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Dissertação

Polimorfismos da metilenotetrahidrofolato redutase e sua associação com fatores de risco para doenças crônicas não transmissíveis na Coorte de 1982, Pelotas, RS, Brasil

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“O conhecimento nos faz responsáveis”

Che Guevara

RESUMO

SILVA, Liziane Pereira da. **Polimorfismos da metilenotetrahidrofolato redutase e sua associação com fatores de risco para doenças crônicas não transmissíveis na Coorte de 1982, Pelotas, RS, Brasil.** 2013. 51f. Dissertação (Mestrado) - Programa de Pós-Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas.

Os polimorfismos do gene Metilenotetrahidrofolato Redutase (*MTHFR*) estão relacionados com a baixa atividade da enzima e aumento dos níveis plasmáticos de homocisteína (Hcy). A hiper-homocisteinemia (HHcy) é um fator de risco para vários processos patológicos incluindo a aterosclerose. O objetivo do estudo foi avaliar o efeito do *MTHFR* C677T e A1298C e fatores comportamentais sobre os níveis de homocisteína em 3.831 amostras biológicas coletadas de indivíduos pertencentes à coorte de nascidos em Pelotas no ano de 1982. Os níveis de homocisteína foram medidos no soro por imunoensaio quimioluminescente. A genotipagem foi realizada pela técnica de discriminação alélica através do uso de sondas pré-desenhadas TaqMan® no equipamento ABI7500 Fast Real-Time PCR System. Os níveis médios de Hcy foram maiores (p <0,001) em indivíduos apresentando a variante *MTHFR* 677T em homozigose do que em indivíduos com genótipos CT e CC, independentemente de sexo, consumo de álcool, tabagismo e atividade física no lazer. No entanto, foi demonstrado um efeito maior em *MTHFR* 677TT fumantes em comparação aos não-fumantes, bem como, em consumidores de álcool do que em não-consumidores, e em indivíduos ativos do que em outros menos ativos (p de interação <0,001, respectivamente). Para *MTHFR* A1298C, os níveis de homocisteína foram maiores no genótipo AA do que nos genótipos AC e CC independente de fatores comportamentais. Homens genotipados como *MTHFR* 1298AA apresentaram aumento de 14% sobre os níveis de homocisteína em relação ao aumento de 4% observado em mulheres (p de interação <0,001). Não houve interação demonstrada entre este polimorfismo e os outros fatores comportamentais analisados. Em conclusão, em adultos jovens da coorte de 1982 foi observado um efeito de interação entre o polimorfismo *MTHFR* C677T com estilo de vida na determinação dos níveis de Hcy, contribuindo para um aumento do risco de doenças crônicas cardiovasculares no futuro.

Palavras-chave: MTHFR, polimorfismos genéticos, interação gene-ambiente, estilo de vida.

ABSTRACT

SILVA, Liziane Pereira da. **Polymorphisms of methylenetetrahydrofolate reductase gene and its association with risk factors for not transmissible chronic disease in cohort 1982, Pelotas, RS, Brasil.** 2013. 51f. Dissertação (Mestrado) - Programa de Pós-Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas.

Methylenetetrahydrofolate Reductase (*MTHFR*) gene polymorphisms are related to low activity of the enzyme increasing homocysteine (Hcy) plasma levels. Hyperhomocysteinemia (HHcy) is a risk factor for several pathological processes including atherosclerosis. The aim of the present study was to evaluate the effect of *MTHFR* C677T and A1298C polymorphisms and behavioral factors on Hcy levels in 3831 biological samples from 1982 Pelotas Birth Cohort individuals. The Hcy levels were measured in serum samples using chemiluminescence immunoassay. The genotyping was performed by allelic discrimination technique using pre-designed TaqMan® assays in the ABI7500 Fast Real-Time PCR System. The mean levels of Hcy were higher ($p<0.001$) in homozygous TT variant of *MTHFR* C677T than in CT and CC genotypes independently of sex, alcohol consumption, smoking and physical activity during leisure time. However it was demonstrated a higher *MTHFR* 677TT effect in smokers compared to non-smokers, as well as, in alcohol consumers than in non-consumers and in active individuals than in less active ones (p for interaction <0.001, respectively). For the *MTHFR* A1298C, the Hcy levels were higher in AA genotype than AC and CC genotypes, independently of behavioral factors. Men genotyped as *MTHFR* 1298AA showed 14% increasing on Hcy levels compared to 4% increase observed in women (p for interaction <0.001). No interactions were demonstrated between this polymorphism and the other behavioral factors analyzed. In conclusion, in young adult from 1982 cohort it was observed an interaction effect between the *MTHFR* C677T polymorphism and lifestyle on Hcy levels, contributing to an increased risk for cardiovascular chronic diseases in the future.

Key words: MTHFR, genetic polymorphisms, gene-environment interaction, lifestyle.

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LISTA DE ABREVIATURAS E DE SIGLAS

A – *Adenina*

Ala - *Alanina*

CβS – *Cistationina β-Sintase*

C – *Citosina*

DNA – *Ácido desoxirribonucleico*

Glu - *Glutamato*

G – *Guanina*

Hcy – Homocisteína

HHcy – Hiper-homocisteinemia

MS – *Metionina Sintase*

MTHFR – *Metilenotetrahidrofolato redutase*

NO – Óxido Nítrico

PCR – *Reação de Cadeia de Polimerase*

RNA – *Ácido ribonucleico*

SNP – *Polimorfismo de Nucleotídeo Único*

THF - *Tetrahidrofolato*

T – *Timina*

Val - *Valina*

SUMÁRIO

1 INTRODUÇÂO

1.1 Introdução Geral

Muitos estudos tem demonstrado que a concentração elevada de homocisteína (Hcy) plasmática é um fator de risco independente para a doença isquêmica coronariana, acidente vascular cerebral, doença vascular periférica e trombose venosa (REFSUM et al., 1998; DEN et al., 1998; ALESSIO et al., 2007). Diversos são os fatores que contribuem para o aumento dos níveis de Hcy, e estes podem ser classificados em duas categorias: fatores de risco modificáveis e fatores de risco não modificáveis (PAPOUTSAKIS et al., 2006). Entre os fatores de risco modificáveis que contribuem para o aumento nos níveis de Hcy, estão as variáveis relacionadas ao estilo de vida, como: tabagismo, dieta e atividade física (MENNEN et al., 2002)

O hábito de fumar tem sido associado aos níveis de Hcy (RASMUSSEN et al., 2000; JACQUES et al., 2001; SOBCZAK et al., 2007; MARSZALL; CZARNOWSKI, 2007). Em diferentes estudos que investigaram essa relação, foi proposto que a fumaça do cigarro, por conter inúmeras substâncias oxidantes, requer muita glutationa, um antioxidante que reduz a Hcy (MANSOOR et al., 1995).

