
Cancer	8 (5.50)	-	4 (8.2)	-	4 (4.2)	-	0.372
Diabetes	35 (24.1)	-	14 (28.6)	-	21 (21.9)	-	0.392

[a]HLA – Human Leukocyte Antigen, [b]MMF – mycophenolate mofetil, [c]standard deviation, [d]*p*-values calculed by Student's t-test for independent samples comparing patients with rejection and without rejection; *p<0.050.

Table 3: Genotypic frequencies of genes *CES1*, *CES2*, *IMPDH1*, *IMPDH2*, *UGT1A9*, *UGT2B7*, *ABCC2* and *SLCO1B1* in 145 kidney transplant patients, and the association between gene polymorphisms and rejection episodes.

Genes (SNP_ID)	**Genotypes**	**With rejection episode(s) n (%)**	**Without rejection episode(s) n (%)**	***Odds Ratio* (IC95%)**	***p*-value[a]**
CES1 (rs62028647)	C/C	4 (8.2)	7 (7.3)	Ref.[b]	
	C/T	45 (91.8)	89 (92.7)	0.92 (0.24-3.6)	0.90
***CES2* (rs2241409)**	C/C	26 (53.1)	64 (66.7)	Ref.	
	T/C	17 (34.7)	30 (31.2)	1.4 (0.61-3.3)	0.42
	T/T	6 (12.2)	2 (2.1)	7.2 (1.1-46)	**0.038***
	T/C and T/T	23 (46.9)	32 (33.3)	1.8 (0.80-4.0)	0.16
IMPDH1 (rs2278293)	G/G	14 (28.6)	32 (33.3)	Ref.	
	A/G	19 (38.8)	43 (44.8)	1.0 (0.38-2.7)	1.0
	A/A	16 (32.6)	21 (21.9)	1.8 (0.61-5.1)	0.30
	A/G and A/A	35 (71.4)	64 (66.7)	1.2 (0.54-2.9)	0.61
***IMPDH2* (rs11706052)**	T/T	45 (91.8)	70 (72.9)	Ref.	
	T/C and C/C	4 (8.20)	26 (27.1)	0.24 (0.071-0.78)	**0.018***
UGT1A9 (rs6714486)	T/T	42 (85.7)	86 (89.6)	Ref.	
	T/A and A/A	7 (14.3)	10 (10.4)	1.4 (0.45-4.2)	0.58
UGT2B7 (rs7438135)	A/A	24 (49.0)	28 (29.2)	Ref.	
	A/G	23 (46.9)	57 (59.4)	0.45 (0.20-1.0)	0.060
	G/G	2 (4.1)	11 (11.4)	0.22 (0.037-1.3)	0.095
	A/G and G/G	25 (51.0)	68 (70.8)	0.41 (0.19-0.92)	**0.030***
ABCC2 (rs717620)	C/C	34 (69.4)	58 (60.4)	Ref.	
	C/T	13 (26.5)	34 (35.4)	0.68 (0.29-1.6)	0.38
	T/T	2 (4.1)	4 (4.2)	0.67 (0.085-5.3)	0.71

	C/T and T/T	15 (30.6)	38 (39.6)	0.68 (0.30-1.5)	0.36
ABCC2 (rs2273697)	G/G	29 (59.2)	59 (61.4)	Ref.	
	G/A	18 (36.7)	31 (32.3)	1.1 (0.46-2.6)	0.84
	A/A	2 (4.10)	6 (6.3)	0.63 (0.085-4.6)	0.65
	G/A and A/A	20 (40.8)	36 (38.6)	1.0 (0.44-2.3)	0.97
SLCO1B1 (rs4149056)	T/T	33 (67.3)	60 (62.5)	Ref.	
	T/C	14 (28.6)	31 (32.3)	0.91 (0.39-2.1)	0.82
	C/C	2 (4.1)	5 (5.2)	1.0 (0.16-6.4)	1.0
	T/C and C/C	16 (32.7)	36 (37.5)	0.92 (0.41-2.1)	0.84

[a]*p*-values for the association test derived from the logistic regression using for time after transplantation, drinker and use of cyclosporine or tacrolimus as covariates, [b]Ref - values taken as a reference, **p*<0.050.

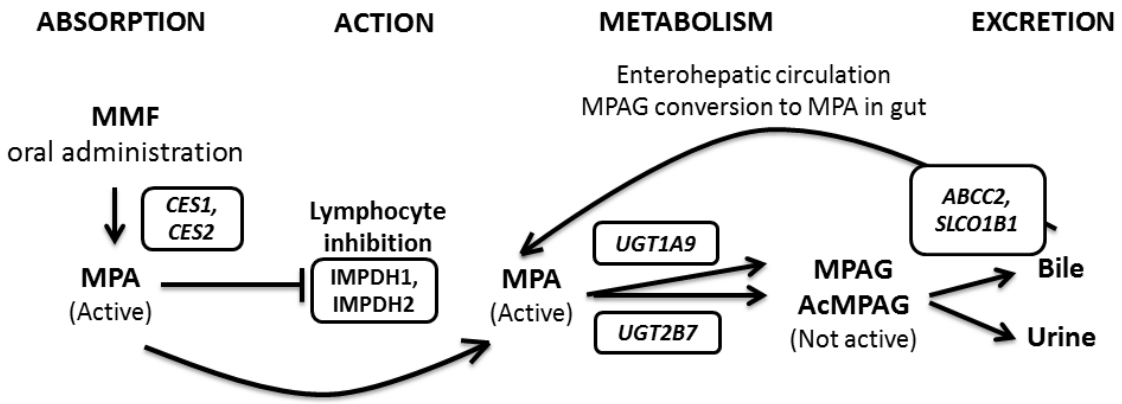

Figure 1: Genes involved in absorption, mechanism of action, metabolism and excretion of mycophenolate of mofetil (MMF). MPA: Mycophenolic acid; AcMPAG: Acyl mycophenolic acid glucuronide; MPAG: 7-*O*-mycophenolic acid glucuronide.

7 ARTIGO IV

Impact of polymorphisms in drug metabolism/transport genes on tacrolimus pharmacokinetics in kidney transplant recipients

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Impact of polymorphisms in drug metabolism/transport genes on tacrolimus pharmacokinetics in kidney transplant recipients

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Abstract

Tacrolimus, an immunosuppressant used widely to prevent solid organ transplant rejection, shows great interindividual pharmacokinetic variability, which may result from polymorphisms in metabolism and drug transport genes that alter its oral bioavailability. As this drug has a narrow therapeutic window and shows large interindividual pharmacokinetic variability, patients are monitored to ensure that the optimal plasma concentration of tacrolimus is maintained to minimize the incidence of rejection episodes and adverse effects. In this study it was evaluated the association between single nucleotide polymorphisms (SNP) in drug metabolism/transport genes (*CYP3A4, CYP3A5, CYP2E1, POR, ABCB1, ABCC2, ABCG2* and *SLCO1B1)* with plasma concentrations of tacrolimus in 55 renal transplant patients, one, two, and three months post-transplantation. Genotyping of the polymorphisms was performed using TaqMan SNP genotyping Assay. Our results indicate that the polymorphisms rs4646437 (*CYP3A4*) and rs776746 (*CYP3A5*) influence tacrolimus pharmacokinetics during the first three months after transplantation. In both polymorphisms the G/A and A/A genotypes were associated with a lower adjusted dose of tacrolimus, compared to the G/G genotypes. Thus, these results suggest that these markers should be assessed in multicenter studies so that they may be used in future clinical practice.

Introduction

The drug tacrolimus is used widely in transplant patients to suppress the immune system, reduce the risk of graft rejection, and improve patient survival (O'Callahhan, 2008). Therapeutic monitoring of this drug has been recommended because of its narrow therapeutic window and wide interindividual pharmacokinetic variability. This variability is pronounced among different ethnicities, African-American patients required a 37% mean higher dose of tacrolimus than Caucasian patients to achieve comparable blood concentrations (Neylan, 1998).

Pharmacokinetic testing has shown that certain *CYP3A4* and *CYP3A5* genetic polymorphisms (Staatz; Goodman; Tett, 2010; Onizuka et al., 2011; Kurzawski et al., 2014) and P-glycoprotein transporters polymorphisms (Staatz; Goodman; Tett, 2010), although not the sole variables, they are responsible for affecting tacrolimus blood concentrations.

Polymorphisms in these genes may contribute to the poor correlation observed between administered doses and plasma concentrations achieved (Zununi et al., 2015), and also interfere with the therapeutic response. Therefore, patients who metabolize the drug more efficiently and eliminate it rapidly from the body, have low plasma levels, which can trigger rejection, whereas poor metabolizers may have supratherapeutic concentrations, resulting in increased adverse effects such as hypertension, nephrotoxicity, neurotoxicity, hypercholesterolemia, diabetes mellitus, and cancer (Elens et al., 2011; Ponticelli, 2005; Fraile et al., 2009).

The polymorphism, rs776746, in *CYP3A5* at position 6986 A>G is well characterized with respect to its influence on tacrolimus pharmacokinetics. The G allele (*CYP3A5*3*) causes a nonfunctional splicing defect (Xie et al., 2004), resulting in a truncated protein and reduced CYP3A5 enzyme activity (Tada et al., 2005). Patients with the G/G genotype (referred to as CYP3A5 "non-expressers") show a higher normalized concentration of tacrolimus than those with at least one allele A (*CYP3A5*1*) (Staatz; Goodman; Tett, 2010; Terrazzino et al., 2012).

The rs776746 polymorphism is currently the most reliable indicator of the required dose of tacrolimus for each patient. However, this SNP can explain only one-third of the interindividual variability in tacrolimus pharmacokinetics (Li et al., 2015), suggesting that other polymorphisms, such as SNPs in *CYP3A4* and *POR*, may affect this process. It has been suggested that patients with the T allele (T/T and T/C) for rs35599367 (*CYP3A4*) require a lower dose of tacrolimus than patients with the C/C genotype (Hesselink et al., 2014). This is associated with reduced mRNA/protein expression promoted by the T allele (Wang; Sadee,

2016). Hence, patients with this allele metabolize tacrolimus more slowly and thus require lower doses of it.

The *POR* gene encodes the protein *cytochrome P450 oxidoreductase*, which donates electrons to all cytochrome P450 enzymes (CYPs), and SNPs in this gene may alter drug metabolism mediated by CYPs (Hart; Zhong, 2008). The best known SNP of this gene is rs1057868, which induces an amino acid substitution (C>T, Ala503Val) and results in a modest decrease in the activity of this protein (Huang et al., 2008). The contribution of this SNP to interindividual variability of tacrolimus is controversial, with some studies showing that patients with the T (T/T and T/C) allele for this SNP require higher doses of tacrolimus, compared to patients with the C/C genotype, in individuals who express *CYP3A5* [14]. However, a lack of association between this SNP and tacrolimus concentration has also been reported (Liu et al., 2016).

In addition to polymorphisms in metabolic genes, those in drug transporter genes may also affect pharmacokinetics, notably, polymorphisms in the P-glycoprotein gene, encoded by the multidrug resistance gene 1 (*MDR1)* (Terrazzino et al., 2012; Saeki et al., 1993).

Studies on the functional effects of major SNPs in this gene (3435C>T, 1236C>T, and 2677G>T/A) on tacrolimus pharmacokinetics showed contradictory results (Staatz; Goodman; Tett, 2010). The TTT haplotype formed by these SNPs reduces ABCB1 protein activity in a significant way when the CYP3A5 activity is low (*CYP3A5**3/*3 nonexpressors). Therefore, carriers of this haplotype require lower doses of tacrolimus to reach the same plasma concentrations than those with CGC haplotypes (Wang et al., 2006; Vanhove et al., 2016). Analysis of the isolated SNP rs1045642, revealed the association of the CT genotype with higher adjusted plasma concentrations of tacrolimus, relative to the CC genotype (Ruiz et al., 2015).

The *ABCC2* and *ABCG2* genes encode apical efflux transport proteins important in the biliary excretion of phase II conjugates (Vanhove et al., 2016; Takano; Yumoto; Murakami, 2006). Ogasawara et al. (2013) evaluated the association of the polymorphisms rs2231142 (*ABCG2*), rs717620, and rs2273697 (*ABCC2*) with tacrolimus concentration and found that the G/A and A/A genotypes of rs2273697, which result in increased ABCC2 enzyme activity, was associated with a lower adjusted concentration of tacrolimus, compared to the G/G genotype.

To evaluate whether polymorphisms in drug metabolism (*CYP3A4, CYP3A5, CYP2E1, POR*) and transport (*ABCB1, ABCC2, ACBG2, SLCO1B1*) genes influence

tacrolimus pharmacokinetics, their association with adjusted plasma concentration of tacrolimus during the first three months after transplantation was investigated.

Materials and Methods

Patients

This was a single center, retrospective, cohort study of kidney transplant patients (N = 55) who received transplants between the years 2004 and 2013 and regularly underwent medical monitoring at the Kidney Institute of Londrina (Paraná State, Brazil).

The experiments were approved by Ethics Committee on Human Research of the State University of Londrina, (CEP/UEL 153/2013 CAAE: 18263413.4.0000.5231). Written informed consent and a questionnaire about their lifestyle were obtained from all study participants. Immunosuppressive therapy consisted of tacrolimus, mycophenolate mofetil and corticosteroids, in the first three months of transplantation. Additional data such as doses and plasma concentrations of tacrolimus, creatinine levels, weight and age were obtained from medical records. Venous blood samples (1 mL) were collected using vacuum tubes - EDTA 6% (Labor Import, Brazil).

Estimative of the glomerular filtration rate (eGFR) and plasma concentration of tacrolimus

The eGFR is used to evaluation of kidney function. The patients'eGFR were calculated in the first 3 months after transplantation, from the abridged Modification of Diet in Renal Disease formula (MDRD-4), modified by Levey et al. (2000). This calculation is based on the serum creatinine; take into account the influences of age, gender, and race.

Plasma concentrations of tacrolimus were obtained by immunoassays conducted by the University Hospital of the State University of Londrina, as part of the routine monitoring of patients and were obtained from the analysis of the medical records. The tacrolimus concentrations were adjusted based on weight, daily dose, and plasma concentrations from each patient after 1, 2 and 3 months of transplantation, dividing the plasmatic concentration (ng mL^{-1}) by the daily dose (mg kg^{-1}).

Analysis of polymorphic allelic variants

Genomic DNA was extracted from 200 µL of blood, using the mini spin extraction

kit (KASVI, Curitiba, Brazil; code K9-0250), following the manufacturer's recommendations. DNA samples were quantified using a NanoDrop 2000 spectrophotometer (ThermoScientific, Waltman, MA, USA). Genotyping was performed by real-time PCR in a Quantica thermocycler (TECHNE, Staffordshire, UK), using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA), TaqMan Genotyping Master Mix (Applied Biosystems), and 5 ng of genomic DNA for each of the analyzed genes. Table 1 shows the genes and their respective SNPs evaluated.

Statistical analysis

All results are expressed as the mean ± standard deviation (SD). The tacrolimus concentration were associated with different genotypes by Mann-Whitney U-test using SPSS®20 statistical software (IBM; Armonk, NY, USA). *P*-value less than 0.05 was considered to be statistically significant. Analysis of Hardy-Weinberg equilibrium and analysis of linkage disequilibrium was performed using HAPLOVIEW version 4.1 (Barrett et al., 2005).

Results

The 55 patients who were taking tacrolimus included 26 women and 29 men with an average age of 40.1 ± 13.0 years. Of these patients, 34 (61.8%) received grafts from living donors - seven with identical Human Leukocyte Antigen (HLA) and 27 with haploidentical HLA. The remaining patients (38.2%) received grafts from deceased donors. Table 2 shows the average values of the main parameters analyzed in the first three months after renal transplantation, such as body weight, serum creatinine level, eGFR, tacrolimus dose (mg/day), tacrolimus plasma concentration (ng/ml), and ajusted plasma concentration (ng ml^{-1}/mg kg^{-1}).

Allele frequencies of the 11 SNPs evaluated are in Hardy-Weinberg equilibrium. Linkage disequilibrium was observed between three SNPs: rs4646450 (*CYP3A5*) and rs776746 (*CYP3A5*) (D′=1.00; r^2=0.57); rs4646450 (*CYP3A5*) and rs4646437 (*CYP3A4*) (D′=0.84; r^2=0.39); and rs4646437 (*CYP3A4*) and rs776746 (*CYP3A5*) (D′=0.88; r^2=0.74) (data not shown).

Of these SNPs, only rs776746 (*CYP3A5*) and rs4646437 (*CYP3A4*) showed a significant association with the adjusted dose of tacrolimus (ng ml^{-1}/mg kg^{-1}) in the first three months after transplantation. In both polymorphisms, the G/A and A/A genotypes were

associated with a lower adjusted dose of tacrolimus, compared to the G/G genotype (Table 3).

Discussion

The wide variability in the effective dose of tacrolimus required to reach optimal therapeutic levels, observed among transplant patients is due to several factors, including age, gender, body weight, drug interactions, and genetic factors, such as polymorphisms that play a critical role in the pharmacokinetics of this drug (Vannaprasaht et al., 2013).

The influence of SNP rs776746 (CYP3A5*3) on the pharmacokinetics of tacrolimus has been well characterized. In this study was performed the analysis of this SNP to confirm its impact in the group evaluated.

Two polymorphisms in *CYP3A5* (rs776746 and rs4646450) were evaluated. As expected, we found an association of rs776746 with the ajusted plasma concentrations of tacrolimus. Patients with the G/G genotype showed higher adjusted plasma concentrations of tacrolimus [(ng mL^{-1})/(mg kg^{-1})], compared to the CYP3A5-expressing genotypes (A/A and A/G). This result is consistent with the results of Kurzawski et al. (2014), who evaluated this association (7 days, and 1, 3, 6, and 12 months after transplantation) in 244 renal transplant patients and found that the CYP3A5-expressing genotypes resulted in lower adjusted plasma tacrolimus concentration.

We analyzed the doses administered to patients expressing CYP3A5 (genotype A/G and A/A of the SNP rs776746) and found that they required higher doses of tacrolimus in the second (14.1 ± 5.5 *vs* 10.0 ± 2.9, $p = 0.002$) and third (13.04 ± 5.7 *vs* 8.69 ± 3.02, $p = 0.002$) month after transplantation, compared to CYP3A5 (G/G) non-expressing patients (data not shown). These findings corroborate the previous finding that CYP3A5-expressing patients, in addition to requiring higher doses of tacrolimus, have lower normalized plasma concentrations (Chen et al., 2014).

The polymorphism rs4646450 (*CYP3A5*) showed a lower influence on tacrolimus pharmacokinetics. Patients with the genotypes G/A and A/A showed adjusted tacrolimus concentration in the three time points examined, although these difference were nor significant when compared using the Mann-Whitney U test.

In this study also was found an association between rs4646437 (*CYP3A4)* and the ajusted tacrolimus concentrations in the three time points examined.Similar results were also observed by Li et al. (2014), that observed in Chinese renal transplant recipients association

between the genotypes G/G of this SNP with the higher concentration/dose of tacrolimus when compared to the genotypes A/A and A/G. This association is probably related to the linkage disequilibrium observed between rs4646437 and the two assessed polymorphisms of the CYP3A5 gene.

There was no significant association between rs35599367 (*CYP3A4*) and the adjusted dose of tacrolimus. It has been shown that rs35599367 is associated with decreased enzyme activity (Pallet et al., 2015). Thus, carriers of this polymorphism (C/T or T/T) may require lower doses of tacrolimus to achieve the desired concentration. Moreover, carriers of the C/T genotype show a significant association with higher adjusted tacrolimus concentrations, only three months after transplantation (Kurzawski et al., 2014). However, our results showed no significant association.

Tacrolimus is transported by the ABCB1 drug efflux pump. Thus, polymorphisms in *ABCB1* may influence its pharmacokinetics. Here, we assessed the association of the SNP rs1045642 (3435C>T) with the adjusted concentration of tacrolimus. This SNP affects mRNA stability and causes its rapid degradation after synthesis (Wang et al., 2005). We found no evidence of an association between rs1045642 and the adjusted concentration of tacrolimus, consistent with previous studies (Kurzawski et al., 2014; Tada et al., 2005; Chen et al., 2014). Thus, rs1045642 does not seem to affect tacrolimus pharmacokinetics in renal transplant patients.

Other drug transport genes evaluated participate in the transport of tacrolimus from the liver to biliary and renal excretion (Sánchez-Lázaro et al., 2015). These genes did not show association with tacrolimus concentrations in the examined time points after transplantation, suggesting that their polymorphic variants have no significant impact on tacrolimus pharmacokinetics.

Conclusion

Individualized pharmacogenetic-based immunotherapy is expected to play a key role in future medical practice, as patients' genetic information may provide additional information for determining the most effective drug dose and minimizing toxicity, to design the most favorable immunosuppressive treatment for each patient.

Our results confirm the impact of rs4646437 (*CYP3A4*) and rs776746 (*CYP3A5*) polymorphisms on tacrolimus pharmacokinetics and suggest that they may be useful markers

for accurately predicting the most effective initial dose of immunosuppressant after renal transplantation .

Conflict of interest

The authors declare no conflicts of interest.

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References

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263-5.

Chen YK, Han LZ, Xue F, Shen CH, Lu J, Yang TH, Zhang JJ, Xia Q. Personalized tacrolimus dose requirement by CYP3A5 but not ABCB1 or ACE genotyping in both recipient and donor after pediatric liver transplantation. *PLoS One* 2014; 9:e109464.

Elens L, Bouamar R, Hesselink DA, Haufroid V, van der Heiden IP, van Gelder T, van Schaik RH. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. *Clin Chem*. 2011; 57:1574-83.

Fraile P, García-Cosmes P, Martín P, García-Bernalt V, Tabernero JM. Non-skin solid tumors as a cause of morbidity and mortality after liver transplantation. *Transplant Proc*. 2009; 41:2433-4.

Hart SN, Zhong XB. P450 oxidoreductase: genetic polymorphisms and implications for drug metabolism and toxicity. *Expert Opin Drug Metab Toxicol*. 2008; 4:439-52.

Hesselink DA, Bouamar R, Elens L, van Schaik RH, van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. *Clin Pharmacokinet*. 2014; 53:123-39.

