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Farmacogenética das estatinas: utilização de escores genéticos para predição de resposta terapêutica.

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Sumário

Lista de abreviaturas utilizadas

Apo A: Apolipoproteína A

Apo B: Apolipoproteína B

Apo E: Apolipoproteína E

ATP: Adenosina tri-fosfato

AVC: Acidente vascular cerebral

CETP: Proteína de transferência de ésteres de colesterol

CT: Colesterol total

DCV: Doença cardiovascular

CYP: Citocromo P450

CYP3A: Citocromo P450 3A

CYP7A1: Citocromo P450 7A1

DNA: Ácido desoxirribonucleico

ERT: Terapia de reposição enzimática

EUA: Estados Unidos da América

GWAS: Estudo de associação do genoma inteiro

HDL-C: Lipoproteína de alto peso molecular

HMG-CoA: 3-hidroxi-3-methyl-glutaril-CoA redutase

IDL: Lipoproteína de densidade intermediária

LDL-C: Lipoproteína de baixo peso molecular

MTP: Proteína de transferência de triglicerídeo microssomal

NCD: Doença não comunicada

OATP: Polipeptídeo de transporte de ânions orgânicos

PCSK9: Pró-proteína convertase subtilisina/kexin tipo 9

SNP: Polimorfismo de nucleotídeo único

SREBP: Proteína de ligação de elementos reguladores de esterol

TG: Triglicerídeos

VLDL: Lipoproteínas de muito baixa densidade

VNTR: Variações no número de sequências repetidas

Resumo da Tese

Introdução:

O tratamento de escolha para tratar a dislipidemia é a utilização de estatinas; porém, ao longo do tempo observou-se uma grande variabilidade na resposta terapêutica. Neste sentido, vários estudos com abordagens de genes candidatos e associação de genoma inteiro (GWAS) tentam elucidar a genética responsável pela variabilidade da resposta a drogas. No entanto, estas abordagens ainda não conseguiram elucidar o componente genético desta variabilidade, uma vez que a grande maioria dos estudos aborda análises de cada variante isoladamente.

Objetivos:

Criar um escore genético de resposta ao tratamento com estatinas, a partir de marcadores genéticos identificados pelo grupo de pesquisa e através de estudos de varredura genômica.

Material e Métodos:

Nossa amostra foi composta por 477 pacientes de descendência européia, com níveis de colesterol de baixa densidade (LDL-C) acima da meta estabelecida e sem tratamento medicamentoso prévio. Selecionamos os genes que apresentam polimorfismos com maior impacto no resultado terapêutico com as estatinas, no caso os genes *ABCB1*, *CYP3A5*, *SLCO1B1*, *SLCO1B3*, *ABCG2*, *APOE*, *CLMN* e *NR1I2*. Depois de identificar os polimorfismos com redução significativa nos níveis de colesterol total (CT) e de LDL-C, um escore genético de resposta à terapia com estatina foi criado a partir dos marcadores

geneticamente identificados *SLCO1B1* (rs2306283), e *NR1/2* (rs3814055, rs7643645 e rs6785049).

Resultados:

Na amostra, 1,7% dos pacientes receberam um escore final de 0 (zero) com redução do CT, 12,6% em um (1), 36,2% em dois (2), 34,5% em três (3) e 14,9% em quatro (4). Dessa forma, o uso de escores ajuda na escolha da terapia a ser utilizada, uma vez que o paciente com escore 4 teve a maior redução dos níveis de CT e de LDL-C (-30,2% e -42,6%, respectivamente), enquanto o paciente com o escore 0 apresentou a menor redução destes níveis (-12,3%, $p<6x10^{-5}$; -14,4%, $p<4x10^{-7}$, respectivamente).

Conclusão:

Nossos resultados mostram a existência de diferentes respostas individuais ao tratamento com estatinas, sendo o uso do escore genético de resposta ao tratamento importante para a eficácia e segurança do tratamento. Os resultados aqui apresentados ainda demonstram que os genes e as variantes a serem incluídos nos escores podem variar conforme a população, o que torna o trabalho relevante em nível nacional, pois é o primeiro a abordar as variantes genéticas conjuntamente.

Palavras-chave: estatina, dislipidemias, colesterol, GWAS, escore de risco.

1. Introdução

1.1 Doenças Cardiovasculares e Dislipidemia

As doenças cardiovasculares (DCVs) afetam o sistema circulatório, incluindo os vasos sanguíneos e o coração. Elas podem ocorrer na forma de infarto agudo do miocárdio (IAM), acidente vascular cerebral (AVC) e arritmias cardíacas, por exemplo. Sua principal característica é a presença de aterosclerose que impede a passagem do sangue (Ministério da Saúde do Brasil, 2013).

As DCVs são a principal causa de morte no mundo. Estima-se que 17,5 milhões de pessoas foram vítimas de problemas coronarianos, como infartos e derrames em 2016 (Ministério da Saúde do Brasil, 2017; Organização Pan-Americana de Saúde, 2017). No Brasil, em um ano as DCVs são responsáveis por 29,4% de todas as mortes registradas no País, representando mais de 308 mil óbitos principalmente por IAM e por AVC (Ministério da Saúde do Brasil, 2013).

A maioria das DCVs pode ser prevenida por meio da abordagem de fatores comportamentais de risco – como uso de tabaco, de dietas não saudáveis, de obesidade, de sedentarismo e de uso nocivo do álcool - utilizando estratégias para a população em geral. Para as pessoas com DCVs, ou com alto risco cardiovascular (devido à presença de um ou mais fatores de risco, como hipertensão arterial sistêmica (HAS), diabete melito, hiperlipedemia ou doença já estabelecida) é fundamental o diagnóstico e o tratamento precoce por meio de

serviços de orientação ou de administração adequada de medicamentos (Organização Pan-Americana de Saúde, 2017, Nayor e cols., 2016).

Uma das metas do plano global sob liderança da Organização Mundial de Saúde - "*Global action plan for the prevention and control of NCDs 2013-2020*", concentra-se na prevenção e no controle das DCVs. Esta meta, prevê que pelo menos 50% das pessoas elegíveis devem receber terapia medicamentosa e aconselhamento para prevenir IAM e AVC, sendo necessário fortalecer componentes-chave dos sistemas de saúde, incluindo financiamento da atenção em saúde com vistas a garantir o acesso a tecnologias básicas e aos medicamentos essenciais para as doenças não transmissíveis (Organização Pan-Americana de Saúde, 2017).

De acordo com os critérios do *National Cholesterol Education Program* (NCEP, 2002), são considerados como os principais fatores de risco para as doenças cardiovasculares os altos níveis de colesterol sérico encontrados nas lipoproteínas de baixa densidade (LDL-C) e os altos níveis de triglicerídeos, bem como os baixos níveis das lipoproteínas de alta densidade (HDL-C), a idade, o sexo, o histórico familiar de doença cardíaca precoce, a HAS, o tabagismo, o diabetes melito, a obesidade, o sedentarismo e a dieta aterogênica. Estudos demonstram que, dos fatores de risco listados, a dislipidemia é uma condição bastante prevalente e que a combinação desta com outros fatores de riscos aumenta de maneira mais que aditiva o risco de uma DCV, em relação a quando um único fator está presente (Organização Pan-Americana de Saúde, 2017).

O perfil lipídico é considerado uma característica multifatorial, que pode ser influenciado pela genética e/ou pelo ambiente. A alta herdabilidade dos níveis do HDL-C de 21 à 79%, do LDL-C de 31 à 68% e dos TG (triglicerídeos) de 19 à

72% indicam a importância de variações genéticas sobre a determinação dos níveis de lipídios e de lipoproteínas (Ober, Loisel e Gilad, 2008; Elder e cols., 2009). Mesmo com o grande número de polimorfismos e genes já estudados com influência sobre as variações do perfil lipídico, os resultados não elucidam a variabilidade desses níveis em sua totalidade.

1.2 Metabolismo e Transporte do Colesterol

Para maior compreensão da terapia hipolipemiante, é importante entender o metabolismo e transporte do colesterol no organismo humano. A gordura consumida por via oral é absorvida no trato gastrointestinal, mediada pela adenosina trifosfato (ATP), que liga as proteínas de cassete. Esses transportadores são codificados por genes, como *ABCB1*, *ABCG2* e *ABCG8* (Mawxwell e cols., 2017). Após absorvidos no fígado, os lipídeos livres são transformados em colesterol através da enzima 3-hidroxi-3-metil-glutaril-CoA redutase (HMG-CoA), codificada pelo gene *HMGCR*. Algumas moléculas de colesterol deslocam-se para o sistema circulatório e são transportadas aos tecidos periféricos, sendo necessária a produção de apolipoproteínas que são codificadas por genes como o *APOA* e o *APOE*. Estas apolipoproteínas são lipoproteínas de muito baixa densidade (VLDL), lipoproteínas de densidade intermediária (IDL), lipoproteínas de baixa densidade (LDL) e lipoproteínas de alta densidade (HDL). Essas lipoproteínas transportam o colesterol até os tecidos periféricos para construção das membranas celulares e para síntese de hormônios. A lipase hepática, a lipase lipoproteína (codificada pelo gene *LPL*), e a proteína de transferência de ésteres de colesterol (codificada pelo gene *CETP*)

mediam o catabolismo das lipoproteínas e também facilitam a transformação do colesterol em um tipo de lipoproteína ou outro. Assim que os tecidos periféricos não necessitam mais de colesterol, este é transportado de volta ao fígado através de um processo chamado de transporte de colesterol reverso, mediado pela proteína de transferência de éteres de colesterol ou ele se deposita na parede dos vasos (aterosclerose). A receptação do colesterol hepatocelular é mediada por receptores de LDL (codificados pelo gene *LDLRP*) e proteínas relacionadas ao receptor de LDL (codificadas por *PCSK9* e outros genes); no entanto, a quantidade de receptores de LDL expressa na superfície dos hepatócitos é mediada por outras proteínas, incluindo as proteínas de ligação de elementos reguladores de esterol - SREBPs (codificadas pelo gene *SREBF*), as proteínas chaperonas SREBP (codificadas pelo gene *SCAP*), e o receptor alfa de estrogênio (codificado pelo gene *ESR1*) (Gotto, 2001; Kostis, 2007; Hutz, Fiegenbaum, 2008; Faludi e cols., 2017; Mawxwell e cols., 2017).

Uma vez que a receptação hepática ocorre, a excreção biliar é mediada pela apolipoproteína E (Apo E), as proteínas cassete *ATPbinding* e os polipeptídeos de transporte de ânions orgânicos (OATPs) (codificados por *SLCO*). Enzimas do citocromo P450 (CYP) como a CYP7A1, também auxiliam na eliminação do colesterol. Assim, existem muitas proteínas envolvidas no transporte de lipídeos que são codificadas por genes que podem levar à variação interindividual nas concentrações de lipoproteínas.

1.3 Terapia Hipolipemiante

Como já citado anteriormente, a dislipidemia é um fator de risco muito 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). Atualmente, os Estados Unidos da América (EUA) e a Europa (*American Heart Association, National Lipid Association, European Atherosclerosis Society*) enfatizam a importância da farmacoterapia das estatinas na prevenção da doença cardiovascular aterosclerótica, expandindo recentemente as indicações de prescrição e as populações-alvo (Stone e cols., 2013; Stroes e cols., 2015; Nayor e cols., 2016 Kitzmiller e cols., 2016).

Em 2013 as diretrizes *American College of Cardiology* e da *American Heart Association* (ACC-AHA) para o tratamento do colesterol expandiram as indicações para a terapia com estatinas na prevenção das DCVs. A recomendação até aquele momento era de iniciar a terapia com estatinas em pacientes com DCV estabelecida ou diabetes e LDL-C ≥100mg/dL (Pencina e cols., 2014). Com a atualização recomenda-se a utilização de estatinas em indíviduos com doença cardiovascular conhecida, independente dos níveis de LDL-C e para prevenção primária a farmacoterapia com estatinas em indivíduos com níveis de LDL-C ≥190mg/dL ou ≥ 70mg/dL (Pencina e cols., 2014).

Com a expansão nas indicações da terapia com estatinas elevou-se o número de adultos elegíveis para o tratamento com estatina em 12,8 milhões, principalmente entre os adultos mais velhos sem doença cardiovascular nos Estados Unidos da américa (Pencina e cols., 2014). No ano de 2017, no Brasil tivemos a Atualização da Diretriz de Brasileira de Dislipidemias e Aterosclerose, expandindo ainda mais os níveis de LDL-C e CT na farmacoterapia, além de

tornar possível a realização dos exames de diagnóstico sem a necessidade do paciente estar em jejum de 12 horas, conforme mostra a tabela 1.

Tabela 1. Valores referência e alvo terapêutico do perfil lipídico (adultos > 20 anos), comparativo V Diretriz Brasileira de Dislipidemias e Prevenção de Aterosclerose versus Atualização da Diretriz Brasileira de Dislipidemias e Prevenção de Aterosclerose - 2017

	V Diretriz			Atualização da Diretriz		
Lipídeos	Com jejum (mg/dL)	Sem jejum (mg/dL)	Categoria Referencial	Com jejum (mg/dL)	Sem jejum (mg/dL)	Categoria Referencial
CT	< 200	NA	Desejável	< 190	< 190	Desejável
HDL-C	> 60	NA	Desejável	> 40	> 40	Desejável
TG	≥ 240	NA	Desejável	< 150	< 175	Desejável
			Categoria de risco			
LDL-C	< 100	NA	Ótimo	< 130	< 130	Baixo
	100-129	NA	Desejável	< 100	< 100	Intermediário
	130-159	NA	Limítrofe	< 70	< 70	Alto
	160-189	NA	Alto	< 50	< 50	Muito alto
	≥ 190	NA	Muito alto			

*CT: Colesterol Total; HDL-C: Lipoproteína de alto peso molecular de colestrol; TG: Triglicerídeos; LDL-C: Lipoproteína de baixo peso molecular; mg/dL: miligrama por decilitro (Xavier e cols., 2013; Faludi e cols., 2017).