A concentração plasmática de Hcy é influenciada por fatores nutricionais, tais como o *status* do ácido fólico e das vitaminas B6 (piridoxina) e B12 (cobalamina) (MUNIZ et al., 2006). É descrito, portanto, que a ingestão de vitamina B6, vitamina B12 e ácido fólico reduzem os níveis de Hcy plasmática (SILASTE et al., 2001) O ácido fólico é um substrato para a produção de tetrahidrofolato (THF), um precursor de 5-metil-THF, que é necessário para a atividade normal da enzima metionina sintase (MARON; LOSCALZO, 2009). O ácido fólico é encontrado em vegetais de

folhas verdes e em alguns produtos de origem animal, como a gema de ovo (KIM, 2007). A necessidade mínima diária de ácido fólico é da ordem de 50 µg, embora a atual ingestão recomendada seja de 400 µg/d para o adulto e 600 µg/d para mulheres grávidas (MCCULLY, 2007).

A vitamina B6 é um cofator necessário para a atividade normal da enzima cistationina β-sintase, participante da via de transsulfuração do metabolismo da Hcy. Sua deficiência nutricional é incomum, devido a sua presença em todos os grupos alimentares (MCCULLY, 2007). A vitamina B12 é um cofator essencial para a atividade normal da metionina sintase (MS), sendo encontrada exclusivamente em carnes de animais ou alimentos lácteos derivados de animais (MCCULLY, 2007).

Indivíduos que realizam atividade física apresentam níveis diminuídos de Hcy em relação aos que não o fazem (PANAGIOTAKOS et al., 2005; NAGHII et al., 2011). Em um estudo de coorte com 620 indivíduos de Israel, foi observado um aumento de 7% nos níveis de Hcy em pessoas sedentárias quando comparadas com indivíduos fisicamente ativos (DANKNER et al., 2007). Um estilo de vida mais ativo pode estar associado a um estilo mais saudável, com ingestão de alimentos que mantém adequadamente o metabolismo da Hcy (DE et al., 2001).

Entre os fatores de risco não modificáveis estão o sexo, a idade e fatores genéticos (MENNEN et al., 2002). Quanto ao sexo, foi demonstrado que mulheres apresentam níveis menores de Hcy devido ao efeito protetor dado pelo estrogênio (GILTAY et al., 1998; MORRIS et al., 2000; HAK et al., 2000). Níveis diferentes de vitaminas do complexo B em homens e mulheres também contribuem para a diferença de Hcy. Além disso, a produção de massa muscular, representada pela creatinina sérica, pode explicar parte da diferença entre os sexos (JACQUES et al., 1999), já que esse metabólito está relacionado à concentração de Hcy em jejum, em pessoas com função renal normal (BRATTSTROM et al., 1994).

Estudos mostram um aumento nos níveis plasmáticos de Hcy de 10,8 mmol/L entre 40-42 anos, para 12,4 mmol/L entre 60-65 anos (NURK et al., 2001). O processo de envelhecimento está associado com a diminuição da capacidade de absorção das vitaminas do complexo B e ácido fólico, o que resulta num gradual aumento da concentração de Hcy com a idade (MCCULLY, 2007). O declínio da função renal também pode contribuir com essa alteração (CASTRO et al., 2006), visto que a insuficiência renal é acompanhada de elevação nos níveis de Hcy (BOSTOM; CULLETON, 1999).

Após o sequenciamento do genoma humano, através do projeto Genoma Humano, muitos estudos voltaram-se ao esclarecimento das funções dos genes, bem como, à caracterização de suas interações com fatores ambientais. Foi descoberto que os genomas dos indivíduos apresentam somente 0,1% de diferença entre suas sequências (Finishing the euchromatic sequence of the human genome, 2004). As principais variações consistem em substituições de uma única base do DNA, sendo esse tipo de variação denominada polimorfismo de nucleotídeo único (*SNP*- single-nucleotide polymorphism), podendo resultar na produção de proteínas com funções alteradas.

1.2 O Metabolismo da Homocisteína

A Hcy é um aminoácido sulfurado, não essencial, ausente da dieta alimentar, mas encontrado na forma de produto intermediário no metabolismo da metionina (BENNOUAR et al., 2007). Este aminoácido foi descrito pela primeira vez por *Butz e Du Vigneaud*, a partir da reação da metionina com ácido sulfúrico concentrado (ELDIBANY; CAPRINI, 2007).

A Hcy plasmática livre pode ser encontrada na forma oxidada, formando dissulfetos (dímeros da Hcy), além de dissulfetos mistos como a homocisteína-cisteína. Dois a cinco por cento estão presentes em sua forma reduzida e 70% a 80% circulam ligados a proteínas plasmáticas, principalmente a albumina. A soma de todas as formas livres e ligadas a proteínas que contenham um grupamento tiol formam a Hcy plasmática total (NEVES et al., 2004).

A Hcy em mamíferos possui dois prováveis destinos metabólicos: a remetilação ou a transsulfuração (Fig.1); na remetilação, via metabólica principal, a Hcy forma metionina pela adição do grupo metil a 5-metiltetrahidrofolato, que é o resultado da conversão do ácido fólico da dieta (5-10-metiltetrahidrofolato) pela enzima 5,10-metiltetrahidrofolato redutase (MTHFR); em condições de excesso de metionina ou necessidade de síntese de cisteína, a Hcy entra na via de transsulfuração, onde é convertida à cistationina pela cistationina β-sintase e, logo, em cisteína com a atuação da vitamina B6 como cofator (BRUSTOLIN; GIUGLIANI; FELIX, 2010).

