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Estudo da influência do polimorfismo rs8014194 do gene *CLMN* (Calmina) na farmacogenética de estatinas

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

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SUMÁRIO

LISTA DE ABREVIATURAS UTILIZADAS

CLMN: Gene que codifica a calmina

DCV: Doença cardiovascular

CVD: *Cardiovascular disease*

DAC: Doença arterial coronariana

GWAS: *Genome-wide association study*

HDL: *High density lipoprotein*

HMG-CoA: *3-hydroxy-3-methylglutaryl-coenzyme A*

LDL: *Low density lipoprotein*

PCR: Reação em cadeia de polimerase

PPAR-α: *Peroxisome proliferator-activated receptor alpha*

SNP: *Single Nucleotide Polymorphism*

TG: Triglicerídeos

RESUMO

Introdução: A doença cardiovascular (DCV) é a principal causa de morte no mundo e é causada principalmente por aumento do perfil lipídico do indivíduo. A classe de hipolipemiantes mais utilizada no mundo é a das estatinas. Em um estudo de varredura genômica feito por Barber e cols., 2010, foi analisada a redução de lipídeos em resposta ao tratamento com estatinas, onde foi constatada a associação do polimorfismo rs8014194 do gene *CLMN* com alterações nos níveis de colesterol total. Devido à importância de investigações farmacogenéticas com relação à resposta dessa classe farmacológica, estudos como o proposto são extremamente relevantes para o conhecimento científico e, futuramente, para sua aplicação na individualização da farmacoterapia com base no conhecimento da variabilidade genética humana. **Objetivos:** O objetivo desse estudo foi verificar a influência do polimorfismo rs8014194 na resposta terapêutica de estatinas. **Material e Métodos:** 384 indivíduos dislipidêmicos descendentes de europeus, habitantes da cidade de Porto Alegre, pacientes do Centro de Diagnóstico Cardiológicoforam inseridos neste estudo de coorte. O DNA genômico dos indivíduos incluídos no estudo foi extraído a partir de amostras de sangue periférico ou raspado bucal. A genotipagem do polimorfismo foi realizada através da técnica de PCR em Tempo Real. A associação dos genótipos com a modificação percentual média de cada variável após o tratamento foi testada comparando-se a alteração média percentual entre os genótipos, após correção por covariáveis. O nível de significância foi estabelecido em 0,05. **Resultados:** Observou-se que na amostra total não houve diferença significativa entre os genótipos e a alteração dos níveis de colesterol total, HDL-C e LDL-C ($p>0,05$). Porém, sujeitos

homozigotos AA tiveram uma redução significativamente maior no nível de triglicerídeos do que portadores dos outros genótipos (p=0,038). Quando a amostra foi extratificada por sexo, se observou uma diferença significativa entre os genótipos e a alteração dos níveis séricos de triglicerídeos somente nos indivíduos de sexo masculino (p=0,036) **Conclusões:** Há evidências de que o polimorfismo rs8014194 do gene *CLMN* está asociado com a modulação nos níveis de triglicerídeos de pacientes em tratamento com estatinas.

Palavras-chave: Estatinas, gene *CLMN,* rs8014194, farmacogenética

ABSTRACT

Introduction: Cardiovascular disease (CVD) is the main cause of death worldwide. The lipid-lowering class most used is the statins. In a genomic wide association study performed by Barber *et al.*, 2010, the lipid lowering in response to treatment with statins was analyzed, and anassociation of rs8014194 polymorphism of the gene *CLMN* was detected with changes in total cholesterol levels. Given the major use of lipid-lowering drugs in the world and the importance of pharmacogenetic investigations regarding the response of this drug class, such as the proposed studies are extremely relevant to the scientific knowledge and the future, for possible application in the individualization of drug therapy based on knowledge of human genetic variability. **Objectives:** The aim of this study was to investigate the influence of the polymorphism rs8014194 in therapeutic response of statins. **Material and Methods**: 384 dylipidemic individuals of European descent, inhabitants of the city of Porto Alegre, patients in the Centro de Diagnóstico Cardiológico of Porto Alegre were inclued on this cohort study.The genomic DNA of individuals included in this study was extracted from peripheral blood samples or oral sowabs. Genotyping of polymorphism was performed by PCR in Real Time. The association of genotypes with the average percent change of each lipid level after treatment was tested by comparing the average percent change between genotypes, after correction for covariates. The level of significance was set at 0.05. **Results:** It was observed that in the total sample there were no significant differences between the genotypes and the change in total cholesterol, HDL-C and LDL-C ($p > 0.05$). However, for triglyceride levels, AA

homozygots subjects had a significantly greater reduction than carriers of other genotypes (p = 0.038). In addition, when the data were extratified by sex, there is a significant difference between genotypes and the change of serum triglyceride levels in male subjects (p = 0.036) **Conclusion:** There is evidence that the rs8014194 polymorphism of *CLMN* gene is associated with the reduction in serum triglycerides in patients treated with statins.

