UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE – UFCSPA
PROGRAMA DE PÓS-GRADUAÇÃO EM PATOLOGIA

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Influência de polimorfismos do gene *PON1* na resposta terapêutica de estatinas

Porto Alegre
2015

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Dissertação submetida ao Programa de Pós-Graduação em Patologia da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre.

Orientadora: Profª. Dra. Marilu Fiegenbaum

Porto Alegre
2015

Catalogação na Publicação

Souza, Jessica Aguiar de
Influência de polimorfismos do gene PON1 na resposta terapêutica de estatinas / Jessica Aguiar de Souza. -- 2015.
62 f. : il., tab. ; 30 cm.

Dissertação (mestrado) -- Universidade Federal de Ciências da Saúde de Porto Alegre, Programa de Pós-Graduação em Patologia, 2015.

Orientador(a): Profa. Dra. Marilu Fiegenbaum.

1. Paraoxonase. 2. Estatinas. 3. Dislipidemia. 4. PON1. 5. HDL-C. I. Título.

Sistema de Geração de Ficha Catalográfica da UFCSPA com os dados fornecidos pelo(a) autor(a).

Agradecimentos

Agradeço à Deus que me amparou e iluminou durante esta caminhada.

À minha orientadora Marilu Fiegenbaum que me acolheu com carinho, agradeço pela compreensão, pelo apoio, orientação e incentivo que tornaram possível a conclusão dessa dissertação.

Dedico esta conquista à minha mãe Deise, minha vó Sedeny e ao meu padrasto Jucimar pela preocupação, torcida, apoio e orações. Agradeço pelas conversas e palavras que serviram de incentivo para seguir em frente. Obrigada por todo amor que me dão e pela oportunidade de ter vocês em minha vida.

Ao meu namorado Guilherme, que dividiu comigo as angústias, frustrações e preocupações. Amigo e companheiro de todas as horas, que me ajudou sempre que possível e quando não era possível, esteve ao meu lado, me incentivando e dando apoio. Dedico este trabalho à você meu amor!

À minha tia Dioni, agradeço por estar sempre disposta a me ajudar, pelo carinho, paciência e pelas horas dispendidas na madrugada para que eu tivesse êxito no meu projeto. Obrigada Tia!

Às minhas amigas Gabriela e Ana Carolina, pelo incentivo, carinho e cumplicidade. Adoro vocês!

Sumário

Lista de abreviaturas utilizadas

DAC- doença arterial coronariana

DCV- doença cardiovascular

PON- paraoxonase

PON1- paraoxonase1

PON2- paraoxonase2

PON3- paraoxonase3

HDL-C- colesterol de lipoproteína de alta densidade

LDL-C- colesterol de lipoproteína de baixa densidade

LDLox- lipoproteína de baixa densidade oxidada

VLDL- lipoproteína de muito baixa densidade

TG- triglicerídeo

CT- colesterol total

HMG-CoA- 3-hidroxi-3-metil-glutaril coenzima A

FR- fator de risco

SNP- polimorfismo de nucleotídeo único

APO A-I- Apolipoproteína A-I

PCR- reação em cadeia de polimerase

Resumo da Dissertação

Introdução: Elevação nos níveis de LDL-C é determinante para o aparecimento da aterosclerose. Como abordagem terapêutica as estatinas se destacam, uma vez que regulam a velocidade de síntese do colesterol. As paraoxonases humanas são uma família de enzimas polimórficas, sintetizadas e secretadas pelo fígado, que se ligam fortemente ao HDL-C, conferindo-lhe maior potencial antioxidante e diminuindo a acumulação de peroxidação lipídica do LDL-C.

Objetivo: Estudar a influência de variantes no gene *PON1* na resposta à hipolipemiante ao tratamento com sinvastatina e atorvastatina em indivíduos habitantes de Porto Alegre.

Material e Métodos: Nossa coorte foi composta por 643 pacientes dislipidêmicos com prescrição médica de uso de estatinas. Níveis séricos de CT, HDL-C, LDL-C e TG foram mensurados antes e após aproximadamente 6 meses de tratamento com sinvastatina ou atorvastatina. As genotipagens foram realizadas através da técnica de PCR em tempo real para dois polimorfismos do gene *PON1*, Q192R (rs662) e L55M (rs854560).

Resultados: Quanto ao polimorfismo Q192R (rs662), pacientes que possuem o alelo Q alcançaram mais a meta lipídica de HDL-C quando comparado aos homozigotos RR (χ^2 P=0.009, análise de ajuste residual P=0.003). Em relação

ao polimorfismo L55M (rs854560), os homozigotos LL foram sub-representados entre os sujeitos que atingiram a meta do HDL-C (χ^2 P=0.026, análise de ajuste residual P=0.008). Através de uma análise logística multivariada observamos que o sexo feminino (OR=1.71, CI95%=1.04-2.83, P=0.036), os níveis basais de HDL-C (OR=1.13, CI95%=1.10-1.16, P<0.001) e os genótipos QQ/QR + MM/ML aumentam a chance de atingir a meta de HDL-C (OR=2.81, CI95%=1.35-5.85, P=0.006).

Conclusões: Nosso estudo observou que os polimorfismos estudados, Q192R (rs662) e L55M (rs854560) possuem um papel importante na resposta interindividual em alcançar a meta dos níveis de HDL-C.

Palavras-chave: Dislipidemia; HDL-C, LDL-C; Paraoxonase; PON1; Estatinas.

1 Introdução

1.1 Doença Cardiovascular e Dislipidemia

A doença arterial coronariana (DAC), juntamente com as outras doenças do aparelho circulatório constituem as principais causas de morbimortalidade nas diversas regiões do mundo (Nicholls e cols., 2012; Go e cols., 2013; Hsu e cols., 2013). No Brasil, dados mostram que 31,8% de mortes em adultos foram ocasionadas por doenças cardiovasculares (Ministério da Saúde, 2013).

O aparecimento dessas doenças se deve à presença da aterosclerose, doença crônica de etiologia multifatorial (Watanabe e cols., 1996; Barton, 2013) que se caracteriza pelo acúmulo de gorduras na parede dos vasos sanguíneos, um processo que tem início numa fase precoce da vida e progride durante anos, apresentando-se em estado já avançado no momento em que surgem as primeiras manifestações clínicas (Stary e cols., 1995). A aterosclerose envolve dois processos, o de aterose, que é a acumulação de gordura formado por vários macrófagos, e a esclerose, que compreende os leucócitos, fibrose das células musculares lisas e tecido conjuntivo (Ross, 1999; Rahimi, 2012). Esta doença é caracterizada como uma inflamação crônica resultante de interações entre elementos normais da parede arterial, células T, macrófagos derivados de monócitos e lipoproteínas modificadas, acarretando em lesões (placas) que migram em direção ao lúmen arterial (Navab e cols., 1996; Ross, 1999; Sposito e cols., 2007) gerando diversas complicações circulatórias, como infarto do

miocárdio, infarto cerebral e aneurisma aórtico (Gimbrone, 1999; Montenegro, 1999).

