Thrombophilia in young patients with acute myocardial infarction

Murat Celik, MD, Abdullah Altintas, MD, Yusuf Celik, PhD, Aziz Karabulut, MD, Orhan Ayyildiz, MD.

ABSTRACT

الهدف: الميل للتجلط من الناحية الجينية يحدد زيادة خطورة التجلط. تبقى إسهامه في تطور مرض الشريان التاجي مثير للجدل. تهدف الدراسة إلى التحقق من اتحاد الميل للتجلط و مرض الشريان التاجي لدى المرضى المصابين باحتشاء في عضلة القلب.

الطريقة: شملت الدراسة ١٢٩ مريضاً تقل أعمارهم عن ٤٥ عاما ويعانون من إحتشاء في عضلة القلب و١٠٧ شخصاً مجموعة التحكم. تم التحقق من عوامل الخطر التقليدية لمرض الشريان التاجي والبروتين ج واس ونقصان مضادات التجلط الثلاثي وعامل في ليدين (في إف ليدين) ومولد الخثرين (بروثرومبين جي ٢٠٢١٠ أيه) وميثيلينتراهيدروفوليت ريدوكتيس (ام تي اتش اف آر).

النتائج: كان هنالك فرقاً ذو دلالة إحصائية من حيث البدانة والتدخين والدهون الثلاثية والكولسترول الكامل والليبروبروتين عالي الكثافة ومنخفض الكثافة والكلوستيرول ذو البيروبروتين منخفض الكثافة جداً والتاريخ العائلي وارتفاع ضغط الدم والسكري وتضخم البطين الأيسر بين المرضى ومجموعة التحكم. ليس هنالك أحداً من المرضى أو مجموعة التحكم يعاني من بروتين ج و إس أو نقصان مضادات التخثر الثلاثية. عشرة مرضى (٧,٨) وأربعة من مجموعة التحكم (٣,٧) يعانون من تحول عامل إف ليدين هيتروزايجوت. تم اكتشاف وجود تحول جين بروثرومبين هوموزايجوس جي ٢٠٢١٠ أيه في مريض واحد (١,١٪). تمت مراقبة تحول هوموزايجوس (ام تي اتش اف آر سي ٦٧٧تي) في ٧,٨٪ من المرضى و٦,٥٪ من مجموعة التحكم. تم اكتشاف تحول هيتروزايجوس (ام تي اتش اف آر سي ٦٧٧تي) في ٣٦,٤٪ من المرضى و٣١,٧٪ من مجموعة التحكم. لم يكن هنالك فرقاً ذو دلالة إحصائية من حيث حمل تحولات التخثر.

خاتمة: وجدنا أن عوامل الخطر التقليدية زادت من عامل خطورة مرض الشريان التاجي. لم يزد بروثرومبين جي ٢٠٢١٠أيه و عامل في ليدين و ام تي اتش اف آر سي ٦٧٧تي و بروتين سي وإس و نقصان مضادات التجلط الثلاثية من خطورة مرض الشريان التاجي بين السكان الشباب.

Objective: To investigate the association of thrombophilia and coronary artery disease (CAD) in patients with myocardial infarction (MI).

Methods: Under the age of 45 years, 129 patients with MI and 107 control subjects were included into the study. Traditional risk factors of CAD and protein C, S, antithrombin III deficiencies, factor V Leiden (FV Leiden), prothrombin G20210A and methylenetetrahydrofolate reductase (MTHFR) C677T mutations were investigated.

Results: There were statistically significant differences in terms of obesity, smoking, triglyceride, total cholesterol, high-density lipoprotein, high-density lipoprotein, and very-low-density lipoprotein cholesterol, family history, hypertension, diabetes, and left ventricular hypertrophy between patients and controls. None of the patients and controls had protein C, protein S, and antithrombin III deficiencies. Ten patients (7.8%) and 4 controls (3.7%) had heterozygote FV Leiden mutation. Homozygous prothrombine G20210A gene mutation was detected in one patient (1.1%). Homozygous MTHFR C677T mutation was observed in 7.8% (patients) and in 6.5% (controls). Heterozygous MTHFR C677T mutation was detected 36.4% in patients and 31.7% in controls. The difference was not statistically significant in terms of carriage of thrombophilic mutations.