Keywords: Statins, rs8014194, *CLMN* gene, pharmacogenetics

1 INTRODUÇÃO

1.1 Doença Cardiovascular e Dislipidemia

A doença cardiovascular (DCV) é a principal causa de morte de homens e mulheres no mundo todo. No Brasil, dos anos de 1996 a 2006, do total de mortes ocorridas, aproximadamente 28% foram ocasionadas por doenças do aparelho circulatório, e destas, 23,3% a 23,9% foram causadas por infarto agudo do miocárdio ou aterosclerose (Datasus, 2009).

As mortes causadas por doenças cardiovasculares acontecem, predominantemente, em países em desenvolvimento, onde a urbanização contribui para a mudança de hábitos, cada vez mais sedentários. A Organização Mundial de Saúde estima que 7 milhões de pessoas morram por ano em virtude de doença arterial coronariana (DAC) e outras 6 milhões em virtude de derrames (Polanscyk e Ribeiro, 2009).

Os principais fatores de risco da DCV são: altos níveis de colesterol sérico encontrado nas lipoproteínas de baixa-densidade (colesterol LDL ou LDL-C) e altos níveis de triglicerídeos (TG), assim como baixos níveis das lipoproteínas de alta densidade (colesterol HDL ou HDL-C), idade, sexo, histórico familiar, hipertensão, tabagismo, *diabetes mellitus*, obesidade, sedentarismo e dieta rica em gorduras (Kostis, 2007). A dislipidemia é bastante frequente na população (Johnson e cols., 2004), sendo que a combinação desta com outros fatores de risco aumenta de maneira considerável o risco de desenvolvimento de uma DCV (Humphries e Higorani, 2006).

O perfil lipídico é considerado uma característica multifatorial, podendo ser influenciado tanto ambientalmente, quanto por fatores genéticos. A alta

herdabilidade dos níveis de HDL-C, LDL-C e TG indicam a importância que variações genéticas podem ter sobre a determinação dos níveis de lipídeos e de lipoproteínas (Kathiersan e cols., 2007).

1.2 Terapia Hipolipemiante

No final da década de 70, houve uma revolução no tratamento farmacológico da hipercolesterolemia, quando os hipolipemiantes foram introduzidos no mercado. Primeiramente desenvolveu-se o uso de sequestradores de ácidos biliares, e, em seguida, houve a descoberta de um produto natural do caldo fermentado de *Aspergillus terreus*, que posteriormente veio a chamar-se lovastatina (Goldberg e cols., 2009).

Atualmente existem várias classes de hipolipemiantes usados no tratamento das dislipidemias, que podem ser usados em monoterapia ou terapia combinada. São eles:

- **Sequestrantes de ácido biliares**: ácidos biliares são derivados do colesterol e são necessários para a digestão e absorção do mesmo, e também de gorduras e vitaminas lipossolúveis. Quando não disponíveis (sequestrados pelo fármaco), o colesterol é dirigido à síntese de ácidos biliares, e a HMG-CoA redutase e os receptores da lipoproteína de baixa densidade (LDL) são modulados, diminuindo a concentração plasmática de colesterol (Stein e Raal, 2014).
- **Fitoesteróis**: o β-sitosterol é o fitosterol mais comum nos vegetais. Eles agem competitivamente em relação ao colesterol na formação de micelas e, portanto, diminuem a absorção de colesterol (Bays e Stein, 2003).

- **Ácido Nicotínico**: a niacina é uma vitamina B que tem demonstrado efeitos favoráveis sobre as lipoproteínas, reduzindo os níveis de colesterol LDL e triglicerídeos (Fazio e cols., 2010).
- **Fibratos**: os fibratos reduzem a produção de triglicerídeos e aumento do colesterol da lipoproteína de alta densidade (HDL) ao ativar o PPAR-α (*peroxisome proliferator activated receptor alpha*) (Bays e Stein, 2003).
- **Ezetimiba**: agentes que bloqueiam a absorção do colesterol da dieta (Nicholls e cols., 2014).
- **Estatinas**: agentes que bloqueiam a síntese do colesterol hepático (Nicholls e cols., 2014).

A escolha da classe está condicionada ao tipo de dislipidemia presente. Estas classes farmacológicas apresentam diversas ações benéficas sobre o sistema cardiovascular e potencialmente sobre outros sistemas, no entanto, alguns pacientes apresentam efeitos adversos graves após utilização e outros não apresentam a eficácia desejada. A variabilidade interindividual na resposta, portanto, é um dos fatores que pode limitar o benefício alcançado (Gaziano e cols., 1999; Nicholls e cols., 2014).