Diversos estudos em humanos e animais apontam que a aterosclerose é uma resposta do organismo à injúria tecidual e sugerem que elevados níveis de lipoproteína de baixa densidade (LDL) (Steinberg, 1997; Tavafi, 2013) contribuem para o desencadeamento da lesão aterosclerótica (Steinberg e cols., 1989; Libby e cols., 2011) e aumento de doenças cardiovasculares (Glass e Witztum, 2001). Os achados de lipídios oxidados em lesões ateroscleróticas mostram uma relação com a progressão da aterogênese (Yla-Herttuala e cols., 1989; Aviram e cols., 1995) e uma das explicações para os eventos iniciais da aterosclerose é a oxidação da LDL (Steinberg e cols., 1989; Esterbauer, 1990; Tavafi, 2013) que desencadeiam eventos pró-inflamatórios que iniciarão todo o processo de formação do ateroma (Steinberg, 1997; Libby, 2002).

Existem vários fatores que podem aumentar a probabilidade da ocorrência das doenças cardiovasculares (DCV), chamados fatores de risco cardiovasculares (FR). Entre os FR bem estabelecidos pela literatura médica, destacam-se: níveis elevados de colesterol sérico (CT) e de triglicerídeos (TG), baixos níveis de lipoproteínas de alta densidade (HDL-C), altos níveis de lipoproteínas de baixa densidade (LDL-C), antecedentes familiares, diabetes *mellitus,* hipertensão arterial sistêmica, idade, sexo, obesidade, sedentarismo, tabagismo, estresse**,** dieta aterogênica, agentes infecciosos, entre outros (Izar e cols., 2003; Piegas e cols., 2003; Avezum e cols., 2005; Balagopal e cols., 2011).

Dos fatores de risco acima elencados, a dislipidemia é predominante e a associação desta com os demais fatores, aumenta de maneira significativa o risco de ocorrência de doenças do aparelho circulatório (Sposito e cols., 2007; Redberg e cols., 2009). Vários estudos mostram que há uma forte relação inversa entre as concentrações séricas de HDL-C e a suscetibilidade de desenvolver aterosclerose e que níveis de HDL-C elevado protegem contra a aterosclerose, mesmo com concentrações elevadas de LDL-C (Durrington, 1989; Nicholls e cols., 2007). Gotto e Brinton (2004) mostraram que a cada aumento de 1mg/dL de HDL, reduz em até 3% o risco de eventos cardiovasculares.

A lipoproteína de alta densidade possui a capacidade de transportar lipídios através do transporte reverso de colesterol, propriedade antiaterogênica (deGoma e cols., 2008; Rader e cols., 2009) onde ocorre o controle da inflamação e do processo oxidativo através da inibição das moléculas de adesão nas células edoteliais, no fator nuclear Kappa B e nas quimiocinas (Navab e cols., 2001; Barter e cols., 2002; Barter e cols., 2004), em que o excesso de colesterol é captado e transportado dos tecidos periféricos para o fígado (Navab e cols., 2004; Rader e cols., 2009), através do plasma, sendo excretado pela bile ou incorporado na lipoproteína de muito baixa densidade (VLDL) (Ansell e cols., 2005). Outra propriedade antioxidante e anti-inflamatória da HDL é realizada pela inibição da LDL oxidada (LDLox) e redução da atividade biológica da LDLox, inibindo desta forma o início ou a progressão da aterosclerose (Navab e cols., 2000 ; Mackness e cols., 2002; Marchegiani e cols., 2008) (Figura 1).

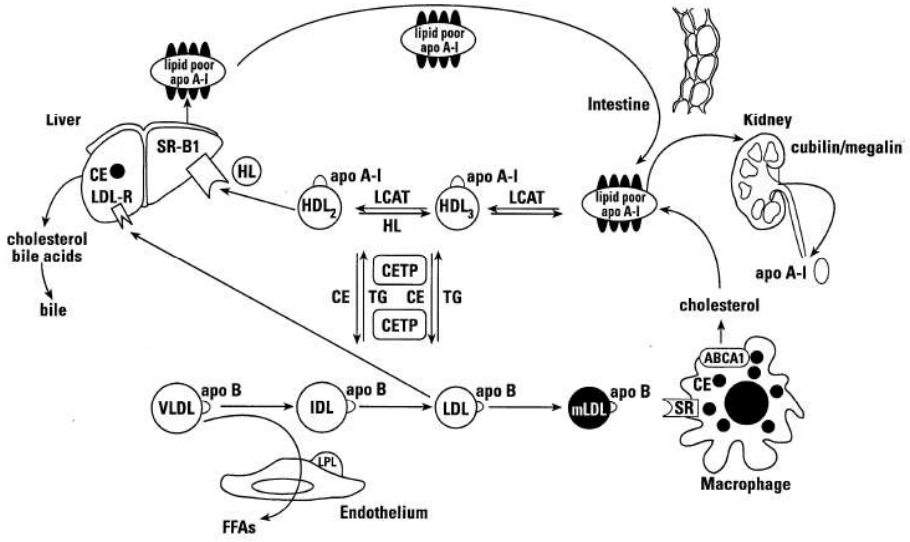

Figura 1. Transporte reverso de colesterol com o transporte de colesterol das células periféricas para o fígado e posterior excreção na bile. O transportador adenosina trifosfato cassete de ligação (ABC) -A1 medeia o efluxo de colesterol livre das células periféricas e macrófagos para apolipoproteína (apo) A-I rica em lipoproteínas nascente de alta densidade (HDL). A esterificação por lecitina: colesterol aciltransferase (LCAT) resulta na formação de partículas esféricas de HDL_2 maduras. Na sequência, a proteína de transferência de colesteril éster (CETP) promove a troca de colesteril éster (CE) por triglicéridos, que são hidrolisados pela lipase hepática (LH). O CE ainda associado com HDL_2 são seletivamente removidos pelo plasma sanguíneo através do receptor scavenger B1 (SR-B1) e transportados até o fígado (transporte reverso de colesterol direto [TRC]), resultando em pequenos lipídios pobres em apo A-I (HDL senescente) que são suscetíveis à filtração glomerular e endocitose pelos receptores cubilina/megalina no túbulo proximal renal. Uma porção do CE é transferido por partículas apo B contendo partículas (lipoproteína de muito baixa densidade [VLDL], lipoproteína de densidade intermediária [IDL] e

lipoproteína de baixa densidade [LDL]) que são transportados para o fígado para absorção através do receptor de LDL (TRC "indireto"). FONTE: Gotto e Brinton, 2004.

1.2 Terapia Hipolipemiante

A hipercolesterolemia se constitui num dos importantes fatores de risco para a aterosclerose e, por conseguinte, para a DCV (Sposito e cols., 2007). Por isso, reduzir os níveis séricos de LDL-C, tem se constituído importante medida de prevenção primária e secundária para a DCV (Dembowski e Davidson, 2009).

Segundo orientação contida na IV Diretriz Brasileira Sobre Dislipidemias e Prevenção da Aterosclerose (2007), se não houver controle desejável dos níveis de colesterol com a mudança no estilo de vida do paciente ou no caso deste não poder esperar os resultados advindos desta mudança, a adição de fármacos hipolipemiantes deve ser empregada como estratégia para reduzir o LDL-C.

Como abordagem terapêutica tem se destacado o grupo farmacológico das estatinas, agentes hipolipemiantes, cuja ação inibe a 3-hidroxi-3-metil-glutaril coenzima A redutase (HMG-CoA), enzima que regula a velocidade de síntese do colesterol, reduzindo seus níveis séricos (Evans e Mcleod, 2003; Tuñón e cols., 2007). Após diversos estudos e evidências sobre a redução de eventos de DAC e redução dos níveis de LDL-C, a terapia com estatinas vem

sendo considerada de primeira linha (Baigent e cols., 2010; Perk e cols., 2012; Anderson e cols., 2013; Grundy, 2013; Stone e cols., 2013).