Huang N, Agrawal V,Giacomini KM, Miller WL. Genetics of P450 oxidoreductase: Sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. *Proc Natl Acad Sci U S A*. 2008; 105:1733-8.

Kurzawski M, Dąbrowska J, Dziewanowski K, Domański L, Perużyńska M, Droździk M. CYP3A5 and CYP3A4, but not ABCB1 polymorphisms affect tacrolimus dose-adjusted trough concentrations in kidney transplant recipients. *Pharmacogenomics*. 2014; 15:179-88.

Levey AS, Green T, Kusek JW, Beck GJ, MDRD Study Group. A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol*. 2000; 11:155A.

Li CJ, Li L, Lin L, Jiang HX, Zhong ZY, Li WM, Zhang YJ, Zheng P, Tan XH, Zhou L. Impact of the CYP3A5, CYP3A4, COMT, IL-10 and POR genetic polymorphisms on tacrolimus metabolism in Chinese renal transplant recipients. *PLoS One*. 2014; 21:9(1):e86206. doi: 10.1371/journal.pone.0086206.

Li JL, Liu S, Fu Q, Zhang Y, Wang XD, Liu XM, Liu LS, Wang CX, Huang M. Interactive effects of CYP3A4, CYP3A5, MDR1 and NR1I2 polymorphisms on tracrolimus trough concentrations in early postrenal transplant recipients. *Pharmacogenomics*. 2015; 16:1355-65.

Liu S, Chen RX, Li J, Zhang Y, Wang XD, Fu Q, Chen LY, Liu XM, Huang HB, Huang M, Wang CX, Li JL. The POR rs1057868-rs2868177 GC-GT diplotype is associated with high tacrolimus concentrations in early post-renal transplant recipients. *Acta Pharmacol Sin*. 2016; 37:1251-8.

Neylan JF. Racial differences in renal transplantation after immunosuppression with tacrolimus versus cyclosporine. FK506 Kidney Transplant Study Group. *Transplantation*. 1998; 65:515-23.

O'Callaghan CA. Kidney transplantation - the long term view. *QJM*. 2008; 101:985-6.

Ogasawara K, Chitnis SD, Gohh RY, Christians U, Akhlaghi F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. *Clin Pharmacokinet*. 2013; 52:751-62.

Onizuka M, Kunii N, Toyosaki M, Machida S, Ohgiya D, Ogawa Y, Kawada H, Inoko H, Ando K. Cytochrome P450 genetic polymorphisms influence the serum concentration of calcineurin inhibitors in allogeneic hematopoietic SCT recipients. *Bone Marrow Transplant*. 2011; 46:1113-7.

Pallet N, Jannot AS, E Bahri M, Etienne I, Buchler M, de Ligny BH, Choukroun G, Colosio C, Thierry A, Vigneau C, Moulin B, Le Meur Y, Heng AE, Subra JF, Legendre C, Beaune P, Alberti C, Loriot MA, Thervet E. Kidney transplant recipients carrying the CYP3A4*22 allelic variant have reduced tacrolimus clearance and often reach supratherapeutic tacrolimus concentrations. *Am J Transplant*. 2015; 15:800-5.

Ponticelli C. Cyclosporine: from renal transplantation to autoimmune diseases. *Ann N Y Acad Sci*. 2005; 1051:551-8.

Ruiz J, Herrero MJ, Bosó V, Megías JE, Hervás D, Poveda JL, Escrivá J, Pastor A, Solé A, Aliño SF. Impact of Single Nucleotide Polymorphisms (SNPs) on Immunosuppressive Therapy in Lung Transplantation. *Int J Mol Sci*. 2015; 16:20168-82.

Saeki T, Ueda K, Tanigawara Y, Hori R, Komano T. Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem*. 1993; 268:6077-80.

Sánchez-Lázaro I, Herrero MJ, Jordán-De Luna C, Bosó V, Almenar L, Rojas L, Martínez-Dolz L, Megías-Vericat JE, Sendra L, Miguel A, Poveda JL, Aliño SF. Association of SNPs with the efficacy and safety of immunosuppressant therapy after heart transplantation. *Pharmacogenomics*. 2015; 16:971-9.

Staatz CE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: Part I. *Clin Pharmacokinet*. 2010; 49:141-75.

Tada H, Tsuchiya N, Satoh S, Kagaya H, Li Z, Sato K, Miura M, Suzuki T, Kato T, Habuchi T. Impact of CYP3A5 and MDR1(ABCB1) C3435T polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. *Transplant Proc*. 2005; 37:1730-2.

Takano M, Yumoto R, Murakami T. Expression and function of efflux drug transporters in the intestine. *Pharmacol Ther*. 2006; 109:137-61.

Terrazzino S, Quaglia M, Stratta P, Canonico PL, Genazzani AA. The effect of CYP3A5 6986A>G and ABCB1 3435C>T on tacrolimus dose-adjusted trough levels and acute rejection rates in renal transplant patients: a systematic review and meta-analysis. *Pharmacogenet Genomics*. 2012; 22:642-5.

Vanhove T, Annaert P, Lambrechts D, Kuypers DR. Effect of ABCB1 diplotype on tacrolimus disposition in renal recipients depends on CYP3A5 and CYP3A4 genotype. *Pharmacogenomics J*. 2016. [Epub ahead of print].

Vannaprasaht S, Reungjui S, Supanya D, Sirivongs D, Pongskul C, Avihingsanon Y, Tassaneeyakul W. Personalized tacrolimus doses determined by CYP3A5 genotype for induction and maintenance phases of kidney transplantation. *Clin Ther*. 2013; 35:1762-9.

Wang D, Johnson AD, Papp AC, Kroetz DL, Sadée W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. *Pharmacogenet Genomics*. 2005; 15:693-704.

Wang D, Sadee W. CYP3A4 intronic SNP rs35599367 (CYP3A4*22) alters RNA splicing. *Pharmacogenet Genomics*. 2016; 26:40-3.

Wang J, Zeevi A, McCurry K, Schuetz E, Zheng H, Iacono A, McDade K, Zaldonis D, Webber S, Watanabe RM, Burckart GJ. Impact of ABCB1 (MDR1) haplotypes on tacrolimus dosing in adult lung transplant patients who are CYP3A5 *3/*3 nonexpressors. *Transpl Immunol*. 2006; 15:235-40.

Xie HG, Wood AJ, Kim RB, Stein CM, Wilkinson GR. Genetic variability in CYP3A5 and its possible consequences. *Pharmacogenomics*. 2004; 5: 243-72.

Zununi VS, Ardalan M, Samadi N, Omidi Y. Pharmacogenetics and drug-induced nephrotoxicity in renal transplant recipients. *Bioimpacts*. 2015; 5:45-54.

Table 1: Genes and respective polymorphisms (SNPs) evaluated in renal transplant patients; the type of mutation that originated each SNP and its chromosomal location.

Gene	SNP	Alternative nomenclature	Change	Chromosome location
CYP3A4	rs35599367	15389C>T (CYP3A4*22)	Intron	7q22.1
	rs4646437	-	Intron	
CYP3A5	rs776746	6986A>G (CYP3A5*3)	Intron, splice acceptor variant	7q21.1
	rs4646450	-	Intron	
CYP2E1	rs3813867	-1295G>C *(CYP2E1*5B)*	Promoter	10q24.3
POR	rs1057868	A503V (POR*28)	Exon (Ala503Val)	7q11.2
ABCB1	rs1045642	3435C>T	Exon (Ile1145Ile)	7q21.12
ABCC2	rs717620	-24C>T	Promoter (5'UTR)	10q24
	rs2273697	1249G>A	Exon (Val417Ile)	
ABCG2	rs2231142	421C>A	Exon (Lys141Gln)	4q22
SLCO1B1	rs4149056	521T>C (SLCO1B1*5)	Exon (Val174Ala)	12p

Table 2: Values (mean ± SD[a]) of different parameters analyzed in the first three months after renal transplantation in patients taking tacrolimus.

Parameters	**Month**		
	1	**2**	**3**
Body weight (kg)	62.3 ± 15.0	63.5 ± 14.5	65.3 ± 14.6
Serum creatinine (mg dL^{-1})	1.68 ± 1.40	1.47 ± 0.53	1.46 ± 0.45
eGFR[b] (ml min^{-1} per 1.73 m^2)	57.1± 23.6	59.7 ± 26.5	58.7 ± 23.6
Tacrolimus dose (mg per day)	12.5 ± 3.70	11.7 ± 4.62	10.5 ± 4.83
Tacrolimus C_0 (ng ml^{-1})	12.8 ± 7.17	13.8 ± 9.30	11.8 ± 4.60
Tacrolimus C_0/dose/W (ng ml^{-1}/mg kg^{-1})	67.9 ± 42.7	83.4 ± 66.8	92.4 ± 69.1

[a]SD: standard deviation; [b]eGFR: glomerular filtration rate estimated by Modification of Diet in Renal Disease formula (MDRD).

Table 3: Comparison of the adjusted concentration of tacrolimus (ng mL^{-1})/(mg kg^{-1}) three months after transplantation with different genotypic variants.

Gene	Genotype	N (%)	Adjusted concentration of tacrolimus					
			Month 1	*p*[a]	Month 2	*p*	Month 3	*p*
***CYP3A4* rs4646437**	G/G	34 (61.8)	79.4 ± 47.0	**0.016***	99.2 ± 77.6	**0.014***	105.2 ± 76.2	**0.039***
	A/G	19 (34.5)	51.6 ± 26.5		59.3 ± 32.4		74.1 ± 52.6	
	A/A	2 (3.64)	27.3 ± 9.84		43.8 ± 14.5		46.9 ± 17.7	
	A/G + A/A	21 (38.2)	49.3 ± 26.3		57.8 ± 31.2		71.5 ± 50.7	
CYP3A4 rs35599367	C/C	51 (92.7)	69.8 ± 43.4	0.234	86.0 ± 68.5	0.290	94.0 ± 70.9	0.767
	C/T	4 (7.30)	43.8 ± 25.3		50.9 ± 20.7		71.9 ± 38.2	
CYP3A5* *rs776746	G/G	32 (58.2)	80.1 ± 48.3	**0.027***	99.8 ± 78.5	**0.019***	107.6 ± 77.6	**0.037***
	G/A + A/A	23 (41.8)	51.0 ± 25.9		60.8 ± 36.5		71.2 ± 49.2	
CYP3A5 *rs4646450*	G/G	23 (41.8)	76.3 ± 50.8	0.413	106.6 ± 90.5	0.091	106.5 ± 77.7	0.195
	G/A	28 (50.9)	63.3 ± 36.0		69.5 ± 37.1		87.0 ± 64.1	
	A/A	4 (7.3)	52.5 ± 34.5		47.1 ± 10.2		48.8 ± 15.3	
	G/A + A/A	32 (58.2)	61.9 ± 35.4		66.7 ± 35.6		82.2 ± 61.4	
CYP2E1 rs3813867	G/G	44 (80.0)	68.5 ± 44.3	0.983	83.4 ± 71.5	0.542	93.8 ± 71.7	0.801
	G/C + C/C	11 (20.0)	65.6 ± 37.4		83.5 ± 45.7		86.5 ± 60.1	
POR rs1057868	C/C	28 (50.9)	73.4 ± 52.8	0.920	87.7 ± 74.0	0.556	92.9 ± 64.9	0.711
	C/T	23 (41.8)	61.0 ± 28.3		76.3 ± 60.8		89.9 ± 74.6	
	T/T	4 (7.30)	69.5 ± 34.7		94.2 ± 56.0		102.80 ± 83.1	
	C/T + T/T	27 (49.1)	62.2 ± 28.8		78.9 ± 59.4		91.8 ± 74.4	
ABCB1 rs1045642	C/C	20 (36.4)	72.2 ± 44.4	0.600	77.8 ± 40.4	0.662	73.0 ± 34.0	0.310
	C/T	27 (49.1)	70.2 ± 46.1		87.1 ± 78.7		99.8 ± 77.0	
	T/T	8 (14.5)	49.4 ± 18.8		84.8 ± 82.4		115.8 ± 98,6	
	C/T and T/T	35 (63.6)	65.5 ± 42.1		86.6 ± 78,3		103.4 ± 81,2	
ABCC2 rs717620	C/C	38 (69.1)	63.1 ± 44.0	0.084	89.9 ± 76.3	0.478	93.6 ± 71.4	0.985
	C/T	13 (23.6)	83.9 ± 40.1		70.5 ± 37.2		92.2 ± 73.9	
	T/T	4 (7.30)	61.2 ± 32.9		64.0 ± 32.2		81.0 ± 30.4	
	C/T + T/T	17 (30.9)	78.6 ± 38.8		69.0 ± 35.2		89.6 ± 65.6	
ABCC2 rs2273697	G/G	38 (69.1)	73.1 ± 46.7	0.222	82.2 ± 67.6	0.899	91.0 ± 58.2	0.244
	G/A	14 (25.5)	56.1 ± 30.4		85.8 ± 68.6		90.5 ± 92.4	
	A/A	3 (5.50)	57.0 ± 34.3		88.2 ± 70.4		118.3 ± 97.7	
	G/A + A/A	17 (30.9)	56.3 ± 30.0		86.2 ± 66.7		95.4 ± 90.8	
ABCG2 rs2231142	G/G	44 (80.0)	71.2 ± 45.6	0.436	84.6 ± 65.6	0.366	89.4 ± 58.9	0.689
	G/T + T/T	13 (22.0)	54.9 ± 25.6		78.5 ± 74.2		104.3 ± 103.3	
SLCO1B1 rs4149056	T/T	36 (65.5)	66.0 ± 40.4	0.763	91.4 ± 78.1	0.513	93.7 ± 67.3	0.633
	T/C	17 (30.9)	65.3 ± 39.3		63.9 ± 32.1		89.2 ± 78.6	
	C/C	2 (3.60)	124.3 ± 99.9		104.5 ± 31.4		95.7 ± 11.4	
	T/C + C/C	19 (34.5)	71.5 ± 47.7		68.2 ± 33.7		89.9 ± 74.2	

[a] *p* value calculated by the Mann-Whitney test; *$p < 0.05$.

8 ARTIGO V

Positive association between *IL-10* allelic variant and cancer development in kidney transplant patients

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Positive association between *IL-10* allelic variant and cancer development in kidney transplant patients

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Abstract

Transplant patients use immunosuppressive drugs to prevent rejection of the transplanted organ. However, immunosuppression is related to cancer development, which is one of the main causes of morbidity and mortality in kidney transplant patients. The aim of this study was to evaluate the association between polymorphisms in DNA repair genes and immune system genes, with the appearance of malignant tumors in kidney transplant recipients. Of the 246 patients evaluated, 25 (10.2%) developed cancer after 15 ± 8.9 years of transplantation, out of which 68.0% were non-melanoma skin carcinomas. Transplant patients who developed cancer were matched (by age, gender, ancestry, smoking status, and alcohol consumption) with neoplasia-free control individuals. Analysis of the polymorphism rs1130409 (*APE1)* was performed using Restriction Fragment Length Polymorphism Analysis of PCR-Amplified Fragment (RFLP-PCR) and those of the polymorphisms rs25487 (*XRCC1*), rs3212986 (*ERCC1*), rs2308321 (*MGMT*), rs1800975 (*XPA*), rs2069762 (*IL-2*), rs1800872 (*IL-10*), rs10889677 (*IL-23R*), rs1800470, and rs1800471 (*TGF-β*) were performed using TaqMan SNP genotyping Assay. Logistic regression analysis showed a significant association between the C/A genotype of rs1800872 (*IL-10*) with increased susceptibility (3.5-fold) for cancer development. These results support further investigation of *IL-10* as a candidate marker for tumor susceptibility in kidney transplant patients.

Introduction

Patients having chronic kidney disease need to replace renal function by hemodialysis or kidney transplantation. Kidney transplantation is considered the best therapeutic choice because it results in increased survival and improved quality of life in the long term, besides being the best financial choice for health services (Schnuelle et al., 1998; Silva et al., 2016). Despite the benefits of transplantation, kidney transplant patients have a higher incidence of opportunistic infections, cardiovascular diseases, and malignant tumors, compared to individuals who do not use immunosuppressive drugs (O'Callaghan, 2008; Rama; Grinyó, 2010).

The overall incidence of neoplasia is two to three times higher in transplant patients than in the general population of the same age and gender (Chapman; Webster; Wong, 2013; Stallone; Infante; Grandaliano, 2015); moreover, patients present more severe symptoms and worse prognosis of the disease (van de Wetering et al., 2010), which is the third most common cause of death in these patients (Rama; Grinyó, 2010). Transplant patients with cancers also run a higher risk of death due to the disease, compared to the general population (Acuna et al., 2016).

The most frequent neoplasia seen among transplant patients is the non-melanoma skin cancer (NMSC) (Gracía et al., 2013), with cutaneous squamous cell carcinoma (SCC) being the most common (Speeckaert et al., 2012). After 20 years of transplantation, about 40-50% of Caucasian patients from Western countries and 70-80% of Australian patients develop NMSC. White-skinned transplant patients are 65-250 times more likely to develop SCC than the general population (Ho; Murphy, 2008).

Piselli et al. (2013) evaluated the incidence of cancer in Italian kidney transplant patients and observed a 4.8% and 9.9% increase in the risk of developing this disease after 5 and 10 years, respectively, of immunosuppression. Immunosuppressive therapy contributes to the development of cancer in transplant patients by favoring oncogenic viral infections, decreasing immunological surveillance (responsible for preventing the development and growth of tumors), causing DNA damage, and decreasing the ability to repair such damages (Rama; Grinyó, 2010; van de Wetering et al., 2010; Engels et al., 2011).

Previously, we showed that immunosuppressive drugs may cause damage to genetic material in normal human cells exposed *in vitro* (Cilião et al, 2015) and that peripheral blood cells of kidney transplant patients, show a direct relationship between the amount of genotoxic damage and time after transplantation (Cilião et al., 2016).

Genetic factors, such as single nucleotide polymorphisms (SNPs), may also influence the risk of developing neoplasia, as they may alter the expression of the gene in question as well as the protein structure itself. Thus, SNPs in DNA repair genes can impair the maintenance of genome integrity, resulting in mutations that contribute to genetic instability and tumor development (Goode; Ulrich; Potter, 2002). Moreover, single base mutations in genes of the immune system, may alter cytokine production thus affecting the regulation of the inflammatory system either directly or indirectly (Suthanthiran, 2000), and may influence the antitumor response.

In this study, we assessed the association of polymorphic allelic variants of DNA damage repair genes (*APE1, XRCC1, ERCC1, XPA*, and *MGMT*) and those of the immune system genes (*IL-2, IL-10, IL-23R, TGF-β*), with the development of cancer in kidney transplant patients.

Methods

Population studied

Among 246 renal transplant patients undergoing post-transplant treatment at the Kidney Institute of Londrina (Londrina - PR, Brazil) 25 developed cancer after transplantation. Tumor presence was confirmed by histopathological analysis. These patients were matched for age, gender, ancestry, smoking status and alcohol consumption with hospital-based controls individuals that not underwent kidney transplantation and not had cancer. The controls samples used were obtained from the DNA stored in the Laboratory of Mutagenesis and Oncogenetics of the State University of Londrina.

The research protocol was approved by the Ethics Committee for Research in Human Beings at the State University of Londrina (CAAE: 18263413.4.0000.5231). Patients who agreed with the research signed a Free and Informed Consent Form, filled a questionnaire about lifestyle and history of environmental exposure. Patients were considered smokers and/or drinkers when they declared in an interview consuming any amount of cigarettes or alcohol.

Peripheral blood samples (1 mL) were collected intravenously from each cancer patient and free-neoplasia controls in vacuum blood collection tubes (EDTA 6%) (Labor Import, Osasco, Brazil).

Polymorphic allelic variants identification

Genomic DNA was extracted from 200 µL of blood, using the mini spin extraction kit (KASVI, Curitiba, Brazil; code K9-0250), following the manufacturer's recommendations. DNA samples were quantified using a NanoDrop 2000 spectrophotometer (ThermoScientific, Waltman, MA, USA).

Selection of SNPs was based on previous reports in the literature that indicated their high frequencies and participation in the development and progression of several malignant tumors.

Genotyping of rs1130409 (*APE1)* was performed by RFLP-PCR in a Veriti 96-well thermocycler (Life Technologies of Brazil Ltda., São Paulo, Brazil), using 2 mM dNTPs, 1.5 mM $MgCl_2$, 12.5 pmol of each primer, 0.5 U of Taq DNA polymerase in 10X PCR buffer (Life Technologies, São Paulo, Brazil), and 50 ng of genomic DNA, in a final volume of 20 µL. The primers sequences used were: sense 5' - CTGTTTCATTTCTATAGGCTA - 3' and antisense 5' - AGGAACTTGCGAAAGGCTTC - 3'. PCR conditions were: 94^{o}C for 5 minutes, 30 cycles consisting of 94^{o}C for 30 seconds, 55^{o}C for 30 seconds, and 72^{o}C for 30 seconds, followed by a final annealing step of 10 minutes at 72^{o}C. PCR product was digested by restriction enzyme *Bfa I* for 12 h at 37^{o}C. T/T genotype is not digested and result in a fragment with 165 pb; G/G genotype is cleaved resulting in two different size fragments (144 and 21 pb). Genotypes were determined by electrophoresis in 10% polyacrylamide gels stained with silver nitrate.

Genotyping of all others was performed by real-time PCR in a Quantica thermocycler (TECHNE, Staffordshire, UK), using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA), TaqMan Genotyping Master Mix (Applied Biosystems) and 5 ng of genomic DNA. Table 1 show the selected markers and the respective SNPs evaluated.

Statistical analysis

The continuous variables age (years) and time after transplantation (years), and the categorical variables (gender, degree of Human Leukocyte Antigen (HLA) compatibility, tobacco and alcohol consumption and diabetes after transplantation) were compared using Student's t-test between groups of patients with and without cancer. Association between cancer episodes and genotypes was performed using univariate logistic regression analysis using SPSS version 20 (IBM, Armonk, NY, USA). Results were presented as Odds Ratios (OR) with a 95% confidence interval (CI95%).