Conclusion: We found that traditional risk factors increased the risk of CAD. Prothrombin G20210A, FV Leiden and MTHFR C677T mutations, protein C, S and AT-III deficiencies did not increase the risk of CAD in our young population.

Saudi Med J 2008; Vol. 29 (1): 48-54

From the Internal Medicine, Clinical of Internal Diseases (Celik M), Nusaybin State Hospital, Departments of Hematology-Oncology (Altintas, Ayyildiz), Biostatistics (Celik Y), and Cardiology (Karabulut) Faculty of Medicine, Dicle University, Diyarbakir, Turkey.

Received 26th May 2007. Accepted 13th August 2007.

Address correspondence and reprint request to: Dr. Abdullah Altintas, Dicle University, Internal Medicine, Department of Hematology-Oncology, 21280, Diyarbakir, Turkey. Tel. +904122488233. Fax. +904122488440. E-mail: draaltintas@dicle.edu.tr

The pathogenesis of cardiovascular disease is multifactorial, and it involves interaction between acquired and inherited risk factors. Diabetes mellitus (DM), hypertension, hyperlipidemia and smoking are the major risk factors of coronary artery disease (CAD). Traditional risk factors are frequently seen and if treated successfully have positive effect on the clinical outcome. Other etiological factors have several different mechanisms implicated in the pathogenesis of CAD especially under the age of 35 years, nonatherosclerotic causes of CAD accounts 8-14%.[1] The prevalence of normal or near normal coronary angiography varies between 1% and 12% after the myocardial infarction (MI), and various etiological factors is responsible from this entity such as coronary spasm, vasculitis, and polycytemia vera.[2-4] Recently, hereditary or acquired thrombotic factors that enhances the risk of thrombosis, called thrombophilia, gain attention in the pathogenesis of CAD. Established causes of thrombophilia including; protein S, protein C and antithrombin III deficiencies (AT-III), prothrombin G20210A mutation, activated protein C resistance (APC-R) mainly due to the factor V Leiden mutation (FV Leiden), high titers of anti-cardiolipin antibodies, and hyperhomocysteinemia.[5] Hereditary prothrombotic states are usually associated with venous rather than arterial thrombosis. However, in association with other risk factors such as smoking, diabetes, previous data suggested that up to 10% of arterial thrombosis are associated with hereditary thrombophilia.[6] Although our country strengthened itself with a younger population Our country have younger population similar to the developing countries, however, mortality of CAD is still as high as developed countries.[7] On the other hand, within the last years, we have seen a significant number of young patients with acute MI. However, no data are available in the southeastern of Turkish population about the frequencies of the thrombophilic mutations in young patients with MI. Therefore, we investigated the traditional risk factors, the frequencies of protein C, protein S, AT-III deficiencies, and FV Leiden, prothrombin G20210A, thermolabile variant of methylenetetrahydrofolate reductase (MTHFR) C677T mutations and results were compared with healthy age matched control subjects.