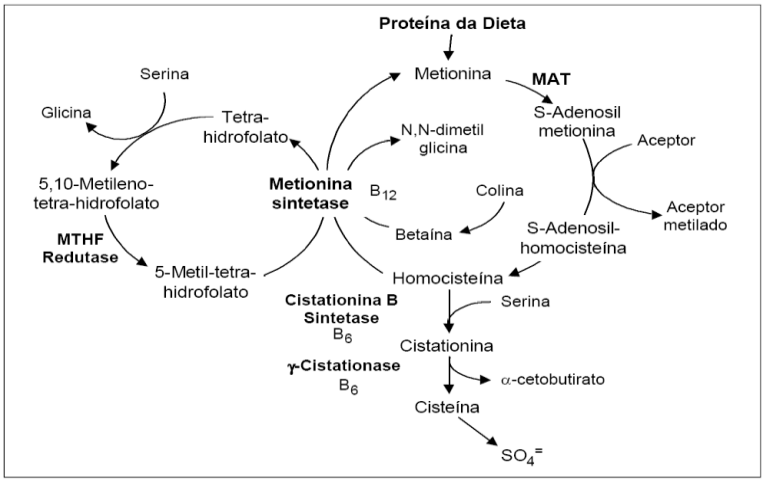

Fig.1 - Metabolismo da Hcy, segundo (BYDLOWSKI; MAGNANELLI; CHAMONE, 1998)

A concentração intracelular de Hcy é controlada pelas enzimas que participam do seu metabolismo. Em decorrência a falhas no mecanismo de conversão de Hcy em metionina, ocorre o transporte ativo desse aminoácido para o meio extracelular e, daí, para a corrente sanguínea. Este mecanismo de exportação, em conjunto com a via da transsulfuração, previne o acúmulo intracelular deste aminoácido sulfuroso potencialmente citotóxico (ELDIBANY; CAPRINI, 2007).

1.3 Hiper-homocisteinemia

Em situações de distúrbios no metabolismo intracelular da Hcy e incapacidade de manutenção das concentrações intracelulares desejáveis, o mecanismo de exportação persiste e a Hcy se acumula na corrente sanguínea, resultando na hiper-homocisteinemia (HHcy) (CASTRO et al., 2006).

Defeitos genéticos em uma das enzimas ou uma deficiência nutricional de cofatores (vitaminas B6, B12 e ácido fólico) nas vias de remetilação ou de transulfuração pode ser associada com níveis elevados de Hcy e HHcy (ELDIBANY; CAPRINI, 2007). Para que sejam mantidos níveis adequados de Hcy, deve haver uma interação entre fatores genéticos, metabólicos e ambientais. O aumento dos níveis pode ser influenciado por um único fator citado ou por uma combinação dos mesmos (STEED; TYAGI, 2011).

Concentração de Hcy até 14,9mmol/L é considerada dentro da faixa de normalidade. A HHcy pode ser diferenciada por categorias de acordo com a concentração plasmática, que incluem causa, prevalência e severidade, sendo classificada como moderada (15–30mmol/L), intermediária (31–100mmol/L) ou severa (>100mmol/L) (tabela 1). Os casos mais severos ocorrem em indivíduos que apresentam dupla homozigose para polimorfismos presentes nos genes que codificam enzimas do metabolismo da Hcy (BRUSTOLIN; GIUGLIANI; FELIX, 2010).

A HHcy é observada aproximadamente em 5% da população geral e é associada com inúmeros transtornos (BRUSTOLIN; GIUGLIANI; FELIX, 2010). Alguns estudos revelaram que as concentrações elevadas de Hcy plasmática estão associadas ao aumento do risco de doenças cardiovasculares e que a HHcy intermediária pode ser um importante fator de risco para algumas patologias, como as doenças vasculares (ALESSIO et al., 2008).

Tabela 1 - Classificação Hiper-homocisteinemia (adaptado (BRUSTOLIN; GIUGLIANI; FELIX, 2010)

Categoria	Concentração plasmática de Hcy total (mmol/L)	Causas
Hiper-homocisteinemia Moderada	15 a 30	- Deficiência nutricional; - Combinação de defeitos genéticos;
Hiper-homocisteinemia Intermediária	31 a 100	- Deficiência nutricional; - Deficiência da enzima MS; - Heterozigose para MTHFR;
Hiper-homocisteinemia Severa	>100	- Deficiência nutricional; - Deficiência das enzimas CβS e MTHFR.

MS- Metionina Sintase;
MTFHR-Metilenotetrahidrofolato Redutase;
CβS- Cistationina β- Sintase

1.3.1 A hiper-homocisteinemia e a doença vascular

A relação entre a Hcy e a doença vascular foi proposta em 1969 por McCully, através de uma observação *postmortem* de concentrações elevadas do aminoácido, em um jovem com doença arterial disseminada e anormalidade do metabolismo da cobalamina (CASTRO et al., 2006). Desde então, inúmeros são os estudos que associam esta enfermidade à HHcy (FROSST et al., 1995; NYGARD et al., 1995; GARCIA et al., 2007; RASSOUL et al., 2008; STEED; TYAGI, 2011).

Existem evidências que a HHcy determina uma lesão vascular por meio de lesão da célula endotelial, iniciando uma cascata negativa de complicações vasculares, incluindo o retardamento e crescimento do endotélio (STEED; TYAGI, 2011).

A doença vascular está intimamente associada à aterosclerose, que é considerada uma doença inflamatória. A Hcy pode influenciar a doença vascular, promovendo o recrutamento de leucócitos. As concentrações patofisiológicas de Hcy regulam a expressão e a secreção da proteína-1 quimioatratora de monócitos (MCP-1) e da interleucina-8 (IL-8), em cultura de células endoteliais humanas. Análises da participação de mecanismos epigenéticos na relação da Hcy e aterosclerose, devido à inibição de reações de transmetilação, também tem sido consideradas (PODDAR et al., 2001).

1.4 Metilenotetrahidrofolato Redutase

A MTHFR é uma enzima envolvida na via de remetilação, reduzindo 5,10-metilenotetrahidrofolato a 5-metilenotetrahidrofolato, que corresponde a forma circulante do folato capaz de doar o grupo metil para conversão da Hcy à metionina (BENNOUAR et al., 2007). A diminuição na atividade enzimática da MTHFR tem sido descrita como causa genética frequente para elevação nos níveis de Hcy (RASLOVA et al., 2000).

O gene *MTHFR* está localizado no cromossomo 1 na região p36.3, compreendendo cerca de 20 kb. É formado por 11 exons com tamanhos entre o 102 e 432 pb e, introns cujas dimensões variam entre 250 pb e 1,5 kb, com exceção de um intron que possui 4,2 kb (VINUKONDA, 2008). Já foram identificadas mais de 15

mutações raras em relação à atividade da enzima do gene *MTHFR*, a maioria delas associadas com grave deficiência enzimática (VINUKONDA, 2008). Por outro lado, dentre os polimorfismos mais frequentes identificados no gene, os mais comuns são os polimorfismos de nucleotídeo-único, o *MTHFR* C677T e o A1298C.

1.5 *MTHFR* C677T e A1298C

O polimorfismo *MTHFR* C677T (rs1801133) localizado no exon 4 do gene, resulta na substituição de uma alanina por uma valina (Ala222Val) (CICEK et al., 2004). Esta substituição é considerada uma mutação termolábil, mutação *missense*, com perda de mais de 60% da atividade enzimática (NAIR et al., 2000). A atividade reduzida da MTHFR resulta em níveis mais baixos de 5-metiltetrahidrofolato, além de um acúmulo de metileno e aumento dos níveis plasmáticos de Hcy (FROSST et al., 1995).