Results

Table 2 shows the main characteristics of transplant patients who either developed or did not develop cancer after transplantation. Out of the 246 patients evaluated in this study, 25 (10.2%) developed cancer after 15 ± 8.9 years of kidney transplantation. The comparison of averages using Student's t test showed a significant difference in the average age, time after transplantation, degree of HLA compatibility, and wart appearance, between patients with and without cancer.

Of the 25 patients who developed malignant tumors, 68% had non-melanoma carcinoma, and included ten cases of basal cell carcinoma and seven cases of squamous cell carcinoma. The remaining 32% comprised cases of prostatic adenocarcinoma, intracranial lymphoma, cervical cancer, Kaposi's sarcoma, plasmablastic lymphoma, gastric carcinoma, intraductal carcinoma associated with Paget's disease, and intestinal colon tumor.

Univariate logistic regression analysis of transplant patients with cancer and matched controls showed only one significative association between the C/A genotype of the SNP rs1800872 (*IL-10*) and a 3.5-fold higher risk of developing cancer (Table 3).

Discussion

Transplant patients have an increased risk of developing malignant tumors compared to the general population (Stallone; Infante; Grandaliano, 2015; Shang et al., 2016). This increased risk may be the effect of several factors, such as chronic use of immunosuppressive drugs, the degree of immunosuppression, viral infections, increased graft survival, advanced age of the patient, and genetic factors among others (Rama; Grinyó, 2010; Chapman; Webster; Wong, 2013).

In this study, the 10.2% of the kidney transplant recipients who developed cancer were older and had longer post-transplantation time periods compared to patients not diagnosed with the disease (Table 2). These data are consistent with the results of Aguiar et al. (2015) who also observed a higher incidence of cancer in older patients (58.0 ± 11.1 years at the time of diagnosis) with longer post-transplantation periods. Furthermore, these data support the view that cancer is a disease that normally develops after years of exposure to mutagenic

agents and compounds that suppress the immune system, such as immunosuppressants in this case.

The decreased immunity caused by immunosuppressants increases the vulnerability of patients to viral infections or reactivation of latent infections (Chapman; Webster; Wong, 2013). Among the cancers evaluated in this study, a case of cervical cancer associated with human papillomavirus (HPV) infections (Chin-Hong, 2016) and one case of Kaposi's sarcoma associated with human herpes virus 8 (HHV8) infections (Rama; Grinyó, 2010), were seen. In immunocompetent patients, Kaposi's sarcoma is a rare, disseminated cutaneous disease. However, in immunosuppressed patients, this disease is more aggressive, affecting several sites and disseminating to other organs (Chapman; Webster; Wong, 2013). In this study, there were no cases of vaginal, vulvar, penile, or anal cancer, which are associated with infections caused by HPV and affect transplant patients more often (Chin-Hong, 2016).

The HPV has been associated with development of warts in kidney transplant patients (Martelli-Marzagão et al., 2016). In this study, 72% of the transplant patients who developed cancer had warts (Table 2). According to Schmook et al. (2003), some of these viral warts have atypical histological features and may evolve to NMSCs.

NMSC is the most prevalent neoplasia among kidney transplant patients (Chapman; Webster; Wong, 2013; Wisgerhof et al., 2011). In this study, 68% of the diagnosed cancers were NMSC. In addition to opportunistic viral infections, the high prevalence of skin neoplasia may be attributed to high exposure to sunlight in Brazil, and the fact that many patients do not follow adequate prevention measures, such as avoiding sun exposure and wearing protective clothing and sunscreen.

Some studies indicate an association between genes involved in DNA repair and immune function and a higher risk of developing cancer (Goode; Ulrich; Potter, 2002; Alamartine et al., 2003). However, the association of SNPs in these genes with the risk of cancer development in Brazilian kidney transplant patients has not been studied yet.

In the present study, patients who developed cancer were matched by age, sex, ancestry, smoking status, and alcohol intake with control subjects who did not undergo kidney transplantation and did not have cancer. This pairing was performed with non-transplanted controls because there are no transplanted patients who developed cancer and who did not develop the disease, who share all these variables and who have the same post-transplantation time. Among the SNPs evaluated in this study, the C/A genotype of rs1800872 (*IL-10*) showed a positive association with a 3.5-fold increase in the risk of tumor development.

The rs1800872 (−592C>A) polymorphism evaluated in this study is in strong linkage disequilibrium with rs1800896 (−1082A>G) and rs1800871 (−819C>T), which are also located in the promoter region of *IL-10*. Hoffmann et al. 2001 evaluated the effect of the haplotype −1082A> G, −819C> T, and −592C> A on protein synthesis in human lymphocytes *in vitro* and found that the prevalent haplotype (GCC) correlates with an increased production of IL-10, compared to the ATA haplotype. Alamartine et al. (2003) evaluated the association of this haplotype with the occurrence of NMSCs in 70 kidney transplant patients paired with 70 controls and observed that the haplotype associated with greater IL-10 production was more frequent in cancer patients. These results contradict the observations of Imko-Walczuk et al. (2016), who assessed the IL-10 concentration in 17 transplant patients with malignant skin tumors and compared them with those of 85 patients without any post-transplant skin changes. They observed lower plasma concentrations of IL-10 in cancer patients, suggesting that the levels of this cytokine can be used to stratify the risk of skin tumor development in transplant patients.

The action of the interleukin IL-10 is complex, as it may have both stimulatory and inhibitory effects on tumors, depending on the cell that produces it, the time of secretion, and the cells of the immune system in which it will act (Geginat et al., 2016). IL-10 secreted by some tumors (lymphomas and melanomas), acts as a tumor growth factor (Geginat et al., 2016), as it induces immunological tolerance thus helping tumor cells to escape immune vigilance (Sato et al., 2011). In other types of cancers, IL-10 secreted by activated monocytes and macrophages hinders the development of the tumor microenvironment thus inhibiting tumor growth, angiogenesis, and metastasis (Kundu et al., 1996; Tanikawa et al., 2012).

In a meta-analysis to detect the association between rs1800872 (*IL-10*) and cancer risk in humans, Ding et al. (2013) showed studies with results conflicting. However in the overall results suggested that the variant homozygote A/A of this SNP was associated with a moderately decreased risk of all cancer type. In the present study it were not found significant association between the A/A genotype and malignant tumor development owing to the low number of carriers of this genotype in the studied population. Nevertheless it was observed a strong association between the C/A genotype and the increased risk of developing cancer.

The overexpression or the deficiency in the production of IL-10 was described under different pathophysiological conditions depending on the cancers analyzed (Asadullah; Sterry; Volk, 2013). In the present study, when was performed the comparative analysis only among patients who developed skin cancer (the most prevalent cancer in our sample) and their respective controls, it was no observed an association between this SNP and the risk of

developing this disease. For this, we suggest that the study with this SNP must be performed in a larger group of transplant patients who developed cancer, analysing each type of cancer separately for, in future, determining the actual contribution of this SNP to the risk of renal transplant patients develop cancer.

Conclusion

Among the types of cancers developed by kidney transplant patients evaluated in this study, the NMSC stands out. The high cancer risk observed can be attributed, along with other factors, by the C/A genotype of the SNP rs1800872 (IL-10). Kidney transplant patients who have this genotype were 3.5 times more likely to develop cancer than the control group. These results warrant further investigation and suggest that this SNP is a potentially reliable marker for the risk of cancer development in kidney transplant patients.

Conflict of interest

The authors declare no conflicts of interest.

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References

Acuna SA, Fernandes KA, Daly C, Hicks LK, Sutradhar R, Kim SJ, Baxter NN (2016) Cancer Mortality Among Recipients of Solid-Organ Transplantation in Ontario, Canada. JAMA Oncol 2:463-9. doi: 10.1001/jamaoncol.2015.5137.

Aguiar B, Santos Amorim T, Romãozinho C, Santos L, Macário F, Alves R, Campos M, Mota A (2015) Malignancy in Kidney Transplantation: A 25-Year Single-center Experience in Portugal. Transplant Proc 47:976-80. doi: 10.1016/j.transproceed.2015.03.039.

Alamartine E, Berthoux P, Mariat C, Cambazard F, Berthoux F (2003) Interleukin-10 promoter polymorphisms and susceptibility to skin squamous cell carcinoma after renal transplantation. J Invest Dermatol 120:99-103. doi: 10.1046/j.1523-1747.2003.12016.x.

Asadullah K, Sterry W, Volk HD. (2003) Interleukin-10 therapy--review of a new approach. Pharmacol Rev 55(2):241-69. doi: 10.1124/pr.55.2.4.

Chapman JR, Webster AC, Wong G (2013) Cancer in the Transplant Recipient. Cold Spring Harbor Laboratory Press. doi: 10.1101/cshperspect.a015677.

Chin-Hong PV (2016) Human Papillomavirus in Kidney Transplant Recipients. Semin Nephrol 36:397-404. doi: 10.1016/j.semnephrol.2016.05.016.

Cilião HL, Camargo-Godoy RB, Barcelos GRM, Zanuto A, Delfino VDA, Cólus IMS (2016) Long-term genotoxic effects of immunosuppressive drugs on lymphocytes of kidney transplant recipients. Mutat Res Genet Toxicol Environ Mutagen 806: 47-52. doi: 10.1016/j.mrgentox.2016.07.001.

Cilião HL, Ribeiro DL, Camargo-Godoy RB, Specian AF, Serpeloni JM, Cólus IM (2015) Cytotoxic and genotoxic effects of high concentrations of the immunosuppressive drugs cyclosporine and tacrolimus in MRC-5 cells. Exp Toxicol Pathol 67: 179-87. doi: 10.1016/j.etp.2014.11.008.

Ding Q, Shi Y, Fan B, Fan Z, Ding L, Li F, Tu W, Jin X, Wang J. (2013) The Interleukin-10 Promoter Polymorphism rs1800872 (-592C>A), Contributes to Cancer Susceptibility: Meta-Analysis of 16 785 Cases and 19 713 Controls. PLoS One. 8(2): e57246. doi: 10.1371/journal.pone.0057246.

Engels EA, Pfeiffer RM, Fraumeni JF Jr, Kasiske BL, Israni AK, Snyder JJ, Wolfe RA, Goodrich NP, Bayakly AR, Clarke CA, Copeland G, Finch JL, Fleissner ML, Goodman MT, Kahn A, Koch L, Lynch CF, Madeleine MM, Pawlish K, Rao C, Williams MA, Castenson D, Curry M, Parsons R, Fant G, Lin M (2011) Spectrum of Cancer Risk Among US Solid Organ Transplant Recipients. JAMA 306:1891-901. doi: 10.1001/jama.2011.1592.

García JB, Suárez-Varela MM, Vilata JJ, Marquina A, Pallardó L, Crespo J (2013) Risk Factors for Non-melanoma Skin Cancer in Kidney Transplant Patients in a Spanish Population in the Mediterranean Region. Acta Dermato-Venereologica 93:422-7. doi: 10.2340/00015555-1525.

Geginat J, Larghi P, Paroni M, Nizzoli G, Penatti A, Pagani M, Gagliani N, Meroni P, Abrignani S, Flavell RA (2016) The light and the dark sides of Interleukin-10 in immune-

mediated diseases and cancer. Cytokine Growth Factor Rev 30:87-93. doi: 10.1016/j.cytogfr.2016.02.003.

Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk (2002) Cancer Epidemiol Biomarkers Prev 11:1513-30. doi: 10.1093/carcin/22.4.593.

Ho WL, Murphy GM (2008) Update on the pathogenesis of post-transplant skin cancer in renal transplant recipients. Br J Dermatol 158: 217-24. doi: 10.1111/j.1365-2133.2007.08363.x.

Hoffmann SC, Stanley EM, Darrin Cox E, Craighead N, DiMercurio BS, Koziol DE, Harlan DM, Kirk AD, Blair PJ (2001) Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. Transplantation 72:1444-50.

Imko-Walczuk B, Piesiaków ML, Trzonkowski P, Pikuła M, Dębska-Ślizień A, Rutkowski B (2016) Associations of Selected Cytokines Levels in Organ Transplant Recipients Without and With Malignant Skin Neoplasms. Transplant Proc 48:1654-9. doi: 10.1016/j.transproceed.2016.03.024.

Kundu N, Beaty TL, Jackson MJ, Fulton AM (1996) Antimetastatic and antitumor activities of interleukin 10 in a murine model of breast cancer. J Natl Cancer Inst 88:536-41.

Martelli-Marzagão F, Santos Junior GF, Ogawa MM, Enokihara MM, Porro AM, Tomimori J (2016) Human papillomavirus detected in viral warts of renal transplant recipients. Transpl Infect Dis 18:37-43. doi: 10.1111/tid.12479.

O'Callaghan CA (2008) Kidney transplantation - the long term view. QJM 101:985-6. doi: 10.1093/qjmed/hcn144.

Piselli P, Serraino D, Segoloni GP, Sandrini S, Piredda GB, Scolari MP, Rigotti P, Busnach G, Messa P, Donati D, Schena FP, Maresca MC, Tisone G, Veroux M, Sparacino V, Pisani F, Citterio F (2013) Immunosuppression and Cancer Study Group. Risk of de novo cancers after transplantation: results from a cohort of 7217 kidney transplant recipients, Italy 1997-2009. Eur J Cancer 49: 336-44. doi: 10.1016/j.ejca.2012.09.013.

Rama I, Grinyó JM (2010) Malignancy after renal transplantation: the role of immunosuppression. Nat Rev Nephrol 6:511-9. doi: 10.1038/nrneph.2010.102.

Sato T, Terai M, Tamura Y, Alexeev V, Mastrangelo MJ, Selvan SR (2011) Interleukin 10 in the tumor microenvironment: a target for anticancer immunotherapy. Immunol Res 51:170-82. doi: 10.1007/s12026-011-8262-6.

Schmook T, Nindl I, Ulrich C, Meyer T, Sterry W, Stockfleth E (2003) Viral warts in organ transplant recipients: new aspects in therapy. Br J Dermatol 149:20-4. doi: 10.1046/j.0366-077X.2003.05627.x.

Schnuelle P, Lorenz D, Trede M, Van Der Woude FJ (1998) Impact of renal cadaveric transplantation on survival in end-stage renal failure: evidence for reduced mortality risk compared with hemodialysis during long-term follow-up. J Am Soc Nephrol 9:2135-41.

Shang W, Huang L, Li L, Li X, Zeng R, Ge S, Xu G (2016) Cancer risk in patients receiving renal replacement therapy: A meta-analysis of cohort studies. Mol Clin Oncol 5:315-25. doi: 10.3892/mco.2016.952.

Silva SB, Caulliraux HM, Araújo CAS, Rocha E (2016) Cost comparison of kidney transplant versus dialysis in Brazil. Cad. Saúde Pública, Rio de Janeiro 32:S0102-311X2016000605005. doi: 10.1590/0102-311X00013515.

Speeckaert R, Brochez L, Lambert J, van Geel N, Speeckaert MM, Claeys L, Langlois M, Van Laer C, Peeters P, Delanghe JR (2012) The haptoglobin phenotype influences the risk of cutaneous squamous cell carcinoma in kidney transplant patients. J Eur Acad Dermatol Venereol 26: 566-71. doi: 10.1111/j.1468-3083.2011.04112.x.

Stallone G, Infante B, Grandaliano G (2015) Management and prevention of post-transplant malignancies in kidney transplant recipients. Clin Kidney J 8:637-44. doi: 10.1093/ckj/sfv054.

Suthanthiran, M (2000) The importance of genetic polymorphisms in renal transplantation. Curr Opin Urol 10:71-5.

Tanikawa T, Wilke CM, Kryczek I, Chen GY, Kao J, Núñez G, Zou W (2012) Interleukin-10 ablation promotes tumor development, growth, and metastasis. Cancer Res 72:420-9. doi: 10.1158/0008-5472.

van de Wetering J, Roodnat JI, Hemke AC, Hoitsma AJ, Weimar W (2010) Patient survival after the diagnosis of cancer in renal transplant recipients: a nested case-control study. Transplantation 90:1542-6. doi: 10.1097/TP.0b013e3181ff1458.

Wisgerhof HC, van der Geest LG, de Fijter JW, Haasnoot GW, Claas FH, le Cessie S, Willemze R, Bouwes Bavinck JN (2011) Incidence of cancer in kidney-transplant recipients: A long-term cohort study in a single center. Cancer Epidemiol 35:105-11. doi: 10.1016/j.canep.2010.07.002.

Table 1: Genes and respective polymorphisms (SNPs) evaluated in renal transplant patients and in control individuals, and the type of mutation that originated each SNP and its chromosomal location.

Gene	SNP	Alternative nomenclature	Change	Chromosome location
XRCC1	rs25487	1196A>G	Exon (Arg399Gln)	14q11.2-q12
ERCC1	rs3212986	8092 C>A	3'ÙTR	19q13.2-q13.3
MGMT	rs2308321	2798995 A>G	Exon (Ile143Val)	10q26
XPA	rs1800975	-4A>G	5'UTR	9q22.3
APE1	rs1130409	-	Exon (Asp148Glu)	19q13.2-13.3
IL-2	rs2069762	-330G>T	Promoter	4q26-q27
IL-10	rs1800872	-592C>A	Promoter (5'UTR)	1q31-q32
IL-23R	rs10889677	-	3'UTR	1p31.3
TGF-β	rs1800470 rs1800471	29T>C 915G>C	Exon (Leu10Pro) Exon (Arg25Pro)	19q13.1-13.3

Table 2: General characteristics of kidney transplant patients.

Characteristics of patients	Patients with cancer		Patients without cancer		*p-value*[b]
	N (%)	Mean ± SD	N (%)	Mean ± SD	
Gender					0.912
Men	15 (60.0)	-	130 (58.8)	-	
Women	10 (40.0)	-	91 (41.2)	-	
Age (years)	25 (100)	60 ± 9.5	221 (100)	47 ± 12.3	**<0.001****
Age of patients at diagnosis of cancer	25 (100)	53 ± 11	-	-	-
Time after transplantation (years)	25 (100)	20 ± 8.7	221 (100)	9.5 ± 7.5	**<0.001****
Interval between transplantation and cancer diagnosis (years)	25 (100)	15 ± 8.9	-	-	-
Degree of HLA[a] compatibility					**0.023***
Living HLA-identical	8 (32.0)	-	46 (20.8)	-	
Living HLA-haploidentical	14 (56.0)	-	95 (43.0)	-	
Deceased unrelated	3 (12.0)	-	80 (36.2)	-	
Rejection episodes (yes)	7 (28.0)	-	79 (35.7)	-	0.443
Smoker (yes)	1 (4.0)	-	17 (7.7)	-	0.504
Drinker (yes)	5 (20)	-	30 (13.6)	-	0.385
Diseases					
Diabetes (yes)	6 (24)	-	47 (21.3)	-	0.767
Warts (yes)	18 (72)	-	73 (33.0)	-	**<0.001****

[a]HLA – Human Leukocyte Antigen, [b]*p*-values calculed by Student's t-test for independent samples comparing patients with rejection and without rejection; *p<0.050; ** p<0.010.

Table 3: Analysis of association between different genotypes of kidney transplant patients with cancer and control individuals.

Genes/SNP		Patients with câncer	Health control	OR (IC95%)[a]	*p* value
		N (%)	N (%)		
XPA rs1800975	G/G	11 (44)	8 (32)	Ref.[b]	
	G/A	11 (44)	17 (68)	0.47 (0.14-1.54)	0.21
	A/A	3 (12)	0 (0.0)	-	-
	A/G + A/A	14 (56)	17 (68)	0.60 (0.19-1.90)	0.38
ERCC1 rs3212986	C/C	16 (64)	14 (56)	Ref.	
	C/A	9 (36)	9 (36)	0.87 (0.27-2.82)	0.82
	A/A	0 (0.0)	2 (8.0)	-	-
	C/A + A/A	9 (36)	11 (44)	0.72 (0.23-2.23)	0.56
XRCC1 rs25487	G/G	15 (60)	11 (44)	Ref.	
	G/A	6 (24)	11 (44)	0.40 (0.11-1.41)	0.15
	A/A	4 (16)	3 (12)	0.98 (0.18-5.28)	0.98
	G/A + A/A	10 (40)	14 (56)	0.52 (0.17-1.61)	0.26
APE1 rs1130409	T/T	9 (36)	8 (32)	Ref.	
	T/G	13 (52)	15 (60)	0.72 (0.22-2.40)	0.60
	G/G	2 (8)	1 (4.0)	1.8 (0.13-23.52)	0.66
	T/G + G/G	15 (60)	16 (64)	0.78 (0.24-2.5)	0.78
MGMT rs2308321	A/A	22 (88)	22 (88)	Ref.	
	G/A	3 (12)	3 (12)	1.00 (0.18-5.51)	1.00
IL-23R rs10889677	C/C	13 (52)	15 (60)	Ref.	
	C/A	9 (36)	10 (40)	1.04 (0.32-3.34)	0.95
	A/A	3 (12)	0 (0.0)	-	-

	C/A + A/A	12 (48)	10 (40)	1.38 (0.45-4.25)	0.57
IL-2 rs2069762	A/A	11 (44)	13 (52)	Ref.	
	A/C	14 (56)	10 (40)	1.66 (0.53-5.18)	0.39
	C/C	0 (0.00)	2 (8.0)	-	-
	A/C + C/C	14 (56)	12 (48)	1.38 (0.45-4.20)	0.57
***IL-10* rs1800872**	C/C	8 (32)	14 (56)	Ref.	
	C/A	16 (64)	8 (32)	3.50 (1.04-11.79)	0.043*
	A/A	1 (4.0)	3 (12)	0.58 (0.052-6.59)	0.66
	C/A + A/A	17 (68)	11 (44)	2.70 (0.85-8.57)	0.091
TGF β rs1800470	C/C	7	8	Ref.	
	C/T	14	15	1.07 (0.31-3.72)	0.92
	T/T	4	2	2.29 (0.32-16.51)	0.41
	C/T + T/T	18	17	1.21 (0.36-4.07)	0.76
TGFβ rs1800471	G/G	21	21	Ref.	
	G/C + C/C	4	4	1.00 (0.22-4.54)	1.00

[a]Odds Ratio value with 95% confidence interval no adjusted, *p<0.050. [b]Ref - values taken as a reference.

9 CONCLUSÕES GERAIS

Os dados obtidos neste trabalho permitem concluír que:

- Os pacientes transplantados renais (N = 246) apresentam maior quantidade de danos genotóxicos e mutagênicos em linfócitos do sangue periférico quando comparados com indivíduos controles saudáveis.