O desenvolvimento das estatinas se deu após a compreensão dos mecanismos moleculares que regulam tanto a biossíntese, quanto os níveis de colesterol sérico (Gould e cols., 1998). Esta potente classe de fármacos, possui a capacidade de reduzir de forma significativa a mortalidade e eventos cardiovasculares em pacientes com hipercolesterolemia ou doença arterial coronariana (Lim, 2013; Stone e cols., 2013). Dados sugerem que em pacientes com mais idade, o tratamento com estatina é seguro quanto aos benefícios clínicos (risco de morbidade e mortalidade por DAC) (Berthold e Gouni-Berthold, 2011; Athyros e cols., 2013).

Estudos randomizados que envolvem a utilização das estatinas mostraram que estes fármacos são os mais eficientes em reduzir os níveis de colesterol total (18 a 25%) e LDL-C (25 a 55%), além de reduzirem moderadamente os níveis de triglicerídeos (10 a 15%) e elevarem os níveis de HDL-C (5 a 10%) (Gotto, 2001; Waters, 2001; Kreisberg e Oberman, 2002).

Estudos também mostraram que estes fármacos apresentam efeitos adicionais além de sua ação hipocolesterolemiante. Estes benefícios independentes do colesterol são chamados efeitos pleitrópicos (Tziomalos e cols., 2014) e incluem a melhoria da função endotelial, a diminuição da migração e proliferação das células musculares lisas vasculares, a atenuação da remodelagem vascular e miocárdica, a inibição da agregação plaquetária, a diminuição da inflamação vascular e da oxidação e a estabilização de placas

ateroscleróticas (Ridker e cols., 1998; Dupuis e cols., 1999; Fuhrman e cols., 2002; Ridker e cols., 2008).

Apesar da comprovação da eficácia das estatinas no combate à dislipidemia, foram relatados alguns efeitos secundários graves, como a miotoxicidade, hepatotoxicidade e neuropatia periférica, levando a suspensão da medicação (Sikka e cols., 2011).

Outro aspecto importante a considerar é o fato de muitos pacientes não atingirem as metas de perfil lipídico estando em tratamento com estes fármacos, o que demonstra haver uma variabilidade interindividual na resposta, fator este que limita a ação benéfica desta classe farmacológica (Evans e Mcleod, 2003; Pang e cols., 2009).

Estudos mostram diversos motivos que levam às variações na resposta ao tratamento, tais como: doenças, diferenças na famacocinética e na farmacodinâmica dos medicamentos, fatores ambientais e fatores genéticos (Shastry, 2006).

1.3 Farmacogenética

Pesquisas revelam que os indivíduos podem reagir diferentemente ao mesmo tipo de medicamento. A resposta poderá ser benéfica, nula ou desfavorável. A razão desta variabilidade nas respostas se deve, em parte, a fatores genéticos (Kurtz, 2004). Essa constatação fez surgir a farmacogenética, ciência que se ocupa em conhecer melhor a influência genética sobre a variabilidade de respostas aos fármacos (Roses, 2001).

Em última análise a farmacogenética visa colaborar para a individualização da terapêutica, isto é, a prescrição do medicamento correto e na dosagem adequada para cada indivíduo, com base nas suas características genéticas (Chowbay e cols., 2005; Shastry, 2006).

Os resultados observados no tratamento farmacológico com estatinas demonstram que estes fármacos são bem tolerados, entretanto podem ocorrer numa minoria de pacientes, efeitos adversos graves (Humphries e Hingorani, 2006; Schmitz e Langmann, 2006) e nem todos os pacientes apresentam a mesma eficácia farmacológica. Parte desta variabilidade de resposta, como já descrito, está associada à variabilidade genética individual. Já foram descritos diversos genes que podem estar associados à farmacogenética de estatinas (Fiegenbaum e cols. 2005a; Fiegenbaum e cols. 2005b; Fiegenbaum e cols. 2005c; Sortica e cols., 2012; Lima e cols., 2013), mas como a resposta terapêutica é uma resposta multifatorial, novos genes candidatos podem ser investigados. Entre eles, estão os genes que codificam as paraoxonases 1, 2 e 3 (*PON1*, *PON2* e *PON3*), responsáveis pela modulação do perfil lipídico e também pela ativação da sinvastatina, que é um pró-fármaco (Draganov e cols., 2005).

1.4 Paraoxonases

A paraoxonase humana é uma família multigênica de enzimas polimórficas, constituída de três diferentes genes: *PON1*, *PON2* e *PON3* (La Du, 1988). Os três membros da família PON se localizam adjacentemente no

braço longo do cromossomo 7 (entre q21.3 e q22.1) em humanos e no cromossomo 6 em ratos (Primo-Parmo e cols., 1996; Perla-Kajan e Jakubowski, 2010) (Figura 2). Nos humanos, os genes compartilham ~70% da sequência de nucleotídeos, possuindo aproximadamente o mesmo peso molecular, sendo muito semelhantes (Primo-Parmo e cols., 1996; Boright e cols., 1998; Mackness e cols., 2002; Campo e cols., 2004). Estes genes possuem também nove éxons, oito íntrons e promotores TATA-less (La Du e cols., 1999; Campo e cols., 2004). Estudos mostram que essa semelhança estrutural entre os genes da *PON*, poderia estar vinculada a existência de um precursor evolutivo comum (Primo-Parmo e cols., 1996; Hegele, 1999; Sorenson e cols., 1999; Draganov e cols., 2000).

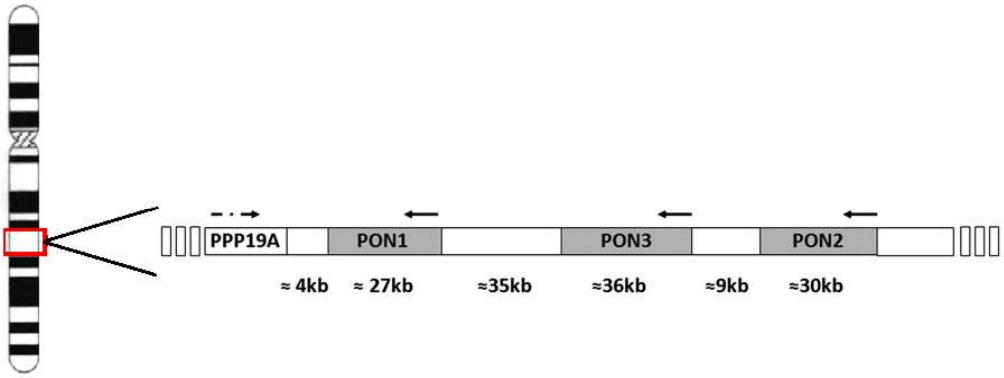

Figura 2. Mapa genético da família de genes *PON* em humanos. FONTE: Li e cols., 2003 (modificado).