Diversos estudos sugerem uma associação do polimorfismo *MTHFR* C677T com aumento do risco para HHcy (CASTRO et al., 2003; ELDIBANY; CAPRINI, 2007; YAKUB et al., 2012), sendo a variante *MTHFR* 677TT associada ao aumento das concentrações plasmáticas de Hcy.

O polimorfismo *MTHFR* A1298C (rs1801131), localizado no exon 7 do mesmo gene, apresenta uma troca do aminoácido glutamina pela alanina (Glu429Ala). Este polimorfismo também pode diminuir a atividade enzimática, porém com menor efeito quando comparado ao *MTHFR* C677T (VAN DER PUT et al., 1998). A dupla heterozigose para os polimorfismos *MTHFR* C677T e A1298C resulta em menor atividade da enzima quando comparada aos polimorfismos separadamente (VAN DER PUT et al., 1998).

1.6 Coorte de nascidos no ano de 1982 em Pelotas-RS, Brasil

Os estudos de coortes de nascimentos são importantes para que se possam pesquisar processos biológicos, comportamentais e psicossociais que operam ao longo do ciclo vital do indivíduo. Existem grandes estudos de coortes realizados em diferentes países, tais como: ALSPAC (NESS, 2004) e o Millenium Cohort Study (SMITH; JOSHI, 2002), ambos no Reino Unido; The Cebu Study Team, 1991, nas

Filipinas (Underlying and proximate determinants of child health, 1991); The National Children's Study, nos EUA (LANDRIGAN et al., 2006) e "Birth to Twenty", na África do Sul (RICHTER et al., 2007). Estudos do ciclo vital permitem avaliar efeitos a longo prazo de diferentes exposições sobre a saúde ou sobre o risco de doenças durante as várias fases da vida do indivíduo, ou seja, a gestação, a infância, a adolescência, a fase adulta e a velhice.

Todos os 5.914 nascimentos ocorridos na zona urbana da cidade de Pelotas-RS, Brasil, no ano de 1982 foram registrados. As mães foram entrevistadas nas maternidades, sendo coletadas informações de caráter demográfico, biológico, socioeconômico e reprodutivo. Desde então, os participantes das coortes têm sido acompanhados em diferentes momentos de suas vidas. Na etapa de acompanhamento da coorte de 82 realizada em 2004/2005, 4.297 participantes foram entrevistados e 3.831 doaram sangue para obtenção de amostras de soro e DNA (BARROS et al., 2008; NAZMI; OLIVEIRA; VICTORA, 2008; NAZMI et al., 2009).

2 JUSTIFICATIVA

A elevação dos níveis de Hcy, hiper-homocisteinemia, é relacionada com diversas patologias crônicas que atingem um grande número de indivíduos, como as doenças cardiovasculares. Avaliar a contribuição de fatores genéticos e comportamentais para o aumento dos níveis de Hcy é de grande valia para o estabelecimento de orientações de saúde preventivas, que visem diminuir o número de afetados por enfermidades relacionadas ao metabolismo desse aminoácido. O presente estudo é baseado na análise de fatores de risco cardiovascular em indivíduos aos 22 anos de idade, que pertencem à coorte de nascimentos ocorridos em Pelotas-RS, no ano de 1982. O estudo permitirá esclarecimentos sobre interações gene-ambiente e sua influência sobre os níveis de Hcy, com base na herança genética e estilo de vida de uma população de adultos-jovens brasileiros.

3 HIPÓTESE

Os níveis de homocisteína em indivíduos com genótipo *MTHFR* 677TT e 1298CC diferem dos níveis observados em indivíduos com genótipo *MTHFR* 677CC/CT e MTHFR 1298AA/AC frente à exposição ao fumo, ao álcool e a atividade física no lazer.

4 OBJETIVOS

4.1 Geral

- Avaliar a associação dos polimorfismos *MTHFR* C677T e A1298C com fatores de risco para doença crônicas não-transmissíveis em indivíduos da coorte de nascimentos da cidade de Pelotas-RS no ano de 1982.

4.2 Específicos

- Avaliar a prevalência dos polimorfismos *MTHFR* C677T e A1298C em indivíduos da coorte de 1982.

- Avaliar a associação dos polimorfismos *MTHFR* C677T e A1298C com níveis de homocisteína sérica em indivíduos da coorte de 1982.

- Avaliar a associação dos polimorfismos *MTHFR* C677T e A1298C com variáveis biológicas e comportamentais em indivíduos da coorte de 1982.

- Avaliar a interação dos polimorfismos *MTHFR* C677T e A1298C com variáveis biológicas e comportamentais na determinação dos níveis de homocisteína em indivíduos da coorte de 1982.

5 ARTIGO

The effect of *MTHFR* C677T and A1298C polymorphisms on homocysteine levels in individuals from a birth cohort

(Artigo científico escrito sob formato do periódico *Plos One*)

Title

The effect of *MTHFR* C677T and A1298C polymorphisms on homocysteine levels in individuals from a birth cohort

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ABSTRACT

Methylenetetrahydrofolate Reductase (MTHFR) gene polymorphisms are related to low activity of the MTHFR enzyme increasing homocysteine (Hcy) plasma levels. Hyperhomocysteinemia (HHcy) is a risk factor for several pathological processes including atherosclerosis. The aim of the present study was to evaluate the effect of *MTHFR* C677T and A1298C polymorphisms and behavioral factors on Hcy levels in 3831 biological samples from 1982 Pelotas Birth Cohort individuals. The Hcy levels were measured in serum samples using chemiluminescence immunoassay. The genotyping was performed by allelic discrimination technique using pre-designed TaqMan® assays in the ABI7500 Fast Real-Time PCR System. The mean levels of Hcy were higher ($p<0.001$) in homozygous TT variant of *MTHFR* C677T than in CT and CC genotypes independently of sex, alcohol consumption, smoking and physical activity during leisure time. However it was demonstrated a higher *MTHFR* 677TT effect in smokers compared to non-smokers, as well as, in alcohol consumers than in non-consumers and in active individuals than in less active ones (p for interaction <0.001, respectively). For the *MTHFR* A1298C, the Hcy levels were higher in AA genotype than AC and CC genotypes, independently of behavioral factors. Men genotyped as *MTHFR* 1298AA showed 14% increasing on Hcy levels compared to 4% increase observed in women (p for interaction <0.001). No interactions were demonstrated between this polymorphism and the behavioral factors analyzed. In conclusion, in young adult from 1982 cohort it was observed an interaction effect between the *MTHFR* C677T polymorphism and lifestyle on Hcy levels, may contribute to an increased risk for cardiovascular chronic diseases in the future.

