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Avaliação da influência de polimorfismos nos genes dos receptores nucleares PPARα, RXRα, PXR e CAR sobre o perfil lipídico e a farmacogenética de inibidores da HMG-CoA redutase

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Orientadora: Silvana de Almeida
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Dedico este trabalho aos meus pais,

Patrícia e Luiz Eduardo.

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“...o melhor de chegar aqui, na saideira, é olhar para trás e concluir que o aconteceu de mais diferente foi eu mesma.(...) estou saindo outra, mesmo que eu pouco perceba essa alteração. (...) Quando olho para o meu passado, encontro uma mulher bem parecida comigo - por acaso, eu mesma - porém essa mulher sabia menos...”

Martha Medeiros

SUMÁRIO

LISTA DE ABREVIATURAS

5’UTR	Região 5’ não traduzida de um gene
ABCB1	Gene codificador da gpP
ABCC2/3/4	Genes codificadores das enzimas MRP2, MRP3, MRP4
APOA1	Gene codificador da apoA-I
apoA-I	Apolipoproteína A-I
apoB	Apolipoproteína B
APOB	Gene codificador da apoB
apoC-III	Apolipoproteína C-III
APOE	Gene codificador da apoE
CAR	Receptor constitutivo de androstano
CETP	Gene codificador da proteína transferidora de ésteres de colesterol
CITCO	6-(4-clorofenil)imidazo[2,1-b][1,3]tiazol-5-carbaldeído-O-3,4-diclorobenzil)oxima
CT	Colesterol total
CYP	Enzima do sistema citocromo p450
CYP2B	Enzimas da subfamília 2B da família CYP
CYP2B	Genes codificadores das enzimas da subfamília CYP2B

CYP2B10	Gene codificador da CYP2B10
CYP2B6	Gene codificador da CYP2B6
CYP2C	Genes codificadores das enzimas da subfamília CYP2C
CYP2C19	Enzima 2C19 da família CYP
CYP2C9	Enzima 2C9 da família CYP
CYP2C9	Gene codificador da CYP2C9
CYP2D6	Gene codificador da CYP2D6
CYP3A	Genes codificadores das enzimas da subfamília CYP3A
CYP3A4	Enzima 3A4 da família CYP
CYP3A4	Gene codificador da CYP3A4
CYP3A5	Enzima 3A5 da família CYP
CYP3A5	Gene codificador da CYP3A5
CYP7A1	Gene codificador da CYP7A1, enzima 7-α hidroxilase de colesterol
DBD	Domínio de ligação ao DNA
DCV	Doença cardiovascular
GST	Gene codificador de glutationa S-transferase
HDL-C	Lipoproteínas de alta densidade
HMG-CoA	3-hidroxi-3-metilglutaril CoA
HMGCR	Gene codificador da HMG-CoA redutase
LBD	Domínio de ligação ao ligante

LDL-C	Lipoproteínas de baixa densidade
LDLR	Gene codificador do receptor de LDL-C
LPL	Gene codificador da lipoproteína lípase
MRP2/3/4	Proteínas associadas à resistência a múltiplas drogas (MRP2, MRP3, MRP4)
NR1B	Nomenclatura oficial do RAR (subfamília 1, grupo B)
NR1C	Nomenclatura oficial do PPAR (subfamília 1, grupo C)
NR1C1	Nomenclatura oficial do PPARα (subfamília 1, grupo C, membro 1)
NR1C2	Nomenclatura oficial do PPARδ/β (subfamília 1, grupo C, membro 2)
NR1C3	Nomenclatura oficial do PPARγ (subfamília 1, grupo C, membro 3)
NR1I2	Gene codificador do PXR (nomenclatura oficial do gene)
NR1I2	Nomenclatura oficial do PXR (subfamília 1, grupo I, membro 2)
NR1I3	Gene codificador do CAR (nomenclatura oficial do gene)
NR1I3	Nomenclatura oficial do CAR (subfamília 1, grupo I, membro 3)
NR2B	Nomenclatura oficial do RXR (subfamília 2, grupo B)
NR2B1	Nomenclatura oficial do RXRα (subfamília 2, grupo B, membro 1)

NR2B2	Nomenclatura oficial do RXRβ (subfamília 2, grupo B, membro 2)
NR2B3	Nomenclatura oficial do RXRγ (subfamília 2, grupo B, membro 3)
OATP	Proteína transportadora de ânions orgânicos
PBREM	Módulo responsivo ao fenobarbital (módulo responsivo ao receptor CAR)
P-gp	Glicoproteína-P
PP2A	Proteína fosfatase 2A
PPAR	Receptor ativado pelo proliferador de peroxissomos
PPARA	Gene codificador do PPARα (nomenclatura oficial do gene)
PPARα	PPAR alfa
PPARδ/β	PPAR delta / PPAR beta
PPARγ	PPAR gama
PPRE	Elemento de resposta ao PPAR
PREM	Módulo responsivo ao PXR
PXR	Receptor pregnano X
RAR	Receptor de ácido retinóico
RXR	Receptor retinóide X
RXRA	Gene codificador do RXRα (nomenclatura oficial do gene)

RXRα	RXR alfa
RXRβ	RXR beta
RXRγ	RXR gama
SLCO1B1	Gene codificador da OATP1B1
SLCO2	Genes codificadores das proteínas OATP2
SLCO2B1	Gene codificador da OATP2B1
SNP	Polimorfismo de variação em uma única base
SULT	Gene codificador de sulfotransferase
SXR	Receptor de esteróides e xenobióticos (nomenclatura também utilizada para o receptor PXR)
TG	Triglicerídeos
UGT	Enzima UDP- glucuronosiltransferase
UGT1A1	Gene codificador da UGT1A1
UGT1A3	Gene codificador da UGT1A3
VNTR	Polimorfismo de variação no número de repetições em tandem

ABSTRACT

Nuclear receptors are a group of ligand-activated transcription factors involved in a vast range of metabolic pathways, including lipid and xenobiotics metabolism. The general aim of this study was to investigate the association of genetic variants in the coding genes of nuclear receptors PPARα (*PPARA* rs1800206; L162V), RXRα (*RXRA* rs11381416), PXR (*NR1I2* rs1523130 and rs2472677), and CAR *(NR1I3* rs2307424 and rs2501873) with HMG-CoA reductase inhibitor pharmacogenetics and with lipid and lipoprotein levels, in South Brazilian individuals of European descent. The first study was conducted in a sample of 286 dyslipidemic patients submitted to simvastatin or atorvastatin treatment. In this study, heterozygous for the *NR1I2* rs1523130 polymorphism showed higher basal levels of total cholesterol (P=0.001) and LDL-cholesterol (P=0.001) than homozygous CC; homozygous AA for the *NR1I3* rs2501873 had a lower LDL-C reduction after statin treatment than G carriers (P=0.026); the heterozygote genotype for the *NR1I3* rs2307424 was the most frequent among the patients that developed adverse reaction to statin (myalgia/ elevation of creatine kinase/ alteration of hepatic function), and T homozygous was not observed in this group, where a significant difference in the genotypic distribution among patients that developed adverse reaction and control group patients was found (P=0.007). In the second study, we analyzed the influence of the polymorphisms on the lipid profile of 620 individuals from general population. Carriers of the A insertion of *RXRA*

rs11381416 polymorphism showed a higher serum triglyceride levels (P=0.020). When analyzed separately by gender, male carriers of the same allele of the *RXRA* rs11381416 also showed lower HDL-cholesterol levels (P=0.049), and male homozygous GG for the *NR1I3* rs2501873 polymorphism showed reduced levels of triglycerides (ANOVA, P=0.009). Among women, carriers of the G (V) allele of *PPARA* rs1800206 polymorphism showed higher LDL-cholesterol levels (P=0.037). In summary, our results show the association of the studied polymorphisms with lipid profile and statin pharmacogenetics, in addition to some gender specific influences on lipid profile; however, further studies should be done to confirm these associations.

Keywords: *PPARA*, *RXRA*, *NR1I2*, *NR1I3*, lipid and lipoprotein levels, HMG-CoA reductase inhibitor (statin) pharmacogenetics

RESUMO

Os receptores nucleares são um grupo de fatores de transcrição ligante-ativados envolvidos em uma vasta gama de vias metabólicas, incluindo vias de metabolização de lipídios e xenobióticos. O objetivo geral deste trabalho foi investigar a associação de variantes genéticas nos genes codificadores dos receptores nucleares PPARα (*PPARA* rs1800206; L162V), RXRα (*RXRA* rs11381416), PXR (*NR1I2* rs1523130 e rs2472677) e CAR *(NR1I3* rs2307424 e rs2501873) com a farmacogenética de inibidores da HMG-CoA redutase e com níveis de lipídios e de lipoproteínas , em indivíduos sul brasileiros descendentes de europeus. O primeiro estudo foi conduzido em uma amostra de 286 pacientes dislipidêmicos submetidos ao tratamento com sinvastatina ou atorvastina. Nesse estudo, heterozigotos para o polimorfismo *NR1I2* rs1523130 apresentaram níveis basais de colesterol total (P=0,001) e LDL-colesterol (P=0,001) mais elevados que homozigotos CC; homozigotos AA para o polimorfismo *NR1I3* rs2501873 tiveram uma menor redução nos níveis de LDL-colesterol após o tratamento com estatina do que indivíduos portadores do alelo G (P=0,026); o genótipo heterozigoto do polimorfismo *NR1I3* rs2307424 foi o mais frequente entre os pacientes que apresentaram reação adversa às estatinas (mialgia, elevação de creatina quinase plasmática, alteração da função hepática), e não foram observados homozigotos T nesse grupo de pacientes, sendo encontrada uma diferença significativa da distribuição genotípica entre os pacientes que desenvolveram efeitos adversos e o grupo de pacientes considerado controle (P=0,007). No segundo estudo,

analisamos a influência dos polimorfismos sobre o perfil lipídico de 620 indivíduos da população em geral. Portadores da inserção A do polimorfismo *RXRA* rs11381416 apresentaram maiores níveis séricos de triglicerídeos (P=0,020). Quando analisados separadamente por sexo, homens portadores do mesmo alelo do polimorfismo *RXRA* rs11381416 também apresentaram menores níveis de HDL-colesterol (P=0,049), e homens homozigotos GG para o polimorfismo *NR1I3* rs2501873 apresentaram níveis reduzidos de triglicerídeos (ANOVA, P=0,009). Entre as mulheres, portadoras do alelo G (V) do polimorfismo *PPARA* rs1800206 apresentaram maiores níveis de LDL-colesterol (P=0,037). Em resumo, nossos resultados demonstram a associação dos polimorfismos estudados com o perfil lipídico e farmacogenética de estatinas, além de algumas influências gênero-específicas sob o perfil lipídico; porém, estudos posteriores devem ser realizados para confirmar tais associações.

Palavras-chave: *PPARA*, *RXRA*, *NR1I2*, *NR1I3*, níveis lipídicos e de lipoproteínas, farmacogenética de inibidores da HMG-CoA redutase (estatinas)

1 INTRODUÇÃO

1.1 Doença Cardiovascular e Dislipidemia

A doença cardiovascular (DCV) é considerada a maior causa de morte na maioria dos países, e, no Brasil, doenças circulatórias são responsáveis por 32,01% da mortalidade (revisado por REDBERG et al.,2009; revisado por POLANCZYK e RIBEIRO, 2009; MINISTÉRIO DA SAÚDE/SVS - SISTEMA DE INFORMAÇÕES SOBRE MORTALIDADE). Entre os fatores de risco classicamente descritos para DCV estão: alterações no perfil lipídico, idade, sexo masculino, história familiar de doença cardíaca precoce, hipertensão, tabagismo, *diabetes mellitus*, obesidade, sedentarismo e dieta aterogênica (NCEP, 2002). Dentre eles, destacam-se níveis de lipoproteínas de baixa densidade (LDL-C: *low-density lipoprotein cholesterol*) elevados e níveis de lipoproteínas de alta densidade (HDL-C: *high-density lipoprotein cholesterol*) reduzidos, os quais são descritos como fatores de risco independentes (NCEP, 2002), e algumas evidências apontam níveis elevados de triglicerídeos (TG) também como potencial fator de risco (revisado por KANNEL e VASAN, 2009).

O perfil lipídico é considerado uma característica multifatorial, podendo ser influenciado tanto ambientalmente, quanto pela genética. Estudos demonstram uma alta herdabilidade dos níveis de HDL-C, LDL-C e TG, indicando a importância que variações genéticas podem ter sobre a determinação dos níveis de lipídios e

de lipoproteínas (KATHIRESAN et al., 2*007;* revisado por OBER, LOISEL e GILAD, 2008).

Uma ampla variedade de genes já foi estudada com relação à influência de suas variações sobre o perfil lipídico. Dentre eles, podemos citar o gene que codifica a apolipoproteína E (*APOE*), descrito como responsável por explicar de 4 a 15% da variabilidade dos níveis de LDL-C (KAMBOH et al.,1993), e também previamente associado a níveis de TG e colesterol total (CT). Apesar da vasta gama de polimorfismos e genes já avaliados nesse sentido, os resultados presentes na literatura não explicam a variabilidade de níveis lipídicos na sua totalidade.

Atualmente, está bem estabelecido que as diferenças entre homens e mulheres vão muito além das diferenças físicas. Ao investigarem 17 características quantitativas, Weiss et al. (2006) demonstraram que níveis de HDL-C e TG são dimórficos entre os dois sexos, e que níveis de LDL-C e HDL-C possuem interação com gênero na herdabilidade. Esses resultados sugerem que muitos genes ou variações genéticas podem desempenhar um papel diferente nos níveis lipídicos em ambos os sexos, e que o gênero é fator importante em estudos que avaliem tais variáveis.

1.2 Os inibidores da HMG-CoA redutase

Como já citado anteriormente, a dislipidemia é um fator de risco importante para a DCV. Dessa forma, o tratamento com terapia hipolipemiante é considerado uma abordagem central na prevenção primária e secundária da DCV (revisado por KOSTIS, 2007). Sabendo-se que dois terços do colesterol total de um indivíduo normal são sintetizados endogenamente a partir de precursores intracelulares, a inibição da biossíntese mostra-se como uma forma eficaz de redução dos níveis séricos de colesterol (revisado por MANZONI e ROLLINI, 2002). A biossíntese de colesterol inicia com reações de condensação de duas moléculas de acetil-CoA, levando a formação de 3-hidroxi-3-metilglutaril CoA (HMG-CoA), seguida pela conversão do HMG-CoA em ácido mevalônico. Esta última reação é catalisada pela HMG-CoA redutase, enzima etapa limitante de todo o processo que culmina com a formação do colesterol (revisado por MANZONI e ROLLINI, 2002).

Inibidores da HMG-CoA redutase são uma classe de moléculas originalmente obtidas do metabolismo secundário de fungos que possuem a propriedade de, como o próprio nome sugere, inibir a síntese de colesterol intracelular através de inibição competitiva da HMG-CoA redutase. Após a descoberta do potencial terapêutico destas moléculas na década de 70, outras substâncias com propriedades semelhantes foram descobertas e sintetizadas e, atualmente 7 inibidores da HMG-CoA redutase, ou estatinas, estão disponíveis farmacologicamente. Algumas estatinas são obtidas diretamente da fermentação

fúngica (como a lovastatina, originada de cultura de *Aspergillus terreus*; e a pravastatina, originada de *Nocardia autrophica*); outras são semi-sintéticas (como a sinvastatina – resultado da adição de um radical metil na estrutura da lovastatina); outras, ainda, são totalmente sintéticas (como atorvastatina, fluvastatina, rosuvastatina e pitavastatina) (revisado por ENDO, 1992; revisado por MANZONI e ROLLINI, 2002; revisado por CAMPO e CARVALHO, 2007; revisado por HUTZ e FIEGENBAUM, 2008).