De acordo com a Atualização da Diretriz Brasileira Sobre Dislipidemias e Prevenção da Aterosclerose (Faludi e cols., 2017), a abordagem farmacológica para o tratamento da dislipidemia envolve fármacos de diversas classes

terapêuticas, sendo elas divididas conforme sua ação, esta classificação consiste naqueles que agem predominantemente nas taxas séricas de colesterol e nos que agem principalmente sobre as taxas de triglicerídeos:

- medicamentos com ação predominantemente na colesterolemia: as resinas ou sequestradores de ácidos biliares, a ezetimiba e os inibidores da 3-hidroxi 3-metilglutaril coenzima-A (HMG CoA) redutase (estatinas ou vastatinas);

- medicamentos que atuam predominantemente sobre os triglicerídeos: os fibratos, o ácido nicotínico (niacina) e os ácidos graxos de ômega 3;

- novos fármacos: os inibidores da proteína de transferência de ésteres de colesterol (CETP), o inibidor da proteína de transferência de triglicerídeo microssomal (MTP), os inibidores da pró-proteína convertase subtilisina/kexin tipo 9 (PCSK9), os inibidores da síntese de apolipoproteína B (antissenso anti-ApoB), os inibidores da síntese de apolipoproteína C-III (antissenso anti-ApoC-III), o antissenso antipolipoproteína C-III (este fármaco ainda não foi aprovado pelas agências regulatórias), a lipase ácida lisossômica recombinante humana (Terapia de Reposição Enzimática – ERT), e a sebelipase alfa (que ainda não foi aprovado pelas agências regulatórias).

Como dito anteriormente, o tipo de dislipidemia é que irá definir a escolha da classe terapêutica a ser utilizada. A terapia com hipolipemiantes apresenta diversas ações benéficas sobre o sistema cardiovascular e potencialmente sobre outros sistemas. Porém, alguns pacientes apresentam efeitos adversos graves após sua utilização e outros não apresentam a eficácia desejada (Faludi e cols., 2017). A variabilidade interindividual na resposta, portanto, é um dos fatores que pode limitar o benefício a ser alcançado.

1.4. Estatinas

As estatinas estão entre os fármacos mais utilizados na medicina e são os mais usados no tratamento da hipercolesterolemia, uma vez que, independentemente da categoria de risco individual, dados dos maiores estudos randomizados envolvendo estas classes farmacológicas mostram que as estatinas são as mais eficientes em baixar os níveis de CT (18 a 25%) e LDL-C (25 a 55%). Além disso, elas também reduzem moderadamente os níveis de TG (10 a 15%) e aumentam da mesma forma os níveis de HDL-C (5 a 10%). Estes estudos também mostram que a utilização de estatinas diminui os riscos de eventos cardíacos fatais e não fatais (24 a 37%) independentemente de outros fatores como idade, sexo, tabagismo, diabetes melito ou HAS (Bhatnagar, 1998; Gotto, 2001; Kreisberg e cols., 2002).

Esses fármacos atuam inibindo parcial e reversivelmente a enzima HMG-Coa redutase, que catalisa uma etapa inicial e limitante de velocidade na biossíntese do colesterol (Brunton e cols., 2012). Com a inibição da HMG-CoA redutase ocorre a redução da secreção de lipoproteínas contendo apolipoproteína B (Apo-B) e aumento da atividade do receptor de LDL, diminuindo os seus níveis plasmáticos (Endo, 1992). Adicionalmente à melhora do perfil lipídico, vários estudos demonstram que as estatinas podem reduzir o risco cardiovascular por mecanismos diferentes, os chamados efeitos pleiotrópicos, diminuindo a inflamação, inibindo a proliferação de células do músculo liso e melhorando a função endotelial (Liao, 2005).

A primeira estatina estudada em seres humanos foi a compactina, renomeada mevastatina, que apresentou o potencial terapêutico dessa classe de fármacos. A primeira estatina aprovada para uso em seres humanos foi a lovastatina, que foi isolada do *Aspergillus terreus.* Existem também outras estatinas, a pravastatina e a sinvastatina ambas derivadas quimicamente modificadas da lovastatina. Além disso, temos atualmente a fluvastatina, a rosuvastatina e a pitavastatina que são compostos sintéticos estruturalmente distintos (Brunton e cols., 2012).

Embora as estatinas possuam normalmente ação satisfatória e sejam amplamente utilizadas na clínica, variações nas respostas ao tratamento são verificadas, e alguns efeitos adversos graves são raramente relatados. Estes últimos já foram relacionados à terapia com estatinas; porém, toxicidade hepática e muscular são os mais importantes (Farmer e Torre-Amione, 2000; Hamilton-Craig, 2001; Kiortsis e cols., 2007; Sewright, Clarkson e Thompson, 2007; Isackson e cols., 2011; Sposito e cols., 2007; Stroes e cols., 2015; Faludi e cols, 2017). A frequência de toxicidade hepática varia entre 1 e 3%, sendo que as 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 (Farmer e Torre-Amione, 2000; Hamilton-Craig, 2001; Sewright, Clarkson e Thompson, 2007; Isackson e cols., 2011; Marciante e cols., 2011).

O real mecanismo envolvido no desenvolvimento dos efeitos adversos das estatinas não é completamente conhecido. Porém, fatores de risco incluem altas doses, idade avançada, sexo feminino, presença de doenças multi-sistêmicas, coadministração com determinados fármacos ou polifármacos, entre outros, além da variabilidade genética (Sewright, Clarkson e Thompson, 2007).

Os estudos de farmacogenética das estatinas já realizados estão concentrados, basicamente, na avaliação da eficácia do tratamento com relação à melhoria do perfil lipídico (Ingelman-Sundberg, 2001; Humphries e cols., 2006; Schmitz e cols., 2006; Hutz e cols., 2008, Barber e cols., 2010; Franke, Gardner, 2010; Chasman e cols., 2012; Deshmukh e cols, 2012; Sortica e cols., 2012; Cuevas e cols., 2016; Ciuculete e cols., 2017). O número de estudos visando à avaliação da influência de polimorfismos genéticos sobre o desenvolvimento de efeitos adversos decorrentes do tratamento com estatinas, porém, não é tão extenso (Ingelman-Sundberg, 2001; Fiegenbaum e cols., 2005a, 2005b, 2005c Humphries e cols., 2006; Schmitz e cols., 2006; Rosendo e cols., 2007; Link e cols., 2008; Ieri, Higuchi, Sugyyama, 2009;Barber e cols., 2010, Deshmukh e cols., 2012; Sortica e cols., 2012; Hopewel e cols., 2013; Postmus e cols., 2014; Kitzmiller e cols., 2016; Leusink e cols, 2016a, Leusink e cols., 2016b; Maxwell e cols., 2017;). Dentre os estudos de farmacogenética das estatinas citados anteriormente podemos salientar três trabalhos desenvolvidos por pesquisadores do nosso próprio grupo de pesquisa, que identificaram os efeitos das variações em genes envolvidos na resposta ao tratamento com as estatinas (Fiegenbaum e cols., 2005a, 2005b, 2005c, Sortica e cols., 2012).

1.5. Farmacogenética das estatinas

Sabe-se que muitos indivíduos apresentam variabilidade de resposta e susceptibilidade à toxicidade a medicamentos/fármacos, conforme descrito anteriormente. Tal fato mostra a importância de conhecer os aspectos que podem influenciar as respostas aos medicamentos, e os fatores que podem levar às reações adversas.

A farmacogenética estuda as influências genéticas sobre as respostas aos fármacos, através da pesquisa em genes, buscando por polimorfismos que predisponham às doenças, modulem respostas aos fármacos, afetem a farmacocinética e farmacodinâmica dos fármacos, e estejam associados a reações adversas de medicamentos (Mukherjee e cols., 2003; Chowbay e cols., 2005).

São considerados polimorfismos genéticos as variações nas sequências de nucleotídeos que compõem o DNA (ácido desoxirribonucleico). Estas variações ocorrem com uma frequência de 1% ou mais na população e de forma estável (Metzger e cols., 2006). Os polimorfismos genéticos ocorrem mais frequentemente nas formas de deleções, mutações, substituições de base única (SNP – *Single Nucleotide Polymorphism*) ou variações no número de sequências repetidas (VNTR), micro e minissatélites (Griffiths e cols., 2002).

As diferenças nas respostas terapêuticas entre os indivíduos podem estar associadas aos polimorfismos genéticos presentes em genes afetando a farmacocinética ou a farmacodinâmica do fármaco (Chowbay e cols., 2005). Conforme visto anteriormente, muitas evidências sugerem que SNPs em genes que codificam transportadores ou enzimas metabolizadoras de medicamentos, bem como na biossíntese e no reparo do DNA, podem determinar a eficácia dos medicamentos e a sua toxicidade (Ingelman e cols., 2001; Maxwell e cols., 2017).

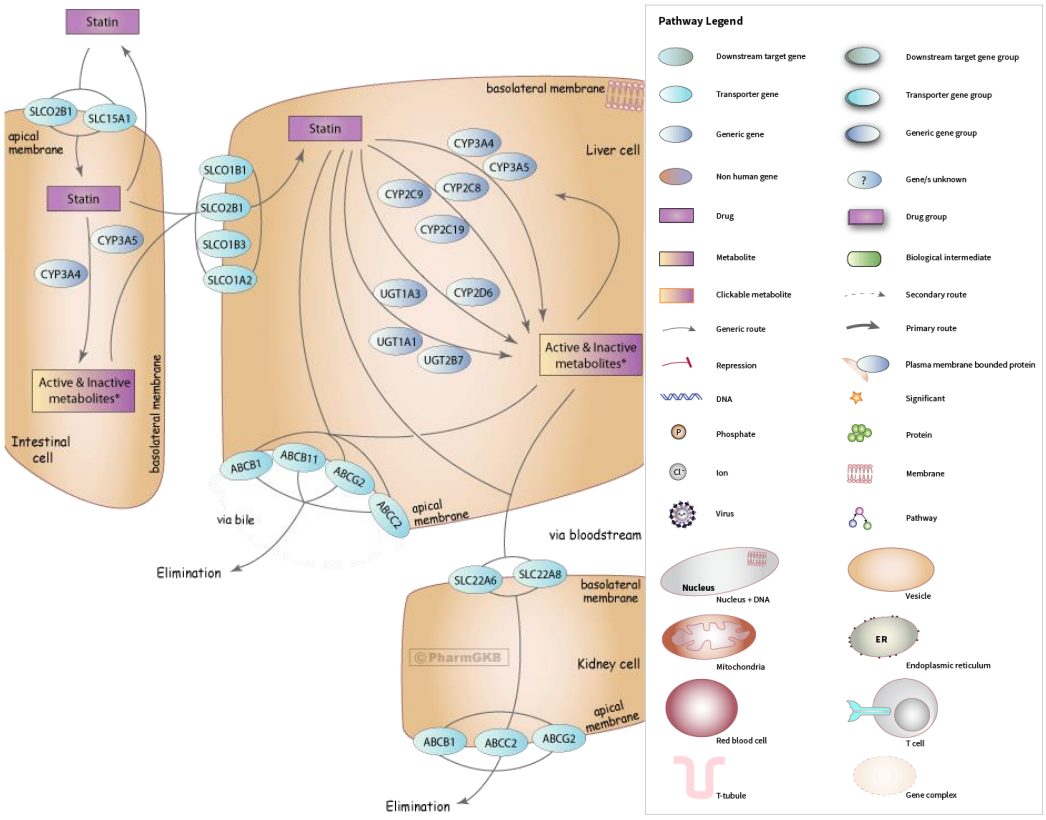

Figura 1. Representação dos genes envolvidos do metabolismo e transporte das estatinas. PharmGK.37 (Reprodução com permissão de Pharmacogenomics Knowledge Base [PharmGKB] e Stanford University, https://www.pharmgkb.org/pathway/PA145011108).

Diversos estudos de genes candidatos e de GWAS “Genome wide association study” – Estudo de associação do genoma inteiro, concentram-se na influência de variantes genéticas na farmacocinética (por exemplo, níveis do fármaco e dos metabólitos no sangue, área sob tempo de concentração) e farmacologia das estatinas (redução de lipídios, frequência de eventos adversos e ocorrência de eventos cardiovasculares) (Kitzmiller e cols., 2016). Desta maneira, existem estudos atuais de revisão que se concentraram nos principais genes que afetam a farmacocinética das estatinas (por exemplo, transportadores

e enzimas metabolizadoras), eficácia das estatinas (por exemplo, alvos e trajeto da droga) e toxicidade dos órgãos finais (por exemplo, miopatia). Na tabela 1, Maxwell e cols. (2017) detalham as variantes farmacogenéticas já estudadas e que estão relacionadas à segurança e à eficácia das estatinas.