Key words: MTHFR, genetic polymorphisms, gene-environment interaction, lifestyle.

INTRODUCTION

The methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in the reduction of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the circulating form of folate, capable of donating a methyl group for conversion of homocysteine (Hcy) to methionine [1]. Hyperhomocysteinemia, which corresponds to a high concentration of plasma Hcy, has been largely associated with numerous disorders such as Neural tube defects, Nonsyndromic oral clefts, Congenital heart defects, Down Syndrome, Alzheimer's disease, Breast cancer, Atherosclerosis and Cardiovascular disorders [2]. Since the mid-eighties, several studies have begun to highlight the relationship of increased plasma levels of Hcy and atherosclerotic diseases, such as myocardial infarction, stroke or peripheral vascular disease [3].

The regulation of plasma Hcy involves nutritional factors, such as the status of folic acid and vitamins B6 and B12 [2], age, sex, behavioral factors [4] as well as genetic factors represented by mutations in the genes of enzymes involved in the metabolism of Hcy [2] [5].

The decrease in the enzymatic activity of MTHFR resulting from genetic alterations has been described as a common cause for elevated levels of Hcy [6]. The *MTHFR* C677T polymorphism (rs1801133), located in exon 4 of the *MTHFR* gene, results in the substitution of alanine for valine (Ala222Val) [7]. This substitution causes reduced activity of MTHFR producing lower levels of 5-methyltetrahydrofolate, and an accumulation of methylene and increased plasma levels of Hcy [8]. Therefore, the *MTHFR* C677T polymorphism is an important genetic determinant of Hcy concentration in plasma [9]. The presence of homozygous *MTHFR* 677T allele renders to enzyme thermolabile [10] and is associated with elevated Hcy concentrations and low serum folate levels [8].

A second polymorphism, the *MTHFR* A1298C (rs1801131), located in exon 7 of the same gene, is an exchange of glutamine amino acid for alanine (Glu429Ala). This polymorphism is also associated with decreased enzyme capacity, but with less effect compared to *MTHFR* C677T polymorphism [10].

The aim of this study was to evaluate the association between *MTHFR* C677T and A1298C with serum levels of Hcy in individuals from 1982 Pelotas Birth Cohort, as well as to study possible interactions between genetic polymorphisms and behavioral factors.

METHODS

Study design

Cross-sectional study based on a sample of individuals from a birth cohort study.

Study population

All births in the urban area of Pelotas, a city of Southern Brazil, in 1982 were recorded and mothers were interviewed in maternity wards to obtain demographic, biological, socioeconomic, and reproductive information. Since birth, the cohort participants have been monitored at different times in their lives. The cohort methods are better detailed elsewhere [11]. In the 2004/2005 follow-up, 4297 people were interviewed, and blood samples were collected from 3831 individuals to obtain serum and DNA samples [11] [13]. The study was approved by the Ethics Committee of the Medical Faculty of Federal University of Pelotas. Written informed consent was obtained from all individuals prior to the interview and to all procedures.

Biological and behavioral variables

Hcy levels of 3821 participants from the 82 cohort were determined by chemiluminescence assay [14] using the Immulite® System (Siemens). Due to insufficient serum samples it was not possible to have Hcy level measured from 5 of 3826 serum samples included in this study.

The biological and behavioral variables used in the present study were as follows: sex [male, female]; smoking [smokers were considered those individuals who reported smoking at least one cigarette every day in the last week]; alcohol consumption [reported by the number of drinks consumed per day: no dose (0 g/d), one dose (0.01 to 14.9 g/d), two doses (15.0 to 29.9 g/d) or more than two doses (≥ 30.0 g/d)]; and physical activity [leisure time physical activity assessed by long version of the International Physical Activity Questionnaire, and calculated by adding the time reported the practice of walking and moderate physical activity and multiplied by two in vigorous activities; were considered less active individuals who had a sum less than 150 minutes of physical activity per week and active ≥150 min/w [15].

All variables described above were obtained from the database of the 82 Cohort.

Genotyping

From 1982 Cohort DNA Bank, 3831 genomic DNA samples were obtained. DNA was extracted from leukocytes of peripheral whole blood by the method of salting-out based on modified Miller's protocol [16]. Genotyping for *MTHFR* C677T (rs1801133) and *MTHFR* A1298C (rs1801131) polymorphisms was performed on 7500Fast Real-Time PCR System (Applied Biosystems, Life Technologies, EUA), using TaqMan pre-designed SNP Genotyping Assays, "C_12028833_20" and "C_850486_20", respectively (Applied Biosystems-Life Technologies, EUA). The reactions were performed in a total volume of 6 µL, as follows: 3 µL of Taqman ® PCR Master Mix (Applied Biosystems, EUA), 0.3 µL of assay mix (Applied Biosystems, EUA), 2.2 µL of DNase/RNase free water (Life Technologies, EUA) and 0.5 µL of DNA [20ng]. The standard reaction conditions were an initial denaturation step at 95°C for 10 minutes followed by 40 cycles of denaturation at 94°C for 15 seconds and annealing and extension at 60°C for 1 minute each. The genotyping repeatability was evaluated from 5% of randomly selected DNA samples, and we observed >99.9% of genotyping concordance for both polymorphisms.

Statistical Analysis

Statistical analyses were performed using Stata (Stata Corporation, College Station, USA), version 12.0. The chi-square ($\chi 2$) was used in the analysis of Hardy-Weinberg Equilibrium (HWE), as well as in the crude analysis for the distribution of *MTHFR* C677T and A1298C genotypes by sex and behavioral variables. The mean levels of Hcy according to the independent variables was analyzed by linear regression and calculation of p-value for interaction. The significance level used in the study was $p<0.05$.

RESULTS

From 3831 DNA samples, it was possible to amplify and genotype 3814 samples for *MTHFR* C677T and 3821 for *MTHFR* A1298C polymorphisms. The

losses of samples in the genotyping process probably occurred by DNA degradation. The observed allele frequency for the *MTHFR* C677T (n=3814) was 0.69 for C allele and 0.31 for the T allele, while the genotype frequencies were 48.0% (n=1831) for the CC genotype, 42.8% (n=1632) for CT genotype and 9.2% (n=351) for the TT genotype. For *MTHFR* A1298C (n=3821), the allele frequency was 0.73 for the A allele and 0.27 for C allele, with the genotype frequency of 54.1% (n=2067) for AA genotype, 38.5% (n=1473) for the AC genotype and 7.4% (n=281) for the CC genotype. In the studied population, all the genotypic distributions were in Hardy-Weinberg Equilibrium: *MTHFR* C677T, χ^2= 0.215 p= 0.643; *MTHFR* A1298C, χ^2= 0.692, p=0.405.