Methods. We investigated 129 consecutive patients (mean age was 39±4.5, age range was between 24-45 years) under the age of 45 years whose hospitalized in the cardiac intensive care unit of Dicle University Medical Faculty with their first acute MI episode between July 2001 and July 2005. One hundred and twenty-nine were male (mean age was 38.75±4.6, age range was between 27-45 years) and 36 were female (mean age was 41.33 ± 4.4, age range was between 24-45 years). Diagnosis of MI was established on the basis of the triad including chest pain, ECG changes, and raised plasma enzymes level. One hundred and seven healthy control subjects (mean age was 38.10±4.8 for all control subjects, 37.23 ± 4.943 for 56 healthy men, and 39.57 ± 4.721 for 51 healthy women) unrelated to the cases but individually matched with patients in terms of age and geographical origin were included into the study. Patients and controls older than 45 years, from different geographical origin and did not have sufficient data about their risk factors were excluded from the study. None of the control subjects had history of thromboembolic diseases. Both patients and control subjects were born in the southeast of Turkey and were living in Diyarbakir province and near Leiden, prothrombin G20210A, MTHFR C677T mutations were investigated for all patients and control subjects. Clinical data included Killip class[8] were recorded and left ventricular hypertrophy (LVH) assessed by echocardiography. A positive family history was defined as the presence of at least one-first degree relative (parent, offspring, or sibling) who had developed CAD before the age of 55 years for men and 65 years for women. The BMI was calculated as weight (kg) and divided by the square of height (meter). Prevalent DM was defined as a fasting serum glucose level >126 mg/dL, non-fasting glucose level >200 mg/dL and current use of any diabetes medication. Prevalent hypertension was defined as seated diastolic blood pressure ≥90 mm Hg, systolic blood pressure ≥140 mm Hg, or use of anti-hypertensive medications within the past 2 weeks. Current smokers were defined on the basis of self-report who smoking currently and regularly.

Biochemical and coagulometric analysis. After fasting for 12 hours venous blood samples collected from all patients and control subjects were analyzed for the following parameters: total cholesterol (112-200 mg/dL), high-density lipoprotein (HDL) (28-63 mg/dL), low-density lipoprotein (LDL) (60-160 mg/dL), triglycerides (50-180 mg/dL), very low-density lipoprotein (VLDL) (10-32 mg/dL), and glucose (70-115 mg/dL) were measured with Abbot Aeroset Toshiba autoanalyser. Protein C (71.8-146.20%), protein S (64.4-128-8%) and AT-III (75-120%) were determined by 2 repetitive measurements subsequent to the recovery of acute thrombotic episode with "instrumentation laboratory coagulation" kit in "ACL advance coagulometer" equipment.

DNA isolation and mutation analysis. Venous blood from all patients and control groups was collected in ethylenediaminetetraacetic acid-containing tubes for genetic analysis. Deoxyribonucleic acid samples were isolated from whole blood with the aid of high pure polymerase chain reaction (PCR) template preparation

kit (Roche Molecular Biochemicals). Deoxyribonucleic acid was stored at -20˚C until the mutation analysis. Factor V Leiden and Prothrombin G20210A mutations were detected with LightCycler-Factor V Leiden and LightCycler-Prothrombin G20210A mutation detection kits (Roche Molecular Biochemicals), MTHFR C677T mutation was detected with LightCycler-DNA Master Hybridizations probes (Roche Molecular Biochemicals), amplifications primers (Metabion), hybridizasyon probes (TIB MOLBIOL). All mutation-related gene regions were amplified in 20 µL PCR capillary tubes. After preparation of the master mixture, 18 µL of the reaction mixture and 2 µL of genomic DNA or control template were added to each LightCycler capillary tube. For negative control, PCR grade water was added instead of template. The capillary tubes were sealed and briefly centrifuged in a microcentrifuge and then placed into the LightCycler carousel. The PCR products were detected using 3'-fluorescein (FLU) labeled and 5'-Red 640 labeled probes. When both probes hybridize in close proximity, fluorescence resonance energy transfer (FRET) occurs, producing a specific fluorescence emission of LightCycler red as a result of fluorescence excitation. The fluorescence intensity depends on the amount of specific PCR products. Amplification per cycle can be monitored with the LightCycler instrument. The LightCycler instrument increases the temperature at the end of the amplification process, and the fluorescence obtained is plotted against the temperature. The mutations are then identified by their characteristic curves. Total assay time is approximately 40 minutes. The resulting melting peaks allow discrimination between the homozygous (wild type or mutant) as well as the heterozygous genotype.