Das classes farmacológicas citadas, as estatinas são as mais amplamente prescritas. Seu mecanismo de ação possiblita a redução do colesterol através da inibição da enzima HMG-CoA redutase, por meio de uma afinidade destes fármacos com o sítio ativo da enzima. Esta inibição é reversível e competitiva com o substrato HMG-CoA (McTaggart e cols., 2001). Estudos mostram que as estatinas são os fármacos hipolipemiantes mais eficientes em baixar os níveis de colesterol total (18 a 25%) e LDL-colesterol (25 a 55%). Além disso, também reduzem moderadamente os níveis de

triglicerídeos (10 a 15%) e aumentam da mesma forma os níveis de HDL-colesterol (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 a idade, sexo, tabagismo, *diabetes* ou hipertensão (Bhatnagar e cols., 1998; Waters e cols., 2001; Gotto, 2002; Kreisberg e Oberman, 2002).

Além de possibilitarem a 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 e Laufs, 2005).

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. Diferentes efeitos adversos já foram relacionados à terapia com estatinas, porém toxicidades hepática e muscular são as mais importantes (Kiortsi e cols., 2007). A incidência de toxicidade hepática varia entre 1 e 3%, sendo que as incidências de reações adversas musculares variam de acordo com a gravidade: 6,2-9,1% para mialgia; 0,1-1,8% para miopatia; e 0,1% para rabdomiólise (Farmer e Torre-Amione, 2002; Hamilton-Craig, 2001).

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

1.3 Farmacogenética

A farmacogenética consiste no estudo da variabilidade genética que está associada a uma variação na resposta a medicações. As pesquisas farmacogenéticas envolvem a procura por polimorfismos em genes que influenciam a resposta ao tratamento, denominados genes candidatos. São considerados genes candidatos os que codificam proteínas que influenciam a farmacocinética dos fármacos, ou seja, a absorção, distribuição, biotransformação e excreção, e genes que codificam as proteínas que influenciam a farmacodinâmica, ou seja, proteínas alvo para a ação do fármaco (Kreisberg e Oberman, 2002). Além disso, a farmacogenética também visa detectar previamente características individuais dos pacientes que possam identificar sua resposta a cada tratamento farmacológico como boa ou ruim.

Os estudos de farmacogenética de estatinas já realizados estão concentrados, basicamente, na avaliação da eficácia do tratamento com relação à melhoria do perfil lipídico (Humphries e Hingorani, 2006; Schmitz e Langmann, 2006; Hutz e Fiegenbaum, 2008). 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 (Talameh e Kitzmiller, 2014). Como exemplos de estudos de farmacogenética de estatinas, podemos citar alguns trabalhos desenvolvidos por pesquisadores do nosso grupo de pesquisa (Fiegenbaum e cols., 2005 a, b, c; Sortica e cols., 2012; Lima e cols., 2013).

Os avanços na biologia molecular e na bioquímica têm evidenciado o real desafio da farmacogenética: cada via fisiológica é composta por dezenas

de proteínas que interagem entre si e cada proteína, codificada por seu gene que apresenta polimorfismos, pode ter sua transcrição regulada por diversas outras proteínas, cujos genes também têm polimorfismos. Além disso, as vias bioquímicas interagem entre si de maneiras complexas, e muitas delas ainda são desconhecidas, de forma que às vezes o real causador de uma alteração na resposta ao fármaco não é óbvio. Tal complexidade leva à necessidade de elaboração e/ou utilização de estudos baseados em varreduras genômicas, do inglês *Genome Wide Association Studies* (GWAs) (Peters e McLeod, 2008).

Os GWAS são grandes estudos que usam técnicas de genotipagem em larga escala, capazes de caracterizar milhares de polimorfismos em todo o genoma em um único experimento. A importância desses estudos na farmacogenética se deve ao fato de que a resposta aos fármacos muitas vezes envolve vias ainda desconhecidas, dificultando a geração de hipóteses *a priori* (Peters e McLeod, 2008). Dessa maneira, tornam-se possíveis estudos clínicos em larga escala sem a necessidade de escolher anteriormente polimorfismos em genes candidatos para associação com determinado fenótipo. Os GWAs têm um enorme potencial de gerar novas hipóteses, e provavelmente serão capazes de alavancar o desenvolvimento do conhecimento na farmacogenética em médio prazo (Gurwitz e McLeod, 2009).

