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Identificação de marcadores moleculares de resposta e falha terapêutica às estatinas: influência de polimorfismos nos genes *SLCO1B1, SLCO1B3 e NR1I2*.

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SUMÁRIO

LISTA DE ABREVIATURAS UTILIZADAS

DCV: doença cardioavascular
IAM: infarto agudo do miocárdio
LDL: *low density lipoprotein* - lipoproteína de baixa densidade
HDL: *high density lipoprotein* - lipoproteína de alta densidade
HMG-COA: *3-hidroxi-3-methyl-glutaril-CoA redutase*
LDL-R: receptores de LDL
VLDL: *very low density lipoprotein* - lipoproteína de muito baixa densidade
CT: colesterol total
SNP: *single nucleotide polymorphism* - substituição de base única
ECA: enzima conversora de angiotensina
OATP: "*organic anion transporter proteins"* - proteínas transportadoras de ânions orgânicos
ABC: "*ATP-binding cassette transporter"* - transportador cassete dependente de ATP
"SLCO1B1": gene do transportador OATP1B1
"SLCO1B3": gene do transportador OATP1B3
"NR1I2": gene do receptor nuclear PXR
PXR/ SXR/PAR: *pregnance X receptor*
NR: *nuclear receptor* - receptores nucleares
"CYP": genes relacionados à enzimas do citocromo P450
GSTS: *glutationa-S-transferase*
UGT: *UDP- glucoronosyltransferases*
SUT: *sulfotransferases*
MDR-1: *multidrug resistance protein 1* - proteína 1 de resistência múltiplas drogas
AAS: *analyse aminoacid substitution* - análise de substituição de aminoácidos
IBGE: Instituto Brasileiro de Geografia e Estatística

RESUMO

Introdução: As doenças cardiovasculares (DCV) são a maior causa de morte em todo o mundo, e um dos principais fatores de risco associados a elas são as dislipidemias, alterações do colesterol circulante e suas frações. Fármacos hipolipemiantes, como as estatinas, são usados amplamente no tratamento e prevenção de agravos cardiovasculares. As estatinas são inibidores da enzima HMG-CoA redutase, responsável pela biossíntese do colesterol, sendo as mais comuns a sinvastatina e a atorvastatina. Apesar da boa tolerabilidade das estatinas e ótimos resultados na redução do perfil lipídico, existe uma grande variabilidade de resposta, que pode ser explicado por variantes polimórficas em genes de transportadores dos fármacos e receptores nucleares que controlam a expressão de proteínas da via metabólica. Dentre esses genes destacam-se o *SLCO1B1* e o *SLCO1B3* com grande variabilidade genética e associações comprovados com a resposta a estatinas, bem como o *NR1I2,* que codifica o receptor nuclear PXR e controla diversos fatores de transcrição importantes, como os genes da família CYP. O objetivo deste estudo foi avaliar a associação de variantes genéticas nos genes descritos com a resposta terapêutica e ocorrência de irregularidades no tratamento.

Materiais e métodos: A amostra consistiu de uma coorte de 477 pacientes com dislipidemia em uso de estatinas. Os pacientes foram avaliados quanto à resposta ao tratamento - medida através da redução percentual do perfil lipídico - e à regularidade do tratamento, caracterizada por intercorrências, como troca de medicações, suspensão e ajustes de dose. Essas variáveis foram associadas através de testes estatísticos por meio do programa SPSS19.0, com os genótipos para polimorfismos nos genes estudados: *SLCO1B1* (rs2306283, rs11045819, rs4149056), *SLCO1B3* (rs41491177) e *NR1I2* (rs3814057, rs6785049, rs3814055, rs7643645, rs2461817). A genotipagem foi realizada por PCR em tempo real com uso de sondas Taqman. Além disso, realizou-se análise de bioinformática com softwares livres, como o Polyphen-2, o SIFT e o Snpinfo, para avaliação de predição funcional dos polimorfismos sobre as proteínas resultantes.

Resultados: Encontrou-se associação da variante rs2306283 (388A>G – *SLCO1B1)* com irregularidade ao tratamento, especificamente com troca de medicamento e suspensão do tratamento. As variantes rs3814055 e rs3804057

do gene *NR1I2* foram associadas com a modulação da redução de colesterol total e LDL-colesterol, mas não com regularidade ao tratamento. A análise de funcionalidade dos polimorfismos do gene *NR1I2* mostraram que a variante rs3814055 está localizada em um sítio de ligação de fatores de transcrição e a variante rs3814057 em sítio de ligação de miRNAs.

Conclusões: Variantes genéticas em transportadores de fármacos e moléculas endógenas, como os *OATPs,* e receptores nucleares, como o PXR, são capazes de modular a resposta ao tratamento, bem como predizer os riscos de intercorrências ao tratamento. O melhor conhecimento dessas variantes é importante na orientação da prescrição médica e entendimento das diferentes respostas apresentadas pelos pacientes.

Palavras-chave: estatinas, polimorfismos, *SLCO1B1, NR1I2,* PXR.

1 INTRODUÇÃO

1.1 Doenças cardiovasculares (DCV)

1.1.1 Epidemiologia

No Brasil, as doenças cardiovasculares (DCV) são a primeira causa de morte, principalmente entre homens com mais de 50 anos (Piuvezam e cols., 2015). Porém, a taxa desses eventos em jovens adultos (20 a 40 anos) também é preocupante, já que soma 12% de todos ocorridos, segundo o Ministério da Saúde (2016). As regiões com maior prevalência são a sul e a sudeste. No entanto, a partir de 2000, todas as regiões têm tido quedas nestes números, com exceção dos Estados da região nordeste, nos quais o número de mortes por DCV aumentou em 13% (Piuvezam e cols., 2015; Guimarães, 2015).

Em âmbito global, as DCVs são responsáveis por 47% dos óbitos registrados em todo o mundo (Kaiser, 2004). Dentre as DCVs, os eventos mais frequentes são o infarto agudo do miocárdio (IAM) e os relacionados com a aterosclerose, que é a base dessas doenças, devido à obstrução do fluxo sanguíneo (Ministério da Saúde do Brasil, 2013).

Willrich (2011) e Mansur e Favarato (2016) trazem dados epidemiológicos da Organização Mundial da Saúde (OMS), mostrando que, desde 1990, as DCVs matam mais do que qualquer outra causa, sendo o número anual de mortes estimado em 17 milhões. Nas últimas décadas, devido ao trabalho de prevenção, essas estimativas diminuíram nos países desenvolvidos, e atualmente 80% dos óbitos são de habitantes de países em desenvolvimento, de baixa e média renda (Wiilrich, 2011; Mansur e Favarato,

2016). Esta situação remete bastante à realidade do Brasil, onde essa é a causa de 33% de todas as mortes registradas, ou seja, cerca de 308 mil mortes por ano, segundo dados do Ministério da Saúde do Brasil (2013).

Em 2009, no estado do Rio Grande do Sul, conforme dados preliminares do Ministério da Saúde, em relatório de situação da Secretaria de Vigilância de 2011, a taxa de mortes por DCV estava próxima de 150 por 100.000 habitantes, uma frequência bastante semelhante às médias da região sul e nacional. Conforme dados da OMS e do IBGE, esse quadro tende a persistir, agravando os dados de mortalidade do nosso Estado e do país. Sposito e cols. (2007), na IV Diretriz Brasileira Sobre Dislipidemias e Prevenção da Aterosclerose, bem como Steffens (2003), trazem estimativas apontando que em 2020 o total de mortes anuais por DCV chegará a 25 milhões, números esses, que já hoje oneram o Sistema Único de Saúde (SUS). No nosso país, este destina 17% do seu orçamento às DCVs, sendo o custo estimado, em 2004, de R$30,8 bilhões (Filho, 2013). Além disso, as DCVs são responsáveis por 10,74 milhões de dias de internações, e também por um terço das aposentadorias por invalidez, trazendo prejuízos à previdência social (Steffens, 2003).

Entre os diversos fatores de risco para o desenvolvimento dessas doenças, como ser fumante, portador de diabetes mellitus ou hipertenso, está também o colesterol alto, sendo que o aumento da lipoproteína de baixa densidade (LDL) e a diminuição dos níveis da lipoproteína de alta densidade (HDL) são comprovadamente prejudiciais à saúde, figurando entre os principais fatores de risco, conforme dados do *National Cholesterol Education Program*, NCEP (2001), e reafirmado em 2013, por Xavier e cols., na V Diretriz Brasileira

de Dislipidemias e Prevenção de Aterosclerose. No Brasil, 35,8% da população é composta de fumantes, 32% de obesos, 15% de hipertensos, 7,6% de diabéticos, e 42% de dislipidêmicos (Gus, 1998; Sposito e cols., 2007).

O entendimento desses fatores de risco, através da pesquisa científica, tem possibilitado o tratamento precoce, aumentando o investimento em prevenção primária e secundária, e a consequente diminuição, nas últimas décadas, do número de mortes. Consoante a isso, com o crescimento da farmacogenética nessa área, permitiu-se também o desenvolvimento de uma visão mais individualizada do tratamento da dislipidemia (Pereira e cols., 2015; Plöthner e cols., 2016).

1.1.2 A dislipidemia e seu tratamento farmacológico

"Doenças cardiovasculares" é um termo amplo para designar alterações fisiológicas dos vasos, plaquetas e coração, tanto estruturais, quanto funcionais. A aterosclerose, processo de formação de placas densas nos vasos, contribui de forma significativa para a maioria das DCV, como a doença arterial coronariana e a doença cerebrovascular e de vasos periféricos, incluindo a aorta, a artéria renal e as artérias presentes nos membros. Existem diversos fatores de risco que contribuem para o aumento do risco para as DCV, como idade, hipertensão e obesidade (Ministério de Saúde, 2006; Xavier e cols., 2013).

A aterosclerose tem origem multifatorial e característica de doença inflamatória crônica, pois ocorre a formação de placas ateroscleróticas no endotélio vascular, alterando a estrutura do tecido que se torna imunogênico. Isto leva ao acúmulo de células de defesa do organismo, o que contribui para o

espessamento da placa. As células fagocitárias ficam repletas de lipídios e são chamadas de células espumosas. Elas continuam secretando citocinas, o que amplifica o processo. A placa aterosclerótica desenvolvida é formada por células, matriz celular, lipídios e massa necrótica de células mortas. Essas placas podem obstruir o vaso, lentificar o fluxo sanguíneo ou desencadear a formação de trombos – já que sua superfície estranha ao organismo é trombogênica – sendo observadas características clínicas decorrentes da aterogênese (Xavier e cols., 2013).

A V Diretriz Brasileira de Dislipidemias e Prevenção de Aterosclerose é a orientação mais atual para o manejo de pacientes dislipidêmicos, ela foi publicada em 2013 e traz mudanças na prática clínica que objetivam inferir sobre os desfechos saúde-doença. A diretriz aborda toda a fisiopatologia das dislipidemias, descreve fatores de risco e busca fazer uma estratificação prática do risco do paciente, para que o médico possa aplicar no seu consultório. O documento traz também alternativas de tratamento não-medicamentosas e com fármacos hipolipemiantes (Xavier e cols., 2013).

A principal alteração desta diretriz em relação à IV Diretriz de 2007 foi a redução dos valores desejáveis para a fração LDL do colesterol, passando de 100mg/dL para 70mg/dL. Dessa forma, aumentou-se o número de pessoas consideradas dislipidêmicas, bem como aquelas em uso de alguma medida farmacológica para redução do colesterol (Xavier e cols., 2013).

A farmacoterapia indicada nos casos de dislipidemia consiste no uso de diferentes fármacos escolhidos entre as classes existentes: sequestradores de ácidos biliares, fitoesteróis, ácido nicotínico, fibratos, inibidores seletivos da absorção de colesterol e inibidores da HMG-CoA redutase, sendo a última

classe formada pelas estatinas (Xavier e cols., 2013; Pereira e cols.,2015; Kotlega e cols.,2016). As estatinas atuam na diminuição da produção endógena de colesterol através da inibição competitiva da 3-hidroxi-3-metil-glutaril-Coenzima A redutase (HMG-CoA), que é responsável pela produção de mevalonato, precursor do colesterol (Sposito e cols., 2007; Willrich, 2011; Kotlega e cols., 2016). A redução do colesterol intracelular promove o incremento da expressão de receptores a LDL (LDL-R) na superfície do hepatócito, captando a LDL circulante e diminuindo consideravelmente o seu nível sérico (Christians e cols., 1998; Sortica e cols., 2012). Além disso, as estatinas promovem uma queda na produção e um aumento do catabolismo das lipoproteínas de muito baixa densidade (VLDL), que, como precursores da LDL, contribuem de forma secundária para a redução da mesma e atenuam a ativação de proteínas pró-inflamatórias e a biodisponibiliade do óxido nítrico (Xavier e cols., 2013). Considerando que a via de biossíntese é responsável por até 70% do colesterol total circulante, torna-se justificável o impacto benéfico desses medicamentos e o fato dos mesmos serem os mais comumente prescritos na prática terapêutica (Kotlega e cols., 2016). Atualmente, existem seis estatinas disponíveis no mercado brasileiro, sendo a mais comumente prescrita a sinvastatina, no entanto existem outras como: a atorvastatina, a fluvastatina, a lovastatina, a pravastatina e a rosuvastatina (Marques, 2011). O governo brasileiro disponibiliza as estatinas para os pacientes que apresentam um alto risco de DCV, sendo o uso crônico destes medicamentos uma preocupação econômica, mesmo com diversos estudos comprovando seu custo-efetividade (Wannmacher, 2004).

Dados de estudos randomizados mostram um efeito redutor de 18 a 25% sobre o colesterol total (CT), e de 25 a 55% sobre os níveis de LDL (Wannmacher, 2004). Fórmulas mais modernas, das estatinas, são capazes também de reduzir o nível de triglicerídeos em até 15% e aumentar os níveis da HDL na mesma proporção (Kajinami e cols., 2003; Pereira e cols., 2015). Os dados compilados pela literatura mostram que as estatinas reduzem os riscos de eventos vasculares, independentemente de outros fatores de risco (Hutz e Fiegenbaum, 2008), sendo que uma meta-análise de cinco estudos, envolvendo 30.817 participantes, mostrou redução de 31% em eventos coronarianos maiores e de 21% da mortalidade total, de forma similar nos diferentes sexos e faixas etárias (Larosa e cols., 1999). Mesmo assim, deve-se considerar que 1 a 5% dos pacientes que fazem uso das estatinas apresentam reações adversas comuns como mialgia, fraqueza, náusea, diarréia ou constipação, e mais raramente, pacientes apresentam reações graves, como dano muscular e hepatotoxicidade, sendo que a frequência destas é dose-dependente (Wiilrich, 2011; Talameh e Kitzmiller, 2014). Cerca de 25 a 50% desses pacientes interrompem o tratamento em um período menor de 12 meses devido a algum tipo de efeito adverso ou falsa de resposta (Jiang e cols., 2016).

1.2 A farmacogenética das estatinas

A ciência que investiga a influência de genes específicos e isolados sobre a atuação de fármacos é chamada de Farmacogenética, ou farmacogenômica, que busca a individualização da prescrição terapêutica

considerando fatores genéticos (Metzger, 2006; Pereira e cols., 2015). O principal alvo de estudo desta área são os polimorfismos genéticos, ou seja, versões diferentes de um mesmo gene que ocorrem de forma aleatória no genoma, sendo que as mais comuns são: a deleção, a inserção, a substituição de base única (SNP) e a variação do número de sequências repetidas, de micro e de minisatélites. Esses polimorfismos podem alterar a atividade da proteína traduzida e esta poderá não exercer corretamente sua função (Metzger, 2006).

Nos Estados Unidos da América, a cada ano são registradas 106.000 mortes por intoxicação medicamentosa e efeitos adversos, fato que suscita o interesse no entendimento maior da relação genótipo-fenótipo, e na individualização das terapias, podendo aumentar sua eficácia e segurança (Plöthner e cols., 2016). Neste contexto, o objetivo da farmacogenética é identificar o fármaco “certo” para o paciente “certo” (Pereira e cols., 2015), permitindo seleção da melhor droga, dose e duração do tratamento, prevenindo efeitos adversos e melhorando a eficácia (Plöthner e cols.,2016).

Os genes de interesse da farmacogenética podem ser divididos em três categorias: genes que codificam proteínas envolvidas no metabolismo e/ou transporte do fármaco, atuando sobre aspectos farmacocinéticos; genes codificantes de proteínas envolvidas no mecanismo de ação do fármaco, atuando sobre parâmetros de farmacodinâmica, ou ainda genes que codificam proteínas envolvidas diretamente na patogênese das DCV (Hutz, Fiegenbaum, 2008). Os marcadores encontrados são capazes de interferir em funções moleculares, genéticas e celulares, e são importantes por possuírem valor diagnóstico, prognóstico e preditivo (Plöthner e cols.,2016).

Dentro da cardiologia, a pesquisa farmacogenética é um campo de muitas apostas. No entanto, seus benefícios clínicos ainda não são totalmente claros. Os estudos objetivam identificar, previamente à prescrição, os pacientes que possuem maior risco de sofrerem com efeitos adversos e de orientar as decisões médicas, que, atualmente, são tomadas de forma empírica (Joseph e cols., 2014). Dentre os grupos de grande interesse estão os anticoagulantes como a varfarina; os β–bloqueadores; o ácido acetilsalicílico; os inibidores da ECA (enzima conversora de angiotensina) e as estatinas (Joseph e cols., 2014). Evidências de variabilidade interindividual (como na varfarina), de falta de resposta (como nas estatinas e nos β-bloqueadores), e de toxicidade ao fármaco (como na toxicidade muscular induzida por estatina), levam à suspeita de uma influência genética, segundo Pereira e cols. (2015).

Apesar dos benefícios comprovados da terapia com estatinas, a resposta ao tratamento apresenta grande variabilidade individual, podendo variar entre 15 e 30% entre diferentes indivíduos submetidos ao mesmo tratamento (Fiegenbaum e cols., 2005; Lima e cols., 2012). Recentemente, essa variabilidade tem sido alvo de muitos estudos de farmacogenética, sendo que, até o momento, cerca de 40 genes foram associados à eficácia da terapia com hipolipemiantes. A grande maioria dos mesmos foi identificada pela abordagem “gene-candidato”, que considera a via de biossíntese do colesterol e aspectos farmacocinéticos e dinâmicos dos fármacos, como por exemplo: transportadores – OATPs e ABC, receptores de LDL, apolipoproteínas, enzimas do citocromo P450, entre outros (Hu e Tomlinson, 2013; Patel e cols., 2014; Pereira e cols., 2015). Os transportadores, como os OATPs, estão envolvidos na absorção intestinal, na distribuição para os tecidos, na excreção

e na concentração sérica, regulando fortemente a exposição sistêmica aos agentes farmacológicos. Assim, eles podem determinar os efeitos farmacológicos e tóxicos do fármaco (Shitara e cols., 2013; Kotlega e cols., 2016).

1.2.1 Os genes *SLCO1B1* e *SLCO1B3*

Os genes da família *SLCO – solute carrier organic anion transport* – codificam transportadores de captação, como o OATP1B1, codificado por *SLCO1B1* e envolvido na captação hepática e intestinal, facilitando a entrada dos fármacos nas células (Kalliokoski e Niemi, 2009; Wiilrich, 2011). Os transportadores do tipo OATPs captam os compostos endógenos, como as estatinas do sangue, e os carregam para dentro das células, como os hepatócitos (Klotega e cols., 2016). Os OATPs são transportadores independentes de sódio que atuam por mecanismo ainda não bem estabelecido na membrana basolateral dos hepatócitos, não sofrendo influência dos gradientes de sódio, cloreto ou potássio, e independentemente do potencial de membrana ou dos níveis de ATP (Klotega e cols., 2016). A família dos OATPs é composta por 11 membros, designados com um número arábico e a subfamília representada por letras do alfabeto, com localizações diversas como cérebro, intestino, fígado, rim e placenta. Eles são codificados por genes localizados nos cromossomos 3, 5, 8, 12, 15 e 20 (Kalliokoski e Niemi, 2009; Shitara e cols., 2013).

