The European Research Journal
http://www.eurj.org
Original Article
e-ISSN: 2149-3189
DOI: 10.18621/eurj.286198

Incidence of the genetic mutations in patients with coronary artery disease

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ABSTRACT

Objectives. Coronary artery disease (CAD) is the leading cause of mortality in the world. It is a complex disorder resulting from the interaction between environmental risk factors and hereditary predisposition. The role of the factor V Leiden (FVL), protrombin gene (PT G20210A) and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms in the development of CAD is controversial. In this study, we investigated the incidence of these polymorphisms in order to delineate their roles in the development of CAD in a tertiary University hospital. ***Methods.*** This study included 58 consecutive CAD patients. Diabetic and hypertensive patients were excluded. FVL, PT G20210A, and MTHFR (C677T, A1298C) mutations were investigated in all patients. Polymerase chain reaction and the amplification refractory mutation system were used to identify these polymorphisms. ***Results.*** Thirty-six men and 22 women were enrolled with an age ranging between 41 to 85 (mean age: 62.75±9.18 years). The heterozygous PT G20210A genotype was identified in 5 (8.6%) patients (2 males, 3 females). The heterozygous FVL genotype was found in 8 (13.8%) patients (6 males and 2 females). The incidence of homozygous MTHFR C677T and homozygous MTHFR A1298 carriers was found to be 17.2% and 8.6%, respectively. There were no significant differences in the distribution of polymorphisms according to gender ($p>0.05$). ***Conclusions.*** The FVL and PT G20210A polymorphisms most likely play a contributory role in the development of CAD. In contrast, the MTHFR C677T and MTHFR A1298C genotypes were not associated with a predisposition to the development of CAD. However, in compound MTHFR C677T/A1298C carriers, the presence of FVL or PT G20210 polymorphism may contribute the development of CAD. Further studies are needed to support these findings.

Eur Res J 2017;3(2):145-151

Keywords: Methylenetetrahydrofolate reductase, polymorphism, genetic mutation, atherosclerosis, coronary artery disease

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Received: January 17, 2016; Accepted: March 14, 2017; Published Online: March 22, 2017

Introduction

Coronary artery disease (CAD) is the leading cause of mortality in the world. CAD is a complex disorder resulting from the interaction between environmental risk factors and hereditary predisposition [1]. In addition to well-known conventional risk factors, increased evidences support that coagulation may be involved in the pathogenesis of atherosclerosis [2]. Myocardial infarction (MI) usually develops as a result of thrombotic occlusion of the coronary artery, which is triggered by the rupturing of atherosclerotic plaque followed by clotting processes [3]. However, MI can develop in patients with normal coronary arteries due to defects of the clotting system [4]. Studies focusing of the prevalence of MI in families suggest a possible genetic basis for the development and progression of CAD [1]. The molecular mechanisms causing coronary artery disease has not been fully elucidated [2].

The factor V Leiden (FVL) and protrombin gene (PT G20210A) polymorphisms have been known as the two most common thrombophilic risk factors leading to venous thrombosis but their roles in the development of CAD remain controversial [5].

FVL is a missense mutation in the factor V gene, which result in the replacement of arginine at amino acid position 506 by glutamine [1]. This polymorphism is known as the most common genetic thrombophilic risk factor of the coagulation system and leads to a reduced effect of activated protein C (APC) known as APC resistance [3]. The FVL polymorphism may also be an important inherited risk factor for the development of thrombotic complications leading to MI and/or digital ischemia [6].

In 1996, a variant of prothrombin gene (PT G20201A) was discovered and associated with increase synthesis and secretion of prothrombin [7]. This mutant allele has a guanine to adenine substitution at the nucleotide 20210 locus in the 3'-untranslated region of the prothrombin gene [4].

Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, a thymidine-cytosine change at nucleotide position 677, especially in the homozygote carriers, leads to decreased enzymatic activity and mild hyperhomocysteinemia. The MTHFR A1298C polymorphism presents a less well-defined effect, with a lesser decrease of the enzyme function [8]. The role of the MTHFR polymorphism in the development of CAD is also controversial [5].