Table 1 shows the mean values of Hcy levels according to the biological, behavioral and genetic variables. As can be observed, men showed higher mean levels of Hcy (p<0.001). In the same way, smokers exhibited higher levels of Hcy when compared to nonsmokers (p<0.001). The Hcy levels increase as the number of alcohol doses consumed by the participant increase (p<0.001); less active individuals showed lower levels of Hcy when compared with active individuals (p<0.001). In relation to the *MTHFR* C677T polymorphism, Hcy levels was associated with the presence of the mutant allele (p<0.001). On the other hand, for the *MTHFR* A1298C, the mutant allele appears to confer a protective effect because a decrease on Hcy levels in the presence of the *MTHFR* 1298C allele was observed.

There was no difference in the genotype frequencies for *MTHFR* C677T and A1298C polymorphisms regarding the variables sex, smoking, alcohol consumption and physical activity (Table 2).

In the analysis according to the *MTHFR* C677T polymorphism, individuals genotyped as *MTHFR* 677TT presented higher Hcy levels than individuals *MTHFR* 677CT and 677CC (p<0.001), and this increase was about 40% in men and 17% in women (p for interaction <0.001). Similarly, the *MTHFR* 677TT genotype was associated with higher levels of Hcy compared to *MTHFR* 677CC and 677CT genotypes, independently of the individual lifestyle. However, the effect of genotype is higher in *MTHFR* 677TT smokers than non-smokers (p for interaction <0.001); in *MTHFR* 677TT individuals which have declared alcohol consuming in comparison with non-users (P for interaction <0.001); and in *MTHFR* 677TT active subjects compared to less active ones (p for interaction <0.001) (Table 3).

Regarding the MTHFR A1298C, the Hcy levels were higher in individuals homozygous for the A allele (AA) than in subjects AC and CC, in both sexes. The presence of MTHFR 1298AA genotype in men determined an increase of 14% in Hcy levels, while in women this increase was about 4.0% (p for interaction <0.001). This difference between genotypes was also observed in the analysis of behavioral variables without, however, to be identified an interaction between genetic polymorphism and each of them (smoking, alcohol consumption and physical activity). *MTHFR* 1298AA individual who were smokers showed Hcy levels 12.3% higher than *MTHFR* 1298CC smokers, whereas among the nonsmokers this increase was 8.9%. With regard to alcohol consumption, the effect of genotype on Hcy levels varied according to the dose of alcohol consumed, and it was not observed among non-users (p=0.178), as well as, among those who have declared an intake of more than two doses (15.9 to 29.9 g/d) (p=0.245). On the other hand in *MTHFR* 1298AA individuals who have declared consuming more than 30 g/d of alcohol, the Hcy levels were about 17.85% higher than in subjects *MTHFR* 1298CC. Among those *MTHFR* 1298AA who have declared a low consumption (0.01 to 14.9 g/d) it was observed an increase about 10.25% in relation to *MTHFR* 1298CC individuals (p<0.001). In respect to physical activity, *MTHFR* 1298AA active individuals had 13.5% higher levels of Hcy than individuals with *MTHFR* 1298CC at the same physical activity group (p<0.001). Among the less active individuals, the difference between MTHFR 1298AA and 1298CC individuals was of 6.4% (p=0.006).

DISCUSSION

It is well known that *MTHFR* polymorphisms determine lower activity of MTHFR enzyme causing Hcy plasma level increase which is associated with long-term cardiovascular outcomes [17]. However, Hcy levels are not only influenced by genetic variants [4] but other important determinants such as sex, age and behavioral factors have been reported in several studies [4] [18] [19]. Some of these factors may be different among populations reinforcing the importance of this analysis.

This study was the first to evaluate the association between MTHFR C677T and A1298C with Hcy levels and possible interactions of polymorphisms with behavioral factors influencing these levels in a large sample of young individuals.

These young people are from 1982 Pelotas birth cohort, which is the longest birth cohort with regular monitoring in developing countries.

According to the HapMap SNP database the MTHFR 677C allele frequency (ss68758613) is most common among Yorubas (a West African ethnic group) than in Caucasians, 0.89 *versus* 0.76, respectively. In respect to *MTHFR* 1298A allele (ss68758611), the frequency observed is 0.89 in Yorubas versus 0.64 in Caucasians. In our target population, the prevalence of the C allele of the MTHFR C677T polymorphism was 0.69 while the A allele prevalence of the MTHFR A1298C polymorphism was 0.73. The literature describing these polymorphisms in Brazilian population is scarce but we found similar frequencies in a study based on 405 Brazilian pregnant women [20]. The differences in frequencies found in our study related to HapMap frequencies need take in account that the Brazilian population was formed by an extensive admixture from three different ancestral roots: Amerindians, Europeans, and Africans [21].

The Hcy levels were found to be higher in men compared to women as described in different studies [4] [22] [23] [24]. This association between sex and Hcy could be related to a muscle mass since creatine/creatinine synthesis is connected with Hcy metabolism [25]. On the other hand, it has been highlighted the role of estrogen since it was found that pregnant, premenopausal, postmenopausal women on estrogen replacement therapy or women treated with partial estrogen agonist (Tamoxifen) show lower levels of Hcy in comparison with menopausal women [26]. This estrogen-homocysteine interaction is based on the mechanism whereby estrogen modulates methionine metabolism by increasing the cystathionine-β synthase activity to forming cysteine and glutathione. Consequently, by interfering in transsulfuration pathway and by promoting glutathione enhancement, estrogen decreases Hcy levels and stabilizes NO, yielding beneficial effects on the vasculature [27].

The main results of this study show interaction effects between *MTHFR* C677T and behavioral factors on Hcy levels from individuals of 22 years old. This is a quite young population compared to those described in the majority of the studies [28] [29] [30] [31]. Although we have found Hcy levels inside the normal rate, we were able to demonstrate an increasing in Hcy levels when an unhealthy lifestyle is adopted, especially by *MTHFR* 677TT subjects.

According to smoking, our results confirm others which describe higher levels of Hcy in smokers compared to non-smokers [18] [19] [33] [32]. Furthermore, the interaction among *MTHFR* 677TT variant and smoking demonstrated in our young population has also been found in other studies [29] [31] .