Tabela 2. Resumo das variantes farmacogenéticas associadas com a eficácia e a segurança das estatinas.

Gene	Alelo	rs ID	Frequência do alelo	N	Eficácia/segurança	Estatina
ABCB1	c.3435T>C p.Ile1145=	rs1045642	52,6%	114	Segurança	Sinvastatina
			54,0%	85	Eficácia	Sinvastatina
			51,9%	344		Atorvastatina
	c.1236T>C p.Gly412=	rs1128503	43,5%	85	Eficácia	Atorvastatina
			44,0%	114		
	c.2677T>A/G p.Ser893Ala/Thr	rs2032582	51,0%	85	Eficácia	Atorvastatina
	c.999+1228C>A	rs3789244	42,3%	1883	Eficácia	Sinvastatina Pravastatina Atorvastatina
	c.2065-76T>A	rs1922242	35,2%		Eficácia	Sinvastatina Pravastatina Atorvastatina
			28,0%	76	Eficácia	Fluvastatina
ABCG8	c.55G>C/A p.Asp19His/Asn	rs11887534	6,7%	338	Eficácia	Atorvastatina
			6,5%	337		
ABCG2/ BCRP	c.34G>A p.Val12Met	rs2231137	27,5%	62	Segurança	Rosuvastatina
	c.532-2387T>C	rs2199936	11,0%	3523	Eficácia	Rosuvastatina
	c.421C>A/G p.Gln141Glu/Lys	rs2231142	9,5%	660	Segurança	Atorvastatina
			9,5%	32	Segurança	Rosuvastatina
			28,0%	62		
			32,0%	386		

			33,7%	276		
			10,9%	165		
			28,0%	100		
			5,5%	601		
			32,0%	386		
			33,7%	276		
			NP	32	Segurança	Fluvastatina
ACE	c.2306-119_2306-118insATACAGTCACTTTTTTTTTTTTTTTGAGACGGAGTCTCGCTCTGTCGCCC	rs1799752	52,0%	364	Eficácia	Fluvastatina
APOA1	c.-113G>A	rs670	20,2%	397	Eficácia	Pravastatina
	c.-113G>A c.-21+67C>T	rs670-rs5069 GG-CC	16,0%	243	Eficácia	Atorvastatina
APOA5	c.-620C>T	rs662799	16,0%	657	Eficácia	Sinvastatina Atorvastatina Lovastatina
Agrupamento gênico APOE/C1/C2/C4	c.*459A>G	rs4420638	19,0%	1976	Eficácia	Sinvastatina Pravastatina
				3936	Eficácia	Atorvastatina
	c.*459A>G	rs4420638	20,3%	966	Eficácia	Sinvastatina
	ε_2 (c.526C>T; p.Arg176Cys)	rs7412	7,0%	97	Eficácia	Pravastatina
		rs429358-rs7412 T-T	6,0%	328	Eficácia	Atorvastatina

	c.-558A>T	rs449647	26,0%	56	Eficácia	Atorvastatina
	ε_2 proxy c.-2127G>A	rs445925	11,0%	2780	Eficácia	Atorvastatina
	c.*459A>G	rs4420638	16,0%	2780	Eficácia	Atorvastatina
	c.-245T>C	rs71352238	10,0%	3523	Eficácia	Rosuvastatina
	ε_4 (vs. ε_2 e ε_3) c.388T>C p.Cys130Arg	rs429358-rs7412	15,0%	98	Eficácia	Lovastatina
	c.*459A>G	rs4420638	12,5%	372	Eficácia	Rosuvastatina
CETP	c.118+279G>A	rs708272	57,0%	212	Eficácia	Atorvastatina
	c.118+279G>A	rs708272	50,0%	99	Eficácia	Sinvastatina
			40,6%	807	Eficácia	Pravastatina
			39,9%	812	Eficácia	Pravastatina
	c.1264G>A	rs5882	27,5%	180	Eficácia	Sinvastatina
	c.-998G>A	rs4783961	32,0%	76	Eficácia	Fluvastatina
CLMN	c.83-24208A>T	rs8014194	24,0%	1976	Eficácia	Sinvastatina Pravastatina Atorvastatina
CYP3A4	c.352A>G p.Ile118Phe	rs55951658	3,3%	16	Eficácia	Sinvastatina
	c.-392G>A	rs2740574	6,3%	340	Eficácia	Atorvastatina
	c.-392G>A	rs2740574	10,6%	142	Eficácia	Atorvastatina
	c.522-191C>T	rs35599367	3,6%	646	Eficácia	Sinvastatina
			4,7%	273	Eficácia	Sinvastatina
			10,6%	80	Eficácia	Sinvastatina
CYP3A5	c.219-237A=	rs776746	8,4%	69	Eficácia	Sinvastatina Atorvastatina
			7,3%	137	Segurança	Atorvastatina

			8,0%	46	Eficácia	Lovastatina
CYP7A1	c.-267C>A	rs3808607	45,8%	337	Eficácia	Atorvastatina
			45,6%	324		
			NP	265		
CYP2C9	c.1075A>C/G p.Ile359=	rs1057910	NP	24	Segurança	Fluvastatina
CYP2D6	c.506-1G>A	rs3892097	17,0%	263	Segurança	Atorvastatina
ESR1	c.453-397T>C	rs2234693	50,0%	338	Eficácia	Atorvastatina
	c.453-351A>G	rs9340799	63,0%	338		
HMGCR	c.118+279G>A	rs708272	6,7%	1536	Eficácia	Pravastatina
	g.74615021C>T	rs17671591	24,0%	101		Atorvastatina
	c.*372T>C	rs12916	NP	26		Atorvastatina
	c.1912A>G p.Ile638Val	rs5908				Atorvastatina
KIF6	c.2104T>C p.Trp702Arg	rs20455	35,0%	4276	Eficácia	Pravastatina
			36,2%	1778	Eficácia	Atorvastatina
LDLR	c.1959T>C p.Val653=	rs5925	42,0%	55	Eficácia	Fluvastatina
LPA	c.3947+467T>C	rs10455872	8,0%	2780	Eficácia	Atorvastatina
			5,0%	3523	Eficácia	Rosuvastatina
LIPC	-514C>T	-	21,8%	236	Eficácia	Pravastatina
LPL	-93 T>G	-	3,6%	419	Eficácia	Pravastatina
	c.1421C>G p.Ser474Ter	rs328	12,0%	378	Eficácia	Rosuvastatina
PCSK9	c.7078+299A>G	rs17111584	5,0%	3523	Eficácia	Rosuvastatina
PON1	c.575A>G p.Gln192Arg	rs662	25,5%	51	Eficácia	Pravastatina
SLCO1B1	c.521T>C p.Val174Ala	rs4149056	15,2%	66	Eficácia	Sinvastatina

						Pravastatina Atorvastatina
			NP	32	Segurança	Sinvastatina Pravastatina Atorvastatina Rosuvastatina
			10,6%	646	Segurança	Sinvastatina
			15,0%	175	Segurança	Sinvastatina
			14,6%	192	Segurança	Sinvastatina
			10,0%	45	Eficácia	Pravastatina
			18,3%	41	Segurança	Pravastatina
			NP	30	Segurança	Pravastatina
		rs4149056	12,8%	141	Segurança	Rosuvastatina
			18,5%	165	Segurança	Rosuvastatina
			11,0%	100	Segurança	Rosuvastatina
			13,2%	272	Segurança	Rosuvastatina
	c.-2585G>A	rs2306281	63,8%	141	Segurança	Rosuvastatina
	c.-910G>A	rs4149015	7,3%	41	Segurança	Pravastatina
	c.521T>C p.Val174Ala	rs4149056	4,9%	41	Segurança	Pravastatina
	c.-2585G>A	rs2306281	15,0%	23	Segurança	Pravastatina
			10,6%	33	Eficácia	Pravastatina
	c.463C>A p.Pro155Ser c.388A>G. p.Asn130Asp	rs11045819 +rs2306283	17,0%	420	Eficácia	Fluvastatina
SLCO1B1	c.521T>C p.Val174Ala	rs4149056	3,6%	41	Segurança	Pravastatina

	c.388A>G. p.Asn130Asp	rs2306283				
	c.-910G>A	rs4149015				
SCAP	c.1627G>A. p.Val543Ile	rs12487736	48,0%	99	Eficácia	Sinvastatina
SOD2	c.47T>C p.Val16Ala	rs4880	50,0%	122	Eficácia	Rosuvastatina
SREBF2	c.1784G>C. p.Gly595Ala	rs2228314	68,8%	142	Eficácia	Atorvastatina
TLR4	c.776A>G p.Asp259Gly	rs4986790	6,6%	655	Eficácia	Pravastatina

Tabela adaptada de Maxwell e cols. (2017).

NP: frequência do alelo não descrita na publicação.

1.6. Estudos de Associação de Genoma Inteiro (GWAS)

Conforme descrito anteriormente, as proteínas transportadoras de fármacos têm sido alvo do aumento de interesse por diversas áreas terapêuticas, devido ao seu importante papel nos processos de regulação das propriedades farmacocinéticas dos fármacos e no desenvolvimento de resistência celular aos fármacos, diminuindo ou aumentando o seu transporte (Franke e cols., 2010).

Muitos estudos de farmacogenética das estatinas mostram que elas sofrem influência de muitos genes transportadores, o que vem fornecendo um maior conhecimento para cada polimorfismo estudado. No entanto, através das abordagens de genes candidatos e de estudos GWAS é possível selecionar alguns genes que estão sendo consistentemente associados à variabilidade de resposta, bem como com seus efeitos adversos.

Os GWAS visam identificar genes envolvidos em doenças humanas, através de pequenas variações, ou polimorfismos no genoma que ocorrem mais frequentemente em pessoas com uma determinada doença do que em indivíduos sem esta enfermidade. Cada estudo pode analisar centenas ou milhares destes polimorfismos ao mesmo tempo.

Sabe-se que os polimorfismos que afetam a eficácia das estatinas têm a capacidade de influenciar a toxicidade das estatinas de forma indireta, visto que os médicos muitas vezes aumentam as doses da estatina em pacientes que não alcançam a redução dos lipídios. Esta atitude pode afetar o órgão final e a toxicidade influenciar diretamente na frequência e na gravidade de eventos adversos das estatinas.

Considerando o impacto destes polimorfismos na saúde do paciente, após analisar estudos de GWAS (Tabela 2) e a estes acrescentar os genes estudados por este grupo de trabalho, foram definidos aqueles que apresentam polimorfismos com maior impacto no resultado terapêutico com estatinas, no caso o *ABCB1,* o *CYP3A5,* o *SLCO1B1,* o *SLCO1B3,* o *ABCG2,* o *APOE,* o *CLMN e* o *NR1I2*. Após esta escolha, foi criado um escore genético para predição da resposta terapêutica, visto que os parâmetros de redução de lipídios são utilizados para a verificação do sucesso do tratamento.

Nosso grupo de trabalho vem realizando pesquisas com os genes *SCAP* (rs12487736), *ABCB1* (rs1128503, rs1045642, rs2032582), *APOE* (rs7412, rs429358), *CETP* (rs708272), *CYP3A5* (rs776746), *SLCO1B1* (rs2306283, rs4149056, rs11045819), *SLCO1B3* (rs4149117), *ABCG2* (rs2231142), *CLMN* (rs8014194) e *NR1I2* (rs3814055, rs2461817, rs7643645, rs6785049, rs3814057).

Tabela 3. Estudos de GWAS com estatinas e os principais genes e polimorfismos identificados para redução de LDL-C.

Estudo	População	Gene	Polimorfismo
Postmus e cols., 2014.	Fase 1: 8421 indivíduos de 6 RCTs e 10175 indivíduos de 10 estudos observacionais	*ABCG2*	rs2199936
		LPA	rs10455872
	Fase 2: 21975.	*APOE*	rs7412
			rs445925
		SORT1/CELSR2/PSRC1	rs646776
			rs12740374
Hopewell e cols., 2013.	18705 indivíduos caucasianos participantes do estudo "*Heart protection Study*".	*SORT1/CELSR2/PSRC1*	rs646776
		LPA	rs10455872
			rs3798220
		ABCG2	rs200242
		SLCO1B1	rs11045819
			rs4149056
		APOE	rs7412
			rs445925
			rs4803750
			rs2075650
			rs4420638
Deshmukh e cols., 2012.	1194 pacientes com diabetes tipo 2, sem doença cardiovascular prévia – participantes do CARDS (*Collaborative Atorvastatins Diabetes Study*).	*LPA*	rs10455872
		APOE/APOC1	rs445925
			rs4420638
	895 pacientes hipertensivos do estudo ASCOT-R (*R*andomized *Anglo-Scandinavian Cardiac Outcomes Trial*).		
	691 pacientes hipertensivos do estudo ASCOT - Obs (Observational *Anglo-Scandinavian Cardiac Outcomes Trial*		

	5804 pacientes da Escócia, Holanda e Irlanda, participantes do estudo PROSPER (*Prospective Study of Pravastatin in the Elderly at Risk*).		
Barber e cols., 2010.	3936 indivíduos caucasianos,sendo 944 participantes do estudo CAP (*Cholesterol and Pharmacogentics*) e 1702 do estudo PRINCE (*PravastatinInflammation/CRP Evaluation*).	*CLMN* *APOE/APOC1* *SORT1/CELSR2/PSRC1*	rs8014194 rs4420638 rs646776
Chasman e cols., 2012.	6989 indivíduos com ancestralidade europeia, participantes do estudo JUPITER (*Justification for the Use of Statins in Prevention: na Intervention Trial Evaluating Rosuvastatin*).	*ABCG2* *LPA* *APOE* *PSCK9* *SLCO1B1*	rs2199936 rs10455872 rs71352238 rs17111584 rs4149056 rs4363657 rs12317268
Marciante e cols., 2011.	732 indivíduos participantes dos estudos *"Cardiovascular Health Study"* e *" Heart and Vascular Health Study".*	*SLCO1B1*	rs4149056
Isackson e cols., 2011.	320 indivíduos, 190 com miopatia causada pelo uso de estatinas dos EUA, Canadá e Austrália e 130 controles.	*SLCO1B1*	rs4149056
Chu e cols., 2012.	6766 participantes com ancestralidade europeia, participantes do estudo JUPITER (*Justification for the Use of Statins in Prevention: na Intervention Trial Evaluating Rosuvastatin*).	*ABCG2* *LPA*	rs2199936 rs10455872
Link e cols., 2008.	12064 indivíduos do Reino Unido.	*SLCO1B1*	rs4149056 rs2306283

1.7. Justificativa

A importância de controlar os níveis de CT e LDL-C nos pacientes dislipidêmicos é fundamental na prevenção de doenças cardiovasculares. Uma das formas de controlar esses níveis é através do tratamento medicamentoso com estatinas. Sabe-se que existe uma variabilidade de resposta às mesmas entre os pacientes, e que fatores genéticos contribuem para esta variação.