A inibição da HMG-CoA redutase restringe a secreção de lipoproteínas contendo apoB e aumenta a expressão de receptores LDL na membrana plasmática dos hepatócitos, aumentando a remoção de partículas LDL do sangue e diminuindo os níveis plasmáticos de LDL-C (revisado por ENDO, 1992). Além de uma redução nos níveis de LDL-C entre 30 e 50%, estatinas são capazes de reduzir entre 5 e 30% os níveis de TG, e de elevar em 5-10% os níveis de HDL-C (revisado por SCHMITZ e LANGMANN, 2006). Adicionalmente ao efeito hipolipemiante, vários estudos demonstram que as estatinas podem reduzir o risco cardiovascular através de seus efeitos pleiotrópicos, diminuindo a inflamação, inibindo a proliferação de células do músculo liso e melhorando a função endotelial (revisado por LIAO, 2005). A redução significativa do risco de eventos cardiovasculares, da morbidade e da mortalidade, justifica o fato de que as estatinas estão entre os fármacos mais utilizados na medicina e são os mais prescritos para o tratamento da dislipidemia (revisado por KAJINAMI et al., 2004).

Apesar de possuírem mecanismo de ação similar, algumas diferenças farmacocinéticas são encontradas entre as estatinas. Com relação ao metabolismo hepático dos fármacos, a lovastatina, sinvastatina e atorvastatina são amplamente metabolizadas através das enzimas 3A4 e 3A5 do sistema citocromo p450 (CYP; CYP3A4 e CYP3A5), a fluvastatina é metabolizada por CYP2C9, a rosuvastatina tem mínima metabolização via CYP2C9/CYP2C19, e a pravastatina e pitavastatina interagem minimamente com as CYPs. Após a metabolização de fase I pelas enzimas CYPs, as estatinas são conjugadas por UDP-glucuronosiltransferases (UGTs), como as UGT1A1 e UGT1A3 (enzimas de fase II). Como transportadores (fase III) envolvidos, respectivamente, com o *uptake* hepático e intestinal e efluxo hepático das estatinas, podemos citar as proteínas transportadoras de ânions orgânicos (OATPs, codificados pelos genes *SLCO1B1* e *SLCO2B1*) e a glicoproteína-P (P-gp, codificada pelo gene *ABCB1*) (revisado por HUTZ e FIEGENBAUM, 2008; revisado por WILLRICH, HIRATA e HIRATA, 2009).

Ensaios clínicos demonstram que estatinas são fármacos bem tolerados e que efeitos adversos graves raramente acontecem. Diferentes efeitos adversos já foram relacionados à terapia com estatinas, porém toxicidades hepática e muscular são as mais importantes (revisado por KIORTSIS et al., *2007*). A incidência de toxicidade hepática varia entre 1 e 3%, sendo que as incidências de reações adversas musculares variam de acordo com a gravidade: 6,2-9,1% para mialgia; 0,1-1,8% para miopatia; e 0,1% para rabdomiólise (revisado por FARMER e TORRE-AMIONE, 2000; revisado por HAMILTON-CRAIG, 2001).

O real mecanismo envolvido no desenvolvimento de efeitos adversos não é completamente conhecido, porém fatores de risco incluem altas doses, idade avançada, sexo feminino, doenças multi-sistêmicas, co-administração com determinados fármacos ou polifarmácia, entre outros, além da variabilidade genética (revisado por SEWRIGHT, CLARKSON e THOMPSON, 2007).

1.3 Farmacogenética

Apesar de ter se popularizado nos últimos anos, a idéia inicial para o estudo da farmacogenética é datada de 1902, quando Garrod propôs que fármacos sofriam biotransformação da mesma maneira que os substratos endógenos, e que defeitos nesta rota poderiam alterar sua concentração e ação. Foi na década de 1940, com a observação de efeitos adversos ocasionados pela utilização de alguns fármacos, que as observações clínicas de que características genéticas poderiam afetar o efeito dos fármacos começaram a ser documentadas. Porém, apenas a partir da década de 1980 as bases moleculares das diferenças farmacogenéticas começaram a ser esclarecidas; e, na década de 1990, os avanços em pesquisa molecular começaram a elucidar os mecanismos dos efeitos farmacológicos e das diferenças genéticas (HUTZ e FIEGENBAUM, 2004).

A farmacogenética consiste no estudo da variabilidade genética que está associada a uma variação na resposta a medicações. As pesquisas farmacogenéticas envolvem a procura por polimorfismos genéticos em genes que

influenciem a resposta ao tratamento, os denominados genes candidatos (HUTZ e FIEGENBAUM, 2004).

Polimorfismos são definidos como variações na seqüência de DNA que ocorrem com uma freqüência superior a 1% na população. Os polimorfismos podem ser classificados como: deleções; inserções; duplicações; variações no número de repetições em tandem (*Variable Number of Tandem Repeats* – VNTRs); e variações em uma única base (*Single Nucleotide Polymorphism* – SNPs), os quais representam a grande maioria dos polimorfismos humanos (HUTZ e FIEGENBAUM, 2004). Segundo revisado por Pang et al. (2009), dados atuais sugerem que polimorfismos genéticos podem responder por 15 e 30% da variabilidade em resposta à fármacos.

Genes candidatos são aqueles que codificam proteínas/enzimas que influenciam a farmacocinética (absorção, transporte, biotransformação e excreção) ou a farmacodinâmica (proteínas alvo para a ação) do tratamento; ou, ainda, genes que são envolvidos na condição da doença subjacente, podendo determinar uma suscetibilidade maior ou menor para resposta à medicação (revisado por KAJINAMI et al., 2004).

A farmacogenética baseada no estudo de variantes de genes codificadores de enzimas metabolizadoras de fármacos está bem estabelecida e existe uma ampla literatura disponível avaliando a funcionalidade *in vitro* e clínica de polimorfismos nesses genes (revisado por JOHNSON, 2003). Considerando que a

maioria dos fármacos é metabolizada hepaticamente por CYPs, inclusive as estatinas, rapidamente compreendemos a importância que estudos sobre variabilidade genética dos genes que as codificam podem representar. Segundo revisado por JOHNSON e LIMA (2003), o estudo de farmacogenética baseado no estudo de genes codificadores de proteínas envolvidas na resposta aos fármacos e seus receptores começou a ser realizado apenas no final da década de 90, mas está em constante ascensão. Além dos receptores acoplados à proteína G, que têm sido bastante estudados em função da sua importância como alvos protéicos de mais de 50% dos fármacos, outros receptores são alvos de estudos farmacogenéticos (revisado por JOHNSON e LIMA, 2003). Dentre esses receptores, podemos citar os receptores nucleares, que são envolvidos em diversas vias de metabolização e capazes de estimular a expressão de múltiplos genes, e que serão melhor discutidos em seção posterior da presente dissertação.

1.3.1 Farmacogenética dos inibidores da HMG-CoA redutase

A ampla utilização de estatinas torna essa classe de fármacos um grande alvo para estudos farmacogenéticos. Embora sejam na maioria das vezes altamente efetivas e geralmente bem toleradas, variações interindividuais com relação à eficácia e segurança ocorrem, sendo que a análise de polimorfismos genéticos pode auxiliar na determinação da predisposição ao desenvolvimento de

efeitos adversos ou a ausência de resposta farmacológica (revisado por SEWRIGHT, CLARKSON e THOMPSON, 2007).

Muitos genes já foram estudados com relação à melhoria do perfil lipídico em resposta ao tratamento com estatinas, e alguns artigos de revisões compilaram os resultados disponíveis na literatura (HUMPHRIES e HINGORANI, 2006; SCHMITZ e LANGMANN, 2006; HUTZ e FIEGENBAUM, 2008). Como exemplos desses genes avaliados, podemos citar: *HMGCR* (HMG-CoA redutase), *LDLR* (receptor de LCL-C), *CETP* (proteína transferidora de ésteres de colesterol), *APOB*, *APOE*, *APOA1, ABCB1, LPL* (lipoproteína lipase)*, CYP3A4*, *CYP3A5*, *CYP2D6*, *CYP2C9*, *CYP7A1* (7-α hidroxilase de colesterol), entre muitos outros. O trabalho realizado por Chasman et al. (2004) é considerado um dos que fornece dados mais consistentes entre os estudos descritos na literatura sobre o assunto; nele, ao estudarem 148 SNPs em 10 genes candidatos, os autores encontraram 2 polimorfismos intrônicos no gene *HMGCR*, e em desequilíbrio de ligação, relacionados com uma menor redução dos níveis de CT e LDL-C após o tratamento com pravastatina 40mg/dia por 24 meses, ou seja, com uma menor eficácia do tratamento.

O número de estudos realizados visando à avaliação da influência de polimorfismos genéticos sobre o desenvolvimento de efeitos adversos decorrentes do tratamento com estatinas, porém, não é tão extenso (revisado por HUMPHRIES e HINGORANI, 2006; revisado por SCHMITZ e LANGMANN, 2006; revisado por ROSENDO et al., 2007). Como exemplo, podemos citar o trabalho

desenvolvido por Fiegenbaum et al. (2005), no qual parte da amostra de pacientes sob tratamento com sinvastatina (20mg/dia) analisada na presente dissertação, foi analisada quanto a eficácia da medicação e ao aparecimento de efeitos adversos (mialgia, constipação, flatulência, dispepsia, náusea, dor abdominal e níveis elevados de transaminases), associando esses parâmetros a 5 polimorfismos genéticos (3 no gene *ABCB1*, 1 no gene *CYP3A4* e outro no gene *CYP3A5*). Estudar fatores predisponentes genéticos ao aparecimento de efeitos adversos a estatinas, mesmo para os efeitos mais brandos, é de suma importância, visto que o aparecimento de tais efeitos pode levar pacientes em alto risco a suspenderem o uso da medicação.

1.4 Receptores Nucleares

Os receptores nucleares são uma superfamília de fatores de transcrição que regulam a expressão de múltiplos genes envolvidos em diversas redes metabólicas. Inicialmente descritos como receptores nucleares órfãos em função da falta de conhecimento sobre suas funções fisiológicas, essa família já conta com mais de 50 receptores identificados em várias espécies (revisado por GIGUÉRE, 1999).

Baseado em suas sequências de aminoácidos, os receptores nucleares podem ser subdivididos em 6 subfamílias (NR1 a 6) (revisado por WOODS, HEUVEL, RUSYN, 2007). De acordo com suas propriedades farmacológicas e

resposta a ligantes, esses receptores podem ser classificados em 3 grandes grupos: receptores de alta afinidade (que se ligam com alta afinidade a hormônios endógenos e vitaminas; por exemplo, NR2B1); receptores nucleares de baixa-afinidade (que se ligam a uma grande variedade de metabólitos intermediários como ácidos graxos, derivados de colesterol, e xenobióticos; por exemplo NR1C1, NR1I2 e NR1I3); e os receptores que pertencem a mesma superfamília mas exercem suas funções independentemente de qualquer ligante (revisado por BERKENSTAM e GUSTAFSSON, 2005).

Apesar de diferentes, os receptores nucleares caracterizam-se, de uma forma geral, pela presença de 3 domínios na sua estrutura: domínio de transativação; domínio de ligação ao DNA (DBD: *DNA-binding domain*) na região N-terminal, através do qual o receptor se liga a sequências de DNA específicas; e, domínio de ligação ao ligante (LBD: *ligand-binding domain*) na região C-terminal, que possui a propriedade essencial de reconhecimento do ligante e confere especificidade e seletividade às respostas fisiológicas (revisado por WOODS, HEUVEL, RUSYN, 2007).

Nas seções que seguem, os receptores nucleares alvo deste trabalho serão explorados.

1.4.1 PPARα

Os receptores ativados pelo proliferador de peroxissomos (PPARs: *peroxissome proliferator-activated receptors*; NR1C) possuem os subtipos PPAR alfa (PPARα; NR1C1), PPAR delta (também conhecido como PPAR beta – PPARδ/β; NR1C2) e PPAR gama (PPARγ; NR1C3). Embora possuam similaridades na estrutura protéica, são codificados por genes diferentes e possuem distribuição tecidual, papéis fisiológicos e especificidades distintas. O PPARα é relativamente bem expresso em tecidos que possuem uma alta taxa metabólica como o fígado, rins, coração, mucosa intestinal, músculo esquelético; e regula a expressão de vários genes envolvidos no metabolismo da glicose, no metabolismo lipídico, na resposta inflamatória e na homeostase energética (revisado por MANDARD, MÜLLER e KERSTEN, 2004).

Os PPARs são fatores de transcrição ligante-ativados e regulam a expressão de genes que possuem um elemento de resposta ao PPAR (PPRE: *PPAR response element*) na sua região promotora. O PPRE consiste de uma repetição direta da seqüência AGGTCA espaçada por uma ou duas bases; já o PPRE seletivo para o PPARα é caracterizado pela seqüência 5'UTR C(C/G)(A/G)A(A/T)(C/T) (revisado por NEVE, FRUCHART e STAELS, 2000). A ativação da transcrição de seus genes alvos, porém, somente ocorre após a ligação do PPAR ao seu agonista (naturalmente ácidos graxos ou eicosanóides) e formação de um heterodímero com um dos outros membros da mesma família de

receptores nucleares, os receptores retinóide X (RXRs: *retinoid X receptor*; NR2B). No caso do PPARα, a heterodimerização ocorre especificamente com o RXR alfa (revisado por MANDARD, MÜLLER e KERSTEN, 2004).

O PPARα é codificado pelo gene *PPARA,* que fica localizado no braço longo do cromossomo 22 (22q12-q13.1). Diversos polimorfismos foram descritos neste gene, porém, um é especialmente interessante: o polimorfismo *PPARA* rs1800206 é uma mutação de sentido trocado causada pela transversão de um nucleotídeo citosina para um nucleotídeo guanina (C>G) na posição 484 do exon 6, levando a uma modificação de um aminoácido leucina (L) para valina (V) na posição 162 da proteína (mais conhecido como polimorfismo L162V). Essa variante fica localizada no DBD do PPARα, e estudos realizados *in vitro* demonstraram que o alelo V162 codifica uma proteína cuja habilidade de ativar transcrição está alterada (FLAVELL et al., 2000; SAPONE et al., 2000). Segundo Flavell et al. (2000), o alelo V162 está associado a um aumento da ativação transcricional em comparação ao alelo L162. Porém, apenas Sapone et al. (2000) demonstraram que essa atividade é dependente da concentração do agonista a que as células estão expostas.

Embora seja o polimorfismo mais amplamente estudado no gene *PPARA,* os resultados disponíveis na literatura com relação à influência de suas variantes sobre o perfil lipídico não são homogêneos. A maioria dos estudos demonstra associação do alelo V162 com um perfil lipídico prejudicial e, dentre eles, alguns podem ser citados: em 2000, Vohl et al. sugeriram uma associação entre o alelo

V162 e maiores níveis de LDL-C e apoB; entre pacientes diabéticos do tipo II, Flavell et al. (2000) demonstraram a associação do alelo V162 com níveis maiores de CT, HDL-C e apoA-I, e Lacquemant et al. (2000) associaram o mesmo alelo com níveis elevados de CT e apoB; em 2006, Tai et al. associaram o alelo V162 com níveis reduzidos de HDL-C e apoA-I.; Manresa et al. (2006) encontraram associação entre o alelo V162 e concentrações menores de HDL-C, maiores taxas de CT/HDL-C e de log TG/HDL-C; em 2007, Sparso et al. demonstraram associação do alelo V162 com níveis elevados de TG, e a homozigozidade do alelo foi associada com maiores concentrações de CT.

Outros pesquisadores, porém, não encontraram associações do polimorfismo L162V com o perfil lipídico, ou, ainda, encontraram associações opostas às descritas acima; além disso, algumas diferenças entre os sexos foram demonstradas em alguns estudos. Em uma amostra de indivíduos normolipidêmicos, Tai et al. (2002) encontraram uma associação entre o alelo V162 e maiores níveis de CT, de LDL-C e de apoC-III apenas em homens, e esse mesmo alelo foi também associado a maiores níveis de apoB em ambos os sexos; adicionalmente, a associação do L162V com os níveis de LDL-C foi maior naqueles indivíduos portadores do alelo E*2 do gene *APOE*. Avaliando pacientes ateroscleróticos, Khan et al. (2004) associaram o alelo V162 a menores níveis de TG entre mulheres, usuárias ou não de estatinas; a mesma associação foi encontrada entre homens usuários de estatinas, porém a associação oposta foi encontrada em homens que não estavam sob o tratamento. Em 2007, Uthurralt et

al. demonstraram a presença do alelo V162 fortemente associada com níveis elevados de TG e níveis reduzidos de HDL-C em adultos jovens saudáveis do sexo masculino, porém o mesmo não foi encontrado em mulheres. Do et al. (2009), relataram níveis reduzidos de TG na presença do alelo V162 em amostra de homens que foram seguidos durante 5 anos e se mantiveram sem o desenvolvimento de DCV. Recentemente, Chen et al. (2010) analisaram a influência de polimorfismos no *PPARA* sobre o risco cardiovascular em uma população brasileira de idosos, não encontrando associação entre o polimorfismo L162V e os níveis lipídicos e de lipoproteínas.