- Quanto maior o tempo após o transplante renal, menor a taxa de filtração glomerular e maior o dano genotóxico observado em linfócitos do sangue periférico, possivelmente devido ao uso prolongado dos medicamentos imunossupressores. Não foi observada associação entre os danos observados no material genético com a função renal.

- Foi observada associação entre os SNPs rs7662029 (*UGT2B7*) e rs2231142 (*ABCG2*) com proteção contra rejeição e dos SNPs rs6714486 (*UGT1A9*) e rs10889677 (*IL-23R*) com aumento de risco para episódios de rejeição.

- Nos pacientes que faziam uso de MMF (N = 145) foi observada associação entre os SNPs rs11706052 (*IMPDH2*) e rs7438135 (*UGT2B7*) com a menor chance de desenvolver rejeição, e do SNP rs2241409 (*CES2*) com aumento de risco de rejeição.

- Os SNPs rs4646437 (*CYP3A4*) e rs776746 (*CYP3A5*) influenciaram a concentração plasmática ajustada do imunossupressor tacrolimo nos três primeiros meses de transplante, confirmando o impacto destes SNPs na farmacocinética do imunossupressor tacrolimo.

- Dos 246 pacientes transplantados 10.2 % desenvolveram câncer, sendo que destes 68% eram NMSC, cujo risco pode ser atribuído, entre outros, pela presença do genótipo C/A do SNP rs1800872 (*IL-10*), que demonstrou um aumento de risco de 3,5 vezes nestes pacientes.

10 REFERÊNCIAS

ABBAS, A. K.; LICHTMAN; A. H. Respostas imunológicas contra tumores e transplantes. In: _____. **Imunologia basica**: Funções e disturbios do sistema imunologico. 2ª Edição, Rio de Janeiro: Elsevier, 2007. p. 195-210.

ABBAS, A. K; LICHTMAN, A. H; POBER, J. S. O Complexo Principal de Histocompatibilidade, **Imunologia Celular e Molecular.** 4ª ed. Rio de Janeiro – RJ: Ed. Revinter, p. 97-111, 2003.

ACUNA, S. A.; FERNANDES, K. A.; DALY, C.; HICKS, L. K.; SUTRADHAR, R.; KIM, S. J.; BAXTER, N. N. Cancer Mortality Among Recipients of Solid-Organ Transplantation in Ontario, Canada. **JAMA Oncol**, v. 2, n. 4, p. 463-9, 2016. doi: 10.1001/jamaoncol.2015.5137.

AGNEZ-LIMA, L. F.; MEDEIROS, S. R. B.; MARQUES, R. C. P.; PINEIROS, M. M.; MENCK, C. F. M. Processos de reparo de DNA: garantindo a estabilidade do material genético. In: RIBEIRO, L. R.; SALVADORI, D. M. F.; MARQUES, E. K. **Mutagênese Ambiental**. 1ª Edição, Rio Grande do Sul: Editora Ultra, 2003. p. 49-79.

AGRAHARKAR, M. L.; CINCLAIR, R. D.; KUO, Y. F.; DALLER, J. A.; SHAHINIAN, V. B. Risk of malignancy with long-term immunosuppression in renal transplant recipients. **Kidney Int**, v. 66, n. 1, p. 383-9, 2004. doi: 10.1111/j.1523-1755.2004.00741.x.

AGUIAR, B.; SANTOS AMORIM, T.; ROMÃOZINHO, C.; SANTOS, L.; MACÁRIO, F.; ALVES, R.; CAMPOS, M.; MOTA, A. Malignancy in Kidney Transplantation: A 25-Year Single-center Experience in Portugal. **Transplant Proc**, v. 47, n. 4, p. 976-80, 2015. doi: 10.1016/j.transproceed.2015.03.039.

ALAMARTINE, E.; BERTHOUX, P.; MARIAT, C.; CAMBAZARD, F.; BERTHOUX, F.; Interleukin-10 promoter polymorphisms and susceptibility to skin squamous cell carcinoma after renal transplantation. **J Invest Dermatol**, v. 120, n. 1, p. 99-103, 2003. doi: 10.1046/j.1523-1747.2003.12016.x.

ALBERTS, B.; JOHNSON, A.; LEWIS, J.; RAFF, M.; ROBERTS, K.; WALTER, P. Sistema Imune Adaptativo. In: _____. **Biologia Molecular da Célula**. Editora Artmed, 5a edição, São Paulo: Artmed, 2010. p. 1551-60.

ALBRING, A.; WENDT, L.; HARZ, N.; ENGLER, H.; WILDE, B.; KRIBBEN, A.; LINDEMANN, M.; SCHEDLOWSKI, M.; WITZKE, O. Relationship between pharmacokinetics and pharmacodynamics of calcineurin inhibitors in renal transplant patients. **Clin Transplant**, 2015. doi: 10.1111/ctr.12504. [Epub ahead of print]

ALLISON, A. C.; EUGUI, E. M. Mycophenolate mofetil and its mechanisms of action. **Immunopharmacology**, v. 47, n. 2-3, p. 85-118, 2000. doi: 10.1016/S0162-3109(00)00188-0.

AL-UZRI, A.; STABLEIN, D. M. A.; COHN, R. Posttransplant diabetes mellitus in pediatric renal transplant recipients: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). **Transplantation**, v. 72, n. 6, p. 1020-4, 2001. doi: 10.1097/00007890-200109270-00007.

ANTIGNAC, M.; BARROU, B.; FARINOTTI, R.; LECHA,T. P.; URIEN, S. Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. **Br J Clin Pharmacol**, v. 64, n. 6, p. 750-7, 2007. doi:10.1111/j.1365-2125.2007.02895.x.

APEL, H.; WALSCHBURGER-ZORN, K.; HÄBERLE, L.; WACH, S.; ENGEHAUSEN, D. G.; WULLICH, B. De novo malignancies in renal transplant recipients: experience at a single center with 1882 transplant patients over 39 yr. **Clin Transplant**, v. 27 p. E30-36, 2013. doi: 10.1111/ctr.12050.

ASADULLAH K, STERRY W, VOLK HD. Interleukin-10 therapy--review of a new approach. **Pharmacol Rev**, v. 55, n. 2, p. 241-69, 2003. doi: 10.1124/pr.55.2.4.

ASTELLAS PHARMA US INC: Prograf Package Insert. Deerfield, IL, Astellas Pharma US Inc, 2005.

AUGUSTINE, J. J.; BODZIAK, K. A.; HRICIK, D. E. Use of sirolimus in solid organ transplantation. **Drugs**, v. 67, n. 3, p. 369-91, 2007. doi: 10.2217/imt.11.143.

AWAD, M. R.; WEBBER, S.; BOYLE, G.; STURCHIOĈ, C.; AHMED, M.; MARTELL, J.; LAW, Y.; MILLER, S. A.; BOWMAN, P.; GRIBAR, S.; PIGULA, F.; MAZARIEGOS, G.; GRIFFITH, B. P.; ZEEVI, A. The effect of cytokine gene polymorphisms on pediatric heart allograft outcome. **J Heart Lung Transplant**, v. 20, n. 6, p. 625-30, 2001. doi:10.1016/S1053-2498(01)00246-7.

AYKANAT, B.; DEMIRCIGIL, G. C.; FIDAN, K.; BUYAN, N.; GULLEROGLU, K.; BASKIN, E.; BAYRAKCI, U. S.; SEPICI, A.; BUYUKKARAGOZ, B.; KARAKAYALI, H.; HABERAL, M.; BURGAZ, S. Basal damage and oxidative DNA damage in children with chronic kidney disease measured by use of the comet assay. **Mutat Res**, v. 9, n. 725, p. 22-8, 2011. doi: 10.1016/j.mrgentox.2011.07.005.

BARLOW, A. D.; NICHOLSON, M. L.; HERBERT, T. P. Evidence for rapamycin toxicity in pancreatic β-cells and a review of the underlying molecular mechanisms. **Diabetes**, v. 62, n. 8, p. 2674-82, 2013. doi: 10.2337/db13-0106.

BARRETT, J. C.; FRY, B.; MALLER, J.; DALY, M. J. Haploview: analysis and visualization of LD and haplotype maps. **Bioinformatics**, v. 21, p. 263-265, 2005. doi: 0.1093/bioinformatics/bth457.

BAUSINGER, J.; SPEIT, G. The impact of lymphocyte isolation on induced DNA damage in human blood samples measured by the comet assay. **Mutagenesis**, v. 31, n. 15, p. 567-72, 2016. doi:10.1093/mutage/gew021.

BENFIELD, M. R.; BARTOSH, S.; IKLE, D. WARSHA,W. B.; BRIDGES, N.; MORRISON, Y.; HARMON, W. A randomized double-blind, placebo controlled trial of steroid withdrawal after pediatric renal transplantation. **Am J Transplant**, v. 10, n. 1, p. 81-8, 2010. doi: 10.1111/j.1600-6143.2009.02767.x.

BENZA, R. L.; COFFEY, C. S.; PEKAREK, D. M.; BARCHUE, J. P.; TALLAJ, J. A.; PASSINEAU, M. J.; GRENETT, H. E. Transforming growth factor-beta polymorphisms and cardiac allograft rejection. **J Heart Lung Transplant**, v. 28, n. 10, p. 1057-62, 2009. doi: 10.1016/j.healun.2009.06.001.

BETONICO, G. N.; ABBUD-FILHO, M.; GOLONI-BERTOLLO, E. M.; PAVARINO-BERTELLI, E. Pharmacogenetics of mycophenolate mofetil: a promising different approach to tailoring immunosuppression? **J Nephrol**, v. 21, n. 4, p. 503-9, 2008.

BLESKESTAD, I. H.; BERGREM, H.; LEIVESTAD, T.; HARTMANN, A.; GØRANSSON, L. G. Parathyroid hormone and clinical outcome in kidney transplant patients with optimal transplant function. **Clinical Transplantation**, v. 28, n. 4, p. 479-86, 2014. doi: 10.1111/ctr.12341

BLOBE, G. C.; SCHIEMANN, W. P.; LODISH, H. F. Role of transforming growth factor beta in human disease. **N Engl J Med**, v. 342, n. 18, p. 1350-8, 2000. doi: 10.1056/NEJM200005043421807.

BRASIL. Ministério da Saúde. Aprova o Protocolo Clínico e Diretrizes Terapêuticas - Imunossupressão no Transplante Renal. Portaria n. 666, de 17 de julho de 2012. Disponivel em: <http://bvsms.saude.gov.br/bvs/saudelegis/sas/2012/prt0666_17_07_2012.html> Acesso em: 26 jul. 2015.

BRASIL. Sociedade Brasileira de Transplante de Orgãos, Registro Brasileiro de transplante 2015. Disponivel em: < http://www.abto.org.br/abtov03/Upload/file/RBT/2015/anual-n-associado.pdf> Acesso em 27 out. 2016.

BRAZELTON, T. R.; MORRIS, R. E. Molecular mechanism of action of new xenobiotic immunosuppressive drugs: tacrolimus (FK-506), sirolimus (rapamycin), mycophenolate mofetil and leftumide. **Transplantation**, v. 8, n. 5, p. 710-20, 1996. doi: 10.1016/S0952-7915(96)80090-2.

BULLINGHAM, R. E.; NICHOLLS, A. J.; KAMM, B. R. Clinical pharmacokinetics of mycophenolate mofetil. **Clin Pharmacokinet**, v. 34, n. 6, p. 429-55, 1998. doi:10.2165/00003088-199834060-00002.

BURCKART, G. J.; FIGG, W. D.; BROOKS, M. M.; GREEN, D. J.; TROUTMAN, S. M.; FERRELL, R.; CHINNOCK, R.; CANTER, C.; ADDONIZIO, L.; BERNSTEIN, D.; KIRKLIN, J. K.; NAFTEL, D.; PRICE, D. K.; SISSUNG, T. M.; GIRNITA, D. M.; ZEEVI, A.; WEBBER, S. A. Multi-institutional Study of Outcomes After Pediatric Heart Transplantation: Candidate Gene Polymorphism Analysis of ABCC2. **J Pediatr Pharmacol Ther**, v. 19, n. 1, p. 16-24, 2014. doi: 10.5863/1551-6776-19.1.16.

BURCKART, G. J.; AMUR, S. Update on the clinical pharmacogenomics of organ transplantation. **Pharmacogenomics.** v.11, n. 2, p. 227-36, 2010. doi: 10.2217/pgs.09.177.

BUXTON, I. L.; BENET, L. Z. Farmacocinética: A dinâmica da absorção, distribuição, ação e eliminação dos fármacos, capítulo 2. In: BRUNTON, L. L.; CHABNER, B. A.; KNOLLMANN, B. C. **As bases farmacológicas da terapêutica de Googman e Gilman.** 12ª edição, São Paulo: AMGH Editora Ltda, 2012. p.17-40.

CAKMAK DEMIRCIGIL, G.; AYKANAT, B.; FIDAN, K.; GULLEROGLU, K.; BAYRAKCI, U. S.; SEPICI, A.; BUYUKKARAGOZ, B.; KARAKAYALI, H.; HABERAL, M.; BASKIN, E.; BUYAN, N.; BURGAZ, S. Micronucleus frequencies in peripheral blood lymphocytes of children with chronic kidney disease. **Mutagenesis**, v. 26, n. 5, p. 643-50, 2011. doi: 10.1093/mutage/ger027.

CARTWRIGHT, N. H.; KEEN, L. J.; DEMAINE, A. G.; HURLOCK, N. J.; MCGONIGLE, R. J.; ROWE, P. A.; SHAW, J. F.; SZYDLO, R. M.; KAMINSKI, E. R. A study of cytokine gene polymorphisms and protein secretion in renal transplantation. **Transpl Immunol**, v. 8, n. 4, p. 237-44, 2001. doi:10.1016/S0966-3274(01)00026-0.

CECKA, M. Clinical outcome of renal transplantation. Factors influencing patient and graft survival. **Surg Clin North Am,** v. 78, n. 1, p. 133-48, 1998. doi:10.1016/S0039-6109(05)70639-3.

CHAPMAN, J. R.; WEBSTER, A. C.; WONG, G. Cancer in the Transplant Recipient, **Cold Spring Harb Perspect Med**, v. 3, n. 7, p. a015677, 2013. doi: 10.1101/cshperspect.a015677.

CHAPMAN, J. R.; O'CONNELL, P. J.; NANKIVELL, B. J. Chronic renal allograft dysfunction. **J Am Soc Nephrol.** v. 16, n. 10, p. 3015-26, 2005.

CHEN, P.; WIENCKE, J.; ALDAPE, K.; KESLER-DIAZ, A.; MIIKE, R.; KELSEY, K.; LEE, M.; LIU, J.; WRENSCH, M. Association of an ERCC1 polymorphism with adult-onset glioma. **Cancer Epidemiol Biomarkers Prev**, v. 9, n. 8, p. 843-7, 2000.

CHEN, Y.; SAMPAIO, M. S.; YANG, J. W.; MIN, D.; HUTCHINSON, I. V. Genetic Polymorphisms of the Transcription Factor NFATc4 and Development of New-Onset Diabetes After Transplantation in Hispanic Kidney Transplant Recipients. **Transplantation**, v. 93, n. 3, p. 325-30, 2012. doi: 10.1097/TP.0b013e31823f7f26.

CHEN, Y. K.; HAN, L. Z.; XUE, F.; SHEN, C. H.; LU, J.; YANG, T. H.; ZHANG, J. J.; XIA, Q. Personalized tacrolimus dose requirement by CYP3A5 but not ABCB1 or ACE genotyping in both recipient and donor after pediatric liver transplantation. **PLoS One**, v. 9, n. 10, p. e109464, 2014. doi: 10.1371/journal.pone.0109464.

CHEN, W.; ROYER, W. E Jr. Strutural insights into interferon regulatory factor activation. **Cell Signal**, v. 22, n. 6, p. 883-7, 2010. doi: 10.1016/j.cellsig.2009.12.005.

CHEUNG, C. Y.; CHAN, H. W.; LIU, Y. L.; CHAU, K. F.; LI, C. S. Long-term graft function with tacrolimus and cyclosporine in renal transplantation: paired kidney analysis. **Nephrology (Carlton),** v. 14, n. 8, p. 758-63, 2009. doi: 10.1111/j.1440-1797.2009.01155.x.

CHIN-HONG, P. V. Human Papillomavirus in Kidney Transplant Recipients. **Semin Nephrol**, v. 36, n. 5, p. 397-494, 2016. doi: 10.1016/j.semnephrol.2016.05.016.

CILIÃO, H. L.; CAMARGO-GODOY, R. B.; BARCELOS, G. R. M.; ZANUTO, A.; DELFINO, V. D. A.; CÓLUS, I. M. S. Long-term genotoxic effects of immunosuppressive drugs on lymphocytes of kidney transplant recipients. **Mutat Res Genet Toxicol Environ Mutagen**, v. 806, p. 47-52, 2016. doi: 10.1016/j.mrgentox.2016.07.001.

CILIÃO, H. L.; RIBEIRO, D. L.; CAMARGO-GODOY, R. B.; SPECIAN, A. F.; SERPELONI, J. M.; CÓLUS, I. M. Cytotoxic and genotoxic effects of high concentrations of the immunosuppressive drugs cyclosporine and tacrolimus in MRC-5 cells. **Exp Toxicol Pathol**, v. 67, n. 2, p. 179-87, 2015. doi: 10.1016/j.etp.2014.11.008.

COLLINS, A.; KOPPEN, G.; VALDIGLESIAS, V.; DUSINSKA, M.; KRUSZEWSKI, M.; MØLLER, P.; ROJAS, E.; DHAWAN, A.; BENZIE, I.; COSKUN, E.; MORETTI, M.; SPEIT, G.; BONASSI, S.; COMNET PROJECT. The comet assay as a tool for human biomonitoring studies: the ComNet project. **Mutat Res Rev Mutat Res**, v.759, p. 27-39, 2014. doi: 10.1016/j.mrrev.2013.10.001.

CRONE, T. M.; PEGG, A. E. A single amino acid change in human O6-alkylguanine-DNA alkyltransferase decrreasing sensitivity to inactivation by O6-benzylguanine. **Cancer Res**, v. 53, n. 20, p. 4750-3, 1993.

COOPER, G. M.; HAUSMAN, R. E. Replicação, manutenção e rearranjos do DNA genômico. In: _____. **A célula: uma abordagem molecular**. 3. ed. Porto Alegre: Artmed, 2007. p. 177-227.

DING Q, SHI Y, FAN B, FAN Z, DING L, LI F, TU W, JIN X, WANG J. The Interleukin-10 Promoter Polymorphism rs1800872 (-592C>A), Contributes to Cancer Susceptibility: Meta-Analysis of 16 785 Cases and 19 713 Controls. **PLoS One**. v. 8, n. 2, p. e57246, 2013. doi: 10.1371/journal.pone.0057246.

DJEBLI, N.; PICARD, N.; RÉROLLE, J. P.; LE MEUR, Y.; MARQUET, P. Influence of the UGT2B7 promoter region and exon 2 polymorphisms and comedications on Acyl-MPAG production in vitro and in adult renal transplant patients. **Pharmacogenet Genomics**, v. 17, n. 5, p. 321-30, 2007. doi: 10.1097/FPC.0b013e32801430f8.

DUGUAY, Y.; BÁÁR, C.; SKORPEN, F.; GUILLEMETTE, C. A novel functional polymorphism in the uridine diphosphate-glucuronosyltransferase 2B7 promoter with significant impact on promoter activity. **Clin Pharmacol Ther**, v. 75, n. 3, p. 223-33, 2004. doi:10.1016/j.clpt.2003.10.006.

ELENS, L.; BOUAMAR, R.; HESSELINK, D. A.; HAUFROID, V.; VAN DER HEIDEN, I. P.; VAN GELDER, T.; VAN SCHAIK, R. H. A new functional CYP3A4 intron 6 polymorphism significantly affects tacrolimus pharmacokinetics in kidney transplant recipients. **Clin Chem**, v. 57, n. 11, p. 1574-83, 2011. doi: 10.1373/clinchem.2011.165613.

ENGELS, E. A.; PFEIFFER, R. M.; FRAUMENI, J. F. JR.; KASISKE, B. L.; ISRANI, A. K.; SNYDER, J. J.; WOLFE, R. A.; GOODRICH, N. P.; BAYAKLY, A. R.; CLARKE, C. A.; COPELAND, G.; FINCH, J. L.; FLEISSNER, M. L.; GOODMAN, M. T.; KAHN, A.; KOCH. L.; LYNCH, C. F.; MADELEINE, M. M.; PAWLISH, K.; RAO, C.; WILLIAMS, M. A.; CASTENSON, D.; CURRY, M.; PARSONS. R.; FANT, G.; LIN, M. Spectrum of Cancer Risk Among US Solid Organ Transplant Recipients. **JAMA-Journal of the American Medical Association**, v. 306, n. 17, p. 1891-901, 2011. doi: 10.1001/jama.2011.1592.

EPPENGA, W. L.; KRAMERS, C.; DERIJKS, H. J.; WENSING, M.; WETZELS, J. F.; DE SMET, P. A. Individualizing Pharmacotherapy in Patients with Renal Impairment: The Validity of the Modification of Diet in Renal Disease Formula in Specific Patient Populations with a Glomerular Filtration Rate below 60 Ml/Min. **A Systematic Review.PLoS One,** v. 10, n. 3, p. e0116403, 2015. doi: 10.1371/journal.pone.0116403.

ESTELLER, M.; HAMILTON, S. R.; BURGER, P. C.; BAYLIN, S. B.; HERMAN, J. G. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. **Cancer Res**, v. 59, n. 4, p. 793-7, 1999.

FADILI, W.; HABIB ALLAH, M.; LAOUAD, I. Chronic renal allograft dysfunction: risk factors, immunology and prevention. **Arab J Nephrol Transplant**, v. 6, n. 1, p. 45-50, 2013.

FENECH, M.; HOLLAND, N.; CHANG, W. P.; ZEIGER, E.; BONASSI, S. The HUman MicroNucleus Project--An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. **Mutat Res**, v. 428, n. 1-2, p. 271-83, 1999. doi:10.1016/S1383-5742(99)00053-8.