Mazur, em 1946, descreveu pela primeira vez a hidrólise enzimática de compostos organofosforados em tecidos animais, e nos anos de 1950, Aldridge e colaboradores (1953a, 1953b) identificaram as esterases de PONs, baseado na capacidade de hidrolisar o substrato paraoxon, produto do catabolismo do inseticida *paration* em soros humanos e de diversas espécies de mamíferos (Costa e cols., 2003, Khersonsky e cols., 2005). Somente em 1996 foi

descoberto que o gene *PON*/atividade arilesterase pertence a uma família multigene de esterases (*PON1*, *PON2* e *PON3*) nomedas conforme a ordem de descoberta. Desde então, a PON é foco de estudo de diferentes áreas da pesquisa (Van Himbergen e cols., 2006; Goswami e cols., 2009) e pode estar associada a eventos fisiopatológicos como a síndrome metabólica (Flekac e cols., 2008; Kordi-Tamandani e cols., 2011), doença aterosclerótica coronariana (Pérez-Herrera e cols., 2008; Shin, 2009; Précourt e cols., 2011) e distúrbios neurológicos (Lawlor e cols., 2007; Kucukali e cols., 2008).

Inicialmente a PON foi caracterizada como uma organofosfato-hidrolase, sendo considerada uma enzima de proteção do sistema nervoso, pois pode desintoxicar os derivados oxon e diazoxon de alguns pesticidas organofosforados (Costa e cols., 2003; Li e cols., 2003; Costa e cols., 2005). Pesquisas revelaram que tanto a PON2 quanto a PON3 possuem apenas a atividade lactonase (Camps, 2009), entretanto, somente a PON1 possui a capacidade de metabolizar organosfosfatos, toxinas nervosas (sarin, soman), ésteres aromáticos (fenilacetato, tiofenilacetato e 2-naftilacetato) e lactonas alifáticas e aromáticas (homocisteína tiolactona, dihidrocumarina (Draganov e cols., 2004; Costa e cols., 2005). Desta forma, alguns estudos consideram o nome PON inadequado, pois não possui ligação com a atividade paraoxonase (Watson e cols., 1995, Aviram e cols., 2000, Draganov e cols., 2004).

A primeira enzima da família descoberta foi a paraoxonase1 em 1946, entretanto, somente no ano de 1991 intensificou-se o interesse em estudá-la, após resultados mostrarem a associação da atividade da PON1 à DCV (Mackness e cols., 1991). Pesquisas têm confirmado que a atividade sérica da

PON1 está inversamente correlacionada com a doença cardiovascular, ou seja, indivíduos portadores de doenças das carotídeas, coronarianas e infarto do miocárdio, apresentam uma menor atividade da PON1 (Jarvik e cols., 2002; Mohamed e cols., 2010). A PON1 é uma esterase sintetizada e secretada pelo fígado e é cálcio-dependente (Gan e cols., 1991; Furlong e cols., 1991; Goswami e cols. 2009), possui 355 aminoácidos com peso molecular de 43-45 kDa formada de três cadeias de carboidratos correspondendo a 15,8% do seu peso (La Du, 1996; Mackness e cols., 1998; Lu e cols., 2006; Marsillach e cols., 2008). Na região central da PON1 encontram-se dois átomos de cálcio, sendo um para estabilização e outro para a sua atividade catalítica (Harel e cols., 2004). A estrutura tridimensional auxilia na determinação do papel fisiológico da PON1 e na análise do modo que a enzima liga-se à HDL-C (Figura 3) (Aviram e cols., 2005), esta lipoproteína contém a apolipoproteína A-I (Apo A-I) e a apoliproteína J, conferindo maior potencial antioxidante ao HDL-C e diminuindo a acumulação de peroxidação lipídica (Mackness e cols., 1991; Kleemola e cols., 2002).

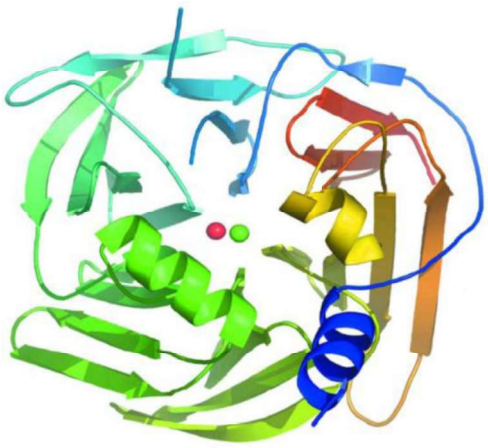

Figura 3: Estrutura tridimensional da PON1 com dois átomos de cálcio na sua região central (vermelho e verde). FONTE: Harel e cols., 2004 (adaptado).

A PON1 não interage com lipoproteínas de baixa densidade ou em lipoproteínas de densidade muito baixas, porém ocorre uma interação específica entre a PON e o HDL-C, possivelmente pela sua associação com a Apo A-I, conforme acima citado (Ahmed e cols., 2001; Blatter e cols., 2006).

A reação de antioxidação promovida pela paraoxonase só é possível a partir da interação específica entre a PON e os lipídios oxidados. Duas hipóteses prováveis foram formuladas para explicar sua atuação: na primeira, a ação protetora seria difundida durante a interação entre HDL-C/LDL-C e a PON, na segunda hipótese, considerada a mais provável, os lipídios oxidados do LDL-C devem ser primeiramente capturados pelo HDL-C (James e Deakin, 2004).

Estudos complementares demonstram que a PON1 não apenas previne a formação de LDL-C oxidada por inativar os fosfolipídios oxidados derivados da LDL-C (Costa e cols., 2005; Navab e cols., 1996), mas também protege os fosfolipídios presentes na HDL do processo de oxidação, ampliando a eficiência em sua ação antioxidante (Aviram e cols., 1998).

Desta forma, o *PON1* é apontado como gene candidato para explicar a propensão para doença cardiovascular, embora se acredite que polimorfismos no gene *PON1* contribuem apenas com um pequeno grau de proteção, pois as modulações da PON1 decorrentes do estilo de vida, dieta, uso de estatinas, terapia de reposição hormonal e outras, alteram os níveis da enzima, podendo agir também como protetores contra DCV (Ferre e cols., 2002; Jarvik e cols., 2002; Costa e cols., 2005). A PON1 em humanos pode sofrer influências de vários fatores, podendo inibir ou estimular sua expressão e atividade. Em

recém-nascidos, a atividade enzimática sérica da PON1 é baixa, aumenta no decorrer da vida e volta a reduzir com o envelhecimento, provavelmente devido ao desenvolvimento de condições relacionadas ao estresse oxidativo (Seres e cols., 2004; Lescai e cols., 2009).

Existem diferenças na atividade e expressão da PON entre os sexos, mulheres apresentam níveis significativamente maiores quando comparadas às médias de atividade em homens (Faggioni, 2003; Costa e cols., 2005; Sumegova e cols., 2006), mostrando a existência de uma relação sexo-dependente justificada por fatores hormonais (Rios e cols., 2007; Costa e cols., 2005). Também existem variações na atividade enzimática da PON1 entre indivíduos de diferentes etnias, podendo variar de 10 a 40 vezes (Cataño e cols., 2006; Elkiran e cols., 2007; Eng e cols., 2009; Eom e cols., 2011).