It has been discussed that the effects of smoking could be biased by concomitant dietary factors. It is known that smokers eat less fruits and vegetables having, as a consequence, a lower intake of folate and vitamin B6. In a metabolic point of view, smoking is related to folate through several mechanisms which smoking might reduce the availability of folate for the remethylation of Hcy to metionine, or might change plasma thiol redox status or might, yet, inhibit enzymes involved in the Hcy metabolism [34]. However, there are controversial results showing that the effects of smoking could remain after correction for folate intake [35]) or they could disappear [26]. This controversy could be due to differences between studies related to folic acid and Hcy baseline levels [36]. On the other hand, it was described that the *MTHFR* 677TT variant of *MTHFR* is associated with 50% increasing of Hcy level when folate intake is suboptimal [34] [37]. In a *MTHFR* C677T mutation model made in *Escherichia coli* was observed that an optimal folate supply prevents the loss of FAD (a cofactor form of vitamin B2) suppressing enzyme inactivation [38]. Therefore the interaction between *MTHFR* C677T polymorphism and smoking on Hcy plasma levels probably involve folate status. A limitation of this study is the lack of data on the folate status of our target population because it could help us to clarify this interaction between smoking and MTHFR C677T variant in determining levels of Hcy.

A positive linear association was found between alcohol consumption and Hcy levels as already published [33] [39]. However, earlier studies have not find this association [40] [41] or have described a J-shaped association [42] [43]. In fact, this association seems not to be only dependent of dose but also dependent of alcoholic beverage type. A moderate dose of beer or red wine may lead to health-promoting effects, probably because the first beverage increases B6 vitamin, whilst decreases B12 vitamin, having no effect on folate levels [44], while the second one contains antioxidants that prevent increase of Hcy [35]. It is well known that alcoholism is related to malnutrition and low levels of several vitamins that participate in the Hcy metabolism [28]. Several mechanisms are proposed to explain the increase of Hcy levels associated to alcohol consumption. An earlier publication has demonstrated

that acetaldehyde generated from ethanol metabolism may increase folate catabolism due to production of superoxide [45]. However, alcohol may interfere with folate metabolism through different mechanisms such as: by inhibiting methionine synthase enzyme which is responsible for the transfer of a methyl group from 5-MTHF to Hcy and preventing remethylation; by promoting ethanol–induced B-vitamin depletion, which is a dietary determinant of Hcy plasma levels and by reducing intestinal absorption of folate [31]. Related to interaction analysis between alcohol consumption and *MTHFR* C677T polymorphism, our findings are also corroborated by other studies [28] [30], suggesting a higher effect when both conditions are present.

We have found more intense physical activity associated with higher levels of Hcy. This finding could be explained by the mean age of our target population which is in a life period of higher muscle mass. Creatine is an important compound of muscular energetic metabolism and is considered a joining factor between physical activity and Hcy. The creatine synthesis consumes about 75% of physiologically labile methyl-groups and contributes to accumulation of reduced Hcy (rHcy). In presence of continuous exercise the rHcy will be accumulated and will determine repercussions on the total fraction level of Hcy [46]. However this finding contradicts an expected association where physical activity is related to a healthy lifestyle with proper eating habits [47]. Reviewing the literature, it is possible to find controversial results related to heterogeneity in the experimental design of physical activity and, also, in the confounding variables control [48]. Some studies reported beneficial effects on Hcy levels in response to exercise [19] [49] but in others the exercise does not reduce Hcy levels [31] [50]. Mennen and colleagues [4] suggested that differences in the categorization of the physical activity variable may be an explanation for the contradictory results found in the association studies. Bree and colleagues [35] suggested an intervention study to elucidate such contradictory results. On the other hand, no interactive effect between MTHFR C677T genotype and physical activity was related by Husemoen and colleagues [29]. A study including other genetic variants of Hcy metabolism genes showed an interaction between MTR 2756A and physical activity, but this response was dependent of physical activity score [31]. Therefore, the genetic effect of interaction with physical activity on Hcy levels remains to be better determined.

Regarding the *MTHFR* A1298C polymorphism, the mutate allele *MTHFR* 1298C seems to have a protector effect, unlike what was observed in other studies where the mutant allele is that confers increased levels of Hcy [51] [52]. A similar result compared to ours was found in a case control study from Italy, where patients with atrial fibrilation and control subjects genotyped as *MTHFR* 1298AA showed higher Hcy levels than *MTHFR* 1298AC e 1298CC. The authors have suggested a linkage disequilibrium between *MTHFR* C677T and *MTHFR* A1298C polymorphisms to explain this result [53]. Furthermore, we have found an effect of MTHFR A1298C polymorphism on Hcy levels in men but not in women and this association was found to be independent of smoking, alcohol consumption and physical activity. On the other hand, it is important to mention that several studies did not find any association between the *MTHFR* A1298C and Hcy levels [10] [54] [55] [56]. In fact, the *MTHFR* A1298C has a small effect on *MTHFR* enzyme activity compared to *MTHFR* C677T, and its influence on Hcy levels is still unclear [51].

In conclusion, the *MTHFR* C677T polymorphism seems to be a stronger determinant on Hcy levels than the *MTHFR* A1298C polymorphism. Interactions effects of *MTHFR* C677T polymorphism and behavioral factors on Hcy levels were demonstrated in young adults from a Brazilian cohort study. Considering that lifestyle could be modified, these determinants could be used as good targets for medical advices in order to avoid chronic disease related to Hcy in the future.

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TABLES

Table 1. Mean Hcy according to sex, behavioral variables and genotypes *MTHFR* C677T and A1298C in the 1982 cohort, Pelotas, RS, Brazil.

Variables	n	Mean (µmol/L)	IC	*p value
Sex				<0.001
Male	1916	9.5	[9.4- 9.6]	
Female	1905	7.4	[7.2- 7.5]	
Smoking				<0.001
No	2833	8.3	[8.1- 8.4]	
Yes	988	8.9	[8.7- 9.1]	
Alcohol consumption				<0.001
0 g/d	1244	8.0	[7.9- 8.2]	
0.01-14.9 g/d	1926	8.5	[8.4- 8.6]	
15.9-29.9 g/d	346	8.9	[8.7– 9.1]	
≥30.0 g/d	305	9.3	[9.1– 9.6]	
Physical activity				<0.001
Active	1337	8.9	[8.8- 9.1]	
Less active	2484	8.2	[8.0- 8.3]	
MTHFR C677T				<0.001
CC	1827	7.7	[7.6- 7.9]	
CT	1628	8.9	[8.8- 9.0]	
TT	349	10	[9.8- 10.3]	
MTHFR A1298C				<0.001
AA	2061	8.6	[8.5- 8.8]	
AC	1469	8.3	[8.1- 8.4]	
CC	281	7.9	[7.6- 8.2]	

* Linear regression

Table 2. Distribution of *MTHFR* C677T and A1298C according to gender and behavioral variables in the 1982 cohort, Pelotas, RS, Brazil.