FENECH, M.; MORLEY, A. A. Measurement of micronuclei in lymphocytes. **Mutat Res**, v. 147, n. 1-2, p. 29-36, 1985. doi:10.1016/0165-1161(85)90015-9.

FENECH, M. Cytokinesis-block micronucleus cytome assay. **Nat Protoc**, v. 2, n. 5, p. 1084-104, 2007. doi: 10.1038/nprot.2007.77.

FISCHEREDER, M.; LUCKOW, B.; HOCHER, B.; WÜTHRICH, R. P.; ROTHENPIELER, U.; SCHNEEBERGER, H.; PANZER, U.; STAHL, R. A.; HAUSER, I. A.; BUDDE, K.; NEUMAYER, H.; KRÄMER, B. K.; LAND, W.; SCHLÖNDORFF, D. CC chemokine receptor 5 and renal-transplant survival. **Lancet**, v. 357, n. 9270, p. 1758-61, 2001. doi:10.1016/S0140-6736(00)04898-4.

FRAILE, P.; GARCÍA-COSMES, P.; MARTÍN, P.; GARCÍA-BERNALT, V.; TABERNERO, J. M. Non-skin solid tumors as a cause of morbidity and mortality after liver transplantation. **Transplant Proc**, v. 41, n. 6, p. 2433-4, 2009. doi:10.1016/j.transproceed.2009.06.022.

FUJIYAMA, N.; MIURA, M.; KATO, S.; SONE, T.; ISOBE, M.; SATOH, S. Involvement of carboxylesterase 1 and 2 in the hydrolysis of mycophenolate mofetil. **Drug Metab Dispos**, v. 38, n. 12, p. 2210-7, 2010. doi: 10.1124/dmd.110.034249.

FUJIYAMA, N.; MIURA, M.; SATOH, S.; INOUE, K.; KAGAYA, H.; SAITO, M.; HABUCHI, T.; SUZUKI, T. Influence of carboxylesterase 2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. **Xenobiotica**, v. 39, n. 5, p. 407-14, 2009. doi: 10.1080/00498250902807338.

FURUKAWA, T.; WAKABAYASHI, K.; TAMURA, A.; NAKAGAWA, H.; MORISHIMA, Y.; OSAWA, Y.; ISHIKAWA, T. Major SNP (Q141K) variant of human ABC transporter ABCG2 undergoes lysosomal and proteasomal degradations. **Pharm Res**, v. 26, n. 2, p. 469-79, 2009. doi: 10.1007/s11095-008-9752-7.

GARCÍA, J. B.; SUÁREZ-VARELA, M. M.; VILATA, J. J.; MARQUINA, A.; PALLARDÓ, L. CRESPO, J. Risk Factors for Non-melanoma Skin Cancer in Kidney Transplant Patients in a Spanish Population in the Mediterranean Region. **Acta Derm Venereol**, v. 93, n. 4, p. 422-7, 2013. doi: 10.2340/00015555-1525.

GARCIA, M.; MACÍAS, R. M.; CUBERO, J. J.; BENÍTEZ, J.; CARAVACA, F.; GERVASINI, G. ABCB1 polymorphisms are associated with cyclosporine-induced nephrotoxicity and gingival hyperplasia in renal transplant recipients. **Eur J Clin Pharmacol**, v. 69, n. 3, p. 385-93, 2013. doi: 10.1007/s00228-012-1355-x.

GE, Y. Z.; WU, R.; LU, T. Z.; JIA, R. P.; LI, M. H.; GAO, X. F.; JIANG, X. M.; ZHU, X. B.; LI, L. P.; TAN, S. J.; SONG, Q.; LI, W.C.; ZHU. J. G. Combined effects of TGFB1 +869 T/C and +915 G/C polymorphisms on acute rejection risk in solid organ transplant recipients: a systematic review and meta-analysis. **PLoS One**, v. 9, n. 4, p.e93938, 2014. doi: 10.1371/journal.pone.0093938.

GEGINAT, J.; LARGHI, P.; PARONI, M.; NIZZOLI, G.; PENATTI, A.; PAGANI, M.; GAGLIANI, N.; MERONI, P.; ABRIGNANI, S.; FLAVELL, R. A. The light and the dark sides of Interleukin-10 in immune-mediated diseases and cancer. **Cytokine Growth Factor Rev**, v. 30, p. 87-93, 2016. doi: 10.1016/j.cytogfr.2016.02.003.

GENSBURGER, O.; VAN SCHAIK, R. H.; PICARD, N.; LE MEUR, Y.; ROUSSEAU, A.; WOILLARD, J. B.; VAN GELDER, T.; MARQUET, P. Polymorphisms in type I and II inosine monophosphate dehydrogenase genes and association with clinical outcome in patients on mycophenolate mofetil. **Pharmacogenet Genomics**, v. 20, n. 9, p. 537-43, 2010. doi: 10.1097/FPC.0b013e32833d8cf5.

GIJSEN, V. M.; VAN SCHAIK, R. H.; SOLDIN, O. P.; SOLDIN, S. J.; NULMAN, I.; KOREN, G.; DE WILDT, S. N. P450 oxidoreductase *28 (POR*28) and tacrolimus disposition in pediatric kidney transplant recipients--a pilot study. **Ther Drug Monit**, v. 36, n. 2, p. 152-8, 2014. doi: 10.1097/FTD.0b013e3182a3f282.

GIRARD, H.; COURT, M. H.; BERNARD, O.; FORTIER, L. C.; VILLENEUVE, L.; HAO, Q.; GREENBLATT, D. J.; VON MOLTKE, L. L.; PERUSSED, L.; GUILLEMETTE, C. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver. **Pharmacogenetics**, v. 14, n. 8, p. 501-15, 2004. doi: 10.1097/01.fpc.0000114754.08559.27.

GLANDER, P.; BRAUN, K. P.; HAMBACH, P.; BAUER, S.; MAI, I.; ROOTS, I.; WAISER, J.; FRITSCHE, L.; NEUMAYER, H. H.; BUDDE, K. Non-radioactive determination of inosine 5'-monophosphate dehydro-genase (IMPDH) in peripheral mononuclear cells. **Clin Biochem**, v. 34, n. 7, p. 543-9, 2001. doi:10.1016/S0009-9120(01)00267-3

GOODE, E. L.; ULRICH, C. M.; POTTER, J. D. Polymorphisms in DNA repair genes and associations with cancer risk. **Cancer Epidemiol Biomarkers Prev**, v. 11, n. 12, p. 1513-30, 2002.

GRINYÓ, J.; VANRENTERGHEM, Y.; NASHAN, B.; VINCENTI, F.; EKBERG, H.; LINDPAINTNER, K.; RASHFORD, M.; NASMYTH-MILLER, C.; VOULGARI, A.; SPLEISS, O.; TRUMAN, M.; ESSIOUX, L. Association of four DNA polymorphisms with acute rejection after kidney transplantation. **Transpl Int,** v. 21, n. 9, p. 879-91, 2008. doi: 10.1111/j.1432-2277.2008.00679.x.

HAN, W.; KIM, K. Y.; YANG, S. J.; NOH, D. Y.; KANG, D.; KWACK, K. SNP-SNP interactions between DNA repair genes were associated with breast cancer risk in a Korean population**. Cancer**, v. 118, n. 3, p. 594-602, 2012. doi: 10.1002/cncr.26220.

HARKENSEE, C.; OKA, A.; ONIZUKA, M.; MIDDLETON, P. G.; INOKO, H.; HIRAYASU, K.; KASHIWASE, K.; YABE, T.; NAKAOKA, H.; GENNERY, A. R.; ANDO, K.; MORISHIMA, Y.; JAPAN MARROW DONOR PROGRAM. Single nucleotide polymorphisms and outcome risk in unrelated mismatched hematopoietic stem cell transplantation: an exploration study. **Blood**, v. 119, n. 26, p. 6365-72, 2012. doi: 10.1182/blood-2012-01-406785.

HART, S. N.; ZHONG, X. B. P450 oxidoreductase: genetic polymorphisms and implications for drug metabolism and toxicity. **Expert Opin Drug Metab Toxicol**, v. 4, n. 4, p. 439-52, 2008. doi: 10.1517/17425255.4.4.439 .

HAUFROID, V. Genetic polymorphisms of ATP-binding cassete transporters ABCB1 and ABCC2 and their impact on drug disposition. **Curr Drug Targets**, v. 12, n. 5, p. 631-46, 2011. doi: 10.2174/138945011795378487.

HEBERT, M. F. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. **Adv Drug Deliv Rev**, v. 27, p. 201-14, 1997.

HENDERSON, C. J,; OTTO, D. M. E.; CARRIE, D.; MAGNUSON, M. A.; MCLAREN, A. W.; ROSEWELL, I.; WOLF, C. R. Inactivation of the Hepatic Cytochrome P450 System by Conditional Deletion of Hepatic Cytochrome P450 Reductase**. J Biol Chem**, v. 278, n. 15, p. 13480-6, 2003. doi:10.1074/jbc.M212087200.

HESSELINK, D. A.; BOUAMAR, R.; ELENS, L.; VAN SCHAIK, R. H.; van GELDER, T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. **Clin Pharmacokinet**, v. 53, n. 2, p. 123-39, 2014. doi: 10.1007/s40262-013-0120-3.

HESSELINK, D. A.; van SCHAIK, R. H.; van DER HEIDEN, I. P.; van DER WERF, M.; GREGOOR, P. J.; LINDEMANS, J.; WEIMAR, W.; van GELDER, T. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. **Clin Pharmacol Ther**, v. 74, n. 3, p. 245-54, 2003. doi:10.1016/S0009-9236(03)00168-1.

HIROUCHI, M.; SUZUKI, H.; ITODA, M.; OZAWA, S.; SAWADA, J.; IEIRI, I.; OHTSUBO, K.; SUGIYAMA, Y. Characterization of the cellular localization, expression level, and function of SNP variants of MRP2/ABCC2. **Pharm Res**, v. 21, n. 5, p. 742-8, 2004.

HO, W. L,; MURPHY, G. M. Update on the pathogenesis of post-transplant skin cancer in renal transplant recipients. **Br J Dermatol**, v. 158, n. 2, p. 217-24, 2008. doi: 10.1111/j.1365-2133.2007.08363.x.

HOFFMANN, S. C,; STANLEY, E. M.; DARRIN COX, E.; CRAIGHEAD, N.; DIMERCURIO, B. S.; KOZIOL, D. E.; HARLAN, D. M.; KIRK, A. D.; BLAIR, P. J. Association of cytokine polymorphic inheritance and in vitro cytokine production in anti-CD3/CD28-stimulated peripheral blood lymphocytes. **Transplantation**, v. 72, n. 8, p. 1444-50, 2001. doi: 0041-1337/01/7208-1444/0.

HOLTHE, M.; RAKVÅG, T. N.; KLEPSTAD, P.; IDLE, J. R.; KAASA, S.; KROKAN, H. E.; SKORPEN, F. Sequence variations in the UDP-glucuronosyltransferase 2B7 (UGT2B7) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients. **Pharmacogenomics J**, v.3, n.1, p.17-26, 2003. doi:10.1038/sj.tpj.6500139.

HU, D. G.; MEECH, R.; LU, L.; MCKINNON, R. A.; MACKENZIE, P. I. Polymorphisms, and haplotypes of the UDP-glucuronosyltransferase 2B7 gene promoter. **Drug Metab Dispos**, v. 42, n. 5, p. 854-62, 2014. doi: 10.1124/dmd.113.056630.

HUANG, N.; AGRAWAL, V.; GIACOMINI, K. M.; MILLER, W. L. Genetics of P450 oxidoreductase: Sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. **Proc Natl Acad Sci USA**, v.105, n. 5, p. 1733–8, 2008. doi: 10.1073/pnas.0711621105.

HUBBARD, P. A.; SHEN, A. L.; PASCHKE, R.; KASPER, C. B.; KIM, J. J. NADPH-cytochrome P450 oxidoreductase. Structural basis for hydride and electron transfer. **J Biol Chem**, v. 276, n. 31, p. 29163-70, 2001. doi:10.1074/jbc.M101731200.

HUTTON, B.; JOSEPH, L.; YAZDI, F.; TETZLAFF, J.; HERSI, M.; KOKOLO, M.; FERGUSSON, N.; BENNETT, A.; BUENAVENTURA, C.; FERGUSSON, D.; TRICCO, A.; STRAUSS, S.; MOHER, D.; KNOLL, G. Checking whether there is an increased risk of post-transplant lymphoproliferative disorder and other cancers with specific modern immunosuppression regimens in renal transplantation: protocol for a network meta-analysis of randomized and observational studies. **Syst Rev**, v. 3, n. 16, 2014. doi: 10.1186/2046-4053-3-16.

IMKO-WALCZUK, B.; PIESIAKÓW, M. L.; TRZONKOWSKI, P.; PIKUŁA, M.; DĘBSKA-ŚLIZIEŃ, A.; RUTKOWSKI, B. Associations of Selected Cytokines Levels in Organ Transplant Recipients Without and With Malignant Skin Neoplasms. **Transplant Proc**, v. 48, n. 5, p. 1654-9, 2016. doi: 10.1016/j.transproceed.2016.03.024.

INNOCENTI, F.; LIU, W.; FACKENTHAL, D.; RAMÍREZ, J.; CHEN, P.; YE, X.; WU, X.; ZHANG, W.; MIRKOV, S.; DAS, S.; COOK, E. JR.; RATAIN, M. J. Single nucleotide polymorphism discovery and functional assessment of variation in the UDP-glucuronosyltransferase 2B7 gene. **Pharmacogenet Genomics**, v. 18, n. 8, p. 683-97, 2008. doi: 10.1097/FPC.0b013e3283037fe4.

INNOCENTI, F.; RATAIN, M. J. "Irinogenetics" and UGT1A: from genotypes to haplotypes. **Clin Pharmacol Ther**, v. 75, n. 6, p. 495-500, 2004. doi:10.1016/j.clpt.2004.01.011.

JACINTO, F. V.; ESTELLER, M. MGMT hypermethylation: a prognostic foe, a predictive friend. **DNA Repair (Amst)**, v. 6, n. 8, p. 1155-60, 2007. doi: 10.1016/j.dnarep.2007.03.013.

JOH, J. W.; LEE, H. H.; LEE, D. S.; LEE, K. W.; LEE, S. K.; KIM, S. J. The Influence of Mycophenolate Mofetil and Azathioprine on the Same Cadaveric Donor Renal Transplantation. **J Korean Med Sci**, v. 20, p. 79-81, 2005. doi: 10.3346/jkms.2005.20.1.79.

KAGAYA, H.; MIURA, M.; SAITO, M.; HABUCHI, T.; SATOH, S. Correlation of IMPDH1 Gene Polymorphisms with Subclinical Acute Rejection and Mycophenolic Acid Exposure Parameters on Day 28 after Renal Transplantation. **Basic Clin Pharmacol Toxicol**, v. 107, p. 631–636, 2010. doi: 10.1111/j.1742-7843.2010.00542.x.

KAINA, B.; CHRISTMANN, M.; NAUMANN, S.; ROOS, W. P. MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. **DNA Repair (Amst)**, v. 6, n.8, p. 1079-99, 2007. doi: 10.1016/j.dnarep.2007.03.008.

KANG, S. H.; KWON, J. Y.; LEE, J. K.; SEO, Y. R. Recent advances in in vivo genotoxicity testing: prediction of carcinogenic potential using comet and micronucleus assay in animal models. **J Cancer Prev**, v. 18, n. 4, p. 277-88, 2013. doi: 10.15430/JCP.2013.18.4.277.

KAPOOR, A. Malignancy in kidney transplant recipients. **Drugs**, v. 68 Suppl 1, p. 11-9, 2008.

KARAHALIL, B.; BOHR, V. A.; WILSON, D. M. Impact of DNA polymorphisms in key DNA base excision repair proteins on cancer risk. **Hum Exp Toxicol**, v. 31, n. 10, p. 981-1005, 2012. doi: 10.1177/0960327112444476.

KARIMI, M. H.; HEJR, S.; GERAMIZADEH, B.; YAGHOBI, R.; SAGHEB, M. M.; KAMALI-SARVESTANI, E. Combined analysis of cytokine gene polymorphism and the level of expression with allograft function in kidney transplant recipients. **Transplant Immunol**, v. 30, n. 1, p. 46-51, 2014. doi: 10.1016/j.trim.2013.09.006.

KAUR, T. B.; TRAVALINE, J. M.; GAUGHAN, J. P.; RICHIE, J. P. JR.; STELLMAN, S. D.; LAZARUS, P. Role of polymorphisms in codons 143 and 160 of the O6-alkylguanine DNA alkyltransferase gene in lung cancer risk. **Cancer Epidemiol Biomarkers Prev**, v. 9, n. 3, p. 339-42, 2000. doi: 10.7916/D8416VT0.

KEPPLER, D. The roles of MRP2, MRP3, OATP1B1 and OATP1B3 in conjugated hyperbilirubinemia. **Drug Metab Dispos**, v. 42, n. 4, p. 651-5, 2014. doi: 10.1124/dmd.113.055772.

KIM, S. K.; PARK, H. J.; SEOK, H.; JEON, H. S.; LEE, T. W.; LEE, S. H.; MOON, J. Y.; IHM, C. G.; KIM, T. H.; KIM, Y. H.; KANG, S. W.; PARK, S. J.; JEONG, K. H.; CHUNG, J. H. Association studies of cytochrome P450, family 2, subfamily E, polypeptide 1 (CYP2E1) gene polymorphisms with acute rejection in kidney transplantation recipients. **Clin Transplant**, v. 28, p. 707–712, 2014. doi: 10.1111/ctr.12369.

KIRSCH-VOLDERS, M.; FENECH, M. Inclusion of micronuclei in non-divided mononuclear lymphocytes and necrosis/apoptosis may provide a more comprehensive cytokinesis block micronucleus assay for biomonitoring purposes. **Mutagenesis**, v. 16, n. 1, p. 51-8, 2001. doi: 10.1093/mutage/16.1.51.

KIRSCH-VOLDERS, M.; PLAS, G.; ELHAJOUJI, A.; LUKAMOWICZ, M.; GONZALEZ, L.; LOOCK, K. V.; DECORDIER, I. The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. **Arch Toxicol**, v. 85, p. 873-99, 2011. doi: 10.1007/s00204-011-0691-4.

KOBASHIGAWA, J. A.; PATEL, J. K. Immunosuppression for heart transplantation: where are we now? Nature Clinical Practice, **Cardiovascular Medicine**, v. 3, n. 4, p. 201-12, 2006. doi:10.1038/ncpcardio0510

KOBAYASHI, H.; SUGIYAMA, C.; MORIKAWA, Y.; HAYASHI, M.; SOFUNY, T. A comparison between manual microscopic analysis and computerized image analysis in the single cell gel electrophoresis assay. **MMS Commun**, v. 3, p. 103-15, 1995.

KOBAYASHI, M.; SAITOH, H.; KOBAYASHI, M.; TADANO, K.; TAKAHASHI, Y.; HIRANO, T. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by multidrug resistance-associated protein 2 in rats. **J Pharmacol Exp Ther**, v. 309, p. 1029-35, 2004.

KRAMER, K.; THYE, T.; TRESZL, A.; PEINE, S.; KOCH, M.; STERNECK, M.; NASHAN, B.; THUDE, H. Polymorphism in NFKBIA gene is associated with recurrent acute rejections in liver transplant recipients. **Tissue Antigens**, v. 84, n. 4, p. 370-7, 2014. doi: 10.1111/tan.12411.

KROETZ, D. L.; PAULI-MAGNUS, C.; HODGES, L. M.; HUANG, C. C.; KAWAMOTO, M.; JOHNS, S. J.; STRYKE, D.; FERRIN, T. E.; DEYOUNG, J.; TAYLOR, T.; CARLSON, E. J.; HERSKOWITZ, I.; GIACOMINI, K. M.; CLARK, A. G. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. **Pharmacogenetics**, n.13, p. 481-94, 2003. doi: 10.1097/01.fpc.0000054113.14659.b9.

KUEHL, P.; ZHANG, J.; LIN, Y.; LAMBA, J.; ASSEM, M.; SCHUETZ, J.; WATKINS, P. B.; DALY, A.; WRIGHTON, S. A.; HALL, S. D.; MAUREL, P.; RELLING. M.; BRIMER, C.; YASUDA, K.; VENKATARAMANAN, R.; STROM, S.; THUMMEL, K.; BOGUSKI, M. S.; SCHUETZ, E. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. **Nat Genet**, v. 27, n. 4, p. 383-91, 2001. doi: 10.1038/86882.

KUNDU, N.; BEATY, T. L.; JACKSON, M. J.; FULTON, A. M. Antimetastatic and antitumor activities of interleukin 10 in a murine model of breast cancer. **J Natl Cancer Inst**, v. 88, n. 8, p. 536-41, 1996.

KURTOGLU, E. L.; YUKSEL, S. Genotoxic effects of tacrolimus on human lymphocyte cells. **Genetika**, v. 48, p. 762-67, 2012. doi: 10.1134/S1022795412050134.

KURZAWSKI, M.; DĄBROWSKA, J.; DZIEWANOWSKI. K.; DOMAŃSKI, L.; PERUŻYŃSKA, M.; DROŹDZIK, M. CYP3A5 and CYP3A4, but not ABCB1 polymorphisms affect tacrolimus dose-adjusted trough concentrations in kidney transplant recipients. **Pharmacogenomics**, v. 15, n. 2, p. 179-88, 2014. doi: 10.2217/pgs.13.199.

KUYPERS, D.R.; VERLEDEN, G.; NAESENS, M.; VANRENTERGHEM, Y. Drug interaction between mycophenolate mofetil and rifampin: possible induction of uridine diphosphate-glucuronosyltransferase. **Clin Pharmacol Ther**.v. 78, n. 1, p. 81-8, 2005. doi:10.1016/j.clpt.2005.03.004.

LANGE, S. S; TAKATA, K; WOOD, R. D. DNA polymerases and cancer. **Nature Reviews Cancer,** v. 11, p. 96-110, 2011. doi:10.1038/nrc2998

LA MANNA, G.; LANCI, N.; DELLA BELLA. E.; COMAI, G.; CAPPUCCILLI, M. L.; NISI, K.; TODESCHINI, P.; CARRETTA, E.; SCOLARI, M. P.; STEFONI, S. Reduction of oxidative damage reflects a better kidney transplantation outcome. **Am J Nephrol**, v. 34, n. 6, p. 496-504, 2011. doi: 10.1159/000329320.