Dentre esses transportadores, os de maior relevância na farmacocinética são o OATPB1 e OATPB3, codificados pelos genes *SLCO1B1* e *SLCO1B3,*

respectivamente, ambos localizados no braço curto do cromossomo 12. Sua importância se dá pela localização no fígado, atuando no transporte de substâncias endógenas e exógenas para o meio intracelular (Kalliokoski e Niemi, 2009; Shitara e cols., 2013). Entre as substâncias exógenas transportadas por esse carreador estão a sinvastatina, a atorvastatina, a pravastatina e a rosuvastatina. A sinvastatina não é transportada em sua forma inativada; porém, a sua forma ativa apresenta grande afinidade pelos OATPs (Sortica e cols*.,* 2012).

Diversos polimorfismos já foram descritos no gene *SLCO1B1* com alguma associação à variação de resposta. No entanto, as variantes 388A>G (rs2306283: Asn130Asp), 463C>A (rs11045819: Pro155Thr) e 521T>C (rs4149056: Val174Ala) são foco de vários estudos sobre a eficácia e a segurança do uso de estatinas (Bailey e cols*.,* 2010; Sortica e cols*.,* 2012; Shitara e cols., 2013). Em especial, os polimorfismos 521T>C e 388A>G já foram associados a uma diminuição da função do transportador, bem como à ocorrência de miopatias em uso de sinvastatina (Bailey e cols., 2010; Sortica e cols*.,* 2012; Shitara e cols., 2013), de pravastatina (Akao e cols., 2012), e de rosuvastatina (Danik e cols., 2013). Contudo, sua influência sobre a efetividade da atorvastatina permanece controversa (Rodrigues e cols., 2011; Santos e cols., 2012).

Recentemente, Keyser e cols. *(*2014), propuseram uma análise mais ampla, onde além de considerar a ocorrência de efeito adverso, os mesmos relacionaram os portadores do alelo C – polimorfismo 521T>C – a um maior risco de falha terapêutica, sendo a falha definida como a necessidade de redução da dose prescrita inicialmente da estatina de escolha. Trompet e cols.

(2016), fizeram uma análise semelhante, sendo que compararam os resultados incluindo e excluindo os não-responsivos. Os autores viram que os resultados eram mais robustos quando excluídos esses pacientes. No entanto, há uma diferença entre eles e aqueles que respondem ao tratamento, o que justifica sua inclusão nos estudos, buscando um maior entendimento e um melhor manejo desses pacientes que apresentam problemas com a terapia.

De modo semelhante, os portadores do alelo G em 388A>G possuem menor atividade do transportador, estando o mesmo associado a uma pior resposta, com menores efeitos sobre o perfil lipídico, e sobre a ocorrência de efeitos adversos (Kalliokoski e Niemi, 2009; Shitara e cols., 2013; Klotega e cols., 2016). Entretanto, os resultados sobre essa variante ainda são controversos, Jiang e cols. (2016), em uma meta-análise, não encontraram uma associação significativa da variante 388A>G com a resposta terapêutica à estatinas, sendo que apontaram como principal limitação o pequeno número de estudos publicados com a variante 388 A>G.

O transportador OATP1B3, codificado pelo gene *SLCO1B3*, é muito similar ao OATP1B1, sendo que suas sequências de aminoácidos apresentam uma homologia de 80%, e seus substratos são essencialmente os mesmos, com a diferença de que o OATP1B3 é menos seletivo, carreando outras moléculas endógenas, como a colecistinina, bem como uma variedade maior de fármacos. No entanto, não há divergências quanto à sua seletividade em relação às estatinas. O gene *SLCO1B3* é polimórfico e algumas sequências já foram associadas com o decaimento da função do transportador (Kalliokoski e Niemi, 2009). Ainda são limitados os estudos sobre esse gene. No entanto, a variante 334 T>G (rs4149117: Ser112Ala) foi associada com concentrações

aumentadas do fármaco no sangue, sendo que os portadores do alelo T apresentaram função diminuída do transportador (Shitara e cols., 2013).

Sortica e cols., 2012, apresentou dados com parte dessa amostra, no entanto no período que se seguiu, a amostra teve um aumento significativo, foram incluídas novas variantes genéticas, e desenvolvida uma nova abordagem estatística, baseada no trabalho de Keyser e cols., 2014, que considera os pacientes irregulares.

As Tabelas 1 e 2, respectivamente, descrevem os dados sobre a frequência dos polimorfismos em *SLCO1B1* e *SLCO1B3* e os estudos já realizados com estatinas.

Tabela 1. Informações de posição, de efeito e de frequência dos polimorfismos estudados nos genes *SLCO1B1* e *SLCO1B3*.

SNPrs#	Posição	Gene/Efeito na proteína	Troca de alelo	Frequência do alelos mais raro (MAF)		
				AFR[1]	EUR[2]	AMR[3]
rs4149117	12:20858546	*SLCO1B3* Ser112Ala	T>G	0,36	0,86	0,84
rs2306283	12:21176804	*SLCO1B1* Asn130Asp	A>G	0,82	0,40	0,47
rs11045819	12:21176879	*SLCO1B1* Pro155Thr	C>A	0,06	0,14	0,10
rs4149056	12:21178615	*SLCO1B1* Val174Ala	T>C	0,01	0,16	0,13

[1] Frequência em Africanos (AFR), [2] Europeus (EUR) e [3] Americanos (AMR).

Tabela 2. Revisão da literatura sobre as variantes polimórficas em *SLCO* e sua relação com as estatinas.

Ano	Autor	Desfechos	Achados	Estatina	Abordagem	População
1999	Hsiang e cols.	Ligação estatina aos OATPs	Observou-se que as estatinas eram transportadas por transportadores do tipo OATPs.	Sinvastatina, Pravastatina, Atorvastatina	Gene Candidato	Animais
2004	Khamdang e cols.	Ligação estatina aos OATPs	Observou-se que as estatinas eram transportadas por transportadores do tipo OATPs.	Pravastatina	Gene Candidato	Animais
2004	Kikuchi e cols.	Efluxo de estatinas da barreira hemato-encefálica	Contribuição de OATPS (tipo OATP2 e OATP3) no efluxo das estatinas	Pravastatina e Pivastatina	Gene Candidato	Animais
2007	Zhang e cols.	Resposta ao tratamento	Portadores do alelo C para a variante rs4149056 (521T>C), gene *SLCO1B1,* apresentaram menor efeito ao tratamento, em relação à medida de colesterol total.	Pravastatina	Gene Candidato	Pacientes chineses com hipercolesterolemia
2009	Voora e cols.	Presença de efeito adverso e indução de mialgia.	Portadores do alelo C para a variante rs4149056 (521T>C) gene *SLCO1B1,* apresentaram maior risco de efeitos adversos, em uso de sinvastatina.	Atorvastatina, Sinvastatina e Pravastatina	Gene Candidato	Estudo STRENGTH[1]
2010	Bailey e cols.	Resposta ao tratamento e efeitos adversos	Foram encontradas associações com genes do Citocromo P450. Não foram encontradas associações com a variante rs4149056 (521T>C) do gene *SLCO1B1.*	Sinvastatina e Rosuvastatina	GWAS[5]	Estudo GEOSTAT-1[2]

2011	Rodrigues e cols.	Resposta ao tratamento	O genótipo GG para a variante rs2306283 (388A>G), gene *SLCO1B1,* apresentou melhor resposta ao tratamento, quanto à redução da LDL.	Atorvastatina	Gene Candidato	Pacientes brasileiros, com dislipidemia, da região Sudeste
2012	Sortica e cols.	Resposta ao tratamento	Pacientes portadores do alelo G para a variante rs2306283 (388A>G) apresentaram maior resposta ao tratamento, quanto a redução de LDL. Para as variantes rs11045819 (463C>A) e rs4149056 (521T>C) não foram encontradas asssociações. Todas variantes estudadas são no gene *SLCO1B1.*	Sinvastatina	Gene Candidato	Pacientes basileiros dislipidêmicos, da região sul
2012	Akao e cols.	Resposta ao tratamento e casos de DCV.	Associação da variante rs4149056 (521T>C) com menor resposta ao tratamento, quanto à redução da LDL, nos genótipos CC. Para a variante rs2306283 (388A>G) não foram encontrados resultados significativos. Ambas no gene *SLCO1B1.*	Pravastatina	Gene Candidato	Estudo PROSPER[3]
2012	Santos e cols.	Presença de efeito adverso e de indução de mialgia.	Não foram encontradas associações para as variantes rs4149056 (521T>C) e rs2306283 (388A>G) do gene *SLCO1B1.*	Atorvastatina	Gene Candidato	Pacientes brasileiros com hipercolesterolemia familiar.
2012	Hu e Tomlinson	Níveis de bilirrubina associados aos níveis de estatinas séricos	Foi encontrada associação com o gene *UGT1A,* mas não com as variantes rs2306283 (388A>G) e rs4149056 (521T>C) do gene *SLCO1B1*	Sinvastatina	Gene Candidato	Pacientes chineses com hipercolesterolemia

2012	Wilke e cols.	Risco para miopatia	O genótipo CC para a variante rs4149056 (521T>C) no gene *SLCO1B1,* foi associado com baixa atividade do transportador e alto risco de miopatia. Essa é uma orientação para o uso da Sinvastatina internacionalmente reconhecida.	Sinvastatina	Gene Candidato	Estudo STRENGTH[1]
2013	Danik e cols.	Ocorrência de miopatia	Não foi encontrada associação entre miopatia em decorrência do uso da rosuvastatina e as variantes rs43657 e rs4149056 no gene *SLCO1B1.*	Rosuvastatina	Gene Candidato	Coorte Jupiter[4]
2013	Chasman e cols.	Resposta ao tratamento e efeitos adversos	Foram encontradas associações entre redução da LDL e os SNPs em *SLCO1B1:* rs4149056, rs4363657 e rs12317268 do gene *SLCO1B1.*	Rosuvastatina	GWAS[5]	6989 pacientes europeus (Coorte Jupiter[4])
2013	DeGorter e cols.	Níveis séricos de estatinas	Encontrada associação entre os níveis séricos de estatina e as variantes rs2306283 (388A>G) e rs4149056 (521T>C) do gene *SLCO1B1*	Rosuvastatina e Atorvastatina	Gene Candidato	Pacientes dislipidêmicos do London Healh Sciences Center (LHSC, London, Canada)
2013	Lee e cols.	Níveis séricos de estatinas e resposta ao tratamento.	Encontrada associação entre os níveis séricos de estatina e a variante rs4149056 (521T>C) do gene *SLCO1B1*, mas não foi encontrada associação para a resposta ao tratamento.	Rosuvastatina	Gene Candidato	Pacientes chineses com hipercolesterolemia

2014	Keyser e cols.	Resposta ao tratamento, diminuição de dose e efeitos adversos.	O genótipo CC para a variante rs4149056 (521T>C) no gene *SLCO1B1,* associado a maior risco de redução de dose em pacientes em uso de Sinvastatina. Para Atorvastatina não foram encontradas associações.	Sinvastatina e Atorvastatina	Gene Candidato	Estudo Rotterdam
2014	Yang e cols.	Expressão quantitativa e locos (EQL) e farmacocinética de drogas.	Influência do gene *SLCO1B1* na bio-transformação de diversos fármacos.	Diversos fármacos	GWAS[5]	Pacientes participantes do International Hap-MapII Project, de diferentes nacionalidades
2015	Hou e cols.	Risco para miopatia	Encontrada associação da variante rs4149056 (521T>C) no gene *SLCO1B1,* com risco para miopatia, sendo o alelo C associado a um maior risco de efeitos adversos.	Várias estatinas	GWAS[5] e Gene Candidato	Meta-análise
2015	Prado e cols.	Resposta ao tratamento.	Não foram encontradas associações com a diminuição da LDL. No entanto, foi encontrada associação da variante rs2306283 (388A>G) com aumento da HDL após o tratamento. Para a variante rs4149056 (521T>C) não foram encontradas associações. Todas variantes no gene gene *SLCO1B1.*	Atorvastatina	Gene Candidato	Pacientes chilenos com hipercolesterolemia

2015	Daí e cols.	Resposta ao tratamento.	Encontrada associação da variante rs4149056 (521T>C) com a efetividade do tratamento em longo prazo, em populações não-asiáticas, referente à alteração da LDL. Não foram encontradas associações com a variante rs2306283 (388A>G). Todas variantes no gene *SLCO1B1.*	Várias estatinas	GWAS[5] e Gene Candidato	Meta-análise
2015	Nagy e cols.	Comparação das frequências alélicas de variantes de interesse farmacogenético em diferentes populações.	Na população de Roma, a frequência do alelo G da variante rs2306283 (388A>G) e do alelo T da variante rs4149056 (521T>C) foi maior do que na população Húngara. Todas variantes no gene *SLCO1B1.*	Não aplicável	Gene Candidato	Italianos (de Roma) e Húngaros
2015	Dou e cols.	Resposta ao tratamento e efeitos adversos	Associação entre a variante rs4149056 (521T>C) do gene *SLCO1B1,* e a resposta ao tratamento foi encontrada somente para a estatina Sinvastatina	Várias estatinas	GWAS[5] e Gene Candidato	Meta-análise
2016	Jiang e cols.	Presença de efeito adverso e indução de mialgia.	Encontrada associação da variante rs4149056 (521T>C) com efeito adverso, mas não com a variante rs2306283 (388A>G). Todas variantes no gene *SLCO1B1.*	Várias estatinas	GWAS[5] e Gene Candidato	Meta-análise

2016	Trompet e cols.	Comparação de perfil lipídico e características de "respondedores" ao tratamento e "não-respondedores".	Não foram encontradas associações genéticas com o grupo de não-respondendores. Viu-se que o grupo é mais jovem, e realiza um maior consumo de álcool e de fumo. O genótipo AA para a variante rs2306283 (388A>G) do gene *SLCO1B1,* foi associado com melhor resposta, quanto a redução da LDL.	Pravastatina	Gene Candidato	Estudo PROSPER[3]
2016	Kadam e cols.	Resposta ao tratamento.		Atorvastatina	Gene Candidato	Pacientes indianos com dislipidemia.

1. STRENGTH – Estudo farmacogenético (*Statin Response Examined by Genetic Haplotype Markers*).
2. GEOSTAT-1 – Estudo farmacogenético (*The Genetic Effects On STATins*).
3. PROSPER – Estudo clínico com estatinas (*Patient-Centered Research Into Outcomes Stroke Patients Prefer and Effectiveness Research Study).*
4. JUPITER – Estudo farmacológico (Ju*stfication for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial)*.
5. GWAS – Estudo de associação por varredura genômica (*Genome Wide Association Study*).

1.2.2 O gene *NR1I2*

O gene *NR1I2,* localizado no braço curto do cromossomo 3 humano, é composto por 10 éxons e codifica o receptor PXR (*pregnance X receptor)*, também conhecido como SXR ou PAR*,* uma proteína de 434 aminoácidos e de aproximadamente 50-kDa, com sítio de ligação bastante flexível (Goodwin e cols., 2001; Tolson e Wang, 2010). O PXR atua como um importante regulador do sistema de defesa do corpo humano contra xenobióticos, expresso de forma intensa no intestino e no fígado. A proteína PXR faz parte de uma superfamília formada por 48 diferentes receptores nucleares, os NRs – *Nuclear Hormone Receptors*- - que desempenham diversas funções reguladoras sobre diferentes genes. Essas proteínas apresentam estrutura de aminoácidos bastante conservada, havendo partes comuns, como a porção N-terminal com função ativadora de transcrição (Garattini e cols., 2016).

Dentre esses receptores, o PXR está envolvido no metabolismo de xenobióticos, controlando genes envolvidos no metabolismo desses ligantes e no transporte de substâncias (Garattini e cols., 2016). A expressão da PXR e sua ativação estão intimamente relacionadas com a expressão de genes da grande família *CYP,* que codifica enzimas do citocromo P450, responsáveis pela detoxificação do organismo (Willson e Kliewer, 2001). Estudos em ratos geneticamente modificados – linhagem *knockout* para os genes codificadores da PXR - demonstraram que a expressão do receptor é um importante regulador das enzimas do citocromo P450 *in vivo*, interferindo em funções como oxidação, conjugação e transporte, sendo que os animais *knockout* não

foram capazes de aumentar a expressão de enzimas do citocromo P450 quando expostos a xenobióticos (Goodwin e cols., 2001; Kliewer e cols., 2002).

O receptor PXR é ativado por diversas moléculas químicas que possuem potencial nocivo ao organismo, que podem ser exógenas ou endógenas, como os sais biliares, os hormônios e muitos medicamentos (Kliewer e cols., 2002; Gotoh e Negish, 2015). O sistema de detecção de xenobióticos no qual o PXR está inserido é muito importante, havendo evidências de que também influencia no metabolismo e na homeostase dos lipídios e de sais biliares, bem como pode explicar os efeitos adversos de muitos fármacos, principalmente quando há politerapia (Willson e Kliewer, 2001; Lima e cols., 2013).

O receptor PXR regula diferentes etapas da metabolização, incluindo a fase I, as enzimas do citocromo P450 (CYP) 2B6, CYP2B9, CYP2C9, CYP3A4 e CYP3A7; na fase II, enzimas como a glutationa-S-transferase (GSTs), a UDP- glucoronosyltransferases (UGTs) e as sulfotransferases (SUTs) e alguns transportadores como o MDR1 *(multidrug resistance protein 1)* e o tipo OATP *(organic anion transporter protein*) (Goodwin e cols., 2001; Wang e cols., 2014).

A indução da CYP3A4 já é bem documentada na literatura, sendo um alvo de grande interesse pela diversidade de compostos que são metabolizados por essa enzima, dentre estas moléculas estão a maioria das estatinas, como a sinvastatina, que é a mais popular. Essa relação já foi estudada em fármacos para depressão e inflamação, imunossupressores, anticoagulantes, hipolipemiantes e anticoncepcionais orais, e as mesmas relações foram encontradas, de modo que é provável que qualquer fármaco metabolizado por ela terá influência da ação do PXR (Kliewer e cols., 2002; Wang e cols., 2014). Por seus efeitos, o PXR é reconhecido como um fator de

variação individual de respostas a medicamentos, com influência no surgimento de algumas doenças (Liu e cols., 2016).

O receptor PXR quando ativado por um ligante se transloca do citoplasma para o núcleo da célula, onde estabelece pontes – através de duas regiões de dedos de zinco - com regiões específicas do DNA promotoras de outros genes. Além disso, ele é capaz de recrutar co-ativadores que intensificam esta ligação (Iunnahah, 2011). Deste modo, o PXR tem a capacidade de interagir com regiões flanqueadoras do gene *CYP3A4* e outros sobre o qual exerce regulação (Goodwin e cols., 2001).

Além disso, estudos demonstram que as estatinas se ligam diretamente aos receptores do tipo PXR, alterando suas vias de sinalização, uma delas que é ativada é a de gliconeogênese no fígado, o que poderia relacionar o uso das estatinas com um aumento da glicose no sangue, e com o risco aumentado de desenvolvimento de diabetes do tipo 2 (Gotoh e Negish, 2015). Outra importante relação é ressaltada por Stanley e cols. (2015), em um estudo onde os autores demonstraram que as estatinas poderiam alterar a produção de uma proteína inflamatória de fase aguda, efeito este mediado por ela. Essa relação, que não está clara se é de inibição ou de ativação, poderia elucidar as causas dos efeitos adversos das estatinas e limitações do seu uso.