Mais de 200 polimorfismos de nucleotídeo único (SNPs) foram identificados no gene *PON1* humano (Richter e cols., 2010), localizados em regiões codificadoras, intrônicas e reguladoras (La Du e cols., 2003; Costa e cols., 2011). Os SNPs mais estudados do *PON1* estão localizados nas posições 192 do gene, onde ocorre uma substituição do aminoácido Glutamina (Gln) por Arginina (Arg) (Q→R) e na posição 55, com a substituição de uma Leucina (Leu) por Metionina (Met) (L→M) (Leviev e James, 2000; Li e cols., 2003; Draganov e La Du, 2004; Ng e cols., 2005). Estudos mostram que no polimorfismo Q192R a aloenzima Q é mais eficiente em proteger as lipoproteínas contra oxidação e consequente associação com menor risco de desenvolver DAC (Durrington e cols., 2001). Desta forma, o alelo Q possui um

papel protetor para as DCV e o alelo R um efeito deletério (Lakshmy e cols., 2010; Liu e cols., 2014).

A substituição L55M está associada de forma independente com a formação de placas ateroscleróticas (Fortunato e cols., 2003), sendo determinante para diferentes concentrações séricas de proteína PON1 (Brophy e cols., 2001). Portadores do alelo M possuem altas concentrações enzimáticas de PON1, portanto o genótipo MM é um preditor significativo de CAD (Oliveira e cols., 2004). Indivíduos homozigotos QQ(192) e MM(55) possuem uma maior eficiência contra a oxidação da LDL (Jaouad e cols., 2003; Lakshmy e cols., 2010).

Conforme exposto, a *PON1* está relacionada com a proteção contra as doenças cardiovasculares, agindo como agente antioxidante e anti-inflamatório. Por esta razão, se justifica o interesse e a relevância da pesquisa relacionada aos polimorfismos deste gene para conhecer a influência genética sobre a variabilidade das respostas ao tratamento com estatinas, objetivando otimizar os efeitos da medicação através da personalização terapêutica.

1.5 Referências Bibliográficas

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2 Objetivos

2.1 Objetivo Geral

Estudar a influência de variantes no gene *PON1* na resposta hipolipemiante ao tratamento com sinvastatina e atorvastatina, por via oral, em amostras de indivíduos habitantes de Porto Alegre.

2.2 Objetivos Específicos

2.2.1 Analisar as frequências genotípicas dos polimorfismos rs662 e rs854560 do gene *PON1* em amostras de pacientes usuários de sinvastatina e atorvastatina.

2.2.2 Investigar a associação dos diferentes polimorfismos no gene *PON1* com a resposta hipolipemiante (eficácia) ao tratamento com sinvastatina e atorvastatina.

3. Artigo científico redigido em inglês

"*PON1* polymorphisms are predictors of ability to attain HDL-C goals in statin-treated patients"

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Enviado para publicação na Revista "Clinical Biochemistry"

PON1 polymorphisms are predictors of ability to attain HDL-C goals in statin-treated patients

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Glossary

CAD- coronary artery disease

CVD- cardiovascular disease

PON- paraoxonase

PON1- paraoxonase1

PON2- paraoxonase2

PON3- paraoxonase3

HDL-C- high-density lipoprotein cholesterol

LDL-C- low-density lipoprotein cholesterol

TG- triglyceride

TC- total cholesterol

HMG-CoA- 3-hydroxymethyl-3-methylglutaryl coenzyme A

SNP- single nucleotide polymorphism

PCR- polymerase chain reaction

ABSTRACT:

Objectives: PON1 plays an important role in inhibiting LDL-C oxidation, which reduces atherosclerosis and cardiovascular disease. Elevated PON1 activity or levels may contribute to increased HDL-C levels, but controversy exists over the hypothesis that genetic variation in the PON1 gene locus modulates HDL-C levels and responses to statin treatment. Therefore, the objective of this study was to investigate the association between two polymorphisms in the *PON1* gene and statin responses in a south Brazilian population.

Design and Methods: The study population included 433 dyslipidemic patients who were prescribed statins. Total cholesterol, triglyceride, HDL-C and LDL-C levels were measured in these patients both before and after approximately 6 months of treatment with simvastatin/atorvastatin. Genotypes were assessed by real-time PCR for two *PON1* polymorphisms, Q192R (rs662) and L55M (rs854560).

Results: Baseline lipid levels were not associated with Q192R or L55M polymorphisms. For the Q192R (rs662) polymorphism, we observed that HDL-C goals were attained less often in patients with RR homozygosity than in Q allele carriers (χ^2 P=0.009, adjusted residual analysis P=0.003). For the L55M (rs854560) polymorphism, LL homozygotes were underrepresented among subjects that achieved the HDL-C goal (χ^2 P=0.026, adjusted residual analysis P=0.008). Analysis by univariate logistic regression confirmed that QQ/QR and MM/ML carriers had an increased chance of attaining HDL-C goals (OR=2.41, CI95%=1.32-4.40, P=0.004 and OR=1.68, CI95%1.15-2.45, P=0.008). In a

multivariate logistic analysis used to assess predictors of attaining an HDL-C goal > 1.55 mmol/L, we observed that gender (OR=1.71, CI95%=1.04-2.83, P=0.036), baseline HDL-C levels (OR=1.13, CI95%=1.10-1.16, P<0.001) and the QQ/QR + MM/ML genotypes increased the chance of achieving HDL-C goals (OR=2.81, CI95%=1.35-5.85, P=0.006).

Conclusions: The results of this study show that the Q192R (rs662) and L55M (rs854560) polymorphisms may play a role in interindividual variation in achievement of HDL-C goals in response to statins.

Keywords: Dyslipidemia; HDL-C, LDL-C; Paraoxonase; PON1; Statins.

1 Introduction

Coronary artery disease (CAD) and other cardiovascular diseases are the leading causes of morbidity and mortality in different regions of the world [1,2]. In Brazil, approximately 32% of all adult deaths are due to cardiovascular disease (CVD) [3]. The onset of these diseases is related to the presence of atherosclerosis, a chronic disease with multifactorial etiology [4,5]. Among the factors contributing to risk for CVD, dyslipidemia has been shown to be prevalent and its association with other factors significantly increases the risk of developing cardiovascular diseases [6].

With regards to genetic influences on coronary heart disease development, there is evidence indicating that changes in paraoxonase family (*PON*) genes contribute to CVD pathogenesis [7,8,9]. Studies have shown that the paraoxonase1 (PON1) enzyme is associated with the inhibition of lipid peroxidation of high-density lipoprotein cholesterol (HDL-C), reduced oxidative modification of low-density lipoprotein cholesterol (LDL-C) [10,11], and preservation of HDL-C and LDL-C function [12,13]. Serum PON1 activity is also inversely correlated with cardiovascular disease in that individuals with diseases of the carotid artery or coronary and myocardial infarction display lower PON1 activity [14,15]. Thus, paraoxonase 1 has been identified as a candidate gene that may explain individual propensity for cardiovascular disease [16,17].

Human paraoxonases are a multigene family of polymorphic enzymes that consists of three different genes, *PON1*, *PON2* and *PON3*, which are located adjacent to each other on the long arm of chromosome 7 (between q21.3 and q22.1) in humans [18]. Although many modulators of PON1 have

been identified, by far the biggest effect on PON1 activity occurs through *PON1* genetic polymorphisms [19]. The purification and cloning of human *PON1* revealed two common polymorphisms (L55M and Q192R) that have been extensively investigated [20]. The Q192R (rs662) polymorphism is more widely recognized and is a missense substitution that changes a glutamine (Q) to an arginine (R) [21]. It has been shown that the 192R allele is less effective at inhibiting the oxidation of LDL and has a substrate-dependent effect on activity [22]. Furthermore, lower HDL-C and higher LDL-C and triglyceride concentrations were observed in RR homozygous patients [23]. The L55M (rs854560) polymorphism involves the conversion of a leucine (L) to a methionine (M). The 55M allele results in significantly higher PON1 mRNA and serum protein levels [24] and has been associated with lipid profiles, but these results have been inconsistent [23,25].