O PPARα intermedia a atividade dos fibratos, outra classe de fármacos hipolipemiantes. Segundo revisado por Paumelle e Sataels (2008), a similaridade de certos efeitos das estatinas e fibratos no metabolismo de lipoproteínas e no processo inflamatório revela que o PPARα pode ser um mediador comum desses efeitos. Ao melhor do nosso conhecimento, apenas Chen et al. (2004) avaliaram a influência do polimorfismo L162V do gene PPARA sobre a resposta à estatina (fluvastatina), e não foi encontrada associação entre os genótipos e os níveis de lipídios e lipoproteínas, basais ou após tratamento.

1.4.2 RXRα

Existem duas famílias de receptores de retinóides (vitamina A e seus derivados), os receptores de ácido retinóico (RARs: *retinoic acid receptors*; NR1B) e os receptores retinóide X (RXRs: *retinoid X receptors*). Os RXRs exercem papel importante no desenvolvimento e na regulação dos processos metabólicos e da fisiologia adulta, e 3 subtipos desses receptores foram identificados em mamíferos: RXR alfa (RXRα; NR2B1); RXR beta (RXRβ; NR2B2); e RXR gama (RXRγ; NR2B3) (revisado por SZANTO et al., 2004). Os RXRs são considerados receptores promíscuos, uma vez que, além de exercerem funções como homodímeros, podem participar da formação de heterodímeros com diversos outros receptores nucleares, o que permite com que possuam efeito sobre várias vias biológicas.

Segundo revisado por Ahuja et al. (2003), através da ativação de complexos de receptores nucleares, os RXRs funcionam como reguladores-chave do metabolismo da glicose, de ácidos graxos e do colesterol, assim como de desordens metabólicas como *diabetes mellitus* tipo II, dislipidemia e aterosclerose. Além da formação de heterodímero com PPARs, como anteriormente citado, os RXRs participam da formação de heterodímeros com outros receptores, como por exemplo o receptor pregnano X (PXR: *pregnane X receptor*) e o receptor constitutivo de androstano (CAR: *constitutive androstane receptor*), sendo também essenciais para que as funções deles sejam exercidas.

O RXRα tem maior expressão em órgãos como fígado, rins, baço, placenta e epiderme (revisado por SZANTO et al., 2004), e é codificado pelo gene *RXRA* localizado no braço longo do cromossomo 9 (9q34.3). Entre outros polimorfismos descritos no *RXRA*, o rs11381416 corresponde a uma mutação causada pela inserção de um nucleotídeo adenina (A) no intron 8 do gene. Até onde alcança o nosso conhecimento, apenas Vasku et al. (2007) estudaram esse polimorfismo em pacientes com duas formas de psoríase, e nenhum estudo foi realizado avaliando a influência dessa variante genética sobre a função ou expressão do RXRα.

Durante a revisão de literatura realizada, encontramos apenas mais um estudo que investigou variantes no gene *RXRA,* evidenciando a escassez de pesquisas sobre o assunto. Nesse estudo, Kölsch et al. (2009) sugerem a influência de outros polimorfismos no gene *RXRA* sobre o risco de desenvolvimento da Doença de Alzheimer e sobre o metabolismo cerebral do colesterol.

1.4.3 PXR

Também conhecido como SXR (receptor de esteróides e xenobióticos; *steroid and xenobiotic receptor*), o receptor pregnano X (PXR: *pregnane X receptor*; NR1I2) foi primariamente descrito por Kliewer et al (1998) e recebeu essa nomenclatura justamente por poder ser ativado por pregnenolona e seus derivados (revisado por XIE et al., 2004). Esse receptor é expresso primariamente

no fígado, com alguma expressão nos tecidos testicular, gastrointestinal e embrionário (revisado por KAKIZAKI et al., 2008).

O PXR não é constitutivamente ativo e o antibiótico rifampicina está entre os seus ligantes mais bem conhecidos. A exposição ao fármaco leva à ativação do receptor presente no citoplasma, resultando na sua dissociação de proteínas citoplasmáticas retentoras e da proteína de choque térmico 90, e na translocação do PXR para o núcleo. No núcleo, acontece a heterodimerização do PXR com RXR, e a posterior ativação da expressão de *CYP3A* pela ligação do heterodímero ao módulo responsivo ao PXR (PREM: *PXR responsive enhancer module*) localizado na região promotora do gene (revisado por KAKIZAKI et al.,2008).

Apesar de mais comumente associado com a regulação dos genes *CYP3A* (*CYP3A4* e *CYP3A5*), outros genes envolvidos na metabolização de xenobióticos e componentes endógenos são também regulados por PXR, como: *CYP2B, CYP2C*, *SULTs* (sulfotransferases)*, GST*s (glutationa S-transferases) *UGT1A, ABCB1, ABCC2/3/4* (proteínas associada à resistência a múltiplas drogas, MRP2/3/4) *e SLCO2* (revisado por LAMBA, LAMBA e SCHUETZ, 2005; revisado por WOODS, HEUVEL, RUSYN, 2007; revisado por WADA, GAO e XIE, 2009). Diferentes xeno e endobióticos foram descritos como ativadores de PXR e as estatinas estão entre os fármacos que possuem a capacidade de ativar esse receptor (revisado por LAMBA, LAMBA e SCHUETZ, 2005; revisado por WILLRICH, HIRATA e HIRATA, 2009).

Além de ser considerado um regulador chave do *clearance* de fármacos, a influência do PXR sobre outros processos metabólicos foi mais recentemente descrita. Genes alvos do PXR também estão envolvidos com o metabolismo e excreção de alguns endobióticos como a bilirrubina, ácidos biliares e hormônios esteroidais. O PXR afeta a homeostase dos lipídios, a β-oxidação de ácidos graxos e a gliconeogênese por diferentes mecanismos que incluem, entre eles, a inibição do PPARα (revisado por WADA, GAO e XIE, 2009).

Zhang et al. (2001) são citados como os primeiros a identificar e caracterizar variantes na sequência do gene que codifica o PXR, o *NR1I2*. Esse gene fica localizado no braço longo do cromossomo 3 (3q12-q13.3) e diversos polimorfismos já foram descritos nessa região do genoma até o presente momento, sendo que a relevância funcional de alguns já foi estudada (revisado por LAMBA, LAMBA e SCHUETZ, 2005).

Em 2008, Lamba et al. analisaram novos SNPs na região promotora e no intron 1 do gene *NR1I2*, e avaliaram a influência dessas variantes genéticas sobre a expressão constitutiva e induzível por rifampicina de CYP3A4. Como resultado, os SNPs *NR1I2* rs1523130 e rs2472677 foram incluídos entre os 5 mais consistentemente associados com o fenótipo de CYP3A4, e correspondem, respectivamente, a uma transição de um nucleotídeo timina para citosina (T>C) na região promotora 5'UTR do *NR1I2*, e a uma transição de um nucleotídeo timina para citosina no intron 1 do gene. Com base na evidência de influência destes genótipos, esses SNPs são alvos de estudo em potencial.

1.4.4 CAR

O CAR foi inicialmente denominado como receptor constitutivamente ativo (CAR: *constitutive active receptor*) por ter a capacidade de ativar a transcrição de determinados genes na ausência de ligantes. Posteriormente, dois ligantes endógenos, androstanol e androstenol, foram identificados como agonistas inversos desse receptor, o que fez com que ele fosse renomeado como receptor constitutivo de androstano (CAR: *constitutive androstane receptor*; NR1I3) (revisado por LAMBA, LAMBA e SCHUETZ, 2005).

O anti-epilético fenobarbital e o xenobiótico 6-(4-clorofenil)imidazo[2,1-b][1,3]tiazol-5-carbaldeído-O-3,4-diclorobenzil)oxima (CITCO) são os ativadores de CAR em humanos melhor conhecidos e mais utilizados em pesquisas. Outros ativadores e repressores de CAR já foram descritos, e estatinas também estão entre os fármacos que parecem ativá-lo (revisado por LAMBA, LAMBA e SCHUETZ, 2005; revisado por WILLRICH, HIRATA e HIRATA, 2009).

O CAR pode estar expresso no núcleo, onde está constitutivamente ativo, ou estar retido no citoplasma da célula ligado a proteínas de choque térmico e apto a responder a sinais citoplasmáticos, e essa propriedade torna o CAR único entre os receptores da subfamília NR1I (revisado por XIE et al., 2004). Como modelo de gene alvo para ativação através do CAR, temos o da subfamília CYP2B e, segundo revisado por Kakizaki et al. (2008), no caso de uma indução por fenobarbital o mecanismo é semelhante ao descrito para o PXR. Como diferenças,

temos a translocação do receptor para o núcleo dependentemente de uma proteína fosfatase (PP2A) e a expressão de *CYP2B* pela ligação do heterodímero CAR/RXR a módulo específico (PBREM: *phenobarbital responsive enhancer module*) na região promotora do gene alvo.

Também semelhantemente ao PXR, a ativação de expressão gênica pelo CAR não está restrita a uma família de genes. Dentre os múltiplos genes regulados pelo CAR e envolvidos nas vias de metabolização de xeno e endobióticos, podemos citar: *CYP2B6*, *CYP2B10, CYP2C*, *CYP3A4*, *GST*s, *SULTs*, *UGT1A1*, *ABCB1*, e *ABCC2/3/4* (revisado por LAMBA, LAMBA e SCHUETZ, 2005; revisado por WOODS, HEUVEL, RUSYN, 2007; revisado por WADA, GAO e XIE, 2009).

A influência do receptor CAR igualmente não se restringe às enzimas de metabolização/transportadores de fase I, II e III. Segundo revisado por Wada, Gao e Xie (2009), o receptor CAR também parece ter um complexo papel no metabolismo energético através da inibição da lipogênese, da β-oxidação de ácidos graxos e da gliconeogênese, e diferentes mecanismos foram descritos como sendo os responsáveis por essas atividades. Um estudo recentemente publicado demonstrou que o CAR regula os níveis de triglicerídeos sob condições de estresse metabólico, e que alguns efeitos metabólicos do receptor são mediados pela regulação negativa que possui sobre a atividade do PPARα (MAGLICH, LOBE e MOORE, 2009). Assim como o PXR, genes alvos do CAR também estão envolvidos com o metabolismo e excreção de alguns endobióticos

como a bilirrubina, ácidos biliares e hormônios esteroidais (revisado por WADA, GAO e XIE, 2009).

O CAR é expresso primariamente no fígado e no rim, com alguma expressão no coração, no cérebro e nos tecidos gastrointestinais (revisado por KAKIZAKI et al.,2008). O gene que o codifica, *NR1I3*, fica localizado no braço longo do cromossomo 1 (1q23.3) e é composto de 9 exons: exons 2, 3 e 4 codificam o DBD; exons 4 a 8 e uma porção 5' do exon 9 codificam o LBD, (revisado por IKEDA et al., 2003). Em 2003, Ikeda et al. identificaram 26 novos polimorfismos no gene *NR1I3*, 3 deles em regiões codificadoras do gene (1 SNP não sinônimo e 2 SNPs sinônimos), e descreveram suas freqüências em indivíduos japoneses. Posteriormente, em 2005, Ikeda et al. identificaram mais 3 SNPs não sinônimos localizados no LBD e, a partir do estudo dos 4 polimorfismos não sinônimos por eles identificados, demonstraram a funcionalidade de 2 variantes com relação a transativação e/ou resposta ao CITCO.

A baixa freqüência dos 4 polimorfismos acima citados, porém, dificulta a realização de estudos de associação como os desenvolvidos na presente dissertação. Dentre as variantes descritas por Ikeda et al. (2003) e com maior frequência na população, podemos citar o polimorfismo *NR1I3* rs2307424: uma transição de um nucleotídeo citosina para um nucleotídeo timina (C>T), localizada no códon 180 do exon 5, e que corresponde a uma mutação sinônima, não alterando o aminoácido prolina naquela posição (P180P). Ao que sabemos, esse

SNP não teve sua funcionalidade testada, porém a sua localização também no LBD é um incentivo para estudos a seu respeito.

Em 2007, Tham et al. avaliaram a influência de alguns polimorfismos em determinados genes sobre o *clearance* de docetaxel, um antineoplásico utilizado no tratamento do câncer de mama. Embora não significativa, existiu uma sugestão de que o genótipo heterozigoto do polimorfismo *NR1I3* rs2307424 tem um efeito negativo no *clearance* da medicação.

Outro polimorfismo descrito no gene *NR1I3* é a transição de um nucleotídeo adenina para um nucleotídeo guanina (A>G) no intron 3. Em nossa revisão da literatura, não encontramos estudos avaliando nem a funcionalidade nem a influência desse polimorfismo *NR1I3* rs2501873 sob qualquer fenótipo, sendo esse um alvo de estudo a ser explorado.

2 JUSTIFICATIVA

A DCV é a principal causa de morte no Brasil e no mundo, e a dislipidemia, uma característica multifatorial, é considerada um importante fator de risco (MINISTÉRIO DA SAÚDE/SVS - SISTEMA DE INFORMAÇÕES SOBRE MORTALIDADE; revisado por POLANCZYK e RIBEIRO, 2009; revisado por REDBERG et al., 2009; NCEP, 2002). Devido à importância dos níveis lipídicos sobre o risco para DCV, terapias hipolipemiantes são amplamente utilizadas para prevenção desta doença, e dentre os medicamentos prescritos para o tratamento da dislipidemia destacam-se as estatinas (revisado por KOSTIS, 2007). Embora na maioria das vezes altamente efetivas e geralmente bem toleradas, existem variações interindividuais com relação à resposta hipolipemiante e ao desenvolvimento de efeitos adversos frente a essa medicação, podendo essa variabilidade ser também influenciada por fatores ambientais ou genéticos (revisado por SEWRIGHT, CLARKSON e THOMPSON, 2007). Dessa forma, fica clara a importância da determinação de variantes genéticas que influenciem tanto os níveis de lipídios e de lipoproteínas, quanto à farmacogenética das estatinas, considerando a sua eficácia e segurança.

De acordo com o que foi descrito na Introdução desta dissertação, o envolvimento dos receptores nucleares em diferentes vias metabólicas, no metabolismo e na homeostase de lipídios, e na metabolização de xenobióticos, torna os genes *PPARA*, *RXRA*, *NR1I2* e *NR1I3*, alvos de estudo deste trabalho,

bons candidatos para investigação da influência de suas variantes tanto sobre o perfil lipídico basal, quanto sobre a resposta hipolipemiante das estatinas e o desenvolvimento de efeitos adversos a essa medicação.

Ao melhor do nosso conhecimento, apesar de inúmeros trabalhos terem avaliado a influência da variabilidade de diferentes genes sobre os aspectos aqui investigados, à exceção do polimorfismo no gene *PPARA*, as associações por nós pesquisadas são inéditas. Somado a esse fato, a inexistência de estudos que tenham sequer descrito as freqüências alélicas e genotípicas dos polimorfismos investigados na população do sul do Brasil torna ainda maior a relevância desse trabalho.

3 OBJETIVOS

3.1 Objetivo Geral

Investigar a influência de variantes genéticas nos genes dos receptores nucleares PPARα, RXRα, PXR e CAR, sobre os níveis de lipídios e de lipoproteínas e sobre a farmacogenética de inibidores da HMG-CoA redutase, em indivíduos sul brasileiros descendentes de europeus e habitantes da região metropolitana de Porto Alegre.