Devido ao fato das estatinas serem metabolizadas por enzimas do citocromo P450 e transportadas tanto pelo MDR1 quanto por transportadores OATPs é plausível inferir que a expressão do PXR possa interferir na resposta ao tratamento com esta classe de medicamentos, bem como estar associada a casos de falha terapêutica e de efeitos adversos. A capacidade de ligação das estatinas aos receptores do tipo PXR chamam atenção para as vias que podem

ser moduladas por esse receptor e, indiretamente, pelo próprio fármaco (Stanley e cols., 2015).

O gene *NR1I2* controla diversas vias, levando à transcrição de enzimas do metabolismo hepático e do ciclo celular, envolvidas na proliferação das células e em rotas de uso de diferentes substratos. Por isso os estudos de suas variantes possuem objetivos bem amplos, não limitando-se apenas à farmacogenética. A variabilidade do *NR1I2* já foi associada com disfunções inflamatórias em animais e humanos, como a doença inflamatória dos ossos, a úlcera crônica e a doença de Chron. Dentre as variantes do PXR estudadas, o genótipo GG da variante rs6785049 (7635A>G) mostrou-se relacionado com indução aumentada da CYP3A4 após o tratamento com rifampicina, e a um maior risco de úlcera crônica (Andersen e cols., 2011).

Variantes polimórficas no *NR1I2* são também estudadas em oncologia, já que controlam diversas rotas do ciclo celular e da proliferação celular. Um estudo com pacientes com câncer de próstata mostrou associação da gravidade dos casos com a variante rs7643645 (A>G), onde pacientes com o alelo variante tiveram alteração do sítio de ligação da proteína e diminuição da expressão basal do mRNA do *CYP3A4* e consequentemente da atividade da enzima. A frequência do alelo G nos controles foi semelhante à da população em geral (0,37), em contraste com a frequência do mesmo nos pacientes com câncer de próstata (0,56) (Reyes-Hernández, 2014).

Em outro estudo, investigou-se a influência de variantes do gene *NR1I2* em casos de hepatoxicidade medicamentosa desencadeada por tratamento para tuberculose, devido à sua relação na indução de enzimas do citocromo P450. Observou-se que pacientes com genótipo AA para a variante rs2461823

apresentavam maior risco de toxicidade, enquanto que pacientes com o genótipo AA para a variante rs7643645 tiveram menor risco de toxicidade em decorrência do tratamento (Wang e cols., 2015). Resistência medicamentosa também foi relacionada com polimorfismos em *NR1I2* em um estudo com colelitíase. Observou-se que para a variante rs6785049 havia diferentes patógenos envolvidos no processo de acordo com o genótipo, e que os portadores do alelo A para essa variante tinham um risco maior de desenvolver cálculos na vesícula. Para as variantes rs2276707 e rs3814055 não foram encontradas associações significativas (Xie e cols., 2016).

Lima e cols. (2013) estudaram a influência de diversos genes, entre eles as variantes rs1523130 e rs2472677 do gene *NR1I2*, na farmacogenética de estatinas, e encontraram associação da variante rs1523130 com os níveis basais de colesterol total e de LDL. Em nosso estudo, tivemos o aumento do número amostral, o incremento de novas variantes genéticas na análise, e o uso de abordagem estatística diferenciada, que considera os pacientes irregulares. São vários os alvos e os objetivos de estudo das variantes de *NR1I2,* e os resultados se mostram controversos, mas sua influência é clara. Por isso, se torna importante o estudo e a validação desses polimorfismos em diferentes situações.

No presente estudo foram escolhidas 5 variantes genéticas distribuídas ao longo do gene (Figura 1 e Tabela 3).

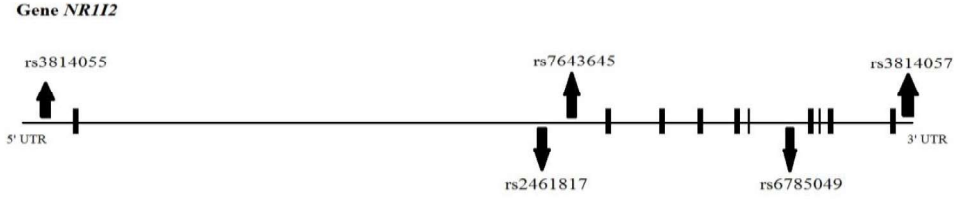

Figura 1. Distribuição dos polimorfismos estudados ao longo do gene *NR1I2 (PXR)*

Tabela 3. Informações de posição, de efeito e de frequência dos polimorfismos estudados no gene *NR1I2*.

SNP rs#	Posição no cromossomo	Posição no gene	Troca de Alelo	Frequência do alelo mais raro (MAF)		
				AFR[1]	EUR[2]	AMR[3]
rs3814055	3:119781188	5'UTR	C>T	0,31	0,37	0,40
rs2461817	3:119805804	2º Intron	A>C	0,39	0,67	0,49
rs7643645	3:119806650	2º Intron	A>G	0,06	0,34	0,50
rs6785049	3:119814886	6º Intron	G>A	0,04	0,62	0,62
rs3814057	3:119818407	3'UTR	A>C	0,52	0,17	0,19

[1] Frequência em Africanos (AFR), [2] Europeus (EUR) e [3] Americanos (AMR).

1.3 Análise de predição de substituição de aminoácidos

Com o acúmulo de sequências de DNA, a informação genética tornou-se muito mais acessível. No entanto, viu-se que apenas identificar variações genéticas não era suficiente para relacioná-las com doenças mendelianas. Para isso, era necessário conseguir correlacionar quais dessas variantes apresentavam um valor funcional, e preferencialmente, fazer isto de uma maneira rápida, eficiente e de baixo custo. Por isso, foram desenvolvidas diversas ferramentas computacionais com esse intuito (Zhou e cols., 2016).

Atualmente, existem várias ferramentas do tipo software livre disponíveis para avaliação de AAS (*Amino Acid Substitution*), que têm como princípio fundamental o alinhamento das sequências, originais e mutadas, avaliando o possível impacto da alteração sobre a funcionalidade da proteína em análise. A maioria das mutações existentes é neutra. No entanto, quando há, na substituição dos aminoácidos, diferenças físico-químicas, isso pode resultar em

dano funcional (Ng e Henikoff, 2006). Estas ferramentas utilizam diferentes algoritmos para avaliar o impacto das alterações, considerando fatores como carga elétrica, polaridade, afinidade com a água, formação de pontes de hidrogênio e sulfeto, alterações conformacionais e rigidez estrutural (Yue e cols., 2006).

Uma comparação das ferramentas disponíveis na Web para este tipo de análise mostra que o Polyphen é o método de maior confiança (81%), seguido pelo snps3d (71%) e pelo SIFT (60%) (Ng e Henikoff, 2006). O Polyphen-2 é uma ferramenta computacional livre disponível em http://genetics.bwh.harvard.edu/pph2/ desenvolvida por Adzhubei e colaboradores, em conjunto com a Universidade de Harvard, com objetivo de predizer o possível impacto funcional causado por uma mutação missense, ou seja, que resulta em troca de aminoácidos na estrutura primária proteica (Adzhubei e cols., 2010; Adzhubei e cols., 2013).

O programa utiliza a sequência FASTA da proteína de interesse ou a identificação do polimorfismo (exemplo: rs2306283). Com os dados de localização da mutação e quais aminoácidos são modificados (Aa^1 e Aa^2), ele realiza um alinhamento das sequências buscando em repositórios de estruturas, e prevê as modificações estruturais e suas consequências para a função da proteína estudada (Adzhubei e cols., 2010). O algoritmo de Polyphen-2 apresenta duas variações, a especificação “HumDiv” – modelo clássico para predição, preferível para análises de alelos raros, de efeito menor, relacionados à seleção natural ou localizados em regiões densas do genoma – e a especificação “HumVar” – sendo esta mais adequada para doenças Mendelianas, onde é necessário distinguir mutações com efeitos

drásticos. De acordo com o algoritmo utilizado pelo software, o mesmo apresenta os resultados na forma de um escore que varia de 0,0 a 1,0, onde 0,0 é considerado uma mutação benéfica e 1,0 representa dano à função da proteína (Adzhubei e cols., 2010).

O Snps3d é um software desenvolvido por Peng Yue e colaboradores, da Universidade de Maryland (USA), disponível em http://www.snps3d.org, que permite a pesquisa de genes candidatos de acordo com a doença de interesse e análise de substituição de aminoácidos. Os escores são dados em números inteiros, sendo que valores positivos representam mutações toleradas e valores negativos, dano funcional. O SIFT (*Human Splicing finder 3.0*), software desenvolvido por Pauline C. Ng e Steven Henikoff, da Universidade de Washington (USA), disponível em sift.bii.a-star.edu.sg, permite a pesquisa de genes candidatos de acordo com a doença de interesse e a análise de substituição de aminoácidos. Os escores são dados em um intervalo entre 0 e 1, sendo que o valor 0 representa dano funcional e o valor 1, mutação tolerável, sem prejuízo (Adzhubei e cols., 2010).

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2 OBJETIVOS

2.1. Objetivo geral:

Investigar a associação das variantes no gene *NR1I2* (rs3814057, rs6785049, rs3814055, rs7643645, rs2461817), *SLCO1B1* (rs2306283, rs11045819, rs4149056) e *SLCO1B3* (rs41491177) com a eficácia ao tratamento com atorvastatina/sinvastatina.

2.2. Objetivos específicos:

- Investigar a associação destas variantes com o desenvolvimento de efeitos adversos ao tratamento com atorvastatina/sinvastatina;
- Investigar a associação destas variantes com o desenvolvimento de falha terapêutica ao tratamento com atorvastatina/sinvastatina;
- Investigar, através de ferramentas de bioinformática, a possível implicação funcional das variantes genéticas estudadas.

3 ARTIGO CIENTÍFICO REDIGIDO EM INGLÊS 1

Molecular markers of treatment irregularity of statins: influence of polymorphisms in *SLCO1B1* and *SLCO1B3.*

Artigo em preparação para submissão à revista *British Journal of Clinical Pharmacology*. Fator de Impacto: 3,83.

Molecular markers of treatment irregularity of statins: influence of polymorphisms in *SLCO1B1* and *SLCO1B3.*

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Summary

Aims: The *SLCO* gene family encodes OATP-like transporters that have affinity for statins and may interfere with their effectiveness. Therefore, they are targets of pharmacogenetic studies, in which *SLCO1B1* associations with the therapeutic response of statins have already demonstrated. The aim of the study was to evaluate the association between rs2306283, rs11045819, rs4149056 in *SLCO1B1* and rs41491177 *SLCO1B3* gene polymorphisms , and regularity of statin treatment. **Methods:** A cohort study carried out by reviewing patients' medical records and genotyping them for the study variants. The measurements of the lipid profile of the patients with the specific genotypes were compared. General linear model (to evaluate the differences between groups) and Cox regression (to evaluate regularity of treatment) were adopted in statistics. **Results:** For rs2306283, G allele carriers had greater risk of irregularities in the treatment (Hazard ratio: 1.50 - 95% CI: 1.05 to 2.13, p = 0.024). For rs4149056, CC homozygotes presented greater risk of statin exchange and treatment withdrawal (Hazard ratio: 2.16 - 95% CI: 1.04 to 4.44, p = 0.037). **Conclusion:** Our results suggest an association between genetic variants and therapeutic efficacy, what may allow definition of response profiles, enabling corroboration with other studies, to reach the therapy individualization.

What is known about this subject

Statins are drugs used in the prevention of cardiovascular diseases, but they present a variability of response associated with the individual genetics of the patient. One of the targets of these studies are the *SLCO* genes, genes known to have influence over the therapeutic response.

What this study adds

This study showed that *SLCO1B1* polymorphisms are not only involved in the lipid profile modulation and adverse drug reaction development, but also can be associated to intercurrences in the treatment. Many patients have irregular treatment, and statin replacement and discontinuation of the treatment may be related to patient-specific genotypes.

Introduction

Cardiovascular diseases (CVD) are at the top of the list of causes of death. It is estimated that, in 2020, the number of deaths will exceed 25 million/year [1-3]. Cardiovascular risk factors are smoking, physical inactivity, diabetes, hypertension, obesity, and with great importance as an independent factor, increased cholesterol, especially the LDL fraction [2,4,5]. Despite the negative estimates, in recent decades the number of deaths decreased in developed countries, in part due to greater understanding of the pathophysiological mechanisms and disease risk factors and also by investing in primary prevention [3,6].

Statins are the most commonly prescribed lipid-lowering drugs due to its high efficacy and tolerability in the general population. Statins are competitive inhibitors of HMG-CoA enzyme - 3-hydroxy-3-methylglutaryl-coenzyme A reductase - a key enzyme of endogenous cholesterol synthesis [7,8]. The inhibition of this pathway prevents the production of mevalonate, the precursor of cholesterol. Furthermore, statins are able to induce increased expression of LDL receptors, decreasing the circulating fraction [6,8].

Despite the proven efficacy, statin treatment may have an individual efficacy variability of 15-30%, which explains the recent interest in the mechanisms that contribute to this variability [2,6]. Among patient factors, many studies have focused on pharmacogenetics, and several genes involved in the metabolism and transport of statins have been associated with therapeutic response and the occurrence of adverse effects [1, 6, 9].

Among the studied genes, special attention is given to the coding genes of organic solute carriers as OATPs, encoded by *SLCO* gene family, since they are directly involved in drug serum and hepatic concentrations [8-13]. The main transporters

responsible for cell influx in the liver are OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*), with polymorphic variants associated with decreased function and statin therapeutic response or induced myopathy. Among the most studied variants are c.388A>G (rs2306283: Asn130Asp), c.462C>A (rs11045819: Pro155Thr) and c.521T> C (rs4149056: Val174Ala) in *SLCO1B1* gene, and c.334T> G (rs4149117: Ser112Ala) in *SLCO1B3* gene. All these alleles have showed decrease protein function on *in vitro* e *in vivo* studies, specifically, the 388A> G variant was associated with increased protein function. [12-16].

In 2012, our research group published results of a study of the association among rs4149056, rs11045819 and rs2306283 polymorphisms in *SLCO1B1* with statin treatment response [17]. In this paper, were analyzed part of the present sample (216 hypercholesterolemic patients) and an association of the variant rs2306283 (SLCO1B1 -388A> G) with the percentage reduction in total cholesterol and LDL-C fraction was found. Based on previous study of our group and literature data, this study aims to test the association between polymorphic variants rs4149056, rs11045819 and rs2306283 in *SLCO1B1* and rs4149117 in *SLCO1B3* genes and the occurrence of adverse effect and treatment irregularity, seeking potential molecular markers of the treatment.

Methods

Subjects and study design

This study is a cohort of hypercholesterolemic patients sampled from a cardiovascular clinic of south Brazil, and included those patients with statin prescription (simvastatin or atorvastatin) that kept the medical follow-up for six months or more. The inclusion criteria were the use of simvastatin or atorvastatin and European ascendancy (ascertained by skin color and morphological characteristics). Exclusion

criteria were uncontrolled hypothyroidism or diabetes, abnormal liver or renal function, irregular treatment adhesion (forgetfulness, carelessness or other reasons). Adopting these criteria, the sample consisted of 477 patients of both sexes. The clinician defined the statin and doses, according to patient's clinical characteristics and existing clinical guidelines.

The therapeutic efficacy was measured by percent change in lipid levels after approximately six months of treatment compared to baseline. A standardized statin dosage variable was created to avoid bias in evaluating the efficacy of lipid-lowering therapy. The daily doses of simvastatin were converted to equivalent doses of atorvastatin at a ratio of 2:1 [18]. The subjects included in the study were taking other medications concomitantly with the statins use. Subjects were maintained on other medication schemes throughout the study without change. Furthermore, as recommended by the Brazilian guidelines of dyslipidemia and prevention of atherosclerosis, were dosed creatine phosphokinase (CPK), glutamic oxaloacetic transaminase/ aspartate transaminase (SGOT/AST) and oxaloacetic pyruvate transaminase/ alanine transaminase (SGPT/ALT) during follow-up. Biochemical analyzes were performed by conventional enzymatic methods in blood samples collected with 12-hour fast at two different times - before prescribing the statin and after six months or more of use. The LDL cholesterol levels were calculated according to the Friedewald formula if triglycerides were lower than 4.52mmol/L [5].

Other data (age, sex, other drugs or concomitant diseases and smoking) were obtained from medical records. The monitoring of patients allowed the creation of groups according to the regularity of treatment and changes over time. Patients were considered on "regular treatment" when the treatment was initiated and sustained over time with the same statin and at the same dosage. The remaining patients were divided

into the following groups: 1) dose increase, 2) dose reduction, 3) treatment withdrawal and 4) statin exchange. Additionally, we evaluated the development of adverse effects, considering one or more myalgia simultaneous events to the use of statins, increased CPK levels, abnormal liver function (measured by SGOT and SGPT levels), or myopathy.

Patients with normalized lipid profiles after statin therapy were evaluated according to the following criteria and recommendations of the V Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis for primary prevention of CVD: Total Cholesterol (TC) < 5.18 mmol/L, LDL-C < 2.59 mmol/L, HDL-C>1.55 mmol/L, and Triglicerydes (TG) <1.70 mmol/L [5].

Parts of this sample have already analyzed in previous studies[1,7,11,17] and all subjects who agreed to participate in this study provided informed consent. This study was approved by the Federal University of Health Sciences of Porto Alegre Ethics Committee, under opinion number 075/05 of 2004.

DNA extraction and genotyping

The molecular analyzes were performed for five polymorphisms covering the *SLCO1B1* and *SLCO1B3* genes, namely: rs2306282 (*SLCO1B1* 388A>G), rs11045819 (*SLCO1B1* 463C>A), rs4149056 (*SLCO1B1* 521T>C) and rs4149117 (*SLCO1B3* 334T>G). Genes were chosen for their relevance in drug transport. All polymorphisms are located in exons leading in amino acid changes with change function. These polymorphisms were also the target of several studies, which justifies its validation as biomarkers of statin therapeutic response. For this analysis, genomic DNA was extracted by salting-out process, described by Lahiri and Nurnberger (1991) and the

genotypes determined by TaqMan genotyping assays, using Real Time Technology PCR (Applied Biosystems, California, USA).

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation. TG levels were log-transformed before analyses due to their skewed distribution, although non-transformed values are shown in the Results section. Allele frequencies were estimated by gene counting and the agreement of the genotype frequencies with the Hardy-Weinberg equilibrium was tested using the chi-square test. The mean percentage of change in plasma lipid levels was obtained by taking the difference between pre- and post-treatment lipid levels, multiplying by 100 and then dividing by the pre-treatment level for each parameter. The distribution of genotypes between groups was evaluated by chi-square test, and adjusted residual values [cell-by-cell analyses] were assessed by WINPEPI 2.1, when appropriate. The comparison between existing groups considering the occurrence of treatment failure was carried out through Cox regression, allowing an estimate of the cumulative risk - hazard - to develop the outcome. The variables age (years), sex, statin dose (mg), treatment time (month), and baseline lipid levels (mmol/L) were included as covariates in all analyzes. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS 19.0 for Windows.