Statins are 3-hydroxymethyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that are widely used to treat dyslipidemic patients, where they have been shown to lower LDL-C and triglyceride levels and slightly increase HDL-C levels [26]. Although statins are usually highly effective and are generally well tolerated, interindividual variation in efficacy has been observed. Considering that 1) lipid and lipoprotein homeostasis gene polymorphisms may be related to drug-response differences, 2) high levels of PON1 activity [27] or levels [28] contribute to increased levels of HDL-C, and 3) Q192R and L55M polymorphisms at *PON1* gene locus are responsible for 48% and 4% of PON1 phenotypic variance, respectively [29], our hypothesis is that these SNPs may modulate lipid levels, and especially HDL-C levels, in patients treated with

statins. Thus, the objective of this study was to evaluate the influence of two functional polymorphisms in the *PON1* gene (Q192R and L55M) on the therapeutic efficacy of simvastatin/atorvastatin in a cohort of Southern Brazil.

2 Methods

2.1 *Study population*

The study population for this analysis consisted of 643 patients with dyslipidemia who received lipid-lowering simvastatin/atorvastatin therapy after medical evaluation. All patients invited to participate in this study were screened with a physical examination, provided a medical history and underwent a clinical laboratory evaluation. The individuals were of European descent and residents in the city of Porto Alegre, Brazil. Exclusion criteria were: age < 20 years, a triglyceride (TG) concentration ≥ 4.52 mmol/L, altered thyroid stimulating hormone levels, impaired hepatic or renal function, the presence of an unstable or uncontrolled disease that influences lipid metabolism, and previous therapy with other lipid-lowering drugs. Physical examinations, clinical data, and clinical laboratory data were obtained by a physician, and the doses administered were determined by a physician according to the clinical characteristics of each patient. Biochemical measurements were analyzed prior to the initiation of treatment with statins (simvastatin/atorvastatin) and approximately six months later to evaluate therapeutic efficacy. Other prescribed medications, such as calcium channel blockers, diuretics, and antithrombotic agents, were continued throughout the study.

After the application of exclusion criteria, four hundred thirty-three patients were analyzed for the lipid-lowering efficacy of the therapy. Therapeutic responses were measured at baseline and after approximately 6 months (6.2 ± 3.2) of treatment. This protocol was approved by the Ethics Committee of Federal University of Health Sciences of Porto Alegre. All participants in the study gave written informed consent.

2.2 *Biochemical analyses*

Peripheral blood samples were collected after 12 h of fasting. Total cholesterol (TC), triglyceride (TG), and glucose concentrations were determined using conventional enzymatic methods on a Mega Merck Analyzer (Merck Darmstadt, Germany), and with specific kits supplied by Labtest (Brazil). HDL-C was determined using a selective immunoseparation-based homogeneous assay, followed by colorimetric quantification. LDL-C was calculated according to Friedewald et al [30].

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

2.3 *DNA extraction and PON1 genotyping*

Genomic DNA was isolated from peripheral blood samples using a technique described by Lahiri and Nurnberger [32]. The genotyping of the

Q192R (rs662) and L55M (rs854560) polymorphisms in the *PON1* gene was performed by allelic discrimination real-time PCR using TaqMan SNP Genotyping assays from Applied Biosystems Inc., California, USA, according to the manufacturer's recommended protocol.

2.4 *Statistical analysis*

Continuous variables are expressed as the mean ± standard deviation. TG levels were log-transformed before analyses due to their skewed distribution, although non-transformed values are shown in the Results section. Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations was tested using chi-square tests. Haplotype frequencies and linkage disequilibrium were estimated with Multiple Locus Haplotype Analysis version 2.0 and ARLEQUIN software version 3.1. Based on the findings of Kivistö et al. [33], the daily doses of simvastatin were transformed to equivalent doses of atorvastatin at a ratio of 2:1. The mean percentage of change in plasma lipid levels was obtained by taking the difference between pre- and post-treatment lipid levels, multiplying by 100 and then dividing by the pre-treatment level for each parameter. To determine the association between genotypes and baseline lipid levels or mean percentage of change in plasma lipid levels, the means for each variable were compared with a General Linear Model using the type III sums of squares. Age (years), gender, smoking status, standardized statin dosage (mg), treatment period (months), and baseline lipid levels (mmol/L) were included in each model as covariates for follow-up lipid levels and for lipid-lowering

response (% of change in plasma lipid levels). For baseline lipid levels, age (years), gender, and smoking status variables were used as covariates. The distribution of genotypes between groups was evaluated by chi-square test, and adjusted residual values [cell-by-cell analyses] were assessed by WINPEPI 2.1, when appropriate. Multiple logistic regression was performed to assess the variables associated with achievement of lipid level goals. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS 19.0 for Windows.

3 Results

3.1 *Characteristics of the study population*

We evaluated a cohort of 433 individuals of European descent who were residents of southern Brazil and who used simvastatin/atorvastatin lipid-lowering therapy. The main characteristics of the study population are presented in Table 1. The subjects in the study were aged between 25 and 88 years (61.6 ± 10.9 years); 33.3% were males, 83.1% of participants were on simvastatin treatment, and 16.9% participants were atorvastatin users. The average treatment duration was approximately 6.2 ± 3.2 months and did not differ between simvastatin users (6.2 ± 3.2 months) and atorvastatin users (6.3 ± 3.3 months) (P = 0.827). The average standardized dose of statins was 10.3 ± 4.6 mg, and this also did not differ between simvastatin users (10.1 ± 4.35 mg, atorvastatin converted range 2.5-20 mg) and atorvastatin users (11.0 ± 5.7 mg, range 10-40 mg) (P = 0.125). Most patients never smoked (79.7%), while 13.2% of patients were ex-smokers and 6.9% were current smokers. A total of

32.6% of subjects had prior cardiovascular disease, 72.1% of subjects had hypertension, and 16.6% had diabetes. Concomitant therapies used by patients throughout the study were calcium channel blockers (18.0%), diuretics (40.4%), antithrombotic drugs (27.5%), beta-blockers (32.6%) and ACE inhibitors (22.6%).

3.2 *Association between Q192R and L55M polymorphisms and statin efficacy*

Genotype frequencies were not significantly different than those expected under Hardy-Weinberg equilibrium. The baseline and mean percent reductions of lipid and lipoprotein levels, by *PON1* Q192R (rs662) and L55M (rs854560) genotypes, are shown in Table 2. Overall, statin treatment significantly reduced plasma levels of total cholesterol (-26.4%, P<0.0001), LDL-C (-36.4%, P < 0.0001) and triglyceride levels (-11.9%, P<0.001), while the increase in HDL-C did not reach statistical significance (+3.7%, P = 0.08). Baseline lipid levels and percentage of change in TC, HDL-C, LDL-C and TG levels were not associated with *PON1* gene polymorphisms.