Até o momento, a maioria dos GWAs se concentrou nos polimorfismos para suscetibilidade a doenças, existindo apenas poucos estudos em farmacogenética, de forma que ainda existe um grande campo para a expansão desse tipo de estudo na resposta diferenciada aos fármacos. Apesar dos resultados interessantes observados por nosso grupo quanto à farmacogenética de estatinas em relação à eficácia, efetividade e

desenvolvimento de efeitos adversos, novos genes sinalizados por GWAS, ainda não foram investigados em nossa população e são restritos a populações da América do Norte e Europa. Um levantamento por nós realizado no *Catalog of Published Genome-Wide Association Studies* (http://www.genome.gov/gwastudies), compreendendo 11 GWAS realizados com tratamento com fármacos da classe dos inibidores da HMG-CoA redutase revelou mais de 50 SNPs associados a um dos fenótipos de interesse em, pelo menos, um estudo.

Em um desses estudos de varredura genômica conduzido por Barber e cols. (2010), foi analisada a redução de lipídeos em resposta ao tratamento com estatinas, onde foi constatada a associação do rs8014194 com alterações nos níveis de colesterol total em resposta ao tratamento. O polimorfismo rs8014194 está localizado no cromossomo 14, em um íntron do gene que codifica a calmina (CLMN). A função da calmina é ainda desconhecida, mas a sequência da proteína contém um domínio semelhante a calponina é altamente expresso em vários tecidos, incluindo fígado e tecido adiposo e parece estar localizada no retículo endoplasmático e no citosol (Hirosawa e cols., 1999).

Embora a calmina não tenha sido previamente implicada no metabolismo do colesterol e de lipoproteínas, no estudo feito por Barber et cols. houve uma diferença de quase 50% na diminuição dos níveis de colesterol, portanto, há evidência para apoiar a conclusão de que rs8014194 foi o primeiro SNP encontrado através de GWAS a ser associado com a resposta ao tratamento com estatinas, embora confirmação definitiva depende de resultados de testes farmacogenéticos adicionais.

Frente ao grande uso de fármacos hipolipemiantes no país e no mundo e a importância de investigações farmacogenéticas com relação à resposta dessas classes farmacológicas, estudos como o proposto são extremamente relevantes para o conhecimento científico e futuramente, para sua aplicação na individualização da farmacoterapia com base no conhecimento da variabilidade genética humana.

Adicionalmente, estudos sugerem que muitos genes ou variações genéticas podem desempenhar um papel diferente nos níveis lipídicos em ambos os sexos, e que o gênero é fator importante em estudos que avaliem tais variáveis (Pellegrini e cols., 2014; Smiderle e cols., 2014).

Dessa forma, fica clara a importância da determinação de variantes genéticas que influenciem tanto os níveis de lipídeos e de lipoproteínas, quanto à farmacogenética dos hipolipemiantes, considerando a sua eficácia e segurança.

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2 OBJETIVOS

2.1 Objetivo geral

Estudar a influência do polimorfismo rs8014194 do gene CLMN em fatores de risco cardiovascular e na resposta terapêutica (eficácia e desenvolvimento de efeitos adversos) ao tratamento com hipolipemiantes em uma amostra de indivíduos descendentes de europeus habitantes da região metropolitana de Porto Alegre.

2.2 Objetivos específicos

2.2.1 Investigar a associação do polimorfismo rs8014194 do gene CLMN com a eficácia ao tratamento com hipolipemiantes.

2.2.2 Investigar a associação do polimorfismo rs8014194 do gene CLMN com o desenvolvimento de efeitos adversos ao tratamento com hipolipemiantes.

3.2.3 Investigar se o polimorfismo rs8014194 do gene CLMN pode influenciar diferencialmente homens e mulheres quanto aos fatores de risco cardiovasculares e quanto à resposta terapêutica hipolipemiante.

3 ARTIGO

Influence of the *CLNM* rs8014194 polymorphism on statin pharmacogenetics

Artigo em preparação para submissão à revista *Current Pharmacogenomics and Personalized Medicine*. Fator de Impacto: 2,05.

TITLE:

Influence of the *CLNM* rs8014194 polymorphism on statin pharmacogenetics

SHORT TITLE: rs8014194 polymorphism and statins

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ABSTRACT

Cardiovascular disease (CVD) is the main cause of death worldwide and can be caused by increased of the lipid profile. In a study of genomic scan, performed by Barber *et al*, in 2010, the association of rs8014194 polymorphism of the gene *CLMN* with changes in total cholesterol levels in individuals treated with statins was found. The aim of this study was to investigate the influence of the polymorphism rs8014194 in therapeutic response (efficacy and development of adverse effects) in the treatment with statins. Sample: 384 dyslipidemic individuals of European descent, inhabitants of the city of Porto Alegre, patients in the Cardiac Diagnostic Center. Study design: Cohort study. Methods and analysis: genotyping of polymorphism was performed by PCR in Real Time. The association of genotypes with the average percent change of each variable after treatment was tested by comparing the average percent change between genotypes, after correction for covariates. Results: No significant difference was observed between the genotypes and the change in total cholesterol, HDL-C and LDL-C ($p > 0.05$). However, homozygous AA individuals had a significantly greater reduction in triglyceride levels than carriers of other genotypes ($p = 0.038$). When the data were extratificated by sex, there was a significant difference between genotypes and the change in triglyceride levels in male subjects ($p = 0.036$). Conclusion: There is evidence that the rs8014194 polymorphism calmina gene is associated with decreased on serum triglycerides in patients treated with statins.