Muitos estudos de farmacogenética avaliam os polimorfismos através de estudos gene candidato ou de GWAS de maneira individual. Na literatura, encontramos pouquíssimos estudos que analisaram o impacto destas variações em conjunto na resposta ao tratamento às estatinas.

Deste modo, se fossem obtidos escores genéticos de predição ao tratamento com estatinas poderíamos melhorar a resposta ao tratamento, e assim reduzir os casos de DCVs causadas pela dislipidemia, justificando a importância da realização deste estudo.

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2. Objetivos

Face à oportunidade de investigar polimorfismos já identificados como preditores de resposta à estatinas através de abordagem gene candidato e de *genome-wide association* (GWAS), assim como marcadores moleculares ainda pouco explorados, em uma coorte de pacientes dislipidêmicos do sul do Brasil, os objetivos específicos do presente estudo foram:

1. Validar a associação de diferentes polimorfismos genéticos em genes previamente associados com eficácia de estatinas: *ABCB1* (rs1045642, rs2032582), *CYP3A5* (rs776746), *SLCO1B1* (rs2306283, rs4149056, rs11045819), *SLCO1B3* (rs4149117), *ABCG2* (rs2231142), *APOE* (rs7412, rs429358), *CLMN* (rs8014194) e *NR1I2* (rs3814055, rs2461817, rs7643645, rs6785049, rs3814057) em uma coorte do sul do Brasil
2. Criar um escore genético de resposta ao tratamento com estatinas, a partir de marcadores genéticos identificados pelo grupo de pesquisa e através de GWAS.

3 Artigo redigido em inglês

Statin pharmacogenetics: use of genetic scores to predict therapeutic response

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Statin pharmacogenetics: use of genetic scores to predict therapeutic response

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Reduced Title: Use of genetic scores to predict a therapeutic response to statins

Abstract

Dyslipidemia is a major risk factor for cardiovascular disease and, the first choice drug treatment is the use of statins. However, significant inter-individual variability in the response to treatment efficacy is observed. Many studies of pharmacogenetic allow understanding this variability, but do not predict the response to the treatment. The present study intend to develop a genetic score of response to treatment with statins, from genetic markers identified by candidate gene and GWAS approaches. Our sample consisted of 477 patients of European descent, with LDL-C levels above the established target and without previous drug treatment. We included polymorphisms in genes that seem to present greater impact on the therapeutic result with statins, namely *ABCB1*, *CYP3A5*, *SLCO1B1*, *SLCO1B3*, *ABCG2*, *APOE*, *CLMN* and *NR1I2*. After identifying polymorphisms with significant reduction in total cholesterol and LDL-C levels, a genetic score of response to statin therapy was created from genetically identified markers *SLCO1B1* (rs2306283) and *NR1I2* (rs3814055, rs7643645 and rs6785049). Our additive model showed a strong association with changes on total cholesterol (6×10^{-5}) and LDL-cholesterol reduction (4×10^{-7}). Among individuals with no responder genotype (score zero), the mean percent reduction of total cholesterol and LDL-cholesterol was -12.3 ± 11.6% and -14.4 ± 19.5%, respectively. For those with score four, the mean percentage reduction of total cholesterol and LDL-cholesterol was -30.2 ± 11.6% and -42.6 ± 14.4%, respectively. Our results show that the use of the genetic score of response to the treatment seems to be important for the efficacy of the treatment, since our data indicate that the genetic score based on statistical significant SNPs could improve the lipid-lowering effect prediction.

Key-words: statins, candidate gene studies, dyslipidemias, genome-wide association studies, LDL-cholesterol.

Introduction

Treatment with lipid-lowering drugs is considered a central approach in primary and secondary prevention of cardiovascular disease [1,4]. Currently the *American Heart Association* (United States), *National Lipid Association, European Atherosclerosis Society* (Europe) and *Brazilian Society of Cardiology* (Brazil) emphasizes the importance of pharmacotherapy with statins in the treatment of atherosclerotic disease prevention, expanding the indications of prescription and target populations [5-9].

Although safe and effective, treatment with statins presents a great variability of response, both in effectiveness and in adverse effects development [6-10]. Several factors may be associated with drug response variability and the genomic variability is one of the important factors. In this sense, several studies with candidate gene and genomic wide association approaches attempt to elucidate the genetics responsible for drug response variability [6,11-13].

Through these studies some genetic variants have been identified more than once as possible predictors of response [2,3,10,14]. However, most studies with either a candidate gene approach or a genomic scan still yield contradictory results because they are done in populations with different genetic backgrounds. In addition, most studies are limited in investigating the isolated effect of each genetic variant, not worrying about the cumulative effect of several associated variants.

Considering the great use of statins worldwide, it is necessary to investigate and validate these genetic markers in different populations, as well as the creation of a genetic response score capable of better predicting the lipid-lowering response. In this way, our aim was to validate previously associated

genetic variants through candidate gene and GWAS approach, as well as to create a genetic response score from the significantly associated markers in a cohort of individuals from South of Brazil.

Material and methods

Study subjects

This study evaluated a cohort of patients attended at a cardiology clinic in the South of Brazil with dyslipidemia (n = 643) and statin prescription who maintained medical follow-up. The inclusion criteria were the use of statins and European offspring (observed by skin color and morphological characteristics). Exclusion criteria were liver disease, renal failure (assessed as creatinine >1.5 mg/dL and/or urea>60 mg/dL), age less than 20 years, triglycerides >400 mg/dL, hypothyroidism, and discontinuation of treatment, forgetfulness or carelessness. After the adoption of the criteria, the final sample consisted of 477 patients of both sexes. Other data such as age, sex, other drugs, concomitant diseases and smoking were obtained from medical records. The physician's choice of statin followed the clinical guidelines of the period and the clinical characteristics of the patient.

Patients with normalized lipid profiles after statin therapy were evaluated according to the following criteria and recommendations of the IV Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis [7] for primary prevention of CVD: total cholesterol (TC) <5.18 mmol/L, LDL-C <2.59 mmol/L, HDL-C >1.55 mmol/L and triglycerides (TG) <1.70 mmol/L.

All subjects who agreed to participate in this study provided informed consent. This study was approved by the Ethics Committee of the Federal University of Health Sciences of Porto Alegre. Parts of this sample have already been analyzed in previous studies.

DNA analyses

Genomic DNA was isolated from peripheral blood leukocytes by a standard salting-out procedure [15]. Molecular analyzes were performed for fifteen polymorphisms that includes the following genes and polymorphisms: *ABCB1* (rs1045642, rs2032582), *CYP3A5* (rs776746), *SLCO1B1* (rs2306283, rs4149056, rs11045819), *SLCO1B3* (rs4149117), *ABCG2* (rs2231142), *APOE* (rs7412, rs429358), *CLMN* (rs8014194) and *NR1I2* (rs3814055, rs2461817, rs7643645, rs6785049, rs3814057). For this analysis, the genotypes were determined by TaqMan hydrolysis probes (Applied Biosystems, California, USA). Approximately 10% of the samples were analyzed twice for all SNPs, aiming for a better quality control.

Statistical analyses

Continuous variables were expressed as mean ± standard deviation. Triglyceride levels were log-transformed prior to the analyzes because of their asymmetry, although the untransformed values are shown in the Results section. Allele frequencies were estimated by gene counting and the Hardy-Weinberg equilibrium was tested using the chi-square test. The efficacy of the therapy was evaluated as the percentage change of the individual lipid levels (TC, HDL-C, LDL-C, and TG) throughout the treatment [(follow-up-baseline) * 100 / baseline].

To determine the association between genotypes and mean percentage of change in plasma lipid levels, the in general linear model was used using the sums of type III squares. Age (years), sex, standard statin dosage (in mg), treatment period (in months) and basal lipid levels (in mmol/L) were included in each model as covariates for lipid-lowering response. A genetic score approach was used to evaluate the combined effects of SNPs on lipid profile changes. The genetic response score (GRS) was constructed using a genotypic score based on the number of favorable alleles (those associated with higher decreases in lipid levels) that were carried by each subject for each SNP. Favorable alleles were defined as previously associated alleles with changes in lipid levels in the literature and according to the date of the present study. A p-value <0.05 was considered as statistically significant. Statistical analyzes were performed using SPSS 19.0 for Windows®.

RESULTS

Characteristics of study population

The main characteristics of the sample are presented in Table 1. After application of the exclusion criteria, the sample comprised of 477 subjects aged between 25 and 89 years (62 ± 10.9 years), of whom 67.4% were females. Three hundred and ninety-eight (83.4%) participants were on simvastatin treatment and 16.6% participants were atorvastatin users. The average treatment duration was approximately 6.2 ± 3.2 months and did not differ between simvastatin and atorvastatin users (p = 0.683). The average standardized dose of statins was 10.5

± 4.7 mg and also did not differ between simvastatin users (10.1 ± 4.5 mg, atorvastatin converted range 2.5-20 mg) and atorvastatin users (11.1 ± 5.6 mg, range 10-40 mg) ($p = 0.0597$). In the sample, 33.5% had prior cardiovascular disease, 73.9% hypertension and 16.6% diabetes. Overall, statin treatment significantly reduced the plasma levels of total cholesterol (-26.1%, $p < 0.0001$), LDL-C (-36.2%, $p < 0.0001$) and triglyceride levels (-11.6%, $p < 0.001$), whilst the increase in HDL-C did not reach statistical significance (+3.9%, $p = 0.07$).

Association of SNPs with statin efficacy

The genotypic proportions were in Hardy-Weinberg equilibrium for all polymorphisms. The mean percent reductions of total cholesterol and LDL-cholesterol according to statistically significant investigated polymorphisms are shown in Supplementary Table1. The results of association of single SNP with statin lipid-lowering effect showed an association of *SLCO1B1* (rs2306283) and *NR1I2* (rs3814055, rs7643645 and rs6785049) with reduction of LDL-cholesterol levels and *SLCO1B1* (rs2306283) and *NR1I2* (rs3814055 and rs6785049) with change of total cholesterol levels. Detailed information on all SNPs investigated, observed minor allele frequencies (MAF) and p-values for each SNP association analysis with changes in total cholesterol and LDL-cholesterol levels are described in Table 2. Considering the associations found in single SNP analysis we constructed a polygenic additive score, in which subjects carriers for lower efficacy genotypes were coded with zero (poor responder) and subjects with higher lipid level reduction genotypes were coded as 1 (responder). The final score of each subject was calculated as the sum of each SNP score. After, a new association test was performed, adjusting for the same covariates previously

included. Only 7 patients (1.7%) received a final score of 0 (zero), 51 (12.6%) of 1 (one), 146 (36.2%) of 2, 139 (34.5%) of 3 (three) and 60 (14.9%) represented the excellent responders with a final score of 4 (four). Our additive model showed a strong association with changes on total cholesterol (6×10^{-5}) and LDL-cholesterol reduction (4×10^{-7}) (Table 3). We observed that as the number of responder genotypes increased, the mean percentage reduction of total cholesterol and LDL-cholesterol also increased. Among individuals with no responder genotype (score of 0), the mean percent reduction of total cholesterol and LDL-cholesterol was -12.3 ± 11.6% and -14.4 ± 19.5%, respectively. For those with score of 4, the mean percentage reduction of total cholesterol and LDL-cholesterol was -30.2 ± 11.6% and -42.6 ± 14.4%, respectively.

Discussion

In this study, we developed a genetic response score in the adult population of European descent, residing in south Brazil to predict changes in total cholesterol and LDL-C with statin therapy. The creation of the genetic score was based on SNPs previously associated with statin response and new selected polymorphisms, totaling 15 polymorphisms in 8 genes. Our results showed that variants in the *SLCO1B1* and *NR1I2* genes were associated with the reduction in LDL-C and total cholesterol levels in adult. Additionally, our data showed that the genetic score based on SNPs with statistical significance could improve the lipid-lowering effect prediction.