When rates of achievement of lipid goals were analyzed (Table 3) for patients with Q192R (rs662) polymorphism, we observed that HDL-C goal was less likely to be achieved in RR homozygous patients than in Q allele carriers (χ^2 P=0.009, adjusted residual analysis P=0.003). The L55M (rs854560) polymorphism was significantly associated with attainment of HDL-C and total cholesterol goals. For HDL-C, LL homozygous patients were underrepresented among subjects that achieved the HDL-C goal (χ^2 P=0.026, adjusted residual analysis P=0.008). For total cholesterol levels, MM homozygous patients were

more frequently observed to achieve goals (χ^2 P=0.040, adjusted residual analysis P=0.017). Univariate logistic regression confirmed that QQ/QR and MM/ML carriers had an increased likelihood of achieving HDL-C goals (OR=2.41, CI95%=1.32-4.40, P=0.004 and OR=1.68, CI95%1.15-2.45, P=0.008, respectively). Considering these results, we performed a multivariate logistic analysis to assess the predictors of achieving HDL-C > 60 mmol/L. In this analysis, gender (female), treatment time (months), age (years), standard statin dose (mg) and baseline HDL-C levels (mmol/L) were entered as covariates and genotypes were grouped into reference group (RR and LL homozygotes), QQ/QR carriers, MM/ML carriers and QQ/QR + MM/ML carriers. In this analysis, we observed that female gender (OR=1.71, CI95%=1.04-2.83, P=0.036), baseline HDL-C levels (OR=1.13, CI95%=1.10-1.16, P<0.001) and being in the QQ/QR + MM/ML group increased the chance of HDL-C goal achievement (OR=2.81, CI95%=1.35-5.85, P=0.006) (Table 4).

4 Discussion

Low levels of HDL-C and high levels of TC, TG and LDL-C are significant predictors of atherosclerosis and CVD throughout the population [34]. In this study, we examined the influence of polymorphisms at Q192R (rs662) and L55M (rs854560) in the *PON1* gene on responses to simvastatin and atorvastatin treatment. Our main finding was an association between polymorphisms Q192R (rs662) and L55M (rs854560) with patient attainment of HDL-C goals.

Statins are widely used, effective drugs for the treatment and prevention of dyslipidemia and CVD [35]. Studies assessing patients treated with statins have demonstrated significant reductions in LDL-C, TG and TC levels and increases in HDL-C levels and a reduced incidence of cardiovascular events and mortality. One study showed that LDL-C was reduced by 55% and that cardiovascular events were decreased from 20-30% [36,37]. There is interindividual variability in extent to which statins are clinically effective at lowering LDL-C levels and reducing cardiovascular events [38,39]. Some of this variability may be due to pharmacogenetic variation, and this is supported by studies that have identified genetic variations that are associated with differences in responses in LDL-C levels to statin therapy [39].

Results of epidemiological studies have also shown that future cardiovascular events can be reduced by 2% to 3% for every 1 mg/dL (0.0259 mmol/L) increase in HDL-C levels [40]. These data show that HDL-C is an important, independent marker for atherosclerosis and for CVD. However, although there is data showing that HDL-C is important, few pharmacological strategies are available to increase HDL-C levels and few studies have identified pharmacogenetic markers useful for tracking statin therapies and HDL-C levels [41-44]. Regarding PON1 gene polymorphisms, the Q192R SNP was associated with HDL-C changes in two studies: Malin et al [41] demonstrated that pravastatin increased HDL-C in R allele carriers but not in QQ homozygous patients ($p=0.095$), and Fu et al [44] demonstrated that RR homozygous patients showed a greater increase in HDL-C levels after

simvastatin therapy. However, this association was not found in patients treated with fluvastatin [43].

PON1 is a calcium-dependent hydrolytic enzyme that is associated with HDL [25,45]. This enzyme plays an important role in the regulation of HDL antioxidant and anti-inflammatory activities [46], and elevated PON1 activity [27] or levels [28] may contribute to increased levels of HDL-C and Apo A-I. PON1 inhibits the oxidation of LDL-C and prevents the accumulation of oxidized lipids in vessels, thereby inhibiting the progression of atherosclerosis and consequently reducing the development of CVDs [10,47]. Studies in animals have shown that in mice that lack the *PON1* gene, atherosclerosis developed more rapidly than in wild-type animals [48]. In humans, results have shown that reduced PON1 activity is associated with increased incidence of atherosclerosis and cardiovascular diseases [49].

Our results demonstrate that, for the Q192R polymorphism, RR homozygous patients less frequently reached their HDL-C lipid target than did carriers of the Q allele. These findings are in apparent contrast with those observed by Malin et al [41] and Fu et al [44], but our results corroborate data regarding CVD risk showing an association between Q192R polymorphisms and CVD, reporting that the Q allele protected against CVD while the R allele had a deleterious effect [8,50,51]. For the L55M polymorphism, we found that carriers of the M allele reached their HDL-C lipid target more often than patients who were homozygous LL, and these data also corroborate the studies supporting an association with CVD [50-53].

Because HDL-C levels and statin responses are complex phenotypes, we also performed a multivariate logistic regression and included covariates in the final model that may have influenced the analyzed outcome. These included age, sex, duration of treatment, baseline HDL-C, standard dose of statin and genotype. As expected, this analysis showed that sex and baseline HDL-C levels are also associated with success in reaching a goal of HDL> 1.55 mmol/L. Our results show that the analyzed polymorphisms maintain their statistical associations with reaching the HDL-C goal even after adjustment for these covariates, indicating that they are markers of the analyzed outcome. Furthermore, analysis of the data revealed a combined effect, where both polymorphisms increase the chance of achieving the outcome analyzed.

This study also has some limitations. It was an observational study with no placebo control. All patients were advised to begin physical activity and diet control, but monitoring and evaluation of these activities were not part of this study. Moreover, while we chose two functional polymorphisms that are often evaluated in cardiovascular outcomes, this study does not cover all variability in the PON1 gene. Other variants and situations may therefore also influence the activity of paraoxonase 1 and its lipid-lowering efficacy. The effectiveness of statins is affected by multifactorial characteristics. We believe that other genes that we have not studied may also be important contributors to the observed variation in patient responses to treatment with statins. Moreover, the results found for TC goal in our study might be spurious, since there is only a direct interaction of PON1 with HDL and LDL molecules and other studies found no relationship between *PON1* polymorphisms and TC or TG levels.

In conclusion, the present study suggests that the Q192R (rs662) and L55M (rs854560) polymorphisms in the PON1 gene contribute to interindividual variation in responses to statin and the ability to achieve HDL-C goals in a Brazilian population. In the literature, one can find several studies that show evidence of a relationship between PON1 and modifications of lipoproteins, as well as the development of CVD. However, studies that specifically address relationships between HDL-C responses in statin-treated patients and *PON1* gene variability are limited. Our results therefore highlight the importance of analyzing patient *PON1* genotypes during statin treatment.

Acknowledgments

The authors are thankful for the financial support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), PROAP-CAPES, FINEP.