Keywords: *CLMN* gene, statins, rs8014194

INTRODUCTION

Cardiovascular disease (CVD) is the worldwide main cause of death of men and women[1], the main risk factors are: high blood cholesterol levels found in low-density lipoproteins (LDL) and high triglyceride levels, age, sex, family history, hypertension, smoking, diabetes mellitus, obesity, sedentary lifestyle and high fat diet[2].

The number of studies that evaluate the influence of genetic polymorphisms on the effectiveness and the development of adverse effects of treatment with statins has been growing and some new genes were identified through GWAS (genome wide association study)[3]. In one of these studies[3], the changing in the lipid profile in response to statin therapy was analyzed in samples of three different studies: Cholesterol and Pharmacogenetics (40 mg/day of simvastatin for 6 weeks), Pravastatin/Inflammation CRP Evaluation (40 mg/day of pravastatin for 24 weeks) and Treating to New Targets (10 mg/day of atorvastatin for 8 weeks), where was detected the association of rs8014194 polymorphism in changes of total cholesterol levels in response to statins treatment. This SNP is located on chromosome 14 in an intron of the gene encoding calmin (CLMN).[4] The calmin function is still unknown, but the sequence of the protein contains a domain similar to calponin and is highly expressed in many tissues including liver and adipose tissue, and appears to be localized in the endoplasmic reticulum and cytosol.[4,5]

Although calmin has not previously been implicated in the metabolism of cholesterol and lipoproteins and neither the variation in CLMN has been associated with metabolic characteristics, in the above quoted study there was

a reduction of almost 50% in cholesterol levels, so there is strong evidence that the rs8014194 is the first SNPs found by GWAS in the response to treatment with statin.[6]

Considering that rs8014194 in the CLMN gene was one of the first markers identified through GWAS for response to statins, and studies in the area of pharmacogenetics need to be replicated in independent populations,[3,6] this study aims to assess the influence of polymorphism rs8014194 in therapeutic response (efficacy and development of adverse effects) to treatment with simvastatin/atorvastatin in a sample of individuals of European descent inhabitants of the metropolitan region of Porto Alegre.

MATERIALS AND METHODS

Patients

Three hundred eighty-four Brazilian hypercholesterolemic patients of European descent were investigated in a cohort study according to simvastatin or atorvastatin treatment. The exclusion criteria were: age < 20 years, triglyceride concentration >400mg/dL, altered thyroid stimulating hormone levels, impaired hepatic or renal function, unstable or uncontrolled disease that influences lipid metabolism and previous therapy with other lipid-lowering drug.

Biochemical analyses

Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TGs) were determined from peripheral blood obtained after 12 h of fasting using standard methods with commercial kits. LDL-C levels were assessed by Friedewald *et al.* (1972)[7]. The percentage of patients with normalized lipid levels after therapy was assessed according to

the V Dyslipidemia Treatment Guideline of Brazil[8] for primary prevention of CVD: TC < 200 mg/dL, LDL-C < 100 mg/dL, HDL-C > 60 mg/dL, and TG < 150 mg/dL.

DNA analyses

Genomic DNA was extracted from peripheral blood samples using the technique described by Lahiri and Nurnberger.[9] DNA was extracted through collected cell scraping the inner cheek side with sputum cytology brush Considering the objectives defined by us, we propose to investigate the polymorphism rs8014194 of CLMN gene in relation to cardiovascular risk factors and pharmacogenetics of statin simvastatin and atorvastatin.

Genotyping of the selected polymorphism was performed by allelic discrimination by PCR in Real Time sets of genotyping TaqMan® SNP Applied Biosystems. The procedures were performed according to the manufacturer's recommendations

Statistical analyses

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

Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with the Hardy-Weinberg equilibrium expectations was tested using chi-square test. Student's *t*-test was performed to assess differences between continuous variables. Categorical variables were compared using chi-square tests (a two-sided p-value of < 0.05 was considered statistically significant) with Yates's correction. When appropriate, adjusted residual values (cell-by-cell analyses) and the power of the tests were assessed

by WINPEPI. Because of lower homozygous genotype frequencies, rs8014194 genotypes of the *CLMN* gene were grouped as A allele homozygotes (A/A) and T allele carriers (A/T and T/T). Considering the possible difference in the lipid-lowering efficacy of simvastatin and atorvastatin therapy in different doses, we created a standardized statin dosage variable transforming the daily doses of simvastatin to equivalent doses of atorvastatin by using the dose equivalence ratio 2:1 for simvastatin:atorvastatin, based on published side-by-side comparisons.[10,11]