Results

Sample description

The sample consists of 477 patients, of whom 67.1% were female, with a mean age 61.9 ± 10.9 years. From all participants, 80.1% were on simvastatin treatment and

19.9% participants were atorvastatin users. The average treatment duration was approximately 16.8 ± 17.0 months and did not differ between simvastatin and atorvastatin users (p=0.083). The average standardized dose of statins was 10.3 ± 4.5mg and was lower between simvastatin users (9.6 ± 3.9mg) than in atorvastatin users (12.9 ± 6.8 mg, p <0.001).

From all patients, 333 have follow-up lipid profile data (average treatment for lipid profile follow-up 6.2 ± 3.2 months). Overall, statin therapy significantly reduced the plasma levels of total cholesterol (-25.6 %, P<0.001), LDL-C (-35.9%, P<0.001) and triglyceride (-13.1%, P<0.001), and increase HDL-C levels (5.9%, P=0.002). Comparing simvastatin and atorvastatin treatment groups, patients on atorvastatin treatment showed higher baseline triglyceride levels than those in the simvastatin group, and also a greater percentage reduction of triglyceride after follow-up (Table 1).

Of the patients, 59.8% showed irregularities in the course of treatment, a fact that appeared closely related to the occurrence of adverse effects, present in 50.5% of patients with irregular treatment (P<0.0001) and with not reach LDL-C goal (53.5 %, P<0.001). The most frequent event in patients with irregularity was the exchange of the prescribed statin (45.6%, Table 2)

Association of polymorphisms with irregular treatment

All genotyped polymorphisms were in agreement with the Hardy-Weinberg Equilibrium. Grouping the patients according treatment regularity in regular statin users (same dose on follow-up) and irregular statin users (patients with dose increase or dose reduction or treatment withdrawal or statin exchange), only rs2306283 (388 A>G, *SLCO1B1*) was associated with treatment irregularity. In a multivariate Cox regression,

the G allele carriers were more likely to develop treatment irregularity (HR=1.50 CI95%=1.05 to 2.13, P=0.024) (Table 3).

Considering that the statin exchange and treatment withdrawal are directly associated with the development of adverse events (Table 2), we carried out a multivariate Cox regression using only statin exchange or treatment withdrawal as the outcome. In this analysis, it was verified that G allele carriers of rs2306283 were more likely to develop adverse side effect (HR=1.55 CI95%=1.03 to 2.33, P=0.036). We also observed the association of these specific events with rs4149056 (521T>C *SLCO1B1*), CC homozygotes showed a higher risk of having statin exchange or treatment withdrawal in the treatment over time (HR=2.16, CI95%=1.04 to 4.44, P=0.037) (Table 3). No found associations with polymorphisms in *SLCO1B3* gene.

Discussion

Genetic testing is being developed to study and validate variants related to pharmacological treatments, but it is still difficult to evaluate the impact of some of these data in clinical outcomes and practice [16]. Additionally, the application of pharmacogenetic in clinical practice might avoid possible damage to individuals with a more vulnerabe genetic profile. It is therefore justified the numerous studies that aim to validate genetic variants as markers of therapeutic response in different populations. In our study, we focused the investigation of two well-studied genes related to statin pharmacogenetics. Our results indicate an association of rs2306283 (*SLCO1B1*) polymorphism with treatment irregularity. We also confirm the association of rs4149056 (*SLCO1B1,* 521T>C) with statin exchange and treatment withdrawal, an indirect measure of adverse drug reaction development.

The *SLCO1B1* and *SLCO1B3* genes encode important carriers in the metabolizing process of drugs that have affinity for statins and are decisive in intra and extracellular concentration of the drug. Their genetic variants have previously been associated with therapeutic response and the induction of myopathy [8,13,16,17,19,20]. In addition, the studied polymorphisms are located in coding regions, being responsible for amino acids changes with a capacity to modify the affinity and transporter function [8,11-13,15,19].

In the present study, carriers of the G allele (rs2306283) had higher risk of irregularity in the treatment (P=0.024) and were more susceptible to statin exchange or treatment withdrawal (P=0.036). Our results are supported by data of OATP1B1 mRNA and protein levels analysis. Nies et al (2013) demonstrated an increased OATP1B1 expression associated with 388A>G variant, and that haplotypes harboring 388A>G but not the functional rs4149056 were associated with myopathy [22].

The rs4149056 polymorphism (*SLCO1B1* - 521T> C) was subject of several studies, however data presents conflicting results, with some able to demonstrate influence of variation on reducing the lipid profile [19,21], while other studies in different samples, did not find such association [11,20,24,25]. Two meta-analyzes, published in 2015, by Dou et al. and Dai et al., analyzed studies with conflicting results and have concluded that there is no significant association for this variant [24,25]. However, both emphasize the possibility that the impact could be influenced by diverse factors: overall small effect on statin treatment, only significant in long-term treatment, subject of differences according to the analyzed drug (simvastatin or atorvastatin) and population. This hypothesis is endorsed by the study of Nagy et al., which demonstrated the existence of significant differences in the frequency of C and T alleles of the variant 521 C> T in the Roman and Hungarian population [26]; this difference may explain the

discrepancy of the results, which are likely the origin of the sample under study [26]. In caucasians the estimated frequency of C allele is about 15%, but varies according to the ancestry of the studied population [13,17,27]. Differing results can also be justified due to statin choice, since associations were found in samples from patients with simvastatin, but not with atorvastatin [28]. Nevertheless, another meta-analysis found an association of the same variant with myopathy induction [29] and Clinical Pharmacogenetics Implementation Consortium has issued a guideline recommending the screening of this variant in myopathy prediction in patients using simvastatin, based on pharmacokinetics and published studies [30]. This justifies and encourages further studies with this polymorphism to elucidate its real relevance in the clinical context. This observation and the literature data are supported by amino acid substitution analysis that predicts functional damage to the protein by mutation, a fact mentioned in other studies [31], and confirms the fact that the carriers of the T allele have a protective effect as regards the irregularities of treatment (P=0.032).

In 2014, Keyser et al. also evaluated the association with the rs4149056 variant (*SLCO1B1* - 521T>C) and found an association with the same therapeutic response with induction to myopathy and also the need to exchange or reduction of the initial statin dose prescribed to patients. In our study, only the relationship with irregularities of the treatment was observed, but the study Keyser et al has great importance because it is the basis of the approach used considering the irregular treatment [32]. In this study,, only the exchange of drug and dose reduction were considered, however, during the review of the medical records of the sample, it was checked for other fault profiles, such as increasing the dose and discontinuation of treatment, and these variables were also included in the study.

This study showed that polymorphisms in genes directly involved with the transport mechanism of statins could determine the presence or absence of adverse effects and irregularities in the course of treatment. In previous study of our research group it was found an association with the variant 388A>G and modification of the lipid profile, and in the present studywe observed a relation with the presence of irregular treatment. These data were similar to the variant 521T> C (rs4149056), both found in *SLCO1B1* gene [17].

This study has some limitations, because it is an observational study, and due to the small sample size, especially for some genotypes, what could affect the visualization of known associations. However, it brings a different statistical approach, which does not exclude irregular patients, as most studies made this because we think it is important to see the genetic profile behind those patients who do not adapt to the treatment. These data are important for the validation of these variants in the population and proposes the use of a broader approach in pharmacogenetic studies, including patients with stroke treated differently than expected.

Conflict of interest

The authors state that there is no conflict of interest in this study.

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Table 1. Sample description according to this used statin.

Variable	Overall	Simvastatin	Atorvastatin	P
Total sample				
n (%)	477	382 (80.1)	95 (19.9)	
Sex, female (%)	320 (67.1)	264 (69.1)	56 (58.9)	0.067
Age (years)	61.9 ± 10.9	61.5 ± 10.6	63.4 ± 12.1	0.129
Follow-up (months)	16.8 ± 17.0	16.1 ± 15.9	19.5 ± 21.3	0.083
Dose (mg)	17.9 ± 7.6	19.2 ± 7.8	12.9 ± 6.8	<0.0001
Standard dose (mg)	10.3 ± 4.5	9.6 ± 3.9	12.9 ± 6.8	<0.0001
Prior CVD (%)	150 (31.4)	94 (24.6)	56 (58.9)	<0.0001
Hypertension (%)	319 (66.9)	288 (75.4)	31 (32.6)	<0.0001
Patients with follow-up lipid levels	333	268	65	
Baseline lipid levels (mmol/L)				
TC	6.33 ± 1.01	6.32 ± 1.03	6.38 ± 0.94	0.669
HDL-C	1.32 ± 0.34	1.32 ± 0.35	1.34 ± 0.31	0.673
LDL-C	4.18 ± 0.92	4.20 ± 0.92	4.11 ± 0.90	0.478
TG	1.79 ± 0.92	1.73 ± 0.86	2.02 ± 1.15	0.023
Follow-up lipid levels (mmol/L)				
TC	4.66 ± 0.99	4.66 ± 0.89	4.64 ± 1.41	0.886
HDL-C	1.37 ± 0.36	1.36 ± 0.34	1.41 ± 0.46	0.324
LDL-C	2.63 ± 0.75	2.64 ± 0.78	2.61 ± 0.64	0.774

TG	1.43 ± 0.64	1.43 ± 0.63	1.41 ± 0.66	0.820
Lipid levels change (%)				
TC	-25.6 ± 12.9	-25.6 ± 13.0	-25.6 ± 12.6	1.000
HDL-C	5.9 ± 22.6	5.5 ± 21.0	7.5 ± 29.0	0.525
LDL-C	-35.9 ± 18.9	-35.9 ± 19.1	-35.8 ± 18.4	0.969
TG	-13.11 ± 31.3	-10.8 ± 33.4	-22.4 ± 22.8	0.008

Table 2. Distribution of the patients according to the type of treatment and association with development of adverse effect and scope of LDL-C goal (mmol/L).

	Total	Adverse effect ocurrence		LDL-C goal (mmol/L)	
		No	Yes	No	Yes
Regular treamtent	190 (40.2%)	171 (90%)	19 (10%)	43 (29.1%)	105 (70.9%)
Irregular treatment	283 (59.8%)	140 (49.5%)	143 (50.5%)	108 (53.5%)	94 (46.5%)
P		<0.0001		<0.001	
Irregular treatment [a]					
Statin exchange	129 (45.6%)	52 (37.1%)	77 (53.8%)[b]	27 (40.3%)	60 (44.7%)
Dose reduction	66 (23.3%)	37 (26.4%)	29 (20.3%)[c]	13 (19.4%)	31 (23.1%)
Dose increase	48 (17.0%)	44 (31.4%)	4 (2.8%)[d]	13 (19.4%)	20 (14.9%)
Treatment withdrawal	40 (14.1%)	7 (5.0%)	33 (23.1%)[e]	14 (20.9%)	23 (17.3%)
P		<0.0001		0.708	

Data presented as n (%). n and% calculated on the total of subjects with treatment irregularity.[a] analysis performed with irregular patients [b]residue +2.82. P=0.005; [c]residue -1.22. P=0.221; [d] residue -6.42. P<0.0001; [e] residue +4.36. P<0.0001.

Table 3. Relationship between studied polymorphisms and occurrence of irregularity in the treatment obtained by Cox regression.

	Irregular treatment			Statin exchange and treatment withdrawal		
	Adjusted Hazard	CI95%	P	Adjusted Hazard	CI95%	P
SLCO1B1 388A>G (rs2306283)						
AA	1.00	-	-	1.00	-	-
AG +GG	1.50	1.05 – 2.13	0.024	1.55	1.03 - 2.33	0.036
SLCO1B1 521 T>C (rs4149056)						
CT+TT	1.00	-	-	1.00	-	-
CC	1.51	0.74-3.08	0.260	2.16	1.04 - 4.44	0.037
SLCO1B1 463C>A (rs11045819)						
AA	1.00	-	-	1.00	-	-
AC+CC	1.32	0.49 – 3.58	0.586	1.33	0.42 – 4.23	0.160
SLCO1B3 334 T>G (rs4149117)						
GG	1.00	-	-	1.00	-	-
GT+TT	1.29	0.92 – 1.80	0.139	1.34	0.89 – 2.01	0.119

4 ARTIGO CIENTÍFICO REDIGIDO EM INGLÊS 2

Association between *NR1I2* (PXR) polymorphisms and lipid-lowering therapy response

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Association between NR1I2 (PXR) polymorphisms and lipid-lowering therapy response

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Abstract: NR1I2 encodes the nuclear receptor PXR (pregnane X receptor), a transcription factor that regulates the expression of various cell pathways, and inducer of CYP3A, the major route of metabolism of statins. This study was composed by a cohort of 477 dyslipidemic patients on statin treatment. Statin effectiveness, which was evaluated according to the therapeutic response - measured by changes in the lipid profile - and the presence of treatment irregularity, was compared between different *NR1I2* genotypes (rs3814055, rs2461817, rs7643645, rs6785049 and rs3814057). When comparing the percentage of changes of Cholesterol Total (TC), HDL-C, LDL-C and Triglycerides (TG), after correcting for multiple comparisons, rs3814055 and rs3804057 were associated with total cholesterol and LDL-cholesterol changes. Additionally, the rs3814057 was associated with a variable reduction of triglyceride levels. Grouping the patients according to treatment regularity in regular statin users (same dose on follow-up) and irregular statin users (patients with dose increase or dose reduction or treatment withdrawal or statin exchange), no association was found between the investigated polymorphisms. These data indicate the possible importance of individual knowledge of genetics in the medical prescription, and the influence of *NR1I2* genetic variability on statin effectiveness.

Introduction

The *NR1I2* gene, located on chromosome 3q13.33, encodes the PXR receptor (pregnane X receptor), a nuclear receptor family (NR) protein that acts as an important regulator of defense of the human body system against xenobiotics, expressed intensely in the intestine and liver[1,2]. The PXR is activated by various endogenous or exogenous chemical molecules, which are potentially harmful to the body, such as bile salts, hormones and many drugs[3,4,5].

The expression of PXR and its activation are closely correlated to the gene expression of the cytochrome P450 enzymes family, responsible for detoxification of the body[3]. The PXR receptor regulates the three stages of metabolism - Phase I, II and III - including phase I enzymes [cytochrome P450 (CYP) 2B6, CYP2B9, CYP2C9, CYP3A4 and CYP3A7), phase II [glutathione-S-transferase (GST), UDP-glucoronosyltransferases (UGTs) and sulfotransferases (SUTs)] and some transporters, as MDR1 (multidrug resistance protein 1) and OATP (organic anion transporter protein)[5,6].

Due to its function within the cells, PXR receptor is recognized as an interindividual variation factor related to responses to drugs, with potential influence over the development of some diseases and adverse effects[7]. Because statins are metabolized by cytochrome P450 enzymes[8] and transported both by MDR1 and OATPs carriers[9,10] is plausible to infer that the PXR expression and genetic variability may interfere with the response to treatment with this class of drugs, as well as be associated with cases of treatment failure and adverse effects.

Several polymorphisms have already been described in the *NR1I2* gene, some of them can alter its function, as well as CYPs, MDR and OATPS expression and therefore alter different pharmacokinetics pathways[11]. Therefore, the present study aims to evaluate the association of polymorphisms in the gene *NR1I2* (rs3814055, rs2461817, rs7643645, rs6785049 and rs3814057) with the effectiveness and the irregularity of treatment with statins in a Brazilian cohort.

Material and Methods

Subjects and study design

This study was composed by a cohort of hypercholesterolemic patients sampled (n=643) from a cardiovascular clinic of South of Brazil, and included those patients with statin prescription that kept the medical follow-up. The inclusion criteria were the use of simvastatin or atorvastatin and European ascendancy (ascertained by skin color and morphological characteristics). Exclusion criteria were uncontrolled hypothyroidism or diabetes, abnormal liver or renal function, irregular treatment due forgetfulness or carelessness). Adopting these criteria, the final sample consisted of 477 patients of both sexes. The clinician defined the statin and doses, according to patient's clinical characteristics and existing clinical guidelines.

The therapeutic efficacy was measured by percent change in lipid levels after approximately six months of treatment compared to baseline for 445 that completed follow-up. For the evaluation of pre and post treatment lipid follow-up data, the lipid profile was measured prior to exchange or suspension of

treatment. A standardized statin dosage variable was created to avoid bias in evaluating the efficacy of lipid-lowering therapy. The daily doses of simvastatin were converted to equivalent doses of atorvastatin at a ratio of 2:1[12]. The subjects included in the study were taking other medications concomitantly with the statins use. Subjects were maintained on other medication schemes throughout the study without change. Furthermore, as recommended by the Brazilian Guidelines of Dyslipidemia and Prevention of Atherosclerosis, were dosed creatine phosphokinase (CPK), glutamic oxaloacetic transaminase / aspartate transaminase (SGOT/AST) and oxaloacetic pyruvate transaminase / alanine transaminase (SGPT/ALT) during follow-up. Biochemical analyzes were performed by conventional enzymatic methods in blood samples collected with 12-hour fast at two different times - before prescribing the statin and on follow-up. The LDL-cholesterol levels were calculated according to the Friedewald formula if triglycerides were lower than 4.52 mmol/L[13].

Other data (age, sex, other drugs or concomitant diseases and smoking) were obtained from medical records. The monitoring of all 477 patients allowed the creation of groups according to the regularity of treatment and changes over time. Patients were considered on “regular treatment” when the treatment was initiated and sustained over time with the same statin and at the same dosage. The remaining patients were divided into the following groups: 1) dose increase, 2) dose reduction, 3) treatment withdrawal and 4) statin exchange. Additionally, we evaluated the development of adverse effects, considering one or more episodes of myalgia simultaneously to the use of statins, increased CPK levels, abnormal liver function (measured by SGOT and SGPT levels), or myopathy.

Patients with normalized lipid profiles after statin therapy were evaluated according to the following criteria and recommendations of the V Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis [31] for primary prevention of cardiovascular disease (CVD): total cholesterol (TC) < 5.18 mmol/L, LDL-C < 2.59 mmol/L, HDL-C > 1.55 mmol/L, and triglycerides (TG) < 1.70 mmol/L.

All subjects who agreed to participate in this study provided informed consent. This study was approved by the Federal University of Health Sciences of Porto Alegre Ethics Committee. Parts of this sample have already analyzed in previous studies[5,14,15,16,17,18].

DNA extraction and genotyping

The molecular analyzes were performed for five polymorphisms covering the *PXR* gene, rs3814055 (Chr.3: 119781188C>T, 5'UTR), rs2461817 (Chr.3: 119805804A>C, 2nd intron), rs7643645 (Chr.3: 119806650A>G, 2nd intron), rs6785049 (Chr.3: 119814886G>A, 6th intron) and rs3814057 (Chr.3:119818407A>C, 3'UTR). For these analyses, genomic DNA was extracted by salting-out process, described by Lahiri and Nurnberger (1991) and the genotypes determined by TaqMan genotyping assays, using Real Time Technology PCR (Applied Biosystems, California, USA). Approximately 10% of the samples were double analyzed for all SNPs, aiming better quality control.

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation. TG levels were log-transformed before analyses due to their skewed

distribution, although non-transformed values are shown in the Results section. Allele frequencies were estimated by gene counting and the agreement of the genotype frequencies with the Hardy-Weinberg equilibrium was tested using the chi-square test. The mean percentage of change in plasma lipid levels was obtained by taking the difference between pre- and post-treatment lipid levels, multiplying by 100 and then dividing by the pre-treatment level for each parameter. To determine the association between genotypes and mean percentage of change in plasma lipid levels, the means for each variable were compared with a General Linear Model using the type III sums of squares. Age (years), sex, smoking status, standardized statin dosage (mg), treatment period (months), and baseline lipid levels (mmol/L) were included in each model as covariates for lipid-lowering response (% of change in plasma lipid levels). The distribution of genotypes between groups was evaluated by chi-square test, and adjusted residual values [cell-by-cell analyses] were assessed by WINPEPI 2.1, when appropriate. The comparison between existing groups considering the occurrence of treatment irregularity was carried out through Cox regression, allowing an estimate of the cumulative risk - hazard - to develop the outcome. The variables age (years), sex, statin dose (mg), treatment time (month), and baseline lipid levels (mmol/L) were included as covariates in all analyzes. A p-value of <0.05 was considered statistically significant. False discovery rate (FDR) procedure was used to adjust p-values for multiple comparisons. Statistical analysis was performed using SPSS 19.0 for Windows.