LAWRENCE, M. C.; BORENSTEIN-AUERBACH, N.; MCGLYNN, K.; KUNNATHODI, F.; SHAHBAZOV, R.; SYED, I.; KANAK, M.; TAKITA, M.; LEVY, M. F.; NAZIRUDDIN, B. NFAT Targets Signaling Molecules to Gene Promoters in Pancreatic Cells. **Mol Endocrinol**, v. 29, n. 2, p. 274-88, 2015. doi: 10.1210/me.2014-1066.

LEE, H.; CLARK, B.; GOOI, H. C.; STOVES, J.; NEWSTEAD, C. G. Influence of recipient and donor IL-1alpha, IL-4, and TNFalpha genotypes on the incidence of acute renal allograft rejection. **J Clin Pathol**, v. 57, n. 1, p. 101-3, 2004. doi: 10.1136/jcp.57.1.101.

LEE, S. J.; GOLDSTEIN, J. A. Functionally defective or altered CYP3A4 and CYP3A5 single nucleotide polymorphisms and their detection with genotyping tests. **Pharmacogenomics**, v. 6, n. 4, p. 357-71, 2005. doi: 10.1517/14622416.6.4.357.

LEGENDRE C, CANAUD G, MARTINEZ F. Factors influencing long-term outcome after kidney transplantation. **Transplant International**. v. 27, p. 19-27, 2014. doi:10.1111/tri.12217

LEVEY, A. S.; GREEN, T.; KUSEK, J. W.; BECK, G. J. MDRD STUDY GROUP. A simplified equation to predict glomerular filtration rate from serum creatinine 2000. J Am Soc Nephrol 11: 155A (A0828). In: EPPENGA, W. L.; KRAMERS, C.; DERIJKS, H. J.; WENSING, M.; WETZELS, J. F.; DE SMET, P. A. Individualizing Pharmacotherapy in Patients with Renal Impairment: The Validity of the Modification of Diet in Renal Disease Formula in Specific Patient Populations with a Glomerular Filtration Rate below 60 Ml/Min. **A Systematic Review.PLoS One**, v. 10, n. 3, p. e0116403, 2015. doi: 10.1371/journal.pone.0116403.

LEWIS, J. P.; HORENSTEIN, R. B.; RYAN, K.; O'CONNELL, J. R.; GIBSON, Q.; MITCHELL, B. D.; TANNER, K.; CHAI, S.; BLIDEN, K. P.; TANTRY, U. S.; PEER, C. J.; FIGG, W. D.; SPENCER, S. D.; PACANOWSKI, M. A.; GURBEL, P. A.; SHULDINER, A. R.The functional G143E variant of carboxylesterase 1 is associated with increased clopidogrel active metabolite levels and greater clopidogrel response. **Pharmacogenet Genomics**, v. 23, n. 1, p. 1-8, 2013. doi: 10.1097/FPC.0b013e32835aa8a2.

LI, C. J.; LI, L.; LIN, L.; JIANG, H. X.; ZHONG, Z. Y.; LI, W. M.; ZHANG, Y. J.; ZHENG, P.; TAN, X. H.; ZHOU, L. Impact of the CYP3A5, CYP3A4, COMT, IL-10 and POR genetic polymorphisms on tacrolimus metabolism in Chinese renal transplant recipients. **PLoS One**, v. 9, n. 1, p. e86206, 2014. doi: 10.1371/journal.pone.0086206.

LI, J. L.; LIU, S.; FU, Q.; ZHANG, Y.; WANG, X. D.; LIU, X. M.; LIU, L. S.; WANG, C. X.; HUANG, M. Interactive effects of CYP3A4, CYP3A5, MDR1 and NR1I2 polymorphisms on tracrolimus trough concentrations in early postrenal transplant recipients. **Pharmacogenomics**, v. 16, n. 12, p. 1355-1365, 2015. doi: 10.2217/pgs.15.78.

LI, M. O.; FLAVELL, R. A. TGF-beta: a master of all T cell trades. Cell, v. 134, n. 3, p. 392-404, 2008. doi: 10.1016/j.cell.2008.07.025.

LIU, J. Y.; YOU, R. X.; GUO, M.; ZENG, L.; ZHOU, P.; ZHU, L.; XU, G.; LI, J.; LIU, D. Tacrolimus versus cyclosporine as primary immunosuppressant after renal transplantation: A meta-analysis and economics evaluation. **Am J Ther**, 2014. doi: 10.1097/MJT.0000000000000150

LIU, S.; CHEN, R. X.; LI, J.; ZHANG, Y.; WANG, X. D.; FU, Q.; CHEN, L. Y.; LIU, X. M.; HUANG, H. B.; HUANG, M.; WANG, C. X.; LI, J. L. The POR rs1057868-rs2868177 GC-GT diplotype is associated with high tacrolimus concentrations in early post-renal transplant recipients. **Acta Pharmacol Sin**, v. 37, n. 9, p. 1251-8, 2016. doi: 10.1038/aps.2016.77.

LOU, Y.; LI, R.; ZHANG, Y.; ZHONG, R.; PEI, J.; XIONG, L.; ZHANG, X.; HAN, B. XPA gene rs1800975 single nucleotide polymorphism and lung cancer risk: a meta-analysis. **Tumour Biol**, v. 35, n. 7, p. 6607-17, 2014. doi: 10.1007/s13277-014-1824-1.

MACPHEE, I. A. M.; FREDERICKS, S.; MOHAMED, M.; MORETON, M.; CARTER, N. D.; JOHNSTON, A.; GOLDBERG, L.; HOLT, D. W. Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. **Transplantation**, v. 79, n. 4, p. 499-502, 2005. doi: 10.1097/01.TP.0000151766.73249.12.

MAGALHÃES, P. S. C; BÖHLKE, M; NEUBARTH, F. Complexo Principal de Histocompatibilidade (MHC): codificação genética, bases estruturais e implicações clínicas. **Revista de Medicina da UCPEL**, Pelotas, v. 2, n. 1, p. 54-5, 2004.

MANOHARAN, K.; BANERJEE, M. R. beta-Carotene reduces sister chromatid exchanges induced by chemical carcinogens in mouse mammary cells in organ culture. **Cell Biol Int Rep**, v. 9, n. 9, p. 783-9, 1985.

MARTELLI-MARZAGÃO, F.; SANTOS JUNIOR, G. F.; OGAWA, M. M.; ENOKIHARA, M. M.; PORRO, A. M.; TOMIMORI, J. Human papillomavirus detected in viral warts of renal transplant recipients. **Transpl Infect Dis**, n. 18, v. 1, p. 37-43, 2016. doi: 10.1111/tid.12479.

MATHEW, T. H. A blinded, long-term, randomized multicenter study of mycophenolate mofetil in cadaveric renal transplantation: results at three years. Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group. **Transplantation,** v. 65, n. 11, p. 1450-4, 1998.

MATIC, M.; NORMAN, E.; RANE, A.; BECK, O.; ANDERSSON; M.; ELENS, L.; TIBBOEL, D.; FELLMAN, V.; van SCHAIK, R. H. Effect of UGT2B7 -900G>A (-842G>A; rs7438135) on morphine glucuronidation in preterm newborns: results from a pilot cohort. **Pharmacogenomics**, v. 15, n. 12, p. 1589-97, 2014. doi: 10.2217/pgs.14.115.

MATTHEW, J. Calcineurin inhibitors in renal transplantation:adverse effects. **Nephrology**, v. 12, p.S66-74, 2007. doi:10.1111/j.1440-1797.2007.00731.x.

MICHELON, H.; KÖNIG, J.; DURRBACH, A.; QUTEINEH, L.; VERSTUYFT, C.; FURLAN, V.; FERLICOT, S.; LETIERCE, A.; CHARPENTIER, B.; FROMM, M. F.; BECQUEMONT, L. SLCO1B1 genetic polymorphism influences mycophenolic acid tolerance in renal transplant recipients. **Pharmacogenomics**, v. 11, n. 12, p. 1703-13, 2010. doi:10.2217/pgs.10.132.

MIURA, M.; KAGAYA, H.; SATOH, S.; INOUE, K.; SAITO, M.; HABUCHI, T.; SUZUKI, T. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. **Ther Drug Monit**, v. 30, p. 559-64. 2008.

NAESENS, M.; KUYPERS, D. R.; SARWAL, M. Calcineurin inhibitor nephrotoxicity. **Clin J Am Soc Nephrol**, v. 4, n. 2, p. 481-508, 2009. doi: 10.2215/CJN.04800908.

NAESENS, M.; KUYPERS, D. R.; VERBEKE, K.; VANRENTERGHEM, Y. Multidrug resistance protein 2 genetic polymorphisms influence mycophenolic acid exposure in renal allograft recipients. **Transplantation**, v. 82, n. 8, p. 1074-84, 2006. doi: 10.1097/01.tp.0000235533.29300.e7.

NEWTON, K.; DIXIT, V. M. Signaling in innate immunity and inflammation. **Cold Spring Harb Perspect Biol**, v. 4, n. 3, p. 1-19, 2012. doi: 10.1101/cshperspect.a006049.

NEYLAN, J. F. Racial differences in renal transplantation after immunosuppression with tacrolimus versus cyclosporine. FK506 Kidney Transplant Study Group. **Transplantation**, v. 65, n. 4, p. 515-23, 1998. doi: 00007890-199802270-00011.

NIEMI, M. Role of OATP transporters in the disposition of drugs. **Pharmacogenomics**, v. 8, n. 7, p.787-802, 2007. doi: 10.2217/14622416.8.7.787.

NOVARTIS PHARMACEUTICALS CORP: Neoral Package Insert. East Hanover, NJ, Novartis Pharmaceuticals Corp, 2004

NUSSBAUM, R. L.; MCINNES, R. R.; WILLARD, H. F. Farmacogenética e Farmacogenômica, capítulo 6. In:_____. **Genética Médica Thompson & Thompson**. 7ª ed. Rio de Janeiro: Editora Elsevier, 2007. p.429-438.

O'CALLAGHAN, C. A. Kidney transplantation - the long term view. **QJM**, v. 101, n. 12, p. 985-6, 2008. doi: 10.1093/qjmed/hcn144.

OGASAWARA, K.; CHITNIS, S. D.; GOHH, R. Y.; CHRISTIANS, U.; AKHLAGHI, F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics

of tacrolimus in kidney transplant recipients. **Clin Pharmacokinet**, v. 52, n. 9, p. 751-62, 2013. doi: 10.1007/s40262-013-0069-2.

OLIVEIRA, V. D.; ZANKL, H.; RATH, T. Mutagenic and cytotoxic effects of immunosuppressive drugs on human lymphocyte cultures. **Exp Clin Transplant**, v. 2, n. 2, p. 273-9, 2004. doi: 10.1159/000244646.

ONG, S. C.; GASTON, R. S. Medical management of chronic kidney disease in the renal transplant recipient. **Curr Opin Nephrol Hypertens**, v. 24, p. 587-93, 2015.

ONEDA, B.; CRETTOL, S.; JAQUENOUD SIROT, E.; BOCHUD, M.; ANSERMO,T. N.; EAP, C. B. The P450 oxidoreductase genotype is associated with CYP3A activity in vivo as measured by the midazolam phenotyping test. **Pharmacogenet Genomics**, v. 19, n. 11, p. 877-83, 2009. doi: 10.1097/FPC.0b013e32833225e7.

ONIZUKA, M.; KUNII, N.; TOYOSAKI, M.; MACHIDA, S.; OHGIYA, D.; OGAWA, Y.; KAWADA, H.; INOKO, H.; ANDO, K. Cytochrome P450 genetic polymorphisms influence the serum concentration of calcineurin inhibitors in allogeneic hematopoietic SCT recipients. **Bone Marrow Transplant**, v. 46, n.8, p. 1113-7, 2011. doi: 10.1038/bmt.2010.273.

OZTÜRK, S.; AYNA, T. K.; CEFLE, K.; PALANDUZ, S.; CIFTÇI, H. S.; KAYA, S. A.; DILER, A. S.; TÜRKMEN, A.; GÜRTEKIN, M.; SEVER, M. S.; CARIN, M. Effect of cyclosporin A and tacrolimus on sister chromatid exchange frequency in renal transplant patients. **Genet Test**, v. 12, n. 3, p. 427-30, 2008. doi: 10.1089/gte.2008.0006.

PALANISAMY, A. P.; SCHILTZ, C. E.; PILCH, N. A.; HUNT, K. J.; NADIG, S. N.; DOWDEN, J. E.; MCGILLICUDDY, J. W.; BALIGA, P. K.; CHAVIN, K. D.; TABER, D. J. Cardiovascular risk factors contribute to disparities in graft outcomes in African American renal transplant recipients: a retrospective analysis. **Blood Press.** v. 24, p. 14-22, 2015.

PALLET, N.; JANNOT, A. S.; EL BAHRI, M.; ETIENNE, I.; BUCHLER, M.; DE LIGNY, B. H.; CHOUKROUN, G.; COLOSIO, C.; THIERRY, A.; VIGNEAU, C.; MOULIN, B.; LE MEUR, Y.; HENG, A. E.; SUBRA, J. F.; LEGENDRE, C.; BEAUNE, P.; ALBERTI, C.; LORIOT, M. A.; THERVET, E. Kidney transplant recipients carrying the CYP3A4*22 allelic variant have reduced tacrolimus clearance and often reach supratherapeutic tacrolimus concentrations. **Am J Transplant**, v. 15, n. 3, p. 800-5, 2015. doi: 10.1111/ajt.13059.

PARK, S. Y.; KANG, Y. S.; JEONG, M. S.; YOON, H. K.; HAN, K. O. Frequencies of CYP3A5 genotypes and haplotypes in a Korean population. **J Clin Pharm Ther**, v. 33, n. 1, p. 61-5, 2008. doi: 10.1111/j.1365-2710.2008.00879.x.

PARKER, R.; ARMSTRONG, M. J.; CORBETT, C.; DAY, E. J.; NEUBERGER, J. M. Alcohol and Substance Abuse in Solid-Organ Transplant Recipients. **Transplantation.** v. 96, n. 12, p. 1015-24, 2013. doi: 10.1097/TP.0b013e31829f7579.

PATTISON, J.; NELSON, P. J.; HUIE, P.; VON LEUTTICHAU, I.; FARSHID, G.; SIBLEY, R. K.; KRENSKY, A. M. RANTES chemokine expression in cell-mediated transplant rejection of the kidney. Lancet, v. 343, n. 8891, p. 209-11, 1994. DOI: 10.1016/S0140-6736(94)90992-X.

PAWLIK, A.; DOMANSKI, L.; ROZANSKI, J.; FLORCZAK, M.; DABROWSKA-ZAMOJCIN, E.; DUTKIEWICZ, G.; GAWRONSKA-SZKLARZ, B. IL-2 and TNF-alpha promoter polymorphisms in patients with acute kidney graft rejection. **Transplant Proc**, v. 37, n. 5, p. 2041-3, 2005. doi: 10.1016/j.transproceed.2005.03.091.

PAZIK, J.; OŁDAK, M.; PODGÓRSKA, M.; LEWANDOWSKI, Z.; SITAREK, E.; PŁOSKI, R.; SZMIDT, J.; CHMURA, A.; DURLIK, M.; MALEJCZYK, J. Lymphocyte counts in kidney allograft

recipients are associated with IMPDH2 3757T>C gene polymorphism. **Transplant Proc**, v. 43, n. 8, p. 2943-5, 2011. doi: 10.1016/j.transproceed.2011.08.037.

PFAFFL, M. A new mathematical model for relative quantification in real-time RT-PCR. **Nucl Acids Res**, v. 29, n. 9, p. e45. 2001.

PICARD, N.; MARQUET, P. Pharmacogenomics of Immunosuppressants. In: LANGMAN LJ, DASGUPTA A. (Ed.). **Pharmacogenomics in Clinical Therapeutics**. 1. Wiley-Blackwell, v.1, 2012. cap. 6, p.70-97. ISBN: 978-0-470-65734-8.

PICARD, N.; RATANASAVANH, D.; PRÉMAUD, A.; LE MEUR, Y.; MARQUET, P. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. **Drug Metab Dispos**, v. 33, n. 1, p. 139-46, 2004. doi: 10.1124/dmd.104.001651.

PISELLI, P.; SERRAINO, D.; SEGOLONI, G. P.; SANDRINI, S.; PIREDDA, G. B.; SCOLARI, M. P.; RIGOTTI, P.; BUSNACH, G.; MESSA, P.; DONATI, D.; SCHENA, F. P.; MARESCA, M. C.; TISONE, G.; VEROUX, M.; SPARACINO, V.; PISANI, F.; CITTERIO, F. Immunosuppression and Cancer Study Group. Risk of de novo cancers after transplantation: results from a cohort of 7217 kidney transplant recipients, Italy 1997-2009. **Eur J Cancer**, v. 49, n. 2, p. 336-44, 2013. doi: 10.1016/j.ejca.2012.09.013.

PONTICELLI, C. Cyclosporine: from renal transplantation to autoimmune diseases. **Ann N Y Acad Sci**, v. 1051, p. 551-8, 2005. doi: 10.1196/annals.1361.099.

RADOMINSKA-PANDYA, A.; POKROVSKAYA, I. D.; XU, J.; LITTLE, J. M.; JUDE, A. R.; KURTEN, R. C.; CZERNIK, P. J. Nuclear UDP-glucuronosyltransferases: identification of UGT2B7 and UGT1A6 in human liver nuclear membranes. **Arch Biochem Biophys**, v. 399, n. 1, p. 37-48, 2002. doi: 10.1006/abbi.2001.2743.

RAHEEM, O. A.; KAMEL, M. H.; DALY, P. J.; MOHAN, P.; LITTLE, D. M.; AWAN, A.; HICKEY, D. P. Mycophenolate mofetil in pediatric renal transplantation: a single center experience. **Pediatr Transplant**, v.15, n.3, p. 240-4, 2011. doi:10.1111/j.1399-3046.2009.01179.x.

RAMA, I.; GRINYÓ, J. M. Malignancy after renal transplantation: the role of immunosuppression. **Nat Rev Nephrol**, v. 6, n. 9, p. 511-9, 2010. doi: 10.1038/nrneph.2010.102.

RAMAMOORTHY A, PACANOWSKI MA, BULL J, ZHANG L. Racial/ethnic differences in drug disposition and response: review of recently approved drugs. **Clin Pharmacol Ther**. v. 97, n. 3, p. 263-73, 2015. doi: 10.1002/cpt.61.

RATH, T.; OLIVEIRA-FRICK, V. Mutagenicity of immunosuppressive medications among renal transplant recipients. **Am J Nephrol**, v. 30, n. 6, p. 514-20, 2009. doi: 10.1159/000244646.

REHM, J.; ROOM, R.; GRAHAM, K.; MONTEIRO, M.; GMEL, G.; SEMPOS, C. T. The relationship of average volume of alcohol consumption and patterns of drinking to burden of disease: an overview. **Addiction**, v. 98, n. 9, p. 1209-28, 2003. doi: 10.1046/j.1360-0443.2003.00467.x

RO, H.; MIN, S. I.; YANG, J.; MOON, K. C.; KIM, Y. S.; KIM, S. J.; AHN, C.; HA, J. Impact of tacrolimus intraindividual variability and CYP3A5 genetic polymorphism on acute rejection in kidney transplantation. **Ther Drug Monit**, v. 34, p. 680-85, 2012.

ROBERTSON, H.; WHEELER, J.; MORLEY, A. R.; BOOTH, T. A.; TALBOT, D.; KIRBY, J. A. Beta-chemokine expression and distribution in paraffin-embedded transplant renal biopsy sections: analysis by scanning laser confocal microscopy. **Histochem Cell Biol**, v. 110, n. 2, p. 207-13, 1998. doi: 9720994.

RODERICK, P.; BYRNE, C.; CASULA, A.; STEENKAMP, R.; ANSELL, D.; BURDEN, R.; NITSCH, D.; FEEST, T. Survival of patients from South Asian and Black populations starting renal replacement therapy in England and Wales. **Nephrol Dial Transplant**, v. 24, n.12, p. 3774-82, 2009. doi: 10.1093/ndt/gfp348.

ROSSI, D.; ZLOTNIK, A. The biology of chemokines and their receptors. **Annu Rev Immunol**, v. 18, p. 217-42, 2000. doi: 10.1146.

RUIZ, J.; HERRERO, M. J.; BOSÓ, V.; MEGÍAS, J. E.; HERVÁS, D.; POVEDA, J. L.; ESCRIVÁ, J.; PASTOR, A.; SOLÉ, A.; ALIÑO, S. F. Impact of Single Nucleotide Polymorphisms (SNPs) on Immunosuppressive Therapy in Lung Transplantation. **Int J Mol Sci**, v. 16, n.9, p. 20168-82, 2015. doi: 10.3390/ijms160920168.

SADLACK, B.; LÖHLER, J.; SCHORLE, H.; KLEBB, G.; HABER, H.; SICKEL, E.; NOELLE, R. J.; HORAK, I. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells. **Eur J Immunol**, v. 25, n. 11, p. 3053-9, 1995. doi: 10.1002/eji.1830251111.

SAEKI, T.; UEDA, K.; TANIGAWARA, Y.; HORI, R.; KOMANO, T. Human P-glycoprotein transports cyclosporin A and FK506. **J Biol Chem**, v. 268, n. 9, p. 6077-80, 1993.

SALLUSTO, F.; BAGGIOLINI, M. Chemokines and leukocyte traffic. **Nat Immunol**, v. 9, n. 9, p. 949-52, 2008. doi: 10.1038/ni.f.214.

SÁNCHEZ-LÁZARO, I.; HERRERO, M. J.; JORDÁN-DE LUNA, C.; BOSÓ, V.; ALMENAR, L.; ROJAS, L.; MARTÍNEZ-DOLZ, L.; MEGÍAS-VERICAT, J. E.; SENDRA, L.; MIGUEL, A.; POVEDA, J. L.; ALIÑO, S. F. Association of SNPs with the efficacy and safety of immunosuppressant therapy after heart transplantation. **Pharmacogenomics**. v. 16, n. 9, p. 971-9, 2015. doi:10.2217/pgs.15.39.