The disposition of statins undergo influence of several cell membrane transporters, such as the OATP1B1 uptake transporter (encoded by *SLCO1B1*)

and efflux transporters BCRP (encoded by *ABCG2*) and Pg-P (encoded by *ABCB1*) [16-20]. OATP1B1 is highly expressed in basolateral membrane of hepatocytes and is also involved in hepatic uptake and systemic clearance of several drugs, including statins as rosuvastatin, atorvastatin and simvastatin [17,20].

Of all genes analyzed in this study, the only gene that has consistently been replicated through candidate gene and GWAS in different populations is the *SLCO1B1* [2,6,8,12,17-23,26-34], also associated in our study. *SLCO1B1* polymorphisms have been studied, with two being the most common, c.388A> G (p.ASn130Asp, rs2306283) and c.521T> C (p.Val174Ala, rs4149056) [19,21]. They were associated with increased and decreased transport activity of OATP1B1, respectively [21]. The *SLCO1B1* c.521T>C (p.Val174Ala) polymorphism greatly increases the plasma concentration of simvastatin acid and moderately increases that of pravastatin [19]. In addition, the *SLCO1B1* g.89595T> C (rs4363657) polymorphism, which has a near complete binding disequilibrium with *SLCO1B1* c.521T> C, has been found to be associated with statin-induced myopathy (increased risk of simvastatin and atorvastatin and lower risk for rosuvastatin and pravastatin), which is the most frequent side effect associated with the use of statins, observed in 1-5% of cases [18,20,22-25].

The *SLCO1B1* c.388A>G polymorphism, that increase the activity of OATP1B1 [21], results in lower statin concentrations [26]. However, we know that the associations of *SLCO1B1* polymorphisms with statin efficacy vary according to the studied population [19,20,21,33]. In Thai patients, treated with simvastatin 20 or 40mg/day, no association between *SLCO1B1* c.388A> G, c.521T> C and g.89595T> C genotypes and the lipid levels reduction was found [21]. In this

study, 74.9% of the population had the *SLCO1B1* c.388A> G allele, similar to other studies with dyslipidemic patients in China and higher than in the Brazilian (32.2%), Hungarian (36.2%), German (36%) and Greek patients (43.3%) [21].

Additionally, Birmingham et al. investigated the influence of *SLCO1B1* c.521T>C or *ABCG2* c.421C>A polymorphisms on Caucasians versus Asian subjects exposure to rosuvastatin. Results indicated that both polymorphisms were associated with higher exposure to rosuvastatin, atorvastatin and simvastatin acid (but not simvastatin) within a population, but only the *ABCG2* c.421C>A polymorphism contributed towards between-population exposure differences. In individual carrying wild-type alleles for both *SLCO1B1* and *ABCG2*, area under the plasma concentration-time curve (AUC) still appeared to be higher for rosuvastatin, atorvastatin and simvastatin acid in Chinese and Japanese subjects compared with Caucasians, respectively [20]. In the same line, another study also did not observe effects of the polymorphism in *SLCO1B1* (rs2306289) on the pharmacokinetics of statins in Chinese populations [19]. As well as, Ciuculete et al., in your context of elderly individuals, *SLCO1B1* SNPs (rs2306283, rs4149056) did not show a major role on LDL-C response to statins as shown in previous studies [14]. However, in our study we found association of the SNP rs2306283 in the response to treatment with statins, in the other SNPs (rs4149056, rs11045819, rs4149117) there was no such association.

Differences in genotypic/allelic frequencies and responses to treatment found and reported by these authors may be related to a number of factors, including age, sex, lifestyle, statin type, underlying patient disease, daily statin dose, baseline lipid level, sample size and ethnicity of the population, which reinforces the importance of knowing the different effects of statins in different

populations. Therefore, these studies confirms the need for studies in different populations, since polymorphisms may influence statin therapy differently, and it is difficult to predict treatment efficacy.

Statins are subject to the oxidation of CYP3A4, largely because of their CYP3A4-mediated first pass metabolism. The effect of *CYP3A4/5* polymorphisms on simvastatin has been reported with contradictory results, possibly because the mechanism that could have resulted in altered enzyme activities remains uncertain [19,20,35]. PXR is also important in statin metabolism as a transcriptional regulator of a wide range of genes that encode enzymes and transporters that metabolize drugs, which may affect the metabolism and response of drug [19]. The *NR1I2* gene is responsible for encoding PXR, which together with PPARα (encoded by *PPARA*) are two important transcription factors and affect the expression of *CYP3A4*. Despite a direct connection between PXR with the *CYP3A4* regulation, few studies addressed it genetic variability with statin efficacy [19,35]. In our study, we found association of *NR1I2* rs3814055, rs7643645 e rs6785049 with lower LDL-C levels, whereas the polymorphisms rs2461817, rs3815057 showed no association with the levels of cholesterol and LDL-C, consequently the patients who presented this responder genotype had expressive reductions in their LDL-C.

Recently, Leusink et al. performed a systematic review of the last 17 years of the pharmacokinetics of statins. They identified 871 articles through Pubmed, 173 of which met the inclusion criteria. Most of the included studies were gene candidate studies and only eigth (5%) were GWAS studies. Most selected studies investigated the effect of generic variation on the LDL-C response statins (128 studies, 74%), whereas 36 (21%) studies investigate the effect on clinical

outcomes such myocardial infarction or stroke. However, ten studies verified a combination of LDL-C and clinical response. In the 166 selected candidate gene studies, were investigated SNPs in 345 different genes. The most commonly investigated were *CETP* (29 studies, 18%), *APOE* (27studies, 16%) and *SLCO1B1* (25 studies, 15%).

Unique SNPs were investigated for their effect on statin response. In 1144 from them, 12% (38) were associated with either LDL-C response or clinical response in at least one study. From studies GWAS investigated, six of them studied the association of SNPs with LDL-C change and two the effects on clinical benefit. In GWAS studies, the SNPs consistently associated differential LDL-C reduction include rs10455872 (*LPA*) and rs71352238, rs7412, rs445925 and rs4420638 (*APOE*). Some of *APOE* investigated SNPs are tagSNPs in linkage disequilibrium with functional variants. The polymorphism rs2900478 in *SLCO1B1*, a gene implicated in statin pharmacogenetics, was also confirmed by Leusink et al. The other new significantly associated SNP was rs646776 in the *SORT1*/*CELSR2*/*PSRC1* locus. Other results from GWAS included rs2199936 and 1481012 in *ABCG2,* in high LD with the main *ABCG2* finding from candidate gene studies [2].

Despite of a great interest in statin pharmacogenetics, few studies evaluated the cumulative effect of genetic variations [14,18], and it is necessary to analyze how the SNPs already identified and the news ones can be behave in the individuals. In one of these studies, two cohorts were evaluated and clinical response to treatment with statins for a long time through cumulative genetic information in elderly individuals was addressed [14].

Ciuculete et al. developed a genetic risk score for lipid-lowering efficacy of statins in elderly patients (aged 70-80 years) in Switzerland. From the polymorphisms studied, four showed a greater impact on the efficacy of statins: *APOE* (rs7412, rs4420638), *ABCC2* (rs2002042) and *CELSR*/*SORT*/*PSRC1* (rs646776). A strong association was found for the *APOE* gene (rs7412) and the ability to lower cholesterol levels [14].

Additionally, Leusink et al. created a risk score for new genetic loci associated with the response to statins through the analysis of multicenter studies already published. They concluded that multiple genes have an effect on changes in LDL-C values [18]. SNPs *ABCG2* (rs2231142), *LPA* (rs10455872) and *APOE* (rs2075650) were studied, and 4% of the European population evaluated had 4 or more risk alleles and more than 95% had between 1 and 3 risk alleles [18]. The authors concluded that the use of statins substantially decreased LDL-C independent of the genotype, and each additional allele in the genetic risk score decreased the LDL-C by only 2% [18]. On other hand, our study showed that each additional “benefical” genotype decreased LDL-C by 7% [18].

In our study population, the polymorphism of the *ABCG2* gene (SNP rs2231142) had no impact on the reduction of LDL-C levels, a result different from that found by Leusink et al. and according to the found by the author Ciuculete et al. showing the diversity of responses according to the study population.

The genetic risk score (GRS) used by Ciuculete et al. [14], as well as our study, shows that response measurement is a powerful tool in assessing the additive effects of genetic variants on statin efficacy and to estimate the magnitude of LDL-C reduction. Other studies use GRS to predict recurrent events and identify patients most likely to respond to statins [37]. GRS may also predict

recurrent events and identify patients most likely to respond to statins. GRSs constructed from the SNPs conducted from these loci are independently associated with incident coronary artery disease (CAD) using standard multivariate regression models [37]. These associations are evident in several ethnic groups. Our study responds to this goal by being able to predict the response to treatment of patients according to their genetic background and thus be able to predict who will best respond to established prevention strategies. Physicians can use scores to identify good and poor responders to statin therapy. However, our results can be only reproduced for populations that are the same as the one studied, since in our review in the literature we found only one study with similar design in which it elaborated risk scores with a significant association with the response to statin therapy in an elderly population.

Our study presented some limitations such as a small number of individuals studied, the non-validation of the calculation of the score and the fact that we did not carry out the analysis in the individuals of all genes strongly associated with the therapy with statins. The indication of statin for the treatment of dyslipidemia is shown to be very effective, but the existence of different responses to the treatment is already proven. Therefore, the use of the genetic response to treatment is extremely important for the efficacy and safety of the treatment.

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Compliance with ethical standards The authors declare that they have no conflict of interest.

Supplementary information is available at *The Pharmacogenomics Journal website".*

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Table 1 Characteristics of patients

Number of patients	477
Age (years)	62.0 ± 10.9
Sex, female (%)	67.4
Statin use (%)	
Simvastatin	83.4
Atorvastatin	16.6
Treatment follow-up (months)	6.2 ± 3.2
Overall statin standard dose (mg)*	10.5 ± 4.6
Simvastatin standard dose (mg)	10.2 ± 4.5
Atorvastatin dose (mg)	11.1 ± 5.6
Smoking (%)	
Past	14.4
Current	7.1
Never	78.5
Prior CVD (%)	33.5
CVD Family history (%)	19.4
Diabetes (%)	16.6
Hypertension (%)	73.9

CVD: cardiovascular disease. Data presented as n (%) or mean ± standard deviation.

Table 2 Statistical associations between SNPs and plasma total cholesterol and LDL-C levels

Nearby gene/locus	SNP	Alleles	Change position	MAF	MAF 1000 genomes *	TC p-value	TC responder genotype	LDL-C p-value	LDL-C responder genotype
ABCB1	rs1045642	G>A	p.Ile1145=	0.470	0.480	0.173	-	0.336	-
	rs2032582	C>A	p.Ser893Thr	0.480	0.410	0.073	-	0.212	-
CYP3A5	rs776746	C>T	c.219-237G>A1	0.070	0.060	0.964	-	0.744	-
SLCO1B1	rs2306283	A>G	p.Asn130Asp	0.460	0.400	**0.018**	AG and GG	**0.015**	AG and GG
	rs4149056	T>C	p.Val174Ala	0.160	0.161	0.072	-	0.471	-
	rs11045819	C>A	p.Pro155Thr	0.170	0.140	0.523	-	0794	-
SLCO1B3	rs4149117	T>G	p.Ser112Ala	0.170	0.140	0.358	-	0.444	-
ABCG2	rs2231142	C>A	p.Gln141Lys	0.100	0.090	0.732	-	0.585	-
APOE	rs7412;rs429358	C>T; C>T	p.Arg176Cys (E*2); p.Cys130Arg (E*4)	0.044	0.063	0.824	-	0.771	-
				0.151	0.155				
CLMN	rs8014194	T>A	c.83-24208A>T	0.240	0.230	0.783	-	0.892	-
NR1I2	rs3814055	C>T	c.-1135C>T2	0.380	0.370	**0.012**	CC and CT	**0.001**	CC and CT
	rs2461817	C>A	c.-22-1425A>C2	0.390	0.330	0.598	-	0.605	-
	rs7643645	A>G	c.-22-579A>G2	0.340	0.340	0.610	-	**0.026**	AG and GG
	rs6785049	A>G	c.795-93G>A2	0.420	0.380	**0.014**	AA	**0.007**	AA
	rs3814057	A>C	c.*1195A>C2	0.220	0.170	0.220	-	0.081	-

SNP: Single nucleotide polymorfisms; MAF: Minor allele frequency; TC: Total cholesterol; LDL-C: Low density lipoprotein
1 NM_000777.4 2 NM_003889.3

Table 3. Total cholesterol (TC) and LDL-C percentage reduction according genetic response score

Score	N (%)	ΔTC%	ΔLDL-C%
0	7 (1.7)	-12.3 ± 11.6	-14.4 ± 19.5
1	51 (12.6)	-21.8 ± 14.1	-28.9 ± 20.3
2	146 (36.2)	-25.5 ± 12.5	-34.0 ± 18.5
3	139 (34.5)	-27.0 ± 11.2	-38.3 ± 16.2
4	60 (14.9)	-30.2 ± 11.6	-42.6 ± 14.4
p		6×10^{-5}	4×10^{-7}

Data presented as mean ± standard deviation.

Supplementary Table 1 Mean reduction of baseline levels of TC, LDL-C at statin treatment for genes *NR1I2* (SNP rs3814055, rs7643645, rs6785049) and *SLCO1B1* (SNP rs2306283).