The mean percentage change in plasma lipid levels was obtained from the difference in pre-and post-treatment lipid levels, multiplied by 100, and divided by the pre-treatment level for each parameter. To analyze the effect of sexual dimorphism on lipid-lowering treatment efficacy according *CLMN* genotypes, the mean percentage change in plasma lipid levels was compared using the general linear model Type III sum of squares. Models were adjusted for age, smoking status, baseline lipid levels, prior CVD, controlled hypothyroidism, and antithrombotic use.

Statistical analysis was performed using SPSS 19.0 for Windows® and the p-value of <0.05 was considered statistically significant.

RESULTS

Characteristics of the study population

We evaluated a cohort of 384 descendants of Europeans, residents in southern Brazil and that made use of simvastatin/atorvastatin lipid-lowering therapy. The main characteristics of the sample are presented in Table 1. The patients in the study were aged between 25 and 88 years (61.1±11.4years) and

33.4% were males; 82.1% participants were on simvastatin treatment and 17.9% participants were atorvastatin users. The average treatment duration was approximately 6.3 ± 3.2 months; the average of standard dose of statins was 10.2 ± 4.6 mg. Most patients never smoked (79.5%), 13.2% patients were ex-smokers and 6.9% were current smokers. 32.7% of patients had prior cardiovascular disease, 72.8% of patients had hypertension and 16.6% diabetes. Patients were use a concomitant therapy throughout the study were calcium channel blockers (18.0%), diuretics (40.4%), antithrombotic (27.5%), beta-blockers (32.6%) and ACE inhibitors (22.6%).

The frequency of the A allele was 24.4% and did not differ from that observed for European populations[6] and between men and women (p=0.929). To evaluate the efficacy of treatment was used the percent change in lipid levels (TC, HDL-C, LDL-C and TG). The data are summarized in Table 2, which shows that in the total sample there are no significant differences between the genotypes and the change in total cholesterol, HDL-C and LDL-C (p>0.05). However, for triglyceride levels, AA homozygotes subjects had a significantly greater reduction than carriers of other genotypes (p=0.038). In addition, when the data were grouped according sex, there is a significant difference between genotypes and the change of serum triglyceride levels only in male subjects (p=0.036). Analysis of genotype*sex interaction, however, revealed no significant interaction triglyceride levels. Furthermore, the interaction analysis revealed in carriers of the T allele, the reduction of total cholesterol levels is similar between men and women. However, for homozygous AA in males there is less hypolipidemic effect and most women (p Genotype*Sex interaction=0.045, Figure 1).

DISCUSSION

The present study analyzes the influence of the polymorphism rs8014194, located in CLMN gene on chromosome 14, in the efficacy of statin therapy in a group of 384 hypercholesterolemic patients of European descent. The possible association of this polymorphism with the reduction of total cholesterol in hypercholesterolemic patients treated with statins has been described by Barber et al. in a study published in 2010, where several analyzes were made to the assessment of change in LDL-C, TC, HDL-C and triglycerides levels, pre and after one year of treatment with statins. The results indicated a strong association between the SNP rs8014194 and the decrease in total cholesterol ($p = 1.8 \times 10^{-8}$).

Our study also showed that AA homozygous patients had a significantly greater reduction in triglyceride levels than carriers of other genotypes. Men carrying the AA genotype showed a significant difference in triglyceride levels of reduction when compared to other male carriers of other genotypes, while women showed no statistically significant difference between the three genotypes. Some studies have shown differences between men and women regarding the response to different drugs, but in the case of statins response difference to simvastatin / atorvastatin may vary 35-40% from one patient to another, regardless of sex.[12] The statin therapy was shown to be more effective in patients who have higher levels of TC starting treatment and the difference in response to treatment with statins may also be correlated to the prevalence, severity and development of autoimmune diseases[13], asthma[14] and cardiovascular disease[15], which may lead to differences in gene regulation

between men and women. Furthermore, individual characteristics influence the pharmacokinetics and pharmacodynamics, and can generate variable responses in different individuals.[16]