Statistical analysis. Continuous variables were expressed as mean ± SD. Numerical data were shown as frequency and percentage (%). Two different kinds of unpaired Student's t test were used for the 2 groups: mean and ratios. Logistic regression models for predicting the odds ratios and 95% confidence intervals (CI) of major cardiovascular and prothrombotic risk factors were constructed using gender, obesity, smoking, hypertension, family history, total cholesterol, HDL and LDL cholesterol, triglyceride, FV Leiden, prothrombin G20210A and MTHFR C677T mutations. Backward stepwise procedure of logistic regression was performed. Two-sided probability values were considered statistically significant at $p<0.05$. Statistical analyses were carried out using the Statistical Package for Social Sciences Version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results. Ninety-three male, 36 female patients with a history of MI, and 107 control subjects were included into the study. Differences of mean age were not statistically significant between patients and control subjects ($p=0.140$). The most common site of infarction was the inferior wall of the heart. There was a statistically significant difference in terms of obesity ($p=0.021$), smoking ($p<0.001$), triglyceride level ($p=0.012$), total cholesterol level ($p=0.018$), HDL level ($p<0.001$), LDL level ($p=0.006$) and VLDL (p=0.027) cholesterol levels, family history of CAD ($p<0.001$), hypertension ($p=0.016$), DM ($p=0.035$) and LVH ($p=0.019$) between patients and control subjects. Family history of CAD, to a lesser extent, hypertension, low level of HDL cholesterol, smoking, high level of total, and LDL cholesterol, high level of triglyceride and obesity were found to be the traditional non-genetic risk factors with the strongest effect. Protein C, protein S, and AT-III deficiences were not detected in both patients and control groups. None of the patients were compound heterozgous for FV-Leiden and prothrombine G20210A or MTHFR C677T. Homozygote FV Leiden mutation was not observed in both patients and control subjects. Ten patients (7.8%) and 4 control subjects (3.7%) had heterozygote FV Leiden mutation. There was not statistically significant difference in terms of carriage of FV Leiden mutation between patients and control subjects (odds ratio, 1.26 [95% CI, 0.84 to 6.89]; $p=0.651$). Heterozygote prothrombine G20210A gene mutation was not

Table 1 - Myocardial infarction (MI) localization and Killip classification[8] of patients.

MI localization	Male patients (n=93)	Female patients (n=36)
Anterior MI	15 (16.1)	7 (19.4)
Widespread anterior MI	12 (12.9)	7 (19.4)
Inferior MI	31 (33.3)	10 (27.7)
Anteroseptal MI	11 (11.8)	2 (5.5)
İnferolateral MI	2 (2.2)	1 (2.7)
İnferoposterolateral MI	7 (1.5)	5 (13.8)
İnferoposterior MI	6 (6.5)	1 (4.2)
High lateral MI	1 (1.1)	-
Anteroposterolateral MI	1 (1.1)	-
Non-ST-elevation MI	7 (7.5)	3 (8.3)
Killip I	71 (76.3)	28 (77.7)
Killip II	15 (16.1)	6 (16.6)
Killip III	4 (4.3)	1 (2.7)
Killip IV	3 (3.2)	1 (2.7)

detected in patients and control subjects. Homozygous prothrombine G20210A mutation was detected in one male patient (1.1%) (odds ratio, 1,65 [95% CI, 0.73 to 5.98]; p=0.862). Homozygous MTHFR C677T mutation was observed in 10 patients (7.8%) and in 7 control subjects (6.5%). Heterozygous MTHFR C677T mutation was detected in 47 patients (36.4%) and in 34 control subjects (31.7%). There were not statistically significant difference in terms of homozygous (odds ratio, 1.02 [95% CI, 0.56 to 1.11]; p=0.693) and heterozygous (odds ratio, 1.23 [95% CI, 0.76 to 1.56]; p=0.259) carriers of MTHFR C677T mutations between patients and control subjects. MI localizations and Killip class[8] of male and female patients were shown in **Table 1**. Major conventional risk factors and mutations analysis were shown in **Table 2**. Multiple logistic regression analysis was performed to determine the effects of conventional coronary risk factors, and FV Leiden, protrombin G20210A and MTHFR C677T mutations on MI. None of the carriage of thrombophilic mutations increases the risk of MI. We found that obesity, smoking, hypertension, positive family history of CAD, high level of triglyceride, high level of total and LDL cholesterol and low level of HDL cholesterol increased the risk of MI. Odds ratios of binary logistic regression analysis of traditional coronary risk factors and thrombophilic mutations were shown in **Table 3**.