In this study, we investigated if the incidence of the FVL, PT G20210A and MTHFR C677T polymorphisms plays a role in the development of CAD.

Methods

This study was conducted in the Bozok University Hospital, in Yozgat, Turkey between April 2015 and October 2016. An informed consent was obtained from all the study participants. The study was approved by the ethic committee of Bozok University and conducted in accordance with the principles of the Declaration of Helsinki.

This study included 58 consecutive patients with angiographically diagnosed CAD. Diabetic and hypertensive patients were excluded from the study. FVL, PT G20210A, and MTHFR (C677T, A1298C) mutations were determined in all patients.

Laboratory Studies

Venous blood (5-8 ml) was drawn from each patient by venipuncture into the vacutainer tube containing ethylenediaminetetraacetic acid (EDTA). Peripheral blood (200 µl) was used to isolate DNA with QIAamp DNA Blood Mini Kit (Qiagen Inc. Germany). The extracted DNA was stored at -200 C. Polymerase Chain Reaction (PCR) and the amplification refractory mutation system were used to identify FVL, PT G20210A, MTHFR C677T, and MTHFR A1298C. Polymorphism screening was carried out with a SNaPshot® multiplex system (Applied Biosystems Inc., Switzerland). Wild, heterozygous, and homozygous genotypic distributions of these polymorphisms were defined as numbers and percent frequencies.

Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). The incidence of polymorphisms was shown as percent (%). Student t-test was used for statistical analysis. A p value of less than 0.05 was considered statistically significant.

Results

Thirty-six males and 22 females with an age ranging from 41 to 85 and a mean age of 62.75±9.18 years were enrolled in the study.

Table 1. Distribution of polymorphisms in patients with coronary artery disease

Polymorphism	Male	Female	Total and %
HET PT G20210A	2 (5.5%)	3 (13.6%)	5 (8.6%)
HET FVL	6 (16.6%)	2 (9.1%)	8 (13.8%)
HET MTHFR C677T	15 (41.6%)	7 (31.8)	22 (37.9%)
HOM MTHFR C677T	7 (19.4%)	3 (13.6%)	10 (17.2%)
HET MTHFR A1298C	22 (61.1%)	8 (36.3)	30 (51.7%)
HOM MTHFR A1298C	4 (11.1%)	1 (4.5%)	5 (8.6%)
No Polymorphism	1 (2.77%)	4 (18.1%)	5 (8.6%)

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET= heterozygous, HOM= homozygous

Table 2. Distribution of co-existence PT G20210A polymorphism and other thrombophilic mutations in patients with coronary artery disease

PT G20210A	FVL	MTHFR C677T	MTHFR A1298C
HET	WT	HET	HET
HET	WT	WT	HET
HET	WT	HET	HET
HET	WT	HET	WT
HET	WT	HET	HET

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET=heterozygous, WT=wild type

Table 3. Distribution of co-existence heterozygous FVL polymorphism and other thrombophilic mutations in patients with coronary artery disease

FVL	PT G20210A	MTHFR C677T	MTHFR A1298C
HET	WT	HET	HET
HET	WT	HET	HET
HET	WT	HET	HET
HET	WT	WT	HOM
HET	WT	WT	HET
HET	WT	WT	HOM
HET	WT	HET	HET
HET	WT	HET	WT

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET=heterozygous, HOM=homozygous, WT=wild type

There were no homozygous carriers for either PT G20210A or FVL polymorphisms (Table 1) and no significant differences in the distribution of polymorphisms according to gender (p>0.05). The heterozygous PT G20210A genotype was identified in 5 (8.6%) patients (2 males, 3 females). All of them had additional defects. A combination of heterozygous PT G20210A and heterozygous MTHFR C677T polymorphisms were detected in 4 (6.9%) patients (Table 2).

The heterozygous FVL genotype was found in 8 (13.8%) patients (6 males and 2 females). Furthermore, 5 (8.6%) patients had a combination of the heterozygous FVL and heterozygous MTHFR C677T polymorphisms (Table 3).