3.2 Objetivos Específicos

a) Descrever as freqüências alélicas, genotípicas e haplotípicas (quando possível) dos polimorfismos *PPARA* rs1800206, *RXRA* rs11381416, *NR1I2* rs1523130 e rs2472677, e *NR1I3* rs2307424 e rs2501873 em duas amostras de indivíduos sul brasileiros descendente de europeus.

b) Investigar a associação dos polimorfismos *PPARA* rs1800206, *RXRA* rs11381416, *NR1I2* rs1523130 e rs2472677, e *NR1I3* rs2307424 e rs2501873 com o perfil lipídico basal em amostra de pacientes dislipidêmicos antes do início do tratamento com os inibidores de HMG-CoA redutase, sinvastatina ou atorvastatina.

c) Investigar a associação dos polimorfismos *PPARA* rs1800206, *RXRA* rs11381416, *NR1I2* rs1523130 e rs2472677, e *NR1I3* rs2307424 e rs2501873 com a resposta hipolipemiante aos inibidores de HMG-CoA redutase, sinvastatina ou atorvastatina em amostra de pacientes submetidos a esse tratamento.

d) Investigar a associação dos polimorfismos *PPARA* rs1800206, *RXRA* rs11381416, *NR1I2* rs1523130 e rs2472677, e *NR1I3* rs2307424 e rs2501873 com a ocorrência de efeitos adversos aos inibidores de HMG-CoA redutase, sinvastatina ou atorvastatina em amostra de pacientes submetidos a esse tratamento.

e) Investigar a associação dos polimorfismos *PPARA* rs1800206, *RXRA* rs11381416, *NR1I2* rs1523130 e rs2472677, e *NR1I3* rs2501873 com o perfil lipídico em amostra de indivíduos da população geral.

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5 ARTIGOS

Os resultados obtidos nesse trabalho serão apresentados na forma de dois artigos científicos. Os artigos estão em fase de preparação e foram devidamente formatados seguindo as orientações aos autores de cada uma das revistas às quais serão submetidos para publicação (Anexos A e B).

5.1 Artigo 1

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Influence of *PPARA, RXRA, NR1I2* and *NR1I3* nuclear receptor gene polymorphisms in lipid levels, and lipid-lowering efficacy and safety of statin therapy

SHORT TITLE: Nuclear receptors, lipid levels and statin response

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Abstract

Nuclear receptors are a group of ligand-activated transcription factors involved in a vast range of metabolic pathways, including lipid and xenobiotic metabolisms. In this study, we investigated the influence of 6 genetic variations in 4 nuclear receptor genes on lipid and lipoprotein levels, and lipid-lowering efficacy and safety of simvastatin and atorvastatin therapy in 286 Brazilian dyslipidemic patients of European descent. The polymorphisms analyzed in *PPARA* (rs1800206; L162V) and *RXRA* (rs11381416) genes did not influence none of the variables analyzed. *NR1I2* rs1523130 polymorphism was associated with baseline total cholesterol and LDL-C levels, with homozygous CC having lower levels than heterozygous. Homozygous AA for *NR1I3* rs2501873 polymorphism had lower LDL-C reduction after statin treatment than G carriers, and the *NR1I3* rs2307424 genotypic distribution was different between subjects with and without adverse drug reaction (myalgia/ elevation of creatine kinase/ alteration of hepatic function). In summary, our results showed the influence of *NR1I2* genetic variant in lipid and lipoprotein levels and *NR1I3* genetic variants with statin pharmacogenetics. To our knowledge, this is the first study describing these associations, therefore additional studies are needed to confirm them.

Keywords: *PPARA*, *RXRA*, *NR1I2*, *NR1I3*, statin pharmacogenetics, lipid and lipoprotein levels

Introduction

In Brazil, as in most part of the world, cardiovascular disease (CVD) is the leading cause of death[1, 2]. Among the classical risk factors for CVD, dyslipidemia is considered important and lipid-lowering therapy is the central approach in the primary and secondary prevention of CVD[3]. The 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) function as competitive inhibitors for the rate-limiting enzyme in the cholesterol pathway biosynthesis and are the most prescribed drugs for dyslipidemia treatment and CVD prevention worldwide[4].

Although statins are most of the time highly effective and generally well tolerated, interindividual variations have been seen in relation to lipid-lowering efficacy and adverse effect occurrence. The same way that genetic variations may influence the plasma lipid and lipoprotein levels, gene polymorphisms are also related to these drug response differences and may account for 15-30% of this variability[5].

Nuclear receptors are a superfamily of more than 50 ligand-activated transcription factors directly involved with gene expression control in different metabolism pathways, and in response to a wide range of developmental, physiological, and environmental stimulus[6]. Peroxissome proliferator-activated receptor alpha (PPARα or NR1C1) regulates a variety of target genes involved in lipid and glucose metabolism, inflammatory response, and energy homeostasis[7].

To bind to DNA and regulate transcription of target genes, this nuclear receptor requires heterodimerization with the retinoid X receptor alpha (RXRα or NR2B1), another member of nuclear receptor superfamily. RXRα has effects in different pathways because of the ability to activate transcription of target genes as homodimers or as obligated partner of other nuclear receptors, as pregnane X receptor (PXR or NR1I2) and constitutive androstane receptor (CAR or NR1I3)[6, 8]. PXR and CAR were originally identified as xenosensors that regulate the expression of drug-metabolizing enzymes/transporters (Phase I, II and III); however, recent studies have shown that they also affect other metabolic pathways as lipid homeostasis and metabolism[9].

Considering the influence of nuclear receptors in lipid metabolism and in recognizing/metabolizing xenobiotic compounds, we hypothesized that genetic variant in genes encoding PPARα, RXRα, PXR, and CAR nuclear receptors could contribute to the interindividual variability in plasma lipid and lipoprotein levels and statin pharmacogenetics. The aim of the present study was to evaluate the association of *PPARA* rs1800206, *RXRA* rs11381416, *NR1I2* rs1523130 and rs2472677, and *NR1I3* rs2307424 and rs2501873 polymorphisms with lipid and lipoprotein levels, and with lipid-lowering efficacy and safety of simvastatin and atorvastatin treatment in a Brazilian population of European ancestry.

Materials and methods

Study subjects and protocol

Two hundred and eighty six Brazilian dyslipidemic patients of European descent from a cardiovascular clinic in southern Brazil were investigated in a cohort study according to simvastatin or atorvastatin treatment. The exclusion criteria were the following: unstable or uncontrolled clinically significant diseases, uncontrolled hypothyroidism, and impaired hepatic or renal function. None of the patients was undergoing previous therapy with statin or other lipid-lowering drug. The statin therapy used (simvastatin or atorvastatin) and the doses administrated were determinated by the physician according to the clinical characteristics of each patient, and the patients remained on other medications throughout the study.

From the initial sample, 240 patients were analyzed for the lipid-lowering efficacy of the therapy, and had their lipid and lipoprotein concentration measured at baseline and after approximately 6 months (5.97 ± 2.44 months) of treatment. Ninety eight patients, who remained on the statin therapy for more than a year (37.70 ± 23.12 months) without presenting adverse drug reaction (ADR), were named as non-ADR group and had genotypes and allele frequencies compared with the 30 patients who developed adverse drug reactions (ADR group). Myalgia with or without elevated creatine phosphokinase serum level, elevation of creatine phosphokinase serum level, and alteration of hepatic function (ascertain by elevated hepatic aminotransferases serum levels) were defined as ADRs when the

patients presented these characteristics unrelated to other conditions. The adverse effects were assessed during clinical follow-up. Decisions about inclusion and exclusion of treatment were made without awareness of the genotypes. The ADRs and the criteria used in the assessment were fully described by Fiegenbaum *et al.*[10].

This protocol was approved by the Ethics Committee of Universidade Federal do Rio Grande do Sul, Universidade Federal de Ciências da Saúde de Porto Alegre, and Centro Universitário Metodista do IPA. A written and signed informed consent was demanded of every subject included in the study.

Biochemical analyses

Blood samples were collected from individuals after a 12-hour fasting. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), and glucose levels were determined by conventional enzymatic methods. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald *et al*.[11], when plasma triglycerides were below than 4.52 mmol/L. Glucose was measured by conventional methods.

DNA analyses

Genomic DNA was isolated from peripheral blood leukocytes by a standard salting out procedure[12].

Polymorphisms c.484C>G (rs1800206; NM/NP_001001928.1; p.Leu162Val) of *PPARA* gene and deletion/insertion polymorphism -/A (rs11381416) of *RXRA* gene were determined using PCR and restriction mapping (PCR-RFLP) as previously described[13, 14].

The polymorphism c.-1663T>C (rs1523130; NM_003889.3) of *NR1I2* gene was determined using an in-house designed PCR-RFLP. The amplification reactions used the following primers: forward 5'-GTCATGAGGATATTGGACCG-3' and reverse 5'-TAGCCATGGCCTTCTGATCT-3'. A mismatch was introduced in the forward primer to create an *Msp*I restriction site that surrounded the polymorphism. Both primers were designed using the Primer3 program (http://frodo.wi.mit.edu/primer3/), and primer specificity was validated against the human genome database of the National Center for Biotechnology Information with BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/). The amplification reactions were performed in final volume of 25 µL containing 0.2 mmol/L of dNTPs, 2.0 mmol/L of MgCl2, 0.4 µmol/L of each primer, 1 U of Taq DNA polymerase and 100 ng of genomic DNA. Samples were denatured at 94ºC for 5 minutes, followed by 35 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds, and 72ºC for 30 seconds, with a final extension at 72ºC for 5 minutes. The *Msp*I digestion resulted in 19 and 141

bp fragments for the G allele or in a single 159 bp fragment for the A allele, and the variants were genotyped using 8% polyacrylamide gel electrophoresis followed by visualization with ethidium bromide staining.

Polymorphisms c.-22-7659T>C (rs2472677; NM_003889.3) of *NR1I2* gene, and c.540C>T (rs2307424; NM_001077469.1; NP_001070937.1: p. Pro180Pro) and c.238+1099A>G (rs2501873; NM_001077469.1) of *NR1I3* gene were determined by allelic discrimination with Taqman 5'-nuclease assays. Genotyping for rs2472677 (ID: C_26079845_10), rs 2307424 (ID: C_25746794_20), and rs2501873 (ID: C_16033320_10) polymorphisms were performed with validated TaqMan genotyping assays (Real Time PCR, Applied Biosystems, California, USA).

For adjustment analytical purpose, the total sample was genotyped for *APOE* allele variants (E*2, E*3 and E*4) as previously described[15].

Statistical analyses

Continuous variables were expressed as mean ± standard deviation. Triglyceride levels were log-transformed before analyses because of their skewed distribution, although non-transformed values are shown in the Results section.

Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested

using chi-square tests. The Graph Pad InStat software version 2.04a (Graph Pad Software, San Diego, California, USA) was used to compare allele and genotype frequencies among groups by Fisher exact test and chi-square test, respectively. When appropriated, chi-square tests as described by Roff and Bentzen[16] (CHITEST.EXE software) were performed. Haplotype frequencies and linkage disequilibrium were estimated with the use of Multiple Locus Haplotype Analysis program (version 2.0; Long, JC (1999)) [17, 18] and ARLEQUIN software (version 3.1)[19]. Dmax (D theoretical maximum) and D' (the relative magnitude of D as compared to its theoretical maximum calculated as D/Dmax) values were calculated as described by Lewontin[20].

Because of lower homozygous genotype frequencies, rs1800206 genotypes of the *PPARA* gene were grouped as homozygous for C allele (CC) and carriers of G allele (C/G and G/G), and rs11381416 genotypes of the *RXRA* gene were grouped as homozygous without the A insertion (-/-) and carriers of the A insertion (-/A and A/A). Considering the possible difference in the lipid-lowering efficacy of simvastatin and atorvastatin therapy in different doses, we created a standardized statin dosage variable transforming the daily doses of simvastatin to equivalent doses of atorvastatin by using the dose equivalence ratio 2:1 for simvastatin:atorvastatin based on published side-by-side comparisons[21] also used by Kivisto *et al*.[22]

To determine the association of the genotypes and diplotypes with baseline lipid levels or response to statin treatment (mean percentage changes in plasma

lipid levels), means of each variable were compared by a General Linear Model using the type III sums of squares. This sum of squares applies to unbalanced study designs and quantifies the effect of an independent variable after adjustment for all other variables included in the model. Age, gender, smoking status, standardized statin dosage, treatment period (months), baseline lipid levels, and dummy variables for the presence of E*2 and E*4 *APOE* alleles (*APOE* dummy variables) were included in each model as covariates for the lipid-lowering response. For baseline lipid levels association analyses, age, gender, smoking status and *APOE* dummy variables were used as covariates. Patients with E*2/E*4 genotype were excluded from analyses. Pairwise comparisons among genotypes were performed by least significant difference with no adjustments. Statistical analysis was performed using the SPSS16.0 statistical package for Windows®.

Results

Characteristics of study population

The characteristics of the total sample, and separately for samples analyzed for lipid-lowering response, ADR and non-ADR groups participants of the present study are presented in Table 1. The total sample correspond of 286 patients aged between 25 and 88 years (62.23±10.68 years), and 68.2% were females. When the groups were compared, only the frequencies of CVD and the use of calcium channel blocker as concomitant therapy showed significant difference ($P<0.05$; CVD: non-ADR group are different from the total sample and patients analyzed for lipid-lowering response; use of calcium channel blocker: non-ADR group is different from the others three groups). All others characteristics did not differ between groups.

The genotype frequencies observed did not reveal statistically significant differences compared to those expected under Hardy-Weinberg equilibrium. The allele and genotype frequencies for the total sample are presented in Table 2. Haplotypic analysis showed that all four expected haplotypes for *NR1I2* (rs1523130 + rs2472677: T+C, T+T, C+T, and C+C) and *NR1I3* (rs2307424 + rs2501873: T+G, T+A, C+A, and C+G) genes were observed. For the *NR1I2* gene, the most frequent haplotypes were C+T and C+C (39.54% and 21.14%, respectively), while for the *NR1I3* gene, the haplotypes C+G and C+A were the most frequent (40.53% and 25.38%, respectively). Linkage disequilibrium was not

detected between the polymorphisms of *NR1I2* and *NR1I3* genes (D'=0.119, P=0.088 and D'=0.109, P=0.108, respectively).

Association between *PPARA, RXRA, NR1I2* and *NR1I3* polymorphisms and baseline lipid and lipoprotein levels

After adjustment for covariates, significant differences were found in TC and LDL-C baseline levels for *NR1I2* rs1523130 polymorphism. CT heterozygous had higher levels of total cholesterol (ANOVA: P=0.005; 6.78 ± 1.03 mmol/L and 6.26 ± 1.07 mmol/L, respectively, Multiple comparasions LSD test: P=0.001) and LDL-C (ANOVA: P=0.004; 4.65 ± 0.93 mmol/L and 4.15 ± 0.91 mmol/L, respectively, Multiple comparasions LSD test: P=0.001) than CC homozygous individuals. No association between the others polymorphisms and baseline mean lipid parameters were observed (data not shown).

Association between *PPARA*, *RXRA*, *NR1I2* and *NR1I3* polymorphisms and statin efficacy and safety

Table 3 shows the mean percent modifications of lipid and lipoprotein levels in all patients analyzed for lipid-lowering response according to polymorphisms genotypes. Overall, statin therapy reduced significantly the plasma levels of TC (-26.36%, P<0.001), LDL-C (-36.44%, P<0.001) and TG (-10.18%, P<0.001),

whereas the increase in HDL-C did not reach statistical significance (3.96%, P=0.321).

After adjustment for covariates, no statistically significant association of the *NR1I3* rs2501873 polymorphism and the LDL-C response to statin treatment was observed (P=0.084); however, when a recessive model was tested, a greater reduction of LDL-C was observed in patients carriers of G allele than AA homozygous (-37.63 ± 16.79 % versus -29.82 ± 21.66 %; P=0.026). No significant difference in lipid and lipoprotein reduction was observed among other polymorphism genotypes.

As showed in Table 4, significant difference was observed for *NR1I3* rs2307424 polymorphism between subjects with or without ADR (P=0.007). Among subjects in the ADR group, no homozygous for the T allele were observed. No other polymorphisms were significantly associated with ADR.

Diplotypes of *NR1I2* and *NR1I3* gene polymorphisms associations with lipid and lipoprotein levels and with statin response

Table 5 shows the baseline lipid and lipoprotein levels in total sample and mean percent reductions of lipid and lipoprotein levels in all patients analyzed for lipid-lowering response according to *NR1I2* (rs1523130 + rs2472677) and *NR1I3* (rs2307424 + rs2501873) diplotypes, respectively.