The GWAS studies have identified untapped variants and validated in other populations. These studies represent a useful tool to identify genes involved in complex human diseases, therefore track the genome of a large number of individuals in search of hundreds to thousands of SNPs involved in a particular phenotype. Only a small number of GWAS studies had previously indentified loci associated with statins responce on a genome-wide level. The JUPITER GWAS trial identified three genetic loci, *ABCG2* (rs2199936), *LPA* (rs10455872) and *APOE* (rs7412), that were associated with percentage LDL-C reduction following rosuvastatin therapy.[17] The SNPs at *LPA* (rs10455872) and *APOE* (rs445925 and rs4420638),[18] that were associated with LDL-C responde to atorvastatin lipid-lowering therapy, were identified in CARDS and ASCOT studies. A combined GWAS in three statin trials identified the SNP rs8014194 within *CLMN*,[3] that is the target of our study but, however two other GWAS identified no genetic determinants of LDL-C response to statin therapy at a genome-wide significant level.[19]

Most of the genetic variations identified so far with these approaches appear to confer moderate effect and few causal alleles have been identified. Evidence has shown that variations in GWAS identified should be validated for each target population, since their effects depend on specific interactions with the environment in which people live and that other variants present in the genome, whose frequency can be very different in each population group.[20,21]

This study has also some limitations. The sample was not equally distributed in terms of the baseline lipid profile, proportion of men and women or age. Some patients had incomplete data, especially on hormonal status, hormone therapy and lifestyle (diet and physical exercise). We also cannot exclude the possibility of a statistical type II error due to the small sample of patients AA homozygous. Despite these limitations, and althought our findings require confirmation in larges and diferent populations, they suggest that rs8014194 polymorphism has a real association with statin's lipid-lowering efficiency.

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Conflits of Interests

None of the authors have any competing interests to disclose.

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Table 1- Patient characteristics

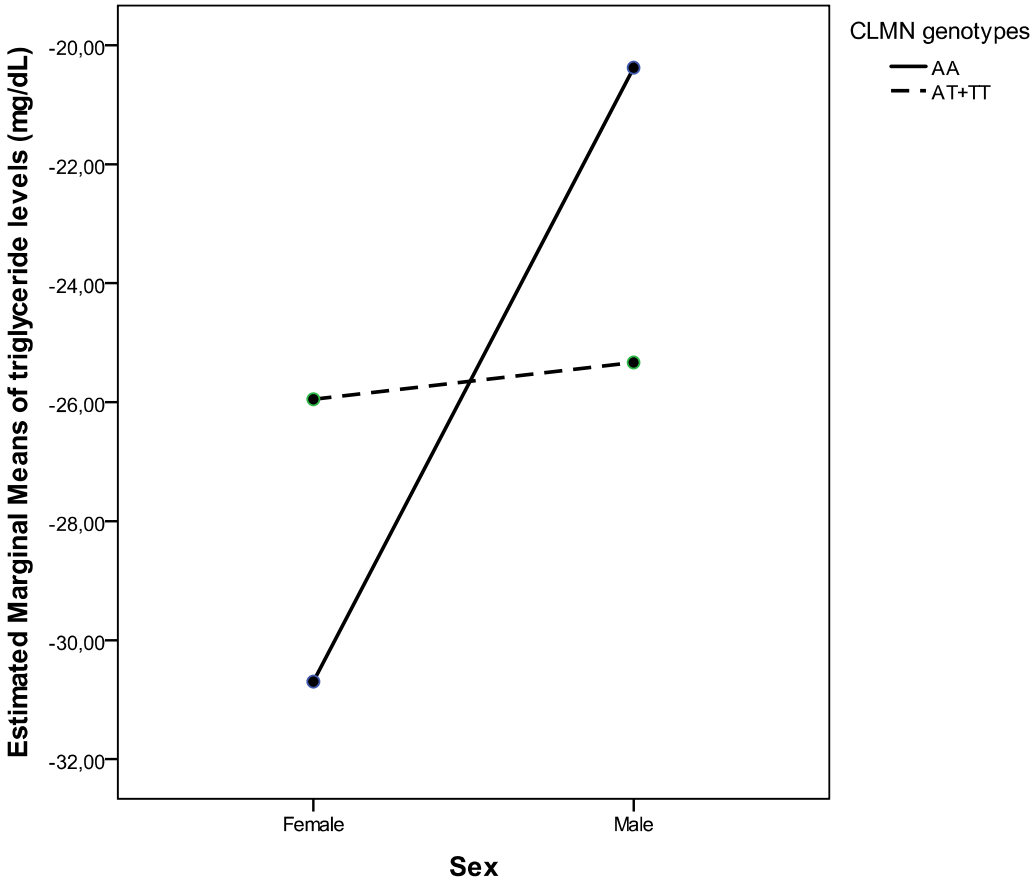
Characteristics	
Number	384
Age (years)	61.1±11.4
Sex, male (%)	33.4
Statin Use (%)	
Simvastatin	82.1
Atorvastatin	17.9
Treatment time (months)	6.3±3.2
Standard dose (mg)	10.2±4.6
Smoking (%)	
Past	13.2
Current	6.9
Never	79.5
Prior CVD (%)	32.7
CVD Family history (%)	15.4
Diabetes (%)	16.6
Hypertension (%)	72.8
Concomitant therapy use (%)	
Calcium channel blockers	18.0
Diuretics	40.4
Antithrombotic	27.5
ACE inhibitor	22.6
β-blocker	32.6
CYP3A4 substrates	17.0
CYP3A4 inducers	1.6
CYP3A4 inhibitors	22.0

Values for age, treatment duration and standard dose are expressed as means ± standard deviations; CVD, Cardiovascular disease; ACE, angiotensin-converting enzyme.