Discussion. Acute MI is caused by a total occlusion of an epicardial coronary artery secondary to an atherosclerotic plaque complicated by thrombus formation; a process called atherothrombosis. Atherosclerotic plaque is not always present and non-atherosclerotic causes of CAD such as thrombophilia should be considered especially in young patients with MI. However, the role of thrombophilia in the pathogenesis of MI is still controversial.[1-2,8-11] High mortality rate of CAD and insufficient representation of younger population of our region in previous reports led us to perform a detailed study in younger patients with a history of MI. Therapeutic consequences of the presence of thrombophilia in patients with MI remain controversial. In patients with MI and high titers of anticardiolipin antibodies, treatment with long-term anticoagulation has been recommended. However, to date no clinical trials have assessed the beneficial effects of anticoagulation among patients with inherited thrombophilia and arterial vascular events, such as MI.[10] In this present study, we found that traditional risk factors including obesity, smoking, hypertension, high level of triglyceride, total and LDL cholesterol, low level of HDL cholesterol, family history of CAD increased the risk of MI. In contrast, presence of prothrombin G20210A, FV Leiden, and MTHFR C677T mutations, protein C, protein S, and AT-III deficiencies did not increase the risk of CAD in patients under the age of 45 years. Activated protein C resistance due to FV Leiden mutation is the most common inherited predisposition to hypercoagulability in Caucasian populations.[12] The prevalence of FV Leiden mutation is 7-9% according to the studies performed in our region.[13-14] Heterozygote carriers of this mutation has 7-fold and homozygote carriers have 80-fold higher risk of venous thrombosis.[15-17] The prothrombin G20210A, a genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin and thrombin levels, thus increasing the risk of thrombotic events. This mutation has a frequency of 1.7% in populations of northern European descent.[18-19] Frequency of this mutation is 1.2-2.7% according to previous reports from our region.[20-21] There is some controversy in the literature about the relation between these 2 mutations, and the development of acute MI. Doggen et al[22] carried out a study in 560 male patients with a first MI episode before the age of 70 years. Authors suggested that the carriage of these 2 mutations increases the risk of MI 1.4-fold, and if there is an additional risk factor such as smoking, DM, obesity or hypertension, the risk of MI increases 3-6 fold. Van de Water et al[23] investigated these 2 mutations in 271 patients with MI. Sixty of these patients had normal or near normal coronary arteries (41 patients <50 years and 19 patients >50 years). The remaining 211 control subjects had angiographically proven as significant coronary artery stenosis (114 patients aged <50 years and 97 patients aged >50 years). When patients <50 years old were investigated, the prevalence of FV Leiden was determined to be 14.6% and 3.6% in the patient and control groups (p=0.04). Seventeen percent of the patients were female and the prevalence of positive family history for CAD was similar in both groups. Similarly, the prevalence of prothrombin G20210A mutation was determined to be 7.3% and 1.8% in the patients and control groups (p=0.04). Italian study group[24] investigated the association between 9 polymorphism of genes encoding hemostasis factors (including FV Leiden and prothrombin G20210A) in patients with MI under the age of 45 years. We found that the traditional non-genetic risk factors for CAD, such as smoking, family history, DM and hypertension were highly associated with premature MI. However, we did not found evidence supporting an association between 9 polymorphisms of genes encoding proteins involved in hemostasis and the occurrence of premature MI. In the other meta-analysis performed by Ye et al,[25] we analyzed 191 studies for 7 gene polymorphism encoding hemostasis factors (including FV Leiden and prothrombin G20210A mutations). As a result

Table 2 - Major conventional risk factors and mutations analysis in patients and control subject.