Considering the associations found between CC genotype of *NR1I2* rs1523130 polymorphism and TC and LDL-C baseline levels (Table 3), *NR1I2* diplotypes were divided as follow: presence C+C in both homologous chromosomes (C+C/C+C – diplotype 1); homozygous rs1523130 C independently of rs2472677 allele (C+C/C+T and C+T/C+T – diplotype 2); homozygous rs1523130 T independently of rs2472677 allele (T+C/T+C, T+C/T+T and T+T/ T+T – diplotype 3); others diplotypes (T+C/C+C, T+C/C+T and T+T/C+T – diplotype 4). Significant differences were found in TC and LDL-C baseline levels between diplotype groups (P=0.014 and P=0.011, respectively), individuals carriers of diplotype 4 had higher levels of TC and LDL-C than carriers of diplotype 2 (P=0.002 for TC and LDL-C).

Also considering previous association found between LDL-C response to statin treatment (Table 4), *NR1I3* genotypes were divided as follow: presence C+A in both homologous chromosomes (C+A/C+A – diplotype 1); homozygous rs2501873 A independently of rs2307424 allele (T+A/C+A and T+A/ T+A – diplotype 2); homozygous rs2501873 G independently of rs2307424 allele (T+G/T+G, C+G/C+G and T+G/C+G – diplotype 3); others diplotypes (T+G/T+A, T+A/C+G and C+G/C+A – diplotype 4). No statistical significant differences were found between diplotype groups.

Discussion

In this study, we examined the association between *PPARA* rs1800206, *RXRA* rs11381416, *NR1I2* rs1523130 and rs2472677, and *NR1I3* rs2307424 and rs2501873 polymorphisms and plasma lipid and lipoprotein baseline, and/or simvastatin and atorvastatin response, considering both the lipid-lowering efficacy and the adverse effect occurrence. The observed allele frequencies found in this study were similar to those reported in other European or European-derived population studies or in PubMed.

The *PPARA* rs1800206 polymorphism is a transversion substitution C>G at codon 162 of exon 6 that results in a missense mutation, leucine-valine (L162V). In the present study, no association was detected of this polymorphism with baseline lipid levels, and lipid-lowering efficacy and safety of statin therapy. Some studies have demonstrated association between G allele (V162) and harmful lipid profile[13, 23-29], others showed opposite findings or negative associations[30-33]. There also appears to be sexual dimorphism in relation to L162V polymorphism associations[25, 28, 30], but the relative moderate sample size in our study did not allow that this was tested. As in our study, Chen et al.[33] recently analyzed the influence of L162V polymorphism in Brazilian elderly population and also did not find association between genotypes and lipid and lipoprotein serum levels, however further investigation should be done to confirm this lack of association in Brazilian populations. To the best of our knowledge, only Chen et al.[31] investigated the association of this polymorphism with the lipid-lowering efficacy of statins, and

did not find association between L162V genotypes neither lipid baseline levels nor lipid lowering response to fluvastatin.

Even though RXRα is a promiscuous nuclear receptor that participates in different metabolic pathways, the influence of genetic variants in *RXRA* is poorly studied. The *RXRA* rs11381416 polymorphism is an adenine nucleotide insertion in intron 8 of gene. This genetic variation did not show influence in lipid levels or statin response in patients of the present study. To our knowledge, only Vasku et al.[14] analyzed the association of this polymorphism with two different psoriasis subtypes, and it is unknown if this polymorphism influence RXRα function or expression. Other genetic variants in *RXRA* were recently associated with Alzheimer's disease risk and cholesterol metabolism[34].

As reviewed by Lamba et al.[35] and Woods et al.[36], PXR and CAR receptors play a key role in xenobiotic sensing and detoxification. Multiple genes have been shown to contain ligand-binding motifs for the heteromiders formed between RXRα-PXR, and RXRα-CAR, and have shown to be directly regulated by these xeno/endo-sensors compounds receptors. Between these genes, we could cite the ones that codify: CYPs enzymes (CYP3A4/3A5, CYP2B, CYP2C); the phase II enzymes as glutathione–Stransferases (GSTs), UDP-glucuronosyltransferases (UGTs) and sulfotransferase (SULTs); and the transporters multi-drug resistance protein 1 (MDR1), organic anion transporter polypeptide (OATP2), and multi-drug resistance associated proteins (MRP2/3/4)[9,35,36]. *In silico* analyses also identified a large number of PXR targets on human genome, including genes involved in drug

metabolism/transport and involved in various cellular process[35]. In addition, statins were described as PXR and CAR activators[37]. Besides the influence of PXR and CAR in expression of drug-metabolizing enzymes and transports genes, they have been shown to affect lipid homeostasis. As reviewed by Wada et al.[9], some different mechanisms of action, and some different cross talks with other nuclear receptors (including PPARα) and proteins, have been described to explain the way that PXR and CAR influenced in energy homeostasis, lipogenesis, fatty acid β-oxidation, gluconeogenesis, and lipid levels.

Lamba et al.[38] demonstrated the association between novel polymorphisms in the promoter and intron 1 of the *NR1I2* gene and the CYP3A4 expression. Two of the five polymorphisms most consistently associated with CYP3A4 phenotypes were the *NR1I2* rs1523130, which correspond to a transition substitution T>C in the promoter region of the gene; and the *NR1I2 rs2472677*, a transition C>T in intron1. *In silico* analyses showed that these two polymorphisms define two clusters of informative SNPs (two different haplotype blocks) and are located in transcription factor binding sites[38]. In our study, *NR1I2* rs1523130 and rs2472677, polymorphisms did not shown to influence neither the lipid-lowering response nor the safety of simvastatin and atorvastatin treatment. Nevertheless the biologic plausibility of the influence of *NR1I2* polymorphisms in pharmacogenetic of statins is wide and should guide novel researches.

As cited above, the relation between PXR and energetic metabolism was more recently described, and something is known about the influence of *NR1I2*

polymorphisms in lipid levels. In our study, homozygous CC for *NR1I2* rs1523130 showed lower levels of TC and LDL-C than heterozygous. Analyzing the diplotypes results (Table 5), homozygous CC (diplotypes 1 and 2) had lower levels of TC and LDL-C than individuals with diplotypes 3 and 4 suggesting that it effect is independent of *NR1I2 rs2472677* genotype.

Another novel finding of our study was the association of *NR1I3* polymorphisms with statin treatment response. Despite the recognized importance of CAR nuclear receptor in xenobiotic/endobiotic metabolism, conjugation, and transport, not much is known about SNPs in the gene. Ikeda et al.[39, 40] have been described some polymorphisms in *NR1I3* and showed the functionality of two non-synonymous of them in transactivation and/or in response to a specific CAR agonist (CITCO); confirming that polymorphisms in this gene may have influence in receptor activity. In our study, however, we analyzed others polymorphisms with a larger frequency of variant occurrence. The *NR1I3* rs2501873 polymorphism is a transition substitution A>G in intron 3 of the gene, and *NR1I3* rs2307424 polymorphism is a transition substitution C>T at codon 180 of exon 5 and corresponds to a silent mutation, proline-proline (P180P), in the ligand-binding domain, the multifunctional domain that, as reviewed by Ikeda et al.[40], mediates, among other actions, the dimerization with RXRα, the interaction with co-ativator proteins, nuclear localization, and transactivation functions.

Our study did not find any significant association between *NR1I3* rs2307424 and rs2501873 polymorphisms and baseline plasma lipid and lipoprotein levels. To

our knowledge, the functionality of these polymorphisms were not tested, but our results encourage a study in this way, since G carriers of *NR1I3* rs2501873 showed higher LDL-C response to simvastatin and atorvastatin, the primary effect of statins, and none patient in the ADR-group was homozygous TT *NR1I3* rs2307424.

Clinical trials have been reported that statins are well tolerated and severe adverse effects rarely occur. However, some different ADRs have been related to statins and hepatic and muscle toxicity are most clinically important ones[41], exactly de ADRs analyzed in this study. Hepatic toxicity have been reported with an incidence of 1% to 3%, and the described incidence of muscular adverse reactions was 6.2% to 9.1% for myalgia, 0.1% to 1.8% for myophathy, and approximately 0.1% for rhabdomyolysi[42, 43]. Although the majority of muscle complaints of statins are clinically benign and tolerable, studies about ADR predisposition are important to increase the life-quality of patients, and because the discomfort may motivate some high-risk patients to stop the treatment[44].

Advanced age, female gender, multisystem diseases and multiple and/or concomitant medications have been described as risk factors for statins ADRs[44]. In our study, comparison of clinical characteristics between ADR and non-ADR groups (Table 1) have shown that these risk factors do not appear to confound the genetic influence of the *NR1I3* rs2307424 polymorphism, since the groups are similar and is the non-ADR group that has more patients using calcium channel blocker.

There were some limitations to our study. First, our investigation addressed the effect of only one or two polymorphisms per gene, and just took into account the APOE genotypes as covariate between all polymorphisms previously related to the variables analyzed. Second, although we have created a standardized statin dosage variable to equate the differences between treatments efficacy, this could not be done to adverse effects analyses and might be a confound variable. Third, due the moderate sample size of the sample analyzed for adverse effects, we could not exclude the possibility of a type II error in the results. Despite these limitations, and although our findings require confirmation in larger and in different populations, they suggest candidate polymorphisms for association with lipid levels and for statins pharmacogenetics studies.

In summary, our study demonstrates that, in a Brazilian population of European descent, *NR1I2* rs1523130 polymorphism may influence baseline lipid levels, *NR1I3* rs2501873 polymorphism may modify the lipid-lowering response, and *NR1I3* rs2307424 polymorphism may influence adverse reactions to simvastatin and atorvastatin, and should be considered as genetic contributors to the interindividual differences. To our knowledge, this is the first study describing these associations; therefore addition studies are warranted to confirm them. Pharmacogenetic studies are an opportunity for a safer and more efficient pharmacotherapy, and although our study does not pretend to explain all the variability determinate by genetic variants, it is an effort to help in this research area.

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Conflict of Interest

None of the authors have any competing interests to disclose.

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Table 1 Main characteristics of patients

Characteristics	Total sample	Lipid-lowering response	Non-ADR-group	ADR-group
No.	286	240	98	30
Age (y)	62.23 ± 10.68	62.07 ± 10.7	65.17 ± 9.54	61.77 ± 8.7
Sex (% male)	31.8	31.7	35.7	30
Smoking				
Never (%)	78.6	80.8	73.2	80
Past (%)	11.6	10.4	15.5	13.3
Current (%)	9.8	8.8	11.3	6.7
CVD (%)[1]	35.1	34.3	53.1	33.3
Hypertension (%)	72	72.1	80.6	70
Diabetes (%)	19.4	19.3	27.6	10
Glucose (mmol/L)	5.57 ±1.34	5.54 ±1.37	5.74 ± 1.72	5.42 ± 0.81
Concomitant therapies				
ACE inhibitor (%)	23.8	22.1	28.6	23.3
β-Blocker (%)	36.5	35.8	49.5	40
Calcium channel blocker (%)[2]	17.1	16.7	28.6	6.7
Diuretics (%)	40.6	41.7	52.0	43.3

Values for age and glucose levels are expressed as mean ± SD. [1] Total sample x Non-ADR group, P=0.003; Lipid-lowering response x Non-ADR group, P=0.002. [2] Total sample x Non-ADR group, P=0.019; Lipid-lowering response x Non-ADR group, P=0.017; ADR group x Non-ADR group, P=0.013. ADR: adverse drug

reaction; CVD: previous coronary heart disease; ACE: angiotensin-converting enzyme.

Table 2 Allele and genotype frequencies of polymorphisms analyzed in the study sample

Polymorphism	Genotypes			Alleles	
PPARA rs1800206	CC	CG	GG	C	G
	251(87.8%)	33 (11.5%)	2 (0.7%)	93.5%	6.5%
RXRA rs11381416	_/_	_/A	A/A	_	A
	238 (83.8%)	44 (15.5%)	2 (0.7%)	91.5%	8.5%
NR1I2 rs1523130	TT	TC	CC	T	C
	34(12.3%)	149 (54.0%)	93 (33.7%)	39.3%	60.7%
NR1I2 rs2472677	CC	CT	TT	C	T
	44 (15.4%)	141(49.3%)	101(35.3%)	40.0%	60.0%
NR1I3 rs2307424	TT	TC	CC	T	C
	40 (14.0%)	115 (40.2%)	131 (45.8%)	34.1%	65.9%
NRI13 rs2501873	GG	GA	AA	G	A
	94 (32.9%)	145 (50.7%)	47 (16.4%)	58.2%	41.8%

Table 3 Baseline and the mean percentage change of lipid and lipoprotein levels after statin treatment

	N	TC	LDL-C	HDL-C	TG
Baseline	240	6.56 ± 1.06	4.46 ± 0.93	1.30 ± 0.31	1.85 ± 0.94
Treatment	240	4.78 ± 0.87	2.76 ± 0.72	1.31 ± 0.35	1.53 ± 0.69
P		<0.0001	<0.0001	0.321	<0.0001
%Change Overall	240	-26.36 ± 12.96	-36.44 ± 17.68	3.96 ± 24.9	-10.18 ± 33.14
PPARA rs1800206					
CC	206	-25.97 ± 13.18	-36.14 ± 18.18	4.60 ± 25.47	-9.99 ± 34.22
CG or GG	30	-28.34 ± 11.99	-38.43 ± 15.05	1.48 ± 22.05	-10.34 ± 25.83
P		0.357	0.623	0.361	0.880
RXRA rs11381416					
_/_	195	-26.13 ± 12.91	-36.42 ± 17.51	4.97 ± 25.5	-10.31 ± 32.57
_/A or A/A	39	-28.16 ± 12.48	-38.66 ± 15.55	0.24 ± 23.17	-7.40 ± 36.90
P		0.865	0.994	0.597	0.739
NR1I2 rs1523130					
TT	27	-23.13 ± 11.66	-34.07 ± 16.47	0.94 ± 18.07	-4.37 ± 37.74
TC	126	-27.39 ± 13.54	-37.37 ± 18.11	4.23 ± 27.37	-10.41 ± 32.70
CC	79	-25.49 ± 12.57	-35.85 ± 17.46	5.20 ± 23.87	-10.39 ± 33.09
P		0.290	0.485	0.407	0.612
NR1I2 rs2472677					
CC	33	-25.90 ± 14.71	-36.17 ± 19.24	0.46 ± 18.30	-8.58 ± 39.90

CT	120	-26.26 ± 13.83	-35.86 ± 18.67	2.44 ± 23.48	-7.78 ± 33.33
TT	83	-26.43 ± 11.19	-37.39 ± 15.97	8.05 ± 28.78	-13.77 ± 30.15
P		0.935	0.742	0.170	0.442
NR1I3 rs2307424					
TT	31	-26.80 ± 11.41	-36.03 ± 16.2	4.70 ± 28.14	-8.38 ± 36.29
TC	89	-28.15 ± 13.07	-37.87 ± 17.46	-0.33 ± 18.82	-13.22 ± 32.67
CC	116	-24.69 ± 13.32	-35.48 ± 18.48	7.45 ± 27.74	-8.11 ± 32.86
P		0.436	0.940	0.255	0.666
NRI13 rs2501873					
GG	79	-27.38 ± 13.52	-37.73 ± 16.92	4.78 ± 22.53	-9.01 ± 36.66
GA	120	-26.42 ± 12.49	-37.57 ± 16.79	2.27 ± 24.73	-7.64 ± 32.57
AA	37	-23.41 ± 13.65	-29.82 ± 21.66	9.20 ± 30.39	-20.03 ± 26.10
P		0.436	0.084[1]	0.563	0.530

Covariates included in the model are: Age, gender, smoking status, standardized statin dosage, treatment period (months), baseline lipid levels, and *APOE* dummy variables; unadjusted and untransformed values are expressed as mean ± SD; [1] G carriers versus AA for *NR1I3* rs2501873, P=0.026 (-37.63 ± 16.79 versus -29.82 ± 21.66). TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides

Table 4 Genotype and allele frequencies of the polymorphisms in non-ADR group and patients that developed adverse drug reaction (ADR group)