The homozygous and heterozygous MTHFR C677T polymorphisms were found in 10 (17.2%) and 22 (37.9%) patients, respectively, giving an overall incidence of 55.1%. The homozygous MTHFR A1298C polymorphism was detected in 8.6% of patients (n=5) and heterozygous MTHFR A1298C polymorphism in 51.7% (n=30) of the patients,

Table 4. Distrubution of the combined heterozygous MTHFR 677/1298 carriers according to additional genetic mutations

MTHFR C677T	MTHFR A1298C	PT G20210A	FVL
HET	HET	WT	WT
HET	HET	WT	HET
HET	HET	WT	HET
HET	HET	WT	HET
HET	HET	WT	WT
HET	HET	HET	WT
HET	HET	WT	WT
HET	HET	WT	WT
HET	HET	WT	WT
HET	HET	WT	HET
HET	HET	WT	WT
HET	HET	WT	WT
HET	HET	WT	WT
HET	HET	HET	WT

FVL=Factor V Leiden, PT 20210A=Prothrombin gene mutation, MTHFR=Methylenetetrahydrofolate reductase, HET=heterozygous, WT=wild type

revealing an overall incidence of 60.3%. There were 14 (24.1%) patients with double heterozygous for the MTHFR C677T and the MTHFR A1298C polymorphisms. Hence, 28.5% (n=4) of them had additional FVL polymorphism and 14.2% (n=2) had additional PT G20210A polymorphism (Table 4). On the other hand, 8.6% (n=5) of patients carried none of these polymorphisms.

Discussion

FVL polymorphism could be related to the development of CAD [9] by two different mechanisms. First, FVL polymorphism causes thrombosis on inner arterial walls leading to a reduction in blood flow. Second, FVL polymorphism can contribute to the enlargement of atherosclerotic plaque due to development of thrombus. Female FVL carriers with of one or more cardiovascular risk factors (smoking, hypertension, diabetes, or dyslipidemia) had increased risk of MI as compared to females with neither FVL nor other risk factor [10]. Risk of MI increased 32-fold in smoking women carrying FVL polymorphism as compared to non-smoking non-carriers women [10]. The FVL polymorphism may also be an important inherited risk factor for the development of thrombotic complications leading to myocardial infarction and/or digital ischemia [6].

Boroumand *et al.* [11] suggested that FVL polymorphism is an important risk factor for the development of CAD and linked it to disease severity. Thus, FVL mutation status may be useful to predict probability of CAD [11]. Gurlertop *et al.* [9] suggested that combinations of FVL mutation and cardiovascular risk factors (hypertension, diabetes mellitus, etc.) might be associated with an enhanced risk of CAD, especially in inhabitants in the northeast part of Turkey. Similarly, Hobikoglu *et al.* [3] detected a higher prevalence of FVL polymorphism in young Turkish males with MI than healthy controls. In contrast, Donmez *et al.* [4] reported that the FVL and PT G20210A polymorphisms are not risk factors for the occurrence of MI in younger patients.

Dunn *et al.* [12] suggested that the heterozygous FVL genotype may not be an independent risk factor for CAD, but its homozygous genotype could play a role in the development of CAD. The prevalence of heterozygous FVL genotype was found in 10.9% of patients undergoing CABG [13]. The rate of heterozygous FVL genotype was found in 13.8% of our patients, which was higher than the prevalence rate of 7.9% in healthy Turkish individuals [14].

It has been speculated that FVL polymorphism might be associated with an increased thrombotic risk and a decreased bleeding risk in patients that undergoing cardiac operations [15]. The incidence of thromboembolic complications during perioperative and postoperative period in cardiac surgical patients was related with heterozygous FVL mutation [16]. In

contrast, Emiroglu *et al.* [13] did not find a statistically significant difference with postoperative thromboembolic complications, which may be due to routine heparin administration postoperatively. In contrast to the report of Emiroglu *et al.* [13], Donahue *et al.* [15] reported that postoperative bleeding drainage was statistically lower in patients with FVL mutation compared with non-carriers.