Alelo	ΔTC%	ΔLDL-C%	Escore
rs3814055			
CC	-25.1±13.0	-35.5±17.9	1
CT	-27.2±11.4	-37.9±16.7	1
TT	-21.3±12.7	-27.9±19.0	0
p	0.012	0.001	
rs7643645			
AA	-25.1±12.6	-33.8±18.5	0
AT	-26.3±12.7	-38.6±17.6	1
TT	-26.6±12.2	-36.7±16.6	1
p	0.610	0.026	
rs6785049			
GG	-25.2±12.6	-34.1±20.9	0
GA	-24.7±13.3	-35.0±18.4	0
AA	-27.3±11.4	-38.7±15.5	1
p	0.014	0.007	
rs2306283			
GG	-21.4±15.1	-29.7±22.8	0
GA	-26.2±11.7	-37.4±16.4	1
AA	-27.7±10.8	-38.0±14.4	1
p	0.018	0.015	

ΔTC: variation of total cholesterol. ΔLDL-C: variation of low density cholesterol.

Summary

This Suplementary Table show the mean reduction of baseline levels of TC, LDL-C at statin treatment for genes *NR1I2* (SNP rs3814055, rs7643645, rs6785049), *SLCO1B1* (SNP rs2306283) and the scores given for the polymorphisms according to the reduction of LDL-C.

4. Considerações finais

Este é o primeiro estudo que desenvolveu um escore genético de predição à resposta terapêutica com estatinas em pacientes dislipidêmicos em uma coorte do sul do Brasil (477 indivíduos), a partir de marcadores genéticos identificados por estudos de genes candidatos e de abordagens de GWAS.

Foram incluídos os polimorfismos em genes que apresentam maior impacto no resultado terapêutico, sendo eles: *ABCB1, CYP3A5, SLCO1B1, SLCO1B3, ABCG2, APOE, CLMN* e *NR1I2*. Após identificarmos os polimorfismos com redução significativa nos níveis de CT e LDL-C (*SLCO1B1*-rs2306283 e *NR1I2* – rs3814055, rs7643645 e rs6785049), foi criado um escore genético de predição de resposta à terapia com estatinas. Com os resultados obtidos, é possível afirmar que o uso do escore pode ser de grande importância para predizer a eficácia do tratamento.

Considerando-se a variabilidade genética da população, futuramente ampliaremos os SNPs que também atuam significativamente na redução dos níveis de CT e de LDL-C. Também se faz necessária a realização da aplicação deste escore em outras populações.

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

Paralelamente ao projeto principal, outras publicações foram realizadas: "*Efficacy of simvastatin versus simvastatin/ezetimibe on a South Brazilian cohort"*, submetido para publicação no periódico *Brazilian Journal of*

Pharmaceutical Sciences; e "*Birth weight, length and head circumference: Progression and impact over the outcome of patients with congenital heart disease*", publicado no periódico *International Journal of Cardiology*.

5. Anexos

5.1. Parecer do Comitê de Ética em Pesquisa em Seres Humanos 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

5.2 Outras Publicações

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Short communication

Birth weight, length and head circumference: Progression and impact over the outcome of patients with congenital heart disease

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ABSTRACT

Background: There are few studies assessing the birth measures of patients with congenital heart disease (CHD). Our aim to evaluate their progression and impact over the outcome.
Methods: The cases consisted of patients with CHD during their first hospitalization in a reference cardiac and pediatric intensive care unit (ICU) from Southern Brazil. Controls were composed of patients with no clinical evidence of CHD hospitalized soon after cases. The cases underwent high-resolution karyotype and fluorescence in situ hybridization (FISH) for 22q11 microdeletion. We analyzed birth weight, length and head circumference of patients of both groups. For CHD patients, we evaluated their progression and impact until hospitalization at ICU.
Results: Our sample was composed of 198 cases and controls. We observe a difference in birth weight of CHD patients only in relation to general population. There was a significant increase in children with CHD and weight below the lower limit from birth until the hospitalization at ICU, and this occurred more in those without complex CHD. Syndromic patients and with an extracardiac malformation also presented a greater difficulty to maintain not only the weight but also the length/height until the hospitalization. Individuals with weight below the lower limit at hospitalization who died had a tendency to present longer stay at ICU.
Conclusions: Some CHD patients, especially without complex defects, and with syndromic aspect and a major extracardiac malformation, present a higher difficult to maintain their weight and growth, and, therefore, may be at risk and should be more closely monitored.

1. Introduction

Congenital heart disease (CHD) is considered the most prevalent malformation, affecting 6–8:1000 live births [1]. Despite the better of survival and quality of life of children with CHDs, these defects still represent a public health problem worldwide [2]. They often require surgery and hospitalization in intensive care units (ICUs) [3], and there is a paucity of these units and centers of expertise in our country [4].

It is noteworthy that there are few studies assessing the birth characteristics and their mean along the life of CHD patients [5,6]. Thus, our aim was to verify the progression and impact of the birth weight, length and head circumference over the outcome of CHD patients.

2. Methods

Cases and controls were consecutively and prospectively allocated during 1 year (August 2005 to July 2006). The cases consisted of patients with CHD during their first hospitalization at cardiac ICU of the Hospital da Criança Santo Antônio (HCSA), a referral pediatric cardiac center in Southern Brazil. The controls were composed of patients with no clinical evidence of CHD who were hospitalized for the first time at same ICU, soon after cases.

The cases underwent high-resolution GTG-Banding karyotype and fluorescence in situ hybridization (FISH) for 22q11 microdeletion, using the DNA probe TUPLE1 (Vysis, Abbott Molecular Inc.). The variables analyzed were sex, gestational age, delivery type, and weight, length and head circumference at birth and at physical exam in the ICU hospitalization. Variables only analyzed in cases were reason for hospitalization, type of cardiac malformation, presence of complex CHD, syndromic aspect, results of karyotype

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and FISH analyses, association with major extracardiac malformations, time of hospitalization and survival during hospitalization at ICU.

For assess the weight, length and head circumference, Margotto [7] curves were used at birth and growth charts present in Jones [8] at physical exam. The measures were adjusted according to the length [9]. Syndromic aspect was determined based on physical exam, especially considering the number and types of dysmorphisms. The classification elaborated by Botto et al. [10] was used for CHD division. CHD were also classified as complex or not.

Data processing was done by creating a database in Statistical Package for Social Sciences (SPSS) version 18.0 for Windows and by PEPI (version 4.0; Artemisia Press, Salt Lake City, UT, USA). For categorical data, qui-square with Yates correction and Fisher's exact tests were used. To evaluate survival, Kaplan-Meier and log-rank tests were used. For control of confounding factors, multivariate Cox proportional hazards model was applied. To compare frequencies derived from paired samples, the McNemar test was used. $P < 0.05$ values were considered as statistically significant. This study was approved by the Ethics Committee of University and Hospital.

3. Results

The sample consisted of 396 patients, 198 cases and 198 controls. For cases, 52% of them were male, ages ranging from 1 to 4934 days (median 220 days). The main reason for hospitalization was cardiac surgery (74.7%). Septal defects (32.3%) and outflow tract defects (18.7%) were the main types of CHDs. Sixty-five patients (32.8%) presented a complex heart defect, 30.8% a syndromic aspect, 16.2% a chromosomal abnormality and 18.7% a major extracardiac malformation. The median time of hospitalization at ICU was 3 days (ranged from 1 to 95 days) and death occurred in 15 cases (7.6%).

As for controls ($N = 198$), most of them were male (55.1%) and their ages ranged from 5 to 6422 days (median 1455 days). The main reasons for hospitalization included respiratory dysfunction (21.1%) and tumor resection postoperative (13.7%). Non-cardiac malformations were described in 29 patients (15.3%).

Birth characteristics observed in cases and controls can be seen in Table 1. No statistically significant differences were observed between the weight, length and head circumference from both groups (Table 1). The cases at birth presented a median weight of 3037.5 g (2688.8;3407.5), length of 48 cm (46;49.5) and head circumference of 34 cm (32;35). On the other hand, the controls at birth had a median weight of 3200 g (2705;3500), length of 49 cm (46;51) and head circumference of 34 cm (32.9;35). We did not verify difference between the medians from both groups.

Table 1
Comparison of the birth findings verified between patients with (cases) and without congenital heart disease (controls).

Variables	Case	Control	P
Gestational age (N)	198	198	0.345
<37 weeks (%)	12.6	17.7	
37–42 weeks (%)	86.9	81.8	
>42 weeks (%)	0.5	0.5	
Sex (N)	198	198	0.614
Male (%)	52	55.1	
Female (%)	48	44.9	
Type of delivery (N)	198	198	0.227
Vaginal (%)	49.5	56.1	
Cesarean section (%)	50.5	43.9	
Weight percentile (N)	196	189	0.070
<P10 (%)	2.6	6.9	
P10–90 (%)	89.2	82	
>P90 (%)	8.2	11.1	
Low weight at birth (<2500 g) (N)	196	189	0.789
Yes (%)	18.3	16.9	
No (%)	81.6	83.1	
Lenght percentile (N)	189	174	0.497
<P10 (%)	13.8	12.1	
P10–90 (%)	77.7	75.8	
>P90 (%)	8.5	12.1	
Head circumference percentile (N)	167	94	0.690
<P10 (%)	2.4	4.3	
P10–90 (%)	88.6	86.1	
>P90 (%)	9	9.6	

N = number of patients.

As for CHD groups, only patients with patent ductus arteriosus (PDA) ($N = 9$) showed weight ($P = 0.011$), length ($P = 0.009$) and head circumference values ($P = 0.002$) below the lower limit at birth. However, this happened due to prematurity (OR = 4.729, CI95% = 1.23–18.14, $P = 0.024$). Patients with syndromic aspect and carriers of chromosomal abnormality had more birth weight below the lower limit ($P = 0.029$ and $P = 0.035$, respectively). Carriers of chromosomal abnormality showed a mean weight ($P = 0.033$) and length at birth ($P = 0.004$) smaller than those without the same.

McNemar difference test revealed an increase of nearly 5-fold in children with CHD and weight below the lower limit when we compared the measures between birth and hospitalization (2.6% versus 12.2%, $P < 0.001$), and this increase more occurred in patients without complex CHD (3.1% versus 15.3%, $P = 0.001$). Syndromic patients presented a greater difficulty in maintaining an adequate weight ($P = 0.021$), as well length/height ($P = 0.002$), similarly to those with a major extracardiac malformation ($P = 0.009$) (Table 2).

Table 2
Comparison of the weight, height/length and head circumference of the patients with congenital heart defect at moment of birth and at physical exam.

Findings	n	At birth				P
		↓ lower limit	↓ lower limit	Normal	Normal	
		At physical exam/hospitalization				
		↓ lower limit	Normal	↓ lower limit	Normal	
		%	%	%	%	
Weight						
Complex heart defect	196					0.269
Yes	65	–	1.5	6.2	92.3	
No	131	0.7	2.3	14.5	82.5	
Syndromic aspect	196					0.021
Yes	59	1.7	5	17	76.3	
No	137	–	0.7	9.5	89.8	
Chromosomal anomaly*	196					0.168
Yes	31	3.2	3.2	13	80.6	
No	165	–	1.8	11.5	86.7	
Major extracardiac malformation	196					0.103
Yes	36	–	2.8	22.2	75	
No	160	0.6	1.8	9.4	88.2	
Length/height						
Complex heart defect	188					0.233
Yes	63	14.3	6.3	17.5	61.9	
No	125	6.4	4	24	65.5	
Syndromic aspect	188					0.002
Yes	55	9	7.3	38.2	45.5	
No	133	9	3.8	15	72.2	
Chromosomal anomaly*	189					0.093
Yes	4	50	–	–	50	
No	185	8.1	4.8	21.6	65.4	
Major extracardiac malformation	188					0.009
Yes	33	12.2	3	42.4	42.4	
No	155	8.4	5.2	17.4	69	
Head Circumference						0.697
Complex heart defect	167					
Yes	59	–	3.4	1.7	94.9	
No	108	–	1.8	3.7	94.5	
Syndromic aspect	167					0.106
Yes	50	–	2	8	90	
No	117	–	2.5	0.9	96.6	
Chromosomal anomaly*	171					–
Yes	8	–	–	50	50	
No	163	–	2.5	3	94.5	
Major extracardiac malformation	167					–
Yes	30	–	–	3.3	96.7	
No	137	–	2.9	2.9	94.2	

* Considered if there was an anomaly in karyotype or FISH for 22q11microdeletion.

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The median time of hospitalization from the patients who died was significantly higher (IQR 5–21 and 2–8, 25, respectively) ($P < 0.001$). Among them, the duration of hospitalization had a tendency to be higher among those with a weight below the lower limit at the time of hospitalization ($P = 0.076$). In Cox regression analysis, considering the death outcome and the covariates syndromic aspect, presence of chromosomal abnormalities and weight below the lower limit at birth, no difference in cumulative risk for death was found.

4. Discussion

Children with CHD have been described with lower body weight when compared to healthy children of the same age group [11,12], and this finding has been associated to a higher risk of mortality [13]. In our study, we did not observe a difference in birth weight between patients with and without CHD. We believe that this lack of difference may be related to the characteristics of our control group, where many patients presented important comorbidities. However, it is noteworthy that when we compare our data with general population (DATASUS database - http://datasus.saude.gov.br/), we observed a very significant difference in the frequency of low birth weight (<2500 g) (18.3% versus 9.5%) ($P < 0.0001$). When evaluating the types of CHD, we found that patients with PDA presented smaller weight, length and head circumference values at birth. However, this was influenced by gestational age.