Polymorphism	Genotypes			Alleles	
PPARA rs1800206	CC		CG or GG	C	G
non-ADR group	85 (86.7%)		13 (13.3%)	92.86%	7.14%
ADR group	29 (96.7%)		01 (3.3%)	98.33%	1.66%
P		0.111		0.204	
RXRA rs11381416	_/_		_/A or A/A	_	A
non-ADR group	80 (81.6%)		18 (18.4%)	89.80%	10.20%
ADR group	25 (83.3%)		05 (16.7%)	91.67%	8.33%
P		1.00		0.807	
NR1I2 rs1523130	TT	TC	CC	T	C
non-ADR group	10 (10.9%)	44 (47.8%)	38 (41.3%)	34.78%	65.22%
ADR-group	06 (20%)	15 (50%)	09 (30%)	45.0%	55.0%
P		0.331		0.169	
NR1I2 rs2472677	CC	CT	TT	C	T
non-ADR group	17 (17.3%)	46 (46.9%)	35 (35.7%)	40.82%	59.18%
ADR group	09 (30.0%)	13 (43.3%)	08 (26.7%)	51.67%	48.33%
P		0.296		0.180	
NR1I3 rs2307424	TT	TC	CC	T	C
non-ADR group	19 (19.4%)	38 (38.8%)	41 (41.8%)	38.78%	61.22%
ADR group	0 (0%)	19 (63.3%)	11 (36.7%)	31.67%	68.33%

P		0.007[1]		0.361	
NR1I3 rs2501873	GG	GA	AA	G	A
non-ADR group	38 (38.8%)	44 (44.9%)	16 (16.3%)	61.22%	38.78%
ADR group	08 (26.7%)	14 (46.7%)	08 (26.7%)	50.0%	50.0%
P		0.366		0.136	

Values are expressed as frequency (percentage). [1]P= 0.007 ± 0.005 (±2 standard error), calculated using the Roff and Bentzen method[16]. ADR: adverse drug reaction

Table 5 Baseline lipid and lipoprotein levels for total sample according *NR1I2* diplotypes and mean percent change in lipid and lipoproteins levels after statin treatment according to *NR1I3* diplotypes

	Diplotype groups				
NR1I2[1] diplotypes	Diplotype 1 (n=12)	Diplotype 2 T (n=76)	Diplotype 3 (n=32)	Diplotype 4 (n=142)	P value[3]
TC (mmol/L)	6.29 ± 1.12[5, 6]	6.25 ± 1.07[5]	6.55 ± 0.97[5, 6]	6.78 ± 1.03[6]	0.014
LDL-C (mmol/L)	4.08 ± 0.85[5, 6]	4.16 ± 0.92[5]	4.36 ± 0.89[5, 6]	4.65 ± 0.93[6]	0.011
HDL-C (mmol/L)	1.31 ± 0.26	1.31 ± 0.36	1.29 ± 0.28	1.31 ± 0.29	0.977
TG (mmol/L)	1.97 ± 1.11	1.91 ± 1.24	1.82 ± 0.71	1.97 ± 1.01	0.968
NR1I3[2] diplotypes	Diplotype 1 (n=19)	Diplotype 2 (n=18)	Diplotype 3 (n=79)	Diplotype 4 (n=120)	P value[4]
TC (mmol/L)	-20.05 ± 14.69	-26.96 ± 11.85	-27.38 ± 13.52	-26.42 ± 12.49	0.271
LDL-C (mmol/L)	-27.35 ± 23.64	-32.44 ± 19.72	-37.73 ± 16.92	-37.57 ± 16.79	0.152
HDL-C (mmol/L)	11.20 ± 27.37	7.08 ± 33.95	4.78 ± 22.53	2.27 ± 24.73	0.693
TG (mmol/L)	-16.69 ± 23.85	-23.55 ± 28.54	-9.01 ± 36.66	-7.64 ± 32.57	0.549

[1] unadjusted and untransformed values are expressed as mean ± SD for the total sample, and diplotypes was grouped as follow: C+C/C+C – diplotype 1; C+C/C+T and C+T/C+T – diplotype 2; T+C/T+C, T+C/T+T and T+T/ T+T – diplotype 3; T+C/C+C, T+C/C+T and T+T/C+T – diplotype 4; [2] unadjusted and untransformed values are expressed as mean percent change of lipid and lipoprotein levels after statin treatment ± SD, and diplotypes was grouped as follow: C+A/C+A – diplotype 1; T+A/C+A and T+A/ T+A – diplotype 2; T+G/T+G,

C+G/C+G and T+G/C+G – diplotype 3; T+G/T+A, T+A/C+G and C+G/C+A – diplotype 4; [3] covariates included in the model are: age, gender, smoking status, and *APOE* dummy variables; [4] covariates included in the model are: age, gender, smoking status, standardized statin dosage, treatment period (months), baseline lipid levels, and *APOE* dummy variables; [5,6]Multiple comparasions LSD diplotype 2 versus diplotype 4, p=0.002 (for TC and LDL-C).

TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol;

HDL-C: high-density lipoprotein cholesterol; TG: triglycerides

5.2 Artigo 2

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***PPARA*, *RXRA*, *NR1I2* and *NR1I3* gene polymorphisms and lipid and lipoprotein levels in South-Brazilian population**

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Abstract

Cardiovascular disease is the main cause of death worldwide, and dyslipidemia is a multifactorial important risk factor. Considering the involvement of nuclear receptors in metabolic pathways, and that some ones act in lipid metabolism and homeostasis, we investigated the influence of genetic variations in *RXRA*, *PPARA*, *NR1I2* and *NR1I3* nuclear receptor genes on lipid and lipoproteins levels in 622 Brazilians of European descent. In general, carriers of the A insertion of *RXRA* rs11381416 polymorphism showed higher levels of TG (1.80 ± 1.20 versus 1.52 ± 1.20; P=0.020). Moreover, some sexual dimorphic associations were find: men carriers of the same *RXRA* variant also had lower levels of HDL-C (1.08 ± 0.27 mmol/L versus 1.01 ± 0.28 mmol/L; P=0.049), and homozygous GG of *NR1I3* rs2501873 polymorphism had lower levels of TG (ANOVA, P=0.009); among women, carriers of the G allele of the *PPARA* rs1800206 (L162V) showed higher levels of LDL-C (3.80 ± 1.13 mmol/L versus 3.50 ± 1.07 mmol/L; P=0.037).The polymorphisms in *NR1I2* gene (rs1523130 and rs2472677) do not show to influence the variables analyzed. Different genes have been related to lipid and lipoprotein levels, and our results suggest that polymorphisms in *RXRA*, *NR1I3*, and *PPARA* genes also influence the lipid profile in a South-Brazilian population. This study might contributes to the comprehension of the interindividual lipid and lipoprotein levels variability; however, these general and by gender associations require confirmation in subsequent studies.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in most part of the world, and, in Brazil, circulatory disease accounts for approximately 32% of all mortality causes (1, 2). Blood lipid levels are the major contributor to atherosclerosis, the complex process that underlies the most cases of CVD development, and lipoprotein disorders, as elevated low-density lipoprotein cholesterol (LDL-C), and low high-density lipoprotein cholesterol (HDL-C), have been described as important independent risk factors for CVD (3). And although somewhat controversial, evidences have also shown serum triglycerides as a potential cardiovascular risk factor (4).

The blood lipid and lipoprotein levels are complex traits influenced by environmental and genetic, and the high heritability of HDL-C, LDL-C and TG levels indicates the important role of genetic variation to explain the interindividual differences of these profiles (5, 6). A large number of polymorphisms in different candidate genes have been associated to lipid and lipoprotein levels, however they did not explain all the variability, and the search for others polymorphisms and candidate genes continues.

Nuclear receptors are a superfamily of more than 50 ligand-activated transcription factors directly involved with control of gene expression in different metabolism pathways, and in response to a wide range of developmental, physiological, and environmental stimulus (7). Peroxissome proliferator-activated

receptor alpha (PPARα or NR1C1) regulates a variety of target genes involved in lipid and glucose metabolism, inflammatory response and energy homeostasis (8). PPARα bind to DNA and regulate transcription of target genes, however, this nuclear receptor requires heterodimerization with the retinoid X receptor alpha (RXRα or NR2B1), another member of nuclear receptor superfamily. RXRα has effects in different pathways because of the hability to activate transcription of target genes as homodimers or as obligate partner of another nuclear receptors, as pregnane X receptor (PXR or NR1I2) and constitutive androstane receptor (CAR or NR1I3) (7, 9). PXR and CAR were originally identified as xenosensors that regulate the expression of drug-metabolizing enzymes/transporters (Phase I, II and III), however, recent studies showed that they also affects other metabolic pathways as lipid homeostasis and metabolism (10).

Considering the influence of nuclear receptors in lipid metabolism, we hypothesized that genetic variants in genes encoding PPARα, RXRα, NR1I2, and NR1I3 nuclear receptors could contribute to the interindividual variability in blood lipid levels. The aim of the present study was to evaluate the influence of *PPARA* rs1800206, *RXRA* rs11381416, N*R1I2* rs1523130 and rs2472677, and *NR1I3* rs2501873 polymorphisms in lipid and lipoprotein levels of a South-Brazilian population of European ancestry.

2. Subjects and methods

2.1. Study Subjects

The study sample consisted of 622 subjects of European ancestry, as ascertained by skin color and morphological characteristics, sampled from a southern Brazilian community recruited at two clinical centers from Universidade Federal do Rio Grande do Sul for free routine blood determinations. The sample collection was performed between 2001 and 2002 and was previously described (11). Pregnant women, individuals with diabetes mellitus and secondary dyslipidemia due to renal, liver or thyroid disease, and on lipid lowering medication were not included in the sample. Information about lifestyle variables such as smoking, alcohol consumption, physical activity, oral contraceptive uptake, and menopausal status were obtained from each individual that consented to participate in the study through an interview. Waist circumference was measured at mid-concentration between the lower rib margin and the iliac crest (12). Body mass index (BMI) was calculated as a ratio of weight in kg and square height in meters (kg/m^2).

The Ethics Committee of the Universidade Federal do Rio Grande do Sul and Universidade Federal de Ciências da Saúde de Porto Alegre approved the study protocol. A written and signed consent was demanded of every subject included in the sample.

2.2. Biochemical analyses

Blood samples were collected from individuals after a 12-hour fasting. Total cholesterol (TC), triglyceride (TG), and glucose concentrations were determined at each clinical center by conventional enzymatic methods, on a Mega Merck Analyzer (Merck Darmstadt, Germany). HDL cholesterol was determined with a selective immunoseparation-based homogenous assay, followed by colorimetric quantification. Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald et al (13), when plasma triglycerides were below than 4.52mmol/L.

2.3. DNA analyses

Genomic DNA was isolated from peripheral blood leukocytes by a standard salting out procedure (14).

SNPs c.484C>G (rs1800206; NM/NP_001001928.1; p.Leu162Val) of *PPARA* gene and deletion/insertion polymorphism -/A (rs11381416) of *RXRA* gene were determined using PCR and restriction mapping (PCR-RFLP) as previously described (15, 16).

The SNP c.-1663T>C (rs1523130; NM_003889.3) of *NR1I2* gene was determined using an in-house designed PCR-RFLP. The amplification reactions used the following primers: forward 5'-GTCATGAGGATATTGGACCG-3' and

reverse 5'-TAGCCATGGCCTTCTGATCT-3'. A mismatch was introduced in the forward primer to create an *Msp*I restriction site that surrounded the polymorphism. Both primers were designed using the Primer3 program (http://frodo.wi.mit.edu/primer3/), and primer specificity was validated against the human genome database of the National Center for Biotechnology Information with BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/). The amplification reactions were performed in final volume of 25 µL containing 0.2 mmol/L of dNTPs, 2.0 mmol/L of MgCl2, 0.4 µmol/L of each primer, 1 U of Taq DNA polymerase and 100 ng of genomic DNA. Samples were denatured at 94ºC for 5 minutes, followed by 35 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds, and 72ºC for 30 seconds, with a final extension at 72ºC for 5 minutes. The *Msp*I digestion resulted in 19 and 141 bp fragments for the G allele or in a single 159 bp fragment for the A allele, and the variants were genotyped using 8% polyacrylamide gel electrophoresis followed by visualization with ethidium bromide staining.

SNPs c.-22-7659T>C (rs2472677; NM_003889.3) of *NR1I2* gene, and c.238+1099A>G (rs2501873; NM_001077469.1) of *NR1I3* gene were determined by allelic discrimination with Taqman 5'-nuclease assays. Genotyping for rs2472677 (ID: C_26079845_10), and rs2501873 (ID: C_16033320_10) SNPs were performed with validated TaqMan genotyping assays (Real Time PCR, Applied Biosystems, California, USA).

The *APOE* allele variants (E*2, E*3 and E*4), previously genotyped by Fiegenbaum et al (11) for this sample, was used for adjustment analytical purpose.

2.4. Statistical analyses

Continuous variables were expressed as mean ± standard deviation. Triglyceride levels were log-transformed before analyses because of their skewed distribution, although non-transformed values are shown in the Results section.

Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested using chi-square tests. The Graph Pad InStat software version 2.04a (Graph Pad Software, San Diego, California, USA) was used to compare allele and genotype frequencies among groups by Fisher exact test and chi-square test, respectively. When appropriated, chi-square tests as described by Roff and Bentzen (17) (CHITEST.EXE software) were performed. Haplotype frequencies and linkage disequilibrium were estimated with the use of Multiple Locus Haplotype Analysis program (version 2.0; Long, JC (1999)) (18, 19) and ARLEQUIN software (version 3.1)(20). Dmax (D theoretical maximum) and D' (the relative magnitude of D as compared to its theoretical maximum calculated as D/Dmax) values were calculated as described by Lewontin (21).

Because of lower homozygous genotype frequencies, rs1800206 genotypes of the *PPARA* gene were grouped as homozygous for C allele (CC) and carriers of G allele (CG and GG), and rs11381416 genotypes of the *RXRA* gene were

grouped as homozygous without the A insertion (-/-) and carriers of the A insertion (-/A and A/A).

To determine the association of the genotypes with lipid and lipoproteins levels, means of each variable were compared by a General Linear Model using the type III sums of squares. This sum of squares applies to unbalanced study designs and quantifies the effect of an independent variable after adjustment for all other variables included in the model. Age, gender, smoking status, body mass index, and dummy variables for the presence of E*2 and E*4 *APOE* alleles (*APOE* dummy variables) were included in each model as covariates. When the statistical analyses were performed separately by gender, menopausal status was also included as a covariate. Subjects with E*2/E*4 genotype were excluded from analyses. Pairwise comparisons among genotypes were performed by least significant difference with no adjustments. Statistical analysis was performed using the SPSS16.0 statistical package for Windows®.

3. Results

3.1. Characteristics of study population

The characteristics of the total sample, and subjects stratified by gender are presented in Table 1. The total sample consist of 622 subjects aged between 15 and 89 (44.72 ± 15.72 years), and 58.5% were females. Men were older than women (P=0.007); and past smoking (P=0.002) and physical activity (P=0.002) were more frequent in men than in women sample. Triglyceride concentrations (P=0.006) and waist circumference measure (P<0.001) were significantly higher in men, whereas HDL-cholesterol concentrations were higher in women (P<0.001). All others characteristics did not differ between gender. Almost 41% of the female sample were post-menoupausal.

Genotype frequency distributions observed for polymorphisms studied did not reveal statistically significant differences when compared to those expected under Hardy-Weinberg equilibrium. Table 2 shows the allele and genotype frequencies of all SNPs analyzed. All four expected haplotypes for *NR1I2* gene (rs1523130 + rs2472677: T+C, T+T, C+T, and C+C) were observed and the most frequent haplotypes were C+T and C+C (35.43% and 23.42%, respectively). A weak linkage disequilibrium was detected between the SNPs of *NR1I2* gene (P=0.006, D'=0.112).

3.2. Association between PPARA, RXRA, NR1I2 and NR1I3 polymorphisms and lipid and lipoprotein levels

As showed in Table 3, after adjustment for covariates, carriers of A allele of *RXRA* rs11381416 polymorphism have higher TG levels (1.80 ± 1.20 mmol/L) than homozygous without the A insertion (1.52 ± 1.02 mmol/L; P=0.020).