Results

Sample description

In the study sample, there was a predominance of women, totaling 67.4% of the sample, and the age range is quite large - 25 to 88 years - however, the average age was 62 ± 10.9 years. The vast majority (76.5%) of the patients used simvastatin doses ranging from 5 to 40 mg/day. The average treatment duration was approximately 6.2 ± 3.2 months and the average of standard dose of statins was 11.1 ± 5.6 mg. The standard dose of statins did not differ between simvastatin users (10.2 ± 4.45 mg) and atorvastatin users (11.0 ± 5.7 mg) (p=0.125). We verified in 60.2% of patients some kind of treatment irregularity (exchange statin, reduction or increase of dose or withdrawal of treatment) and only 50% reached the desirable lipid levels (measured by LDL-C levels). The target of LDL-C was used as a variable to define effectiveness of treatment, established as 2.586 mmol/L (100 mg/dL) for all patients, except for patients with a history of cardiovascular disease and diabetes, and for these the ideal goal was of 1.810 mmol/L (70 mg/dL) (Table 1).

NR1I2 polymorphisms, linkage disequilibrium and haplotype frequencies

NR1I2 is about 38kb in length, and five SNPs, including rs3814055 (located in 5'UTR), rs2461817, rs7643645 and rs6785049 (located in intronic regions) and rs3814057 located in 3'UTR region of *NR1I2,* were genotyped. The minor allele frequencies for the investigated polymorphisms were 0.38 (rs3814055), 0.39 (rs2461817), 0.34 (rs7643645), 0.42 (rs6785049) and 0.22 (rs3814057). The current study showed similar Minor Allele Frequency (MAF) to Europeans according to the 1000 genomes data. The genotype frequencies did not show statistically significant differences compared with those expected

under the Hardy–Weinberg equilibrium (p>0.05 for all SNPs). Only rs6785049 and rs3814057 SNPs were in linkage disequilibrium (D'=0.98), but the determination coefficient was low (r^2=0.34). Three haplotypes were derived according to the linkage disequilibrium structure between rs6785049-rs3814057: AA (55.7%), GA (22.2%) and GC (22.1%), respectively.

Association between NR1I2 polymorphisms and statin efficacy

Table 2 shows the mean change in percentage on lipid levels concentration in all patients investigated for lipid-lowering response after simvastatin/atorvastatin treatment, according to *NR1I2* genotypes. When comparing percentage of changes of TC, HDL-C, LDL-C and TG, after correcting for multiple comparisons, rs3814055 and rs6785049 were associated with total cholesterol and LDL-cholesterol changes. For rs3814055, CC and CT carriers presented an increased response than TT homozygotes for total cholesterol (CC: -25.1% ± 13.0%; CT: -27.2% ± 11.4% and TT: -21.3% ± 12.7, corrected p-value=0.024) and for LDL-cholesterol levels (CC: -35.5% ± 17.9%; CT: -37.9% ± 16.7% and TT: -27.9% ± 19.0, corrected p-value=0.004). For rs6785049, homozygous AA showed a more effective reduction than GG or GA genotypes of total cholesterol (AA: -27.3% ± 11.4%, GA: -24.7% ± 13.3%, GG: -25.2% ± 12.6%, corrected p-value=0.028) and LDL-cholesterol (AA: -38.7% ± 15.5%, GA: -35.0% ± 18.4%, GG: -34.1% ± 20.9%, corrected p-value=0.028). Additionally, the rs3814057 was associated with a variable reduction of triglyceride levels, with CC homozygotes showing an increased response (corrected p-value=0.044). The association of *NR1I2* with baseline and follow-up lipid and lipoprotein levels is shown in Supplementary Table 1.

Association of polymorphisms with irregular treatment

Grouping the patients according to treatment regularity in regular statin users (same dose on follow-up) and irregular statin users (patients with dose increase or dose reduction or treatment withdrawal or statin exchange), no association was found among the investigated polymorphisms (Table 3).

Functionality prediction analysis

We also performed a functionality prediction analysis through SNPinfo tool and rs3814055 and rs3814057 were predicted as functional. The rs3814055 was predicted as a potential transcription factor binding site (TFBS). It is possible that changes in the ligation of several transcription factors, such as *GATA4* (GATA binding protein 4), *HAND1* (heart and neural crest derivatives expressed 1), *HNF4A* (hepatocyte nuclear factor 4 alpha), *PPARA/G* (peroxisome proliferator activated receptor alpha and gama), and SREBF1 (sterol regulatory element binding transcription factor 1), related to cholesterol and drugs metabolism and diseases such as atherosclerosis. The rs3814057, located in the 3'UTR was predicted as potential microRNA binding site for several described miRNAs (hsa-miR-1244, hsa-miR-1323, hsa-miR-384, hsa-miR-548a-3p, hsa-miR-548o and hsa-miR-221).

Discussion

The present study sought associations between the effectiveness of statin therapy, as measured by altered lipid profile, and polymorphisms in the *NR1I2* gene, which encodes the nuclear PXR receptor. These same variants

were also tested with treatment irregularity development. In addition, we used bioinformatics tools to perform a functional prediction analysis to describe the impact of these variants on the action of the normal protein. This approach allowed us to find associations of the polymorphic variants with clinical aspects of the treatment with statins. We found an association of rs3814055 and rs6785049 with reduction of the total cholesterol and LDL-cholesterol and the variant rs3814057 with reduction of triglycerides. These same variants were not associated with irregularity of treatment, defined as the change of statin, dose adjustment or treatment suspension. In addition, the variant rs3814055 may be associated with the modification of binding of various transcription factors to the *NR1I2* gene, some involved in cardiac system development, and drug metabolism; and variant the rs3814057 demonstrates association with the modification of the binding of micro RNAs to the gene.

The associations between *NR1I2* polymorphisms and changes in the lipid profile can be explained by the potent induction of cytochrome P450 enzymes produced by PXR, especially on CYP3A4, and this is a widely studied association[1,19,20]. The *CYP3A* gene has a basal expression, which can be modulated or induced by xenobiotics. It was observed that PXR has the capacity to bind to the promoter region of the gene amplifying the expression of *CYP3A4*[21]. This action also depends on a balance of nuclear receptors and other chemical mediators, which showed that the PXR activation action can be balanced by inhibitory action of the type CAR proteins, which also complexed to RXR and interact with the promoter CYP3A. PXR is also involved in the regulation of drug transporters, such as ABC family (specially MDR1) and SLCO family, all carriers that have high affinity for statins[1]. It was observed that

PXR activation decreases the function of ABC type transporters in hepatocytes and that drugs that activate PXR could cause dyslipidemias[1,22]. Therefore, the understanding of cytochrome P450 and drug carriers modulation by PXR is key to understand the metabolism and transport of drugs[2,21].

Besides acting regulating statin metabolism and transport, the *NR1I2* gene also acts on cholesterol metabolism and can regulate the expression of HMG-CoA reductase, enzyme responsible for endogenous cholesterol synthesis and the main target of statins[22]. Hoffart et al. (2011) showed the effect of atorvastatin on PXR, stating that hypolipidemic metabolites are capable of increasing the expression of the nuclear receptor and consequently CYP3A[23]. Korhonova et al. (2015) showed that not only atorvastatin, but also optical isomers of fluvastatin and rosuvastatin interfere with the expression of PXR, which is directly related to the effectiveness of the treatment and also with the occurrence of adverse effects[24].

Lamba et al. (2008) identified about 90 polymorphisms in the promoter and intron 1 of *NR1I2* gene and showed that the majority were associated with expression of inducible and constitutive system CYP3A4[25]. According to Lamba et al. (2008), the rs3814055 polymorphism is associated with the regulation of inflammatory proteins, rs2461817 is associated with the expression of DR3 and EBP and the rs7643645 polymorphism with the expression of HNF4, where the carrier of the G allele loses the transcription control site of that protein[25]. In our study, through computational prediction made by Snpinfo.org tool, rs3814055 is a transcription factors binding site (TFBS) and, therefore, could affect the transcription of *NR1I2*. In this analysis was also found the rs3814057 as potential miRNA binding site, possibly affecting *NR1I2* mRNA stability.

The study presents limitations inherent to its design, because it is an observational study based on information obtained from medical records. Inadequate filling of medical records leads to data loss, and, therefore, exclusion of some patients. In addition, we should consider the limited sample size and the limitations of the used technique, which uses the candidate gene approach, investigating only a limited number of variants previously selected.

The knowledge of new genes that influence the metabolism of statins is very important for the pharmacogenetic study of these drugs. Nuclear receptors have great importance in the control of gene expression, and therefore indirectly influence several protein routes. The PXR receptor is capable of altering the expression of cytochrome P450 enzymes and therefore influences the metabolism of statins. With this study, we could show that polymorphisms in the *NR1I2* gene seemd to be important variables in the final effectiveness of the treatment with statins, being able to be related to the reduction of the lipid profile. Currently, there are several bioinformatics tools that allow us to further explore the gene sequences and their variations, simulating the effect of these polymorphisms on the final protein and its function, elucidating the mechanisms by which they cause clinical changes. Further studies in the field of pharmacogenetics of statins with different populations and polymorphisms are very important for the validation of these variants and the demonstration of their correlation with the clinic.

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Conflict of interest

The authors state that there is no conflict of interest in this study.

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Table 1. Patient characteristics

Characteristics	
Number	477
Age (years)	62.0 ± 10.9
Sex, male (%)	67.4
Statin Use (%)	
Simvastatin	83.4
Atorvastatin	16.6
Treatment time (months)	6.2 ± 3.2
Overall statin standard dose (mg)*	10.5 ± 4.62
Simvastatin standard dose (mg)	10.2 ± 4.45
Atorvastatin dose (mg)	11.1 ± 5.60
Smoking (%)	
Past	14.4
Current	7.1
Never	78.5
Prior CVD (%)	33.5
CVD Family history (%)	19.4
Diabetes (%)	16.6
Hypertension (%)	73.9
Concomitant therapy use (%)	
Calcium channel blockers	17.5
Diuretics	42.6
Antithrombotic	23.5
ACE inhibitor	24.3
β-blocker	31.6
LDL-C (mmol/L) goal achievement (%)	50

Irregularity on statin treatment (%)	60.2
CYP3A4 substrates	17.0
CYP3A4 inducers	2.3
CYP3A4 inhibitors	21.0

Values for age, treatment duration and statin standard dose were expressed as mean ± standard deviation; CVD, Cardiovascular disease; ACE, angiotensin-converting enzyme. *Simvastatin dose was transformed to equivalent doses of atorvastatin at a ratio of 2:1.

Table 2. Mean percentage change of lipid and lipoprotein levels after statin treatment according to the *NRI12* genotypes.

	n	TC	LDL-C	HDL-C	TG
rs3814055	414				
CC	155	-25.1 ± 13.0	-35.5 ± 17.9	2.2 ± 18.9	-12.3 ± 30.7
CT	207	-27.2 ± 11.4	-37.9 ± 16.7	4.6 ± 24.7	-11.4 ± 33.3
TT	52	-21.3 ± 12.7	-27.9 ± 19.0	4.2 ± 22.3	-9.0 ± 39.6
P		0.012	0.001	0.435	0.703
P^c		0.024	0.004	0.580	0.703
rs2461817	442				
AA	68	-24.6 ± 12.6	-34.5 ± 18.6	2.6 ± 18.6	-14.6 ± 32.4
AC	212	-26.4 ± 12.9	-37.4 ± 16.6	1.0 ± 20.2	-7.6 ± 36.2
CC	162	-25.4 ± 12.2	-35.3 ± 19.7	7.6 ± 25.1	-15.2 ± 28.7
P		0.598	0.605	0.017	0.135
P^c		0.797	0.605	0.068	0.270
rs7643645	444				
AA	198	-25.1 ± 12.6	-33.8 ± 18.5	4.4 ± 21.4	-13.2 ± 32.6
AT	187	-26.3 ± 12.7	-38.6 ± 17.6	3.9 ± 23.5	-9.7 ± 33.5
TT	59	-26.6 ± 12.2	-36.7 ± 16.6	1.4 ± 19.7	-12.8 ± 32.3
P		0.610	0.026	0.852	0.561
P^c		0.813	0.104	0.852	1.000
rs6785049	440				
GG	84	-25.2 ± 12.6	-34.1 ± 20.9	4.4 ± 24.1	-12.7 ± 33.1
GA	205	-24.7 ± 13.34	-35.0 ± 18.4	4.4 ± 20.3	-11.6 ± 33.4
AA	151	-27.3 ± 11.4	-38.7 ± 15.5	2.6 ± 23.4	-10.6 ± 32.6

P		0.014	0.007	0.812	0.939
P^c		0.028	0.028	1.000	0.939
rs3814057	445				
AA	270	-26.6 ± 11.9	-37.8 ± 16.2	4.0 ± 21.3	-12.8 ± 32.6
AC	156	-24.1 ± 13.1	-34.4 ± 19.0	2.9 ± 22.0	-7.4 ± 33.4
CC	19	-25.7 ± 16.8	-27.5 ± 28.7	8.9 ± 31.9	-28.6 ± 26.8
P		0.220	0.081	0.665	0.011
P^c		0.293	0.162	0.665	0.044

Covariates included in the model: Age, gender, standardized statin dosage (mg), treatment period (months), baseline lipid levels (mmol/L). Values are expressed as mean ± SD; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides. P^c: multiple comparisons corrected P-value

Table 3. Relationship between studied polymorphisms and occurrence of irregularity in the treatment obtained by Cox regression.

	Irregular treatment			Statin exchange and Treatment withdrawal		
	Adjusted Hazard	CI95%	P	Adjusted Hazard	CI95%	P
rs3814055						
CC	1.00	-	-	1.00	-	-
CT	0.99	0.69-1.42	0.958	0.93	0.64-1.35	0.704
TT	1.54	0.93-2.54	0.094	1.19	0.53-2.66	0.674
rs2461817						
AA	1.00	-	-	1.00	-	-
AC	0.79	0.50-1.24	0.308	0.75	0.45-1.27	0.287
CC	0.78	0.49-1.24	0.779	0.73	0.43-1.25	0.251
rs7643645						
AA	1.00	-	-	1.00	-	-
AG	1.05	0.75-1.47	0.767	1.08	0.75-1.58	0.670
GG	1.05	0.62-1.78	0.864	0.95	0.49-1.82	0.884
rs6784049						
GG	1.00	-	-	1.00	-	-
GA	1.07	0.71-1.61	0.737	0.92	0.58-1.45	0.711
AA	1.01	0.65-1.58	0.962	0.92	0.56-1.51	0.742
rs3814057						
AA	1.00	-	-	1.00	-	-
AC	1.05	0.76-1.45	0.770	0.93	0.64-1.35	0.704
CC	0.89	0.41-1.96	0.777	1.19	0.53-2.66	0.674

Supplementary table 1. Baseline and follow-up of lipid levels according to the *NR1I2* genotypes

	Baseline lipid levels (mmol/L)				Follow-up lipid levels (mmol/L)			
rs3814055	TC	HDL-C	LDL-C	TG	TC	HDL-C	LDL-C	TG
CC	6.35 ±1.03	1.33 ± 0.32	4.21 ± 0.91	1.77 ± 0.89	4.62 ± 0.86	1.31 ± 0.31	2.66 ± 0.70	1.40 ±0.60
CT	6.52 ± 1.03	1.34 ± 0.36	4.33 ± 0.95	1.82 ± 0.94	4.68 ± 0.82	1.36 ± 0.39	2.64 ± 0.71	1.47 ± 0.69
TT	6.45 ± 1.14	1.26 ± 0.28	4.13 ± 1.03	1.93 ± 0.89	4.94 ± 0.83	1.29 ± 0.34	2.96 ± 0.72	1.54 ± 0.63
p	0.255	0.263	0.417	0.478	0.055	0.212	0.017	0.403
rs2461817								
AA	6.48 ±0.96	1.33 ± 0.28	4.31 ± 0.88	1.83 ± 0.77	4.76 ± 0.87	1.32 ± 0.30	2.73 ± 0.68	1.49 ± 0.66
AC	6.48 ± 1.08	1.32 ± 0.35	4.34 ± 0.96	1.78 ± 0.89	4.65 ± 0.88	1.32 ± 0.35	2.66 ± 0.73	1.47 ± 0.67
CC	6.42 ± 1.03	1.33 ± 0.34	4.17 ± 0.94	1.81 ± 0.99	4.64 ± 0.83	1.37 ± 0.39	2.66 ± 0.73	1.37 ± 0.59
P	0.238	0.996	0.159	0.805	0.571	0.332	0.753	0.255
rs7643645								
AA	6.42 ± 1.07	1.31 ± 0.32	4.28 ± 0.99	1.82 ± 0.93	4.72 ± 0.90	1.32 ± 0.31	2.74 ± 0.77	1.43 ± 0.64
AG	6.45 ± 1.00	1.33 ± 0.35	4.29 ± 0.90	1.80 ± 0.93	4.62 ± 0.82	1.36 ± 0.41	2.61 ± 0.72	1.46 ± 0.66
GG	6.36 ±1.05	1.37 ± 0.31	4.19 ± 0.91	1.76 ± 0.72	4.63 ± 0.86	1.35 ± 0.33	2.60 ± 0.68	1.47 ± 0.61
P	0.822	0.468	0.768	0.988	0.510	0.483	0.165	0.795
rs6785049								
GG	6.42 ± 1.03	1.30 ± 0.32	4.24 ± 0.99	1.87 ± 0.95	4.66 ± 0.88	1.29 ± 0.30	2.66 ± 0.74	1.50 ± 0.69
GA	6.52 ± 0.97	1.32 ± 0.32	4.38 ± 0.89	1.82 ± 0.96	4.79 ± 0.87	1.35 ± 0.33	2.79 ± 0.75	1.41 ± 0.61
AA	6.32 ± 1.11	1.35 ± 0.36	4.16 ± 0.95	1.74 ± 0.79	4.67 ± 0.85	1.35 ± 0.41	2.50 ± 0.68	1.46 ± 0.66
P	0.160	0.379	0.071	0.435	0.006	0.441	0.001	0.821
rs3814057								
AA	6.47 ± 1.02	1.33 ± 0.33	4.31 ± 0.90	1.80 ± 0.83	4.65 ± 0.83	1.35 ± 0.37	2.65 ± 0.72	1.44 ± 0.63

AC	6.38 ± 1.05	1.33 ± 0.34	4.24 ± 0.97	1.77 ± 0.92	4.71 ± 0.88	1.33 ± 0.35	2.70 ± 0.77	1.47 ± 0.68
CC	6.24 ± 0.94	1.27 ± 0.31	4.00 ± 1.07	1.99 ± 1.53	4.50 ± 1.04	1.29 ± 0.22	2.64 ± 0.81	1.18 ± 0.47
P	0.451	0.701	0.281	0.793	0.518	0.750	0.811	0.186

Values were expressed as mean ± SD; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides.

CONSIDERAÇÕES FINAIS

O presente estudo faz parte de um projeto maior intitulado **"Estudo da influência da variabilidade genética em fatores de risco cardiovascular e na farmacogenética de hipolipemiantes",** aprovado pelo Comitê de Ética em Pesquisa (CEP) da UFCSPA (Parecer N° 075/05). Deste projeto maior estão sendo desenvolvidos diversos outros trabalhos e projetos, incluindo essa dissertação de mestrado.