	MTHFR C677T, n(%)				MTHFR A1298C, n(%)			
Variables	**CC**	**CT**	**TT**	***p value**	**AA**	**AC**	**CC**	***p value**
Sex				0.14				0.43
Male	949 (49.6)	794 (41.5)	170 (8.9)		1018 (53.1)	758 (39.6)	140 (7.3)	
Female	882 (46.9)	838 (44.1)	181 (9.5)		1049 (55.1)	715 (37.5)	141 (7.4)	
Smoking				0.29				0.51
No	1342 (47.4)	1232 (43.5)	256 (9.1)		1518 (53.6)	1103 (38.9)	213 (7.5)	
Yes	489 (49.7)	400 (40.7)	95 (9.7)		549 (55.6)	370 (37.4)	68 (6.9)	
Alcohol consumption				0.72				0.95
0 g/d	616 (49.6)	507 (40.8)	118 (9.5)		678 (54.4)	471 (37.8)	97 (7.8)	
0.01-14.9 g/d	906 (47.1)	842 (43.8)	176 (9.1)		1029 (53.5)	755 (39.2)	140 (7.3)	
15.9-29.9 g/d	165 (48.1)	151 (44.0)	27 (7.9)		193 (55.9)	128 (37.1)	24 (7.0)	
≥30.0 g/d	144 (47.1)	132 (43.1)	30 (9.8)		167 (54.6)	119 (38.9)	20 (6.5)	
Physical activity				0.20				0.09
Active	667 (49.9)	556 (41.6)	114 (8.5)		699 (52.2)	547 (40.9)	92 (6.8)	
Less active	1164 (47.0)	1076 (43.4)	237 (9.6)		1368 (55.1)	926 (37.3)	189 (7.6)	

* Chi-square ($\chi 2$)

Table 3 - Mean Hcy according to *MTHFR* C677T and A1298C, gender and behavioral variables in the 1982 cohort, Pelotas, RS, Brazil

	Hcy (µmol/L)									
	***MTHFR* C677T**					***MTHFR* A1298C**				
Variables	**CC**	**CT**	**TT**	***p value**	****p for interaction**	**AA**	**AC**	**CC**	***p value**	****p for interaction**
Total population	7.7 (7.6, 7.9)	8.9 (8.8, 9.0)	10.0 (9.8, 10.3)	<0.001		8.6 (8.5, 8.8)	8.3 (8.1, 8.4)	7.9 (7.6, 8.2)	<0.001	
Sex										
Male	8.4 (8.2, 8.7)	10.2 (10.0, 10.4)	12.0 (11.6, 12.4)	<0.001	<0.001	9.8 (9.6, 10.1)	9.2 (9.0, 9.4)	8.6 (8.1, 9.0)	<0.001	0.004
Female	7.0 (6.9, 7.2)	7.6 (7.5, 7.7)	8.2 (8.0, 8.4)	<0.001		7.4 (7.3, 7.6)	7.3 (7.2, 7.4)	7.1 (6.9, 7.4)	0.050	
Smoking										
No	7.7 (7.5, 7.9)	8.6 (8.5, 8.8)	9.6 (9.3, 9.9)	<0.001	<0.001	8.5 (8.3, 8.6)	8.1 (8.0, 8.3)	7.8 (7.5, 8.1)	<0.001	0.368
Yes	7.9 (7.6, 8.2)	9.6 (9.3, 9.8)	11.3 (10.8, 11.8)	<0.001		9.1 (8.9, 9.4)	8.6 (8.4, 8.9)	8.1 (7.6, 8.7)	0.007	
Alcohol consumption										
0 g/d	7.8 (7.5, 8.0)	8.4 (8.2, 8.6)	9.0 (8.6, 9.4)	<0.001	<0.001	8.2 (8.0, 8.5)	8.0 (7.8, 8.3)	7.9 (7.4, 8.3)	0.178	0.083
0.01-14.9 g/d	7.6 (7.4, 7.8)	8.9 (8.7, 9.0)	10.1 (9.9, 10.4)	<0.001		8.6 (8.4, 8.8)	8.2 (8.0, 8.4)	7.8 (7.4, 8.1)	<0.001	
15.9-29.9 g/d	7.7 (7.1, 8.3)	9.7 (9.2, 10.1)	11.6 (10.7, 11.6)	<0.001		9.1 (8.5, 9.6)	8.7 (8.2, 9.2)	8.3 (7.2, 9.4)	0.245	
≥30.0 g/d	8.5 (8.0, 9.0)	10.2 (9.7, 10.6)	11.9 (11.0, 12.8)	<0.001		9.9 (9.4, 10.5)	9.2 (8.7, 9.7)	8.4 (7.3, 9.5)	0.023	
Physical activity										
Active	8.1 (7.9, 8.3)	9.5 (9.3, 9.7)	11.0 (10.5, 11.4)	<0.001	0.016	9.2 (9.0, 9.5)	8.7 (8.4, 8.9)	8.1 (7.6, 8.6)	<0.001	0.090
Less active	7.5 (7.4, 7.7)	8.6 (8.4, 8.7)	9.6 (9.3, 9.9)	<0.001		8.3 (8.2, 8.5)	8.0 (7.9, 8.2)	7.8 (7.4, 8.1)	0.006	

* Linear regression

** Interaction test

6 CONCLUSÕES

- O polimorfismo *MTHFR* C677T está associado com os níveis de Hcy, na coorte de nascidos em 1982; indivíduos com genótipo TT apresentaram maiores níveis de Hcy, quando comparados aos de genótipos CC e CT.

- O polimorfismo *MTHFR* A1298C está associado com os níveis de Hcy, na coorte de nascidos em 1982, e indivíduos com genótipo AA, apresentaram maiores níveis, quando comparados aos de genótipos AC e CC.

- As variáveis sexo, fumo, consumo de álcool e atividade física estão associadas com os níveis de Hcy em indivíduos da coorte de nascidos em 1982; homens apresentaram maiores níveis de Hcy em relação às mulheres; indivíduos fumantes exibiram níveis maiores de Hcy quando comparados aos não fumantes; os níveis de Hcy são maiores conforme o aumento do número de doses de álcool consumidas; e em relação a atividade física, indivíduos menos ativos apresentaram níveis de Hcy menores quando comparados aos com comportamento ativo.

- O estudo apresentou um efeito interativo do polimorfismo *MTHFR* C677T com sexo, fumo, consumo de álcool e sedentarismo, e do polimorfismo *MTHFR* A1298C com a variável sexo na coorte de nascidos em 1982, reforçando a atuação conjunta de fatores genéticos e fatores comportamentais na determinação de níveis de Hcy.

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