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Table 1- Patient characteristics

Characteristics	
Number	433
Age (years)	61.6 ± 11.0
Sex, male (%)	33.3
Statin Use (%)	
Simvastatin	83.1
Atorvastatin	16.9
Treatment time (months)	6.2 ± 3.2
Overall statin standard dose (mg)*	10.3 ± 4.60
Simvastatin standard dose (mg)	10.1 ± 4.35
Atorvastatin dose (mg)	11.0 ± 5.70
Smoking (%)	
Past	13.2
Current	6.9
Never	79.7
Prior CVD (%)	32.6
CVD Family history (%)	16.4
Diabetes (%)	16.6
Hypertension (%)	72.1
Concomitant therapy use (%)	
Calcium channel blockers	18.0
Diuretics	40.4
Antithrombotic	27.5
ACE inhibitor	22.6
β-blocker	32.6
CYP3A4 substrates	18.0
CYP3A4 inducers	1.4
CYP3A4 inhibitors	21.0

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

Table 2- Baseline, follow-up and mean percent change in lipid and lipoprotein levels after statin treatment, according *PON1* polymorphisms

SNP	n	Total cholesterol	HDL-C	LDL-C	Triglycerides
rs662 (Q192R)					
Baseline lipid levels (mmol/L)					
TT (QQ)	200	6.43 ± 1.02	1.35 ± 0.36	4.26 ± 0.92	1.79 ± 0.95
CT (QR)	178	6.37 ± 1.06	1.33 ± 0.33	4.25 ± 0.92	1.70 ± 0.73
CC (RR)	55	6.35 ± 0.98	1.26 ± 0.27	4.29 ± 0.84	1.76 ± 0.73
p		0.949	0.305	0.931	0.882
Lipid change (%)					
TT (QQ)	200	-26.3 ± 12.8	2.9 ± 22.5	-35.5 ± 18.9	-12.7 ± 30.8
CT (QR)	178	-25.8 ± 12.3	5.3 ± 21.9	-37.0 ± 17.7	-11.3 ± 31.3
CC (RR)	55	-27.7 ± 9.9	2.8 ± 19.3	-37.2 ± 13.6	-16.6 ± 28.4
p		0.552	0.366	0.658	0.583
rs854560 (L55M)					
Baseline lipid levels (mmol/L)					
AA (LL)	194	6.38 ± 0.99	1.32 ± 0.33	4.24 ± 0.92	1.75 ± 0.96
AT (LM)	187	6.42 ± 1.08	1.34 ± 0.36	4.28 ± 0.90	1.75 ± 0.77
TT (MM)	52	6.47 ± 0.96	1.35 ± 0.27	4.33 ± 0.92	1.73 ± 0.56
p		0.770	0.786	0.766	0.968
Lipid change (%)					
AA (LL)	194	-25.8 ± 12.5	3.4 ± 24.0	-35.4 ± 17.9	-10.2 ± 32.6
AT (LM)	187	-26.1 ± 11.8	4.8 ± 20.3	-36.1 ± 17.4	-14.6 ± 30.6
TT (MM)	52	-29.5 ± 13.0	2.1 ± 21.9	-40.8 ± 19.0	-14.9 ± 23.9
p		0.146	0.572	0.142	0.163

Values are means ± standard deviations; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

Table 3 - Percentage of patients reaching lipid goals, according to genotype

	Lipid goal							
SNP	Total cholesterol		HDL-C		LDL-C		Triglycerides	
	< 5.18 mmol/L	≥ 5.18 mmol/L	> 1.55 mmol/L	≤ 1.55 mmol/L	< 2.59 mmol/L	≥ 2.59 mmol/L	< 1.70 mmol/L	≥ 1.70 mmol/L
rs662 (Q192R)								
TT (QQ)	153 (47.5%)	48 (41.0%)	106 (46.5%)[a]	96 (45.7%)	92 (43.6%)	106 (47.1%)	142 (44.9%)	56 (47.1%)
CT (QR)	130 (40.4%)	53 (45.3%)	104 (45.6%)[b]	78 (37.1%)	91 (43.1%)	91 (40.4%)	133 (42.1%)	49 (41.2%)
CC (RR)	39 (12.1%)	16 (13.7%)	18 (7.9%)[c]	36 (17.1%)	28 (13.3%)	28 (12.4%)	41 (13.0%)	14 (11.8%)
p	0.483		0.009		0.763		0.904	
rs854560 (L55M)								
AA (LL)	136 (42.6%)[d]	60 (51.3%)	87 (38.5%)[g]	107 (51.2%)	96 (46.2%)	99 (44.0%)	142 (45.4%)	52 (43.7%)
AT (LM)	137 (42.9%)[e]	50 (42.7%)	110 (48.7%)[h]	78 (37.3%)	86 (41.3%)	101 (44.9%)	134 (42.8%)	51 (42.9%)
TT (MM)	46 (14.4%)[f]	7 (6.0%)	29 (12.8%)[i]	24 (11.5%)	26 (12.5%)	25 (11.1%)	37 (11.8%)	16 (13.4%)
p	0.040		0.026		0.740		0.887	

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
[a] Adjusted residual=+0.16, P=0.871; [b] Adjusted residual=+1.80, P=0.072; [c] Adjusted residual=-2.94, P=0.003
[d] Adjusted residual=-1.61, P=0.108; [e] Adjusted residual=+0.04, P=0.968; [f] Adjusted residual=+2.39, P=0.017
[g] Adjusted residual=-2.66, P=0.008; [h] Adjusted residual=+2.39, P=0.017; [i] Adjusted residual=+0.43, P=0.667

Table 4 – Predictors for HDL >1.55 mmol/L.

Variable	OR	CI 95%	p
Age (years)	0.99	0.97-1.02	0.603
Sex, female	1.71	1.04-2.83	0.036
Treatment time (months)	1.02	0.94-1.10	0.590
Baseline HDL-C levels (mmol/L)	1.13	1.10-1.16	<0.001
Standard dose (mg)	1.01	0.96-1.06	0.746
QQ/QR genotype	1.72	0.80-3.71	0.165
MM/ML genotype	1.33	0.14-12.31	0.800
QQ/QR and MM/ML genotypes	2.81	1.35-5.85	0.006

HDL-C, high-density lipoprotein cholesterol.

4. Considerações finais

O presente estudo faz parte de um projeto maior intitulado "**Estudo da influência da variabilidade genética em fatores de risco cardiovascular e na farmacogenética de hipolipemiantes",** aprovado pelos Comitês de Ética em Pesquisa (CEP) da UFCSPA (Parecer N° 075/05).

É sabido que a dislipidemia é um fator de risco clássico para o desenvolvimento de doenças cardiovasculares, responsável por uma grande morbimortalidade. As estatinas são amplamente utilizadas no tratamento da dislipidemia e é observado uma ampla variação na resposta à estes fármacos. Dentre os fatores responsáveis por estas variações na resposta, estão os fatores genéticos. A farmacogenética estuda polimorfismos genéticos que podem afetar as respostas à terapia medicamentosa, podendo o paciente apresentar uma maior eficácia e menor risco de efeitos adversos. Nosso trabalho visa contribuir para um maior conhecimento da farmacogenética no tratamento com estatinas, onde analisamos dois polimorfismos do gene *PON1* e observamos que o genótipo possui influência na resposta ao tratamento com estatinas. A identificação de polimorfismos, bem como o conhecimento do genótipo dos pacientes, contribui para uma terapia mais individualizada, buscando encontrar a melhor dose, o melhor tratamento e evitando efeitos adversos para o indivíduo.

5. Anexo

5.1. Parecer do Comitê de Ética da UFCSPA

UFCSPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMITÊ DE ÉTICA EM PESQUISA - CEP
UFCSPA

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

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

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

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

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

Porto Alegre, 28 de outubro de 2010.

José Geraldo Vernet Taborda
Coordenador do CEP/UFCSPA