Risk factors (normal range)	Patients (n=129)	Controls (n=107)	*P*-value
Age (mean±SD)	39.2 ± 4.5	38.1 ± 4.8	0.140
Obesity* (%)	11.6	3.7	0.021*
Smoking status† (%)	65.1	19.6	<0.001*
Total cholesterol (112-200 mg/dL)	207.15 ± 52.4	184.8 ± 34.21	0.018*
High-density lipoprotein (28-63 mg/dL)	29.65± 9.15	38.56 ± 8.22	<0.001*
Low-density lipoprotein (60-160 mg/dL)	140.1± 45.55	118.7 ± 29.71	0.006*
Triglyceride (50-180 mg/dL)	184.4 ± 108.4	150.7 ± 63.84	0.012*
Very-low-density lipoprotein (10-32 mg/dL)	36.85 ± 20.95	30.22 ± 13.53	0.027*
Protein C (71.8-146.20%)			-
Protein S (64.4-128-8%)			-
AT-III (75-120%)			-
Familial history coronary artery disease	37.2	4.7	<0.001*
Hypertension‡	21.7	5.6	0.016*
Diabetes mellitus§	10.8	3.7	0.035*
Left ventricular hypertrophy†	17.1	3.7	0.019*
Factor V Leiden Mutation			
Homozygote			
Heterozygote	7.8	3.7	0.531
Prothrombin G20210A Mutation			
Homozygote	1.1		
Heterozygote	None		
Methylenetetrahydrofolate reductase Mutation			
Homozygote	7.8	6.5	0.413
Heterozygote	36.4	31.7	0.355

*body mass index ≥30, †Smoking - current smokers was defined on the basis of self-report who smoking currently and regularly, ‡blood pressure ≥140/90 mm Hg, §fasting glucose level ≥126 mg/dL, FH CAD - familial history of coronary arterial disease, LVH - left ventricular hypertrophy,

Table 3 - Odds ratİos of bİnary logİstİc regressİon analysİs of conventİonal coronary rİsk factors.

Parameters	Odds ratio	CI (95 %)	*P*-value
Age	0.97	0.89 - 1.02	0.456
Gender (male/female)	1.76	0.96 - 2.28	0.375
Obesity	1.32	1.05 - 2.24	0.026*
Smoking	2.85	1.13 - 3.43	0.039*
Hypertension	3.56	2.24 - 7.21	0.012*
Family history of coronary artery disease	4.24	2.87 - 8.65	0.001*
Total cholesterol (mg/dL)	2.77	1.96 - 3.85	0.002*
High-density lipoprotein (mg/dL)	2.99	1.87 - 4.24	0.001*
Low-density lipoprotein (mg/dL)	2.17	1.54 - 3.12	0.023*
Triglyceride	1.98	1.23 – 2.56	0.013*
Prothrombin G20210A	1.65	0.73 - 5.98	0.862
Factor V Leiden	1.26	0.84 - 6.89	0.651
Methylenetetrahydrofolate reductase Mutation C677T			
Homozygote	1.02	0.56 – 1.11	0.693
Heterozygote	1.23	0.76 – 1.56	0.259

*Statistically significant, classification rate: 95.2 %, CI - confidence interval