Em paralelo à realização do projeto de mestrado, foi conduzida uma pesquisa de variantes polimórficas em uma amostra de pacientes HIV+. O objetivo dessa pesquisa foi relacionar variantes polimórficas em *SEP15* e *NR1I2* com o tempo de progressão à AIDS. Apesar da pesquisa não abordar o mesmo tema do estudo apresentado, trazemos em anexo (anexo 3) as publicações relacionadas, já que as mesmas também se referem a variantes polimórficas e utilizam as mesmas técnicas de análise.

O aprimoramento do conhecimento genético sobre variantes polimórficas comuns permite uma melhor compreensão dos mecanismos de variação de resposta e da ocorrência de efeitos adversos em pacientes, bem como também dos fatores prognósticos, e efetividade da intervenção, o que pode contribuir para uma prescrição médica melhor orientada e melhores resultados para o paciente.

ANEXOS

Anexo 1: Instruções para submissão revista à British Journal Of Clinical Pharmacology

Instructions to Authors: British Journal of Clinical Pharmacology (BJCP)

Papers will be considered for publication if they are relevant to any aspect of drug action in humans. Submission of a manuscript to BJCP will be taken to indicate that

the content of the manuscript is original and that it has not been published or accepted for publication, either in whole or in part, other than as short abstracts, communications or conference proceedings;
no part of the manuscript is currently under consideration for publication elsewhere;
all authors have seen and approved the final version of the submitted paper;
authors have, if necessary, obtained permission to publish from their employers or institutions;
approvals are held from any persons acknowledged, or cited as having provided personal communication; and
permission has been obtained to use any copyrighted material, such as reproducing a figure from another article, in print and electronic forms, and that the source of the material has been acknowledged.
BJCP publishes papers of various kinds including Original Research Articles, Methods in Clinical Pharmacology, Reviews (including Systematic Reviews), Commentaries, Opinion, Education papers and Letters to the Editor.

Original research articles are grouped under headings, including Clinical trials, Drugs in Pregnancy and Lactation, Drug Interactions, Drug Metabolism, Drug Safety, Human Toxicology,Methods in Clinical Pharmacology, New Drug Mechanism, Paediatric Clinical Pharmacology, Pharmacodynamics (PD), Pharmacoeconomics, Pharmacoepidemiology, Pharmacogenetics, Pharmacokinetics (PK), PK-PD relationships, Therapeutics, and Translational Research. Authors are invited to suggest which heading they feel their manuscript would best fit, and may suggest additional headings if they so wish.

Investigations of human subjects will usually be expected to have been conducted in accordance with good clinical practice, which defines investigator responsibilities, including, among others, qualifications, resources, providing medical care to trial participants, communication with the research ethics committee, protocol, informed consent, data integrity and reporting (http://ichgcp.net/4-investigator). Where this is the case we would expect the principal investigator to be an author.

Tips for authors on how to navigate the peer review process can be found here.

PREPARING AND SUBMITTING A MANUSCRIPT
Once you have read these guidelines in full, please go here to submit your manuscript.

You will need to submit three documents:
a. manuscript;
b. covering letter that includes specific statements; and

c. a competing interests statement formatted as per our guidelines.

If your paper is accepted, you will subsequently be required to submit both a table of key links (targets and ligands) to the BPH/IUPHAR Guide to Pharmacology, and a copyright transfer agreement form signed by the corresponding author. You will be taken to the Wiley Author Licensing Service to complete this, and full guidance is given there. Please see more below.

Papers must be written in clear, concise UK English. Avoid jargon and neologisms.

Regrettably, the Journal is unable to undertake major corrections to language, which is the responsibility of the author. Because there should be no barriers to getting your research published, however, manuscripts are often returned for English language and formatting issues. Thus, a pre-acceptance Editing Service (comprising English language editing, translation services, manuscript formatting and preparation) is available and can provide you, the author, with expert help to ensure your manuscript is ready for submission. Japanese authors can also find a list of local English improvement services at http://www.wiley.co.jp/journals/editcontribute.html . All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

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Manuscript documents should be typed in double spacing and should be page numbered. Line numbers must also be added to your manuscript before submission.

A separate title page should be included (see below). The submitting author need not be the same as the corresponding author.

Authors must have contributed directly to the paper submitted, according to ICMJE guidelines on authorship. For a description of the roles and responsibilities of authors and contributors see here.

Authors may find this resource useful.

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Authors should ensure that they have provided the following information, when appropriate:

A cover letter detailing originality of the manuscript and agreement of all authors and also identifying the Principal Investigator who undertook any human studies reported.
A title page including a title of no more than 150 characters, all author names and affiliations, and the corresponding author contact information. Also including a running head, keywords and word count.
A structured summary of no more than 250 words, including estimates of relevant treatment effects, along with 95% CIs or other measures of variability, when appropriate.

‘What is known about this subject’ and ‘What this study adds’ statements (up to three bullet point sentences for each).
A statement (in Methods) of ethics committee approval and subject consent including the name of the ethics committee and the approval number or identification code.
Confidence intervals (CI) of relevant differences between major end points.
Details of precision, accuracy, sensitivity, and specificity for drug/metabolite assays.
A statement declaring any competing interests, or declaring that there are no competing interests.
Acknowledgement of financial and other support.
Preprints of relevant unpublished papers.
Figures and tables must be submitted as separate files, and guidance on preparing artwork can be found here. The following formats are accepted: gif, jpg, bmp, tif, pic (figures) and as Word and Excel files (tables). PDF and Powerpoint files are not accepted.

The main text file must contain the title page, summary, text, references, and figure legends.

Manuscripts in rich text format should not contain word processor styles (for example bold, italics, justification).

For more information about the online submission system, please see here or email the Editorial Assistant.

Covering letter

Please include in your covering letter the following:

Statements regarding the originality of the work and agreement of all authors to its publication

Papers will not be approved for review unless accompanied by a statement, in the covering letter, that they have not been, and will not be published, in whole, or in part, in any other journal, and that all the authors have agreed to the contents of the manuscript in its submitted form. If authors have a problem in contacting a co-author for approval, the reason for this should be explained in the letter and may subsequently be discussed via email with the relevant Senior Editor.

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For ALL papers describing investigations of human subjects (e.g. Research Papers) please indicate which of the authors was the Principal Investigator. (If the Principal Investigator is not an author of the paper, please explain why not.) BJCP has a policy of not accepting papers of which a person with final medical responsibility is not an author: please see the recent Editor's View.

Reviewers

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Employment: Recent, present or anticipated employment of you or a family member by any organization that may gain or lose financially through publication of the paper.
Gifted drugs, materials or devices not commercially available
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Examples of appropriate conflicts of interest statements would be

This work was supported by the NIH (grant to AB and CD).

This work was supported by a grant from Big Pharma Inc. (to AB) and equipment was donated by Small Pharma Inc. EF received a graduate studentship award from the University of xxxxx.
AB declared no other relationships or activities that could appear to have influenced the submitted work.
This work was supported by a grant from the National Institutes of Health, USA (DKxxxx to AB).
This work was supported by the NIH (grant to AB and CD).
This work was supported by a grant from Big Pharma Inc. (to AB) and equipment was donated by Small Pharma Inc. EF received a graduate studentship award from the University of xxxxx.
For papers where no conflicts of interest or funding are declared, a default statement is added to that paper.'

The peer and publication process
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Please note submissions are checked using the iThenticate® anti-plagiarism software. Please seehere for a quick guide on plagiarism, and here for a full guide.

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Papers should be concise and consideration should be given to using supporting information (see http://authorservices.wiley.com/bauthor/suppmat.asp for more information about our Supporting Information service). Most original research articles published in BJCP are between 3000 and 4000 words (for Introduction, Methods, Results and Discussion only). Please refer to a recent issue for examples of length that we regard as appropriate, which varies with the complexity and importance of the study. If you believe your original research paper is going to significantly exceed 4000 words, we require justification for this with your submission letter.

When writing your paper, original research papers should generally be divided into the following sections: Title Page (including PI Statement), Structured Summary, Introduction, Methods, Results, Discussion, Acknowledgements, References, tables and legends to figures (Please remember that figures must be submitted as separate files). In addition authors should provide material for the following 2 sections, placing this after the Structured Summary:

Section 1: What is already known about this subject: In up to three short bullet point sentences (not more than 50 words in total) summarise the state of scientific knowledge on this subject before you did your study and say why this study needed to be done.

Section 2: What this study adds: In up to three short bullet point sentences (not more than 50 words in total) give a simple answer to the questions "What do we now know as a result of this study that we did not know before?" and "What take-home-message do you want to impart to the readers?"

These two statements should be succinct, accurate and specific.

All Research Papers require a list of author contributions.

Review articles
Review articles on a wide range of topics appear regularly in the Journal. Articles may be unsolicited, or may be commissioned by the Reviews Editor. Either kind may be single papers or, by prior agreement with the Editor, part of a themed series. Contributors are welcome to submit single review articles directly (systematic reviews are especially welcome). Most reviews should be between 2500 and 3000 words, should be fully referenced, and if judged potentially suitable will undergo peer review. Each review should include a summary but not the boxes ("what is known"/ "what this adds") that are required only for original research articles. They will be subject to the other requirements of an original research paper.

From time to time the Journal will publish themed issues, including review articles and related original research papers. Authors who want to suggest a theme for a special issue should contact the Editor-in-Chief.

Systematic reviews
The Journal welcomes systematic reviews. The manuscript should provide a concise account of the methods used, and concentrate on highlighting key aspects of interest and relevance to clinical pharmacologists, under the following headings: Structured Summary, Introduction, Methods, Results, Discussion, and Conclusion.

Introduction This should describe the background (e.g. relevant clinical and pharmacological issues) and the scope and aim of the review. What was the reason for the review? The strengths and weaknesses of the existing literature should be briefly defined, earlier reviews identified and the need for the present paper explained.
Methods Study selection is particularly important (search strategy, type of intervention/exposure, types of studies included, types of outcomes, types of participants); include information on any data extraction and synthesis (statistical techniques and use of a quality assessment tool, if any).
Results The key characteristics of the included studies and the main outcome measures; discuss variation within and between studies.
Discussion Compare the findings with existing knowledge; outline the limitations of the review.
Conclusion Summarize the key findings and the implications for clinical pharmacology and/or practical drug therapy.
Letter to the Editor
Comments on previously published papers, items of topical interest, and brief original communications will be considered under this heading. The length, including references, should not exceed 800 words, plus one figure or table. The letter should NOT be divided into sections.

Case reports (must be submitted as Letter to the Editor)
The Journal generally does not publish case reports as full papers but may do so as Letters to the Editor. As for other letters the length, including references, should not exceed 800 words, plus one figure or table. Such case reports (for example adverse drug reactions or interactions) should include some novel aspect of drug action in man (for example a new adverse reaction or one that gives insight into a mechanism or method of management). Such reports may include single cases or short case series. Notes and guidelines on the format for publishing such reports, including a structured summary, will be found at http://www.bmj.com/content/suppl/2003/06/19/326.7403.1346.DC1.

Short Reports are published by the journal. These are short original articles, with a maximum word count of 1.500 to 2,000 words. The summary should be unstructured and comprise no more than 150 words. There should be no more than 3 figures and tables (in any combination). Short Reports should be structured in the same way as original articles (Introduction, Methods, Results, Discussion) and should include statements on What is already known about this subject and What this study adds.

TERMINOLOGY
Stereoisomers

When a drug can exist as stereoisomers or diastereomers (for example geometrical isomers), the form of compound studied must be designated as follows in the methods section.

In the case of racemates the prefix rac- should precede the drug name (for example rac-propranolol).

When possible the absolute configuration of enantiomers should be indicated (for example (S)-warfarin).

Similarly, geometrical isomerism should be indicated by the prefixes Z/E or cis/trans. When appropriate, the interpretation of data obtained using mixtures of isomers should take account of stereochemical aspects.

Drug names
Prescribed drugs should be designated by an International Non-proprietary Name (recommended, rINN, or proposed, pINN). If such a name is not available, a drug should be designated by its British Approved Name (BAN; for example hyoscyamine) or its chemical name (for example glyceryl trinitrate).

When a mixture of drugs has a combination BAN (for example co-trimoxazole, co-fluampicil), that should be used.

For brevity, a company's code name may be used, but in that case the full chemical name or a figure showing the structure of the drug should be given in the introduction or a reference provided that gives this information.

Some mediators with well established common names (e.g. prostacyclin) are also prescribed as licensed preparations with an rINN (e. g. epoprostenol). In such cases the rINN should be used in the context of therapeutic use. Sometimes English and American usage varies, as with adrenaline / epinephrine and noradrenaline / norepinephrine. “Adrenaline / noradrenaline” relate clearly to terms such as “noradrenergic”, “adrenergic” and “adrenal gland” but we will accept the term preferred by authors.

Units
SI units (mass or molar units) should be used. If other units are used, a conversion factor should be included in the Methods section.

Symbols
A set of standard symbols in pharmacokinetics and pharmacodynamics can be found here.

STRUCTURE
Title page
The title page should include

the title giving an informative and accurate indication of the content of the paper. It should be no longer than 150 characters, including spaces;
the names, positions, and addresses of the authors;

the name and e-mail address of the submitting author and the corresponding author, if different;
a running head of no more than 75 characters, including spaces;
keywords (these are used to identify potential referees and as indexing terms);
the word count, excluding the title page, summary, references, tables, and figures;
the numbers of tables and figures.
In order to ensure correct citation of your article on PubMed, should your article be accepted for publication, please include spaces in-between author's initials on the title page of your manuscript, e.g. A E Smith. If author's initials do not appear on the title page of your manuscript according to these guidelines they may appear incorrectly on PubMed until your article has been typeset and published in an issue.

Summary
The text must be preceded by a structured summary, including the following headings:

Aim(s)
Methods
Results (some numerical data, including confidence intervals on differences, when appropriate, must be included)
Conclusions
The summary should be a maximum of 250 words; abstracting services truncate summaries that are longer. It should be couched in terms that will be understood by most readers of the Journal.

Analytical methods
Authors should include details of the precision, accuracy, sensitivity, and specificity of an analytical method used to measure drugs, metabolites or biomarkers or refer to other publications in which the information is given.

Precision is a measure of random error, usually expressed as the coefficient of variation.
Accuracy is a measure of systematic error, also called bias; it can be expressed as the percentage difference between the result for a test sample and the reference value for that compound.
Sensitivity or lower limit of quantification.
Specificity is the extent to which the method does not detect compounds other than those intended.
Statistical methods

In the Methods section statistical methods should be described clearly, in particular for the main analyses. Authors should state which data they had planned to analyse as primary endpoints to investigate their primary objective, and whether they had been analysed as inferential analysis (i.e. with a pre-specified statistical hypothesis) or for exploratory reasons.

Derivations of endpoints, e.g. change from baseline or relative frequencies, should be clearly specified. Covariates that have been used in the statistical analysis (e.g. ANCOVA) should be identified, with a justification for their inclusion (e.g. a reference).

The statistical section should clarify whether the aim of the study was exploratory or confirmatory. In confirmatory trials, a detailed sample size determination should be provided. Strategies to account for multiple testing should be outlined where relevant.

In exploratory trials, the sample size may be justified in terms of expected precision of the estimates parameters of interest. If study size has been chosen based on other criteria (e.g. practical constraints), this should be stated.

In the Results section, measures of variability/uncertainty, such as confidence intervals, should be provided, where relevant for important endpoints. The use of p-values to demonstrate statistical significance should be limited to key endpoints.

Equivalence or non-inferiority of treatments should only be claimed if a statistical margin (e.g. bioequivalence margins of 80%-125%) had been pre-specified in the protocol, and the study had been powered to demonstrate these objectives. It should be avoided to interpret the absence of statistically significant differences as equivalence between treatments.

The Editors may utilize specialist statistical referees for manuscript review where necessary.

Tables
Tables should generally not have more than 85 characters to a line (counting spaces between columns as 4 characters) and certainly not more than 110 characters to a line, unless absolutely unavoidable. Each table should be typed on a separate page and be in an editable format (doc or xls).

Figures and Use of Colour
Please provide high quality figures suitable for the journal's production processes. Use of colour (free to authors) is encouraged when it improves the clarity of the message conveyed.

Authors should consider presenting data on the same scale as the statistical analysis, i.e. when the logarithmic transformation was used during the analysis, the corresponding data should also be presented on logarithmic scale. For concentration-time profiles it might sometimes be useful to present both the initial and the terminal phase using a larger graph as well as an inset. In multi-panel figures it is recommended to align the corresponding axes.

To enhance the presentation of their articles, authors are encouraged to use colours as presented in the examples. The suggested colours all work together and should be clear to those readers who are colour-blind. With certain figures when it is helpful to strongly differentiate between sets of results, the first colour of each pair should initially be used (example), with the corresponding extra colours introduced as necessary. Although white backgrounds are generally advisable, if necessary a suggested background colour that works both with the overall look of the journal, and the proposed colour palette is illustrated (example).

Digital files should be prepared in accordance with the instructions that will be found at http://authorservices.wiley.com/bauthor/illustration.asp. The eps format (illustrations,

graphs, annotated artwork; minimum resolution 800 dpi) and tif format (micrographs, photographs; minimum resolutions 300 dpi) are recommended, although in some cases other formats can be used.

References

References should be cited using the Vancouver style (see examples below; for detailed instructions see http://www.icmje.org).
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The reference list should show the references in numerical and not alphabetical order, and should include the names of the first six authors (plus et al if there are more than six), the full title of the article, the title of the publication (abbreviated as in Medline or PubMed), the year, the volume number, and the first and last page numbers.
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Examples:

Johnson TN, Rostami-Hodjegan A, Tucker GT. A comparison of methods to predict drug clearance in neonates, infants and children. Br J Clin Pharmacol 2004; 57: 677-8.
Metters J (editor). Independent Steering Committee Report of an Independent Review of Access to the Yellow Card Scheme. London: The Stationery Office, 2004.
Hoffman BB, Lefkowitz RJ. Beta-adrenergic receptor antagonists. In: The Pharmacological Basis of Therapeutics, Eighth Edition, eds Gilman AG, Rail TW, Nies AS, New York: Pergamon Press, 1990: 229-43.
Accepted articles and Early view
References to papers published as Accepted Articles should be cited thus: Ferro A. Paediatric prescribing: why children are not small adults. Br J Clin Pharmacol 2015. doi: 10.1111/bcp.12540.

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Artigo publicado na revista Genetic Testing and Molecular Biomarkers

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ORIGINAL ARTICLE

Role of *SEP15* Gene Polymorphisms in the Time of Progression to AIDS

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Aims: HIV infection is a chronic disease that requires intensive treatment in its later phases, including dietary supplementation. Several studies have suggested clinical improvements in patients with high levels of selenium, linking these levels with a longer progression to AIDS. The objective of this study was to verify the association of two polymorphisms in the *SEP15* gene, which encodes a selenoprotein that is responsible for the transport of selenium in cells, with the time of progression to AIDS in HIV-1-infected patients. *Methods:* Blood samples were obtained from 139 HIV-1-positive individuals after they provided informed consent. DNA was isolated and genotyped using real-time polymerase chain reaction for the presence of *SEP15* single nucleotide polymorphisms (rs5859 and rs561104). Questionnaires on sociodemographic features and behavior were answered, and the time of progression to AIDS was estimated based on a medical chart analysis. *Results:* The allelic and genotypic frequencies did not differ between rapid and nonrapid progressors; however, the presence of the AA genotype of the rs5859 polymorphism was associated with a shorter time of progression to AIDS compared with GG homozygotes (hazard ratio = 3.62, 95% CI = 1.55–8.43, $p = 0.003$). *Conclusion:* These findings show the importance of genetic analysis of the *SEP15* gene in individual patients with regard to predicting time of progression to AIDS.