Chromosomal abnormalities have been described in 3 to 18% of patients with CHD [14], and in our sample this rate was 16.2%. It is known that carriers of chromosomal abnormalities often are syndromic, present other extracardiac malformations and have low birth weight [15]. This was in accordance with our results, where patients with chromosomal abnormality ($P = 0.035$) and syndromic aspect ($P = 0.029$) presented a lower birth weight.

The degree of growth lack at preoperative period has been associated with some morbidities, as longer hospital stays and increased mortality [5,12,16]. In our sample, we verified a significant increase (of nearly 5-fold) in children with CHD and weight below the lower limit when we compared the measures between birth and physical exam. Some authors suggest that this decrease could happen secondary to hemodynamic instability [11]. It is noteworthy that this decrease in the growth especially occurred in our sample among patients without complex CHD. Our findings suggest that these patients may be being neglected and should be better monitored. This difficulty to maintain the weight was also verified among patients with syndromic aspect ($P = 0.021$). Perhaps, this finding had been influenced by the presence of some associated extracardiac features, as swallowing disorders.

As for the length at birth, we did not find differences when comparing patients with and without CHDs ($P = 0.117$). However, we observed that patients with complex heart defect ($P = 0.233$) and chromosomal abnormalities ($P = 0.093$) had lower birth lengths. Moreover, syndromic patients and individuals with major extracardiac malformations had a greater difficulty in maintaining adequate measures ($P = 0.002$ and $P = 0.009$, respectively), what may be related to a chronic malnutrition status secondary, perhaps, to some features as feeding difficulties.

We did not observe a difference in relation to head circumference at birth between patients with and without CHD. No differences were also noted when we compared the types of CHDs, the presence or not of complex defects ($P = 0.697$) and the syndromic aspect ($P = 0.106$) (Table 2). In literature, some CHDs, such as tetralogy of Fallot, have been associated with microcephaly which strengthens the evidence for possible central nervous system damage due to intrauterine hypoxia [17].

The patients who died had a median time of hospitalization significantly higher. Moreover, it was observed that among them the median length of hospitalization had a tendency to be higher among those with a weight below the lower limit at time of hospitalization. These findings alert to the need for a better care for factors that may influence the weight and growth of CHD patients, such as syndromic aspect and presence of a major extracardiac malformation. Patients without complex defects may also be at risk. All this is important since they may have an influence over the outcomes after cardiac surgery and life span.

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Conflict of interests

None.

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Efficacy of simvastatin versus simvastatin/ezetimibe on a South Brazilian cohort

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Abstract

Objectives: To compare reductions in LDL-C, non-HDL-C, triglycerides and total cholesterol levels after treatment with simvastatin only (20 mg) or 20 mg simvastatin with 10 mg ezetimibe.

Methods: Our sample comprised 253 patients with European ancestry, with LDL-C exceeding target levels who had not previously been treated using drugs. During an initial phase, these patients were treated with 20 mg simvastatin for 6 months. Fifty-seven patients who did not achieve their target levels were then enrolled on a second phase of the trial and treated with a combination of 20 mg simvastatin and 10 mg ezetimibe for 4 months.

Results: After the first treatment phase we achieved a significant reduction in LDL-C levels (-37.5%, $p<0.001$) and 20.6% of the patients achieved their target LDL-C levels. In the second phase of the trial, 57 patients were treated with a combination of 20mg simvastatin and 10 mg ezetimibe and after 4 months an additional -31.7% ($p<0.001$) reduction in LDL-C levels had been achieved and 56.1% of the patients had hit their LDL-C targets.

Conclusions: Treatment with simvastatin proved effective, but the combination of simvastatin with ezetimibe proved to be very effective for reducing LDL-C levels in patients who did not respond well to monotherapy, with a positive impact on health indicators related to cardiovascular diseases.

Running Title: Eficácia de sinvastatina e sinvastatina/ezetimiba

Key words: simvastatin, ezetimibe, dyslipidemias, cholesterol, Cholesterol-LDL

Introduction

Statins play a fundamental role in treatment of dyslipidemias, which are considered one of the risk factors for cardiovascular diseases (Faludi et al., 2017).Treatment guidelines state that one of the objectives for patients with hypercholesterolemia is reduction of the levels of low density lipoprotein cholesterol (LDL-c) (Faludi et al., 2017; Xavier et al., 2013; Farnier et al., 2013). Reduction of LDL-C levels with statins has significantly lowered cardiovascular morbidity and mortality, in both primary and secondary prevention (Xanthopoulou et al., 2013; Teramoto, Taneyama, 2013). However, some patients may respond differently to treatment with statins, resulting in a considerable number of people with LDL-C concentrations exceeding target levels (Thongtang et al., 2013; Gudzune et al., 2012) particularly among high risk patients (Robinson et al.,2013).

Several recent studies have shown that from 40 to 80% of patients treated with statins do not achieve the recommended LDL-C or total cholesterol levels, with lower rates of on-target levels more frequently observed among higher-risk patients (Morrone et al., 2012; Kondo et al., 2014). Treatment with statins can reduce LDL-C levels by 30 to 50% and if the statin dose is doubled there is an additional reduction of 5 to 7%. However, to improve treatment efficacy it may be necessary to use agents such as ezetimibe that complement the inhibition of cholesterol synthesis provided by statins (Morrone et al., 2012).

Ezetimibe is a selective inhibitor of cholesterol absorption that blocks transport of cholesterol and phytosterol in the intestine and significantly reduces plasma levels of LDL-C (~15 to 20%, total cholesterol (~13%), and triglycerides (~6%), in addition to increasing the HDL-C fraction (~2%) (Ziada et al., 2013). Co administration of ezetimibe with statins provokes a significant reduction in LDL-C (approximately -6 to -21%).

According to the V Brazilian Directive on Dyslipidemias and Atherosclerosis Prevention (Xavier et al., 2013), supplementing with ezetimibe is recommended when target LDL-c is not achieved with treatment with statins (recommendation IIa, evidence C). The aim of this study is to compare reductions in levels of LDL-C, non-HDL-C, triglycerides (TG), and total cholesterol (TC) achieved with simvastatin alone (20 mg) or simvastatin

combined with ezetimibe (20/10 mg), in a population of 253 patients with primary dyslipidemia.

Study materials and methods

This is an observational study of a cohort recruited for previous studies that were designed to investigate the influence of genetic factors on response to treatment (Fiegenbaum et al., 2005a; Fiegenbaum et al., 2005b; Fiegenbaum et al., 2005c; Sortica et al., 2012; Lima et al., 2013) with data collection from 2003 to 2008. In compliance with recommendations contained in the V Brazilian Directive on Dyslipidemias and Atherosclerosis Prevention, published by the Atherosclerosis Department of the Cardiology Brazilian Society (Xavier et al., 2013), the LDL-c target for patients with cardiovascular disease and/or diabetes is <70 mg/dL, whereas for other patients it is <100 mg/dL. The present study was split into two phases. The sample for the first phase comprised 253 patients with dyslipidemia and European ancestry who were treated with 20 mg simvastatin for a mean period of 6 months. The second phase recruited a subset of patients who had not achieved their target LDL-c and were treated with a combination of 20 mg simvastatin and 10mg ezetimibe per day for a further 4 months. This study was approved by the Research Ethics Committees at the Federal University of Health Sciences of Porto Alegre and the Federal University of Rio Grande do Sul and all patients signed free and informed consent forms.

All patients had indications for antihyperlipidemics as per the V Brazilian Directive on Dyslipidemias and Atherosclerosis Prevention, published by the Atherosclerosis Department of the Cardiology Brazilian Society.[2] To be eligible for the study, patients could not have: abnormal thyroid stimulating hormone (TSH) levels; renal failure, defined as creatinine >1.5 mg/dL and/or urea >60 mg/dL; liver conditions with abnormal glutamic-oxalacetic transaminase (GOT) or alanine aminotransferase (ALT); or age <20 years.

Recruitment and follow-up of patients were undertaken by physicians from the Centre of Diagnostic Cardiology (CDC), a private cardiology clinic located in the city of Porto Alegre, Rio Grande do Sul, Brazil, which treats patients from the state of Rio Grande do Sul. Laboratory test results were acquired and data were collected on age, sex, smoking, comorbidities, family

history of cardiovascular diseases, concurrent treatments, and other relevant clinical data. This information was input to a spreadsheet and used to construct a database.

Laboratory analyses

Samples for phase 1 laboratory analyses were taken at study outset and after 6 months of treatment with simvastatin, while the phase 2 interval was 4 months. All blood samples for analyses were taken after 12 hours fasting. Tests for total cholesterol (TC), high density lipoprotein (HDL-C), triglycerides (TG), and other relevant biochemical parameters were conducted using conventional enzymatic methods. Low density lipoprotein (LDL-C) and non-HDL-C cholesterol levels were calculated using the Friedewald formula (Friedewald, Fredrickson, 1972).

Statistical analysis

Data processing and analysis were performed using the Statistical Package for Social Sciences (SPSS), version 19.0 for Windows. Qualitative variables are expressed as absolute and relative frequencies and quantitative variables as means and standard deviations. Triglyceride levels were log-transformed (lntg) to eliminate asymmetry in distributions and non-transformed values are shown in the tables. Pre and post treatment means were compared with the *t* test for paired samples. Values of $p<0.05$ were considered significant.

Results

Characteristics of the sample

The first phase of the study enrolled 253 patients with dyslipidemia, none of whom had had any prior contact with statins to treat dyslipidemia, i.e. for all of them this was their first statin prescription. There were 173 women in the sample. The following risk factors for cardiovascular disease were observed: smokers and ex-smokers (21.4%), systemic arterial hypertension (71%), diabetes (17.1%), treated hypothyroidism (14%), and family history of cardiovascular disease (19.9%). The medications most frequently used concomitantly with simvastatin were diuretics, at 40.5%, and angiotensin-converting enzyme inhibitors (ACEI), at 22.2%. After treatment with 20 mg

simvastatin it was found that 155 patients (61.3%) had not achieved their target LDL-c levels. Comparison of the subset that achieved their targets and the subset that did not showed that there was a higher prevalence of coronary artery disease among patients who did hit their targets (92% vs. 35.5%, p<0.0001). None of the other variables differed between the groups.

In the second phase of the study, 57 patients from the subset who had not met the target with 20mg simvastatin/day were treated with a combination of 20 mg simvastatin and 10 mg ezetimibe, once per day, for a period of 4 months. In the second phase, 56.1% of the patients treated achieved their LDL-c targets. Comparison of those who reached their target in the second phase and those who still did not achieve their target levels revealed significant differences for: family history of cardiovascular disease (p=0.004), hypothyroidism and use of levothyroxine sodium (p=0.013), use of diuretics (p=0.002), use of ECA inhibitors (p=0.013), and use of vasodilators (p=0.007).

Changes to lipid profile

Table 2 shows changes in total cholesterol, HDL, LDL, and triglycerides after each study phase in the entire sample and subsets. For the whole sample, at the end of the first phase of the study it was observed that there had been significant reductions in cholesterol (-27.3%, p<0.001), LDL (-37.5%, p<0.001), and triglycerides leves (-18.4%, p<0.001). When the sample was subdivided into those who achieved the LDL target and those who did not, it was found that the reductions in total cholesterol and LDL levels were significantly greater in the group that did achieve the target (-38.9% vs. -22.0% for total cholesterol and -52.5% vs. -31.0% for LDL levels), although the reductions in these plasma levels were significant in both of these subsets (p<0.001). Triglycerides only exhibited a statistically significant reduction in the subset that did achieve the LDL target (-24.3%, p=0.003) and HDL levels did not change significantly in any of the groups. Among the patients who were enrolled on the second phase of the study, it was observed that total cholesterol and LDL levels had reduced by 19.7% and 31.7%, respectively, after 4 months of treatment with the combination of 20 mg simvastatin and 10 mg ezetimibe. The overall reductions in total cholesterol and LDL-c levels were 33.7% and 50.6%, respectively, which is comparable to the subset that had achieved the target with 20 mg simvastatin

monotherapy and significantly greater than the reduction observed in the subset that did not.

Discussion

The majority of the reductions in LDL-C levels observed in this study are similar to reductions achieved in studies with different populations. In the first phase of our study, when patients had their first contact with simvastatin, we obtained a significant reduction in LDL-C levels, of -37.5%, which is in line with the literature that predicts reductions of approximately 30% (Faludi et al., 2017; Xavier et al., 2013).

Clinical practice shows us that there is a need for additional treatment options, considering that many patients do not achieve the desired LDL-C levels or cannot tolerate statins. In our study, 20.6% of patients achieved target LDL-C after first contact with simvastatin and the remaining 79.4% of the patients were put on other treatment strategies with the aim of treating them. The physicians defined the dosages and medications employed on the basis of the guidelines current at the time of the study, and it is important to point out that this is an illustration of what takes place in clinical practice in Brazil.

