The Egyptian Journal of Medical Human Genetics xxx (2017) xxx–xxx

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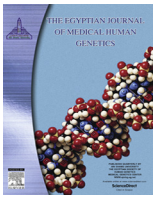
Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com



Original article

Common variant of 5,10-methylenetetrahydrofolate reductase may increase risk of coronary artery disease in the Iranian population

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ARTICLE INFO

Article history:
Received 7 November 2017
Accepted 28 November 2017
Available online xxxx

Keywords:
Coronary artery disease (CAD)
Methylene tetra hydrofolate reductase (MTHFR)
Polymorphism

ABSTRACT

Background: Coronary artery disease (CAD) is the most prevalent form of cardiovascular disease that is caused by the formation of plaque in the arteries walls. Both genetic and environmental factors play an important role in the development of CAD.
Aim: The aim of this study was to determine the association of 5,10-methylenetetrahydrofolate reductase (*MTHFR*) C677T gene polymorphism with CAD in an Iranian population.
Subjects and methods: In this case/control study, sequence specific primer-polymerase chain reaction (SSP-PCR) method was used for genotyping of 310 patients with CAD and 367 healthy controls.
Results: Frequency of C/T genotype was significantly higher in the patients group than the control group *(P = .03, OR: 1.6, 95% CI: 1.04–2.47).* Based on the assumption that T is a risk allele, dominant model compares C/C genotypes to C/T + T/T genotypes. A significant association was observed in *MTHFR* C677T when the effect of the polymorphism was considered under a dominant genetic model *(OR = 1.59; 95% CI = 1.03–2.46; P = .02).* Evaluating genotype frequencies in 4 different ethnic groups (Fars, Turkmen, Sistani, and others) demonstrated significant statistical association of C/T genotype in Fars sub-groups *(OR = 1.8; 95% CI = 1.11–3.06; P = .01)* but this association is not observed in other populations. Significant association of C/T *(P = .01, OR: 2.21, 95% CI: 1.15–4.4)* genotype was found in women, but this association was not observed in men.
Conclusion: The results of this study showed that C/T genotype in *MTHFR* C677T position is a causative factor, especially in women, and might be associated with susceptibility to CAD in the Iranian population.

1. Introduction

Coronary artery disease (CAD) is caused by atherosclerotic plaque build-up in the wall of coronary arteries and with decreased blood flow [1]. CAD is the most common type of cardiovascular disease and the most important cause of death globally [2].

Previous studies have shown that both genetic and environmental factors affect the incidence of this disease [3]. Factors such as age, smoking, high blood pressure, family history, diabetes, lack of exercise, obesity, stress, high blood cholesterol, poor diet and excessive alcohol increase the risk of developing CAD [4,5]. Genetic factors play a major role in CAD progression in younger people [6].

5,10-Methylenetetrahydrofolate reductase (*MTHFR*) gene is located on the short (p) arm of chromosome 1 at position 36.3 (1p36.3). *MTHFR* reduces 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the carbon donor for remethylation of homocysteine to methionine [7].

Wilcken et al. reported that CAD patients have higher concentrations of homocysteine than healthy individuals [8]. In subsequent studies, Hyperhomocysteinaemia has been recognized as a risk factor for coronary heart disease [9]. Previous investigations reported reduced *MTHFR* activity with a thermo-unstable enzyme in patients with CAD [10].

Goyette et al. found nine polymorphisms in the *MTHFR* gene via single-strand conformation polymorphism and direct sequencing of PCR fragments [11]. Using the same procedures, Frosst et al. reported that C to T substitution at nucleotide 677 leads to alanine to valine amino acid change at the 222nd position of *MTHFR* protein (Ala222Val). In most populations, the C677T polymorphism (rs1801133) in *MTHFR* is an even more common polymorphism with an allele frequency of 30–40% [7,12].

Some studies reported an association between C677T polymorphism and risk of cardiovascular disease, while others did not find such association [13].

Peer review under responsibility of Ain Shams University.
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https://doi.org/10.1016/j.ejmhg.2017.11.004
1110-8630/

This study aimed to determine and compare the prevalence of *MTHFR* (C677T) polymorphism in patients with CAD and healthy individuals to provide baseline epidemiological data for future clinical investigations of CAD and other diseases in Iran.

Iran is the 17th most populous nation in the world and has central location in Eurasia and Western Asia [14]. Different ethnicities live in Iran including the Persians (the largest groups) Turkic groups (include the Turkmen and the Qashqai peoples) Kurds, Lurs, Sistanis, Balochis, Arabs and others. Because of the ethnic diversity, evaluating genetic variation in Iran is valuable and it may be used in future studies.

2. Subjects and methods

2.1. Patients

In this case-control study, the frequency of *MTHFR* C677T gene polymorphism was evaluated in 310 patients with CAD and 367 healthy controls. The mean age of subjects in the case and control group was 59.25 ± 10.54 and 51.94 ± 10.88 years respectively. The percentages of men and female was 60.32% and 39.68% in the cases and 35.62% and 64.38% in the controls respectively. Control subjects were matched with patients by age, ethnicity, and geographical area.