SANKARAN, D.; ASDERAKIS, A.; ASHRAF, S.; ROBERTS, I. S.; SHORT, C. D.; DYER, P. A.; SINNOTT, P. J.; HUTCHINSON, I. V. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. **Kidney Int**, v. 56, n. 1, p. 281-8, 1999. doi: 10.1046/j.1523-1755.1999.00536.x.

SATO, T.; TERAI, M.; TAMURA, Y.; ALEXEEV, V.; MASTRANGELO, M. J.; SELVAN, S. R. Interleukin 10 in the tumor microenvironment: a target for anticancer immunotherapy. **Immunol Res**, v. 51, n. 2-3, p. 170-82, 2011. doi: 10.1007/s12026-011-8262-6.

SATTLER, M.; GUENGERICH, F. P.; YUN, C. H.; CHRISTIANS, U.; SEWING, K. F. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. **Drug Metab Dispos**, v. 20, p. 753-61, 1992.

SCHMOOK, T.; NINDL, I.; ULRICH, C.; MEYER, T.; STERRY, W.; STOCKFLETH, E. Viral warts in organ transplant recipients: new aspects in therapy. **Br J Dermatol**,v. 149, Suppl 66, p. 20-24, 2003.

SCHNUELLE, P.; LORENZ, D.; TREDE, M.; van DER WOUDE, F. J. Impact of renal cadaveric transplantation on survival in end-stage renal failure: evidence for reduced mortality risk compared with hemodialysis during long-term follow-up. **J Am Soc Nephrol**, v. 9, n. 11, p. 2135-41, 1998.

SEDER, R. A.; PAUL, W. E. Acquisition of lymphokine-producing phenotype by CD4+ T cells. **J Allergy Clin Immunol**, v. 94, n. 6, p. 1195-202, 1994. doi: 10.1146/annurev.iy.12.040194.003223

SEYHUN, Y.; MYTILINEOS, J.; TURKMEN, A.; OGUZ, F.; KEKIK, C.; OZDILLI, K.; NANE, I.; AYDIN, F.; CARIN, M. Influence of cytokine gene polymorphisms on graft rejection in Turkish

patients with renal transplants from living related donors. **Transplant Proc**, v. 44, n. 6, p. 1670-8, 2012. doi:10.1016/j.transproceed.2012.01.125.

SHAH, S.; HARWOOD, S. M.; DÖHLER, B.; OPELZ, G.; YAQOOB, M. M. Inosine monophosphate dehydrogenase polymorphisms and renal allograft outcome. **Transplantation**, v. 94, n. 5, p. 486-91, 2012. doi: 10.1097/TP.0b013e31825b7654.

SHANG, W.; HUANG, L.; LI, L.; LI, X.; ZENG, R.; GE, S.; XU, G. Cancer risk in patients receiving renal replacement therapy: A meta-analysis of cohort studies. **Mol Clin Oncol**, v. 5, n. 3, p. 315-25, 2016. doi: 10.3892/mco.2016.952.

SHEN, A. L.; O'LEARY, K. A.; KASPER, C. B. Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase. **J Biol Chem**, v. 277, n. 8, p. 6536-41, 2002. doi:10.1074/jbc.M111408200.

SHEN, Z. T.; WU, X. H.; LI, B.; SHEN, J. S.; WANG, Z.; LI, J.; ZHU, X. X. CYP2E1 Rsa I/Pst I polymorphism and lung cancer susceptibility: a meta-analysis involving 10,947 subjects. **J Cell Mol Med**, v. 19, n. 9, p. 2136-42, 2015. doi: 10.1111/jcmm.12579.

SHIPKOVA, M.; ARMSTRONG, V. W.; WIELAND, E.; NIEDMANN, P. D.; SCHÜTZ, E.; BRENNER-WEISS, G.; VOIHSEL, M.; BRAUN, F.; OELLERICH, M. Identification of glucoside and carboxyl-linked glucuronide conjugates of mycophenolic acid in plasma of transplant recipients treated with mycophenolate mofetil. **Br J Pharmacol**, v. 126, p. 1075-82. 1999.

SHUKER, N.; BOUAMAR, R.; WEIMAR, W.; van SCHAIK, R. H. N.; van GELDER, T.; HESSELINK, D. A. ATP-binding cassette transporters as pharmacogenetic biomarkers for kidney transplantation. **Clin Chim Acta**, v. 413, p. 1326-37, 2012. doi:10.1016/j.cca.2011.09.040.

SILVA, S. B.; CAULLIRAUX, H. M.; ARAÚJO, C. A. S.; ROCHA, E. Cost comparison of kidney transplant versus dialysis in Brazil. **Cad. Saúde Pública**, Rio de Janeiro, v. 32, n. 6, pii: S0102-311X2016000605005, 2016. doi: 10.1590/0102-311X00013515.

SINGH, N. P.; MCCOY, M. T.; TICE, R. R.; SCHNEIDER, E. L. A simple technique for quantitation of low levels of DNA damage in individual cells. **Exp Cell Res**, v. 175, n. 1, p. 184-91, 1988. doi:10.1016/0014-4827(88)90265-0.

SISSUNG, T. M.; GOEY, A. K.; LEY, A. M.; STROPE, J. D.; FIGG, W. D. Pharmacogenetics of membrane transporters: a review of current approaches. **Methods Mol Biol**, v. 1175, p. 91-120, 2014. doi: 10.1007/978-1-4939-0956-8_6.

SOMBOGAARD, F.; van SCHAIK, R. H.; MATHOT, R. A.; BUDDE, K.; van DER WERF, M.; VULTO, A. G.; WEIMAR, W.; GLANDER, P.; ESSIOUX, L.; van GELDER, T. Interpatient variability in IMPDH activity in MMF-treated renal transplant patients is correlated with IMPDH type II 3757T > C polymorphism. **Pharmacogenet Genomics**, v. 19, n. 8, p. 626-34, 2009. doi: 10.1097/FPC.0b013e32832f5f1b.

SPEECKAERT, R.; BROCHEZ, L.; LAMBERT, J.; van GEEL, N.; SPEECKAERT, M. M.; CLAEYS, L.; LANGLOIS, M.; van LAER, C.; PEETERS, P.; DELANGHE, J. R. The haptoglobin phenotype influences the risk of cutaneous squamous cell carcinoma in kidney transplant patients. **J Eur Acad Dermatol Venereol**, v. 26, n. 5, p. 566-71, 2012. doi: 10.1111/j.1468-3083.2011.04112.x.

SPEIT, G. Does the recommended lymphocyte cytokinesis-block micronucleus assay for human biomonitoring actually detect DNA damage induced by occupational and environmental exposure to genotoxic chemicals? **Mutagenesis**, v. 28, n. 4, p. 375-80, 2013. doi: 10.1093/mutage/get026.

SPIVEY, T. L.; UCCELLINI, L.; ASCIERTO, M. L.; ZOPPOLI, G.; DE GIORGI, V.; DELOGU, L. G.; ENGLE, A. M.; THOMAS, J. M.; WANG, E.; MARINCOLA, F. M.; BEDOGNETTI, D. Gene expression profiling in acute allograft rejection: challenging the immunologic constant of rejectionhypothesis**. J Transl Med**, v. 9, n. 174, p. 1-22, 2011. doi: 10.1186/1479-5876-9-174.

STAATZ, C. E.; GOODMAN, L. K.; TETT, S. E. Effect of CYP3A and ABCB1 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of calcineurin inhibitors: **Part I. Clin Pharmacokinet**, v. 49, n. 3, p. 141-75, 2010. doi: 10.2165/11317350-000000000-00000.

STAATZ, C. E.; TETT, S. E. Pharmacology and toxicology of mycophenolate in organ transplant recipients: an update. **Arch Toxicol**, v. 88, p. 1351-89, 2014. doi 10.1007/s00204-014-1247-1.

STALLONE, G.; INFANTE, B.; GRANDALIANO, G. Management and prevention of post-transplant malignancies in kidney transplant recipients. **Clin Kidney J**, v. 8, n. 5, p. 637-44, 2015. doi: 10.1093/ckj/sfv054.

STEPHENS, M.; DONNELLY, P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. **Am J Human Genet**, v. 73, p. 1162-9, 2003.

STOJANOVA, J.; POUCHÉ, L.; PICARD, N. Genetic polymorphisms in the immune response: A focus on kidney transplantation. **Clin Biochem**, v. 49, n. 4-5, p. 363-76, 2016. doi: 10.1016/j.clinbiochem.2015.07.016.

STRASSBURG, C. P.; BARUT, A.; OBERMAYER-STRAUB, P.; LI, Q.; NGUYEN, N.; TUKEY, R. H.; MANNS, M. P. Identification of cyclosporine A and tacrolimus glucuronidation in human liver and the gastrointestinal tract by a differentially expressed UDP-glucuronosyltransferase: UGT2B7. **J Hepatol**, v. 34, n. 6, p. 865-72, 2001. doi:10.1016/S0168-8278(01)00040-X.

STRATTON, M. R.; CAMPBELL, P. J.; FUTREAL, P. A. The cancer genome. **Nature**, v. 458, n. 9, p. 719-24, 2009. doi:10.1038/nature07943.

SUTHANTHIRAN, M. The importance of genetic polymorphisms in renal transplantation. **Curr Opin Urol**, v. 10, n. 2, p. 71-5, 2000.

TADA, H.; TSUCHIYA, N.; SATOH, S.; KAGAYA, H.; LI, Z.; SATO, K.; MIURA, M.; SUZUKI, T.; KATO, T.; HABUCHI, T. Impact of CYP3A5 and MDR1(ABCB1) C3435T polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. **Transplant Proc**, v. 37, n. 4, p. 1730-2, 2005. doi: 10.1016/j.transproceed.2005.02.073.

TAKANO, M.; YUMOTO, R.; MURAKAMI, T. Expression and function of efflux drug transporters in the intestine. Pharmacol Ther, v. 109, n. 1-2. p. 137-61, 2006. doi: 10.1016/j.pharmthera.2005.06.005.

TAMURA, A.; WAKABAYASHI, K.; ONISHI, Y.; TAKEDA, M.; IKEGAMI, Y.; SAWADA, S.; TSUJI, M.; MATSUDA, Y.; ISHIKAWA, T. Re-evaluation and functional classification of non-synonymous single nucleotide polymorphisms of the human ATP-binding cassette transporter ABCG2. **Cancer Sci**, v. 98, n. 2, p. 231-9, 2007. doi: 10.1111/j.1349-7006.2006.00371.x.

TANAKA, E.; TERADA, M.; MISAWA, S. Cytochrome P450 2E1: its clinical and toxicological role. **J Clin Pharm Thers**, v. 25, p. 165-75, 2000. doi: 10.1046/j.1365-2710.2000.00282.x.

TANIKAWA, T.; WILKE, C. M.; KRYCZEK, I.; CHEN, G. Y.; KAO, J.; NÚÑEZ. G.; ZOU, W. Interleukin-10 ablation promotes tumor development, growth, and metastasis. **Cancer Res**, v. 72, n. 2, p. 420-9, 2012. doi: 10.1158/0008-5472.CAN-10-4627.

TAYLOR, A. L.; WATSON, C. J.; BRADLEY, J. A. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. **Crit Rev Oncol Hematol**, v.56, n.1, p. 23-46, 2005. doi: 10.1016/j.critrevonc.2005.03.012.

TERRAZZINO, S.; QUAGLIA, M.; STRATTA, P.; CANONICO, P. L.; GENAZZANI, A. A. The effect of CYP3A5 6986A>G and ABCB1 3435C>T on tacrolimus dose-adjusted trough levels and acute rejection rates in renal transplant patients: a systematic review and meta-analysis. **Pharmacogenet Genomics**, v. 22, n. 8, p. 642-5, 2012. doi: 10.1097/FPC.0b013e3283557c74.

TIAN, J. N.; HO, I. K.; TSOU, H. H.; FANG, C. P.; HSIAO, C. F.; CHEN, C. H.; TAN, H. K.; LIN, L.; WU, C. S.; SU, L. W.; HUANG, C. L.; YANG, Y. H.; LIU, M. L.; CHEN, Y. T.; LIU, S. C.; HSU, Y. T.; KUO, H. W.; LIU, C. T.; YANG, Y. T.; CHEN, A. C. H.; SHIH, Y. H.; LIU, Y. L. UGT2B7 genetic polymorphisms are associated with the withdrawal symptoms in methadone maintenance patients. **Pharmacogenomics**, v. 13, n. 8, p. 879-88, 2012. doi: 10.2217/pgs.12.69.

TICE, R. R.; AGURELL, E.; ANDERSON, D.; BURLINSON, B.; HARTMANN, A.; KOBAYASHI, H.; MIYAMAE, Y.; ROJAS, E.; RYU, J. C.; SASAKI, Y. F. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. **Environ Mol Mutagen**, v. 35, n. 3, p. 206-21, 2000. Doi: 10.1002/(SICI)1098-2280(2000)35:3<206::AID-EM8>3.0.CO;2-J.

TIERCY, J. M. Molecular basis of HLA polymorphism: Implications in clinical transplantation. **Transpl Immunol.** v. 9, p. 173-80, 2002.

TSAI, J. P.; YANG, S. F.; WU, S. W.; HUGN, T. W.; TSAI, H. C.; LIAN, J. D.; CHANG, H. D. Association between interleukin 23 receptor polymorphism and kidney transplant outcomes: A 10-year Taiwan cohort study. **Clin Chim Acta**. v. 412, p. 958-62, 2011.

TONELLI, M; WIEBE, N; KNOLL, G; BELLO, A; BROWNE, S; JADHAV, D; KLARENBACH, S; GILL, J. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. **Am J Transplant**. v. 11, n. 10, p. 2093-109, 2011. doi: 10.1111/j.1600-6143.2011.03686.x.

TUKEY, R. H.; STRASSBURG, C. P. Human UDP-glucuronosyltransferases: metabolism, expression, and disease**. Annu Rev Pharmacol Toxicol**, v. 40, p. 581-616, 2000. doi:10.1146/annurev.pharmtox.40.1.581.

van DE WETERING, J.; ROODNAT, J. I.; HEMKE, A. C.; HOITSMA, A. J.; WEIMAR, W. Patient survival after the diagnosis of cancer in renal transplant recipients: a nested case-control study. **Transplantation**, v. 90, n. 12, p. 1542-6, 2010. doi: 10.1097/TP.0b013e3181ff1458.

van SCHAIK, R. H.; van AGTEREN, M.; DE FIJTER, J. W.; HARTMANN, A.; SCHMIDT, J.; BUDDE, K.; KUYPERS, D.; LE MEUR, Y.; van DER WERF, M.; MAMELOK, R.; van GELDER, T. UGT1A9 -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. **Clin Pharmacol Ther**, v. 86, n. 3, p. 319-27, 2009. doi: 10.1038/clpt.2009.83.

VANDEN EIJNDEN, S.; GORIELY, S.; DE WIT, D.; WILLEMS, F.; GOLDMAN, M. IL-23 up-regulates IL-10 and induces IL-17 synthesis by polyclonally activated naive T cells in human. **Eur J Immunol**. v. 35, p. 469-75, 2005.

VANHOVE, T.; ANNAERT, P.; LAMBRECHTS, D.; KUYPERS, D. R. Effect of ABCB1 diplotype on tacrolimus disposition in renal recipients depends on CYP3A5 and CYP3A4 genotype. **Pharmacogenomics J.** 2016. doi: 10.1038/tpj.2016.49. [Epub ahead of print].

VANNAPRASAHT, S.; REUNGJUI, S.; SUPANYA, D.; SIRIVONGS, D.; PONGSKUL, C.; AVIHINGSANON, Y.; TASSANEEYAKUL, W. Personalized tacrolimus doses determined by CYP3A5 genotype for induction and maintenance phases of kidney transplantation. **Clin Ther**, v. 35, n. 11, p. 1762-9, 2013. doi:10.1016/j.clinthera.2013.08.019.

VIAL, T.; DESCOTES, J. Immunosuppressive drugs and cancer. **Toxicology**, v. 185, n. 3, p. 229-40, 2003. doi:10.1016/S0300-483X(02)00612-1.

WALSH, M.; SOLOMONS, N.; LISK, L.; JAYNE, D. R. Mycophenolate mofetil or intravenous cyclophosphamide for lupus nephritis with poor kidney function: a subgroup analysis of the Aspreva Lupus Management Study. **Am J Kidney Dis**, v. 61, n. 5, p. 710-5, 2013. doi: 10.1053/j.ajkd.2012.11.042.

WALTER SORIA, N.; BELAUS, A.; GALVÁN, C.; ANA PASQUALI, M.; VELEZ, P.; DEL CARMEN MONTES, C.; BELTRAMO, D. M. A simple allele-specific polymerase chain reaction method to detect the Gly143Glu polymorphism in the human carboxylesterase 1 gene: importance of genotyping for pharmacogenetic treatment. **Genet Test Mol Biomarkers**, v. 14, n. 6, p. 749-51, 2010. doi: 10.1089/gtmb.2010.0037.

WANG, D.; GUO, Y.; WRIGHTON, S. A.; COOKE, G. E.; SADEE, W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. **Pharmacogenomics J**, v. 11, n. 4, p. 274-86, 2011. doi: 10.1038/tpj.2010.28.

WANG, D.; JOHNSON, A. D.; PAPP, A. C.; KROETZ, D. L.; SADÉE, W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. **Pharmacogenet Genomics**, v. 15, n. 10, p. 693-704, 2005. doi: 10.1097/01.fpc.0000178311.02878.83.

WANG, D.; SADEE, W. CYP3A4 intronic SNP rs35599367 (CYP3A4*22) alters RNA splicing. **Pharmacogenet Genomics**, v. 26, n. 1, p. 40-43, 2016. doi: 10.1097/FPC.0000000000000183.

WANG, J.; YANG, J. W.; ZEEVI, A.; WEBBER, S. A.; GIRNITA, D. M.; SELBY, R.; FU, J.; SHAH, T.; PRAVICA, V.; HUTCHINSON, I. V.; BURCKART, G. J. IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. **Clin Pharmacol Ther**, v. 83, n. 5, p. 711-7, 2008. doi:10.1038/sj.clpt.6100347.

WANG, J.; ZEEVI, A.; MCCURRY, K.; SCHUETZ, E.; ZHENG, H.; IACONO, A.; MCDADE, K.; ZALDONIS, D.; WEBBER, S.; WATANABE, R. M.; BURCKART, G. J. Impact of ABCB1 (MDR1) haplotypes on tacrolimus dosing in adult lung transplant patients who are CYP3A5 *3/*3 nonexpressors. **Transpl Immunol**, v. 15, n. 3, p. 235-40, 2006. doi: 10.1016/j.trim.2005.08.001.

WAVAMUNNO, M. D.; CHAPMAN, J. R. Individualization of immunosuppression: concepts and rationale. **Curr Opin Organ Transplant**, v. 13, n. 6, p. 604-8, 2008. doi: 10.1097/MOT.0b013e3283193bc5.

WIELAND, E.; SHIPKOVA, M.; SCHELLHAAS, U.; SCHÜTZ, E.; NIEDMANN, P. D.; ARMSTRONG, V. W.; OELLERICH, M. Induction of cytokine release by the acyl glucuronide of mycophenolic acid: A link to side effects? **Clin Biochem**, v. 33, p. 107-13, 2000.

WILSON, A. G.; DE VRIES, N.; POCIOT, F.; DI GIOVINE, F. S.; van DER PUTTE, L. B.; DUFF, G. W. An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles**. J Exp Med**, v. 177, n. 2, p. 557-60, 1993.

WILSON, A. G.; SYMONS, J. A.; MCDOWELL, T. L.; MCDEVITT, H. O.; DUFF, G. W. Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. **Proc Natl Acad Sci USA**, v. 94, n. 7, p. 3195-9, 1997.

WISGERHOF, H. C.; van DER GEEST, L. G.; DE FIJTER, J. W.; HAASNOOT, G. W.; CLAAS, F. H.; LE CESSIE, S.; WILLEMZE, R.; BOUWES BAVINCK, J. N. Incidence of cancer in kidney-transplant recipients: a long-term cohort study in a single center. **Cancer Epidemiol**, v. 35, n. 2, p. 105-11, 2011. doi: 10.1016/j.canep.2010.07.002.

WOILLARD, J. B.; KAMAR, N.; COSTE, S.; ROSTAING, L.; MARQUET, P.; PICARD, N. Effect of CYP3A4*22, POR*28, and PPARA rs4253728 on Sirolimus In Vitro Metabolism and Trough Concentrations in Kidney Transplant Recipients. **Drug Monit Toxicol Clinical Chem**, v. 59, n. 12, p. 1761–9, 2013. doi: 10.1373/clinchem.2013.204990.

WOLFE, R. A.; ASHBY, V. B.; MILFORD, E. L.; OJO, A. O.; ETTENGER, R. E.; AGODOA, L. Y.; HELD, P. J.; PORT, F. K. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. **N Engl J Med**, v. 341, n. 23, p. 1725-30, 1999. doi: 10.1056/NEJM199912023412303.

WONG, G.; HAYEN, A.; CHAPMAN, J. R.; WEBSTER, A. C.; WANG, J. J.; MITCHELL, P.; CRAIG, J. C. Association of CKD and Cancer Risk in Older People**, J Am Soc Nephrol**, v. 20, n. 6, p. 1341-50, 2009. doi: 10.1681/ASN.2008090998.

WOOD, K. J.; GOTO, R. Mechanisms of rejection: current perspectives. **Transplantation**, v. 93, n. 3, p. 1-10, 2012. doi: 10.1097/TP.0b013e31823cab44.

XIE, H. G.; WOOD, A. J.; KIM, R. B.; STEIN, C. M.; WILKINSON, G. R. Genetic variability in CYP3A5 and its possible consequences. **Pharmacogenomics**, v. 5, n. 3, p. 243-72, 2004. doi:10.1517/phgs.5.3.243.29833.

XUE, M. H.; LI, G. Y.; WU, X. J.; ZHANG, C. X.; ZHANG, C. F.; ZHU, K. X. Genetic variability of genes in NER pathway influences the treatment outcome of gastric cancer. Int J Clin Exp Pathol, v. 8, n. 5, p. 5563-9, 2015. ISSN:1936-2625/IJCEP0007176.