Introduction

HIV INFECTION IS A CHRONIC DISEASE that requires intensive treatment and can present a variable clinical course. Several factors are involved in the progression to AIDS. For example, the patient's condition is aggravated by nutritional deficiencies that can affect the responsive capacity of the immune system. Among the essential micronutrients, selenium plays a key role in the activation of immune cells (Hoffmann *et al.*, 2010; Huang *et al.*, 2012). HIV infection simultaneously increases the demand for micronutrients and causes their loss, which leads to a deficiency that can be improved with micronutrient supplementation (Bogden and Oleske, 2007; Chandrasekha and Gupta, 2011). Low selenium levels are associated with a lower number of $CD4^+$ cells, faster progression to AIDS, and 19.9% increase in the risk of death (Bogden and Oleske, 2007; Pitney *et al.*, 2009).

In animal studies, it was shown that selenium inhibits HIV reverse transcriptase, thus reducing the ability of the virus to replicate (Schrauzer and Sacher, 1994). Lower levels of viral RNA have been found in the plasma of infected children with high levels of selenium (Bunupuradah *et al.*, 2012). Furthermore, selenium is required for the proliferation and differentiation of lymphocytes and for the activation of macrophages and NK cells (Schrauzer and Sacher, 1994; Huang *et al.*, 2012). However, an excessive amount of selenium has adverse effects (Bogden and Oleske, 2007), such as an increased risk of type 2 diabetes and acute intoxication (Pitney *et al.*, 2009). These data underscore the importance for an appropriate micronutrient supplementation that targets each patient's condition by considering both nutritional status and genetic background and that modulates the activity of selenium (Schrauzer and Sacher, 1994).

Studies have shown that single nucleotide polymorphisms (SNPs) in gene encoding selenoproteins modify the expression or function of these proteins, thus affecting the clinical characteristics of patients with different diseases (Penney *et al.*, 2010). The *SEP15* gene encodes a 15-kDa human selenoprotein (Hu *et al.*, 2001) that is the fourth most abundant selenoprotein in the body. The expression of *SEP15* is

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proportional to the amount of selenium circulating in plasma (Huang *et al.*, 2012).

Considering that selenium levels affect viral replication and the expression of *SEP15* is associated with selenium levels, our hypothesis is that genetic variants in *SEP15* can affect the progression to AIDS in HIV-positive patients. The *SEP15* gene is located on chromosome 1, and among the polymorphisms described, rs5859, which is located in the 3′UTR region, and rs561104, which is located in an intron, were the targets of this study because of their effects in decreasing the efficiency of the selenocysteine insertion sequence element at high selenium concentrations (Hu *et al.*, 2001). Our research group also demonstrated that rs561104 is associated with the serum selenium concentration. In addition, we have also demonstrated a genotype-dependent correlation of micronutrient intake and the serum selenium concentration for rs5859 (da Rocha *et al.*, 2014). Therefore, this study aims to investigate the association between these two well-characterized SNPs and the time of progression to AIDS.

Materials and Methods

Study sample

Following a stringent review of 3500 medical charts in a hospital located in Porto Alegre, Brazil, 139 blood samples were obtained from HIV-positive patients. For these 139 patients, it was possible to clearly characterize the time of progression to AIDS (time from the date of negative serology to the start date of antiretroviral treatment) through retrospective analysis of their medical records. It is important to note that in Brazil, the Ministry of Health Guidelines for Antiretroviral Treatment indicates that the initiation of Highly Active Antiretroviral Therapy (HAART) should occur for both symptomatic and asymptomatic HIV-1 patients with $CD4^+$ T-cell counts below 350 cells/mm^3 and/or a persistently high viral load (National Program for STD and AIDS, 2012).

Data obtained from medical records, such as the date of seroconversion, HIV-negative serology date, HIV-positive serology date, date of antiretroviral treatment initiation, and lymphocyte count $CD4^+$, were used to classify patients according to the following criteria: (1) rapid progressors, the time between seroconversion and AIDS diagnosis was less than three years, with at least two consecutive counts of $CD4^+ < 350$ cells/mm^3 before data collection; and (2) nonrapid progressors, time after seroconversion was greater than 9 years without a diagnosis of AIDS, with at least three consecutive counts of $CD4^+$ T lymphocytes >500 cells/mm^3 before data collection. In addition, the time of progression to AIDS (in years) was estimated for these patients using the dates of the antiretroviral treatment initiation and seroconversion. Subsequent analyses were performed for the time of progression to AIDS and the type of progression (rapid or nonrapid).

Data regarding the individual features of subjects were obtained through a sociodemographic questionnaire. The Ethics Committee of the Conceição Hospital Group (Porto Alegre City) approved the study protocol. Informed consent was obtained from each subject included in the study.

DNA extraction and genotyping

Total DNA extraction from blood samples was performed using the salting-out method, as described by Lahiri and Nurnberg (1991). From the extracted DNA, genotypes were determined using real-time polymerase chain reaction (PCR) with validated TaqMan assays to assess the following two SNPs located in *SEP15*: rs5859 (A>G) and rs561104 (C>T) (Applied Biosystems). This method uses the 5′ nuclease activity of Taq polymerase to cleave a nonextendible hybridization probe during the extension phase of PCR. The *CCR5del32* (rs333) variant was evaluated by PCR and visualized on 3% agarose gel, as described by Chies and Hutz (2003), and used to control for known genetic factors involved in the progression to AIDS.

Statistical analyses

Categorical variables are described in proportions and continuous variables are expressed as the mean (standard deviation [SD]) or median (interquartile range [IQR]). Allelic frequencies were calculated using the gene counting method. The agreement of the genotypic frequencies with the Hardy–Weinberg equilibrium expectations was tested using chi-square tests.

After the subjects were classified as rapid or nonrapid progressors according to the initiation of HAART and the seroconversion date, the $CD4^+$ T-cell slope values before HAART for each group were estimated using linear regression with the square root of the $CD4^+$ T-cell count (Wolbers *et al.*, 2010). The nonparametric Mann–Whitney test and Fisher's exact test were used to characterize samples according to the AIDS progression classification (rapid and nonrapid progression). The genotype and allelic distributions among rapid and nonrapid progressors were compared using Fisher's exact test.

In addition, a Cox regression analysis was performed using the time of progression to AIDS in years as the dependent variable. To estimate the hazard ratio (HR), variables that could influence the course of the disease were tested using Cox regression in a univariate model. The following variables were included: viral coinfection (hepatitis C/B and/or HTLV), sex, ethnicity, and the genotypes for *CCR5del32*. Variables with a $p < 0.1$ were maintained in the multivariate model to address the effect of *SEP15* polymorphisms. All statistical analyses were performed with IBM SPSS Statistics for Windows, version 19.0 (IBM Corp.), and a $p < 0.05$ indicated statistical significance.

Results

Characteristics of the study population

The characteristics of the study population are shown in Table 1. The median time of progression to AIDS in the rapid progressors group was 2 years (IQR = 1–3 years), whereas the nonrapid group was 11 years (IQR = 9–17 years). Twenty-three patients in the nonrapid group did not start antiretroviral therapy at the time of study completion (follow-up median = 9.75 years; IQR = 8.25–12.25 years). The characterization of the disease progression included significant differences in the values of $CD4^+$ T-cell slopes, baseline $CD4^+$ T-cell counts, and baseline HIV-RNA copies ($p < 0.001$ for all variables). The number of $CD4^+$ T cells decreased more abruptly in the rapid progressors (median slope = −0.65; IQR = −1.28 to −0.27) than they did in the nonrapid

TABLE 1. SOCIODEMOGRAPHIC AND CLINICAL FEATURES OF THE STUDY SAMPLE

Variable	*Type of progression*		p
	Rapid (47)	*Nonrapid (92)*	
Time to AIDS (years)[a]	2 (1–3)	11 (9–17)[b]	
Female	68.1%	76.1%	0.319
Age (years)[c]	40.02 (11.33)	42.72 (10.62)	0.169
Baseline CD4$^+$ T cell (cells/μL)[a]	474.5 (306; 577.5)	588 (463; 783)	<0.001
Baseline HIV-RNA log10 (copies/mL)[a]	4.28 (3.90; 4.70)	3.71 (2.67; 4.20)	<0.001
CD4 slope[a]	−0.65 (−1.28; −0.27)	0.40 (0.22; 0.53)	<0.001
Transmission			0.647
Het (%)	80.9	76.1	
MSM (%)	12.8	9.8	
IDU (%)	4.3	7.6	
Others (%)	2.1	6.5	
Ethnicity			0.096
White (%)	27.6	43.4	
Non-white (%)	72.4	56.6	
Coinfection (yes)	14.9	24.4	0.272

[a]Median (interquartile range).
[b]Data for 69 patients who progressed to AIDS.
[c]Mean (standard deviation).
Exposure category: Het, heterosexual; MSM, men who have sex with men; IDU, injection drug user; others include vertical transmission and occupational accident. Coinfections include hepatitis C/B and HTLV.

progressors (median slope = 0.40; IQR = 0.22–0.53). Other variables did not differ between rapid and nonrapid progressors.

Association of SEP15 gene polymorphisms and the time of progression to AIDS

The genotype frequencies did not show statistically significant differences compared with those expected under the Hardy–Weinberg equilibrium ($p = 0.07$ for rs5859 and $p = 0.84$ for rs561104). No association was found when the genotypic and allelic frequencies were compared between rapid and nonrapid progressors ($p > 0.05$, data not shown). Considering that sex (HR = 0.57, 95% CI = 0.35–0.93, $p = 0.025$) and ethnicity (HR = 0.62, 95% CI = 0.39–0.99, $p = 0.044$) were significant factors in the univariate Cox regression, a multivariate Cox regression was performed using these variables and each polymorphism. Through this analysis, we observed an association between the rs5859 polymorphism (Table 2 and Fig. 1) and the time of progression to AIDS, in which the progression was faster for AA homozygotes than for GG homozygotes (HR = 3.62, 95% CI = 1.55–8.43, $p = 0.003$). GA heterozygotes showed no difference from the reference group (GG homozygotes; HR = 1.21, 95% CI = 0.80–1.83, $p = 0.360$). No associations were found for the other polymorphism analyzed.

Discussion

The purpose of the present study was to determine whether polymorphisms on the *SEP15* gene, which encodes a selenoprotein that is responsible for the transport of selenium in

TABLE 2. MULTIVARIATE COX PROPORTIONAL HAZARDS REGRESSION ANALYSIS TO ASSESS THE INDEPENDENT EFFECTS OF THE *SEP15* RS5859 AND RS561104 POLYMORPHISMS ON THE TIME TO PROGRESSION TO AIDS

	HR[a]	*95% CI*	p	*Adjusted HR*[b]	*95% CI*	p
rs5859						
Ethnics (white)	0.62	0.39–0.99	0.044	1.92	1.26–2.95	0.003
Sex (male)	0.57	0.35–0.93	0.025	1.75	1.11–2.75	0.015
GA genotype	1.23	0.83–1.82	0.308	1.21	0.80–1.83	0.360
AA genotype	2.40	1.06–5.43	0.035	3.62	1.55–8.43	0.003
rs561104						
Ethnics (white)	0.62	0.39–0.99	0.044	1.71	1.11–2.64	0.015
Sex (male)	0.57	0.35–0.93	0.025	2.14	1.32–3.47	0.002
TC genotype	0.84	0.55–1.28	0.417	0.79	0.52–1.23	0.309
CC genotype	0.75	0.38–1.49	0.412	0.75	0.36–1.56	0.440

[a]Univariate model.
[b]Multivariate model.
HR, Hazard ratio; CI, Confidence interval.

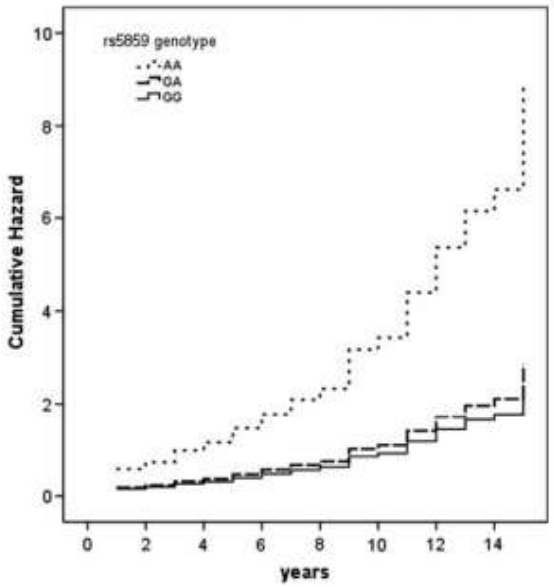

FIG. 1. Cox regression analysis for rs5859 showing the time of progression to AIDS in individuals with the AA genotype (Hazard ratio = 3.62, 95% CI = 1.55–8.43, $p = 0.003$) and GA genotype (Hazard ratio = 1.21, 95% CI = 0.80–1.83, $p = 0.360$) compared to GG homozygotes.

cells, are associated with the time of progression to AIDS in HIV-1-infected patients. Our major finding indicates that rs5859 is associated with a faster progression to AIDS. To the best of our knowledge, there are no studies that have identified an association between the genotypes of *SEP15* and the time of progression to AIDS in humans. However, it is known that the rs5859 polymorphism can functionally modify the 15-kDa human selenoprotein (Geybels *et al.*, 2013). Furthermore, it has been broadly illustrated in the literature that low selenium concentrations are associated with increased morbidity and mortality more than other micronutrients and that selenium deficiency is more common due to unknown mechanisms (Singhal and Austin, 2002; Xu *et al.*, 2002).

Several studies have established a relationship between a rapid progression of the disease and micronutrient deficiencies or a decline in the number of $CD4^+$ cells (Kupka *et al.*, 2004; Pitney *et al.*, 2009). An antioxidant deficiency in HIV infection facilitates the development of injury by oxidative stress, which contributes to deregulation of the immune system, T lymphocyte death, and viral replication (Hurwitz *et al.*, 2007; Stone *et al.*, 2010; Baum *et al.*, 2013). In this context, selenium plays a key role in antioxidant defense, acting as a cofactor of glutathione peroxidase (Kupka *et al.*, 2004; Reshi *et al.*, 2014).

In North America, ~70% of HIV-infected patients are receiving vitamin supplements; however, ~30% of the patients still have a deficiency of at least one micronutrient, which demonstrates the importance of individualized treatment and raises questions about the mechanisms that lead to a disability in nutritional deficiency (Chandrasekha and Gupta, 2011). A micronutrient deficiency in patients with HIV has a synergistic effect, with one exacerbating the other (Kupka *et al.*, 2004). However, when this deficit can be corrected, there is evidence that the disease progression is slowed (Singhal and Austin, 2002; Hurwitz *et al.*, 2007). The beneficial effects of selenium supplementation were recently demonstrated by an increase in the time of progression to AIDS and an indirect increase in the number of $CD4^+$ cells, which reinforce the benefits of using this simple and inexpensive therapeutic measure in patients with HIV (Hurwitz *et al.*, 2007; Baum *et al.*, 2013; Kamwesiga *et al.*, 2015). Despite the evidence of the benefits of selenium supplementation, Hurwitz *et al.* reported the existence of two groups that included individuals who were either responders or nonresponders to selenium. The nonresponders group had a lower concentration of selenium, and thus, experienced fewer benefits from the supplementation than did the responders, even though the groups received the same therapeutic dose. This result suggests and we hypothesize that selenium deficiency is due to poor nutrition and absorption and to the association of polymorphisms in gene encoding selenoproteins, which could have deleterious effects on the transport and incorporation of this micronutrient.

Data from genetic association studies connecting variants in the *SEP15* gene with the risk of diseases have shown that the investigated SNPs altered the expression or function of selenoproteins and may increase the risk of developing diseases, such as breast cancer risk (Hu *et al.*, 2001), lung cancer in smokers (Jablonska *et al.*, 2008), rectal cancer risk in men (Sutherland *et al.*, 2010), and prostate cancer risk and survival (Penney *et al.*, 2010). However, the only published study that found a relationship among the *SEP15* gene, selenium levels, and the development of acquired immunodeficiency was performed in Rhesus monkeys. In that study, infected monkeys showed selective expression of selenoproteins and decreased expression of *SEP15*, which is possibly the mechanism responsible for the reduction in selenium in the body (Xu *et al.*, 2002).

The present study has some limitations, which includes a small sample size, limited number of SNPs, and lack of selenium concentration and intake data. However, the findings of the present study support the hypothesis that selenium and, possibly, genetic variants affecting selenium levels are involved in the progression to AIDS. Therefore, consistent with the findings of other studies, a patient's treatment can be tailored to their specific genetic profile and risk of progression to AIDS, thus allowing an improvement in the patient's survival time. Other studies may help to establish the genetic profiles in the different categories so that we can better understand the mechanisms of slow progressors and try to apply these mechanisms in patients with a higher risk of progression to AIDS. Genes involved in encoding selenoproteins, such as *SEP15*, are potential candidates for the continuation of this research.

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Author Disclosure Statement

No competing financial interests exist.

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Association of *NR1I2* gene polymorphisms and time of progression to AIDS

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BACKGROUND The time of progression towards AIDS can vary greatly among seropositive patients, and may be associated with host genetic variation. The *NR1I2* (PXR) gene, a ligand-activated transcription factor, regulates the transcription immune pathway genes and can therefore be targets of viral replication mechanisms influencing time of progression to AIDS.

OBJECTIVE To verify the association of single nucleotide polymorphisms (SNPs) rs3814057, rs6785049, rs7643645, and rs2461817 in the *NR1I2* (PXR) gene with progression to AIDS in HIV-1 infected patients.

METHODS Blood samples were obtained from 96 HIV-1 positive individuals following informed consent. DNA was isolated and genotyped through real time polymerase chain reaction (PCR) for the presence of SNPs in the *NR1I2*. Questionnaires on socio-demographic features and behaviors were answered and time of progression to AIDS was estimated based on medical chart analysis.

FINDINGS Patients with the GG genotype for rs7643645 were shown to be related with a more rapid disease progression when compared to GA and AA genotypes. This result was maintained by the Multivariate Cox Regression considering sex, ethnicity, and presence of *HLA-B*57*, *HLA-B*27*, and *CCR5del32* polymorphisms.

MAIN CONCLUSIONS Recent studies reported the expression of the nuclear receptors in T-Lymphocytes, suggesting their possible role in the immune response. In addition, nuclear receptors have been shown to inhibit the HIV replication, although no such mechanism has been thoroughly elucidated to date. This is the first time an association between *NR1I2* polymorphism and time of progression to AIDS is reported and supports an apparent relationship between the gene in the immune response and identifies another genetic factor influencing AIDS progression.