Table 2 - Lipid Change (%) according *CLNM* rs8014194 genotypes

SNP rs8014194	n	Total cholesterol	HDL-C	LDL-C	Tryglicerides
AA	28	-28.6 ± 10.7	-1.55 ± 19.8	-36.7 ± 15.6	-19.2 ± 22.6
AT	130	-25.1 ± 12.5	-4.1 ± 21.1	-35.6 ± 19.1	-6.09 ± 35.1
TT	224	-26.3 ± 11.9	-4.4 ± 23.7	-35.8 ± 17.6	-14.4 ± 28.9
p		0.611	0.470	0.956	0.038
Mulheres					
AA	19	-33.4 ± 7.1	-0.1 ± 20.0	-44.0 ± 8.9	-21.3 ± 17.7
AT	87	-26.1 ± 12.9	-3.5 ± 19.5	-36.8 ± 20.6	-8.3 ± 31.5
TT	150	-26.6 ± 12.2	-3.1 ± 22.8	-36.7 ± 17.6	-13.9 ± 30.5
p		0.280	0.213	0.586	0.279
Homens					
AA	9	-18.4 ± 10.2	-4.3 ± 20.2	-22.0 ± 16.0	-14.6 ± 31.3
AT	43	-22.9 ± 11.2	-5.3 ± 24.2	-33.3 ± 15.6	-1.6 ± 41.2
TT	76	24.7 ± 12.4	-6.6 ± 25.2	-32.3 ± 17.9	-13.5 ± 28.5
p		0.251	0.374	0.448	0.036

Figure 1 – Estimated Means of trigliceryde levels according sex and *CLMN* genotypes (Genotype*Sex interaction=0.045).



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- [1] Özdemir V, Fisher E, Dove ES, et al. End of the beginning and public health pharmacogenomics: Knowledge in 'mode 2' and P5 medicine. Curr Pharmacogenomics Person Med 2012; 10(1): 1-6.
- [2] Funtowicz SO, Ravetz JR. Risk management as a postnormal science. Risk Anal 1992; 12(1): 95-7.
- [3] Ravetz J. The post-normal sciences of precaution. Water Sci Technol 2005; 52(6): 11-7.

Book Chapter:

- [4] Barben D, Fisher E, Selin C, *et al*. Anticipatory governance of nanotechnology: Foresight, engagement and integration. In: The Handbook of Science and Technology Studies, Third Edition. Hackett EJ, Amsterdamska O, Lynch M, Wajcman J, Eds. Cambridge, MA: MIT Press, 2008; pp. 979-1000.

Book:

- [5] Rose N. The Politics of Life Itself: Biomedicine, Power, and Subjectivity in the Twenty-First Century. Princeton: Princeton University Press 2006.

World Wide Web:

- [6] Dove ES. The genetic privacy carousel: A discourse on proposed genetic privacy bills and the co-evolution of law and science. Curr Pharmacogenomics Person Med 2011; 9(4): 252-63. Available from: (http://www.benthamdirect.org/pages/content.php?CPPM/2011/00000009/00000004/003AF.SGM) [Accessed April 26, 2012].
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- Paraphrasing poorly: Copying complete paragraphs and modifying a few words without changing the structure of original sentences or changing the sentence structure but not the words.

- Verbatim copying of text without putting quotation marks and not acknowledging the work of the original author.
- Properly citing a work but poorly paraphrasing the original text is considered as unintentional plagiarism. Similarly, manuscripts with language somewhere between paraphrasing and quoting are not acceptable. Authors should either paraphrase properly or quote and in both cases, cite the original source.
- Higher similarity in the abstract, introduction, materials and methods, and discussion and conclusion sections indicates that the manuscript may contain plagiarized text. Authors can easily explain these parts of the manuscript in many ways. However, technical terms and sometimes standard procedures cannot be rephrased; therefore Editors must review these sections carefully before making a decision.

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Published manuscripts which are found to contain plagiarized text are retracted from the journal website after careful investigation and approval by the Editor-in-Chief of the journal. A 'Retraction Note' as well as a link to the original article is published on the electronic version of the plagiarized manuscript and an addendum with retraction notification in the journal concerned.

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ANEXO B: Aprovação Comitê de Ética em Pesquisa

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