YENMIS, G.; ONER, T.; CAM, C.; KOC, A.; KUCUK, O. S.; YAKICIER, M. C.; DIZMAN, D.; KANIGUR SULTUYBEK, G. Association of NFKB1 and NFKBIA polymorphisms in relation to susceptibility of Behçet's disease. **Scand J Immunol**, v. 81, n.1, p. 81-6, 2015. doi: 10.1111/sji.12251.

YU, X.; WEI, B.; DAI, Y.; ZHANG, M.; WU, J.; XU, X.; JIANG, G.; ZHENG, S.; ZHOU, L. Genetic polymorphism of interferon regulatory factor 5 (IRF5) correlates with allograft acute rejection of liver transplantation. **PLoS One**, v. 9, n.4, p. e94426, 2014. doi: 10.1371/journal.pone.0094426.

YUZAWA, K.; KONDO, I.; FUKAO, K.; IWASAKI, Y.; HAMAGUCHI, H. Mutagenicity of cyclosporine. Induction of sister chromatid exchange in human cells. **Transplantation**, v. 42, n. 1. P. 61-3, 1986.

ZHANG, J. J.; ZHANG, H.; DING, X. L.; MA, S.; MIAO, L. Y. Effect of the P450 oxidoreductase 28 polymorphism on the pharmacokinetics of tacrolimus in Chinese healthy male volunteers. **Eur J Clin Pharmacol**, v. 69, n. 4, p. 807-12, 2013. doi: 10.1007/s00228-012-1432-1.

ZHENG, H.; WEBBER, S.; ZEEVI, A.; SCHUETZ, E.; ZHANG, J.; BOWMAN, P.; BOYLE, G.; LAW, Y.; MILLER, S.; LAMBA, J.; BURCKART, G. J. Tacrolimus dosing in pediatric heart transplant patients is related to CYP3A5 and MDR1 gene polymorphisms. **Am J Transplant**, v. 3, n. 4, p. 477-83, 2003. doi: 10.1034/j.1600-6143.2003.00077.x.

ZHENG, J.; JIANG, L.; ZHANG, L.; YANG, L.; DENG, J.; YOU, Y.; LI, N.; WU, H.; LI, W.; LU, J.; ZHOU, Y. Functional genetic variations in the IL-23 receptor gene are associated with risk of breast, lung and nasopharyngeal cancer in Chinese populations. **Carcinogenesis**, v. 33, n. 12, p. 2409-16, 2012. doi: 10.1093/carcin/bgs307.

ZHOU, S.; RUAN, Y.; YU, H.; CHEN, Y.; YAO, Y.; MA, Y.; GAO, Y. Functional IL-23R rs10889677 genetic polymorphism and risk of multiple solid tumors: a meta-analysis. **PLoS One**, v.8, n. 11, p. e80627, 2013. doi: 10.1371/journal.pone.0080627.

ZHU, H. J.; PATRICK, K. S.; YUAN, H. J.; WANG, J. S.; DONOVAN, J. L.; DEVANE, C. L.; MALCOLM, R.; JOHNSON, J. A.; YOUNGBLOOD, G. L.; SWEET, D. H.; LANGAEE, T. Y.; MARKOWITZ, J. S. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: clinical significance and molecular basis. **Am J Hum Genet**, v. 82, n. 6, p. 1241-8, 2008. doi: 10.1016/j.ajhg.2008.04.015.

ZIMMERMANN, A. G.; SPYCHALA, J.; MITCHELL, B. S. Characterization of the human inosine-5'-monophosphate dehydrogenase type II gene. **J Biol Chem**, v. 270, n. 12, p. 6808-14, 1995.

ZUNUNI VAHED, S.; ARDALAN, M.; SAMADI, N.; OMIDI, Y. Pharmacogenetics and drug-induced nephrotoxicity in renal transplant recipients. **Bioimpacts**, v. 5, n. 1, p. 45-54, 2015. doi: 10.15171/bi.2015.12.

11 ANEXOS

ANEXO A – Aceite do comitê de ética

UNIVERSIDADE ESTADUAL DE LONDRINA

PARANÁ
GOVERNO DO ESTADO

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina
Registro CONEP 5231

Parecer CEP/UEL:	153/2013
CAAE:	18263413.4.0000.5231
Data da Relatoria:	26/09/2013
Pesquisador(a):	Ilce Mara de Syllos Cólus
Unidade/Órgão:	CCB - Departamento de Biologia Geral

Prezado(a) Senhor(a):

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina**" (Registro CONEP 5231) – de acordo com as orientações da Resolução 466/12 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"Polimorfismos e análise da expressão de genes do metabolismo de drogas e da resposta imune na avaliação do risco de rejeição ao enxerto em pacientes transplantados renais."

Situação do Projeto: **Aprovado**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL, via Plataforma Brasil, relatório final da pesquisa.

Londrina, 07 de outubro de 2013.

Profa. Dra. Alexandrina Aparecida Maciel Cardelli
Coordenadora do Comitê de Ética em Pesquisa Envolvendo Seres Humanos
Universidade Estadual de Londrina

Comitê de Ética em Pesquisa Envolvendo Seres Humanos - UEL

ANEXO B – Aceite comitê de ética, emenda ensaios de mutagenicidade.

UNIVERSIDADE ESTADUAL DE LONDRINA - UEL/ HOSPITAL REGIONAL DO NORTE DO

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Polimorfismos e análise da expressão de genes do metabolismo de drogas e da resposta imune na avaliação do risco de rejeição ao enxerto em pacientes transplantados renais

Pesquisador: Ilce Mara de Syllos Cólus

Área Temática: Genética Humana:
(Haverá armazenamento de material biológico ou dados genéticos humanos no exterior e no País, quando de forma conveniada com instituições estrangeiras ou em instituições comerciais;);

Versão: 3

CAAE: 18263413.4.0000.5231

Instituição Proponente: CCB - Departamento de Biologia Geral

Patrocinador Principal: Financiamento Próprio
CNPQ

DADOS DO PARECER

Número do Parecer: 1.052.399

Data da Relatoria: 06/05/2015

Apresentação do Projeto:

Trata-se de emenda do projeto intitulado "Polimorfismos e análise da expressão de genes do metabolismo de drogas e da resposta imune na avaliação do risco de rejeição ao enxerto em pacientes transplantados renais" sob responsabilidade da Profª. Drª. Ilce Mara de Syllos Cólus.

A pesquisadora informa que a primeira etapa deste projeto, composta por realização de entrevista e coleta de amostra de sangue periférico, já foi realizada e que durante esta etapa foi observado que pacientes transplantados apresentaram alguns efeitos adversos, provavelmente decorrentes da terapia imunossupressora. Desta forma a pesquisadora solicita a realização dos ensaios do cometa (detecta quebras de fita simples e dupla, sítios de reparo por excisão e/ou lesões álcali-lábeis no DNA de células individuais) e do micronúcleo (detecta danos aneugênicos e clastogênicos) para que possa ser realizada uma melhor interpretação dos dados levantados em entrevistas e possíveis associações entre a utilização de medicamentos, tempo de transplante e efeitos adversos.

Para isso, quando coletadas amostras de sangue para a genotipagem, 600 µL de sangue serão

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utilizados para a realização do ensaio do micronúcleo e 20 µL de sangue para o ensaio do cometa. Para realização destes testes todos os pacientes devem ter aceitado participar do referido trabalho, assinado o TCLE e respondido ao questionário (já aprovados por este Comitê).

No TCLE já consta a autorização dos pacientes para utilização de moléculas obtidas a partir do sangue coletado, e autorização dos participantes para utilização deste material (DNA, RNA ou proteínas) para armazenamento das amostras para a realização de estudos futuros no Laboratório de Mutagênese e Oncogenética da Universidade Estadual de Londrina (UEL).

Objetivo da Pesquisa:

O objetivo principal deste projeto é avaliar e comparar a frequência de variantes genéticas polimórficas responsáveis pelo metabolismo de drogas e resposta imune em pacientes em monitoramento pós transplante renal que apresentaram ou não episódios de rejeição.

Avaliação dos Riscos e Benefícios:

A emenda não trará riscos adicionais aos participantes da pesquisa além dos já aprovados anteriormente, uma vez que será utilizado o mesmo material biológico previamente obtido para a realização da primeira etapa do estudo.

Comentários e Considerações sobre a Pesquisa:

As metodologias dos ensaios do micronúleo e do cometa foram detalhadas e anexadas.

Considerações sobre os Termos de apresentação obrigatória:

No TCLE já consta a autorização dos pacientes para utilização de moléculas obtidas a partir do sangue coletado, e autorização dos participantes para utilização deste material (DNA, RNA ou proteínas) para armazenamento das amostras para a realização de estudos futuros no Laboratório de Mutagênese e Oncogenética da Universidade Estadual de Londrina (UEL).

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Emenda aprovada.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

LONDRINA, 06 de Maio de 2015

Assinado por:
Paula Mariza Zedu Alliprandini
(Coordenador)

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ANEXO C – Termo de consentimento livre e esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Nós, Ilce Mara de Syllos Cólus, Heloísa Lizotti Cilião e Rossana Batista de Oliveira Gogoy Camargo da Universidade Estadual de Londrina o(a) convidamos para nossa pesquisa e solicitamos sua colaboração e o seu consentimento para incluí-lo(a) em nosso projeto de pesquisa "Polimorfismos e análise da expressão de genes do metabolismo de drogas e da resposta imune na avaliação do risco de rejeição ao enxerto em pacientes transplantados renais" O objetivo deste estudo é avaliar genes relacionados com a rejeição na busca de marcadores moleculares, que possam auxiliar na escolha do medicamento e das doses do imunossupressor mais adequado para cada paciente, individualizando a terapia imunossupressora e reduzindo os episódios de rejeição e os efeitos adversos causados pela super dosagem.

Assim, solicitamos a sua autorização para que uma pequena quantidade de seu sangue (10 ml) seja coletado via punção venosa (picada na veia) com seringa e agulha descartáveis. Esclarecemos que não haverá desconforto físico adicional para a sua pessoa, além da picada da agulha. Pedimos sua autorização para que moléculas obtidas a partir deste material (DNA, RNA ou proteínas) possam ser armazenadas para estudos futuros no Laboratório de Mutagênese e Oncogenética da UEL, quando será solicitada nova autorização do Comitê de Ética em Pesquisa com Seres Humanos para a realização das pesquisas posteriores. O material obtido ficará armazenado no Laboratório de Mutagênese e Oncogenética da UEL, sob responsabilidade dos pesquisadores responsáveis por esta pesquisa.

Solicitamos também sua autorização para que possamos consultar seu prontuário médico e obter alguns dados clínicos do seu tratamento imunossupressor, compatibilidade com o doador e a evolução clínica pós transplante.

Solicitamos-lhe o preenchimento de um questionário sobre seu estilo de vida, histórico de exposição e evolução do transplante, onde o(a) sr(a) será identificado(a) apenas por um código, preservando sua identidade. Estes questionários ficarão armazenados no laboratório de Mutagêneses e Oncogenética da Universidade Estadual de Londrina e somente poderão ter acesso a ele os pesquisadores responsáveis por esta pesquisa.

Sua identidade não será revelada e será mantido o caráter confidencial de todas as informações obtidas. Esclarecemos que o(a) sr(a) a qualquer momento tem a liberdade de se recusar em contribuir com o estudo, sem ser prejudicado(a) no seu tratamento e acompanhamento médico. Os resultados do estudo serão divulgados em congressos científicos e publicados em revistas especializadas, preservando sua identidade. Os mesmos provavelmente não trarão benefícios para a sua pessoa, mas poderão contribuir, no futuro, para a redução do número de casos de rejeição de transplantes, assim como para a diminuição de tumores em indivíduos submetidos a drogas imunossupressoras.

No caso de autorizado, o(a) sr(a). deverá assinar este Termo de Consentimento.

Desde já agradecemos sua colaboração.

_____ _____

Nome do voluntário Assinatura

RG: _____

Pesquisador responsável: _____

Londrina, ____ de _____ de 2014.

Ressaltamos que nem os pesquisadores e nem o paciente receberão qualquer remuneração financeira por participar desta pesquisa.

Os pesquisadores responsáveis por este estudo (Ilce Mara de Syllos Cólus, Heloísa Lizotti Cilião e Rossana Batista de Oliveira Gogoy Camargo, telefone 3371.4608, sempre que solicitados, estarão à sua disposição para esclarecimento de qualquer questão relacionada a esta pesquisa.

Telefone do Comitê de Ética em Pesquisa 3371-2490.

Agradecemos-lhe a valiosa colaboração.

_____	_____
Profª. Drª. Ilce Mara de Syllos Cólus	Heloísa Cilião /Rossana Camargo
Assinatura do pesquisador responsável	Coletor / Entrevistador

CONSENTIMENTO PÓS-INFORMADO

Eu, _____, abaixo assinado, declaro que fui esclarecido sobre o objetivo do presente estudo sobre os eventuais desconfortos que poderei sofrer, assim como sobre os benefícios da pesquisa. Concordo, portanto, em participar na qualidade de voluntário, do referido Projeto de Pesquisa, sob livre e espontânea vontade, autorizando a coleta e o armazenamento de amostras de moléculas obtidas a partir do meu sangue para pesquisas futuras bem como os dados do questionário que respondi. Por ser expressão de verdade firmo o presente termo.

Data ____ /____ /_____

Assinatura:

_____

ANEXO D – Entrevista pacientes transplantados renais.

Entrevista para pacientes transplantados renais
Laboratório de Mutagênese e Oncogenética da UEL

Código n°_____

HISTÓRICO PESSOAL:

1- Registro Institucional: Instituto do Rim

2- Sexo: Masculino ( ) Feminino ( )

3- Idade: ____

4- Data de nascimento: ____ /____ /_____

5- Local de nascimento: _____UF:_____

6- Local de residência
Cidade: _____UF:_____
Rua: _____n°_____
Telefone: _____ e-mail: _____

7- Sua moradia é na zona rural ou urbana ?
( ) RURAL ( )URBANA

8- Há quanto tempo reside neste local ?
_____anos_____meses.

9- Profissão: _____

10- Grau de instrução:

Ensino Fundamental (antigo 1º grau)	incompleto ( ) completo ( )
Ensino Médio (antigo 2º grau)	incompleto ( ) completo ( )
Ensino Superior/Graduação	incompleto ( ) completo ( )
Pós Graduação	incompleto ( ) completo ( )
Curso Técnico	incompleto ( ) completo ( )

11- A qual grupo étnico você pertence ?

Negro ( ) Caucasiano ( ) Asiático ( ) Indígena ( ) Outros ( )

12- Ancestralidade (assinalar e descrever)
( ) Européia (qual ?) _____
( ) Africana (qual ?) _____
( ) Indígena (qual ?) _____
( ) Oriental (qual ?) _____
( ) Mestiça (qual ?) _____

13- Informações dos familiares do indivíduo:

Nome completo do pai:_____
Nome da mãe:_____

Tamanho da irmandade (incluindo você):
Total:_____ Vivos:_____

14 - Você possui algum irmão gêmeo idêntico?
( ) SIM ( ) NÃO

HISTÓRICO DE EXPOSIÇÃO RELACIONADO OU NÃO AO TRABALHO:

15- Você já se expôs a algum destes agentes/substâncias listados abaixo EM SEU TRABALHO OU EM ALGUMA OUTRA ATIVIDADE QUE EXERCE ?
Se SIM, por quanto tempo e há quanto tempo foi isso:_____

Derivados de petróleo
(querosene, gasolina, solventes, etc...)_____( ) SIM ( ) NÃO
Tintas e/ou corantes_____( ) SIM ( ) NÃO
Compostos usados em Indústrias têxteis ou de tecelagem _____( ) SIM ( ) NÃO
Praguicidas e/ou herbicidas_____( ) SIM ( ) NÃO
Radiação_____( ) SIM ( ) NÃO
Metais pesados
(Chumbo, Níquel, Cromo, etc...)_____( ) SIM ( ) NÃO
Substâncias usadas no processamento de madeira ____( ) SIM ( ) NÃO
Substâncias usadas na indústria de papelo ou celulose ( ) SIM ( ) NÃO
Substâncias usadas em Mineração_____( ) SIM ( ) NÃO
Substâncias usadas na indústria de calçados ou curtume ( ) SIM ( ) NÃO
Substâncias usadas em Metalurgia _____( ) SIM ( ) NÃO
Substâncias usadas em Usina de açúcar ou álcool ___( ) SIM ( ) NÃO
Substâncias usadas em processamento de Plástico ou borracha _____( ) SIM ( ) NÃO

Sol _____ ( )SIM ( ) NÃO
Se SIM, especifique tempo e período do dia:_____

Outras substâncias químicas ou agentes físicos_____ ( ) SIM ( ) NÃO
Se SIM, especifique:_____

16- Você utiliza ou utilizava equipamentos de proteção individual (máscaras, luvas, óculos, chapéu, roupas apropriadas, protetor solar, etc...):
( ) SIM ( ) NÃO

HISTÓRICO TABAGISTA:

17- Você fuma atualmente ? ( ) SIM ( ) NÃO

18- Se SIM, qual a quantidade de cigarros que fuma por dia?
( ) menos de meio maço ( ) de meio a um maço ( ) mais de um maço

19- Se NÃO, já foi fumante ? Há quanto tempo parou de fumar ?
( ) nunca fumei parei de fumar há ( ) 0-5 anos
( ) 5-10 anos
( ) mais de 10 anos

20- Você convive ou conviveu em seu trabalho ou em casa com pessoas que fumam?
( ) SIM ( ) NÃO

HISTÓRICO DE ETILISMO:

21- Você consome bebidas alcoólicas ? ( ) SIM ( ) NÃO

22- Se SIM, que tipo de bebida alcoólica você costuma consumir?
( ) Destiladas ( ) Não-destiladas ( ) Outras ( ) Ambas

23- Quanto você costuma beber por semana?
( ) no máximo 1 copo ( ) de 2 a 5 copos ( ) de 6 a 10 copos ( ) mais de 10 copos

24- Se já parou há quanto tempo não consome mais esta bebida?
( ) 0-5 anos ( ) 6-10 anos ( ) mais de 10 anos

25- Quanto você costumava beber por semana?
( ) no máximo 1 copo ( ) de 2 a 5 copos ( ) de 6 a 10 copos ( ) mais de 10 copos

26- Durante a sua vida, já consumiu ou consome alguma bebida diariamente por mais de 6 meses continuamente ?
( ) SIM ( ) NÃO

HISTÓRICO DE SAÚDE:

27- Nos últimos 2 anos você tomou algum medicamento diariamente?
( ) SIM ( ) NÃO ( ) NÃO SABE

Se SIM, especifique:

( ) Hormônio	( ) Anti-inflamatório
( ) Analgésicos	( ) Anti-hipertensivos
( ) Anabolizantes	( ) Imunossupressores
( ) Antibióticos	( )Antiarrítmicos
( ) Antianêmicos	( ) Tranquilizantes
( ) Vitaminas	( ) Antifúngicos
( ) Antialérgicos	( ) Tranquilizantes

( ) Outros:_____

28- Depois que você iniciou seu tratamento com imunossupressor:
A - Observou o surgimento de alguma lesão na pele?
( ) SIM ( ) NÃO

B - Observou o surgimento de verrugas?
( ) SIM ( ) NÃO

C - Apresentou gastrite ou alguma complicação digestiva?
( ) SIM ( ) NÃO
Se sim, qual? _____

D - Desenvolveu catarata?
( ) SIM ( ) NÃO

E - Teve algum tipo de micose?
( ) SIM ( ) NÃO
Se sim, qual? _____

F – Desenvolveu diabetes?
( ) SIM ( ) NÃO

G – Desenvolveu Hipertensão arterial?
( ) SIM ( ) NÃO

H – Desenvolveu alguma doença cardiovascular?
( ) SIM ( ) NÃO

I – Desenvolveu Osteoporose?
( ) SIM ( ) NÃO

J- Desenvolveu anemia?
( ) SIM ( ) NÃO

L – Desenvolveu sopro?
( ) SIM ( ) NÃO

M- Desenvolveu Hepatite?
( ) SIM ( ) NÃO

Outros problemas ( ) Qual: _____

29- Você já foi submetido a algum tipo de cirurgia?
( ) SIM ( ) NÃO
Se SIM, especifique:
Tipo: _____
Ano: _____

30- Você já foi submetido a algum tipo de transplante?
( ) SIM ( ) NÃO
Se SIM, especifique:
( ) Rim ( ) Fígado ( ) Pulmão ( ) Coração ( ) Córnea ( ) Medula óssea

( ) 1 vez ( ) 2 vezes ( ) 3 vezes

Qual a data do transplante:_____

31- Você já recebeu transfusão sanguinea?
( ) SIM ( ) NÃO
Quantas? _____

32- Você tem antecedentes de câncer em sua família?
( ) SIM ( ) NÃO

33- Em casos de câncer na família, qual era o vínculo de parentesco?
( ) Pai ( ) Mãe ( ) Filho ( ) Tio ( ) Primo ( ) Outro
Se OUTRO, especifique: _____

34- Qual foi a localização do u tumor?
Especifique:_____

EVOLUÇÃO CLINICA DO TRANSPLANTE

35- Quem foi o doador:
( ) Parente vivo
Especifique o grau de parentesco _____
( ) não parente vivo
( ) Cadáver

36- Você já teve alguma crise de rejeição?
( ) SIM ( ) NÃO
Se SIM, especifique quantas vezes:
( ) 1 vez ( ) 2 vezes ( ) 3 vezes ( ) acima de 3 vezes

37- Você já foi tratado anteriormente devido a algum tipo de câncer?
( ) SIM ( ) NÃO
Se SIM, especifique o tipo: _____

38 – Se teve câncer, o tratamento ocorreu:
( ) antes do transplante
( ) depois do transplante

Histórico Alimentar: (refira-se somente aos hábitos frequentes):

39- Você se alimenta apenas de vegetais?
( ) SIM ( ) NÃO

40- Com que freqüência você come as carnes listadas abaixo:

	1-2 dias/semana	3-4 dias/semana	5-6 dias/semana	diariamente
Carne bovina				
Peixe				
Frango				
Porco				