Key words: AIDS progression - genetic polymorphisms - nuclear receptors - *NR1I2*

The time of progression towards AIDS can vary greatly among seropositive patients, ranging from as little as three to as long as 20 years after infection. Previous studies estimated that 10% of HIV positive patients develop AIDS within two to three years (rapid progressors), and 5-15% will remain AIDS-free after 20 years (slow progressors) (Casado et al. 2010). Several attempts to explain differential progression towards AIDS have been made, and so far multiple genes have been shown to play a role in shaping individual time of progression, affecting susceptibility, transmission, and course of disease, ultimately determining viral set points, rate of decline of CD4+ T-cells, level of viremia and cytotoxic T lymphocytes escape (Kaur & Mehra 2009). The extent to which each of those genes influences AIDS onset is not clear, but it is understood to be the result of multiple small factors combined. Nuclear receptors (NR), a family of transcription factors (TF) which comprise the so called orphan receptors, are among the host factors currently known to have some influence in AIDS progression (Hanley et al. 2004). Hanley et al. (2010) and his team have shown two specific NRs that play an import role in diminishing HIV replication through specific mechanisms in vitro. The *NR1I2* (PXR) gene, ligand-activated TFs, regulate the transcription of a wide range of genes (Xie & Evans 2001, Urquhart et al. 2007), including immune pathways (Martínez et al. 2007) and can therefore be targets of viral replication mechanisms influencing time of progression to AIDS. The *NR1I2* (PXR) is also a key to gene regulation, influencing morphogenesis and differentiation, and it may also play a role in the cell cycle progression (Schote et al. 2007, Zhou 2016). Currently available studies also indicate that PXR regulates CYP3A4 expression (Urquhart et al. 2007), which is involved in the metabolism of

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antiretrovirals and therefore could be a key predictor of drug responsiveness and toxicity (Schipani et al. 2010, Sinxadi et al. 2015, Cusato et al. 2016). This study aims to investigate an association of polymorphisms in the PXR gene with time of progression to AIDS.

MATERIALS AND METHODS

Study sample - Ninety-six blood samples were obtained from HIV-positive patients following stringent review of 3500 medical charts between 2011-2013 in the hospital located in Porto Alegre city, Southernmost Brazil.

For these patients, it was possible to clearly determine the time of progression for AIDS, considering the middle time between HIV-negative and HIV-positive serology, using the retrospective data of their medical records. It is important to note that this parameter is in accordance with the prevailing Brazilian guidelines for the management of HIV+ patients in the country affective in 2013. The Ministry of Health Guidelines for Antiretroviral Treatment indicates that the initiation of Highly Active Antiretroviral Therapy (HAART) should occur for both symptomatic and asymptomatic HIV-1 patients with CD4+ T-cell counts below 350 cells/mm3 and/or a persistently high viral load (NP 2012).

Data available in the medical records, such as the date of seroconversion, HIV-negative serology date, HIV-positive serology date, date of antiretroviral treatment initiation, and lymphocyte count CD4+, were used to classify patients according to the following criteria: (i) rapid progressors, the period from seroconversion (considering middle time between HIV-negative and HIV-positive serology) to the beginning of treatment for AIDS of up to three years, with at least two consecutive CD4+ counts < 350 cells/mm³ before data collection and HAART medical recommendation; (ii) typical progressors, the period from HIV-positive serology (with clinical monitoring) to the treatment for AIDS of over four years, with at least three consecutive CD4+ T lymphocytes counts > 500 cells/mm³ before data collection and HAART medical recommendation; (iii) slow progressors, the period from HIV-positive serology exceeding nine years, with CD4+ T lymphocytes counts > 500 cells/mm³ and without requiring treatment for AIDS. The analyses were performed using this classification in rapid, chronic, and slow progressors.

Other data concerning the individual features of patients were obtained through a sociodemographic questionnaire. The individuals were classified as of European or African ancestry, according to the phenotypic characteristics of individuals, as judged by the researcher at the time of data collection, and from data about the ethnicity of the parents/grandparents reported by the participants. According to Pena et al. (2011), the Brazil Southernmost Region showed reduced mixing compared to the rest of the country, due to great geographical variances and cultural differences in the country. The issue concerning the skin-color-based classification criteria used in this geographical area of Brazil is well documented and has already been assessed in previous studies (da Silva et al. 2011).

DNA extraction and genotyping - Total DNA extraction from blood samples collected was performed through the well-established technique of salting out described by Lahiri and Nurnberger (1991). Genotyping of four single nucleotide polymorphisms (SNPs) in the *NR1I2* gene rs3814057 (A > C; mRNA 3'UTR), rs6785049 (G > A; intronic region), rs7643645 (A > G; intronic region), and rs2461817 (A > C; intronic region), were performed by real time polymerase chain reaction (PCR) and validated by TaqMan assays (Applied Biosystems, California, USA). The SNPs evaluated here were selected using the HapMap tool (www.hapmap.org) with the following annotate TagSNP Picker features: European population (CEU), minimum frequency of the rarest allele of 10% and a determination coefficient (R^2) equal to 80%. *HLA-B*27*, and *HLA-B*57* and *CCR5del32* genotypes were available for the sample and used to control for known genetic factors involved in the progression to AIDS. The *CCR5del32* (rs333) variant was previously evaluated by PCR and visualised on 3% agarose gel, as described by Chies and Hutz (2003). The presence of *HLA-B*27* and *HLA-B*57* were previously genotyped according Cesbron-Gautier et al. (2004).

Statistical analysis - Categorical variables are described in proportions and continuous variables and are expressed as the mean [standard deviation (SD)] or median [interquartile range (IQR)]. Allelic frequencies were estimated through gene counting. The agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested using Chi-square tests.

Initially, patients enrolled were grouped as rapid, chronic, and slow progressors according to the data obtained from the questionnaire and review of the subjects' medical charts. Their CD4+ T-cell slope values before HAART were estimated by linear regression with the square root of the CD4+ T-cell count and a non-parametric Mann-Whitney and exact Fisher test were used to characterise the progression of the patients according socio-demographic characteristics of patients. After characterisation of the sample, all variables were tested using Cox regression in a univariate model. Multivariate Cox regression was performed considering a forward stepwise model and all three models with statistical significance are shown on results. Genetic recessive models for *NR1I2* polymorphisms were assumed. All statistical analyses were performed with IBM SPSS Statistics for Windows, version 19.0 (IBM Corp, Armonk, NY) and $p < 0.05$ was considered to indicate statistical significance.

Ethics - The study protocol was approved by the Ethical Committees of the Conceição Hospital Group (Porto Alegre) (approval number 10-213) and Fundação Estadual de Produção e Pesquisa (FEPPS) (approval number 13/2010). Written informed consent was obtained from each subject included in the study.

RESULTS

Characteristics of study population - Table I contains data regarding sample description and comparison of clinical and socio-demographic variables between slow, typical, and rapid progressors. Female sex was slightly more frequently observed among slow and typical progressors than in rapid progressors ($p = 0.067$). Characterisation of disease progression included sig-

TABLE I

Socio-demographic and clinical features of study sample

Variable	Total (96)	Type of progression			p value
		Rapid (20)	Typical (40)	Slow (36)	
Time to AIDS (years)	7.0 (1-15)	1.55(1-3)	5.89(4-8)	11.21(9-15)	< 0.001
Slope CD4+ (cell/mm³)	-	-0.988 (-2.424; -0.399)	-1.090 (-2.649; -0.427)	-0.473 (-0.881; -0.160)	< 0.001
Female	75.0%	55.0%	80.0%	80.5%	0.067
Age (years)	40.81(10.21)	42.72 (11.06)	40.07 (11.83)	40.52 (8.43)	0.640
Transmission					
Het	76.0%	75.0%	80.0%	72.2%	0.725
MSM	8.3%	15.0%	7.5%	5.5%	0.458
IDU	8.3%	5.0%	5.0%	14.0%	0.312
Others	6.3%	5.0%	5.0%	8.3%	0.808
Ethnicity					
White	58.3%	57.9%	74.3%	45.0%	0.025
Non-white	41.7%	42.1%	25.7%	55.0%	
Co-infection (yes)	38.5%	55.0%	37.1%	31.8%	0.194

Except for age (mean/standard deviation) and time to AIDS (median/minim and maxim), data are shown as a percentage (%). Exposure category: Het = heterosexual; MSM, = men who have sex with men; IDU = injection drug user; others includes vertical transmission and occupational accident. Co-infections include hepatitis C/B and HTLV.

TABLE II

Genotypic distribution of *NR1I2* single nucleotide polymorphisms (SNPs) among rapid, typical, and slow progressors

SNP	Type of progression			p value
	Rapid	Typical	Slow	
rs2461817				
AA	4 (21.1%)	7 (17.9%)	3 (10.7%)	
AC	10 (52.6%)	19 (48.8%)	15 (53.7%)	0.866
CC	5 (26.3%)	13 (33.3%)	10 (35.6%)	
rs3814057				
AA	7 (35.0%)	16 (40.0%)	15 (51.8%)	
AG	11 (55.0%)	20 (50.0%)	11 (37.9%)	0.781
GG	2 (10.0%)	4 (10.0%)	3 (10.3%)	
rs6785049				
AA	1 (5.0%)	8 (20.5%)	8 (27.6%)	
AG	13 (65.0%)	18 (46.2%)	12 (41.4%)	0.321
GG	6 (30.0%)	13 (33.3%)	9 (31.0%)	
rs7643645*				
AA	8 (40.0%)	14 (35.0%)	17 (58.6%)	
AG	10 (50.0%)	18 (45.0%)	12 (41.4%)	0.077*
GG	2 (10.0%)	8 (20.0%)	0 (0%)	

*: rapid plus typical *versus* slow progressors, p-value = 0.026.

nificant differences ($p < 0.001$) in the values of CD4+ T-cells slopes: the number of CD4+ T-cells decreased more abruptly in rapid progressors (median slope -0.988; IQR -2.424 to -0.399) and typical progressors (median slope -1.090; IQR -2.649 to -0.427) than in the slow progressors (median slope -0.473; IQR -0.881 to -0.160). Twenty-three patients in the slow group did not start antiretroviral therapy at the time of study completion (follow-up median = 9.75 years; IQR = 8.25-12.25 years).

Association of NR1I2 gene polymorphisms and time of progression to AIDS - Table II shows the comparison of *NR1I2* SNPs among rapid, typical, and slow progressors. Although no association was detected for any SNP, an association with the rs7643645 GG genotype was observed when the comparison of rapid plus typical versus slow progressors was performed ($p = 0.0216$). Considering that sex [hazard ratio (HR) = 1.608, confidence interval (CI)95% 1.000-2.587, $p = 0.050$] and ethnicity (HR = 1.657, CI95% 1.054-2.606, $p = 0.029$) showed significance in Cox Regression Univariate test (Table III), and that *HLA-B*57*, *HLA-B*27*, and *CCR5 del32* polymorphisms are well known factors that affect time to progress to AIDS, a multivariate stepwise Cox regression was performed. In this analysis, three models were generated. In model three (step 3 on Table III) our results evidenced an association of rs7643645 GG genotype to a shorter time of progression (HR = 2.029, CI95% 1.036-3.975, $p = 0.039$) when compared to GA and AA genotypes (Table III). No other polymorphisms were significantly associated with time of progression towards AIDS.

DISCUSSION

This study evaluated the possible influence of four common SNPs from *NR1I2* gene in the time of progression to AIDS. The major finding of this study was the significant association of the rs7643645 polymorphism, gender, and ethnicity with a shorter time of progression

TABLE III

Univariate and multivariate Cox proportional hazards regression analysis concerning rs7643645 SNP and risk for progression to AIDS

	Univariate analysis			
	HR	95%CI		p value
Sex (male)	1.608	1.000	2.587	0.050
Ethnicity (white)	1.657	1.054	2.606	0.029
G homozygosis	1.810	0.915	3.789	0.083
CCR5del32	0.792	0.352	1.742	0.588
HLA-B*57/B*27	0.948	0.452	2.189	0.803
	Forward stepwise multivariate analysis			
	HR	95%CI		p value
*Step 1**				
Ethnicity (white)	1.631	1.041	2.789	0.037
Step 2 **				
Sex (male)	1.736	1.053	2.915	0.028
Ethnicity (white)	1.710	1.136	2.803	0.021
Step 3 ***				
Sex (male)	1.945	1.161	3.258	0.011
Ethnicity (white)	1.710	1.003	2.614	0.049
G homozygosis	2.029	1.036	3.975	0.039

CI: confidence interval; HR: hazard ratio; *: -2 Log likelihood = 576.99, chi-square = 4.01, p-value = 0.055; **: -2 Log likelihood = 572.50, chi-square = 8.89, p-value = 0.017; ***: -2 Log likelihood = 568.50, chi-square = 12.77, p-value = 0.007.

towards AIDS. In the rs7643645 polymorphism, the genotype GG has a major risk to progression for disease.

The characterisation of AIDS progression is diverse in literature (Kaur & Mehra 2009, Casado et al. 2010), and presence of AIDS symptoms, CD4+ T-cell counts and plasma HIV RNA levels already were considered by different authors to classify the AIDS progression (Nakaiama et al. 2002, Okulicz et al. 2009). This study used retrospective CD4+ T-cell counts to assess the progression to AIDS, so initially the patients' progression was validated by the significant difference in the slope of decline in CD4+ T-cell numbers between rapid progressors (-0.988, steep slope) and slow progressors (-0.473, gentle slope).

The analysis on the socio-demographic features of patients showed a positive association between female gender and slow progression to AIDS (p-value = 0.019). This finding is in agreement with other studies, which found higher CD4+ TL counts in females than in male HIV-patients (Jarrin et al. 2008). However, a recent study by Thorsteinsson et al. (2012) found no association between sex and onset of AIDS. It is important to also consider the influence of the patient's selection mode to reach our results. Patients enrolled were those who voluntarily sought health care, which can constitute a bias, since usually in Brazil more women look for health care services than men (Ribeiro et al. 2006) and our collection site (Conceição Hospital Group) has specific health service for pregnant women. Moreover, a positive association between non-white individuals and slow progression to AIDS was also encountered (p-value = 0.053). Okulicz et al. (2009) showed an increased frequency of African-American individuals among long-term non-progressors in a USA cohort. Further, an earlier study of Anastos et al. (2000) had observed an association between slower decline in CD4+ TL and non-white individuals. Despite of the small sample size investigated due the limited data about progression to AIDS and the ethnic composition of Brazilian population, the results show that investigations including populations of different ancestries and geographic regions are important to understand how genetic background can influence immune responses and modulate HIV infection and progression. HIV affects the entire immune system of the patient, so the pathways and genes studied are those associated with the immune system. However, it is increasingly recognised that gene regulation should be mediated, for example, for nuclear transcription factors such as PXR family receptors encoded by *NR1I2*. These receptors form a network, which influences the expression of many other genes. These interactions are complex and may be simulated using function prediction analysis tools made with an online tool (available at http://snpinfo.niehs.nih.gov). The interactions of *NR1I2* involve tumor suppressor genes, cell cycle regulators and differentiation, inflammatory response promoters, genes involved in production of blood cells and the response of cells to retroviral infection. A recent study demonstrated that PXR expression on peripheral blood cells is different in tissue expression, and this variable in combination with other markers can modulate the immune response of human body to the tumor cells. Following this reasoning, considering that the receptor PXR is able to modulate the recognition of abnormal cells, it is questionable and important to study its real relevance in cells infected with HIV (Kong et al. 2016).

The findings in this study regarding the association of allele G homozygosis in rs7643645 and a rapid progression to AIDS are in agreement with Schote et al. (2007), who suggested an apparent role for PXR in immune response. In that study, it was observed that PXRs, among others NRs, are expressed in CD4+ TL, CD8+ TL, CD14+ TL and CD19+ TL, which suggests their function influences expression of molecules involved in immune mechanisms. The mechanism through which PXR correlates to AIDS progression is not all unraveled to date. However, it is known that it is related to the metabolism of the drugs used to treat AIDS patients, given its role in Phase I and II metabolism of xenobiotics and also being suppressed by some drugs. These studies relate the amount of antiretroviral drugs available in the plasma of patients with polymorphisms in *NR1I2* and *NR1I3*, and consequently the resistance to drugs and prognosis of these patients (Healan-Greenberg et al. 2008, Svärd et al. 2010).

It is known, though, that metabolism and inflammation are related mechanisms. In the presence of cytokines and of a specific types of inflammation (acute phase response by lipopolysaccharide), for instance, a decreased

expression of PXR and of its target genes is seen (Moore et al. 2000). It has been suggested that a relationship between PXR and NFkappa-beta functions exists, with NF-kb increased action in PXR null mice as well as a decrease of PXR function with inhibition of NF-kbs, demonstrating a potential for immunosuppressive activity of PXR's ligands (Wahli 2008). This leads to the belief of a possible influence of this NR in the course of immunosuppressive diseases, such as AIDS, as we have found.

Our study presents some limitations and the results must be interpreted considering some issues: (i) we analyse a small sample of HIV+ patients due the limited data about progression to AIDS; (ii) we genotype a small number of polymorphisms and other *NR1I2* SNPs could be associated with the progression to AIDS; (iii) our data do not represent the actual distribution of slow, typical and rapid progressors in HIV + patients, since a high frequency of slow progressors is observed in the sample; (iv) a group of patients who had not started antiretroviral therapy at the time of inclusion in the study was classified as slow progressor. This group was not fully characterised and potentially includes LTNPs patients (long-term non-progressors patients). Despite this, this is the first time that a *NR1I2* gene polymorphism was found to be related to time of progression to AIDS and even though our sample was not an extensive representative, it can be hypothesized that the absence of the rs7643645 SNP in homozygosis (presence of the A allele) is associated with a delayed progression towards AIDS, when also considering ethnicity and gender. This agrees with the widely accepted belief of several different factors influencing AIDS progression and illustrates the importance of considering them all together (McLaren & Fellay 2015). In addition, this finding is in accordance to several recent studies that suggest a role of NR in immune mechanisms and HIV infection. The presented results support the evidence and stress the importance of continuation of the search for additional genes' activities that might be related to the development of this widespread disease. It also claims for the elucidation of mechanisms through which it occurs and investigation of other NRs in the matter.

AUTHORS' CONTRIBUTIONS

RMM - Conception and design of study, acquisition of data, analysis and/or interpretation of data, drafting the manuscript, revising the manuscript critically for important intellectual content, approval of the version of the manuscript to be published; CFM - acquisition of data, analysis and/or interpretation of data, drafting the manuscript, revising the manuscript critically for important intellectual content, approval of the version of the manuscript to be published; JLB - analysis and/or interpretation of data, drafting the manuscript, approval of the version of the manuscript to be published; MCCM - conception and design of study, acquisition of data, approval of the version of the manuscript to be published; MGM - acquisition of data, approval of the version of the manuscript to be published; SEMA - conception and design of study, drafting the manuscript, revising the manuscript critically for important intellectual content, approval of the version of the manuscript to be published; MF - conception and design of study, analysis and/or interpretation of data, drafting the manuscript, revising the manuscript critically for important intellectual content, approval of the version of the manuscript to be published.

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Anexo 4: Aprovação do Comitê de Ética da UFCSPA

UFCSPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMITÊ DE ÉTICA EM PESQUISA - CEP
UFCSPA

O Comitê de Ética em Pesquisa da UFCSPA, registrado na Comissão Nacional de Ética em Pesquisa (CONEP) sob o nº 075/05 em 23/07/04, analisou o Projeto:

Projeto: 10-686 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
MARILU FIEGENBAUM
SILVANA DE ALMEIDA
MARA HELENA HUTZ
LISIANE SMIDERLE
LUCIANA OTERO LIMA
RENATA BIANCHI MARIAN

Título: ESTUDO DA INLFUÊNCIA DA VARIABILIDADE GENÉTICA EM FATORES DE RISCO CARDIOVASCULAR E NA FARMACOGENÉTICA DE HIPOLIPEMIANTES.

Esse projeto foi aprovado em seus aspectos éticos e metodológicos conforme as Resoluções 196/09 e demais Resoluções complementares. Toda e qualquer alteração do projeto, assim como eventos adversos graves, deverão ser comunicados a este CEP. Os TCLE, quando necessários, somente poderão ser utilizados após prévia e explícita aprovação (carimbo) de sua redação por este CEP".

Porto Alegre, 28 de outubro de 2010.

José Geraldo Vernet Taborda
Coordenador do CEP/UFCSPA