Franco *et al.* [17] confirmed that the heterozygous PT G20210A polymorphism was associated with 25% increase in prothrombin levels and increased thrombin formation. In addition, the increased levels of prothrombin could lead to an increase in thrombin-active fibrinolysis inhibitor (TAFI), which is an inhibitor of the fibrinolysis and therefore allows for increased clotting [18]. Thrombin is also involved in the regulation of endothelial cell proliferation and fibroblast mutagenesis. Thus, it may attribute the development of atherosclerotic plaques [19]. Based on this, the PT G20210A polymorphism may lead to increased risk of CAD [1]. Additionally, PT G20210 mutation in association with other major risk factors may amplify the risk of CAD [19].

The prevalence of PT G20210 polymorphism was reported as 7.3% in the patients with MI as compared to 1.8% in healthy controls [4]. Similarly, Rahimi et al. [5] found a high prevalence of PT G20210A polymorphism (3.1%) in CAD patients with diabetes mellitus. In our study, a high rate (8.6%) of PT G20210A polymorphism was detected, suggesting a relationship with coronary artery disease. However, the association of CAD with high prothrombin activity may be a biomarker for disease development of CAD [19].

A number of genetic polymorphisms and nutritional factors can affect the homocysteinemia levels [20]. Increased homocysteinemia levels were associated with progressive changes of atherosclerotic coronary plaques [21]. The MTHFR C677T polymorphism has a clear effect on homocysteinemia levels, however contradicted results are reported regarding the association with CAD. Yilmaz *et al.* [22] reported that MTHFR C677T polymorphism was not associated with the development of CAD in the Turkish people.

Unlike homozygous MTHFR C677T carriers, homozygous MTHFR A1298C carriers are not associated with increased homocysteinemia levels [23]. The frequency of homozygous MTHFR A1298C carriers is approximately 9% in Canadian and Dutch populations, and 10% in Turkish populations [24]. In our current study, the frequency of the homozygous MTHFR A1298C mutation in patients with CAD was 8.6%.

Hyperhomocysteinemia may cause oxidative DNA damage leading to coronary artery lesions [25]. Despite the influence on homocysteinemia levels, the role of MTHFR C677T polymorphism as a genetic risk factor of CAD is still controversial [25]. Yenilmez *et al.* [26] suggested that FVL and MTHFR A1298C polymorphisms could play important roles in the progression of coronary lesions. The MTHFR C677T polymorphism was reported as an important risk factor for early fatal coronary occlusion in Hungarian people [27]. Furthermore, the MTHFR C677T polymorphism was associated with hyperhomocysteinemia and CAD in Asian patients but not in European counterparts [28]. Kawashiri *et al.* [29] reported a relation between MTHFR C677T mutation and the risk of CAD in male patients with heterozygous familial hypercholesterolemia. In contrast, Kim *et al.* [30] did not found associations among the MTHFR C677T polymorphism, the risk of CAD, and homocysteinemia level in Koreans. They suggest that MTHFR gene is related to homocysteine metabolism but is not a predictor for the risk of CAD [30]. Furthermore, meta-analysis did not support the hypothesis that the MTHFR C677T mutation is an independent predictor of developing CAD [31].

The frequency of MTHFR C677T genotype for homozygous and heterozygous carriers was reported 9.6% and 47.4%, respectively in healthy Turkish people [24]. Similarly, the rate of C677T variant for homozygous and heterozygous carriers in this study was 17.2% and 37.9%, respectively. The prevalence of compound MTHFR C677T/A1298C genotypes was 21.6% in healthy Turkish population [24], similar to the rate (24.1%) our study. Based on this data, we speculate that these genotypes are not playing an important role in the development and progression of CAD.

Conclusions

FVL and PT G20210A polymorphisms could contribute to the development of CAD. MTHFR C677T and MTHFR A1298C genotypes were not associated with the predisposition of the development of CAD. However, in compound MTHFR C677T/A1298C carriers, the presence of FVL or PT

G20210 polymorphism may contribute to the development of CAD. Further studies are needed to confirm the importance of these combinations as hereditary risk factors for the development of CAD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

This study was supported by a grant from Bozok University, The Directorate of Scientific Research Projects (2016, 6602c-TF/16-23).

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