When the statistical analyses were performed separately by gender, only among men, the presence of A allele of *RXRA* rs11381416 polymorphism also showed a borderline association with HDL-C levels (homozygous without the A insertion: 1.08 ± 0.27 mmol/L; A carriers: 1.01 ± 0.28 mmol/L; P=0.049). In male sample, *NR1I3* rs2501873 polymorphism was associated with TG levels (ANOVA, P=0.009), and multiple comparasions showed that GG homozygous had lower levels of TG (1.41 ± 0.97 mmol/L) than GA heterozygous (1.77 ± 1.27 mmol/L; P=0.004) and AA homozygous (1.72 ± 0.91 mmol/L; P=0.011) (data not shown).

In women sample, carriers of the G allele of the *PPARA* rs1800206 showed higher levels of LDL-C (3.80 ± 1.13 mmol/L) than homozygous CC (3.50 ± 1.07 mmol/L; P=0.037) (data not shown).

4. Discussion

To our knowledge, this is the first study that described the allele/genotype frequencies of *RXRA* rs11381416, N*R1I2* rs1523130 and rs2472677, and *NR1I3* rs2501873 polymorphisms in a Brazilian population, and is the second one describing the *PPARA* rs1800206 polymorphism in the same population (but the first in a south population of European descent). The allele frequencies observed were similar to those reported in SNP databases (Entrez SNP and International HapMap Project), and previous studies analyzing European populations. The small relative magnitude of linkage disequilibrium (D'=0.112) detected between the SNPs in *NR1I2* gene is in accordance to the HapMap data and Lamba et al (22), that describe these SNPs in different haplotype blocks, however we believe that significant p value for linkage disequilibrium (P=0.006) detected in this study to apply the large sample size.

Even though RXRα is a promiscuous nuclear receptor that participates in different metabolic pathways, the influence of genetic variants in *RXRA* is poorly studied. The *RXRA* rs11381416 polymorphism is an adenine nucleotide insertion in intron 8 of gene, and our results showed the presence of this A insertion associated to a worse lipid profile: in the role population analyzed, carriers of A insertion had higher TG levels; and in the male sample, the presence of the same allele was associated to lower HDL-C levels. To our knowledge, only Vasku et al (16) analyzed the association of this polymorphism with two different psoriasis subtypes, and it is unknown if this polymorphism influence RXRα function or

expression. Other genetic variants in *RXRA* were recently associated with Alzheimer's disease risk and cerebral cholesterol metabolism (23). As reviewed by Ahuja et al (24), the RXRs function as key regulators of glucose, fatty acid and cholesterol metabolism and metabolic disorders such as type 2 diabetes, hyperlipidemia and atherosclerosis through the activation of multiple nuclear receptor complexes. Thus, genetic variations in RXRs coding genes may affect a large number of metabolic signaling pathways and deserve more attention.

The *NR1I3* rs2501873 polymorphism is a transition substitution A>G in intron 3 of the gene, and to our knowledge, the functionality of these polymorphisms was not tested. In this study, when the statistical analyzes were performed for all the sample, the *NR1I3* rs2501873 polymorphism did not show to influence the lipid and lipoprotein levels; however, in male sample, the polymorphism was highly associated with TG levels, with homozygous GG having lower TG levels. Testosterone metabolites, androstanol and androstenol, have been described to inhibit the constitutive transcriptional activity of CAR, suggesting that differences in receptor activation and function might exist between genders (25). Jointly, a recent study provided strong evidence that CAR regulates the triglyceride levels under conditions of metabolic stress, and that some of metabolic effects of the CAR are due via negative regulation of PPARα activity (26). To summarize, some evidences point biological plausibility in our result, although the confirmation of this association is required.

The *PPARA* rs1800206 polymorphism is a transversion substitution C>G at codon 162 of exon 6 that results in a missense mutation, leucine-valine (L162V). Some studies have demonstrated association between G allele (V162) and harmful lipid profile (15, 27-33), others showed opposite findings or negative associations (34-37). In the present study, as in recent one that analyzed the influence of L162Vpolymorphism in Brazilian elderly population (37), no association was detected of this polymorphism with lipid and lipoprotein levels considering the total sample; however, our results showed that the presence of V162 was associated to higher LDL-C levels in female sample. Sexual dimorphic associations have been previously demonstrated (29, 32, 34), and our result corroborates that the influence of this polymorphism may be different between genders. Additionally, genetic variation at the *APOE* locus have been described to modulated the impact of the L162V polymorphism exactly on LDL-C concentrations (29), justifying further the adjustment for *APOE* genotype in our statistical analyses. The functionality of this polymorphism have been tested and opposite effects on gene transcription have been found depending of the exposure concentration to a PPAR ligand (38). This way, additional gene-gene or gene-environmental interactions may modulate the effect of the L162V polymorphism.

The *NR1I2* rs1523130 polymorphism corresponds to a transition substitution T>C in the promoter region of the gene; and the N*R1I2* rs2472677 polymorphism, a transition C>T in intron 1. Despite the consistent association of both polymorphisms with CYP3A4 phenotypes recently described by Lamba et al (22),

our results suggest that *NR1I2* rs1523130 and rs2472677 polymorphisms did not influence the lipid and lipoprotein levels. The PXR activity is also modulated by steroids (39), besides the analyses performed separately by gender did not find any significant association. As cited above, the relation between PXR and energetic metabolism was more recently described, and something is known about the influence of *NR1I2* polymorphisms in lipid levels. In view of this, others exploratory and confirmatory studies should be made.

As discussed by Weiss et al (40), gender can be considered an environmental factor that can modify both penetrance and expressivity of a wide variety of traits. The influence of sex-steroid hormones, and also the lifestyle differences between genders, might explain some differences found between men and women (6). Evaluating the distribution of lipids levels between genders, and the sex interaction in heritability, Weiss et al (40) showed HDL-C and TG levels strongly sexually dimorphic, agreeing with our finds (Table 1); they also showed LDL-C and HDL-C with a significant sex interaction in heritability, what is in accordance to the gender specific associations found in our study. Since the steroid hormones have been described to modulate the transcription factors activity of some nuclear receptors (25, 41, 42), it is not surprising that polymorphisms in these receptors coding genes might contribute to the sex-specific gene-environment interactions.

The investigation of the effect of only one or two SNPs per gene, and the adjust taking into account only *APOE* genotypes as covariate between all

polymorphisms previously related to the lipid and lipoprotein levels, are limitations to our study. However, the relatively wide sample size gives power to this investigation, and allowed that differences between genders were explored.

Taking into account the high morbidity and mortality of CVD, the importance of the dyslipidemia as risk factor for CVD, and that dyslipidemia is a multifactorial characteristic, elucidation of the genetic predisposition factors is crucial. Our study suggests that polymorphisms in *PPARA, RXRA* and *NR1I3* genes are between the ones that influence blood lipid and lipoproteins levels and, this way, might contribute to the comprehension of this variability.

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Thanks are due to Ana Lúcia S. Antunes and Maria Perpétua de O. Pinto from the Clinical Analysis Laboratory of the Pharmacy College and to Gledison Gastaldo from the Biochemical Laboratory of the Clinical Hospital of Porto Alegre. We are also grateful to André Vargas, Marsel Arsand, Fabiana M. de Andrade and Vanessa S. Mattevi for their help in sample collection.

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Table 1

Characteristics	Total sample	Female	Male	P[a]
No.	622	364	258	
Age (years)	44.72 ± 15.72	43.29 ± 16.20	46.74 ± 14.92	0.007
TC (mmol/L)	5.41 ± 1.29	5.43 ± 1.37	5.37 ±1.18	0.504
LDL-C (mmol/L)	3.54 ± 1.10	3.54 ± 1.15	3.55 ± 1.03	0.974
HDL-C (mmol/L)	1.16 ± 0.30	1.22 ± 0.31	1.07 ± 0.27	<0.001
TG (mmol/L)	1.55 ± 1.04	1.47 ± 0.98	1.67 ± 1.12	0.006
BMI (kg/m^2)	26.15 ± 4.65	26.08 ± 4.98	26.25 ± 4.14	0.633
Waist circumference (cm)	91.12 ± 12.31	87.93 ±12.41	95.47 ± 10.78	<0.001
Smoking				
Never (%)	64.1	69.0	57.2	0.003
Past (%)	9.1	5.9	13.6	0.002
Current (%)	26.8	25.1	29.2	0.270
Sedentary/Physically inactive (%)	59.1	64.7	51.5	0.002
Postmenopausal (%)		40.9		

Table 2

SNP	Genotypes			Alleles	
PPARA rs1800206	CC	CG	GG	C	G
	524 (84.5%)	92 (14.8%)	4 (0.7%)	91.9%	8.1%
RXRA rs11381416	_/_	_/A	A/A	_	A
	530 (86.2%)	82 (13.3%)	3 (0.5%)	92.9%	7.1%
NR1I2 rs1523130	TT	TC	CC	T	C
	116(19.0%)	271 (44.3%)	224 (36.7%)	41.2%	58.8%
NR1I2 rs2472677	CC	CT	TT	C	T
	120 (19.3%)	308 (49.6%)	193 (31.1%)	44.1%	55.9%
NRI13 rs2501873	GG	GA	AA	G	A
	166 (26.7%)	297 (47.7%)	159 (25.6%)	50.6%	49.4%

Table 3

	N	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)
PPARA rs1800206					
CC	512	5.41 ± 1.30	3.54 ± 1.11	1.17 ± 0.31	1.53 ± 0.95
CG or GG	94	5.56 ± 1.24	3.69 ± 1.02	1.13 ± 0.25	1.70 ± 1.44
P		0.477	0.386	0.153	0.439
RXRA rs11381416					
_/_	519	5.44 ± 1.31	3.58 ± 1.12	1.17 ± 0.31	1.52 ± 1.02
_/A or A/A	82	5.43 ± 1.17	3.52 ± 0.98	1.13 ± 0.28	1.80 ± 1.20
P		0.436	0.236	0.192	0.020
NR1I2 rs1523130					
TT	112	5.41 ± 1.23	3.56 ± 1.02	1.14 ± 0.28	1.58 ± 1.06
TC	265	5.43 ± 1.34	3.56 ± 1.14	1.17 ± 0.30	1.56 ± 1.03
CC	220	5.48 ± 1.29	3.60 ± 1.11	1.17 ± 0.31	1.56 ± 1.07
P		0.570	0.707	0.712	0.850
NR1I2 rs2472677					
CC	117	5.30 ± 1.27	3.49 ± 1.06	1.15 ± 0.28	1.42 ± 0.97
CT	298	5.45 ± 1.37	3.55 ± 1.16	1.17 ± 0.31	1.61 ± 1.01
TT	192	5.48 ± 1.18	3.64 ± 1.02	1.16 ± 0.30	1.56 ± 1.14
P		0.809	0.612	0.728	0.070
NRI13 rs2501873					
GG	160	5.49 ± 1.42	3.61 ± 1.16	1.18 ± 0.33	1.54 ± 1.03
GA	289	5.43 ± 1.30	3.57 ± 1.12	1.16 ± 0.28	1.54 ± 1.10
AA	159	5.37 ± 1.16	3.50 ± 1.01	1.15 ± 0.30	1.60 ± 0.96
P		0.738	0.665	0.680	0.516

Table 1 **Main characteristics of subjects.**

Continuous variables are expressed as mean ± SD; [a]Female vs. male P value.TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; BMI: body mass index

Table 2 **Allele and genotype frequencies of SNPs analyzed in the study sample.**

Table 3 **Association between PPARA, RXRA, NR1I2 and NR1I3 SNPs and lipid and lipoprotein levels.**

Covariates included in the model are: Age, gender, smoking status, body mass index, and *APOE* dummy variables; unadjusted and untransformed values are expressed as mean ± SD. TC: total cholesterol. LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides

ANEXOS

Anexo A – Orientações aos autores para submissão de artigo para publicação na revista *The Pharmacogenomics Journal* (Artigo 1)

Disponível em: <http://mts-tpj.nature.com/cgi-bin/main.plex?form_type=display_auth_instructions>. Acesso em: 05 nov. 2009.

The Pharmacogenomics Journal

Guide to Authors – FORMAT OF PAPERS

Article Types Table

Article Type	**Description**	**Word Count**
Original Article	These are reports whose conclusions represent a substantial advance in the understanding of an important problem.	Original articles should have a title; running title of less than 50 characters; abstract of no more than 150 words; and main text of no more than 3500 words (excluding references).
Review	These survey recent developments in topical areas of scientific research or, on occasion, more wide-ranging subjects. Authors are encouraged to include tables and figures, as well as suitable material to be used in text boxes (to highlight salient points of the paper or expand on certain concepts) and a glossary of specialist terms.	Review articles should have a title; short title; abstract of no more than 150 words; and main text of between 3000 and 5000 words (excluding references).
News and Commentary	Inform readers about new scientific advances, as reported either in recently published papers or at scientific meetings.	The opening paragraph should consist of a precis of 20-30 words. News and Commentary articles should not exceed 1500 words and 15 references. Up to two tables and two figures may be included.
Perspectives	Provide an update on developments in fast-moving	Perspectives articles should not exceed 1500 words and 15

	areas of interest.	references. Up to two tables and two figures may be included.
Ethical, Economics, Legal and Social (EELS)	Present well-argued, thought-provoking analysis of issues and considerations involved in both basic research and in practice.	EELS articles should not exceed 2000 words and 30 references. Up to two tables and two figures may be included.
Clinical Implications	Are either hypothetical or based on current clinical trials and encourage the transition from basic research to clinical practice by presenting a diagnostic or treatment challenge.	Clinical Implications articles should not exceed 2000 words and 30 references. Up to two tables and two figures may be included.

Preparation of Original Articles

1. Cover letter (must include a Conflict of Interest statement)
2. Title page (excluding acknowledgements)
3. Abstract and keywords
4. Introduction
5. Materials (or patients) and methods
6. Results
7. Discussion
8. Acknowledgements
9. Conflict of Interest
10. References
11. Tables
12. Figures

Cover letter

The uploaded covering letter must state the material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration. The covering letter must also contain a Conflict of Interest statement (see Editorial Policy section).

Title Page

The title page should bear the title of the paper, the full names of all the authors, highest academic degree obtained, and their affiliations, together with the name, full postal address, telephone and fax numbers and e-mail address of the author to whom correspondence and offprint requests are to be sent (This information is also asked for on the electronic submission form). The title should be brief, informative, of 150 characters or less and should not make a statement or conclusion. The running title should consist of not more than 50 letters and spaces. It should be as brief as possible, convey the essential message of the paper and contain no abbreviations. Authors should disclose the sources of any support for the work, received in the form of grants and/or equipment and drugs.

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Materials / subjects and Methods

This section should contain sufficient detail, so that all experimental procedures can be reproduced, and include references. Methods, however, that have been published in detail elsewhere should not be described in detail. Authors should provide the name of the manufacturer and their location for any specifically named medical equipment and instruments, and all drugs should be identified by their pharmaceutical names, and by their trade name if relevant.

Results and Discussion

The Results section should briefly present the experimental data in text, tables or figures. Tables and figures should not be described extensively in the text, either. The discussion should focus on the interpretation and the significance of the findings with concise objective comments that describe their relation to other work in the area. It should not repeat information in the results. The final paragraph should highlight the main conclusion(s), and provide some indication of the direction future research should take.

Acknowledgements

These should be brief, and should include sources of support including sponsorship (e.g. university, charity, commercial organization) and sources of material (e.g. novel drugs) not available commercially.

Conflict of interest

Authors must declare whether or not there is any competing financial interests in relation to the work described. This information must be included at this stage and will be published as part of the paper. Conflict of interest should also be noted on the cover letter and as part of the submission process. See the Conflict of Interest documentation in the Editorial Policy section for detailed information.

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Complete book:

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Hood E. Pharmacogenomics: the promise of personalized medicine. *Environ Health Perspect* 2003; **111**(Suppl 1): 581 (abstract 123).

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Anexo B – Orientações aos autores para submissão de artigo para publicação na revista *Metabolism: Clinical and Experimental* (Artigo 2)

Disponível em: <http://www.elsevier.com/wps/find/journaldescription.cws_home/623319/authorinstructions>. Acesso em: 29 nov. 2009.