CAD patients were diagnosed by angiography test and approved by a cardiologist, from March 2013 to March 2014, at Kowsar Heart Center in Kordkuy, Iran. Inclusion criteria for patients group was coronary stenosis of 70% and more in at least one major coronary artery. Patients with coronary stenosis below 70% were excluded from the study. None of the patients had major autoimmune or hematologic disease.

Individuals with normal angiography at rest, without symptoms of myocardial ischemia during exercise were selected as controls.

All of subjects (including case and control) were divided into four main ethnic groups of Fars, Turkmen, Sistani and others.

In agreement with the Helsinki Declaration, all participants signed the informed consent and the Ethics Committee of Golestan University of Medical Sciences approved this study. The sample size was determined by the Quanto software V-1.2 with a statistical power of 80%.

2.2. DNA extraction and genotyping

Five ml peripheral whole blood was used for extracting genomic DNA by a standard protocol [15].

C677T gene polymorphism of *MTHFR* was detected based on the specific sequence primer polymerase chain reaction (SSP-PCR) method. Human growth hormone (*HGH*) gene was used as an internal control for false-negative reactions. The polymorphism was identified by the sequence-specific forward primers 5-GAGAAGGTGTCTGCGGGAGC-3′ and 5-GAGAAGGTGTCTGCGGGAGT-3′ in combination with the consensus reverse primer 5-CTCAATCACGTCCTTGATCTC-3′ [16]. The following control primer pairs that amplify a conserved region of the HGH were used: HGH-F: 5′-GCCTTCCCAACCATTCCCTTA-3′ and HGH-R: 5′-TCACGGATTTCTGTTGTGTTTC-3′.

SSP-PCR amplification was done using a 15 ml reaction mixture containing one ml of genomic DNA, 0.9 ml of 25 mm MgCl2 (Qiagen, USA), 1.5 ml of each 10X reaction buffer, 1.5 ml of 10 mM dNTP, 2.2 ml of sucrose 60%, 0.5 VL-for-specific primers (10 pM), 0.5 ml of 10 pM HGH primers, and 0.2 of Taq polymerase (Qiagen, USA).

PCR was carried out by a thermal cycler (Techne, UK), using the following program: 1 min at 95 °C followed by 10 cycles of 15 s at 94 °C, 15 s at 65 °C, 40 s at 72 °C, followed by 20 cycles of 10 s at 94 °C, 50 s at 59 °C and 40 s at 72 °C, with 5 min at 72 °C as final extension.

The PCR products were electrophoresed on 1.5% agarose gel (Merck, Germany) stained with ethidium bromide. Photographs were taken using a gel documentation system (UVITEC, UK) and DNA bands were visualized under the UV radiation.

2.3. Statistical analysis

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS, version 17.0, IBM, USA) and the mean of parametric variables was calculated. Allele and genotype frequencies were calculated and compared between groups using non-parametric tests, followed by exact Fisher's analysis by STATA V-8 (California, USA). The risk associated with genotypes/alleles was calculated as the odds ratio (OR) with 95% confidence interval (CI). P-value less than .05 were considered as statistically significant.

3. Results

The allelic and genotypic distributions of the *MTHFR* gene C677T in healthy controls and patients with CAD under co-dominant, dominant and recessive models are shown in Table 1.

Frequency distributions of genotypes C/C, C/T and T/T in the control subjects were about 20.4%, 72.8% and 6.8%, respectively.

Table 1
Frequency of the *MTHFR* C677T allele and genotypes among patients (N = 310) and controls (N = 367) under Co-dominant, Dominant and Recessive model.

Alleles and Genotype	Controls	CAD patients	OR (95% CI)*	P-value
C/C	75 (20.4 %)	43 (13.9 %)	1 (–)**	–
C/T	267 (72.8%)	244 (78.7 %)	1.6 (1.04–2.47)	**.03**
T/T	25 (6.8%)	23 (7.4 %)	1.6 (0.77–3.34)	NS* (.2)
C	417 (57%)	330 (53%)	1 (–)	–
T	317 (43%)	290 (47%)	1.16 (0.93–1.44)	NS (.2)
Model of Inheritance				
Dominant				
C/C	75 (20.4 %)	43 (13.9 %)	1 (–)	–
C/T + T/T	292 (79.6%)	267 (86.1%)	1.59 (1.03–2.46)	**.02**
Recessive				
C/T + C/C	342 (93.2%)	287 (92.6%)	1 (–)	–
T/T	25 (6.8%)	23 (7.4 %)	1.1 (0.58–2.06)	NS (.8)
Co-dominant				
C/C + T/T	100 (27.2 %)	66 (21.3)	0.72 (0.5–1.0)	NS (.07)
C/T	267 (72.8%)	244 (78.7 %)	1 (1)	–

*OR: Odds Ratio, CI: Confidence Interval, NS: Not Significant.
**The frequency of CC genotype was more in the control group and was selected as reference group.

Please cite this article in press as: Attar M et al. Common variant of 5,10-methylenetetrahydrofolate reductase may increase risk of coronary artery disease in the Iranian population. Egypt J Med Hum Genet (2017), https://doi.org/10.1016/j.ejmhg.2017.11.004