Although the combination of a statin with ezetimibe has been in use for several years, there have been many studies published recently attempting to test the efficacy with respect to the lipid profile of supplementing with ezetimibe in comparison with statin monotherapy (Table 3). As for the statins employed in these studies, all were investigated in combination with ezetimibe, but the most often described statin was atorvastatin, followed by simvastatin. This preference for atorvastatin is possibly because it is more accessible for patients in other countries, in contrast to Brazil, where simvastatin is available as part of the Popular Pharmacy Program at a much lower price than on the open market, making the drug more accessible for the population. It can also be observed that in the majority of studies supplementing with ezetimibe increased the reduction in LDL-C levels, with results varying from -22.4% to -56.4%. Some studies have also assessed LDL-C reductions when ezetimibe is used in isolation and found that the reduction in LDL-C is less than when it is combined with statins (Farnier et al., 2013; Ziada et al., 2013; Toth et al., 2014; Yamazaki et al., 2013; Leiter et al., 2008; Yu et al., 2012; Barbaosa et al., 2013).

Although adding ezetimibe amplifies the antihyperlipidemic effect of statins, the studies that have observed greatest reductions are those that have employed higher statin doses. In a study conducted by Robinson et al. (Robinson et al., 2013), a larger reduction in LDL-C levels (-56.4%) was observed after combining 10 mg ezetimibe and 40 mg simvastatin, whereas a smaller reduction in LDL-C (-22.4%) was observed in a study by Yamazaki et al. (2013), who used a combination of 10 mg ezetimibe with 2.5 mg rosuvastatin. Still with relation to LDL-C reductions, the smallest result observed using medication (not placebo) in any of the studies was a reduction of -11.0% with 80 mg atorvastatin monotherapy. However, in that study all patients had been treated for 4 weeks with 40 mg atorvastatin, which could have made it less likely that a greater reduction in LDL-C would be achieved by doubling the dose (Leiter et al., 2008).

Comparing the present study with others conducted recently in Brazil (Table 3), it will be observed that there are differences in the study designs employed and that only this study was retrospective. With regard to sample size, the present study had the largest number of patients, with 253 vs. 122 and 112 in the others (Moreira et al., 2014; Barbosa et al., 2013) and the statins employed were different. All studies reported reductions in LDL-c with the combined scheme, but one study that compared monotherapy with 40mg rosuvastatin to a combination of 10 mg ezetimibe and 40 mg simvastatin observed a greater reduction in response to monotherapy, -58% vs. -53% respectively. This result probably occurred because rosuvastatin is a more powerful statin than simvastatin and, as mentioned earlier, the dose and type of statin used in combined therapy are important factors in the final benefit observed. Another study (Barbosa et al., 2013) initially employed atorvastatin as monotherapy before randomizing the patients and then assessing patients treated with 40 mg atorvastatin monotherapy, 10 mg ezetimibe, or a combination of 10 mg ezetimibe and 40 mg atorvastatin, and also achieved reductions in LDL levels with the combined treatment. The present study is the only one that analyzed the combination of ezetimibe with simvastatin after monotherapy with simvastatin in a single population, comparing the same patients at two points in time and analyzing the effect of the combination in patients who did not achieve the hoped-for reductions in LDL-C levels.

Over the years, management and awareness of dyslipidemia has improved, but several different recent studies indicate that 30 to 35% of high risk patients still go untreated, and many others do not achieve the targets set out in guidelines (Toth et al., 2014). Based on the analyses conducted and on clinical practice, there is a need to emphasize that for patients who do not achieve the desired LDL-C levels with statin monotherapy, supplementing with ezetimibe is beneficial in all aspects (Toth et al., 2014), particularly in high risk patients with LDL-C targets <70 mg/dL.

Not only have the clinical results proven greater efficacy with ezetimibe combined with statins, but the treatment compliance rates were also found to be higher in a study conducted with a population in Italy (Guglielmi et al., 2017). In that study, it was observed that using doses of statins with low treatment response had a negative impact on compliance with treatment, which is a problem that will have repercussions for overall health, since dyslipidemia is a risk factor for cardiovascular diseases.

Considering that in Brazil part of the population has greater access to simvastatin, as mentioned above, it may not appear that combining it with ezetimibe is a financially beneficial treatment, but a study by Almaki et al. (Almaki et al., 2017) demonstrated that over a lifetime, in comparison with monotherapy with simvastatin, this combination is an economically attractive treatment, particularly for patients with acute coronary disease.

Conclusions

The results of this analysis allow for the conclusion that the combination of simvastatin and ezetimibe is beneficial for patients in whom it is difficult to achieve LDL-C targets with monotherapy, enabling LDL-C levels to be reduced in a shorter treatment time and the recommended target to be met, achieving rational use of medications and increased treatment compliance in part of the population, and corresponding reductions in rates of cardiovascular diseases and their consequences.

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Table 1. General characteristics of the sample

Characteristics	**Entire sample**	**Achieved target with 20 mg simvastatin/day**		**p**	**Achieved target with 20 mg simvastatin and 10 mg ezetimibe/day**		**p**
	n	**No**	**yes**		**No**	**yes**	
	253	**155**	**98**		**25**	**32**	
Female	68.4	67.1	77.5	0.088	24.0	21.2	0.767
Smoker				0.191			0.583
No	78.7	75.1	84.7		72.0	81.2	
Yes	9.1	11.9	5.1		20.0	19.4	
Ex-smoker	12.2	13.0	10.2		8.0	9.4	
CVD	26.5	35.5	92.0	<0.0001	40.0	34.4	0.784
SAH	71.0	72.2	68.3	0.118	56.0	68.7	0.816
Family history	19.9	18.1	26.5	0.118	24.0	0	0.004
Diabetes Mellitus	17.1	9.3	12.2	0.166	28.0	25.0	1.000
Hypothyroidism	14.0	14.1	13.2	1.000	20.0	0	0.013
Concomitant medications							
β- blockers	30.6	30.3	32.6	0.781	53.0	62.5	0.299
Antithrombotics	21.5	27.1	20.4	0.294	56.0	50.0	0.802
Levothyroxine sodium	14.3	14.2	14.3	1.000	20.0	0	0.013
Diuretics	40.5	40.0	35.7	0.510	32.0	75.0	0.002
ACEI	22.2	25.2	18.4	0.221	6.0	37.5	0.013
Calcium blockers	15.5	17.4	16.3	0.865	16.0	25.0	0.520
Vasodilators	15.9	14.8	16.3	0.858	0	25.0	0.007
Nitrates	4.0	5.2	6.1	0.782	12.0	25.0	0.315

CVD: cardiovascular disease; SAH: hypertension; ACEI: Angiotensin-converting enzyme inhibitors.

Table 2. Lipid levels before and after treatment with 20 mg simvastatin and after simvastatin combined with ezetimibe (20 mg/10 mg)

	Baseline lipid levels	**Lipid levels after treatment with 20 mg simvastatin/day**	**Changes in lipid levels (%)**	**p**
Treatment with 20 mg simvastatin/day				
Entire sample (n=253)				
TC (mg/dL)	255.3 ± 39.2	185.5 ± 34.0	-27.3	<0.001
HDL-C (mg/dL)	50.8 ± 12.3	51.6 ± 12.1	1.6	0.255
LDL-C (mg/dL)	172.7 ± 35.9	107.9 ± 28.3	-37.5	<0.001
TG (mg/dL)	157.9 ± 76.7	128.9 ± 55.1	-18.4	<0.001
Achieved LDL target (n=98)				
TC (mg/dL)	260.9 ± 38.9	157.2 ± 21.1	-38.9	<0.001
HDL-C (mg/dL)	52.1 ± 13.1	50.8 ± 10.9	-2.3	0.653
LDL-C (mg/dL)	179.3 ± 30.4	84.1 ± 14.7	-52.5	<0.001
TG (mg/dL)	147.7 ± 76.5	111.8 ± 43.9	-4.8	0.003
Did not achieve LDL target (n=155)				
TC (mg/dL)	269.8 ± 36.6	204.9 ± 27.1	-22.0	<0.001
HDL-C (mg/dL)	49.2 ± 11.2	50.3 ± 10.8	4.9	0.452
LDL-C (mg/dL)	190.5 ± 34.4	125.7 ± 22.7	-31.0	<0.001
TG (mg/dL)	149.1 ± 65.3	142.1 ± 63.0	-7.4	0.350
Treated with 20 mg simvastatin and 10 mg ezetimibe/day				
(n=57)				
TC (mg/dL)	222.6 ± 44.7	178.8 ± 42.3	-19.7	<0.001
HDL-C (mg/dL)	52.0 ± 11.8	53.4 ± 11.7	2.7	0.422
LDL-C (mg/dL)	137.7 ± 36.0	94.0 ± 27.3	-31.7	<0.001
TG (mg/dL)	165.0 ± 108.7	154.5 ± 93.2	6.4	0.271

TC: total cholesterol; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides.

Table 3. Studies of statins with ezetimibe, assessing % reduction of LDL-C.

Study	Country	Type of study	N	Treatment	Duration of Treatment (months)	LDL-C reduction (%)	Observation
This study	Brazil	retrospective	253	20 mg simvastatin (n= 253)	6	- 37.5	
				Ezetimibe+20 mg simvastatin (n= 57)	4	- 31.7	
Moreira et al., 2014[18]	Brazil	prospective	112	10 mg ezetimibe + 40 mg simvastatin	3	-53.0	
				40 mg rosuvastatin		-58.0	
Toth et al., 2014[19]	United States	retrospective	27,919	Statins at same dose	Variable	4.9	All patients were already taking statins prior to study
				Ezetimibe + simvastatin		-24.0	
				Statins at double dose		-9.6	
Ziada et al., 2013[11]	England	retrospective	27	10 mg ezetimibe without statin (n= 12)	NI	- 28.1	
				Increased to 20mg ezetimibe without statin (n= 12)		Additional reduction of 1.09	
				10 mg ezetimibe with statin (40 mg, 20 mg, 5 mg rosuvastatin; 40 mg atorvastatin; 20 mg fluvastatin; 40 mg pravastatin)		-33.3	
				Increased to 20mg ezetimibe with statin (40mg, 20 mg, 5 mg rosuvastatin; 40mg atorvastatin; 20mg fluvastatin; 40mg pravastatin) (n=15)		Additional reduction of 10.3	
Farnier et al., 2013[3]	multicenter	retrospective	2990	Placebo (n= 302)	12	-0.2	
				10 mg ezetimibe (n= 291)		-19.3	
				10, 20, 40, 80 mg simvastatin (n= 1193)		-38.0	
				Ezetimibe+simvastatin at 10/10, 10/20, 10/40, or 10/80 mg (n= 1204)		-52.4	

Moutzouri et al., 2013[19]	Greece	prospective	153	40 mg simvastatin (n=55)	3	-41.0	Analyzed oxLDL
				10 mg Rosuvastatin (n= 45)		-39.0	
				10 mg ezetimibe+simvastatin 10 mg (n= 53)		-40.0	
Yamazaki et al., 2013[20]	Japan	prospective	46	10 mg Rosuvastatin (n=24)	3	-19.4	
				10 mg ezetimibe + 2.5 mg rosuvastatin (n= 22)		-22.4	
Thongtang et al., 2012[6]	United States (multicenter)	prospective	874	Low potency: ≤10 mg simvastatin; ≤20 mg lovastatin; ≤20 mg pravastatin; ≤40 mg fluvastatin (n=133)	1.5	-22.7	
				Medium potency: > 10-40 mg simvastatin; ≤20 mg atorvastatin; > 20-80 mg lovastatin; > 20-80 mg pravastatin; > 40-80 mg fluvastatin (n=582)		-25.0	
				High potency: > 40-80 mg simvastatin; >20-80 mg atorvastatin (n=159)		-29.1	
				10 mg ezetimibe +atorvastatin		-26.4	
				10 mg ezetimibe+pravastatin		-23.1	
				10 mg ezetimibe+simvastatin		-26.4	
				10 mg ezetimibe+lovastatin or fluvastatin		-24.1	
Robinson et al., 2013[8]	United States	prospective	678	10 mg atorvastatin (n= 161)	1.5	-39.0	
				20 mg atorvastatin (n=168)		-42.1	
				40 mg atorvastatin (n= 161)		-48.3	
				10 mg ezetimibe+20mg simvastatin (n= 156)		-52.3	
				10 mg ezetimibe+40 mg simvastatin (n=163)		-56.4	

Leiter et al., 2008[21]	United States and Canada multicenter	retrospective	579	10 mg ezetimibe+40 mg atorvastatin (n= 288)	1.5	-27.0	In phase 1, all patients took 40 mg atorvastatin for 4 weeks
				80mg atorvastatin (n= 291)		-11.0	
Yu et al., 2012[22]	Japan	prospective	63	Double dose statin (n= 34)	2	-17.9	
				10 mg ezetimibe+ 20 mg atorvastatin; 10 mg ezetimibe + 10 mg simvastatin; 10 mg ezetimibe + 20 mg pravastatin (n= 29)		-26.2	
Barbosa et al., 2013[23]	Brazil	prospective	122	40 mg atorvastatin (n= 45)	1	-11.0	Were initially taking 10mg atorvastatin
				10 mg ezetimibe (n= 40)		-19.0	
				10 mg ezetimibe/40 mg atorvastatin (n= 37)		-52.0	
Foody et al., 2013.	US	retrospective	43,492	Simvastatin (n=4,170)		-13.3	Initially given monotherapy
				Atorvastatin (n=7,653)		-13.3	
				Rosuvastatin (n=1,230)		-19.3	
				Simvastatin + Ezetimibe (n=540)		-33.4	
				Atorvastatin + Ezetimibe (n=1,504)		-32.6	
				Rosuvastatin + Ezetimibe (n=268)		-32.2	

LDL-C: low density lipoprotein cholesterol; NI: information not provided.