Metabolism: Clinical and Experimental

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The title page of the manuscript should be uploaded as a separate file and include (1) the name of the institution where the work was done ("From the Fels Research Institute . . ."); (2) acknowledgments for research support the authors wish to publish; (3) Conflicts of Interest; (4) Institutional Approval (if applicable); and (5) email address of the corresponding author.

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Manuscripts describing research on human subjects must include a statement that the research was approved by the appropriate committee of the institution. Consent of human subjects is required. For research on experimental animals, authors are expected to have followed the institutional guidelines for the care and use of laboratory animals and indicate institutional approval. This statement must be included in the methods section of the manuscript and disclosed in the cover letter.

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Illustrations and tables

All tables and figures must be cited in order in the text using arabic numerals. Figure legends should be compiled in a separate list at the end of the paper.

TIFF and EPS are the preferred formats for artwork. Graphics software such as Photoshop and Illustrator, not presentation software such as PowerPoint, CorelDraw, or Harvard Graphics, should be used to create the art. All type fonts used in studio-created artwork must be either "embedded" in the file or supplied separately. All graphic files supplied as bitmap format (not vector format) in TIFF, JPEG or GIF must be submitted in sufficiently high resolution (240-300 dpi for grayscale or color images and 600-1000 dpi for line art) to allow for printing. See Elsevier's website for guidelines for preparing electronic artwork: ⊡→http://www.elsevier.com/artworkinstructions

References should be compiled numerically according to the order of citation in the text and typed double-spaced in the Vancouver style, **giving inclusive pages:**

Journal article:

1. Chick WL, Lauris V, Soeldner JS, et al. Monolayer culture of a human pancreatic beta-cell adenoma. *Metabolism* 1973;22:1217-24.

2. Katz A, Bogardus C. Insulin-mediated increase in glucose 1,6-bisphosphate is attenuated in skeletal muscle of insulin-resistant man. *Metabolism* (in press).

Complete book:

3. Wesson LG. Physiology of the human kidney. Philadelphia: Grune & Stratton; 1969.

Chapter in book:

4. Young VR. The role of skeletal and cardiac muscle in the regulation of protein metabolism. In: Munro HN, editor. Mammalian protein metabolism, vol 4. San Diego: Academic; 1970. p. 585-674.

Epub Ahead of Print:

5. Jones B, Smith A. The relationship of immunoreactivity and HPA-axis measurements. Ann Emerg Med. 2007 Feb 8; [Epub ahead of print].

URL:
6. The American Academy of Pain Medicine.The use of opioids for the treatment of chronic pain: a consensus statement. Available at: http://www.painmed.org. Accessed March 9, 2006.

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Anexo C – Avaliações dos Comitês de Ética para a realização da pesquisa apresentada no Artigo 1

ANEXO 5 – PARECER DO CONSELHO DE ÉTICA DA UNIVERSIDADE

UFRGS
UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA PROPESQ

COMITÊ DE ÉTICA EM PESQUISA

RESOLUÇÃO

O Comitê de Ética em Pesquisa da Universidade Federal do Rio Grande do Sul analisou o projeto:

Número:200390

Título do projeto: Estudo da influência da variabilidade genética na resposta ao tratamento com sinvastatina (proc. 23078. 001196/03-78)

Investigador(es) principal(ais): Mara Helena Hutz/Marilu Fiegenbaun e outros.

O mesmo foi aprovado na reunião nº 22, ata nº 43 do Comitê de Ética em Pesquisa da UFRGS, por estar adequado ética e metodologicamente e de acordo com a Resolução 196/96 do Conselho Nacional de Saúde.

Porto Alegre, 10 de setembro de 2003.

José Roberto Goldim
Coordenador CEP/UFRGS

IPA
Centro Universitário
Metodista

COMITÊ DE ÉTICA EM PESQUISA

PARECER DE PROJETO DE PESQUISA

CADASTRO C.E. 1527	**DATA DO PARECER** 01/12/05
TÍTULO DO PROJETO Farmacogenética de fármacos hipolipemiantes: avaliação da influência de variantes genéticas sobre o desenvolvimento de efeitos adversos.	
CURSO/ FACULDADE **Farmácia**	

ITEM	**SITUAÇÃO**
Respeito às normas éticas de pesquisa	(X) SIM ( ) NÃO
Termo de consentimento apresenta	(X) SIM ( ) NÃO
Linguagem acessível	(X) SIM ( ) NÃO
Justificativa, objetivos e procedimentos	(X) SIM ( ) NÃO
Desconfortos e riscos	(X) SIM ( ) NÃO
Benefícios esperados	(X) SIM ( ) NÃO
Medidas de proteção de riscos	(X) SIM ( ) NÃO
Formas de assistência e responsável	(X) SIM ( ) NÃO
Esclarecimentos antes e depois da pesquisa	(X) SIM ( ) NÃO
Possibilidade de inclusão em grupo controle ou placebo	(X) SIM ( ) NÃO
Liberdade de recusar ou retirar o consentimento sem penalização	(X) SIM ( ) NÃO
Garantia de sigilo e privacidade	(X) SIM ( ) NÃO

Comentários

Apesar de não constar claramente esclarecimentos antes e depois da pesquisa, o Projeto apresenta oficialmente a responsável pela pesquisa para solucionar qualquer dúvida. Sendo assim, fez sustentação ao Projeto sob o ponto de vista ética.

Avaliação
(X) Recomenda Aprovação
( ) Recomenda Pendência

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DE CIÊCIAS DA SAÚDE DE PORTO ALEGRE
COMITÊ DE ÉTICA EM PESQUISA
RUA SARMENTO LEITE, 245 – FONE: (51) 33038804
CEP 90050-170 – PORTO ALEGRE – RS - cep@ffcmpa.edu.br

Of. 570/08-CEP

Porto Alegre, 13 de março de 2008.

Ilma. Sra.
Profª Silvana de Almeida
Nesta Universidade

Prezada Pesquisadora

Informamos que seu projeto "Farmacogenética dos Inibidores da HMG-COA Redutase: avaliação da Influência de variantes dos genes receptores nucleares na resposta hipolipemiante e no desenvolvimento de efeitos adversos.", Processo nº334/07, foi avaliado pelo Comitê de Ética e Pesquisa, na reunião do dia 13 de março de 2008, sendo o projeto considerado aprovado, conforme parecer consubstanciado nº 554/08, anexo. Outrossim, informamos que de acordo com o art. 4º, letra c do Regulamento do CEP, V.Sa. deverá nos encaminhar relatórios semestrais do desenvolvimento do projeto.

Atenciosamente,

Prof. José Geraldo Vernet Taborda
Coordenador do CEP/UFCSPA

Anexo D – Avaliações dos Comitês de Ética para a realização da pesquisa apresentada no Artigo 2

HOSPITAL DE CLÍNICAS DE PORTO ALEGRE Anexos
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO
COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

RESOLUÇÃO

As Comissões Científica e a Comissão de Pesquisa e Ética em Saúde, que é reconhecida pela CONEP como Comitê de Ética em Pesquisa do HCPA, reanalisaram o projeto:

Número: 97308

Título: "DETERMINAÇÃO DA VARIABILIDADE GENÉTICA DAS APOLIPOPROTEÍNAS COMO FATOR DE SUSCETIBILIDADE À DOENÇA CARDIOVASCULAR ISQUÊMICA ATEROSCLERÓTICA".

Autores: Mara Helena Hutz, Alcides Zago, Marco Antonio R. Torres, André F. Vargas, Marilu Fiegenbaum, Fabiana M. de Andrade, Gisele M. Ewald.

- O mesmo foi aprovado, por estar adequado ética e metodologicamente, de acordo com as Diretrizes e Normas Regulamentadoras de Pesquisa envolvendo Seres Humanos (Resolução 196/96 do Conselho Nacional de Saúde) e às Resoluções Normativas do GPPG/HCPA. Os autores deverão encaminhar relatórios semestrais sobre o andamento do Projeto.

Porto Alegre, 18 de março de 1998.

Profª Themis Reverbel da Silveira.
Coordenadora do GPPG e CEP/HCPA

95

Ja Ramiro Barcelos, 2350 - Largo Eduardo Zaccaro Faraco - 90035-003 - Porto Alegre, RS - Brasil
e (55) 51 - 316 8000 - Telefax (55) 51 - 316.8001 - Caixa Postal 1247 - E-mail:hcpa@dpx1.hcpa.ufrgs.br

Parecer Consubstanciado de Projeto de Pesquisa

Título do Projeto: " ADENDO Farmacogenética dos inibidores da HMG-COA redutase: avaliação da influência dos genes de receptores nucleares na resposta hipolipemiante e no desenvolvimento de efeitos adversos"

Pesquisador Responsável Silvana de Almeida **Parecer nº 662/08**

Data da Versão 03/07/2008 | **Cadastro 334/07** | **Data do Parecer 14/08/2008**

Grupo e Área Temática III - Projeto fora das áreas temáticas especiais

Objetivos do Projeto
Estudar a influência de variantes nos genes PPARA e RXPR na resposta a hipolipemiante e no desenvolvimento de efeitos adversos ao tratamento com sivastatina, por via oral, em uma amostra de indivíduos de descendência européia.

Sumário do Projeto
O presente estudo propõem-se a avaliar a influência das varientes dos genes PPARA e RXPR na resposta a hipolipemiante e no desenvolvimento de efeitos adversos ao tratamento com sivastatina, por via oral, em uma amostra de indivíduosde descendência européia. Uma vez determinada as variantes para os genes que codificam esses receptores, essa informação poderá auxiliar na determinação de indivíduos com maior ou menos risco para o desenvolvimento de efeitos adversos.

Itens Metodológicos e Éticos	Situação
Título	Adequado
Autores	Adequados
Local de Origem na Instituição	Adequado
Projeto elaborado por patrocinador	Não
Aprovação no país de origem	Não necessita
Local de Realização	Própria instituição
Outras instituições envolvidas	Sim
Condições para realização	Adequadas

Comentários sobre os itens de Identificação

Introdução	Adequada

Comentários sobre a Introdução

Objetivos	Adequados

Comentários sobre os Objetivos

Pacientes e Métodos	
Delineamento	Adequado
Tamanho de amostra	Total 673 Local
Cálculo do tamanho da amostra	Adequado
Participantes pertencentes a grupos especiais	Não
Seleção eqüitativa dos indivíduos participantes	Adequada
Critérios de inclusão e exclusão	Adequados
Relação risco- benefício	Adequada
Uso de placebo	Não utiliza
Período de suspensão de uso de drogas (wash out)	Não utiliza
Monitoramento da segurança e dados	Adequado
Avaliação dos dados	Adequada - quantitativa
Privacidade e confidencialidade	Adequada
Termo de Consentimento	Adequado
Adequação às Normas e Diretrizes	Sim

Comentários sobre os itens de Pacientes e Métodos

Cronograma	Adequado
Data de início prevista	03/ 2008
Data de término prevista	12/2009
Orçamento	Adequado
Fonte de financiamento externa	Agência de fomento

Comentários sobre o Cronograma e o Orçamento

Referências Bibliográficas	Adequadas

Comentários sobre as Referências Bibliográficas

Recomendação

Aprovar

Comentários Gerais sobre o Projeto

Anexo E - Termo de Consentimento Livre e Esclarecido assinado pelos participantes da pesquisa apresentada no Artigo 1

TERMO DE CONSENTIMENTO PÓS-INFORMAÇÃO

ESTUDO DA INFLUÊNCIA DA VARIABILIDADE GENÉTICA NA RESPOSTA AO TRATAMENTO COM SINVASTATINA.

Antes de sua participação neste estudo, é preciso esclarecer alguns detalhes importantes, para que possíveis dúvidas sejam resolvidas. Em caso de qualquer outra dúvida quanto à pesquisa ou sobre os seus direitos, você poderá contatar com Marilu Fiegenbaum, farmacêutica e Mestre em Genética e Biologia Molecular, responsável pelo estudo, pelo telefone (051) 3316-6735.

Qual o objetivo desta pesquisa?

Apesar de seguro e eficaz, a eficiência do tratamento com medicamentos que diminuem os níveis de colesterol é variável, ou seja, algumas pessoas têm seus níveis de colesterol diminuídos mais rapidamente e com menores doses do que outras. Recentes investigações têm mostrado que esta variabilidade na resposta (o quanto os níveis de colesterol diminuem) pode ser influenciada por fatores genéticos. Desta forma, o estudo tem por objetivo verificar se dieferenças genéticas estão associadas a respostas adequadas no uso de sinvastatina 20 mg.

Como vamos fazer isso?

Através da análise de DNA, observaremos quais são as variantes genéticas de cada indivíduo.

Como é feita esta análise de DNA?

Com o uso de agulhas e seringas descartáveis será coletada de você uma amostra de sangue (quantidade aproximada de uma colher de sopa). Esta coleta será feita por um indivíduo treinado. Após isto, o sangue será examinado para determinar variações genéticas que potencialmente podem modular sua resposta ao tratamento com sinvastatina 20mg por dia. As amostras serão identificadas por números diferentes daqueles utilizados pelo Centro de Diagnóstico Cardiológico. Ao final desse trabalho as amostras de DNA serão preservadas de forma que possam eventualmente ser utilizadas em futuras pesquisas sobre o mesmo assunto. Todos os dados que vinculem sua identidade com os dados obtidos serão separados e preservados em diferentes bancos de dados.

Quais os riscos em participar?

Não há riscos em participar do projeto. No entanto, poderá haver formação de um hematoma no braço em função da coleta de sangue. Além deste, não há qualquer outro risco para a paciente em participar deste projeto.

O que a paciente ganha com este estudo?

Embora este trabalho não possa gerar nenhum benefício imediato aos participantes, este estudo poderá trazer vários benefícios a longo prazo, quando o conhecimento de quais pessoas respondem melhor ao tratamento com este tipo de medicamento será possível. Tendo-se observado isto, poderemos determinar com mais eficácia qual o tipo de terapia é mais aconselhado para cada paciente. Por fim, a sua participação ajudará no desenvolvimento de novos conhecimentos, que poderão eventualmente beneficiar você e outras pessoas no futuro.

Quais são os seus direitos?

Os seus registros médicos serão sempre tratados confidencialmente. Os resultados deste estudo poderão ser usados para fins científicos, mas você não será identificada por nome.

Sua participação no estudo é voluntária, de forma que, caso você decida não participar, isto não afetará no tratamento normal que você tem direito.

FORMULÁRIO DE CONSENTIMENTO PARA PACIENTES

ACORDO EM PARTICIPAR DE UM ESTUDO EM GENÉTICA

Número do estudo: _____ Cód. de ident. do indivíduo:_____

Nome do indivíduo:

_____

Data de nascimento: ___/___/_____

Médico supervisor:

_____

Assinatura da paciente:

_____

Assinatura do médico supervisor:

_____

Assinatura da testemunha:

_____

Data: ___/___/_____

Anexo F - Termo de Consentimento Livre e Esclarecido assinado pelos participantes da pesquisa apresentada no Artigo 2

TERMO DE CONSENTIMENTO PARA A PARTICIPAÇÃO NO ESTUDO SOBRE FATORES GENÉTICOS QUE AFETAM OS NÍVEIS DE COLESTEROL E TRIGLICERÍDEOS NA POPULAÇÃO DE PORTO ALEGRE

Através deste documento, consinto em ser entrevistado e em doar uma quantidade de 5 (cinco) ml de sangue, a ser obtida juntamente com a retirada de sangue para os exames dos quais necessito, no Laboratório de Análises Clínicas da Faculdade de Farmácia da UFRGS.

Estou ciente de que os objetivos deste estudo são conhecer um pouco mais sobre algumas das causas do aumento dos níveis de colesterol e triglicerídeos. Além disso, sei que os benefícios virão somente a longo prazo, quando será possível prever o risco genético de um indivíduo ter problemas cardiovasculares.

Os resultados do presente estudo estarão a minha disposição tão logo forem obtidos.

Tenho a garantia de que meus dados serão mantidos em sigilo, e que meu nome daqui para frente não será revelado.

Porto Alegre, / / . _____

Assinatura do Paciente

Pesquisadora Responsável:
Dra. Mara Helena Hutz
Departamento de Genética da UFRGS
Telefone: 316 - 6720

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