of this meta-analysis, we showed that FV Leiden and prothrombin G20210A mutations might be moderately associated with the risk of CAD. In this present study, the prevalence of these 2 mutations was similar in patients and control subjects and similar with previously reported data from our region. We suggested that this 2 mutations are not associated with an increased risk of CAD in our young population with MI. Homocysteine is an intermediary metabolite of methionine. The adverse effects of hyperhomocysteinemia are endothelial injury, smooth muscle proliferation, increased production of platelet aggregating substances such as thromboxane A2, inhibition of natural anticoagulation pathway via protein C. One of the genetic causes for mild hyperhomocysteinemia is associated with homozygosity for thermolabile variant of MTHFR that reduced specific activity has been described. A substitution of cytosine (C) by thymine (T) at nucleotide 677 of the MTHFR that converts an alanine to valine residue was responsible for the thermolability of MTHFR.[26-27] Heterozygous carrier of the MTHFR C677T have normal plasma homocysteine levels unless folat levels are reduced.[28] Homocysteine studies collaboration[29] performed a meta-analysis. Data from 5073 patients with CAD and 1113 patients with stroke were analyzed. We showed that 25% decreased of homocysteine level diminishes the risk (11% in CAD and 19% in stroke). El-Sammak et al[30] analyzed the plasma homocysteine, folat level and MTHFR C677T mutation. Their study group consisted of 50 healthy control males, 50 elderly males age ranged between 50-75 years without any cardiovascular diseases and 50 aged matched elderly male patients with MI. We showed that elevated plasma homocysteine level positively correlates with age and MTHFR C677T mutation did not associate with either high homocysteine or the occurrence of CAD. In another meta-analysis, Lewis et al,[31] found that no strong evidence existed to support an association of the MTHFR C677T polymorphism and coronary heart disease in Europe, North America, or Australia. In the present study, there were no statistically significant differences between patients and control subjects regarding the distribution of the MTHFR C677T mutations (p=0.693 for homozygous, p=0.259 for heterozygous). This result is close to the results of previous studies[29-31] and we suggested that heterozygosity or homozygosity of MTHFR C677T are not associated with an increased risk of MI. Hereditary deficiencies of antithrombin III, protein C and protein S are risk factors for venous thrombosis.[32-35] Several case reports have been described an association between MI and protein C, protein S or AT-III deficiencies. These protein deficiencies when coupled with smoking, factor VII hyperactivity, and family history, there is higher incidence of premature MI.[36-38] In the present study, AT-III, protein C and protein S deficiencies were not observed in patients and control subjects. Our data did not support an association between these deficiencies and increased risk of CAD. There are some limitations in this study, firstly, number of patients and controls are relatively small. Another important limitations were the lack of coronary angiographic evaluation, homocysteine and folat levels. However, we investigated many traditional and hereditary thrombophilic risk factors and we found a small number of articles containing large data like our study.

In conclusion, it is important to recognize thrombophilic risk factors in young patients with MI. To the best of our knowledge, our report is the first to describe the prevalence of thrombophilic mutations in patients with young MI in southeast of Turkish people. We found that traditional risk factors were increased the risk of MI. In contrast, presence of thrombophilic factors did not increase the risk of MI. The screening policy in a given country should be based on that country's own population data, and moreover, we know that there may be major racial variation in gene polymorphisms. We think that these mutations and protein deficiencies do not contribute much to MI in our young population. However, further large-scale studies are necessary for confirming our results.

References

1. Eliot RS, Baroldi G, Leone A. Necropsy studies in myocardial infarction with minimal or no coronary luminal reduction due to atherosclerosis. ***Circulation*** 1974; 49: 1127-1131.
2. Sharifi M, Frohlich TG, Silverman IM. Myocardial infarction with angiographically normal coronary arteries. ***Chest*** 1995; 107: 36-40.
3. Lie JT, Failoni DD, Davis DC. Temporal arteritis with giant cell aortitis, coronary arteritis, and myocardial infarction. ***Arch Pathol Lab Med*** 1986; 110: 857-860.
4. Wirth L. Myocardial infarction as the initial manifestation of polycthemia vera. ***Mil Med*** 1960; 125: 544.
5. Lane DA, Mannucci P, Bauer K, Bertina RM, Bochkov NP, Boulyjenkov V, et al. Inherited thrombophilia: Part 2. ***Thromb Haemost*** 1996; 76: 824-834.
6. Goodnight SH, Griffin JH. Hereditary thrombophilia. In: Beutler E, Lichtman BA, Coller BS, Kipps TJ, Seligsohn U, editors. Williams Hematology. 6th ed. USA: McGraw-Hill; 2001; p. 1215-1235.
7. Onat A. The prevalence of heart diseases in our adult patients, new coronary event and death frequency. In: Onat A, editor. Tekharf. Istanbul: ARGOS; 2005. p. 20-27. (Turkish).
8. Killip T 3rd, Kimball JT. Treatment of myocardial infarction in a coronary care unit. A two year experience with 250 patients. ***Am J Cardiol*** 1967; 20: 457-464.
9. Alpert JS. Myocardial infarction with angiographically normal coronary arteries. ***Arch Intern Med*** 1994; 154: 265-269.
10. Segev A, Ellis MH, Segev F, Friedman Z, Reshef T, Sparkes JD, et al. High prevalence of thrombophilia among young patients with myocardial infarction and few conventional risk factors. ***Int J Cardiol*** 2005; 98: 421-424.

11. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Buller HR, Peters RJ. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction. A systematic review. ***Circulation*** 2001; 104: 3063-3068.
12. Deitcher SR, Caiola E, Jaffer A. Demystifying two common genetic predisposition to venous thrombosis. ***Cleve Clin J Med*** 2000; 67: 825-836.
13. Kalkanli S, Ayyildiz O, Tiftik N. Factor V Leiden mutation in venous thrombosis in southeast Turkey. ***Angiology*** 2006; 57: 193-196.
14. Gurgey A, Mesci L. The prevalence of Factor V Leiden (1691G → A) mutation in Turkey. ***Turk J Pediatr*** 1997; 39: 313-315.
15. Pineo GF, Hull RD. Thrombophilia: disorders predisposing to venous thromboembolism. ***Clin Haematol*** 1998; 11: 525-540.
16. Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden thrombophilia study. ***Lancet*** 1993; 342: 1503-1506.
17. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High-risk of thrombosis in patients homozygous for factor V Leiden (APC-resistance). ***Blood*** 1995; 85: 1504-1508.
18. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. ***Blood*** 1996; 88: 3698-3703.
19. Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, et al. Geographic distribution of the 20210 G to A prothrombin variant. ***Thromb Haemost*** 1998; 79: 706-708.
20. Ayyildiz O, Kalkanli S, Batun S, Aybak M, Isikdogan A, Tiftik N, et al. Prothrombin G20210A gene mutation with lightCycler polymerase chain reaction in venous thrombosis and healthy population in the southeast of Turkey. ***Heart Vessels*** 2004; 19: 164-166.
21. Akar N, Mısırlıoglu M, Akar E, Avcu F, Yalcin A, Sozuoz A. Prothrombin gene 20210 G-A mutations in Turkish population. ***Am J Hematol*** 1998; 58: 49.
22. Doggen CJ, Cats VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. ***Circulation*** 1998; 97: 1037-1041.
23. Van de Water NS, French JK, Lund M, Hyde TA, White HD, Browett PJ. Prevalence of Factor V Leiden and prothrombin variant G20210A in patients age <50 years with no significant stenoses at angiography three to four weeks after myocardial infarction. ***J Am Coll Cardiol*** 2000; 36: 717-722.
24. Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group. No evidence of association between prothrombotic gene polymorphisms and the development of acute myocardial infarction at a young age. ***Circulation*** 2003; 107: 1117-1122.
25. Ye Z, Liu EH, Higgins JP, Keavney BD, Lowe GD, Collins R, et al. Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. ***Lancet*** 2006; 367: 651-658.
26. Townend J, O'Sullivan J, Wilde JT. Hyperhomocysteinaemia and vascular disease. ***Blood Rev*** 1998; 12: 23-34.
27. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. ***Lancet*** 1999; 354: 407-413.
28. Hanson NQ, Aras O, Yang F, Tsai MY. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. ***Clin Chem*** 2001; 47: 661-666.
29. Homocysteine studies collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. ***JAMA*** 2002; 16: 2015-2022.
30. El-Sammak M, Kandil M, El-Hifni S, Hosni R, Ragab M. Elevated plasma homocysteine is positively associated with age independent of C677T mutation of the methylenetetrahydrofolate reductase gene in selected Egyptian subjects. ***Int J Med Sci*** 2004; 1: 181-192.
31. Lewis SJ, Ebrahim S, Davey Smith G. Meta-analysis of MTHFR 677C->T polymorphism and coronary heart disease: does totality of evidence support causal role for homocysteine and preventive potential of folate? ***BMJ*** 2005; 331: 1053.
32. Nachman RL, Silverstein R. Hypercoagulable states. ***Ann Intern Med*** 1993; 119: 819-827.
33. Macik BG, Ortel TL. Clinical and laboratory evaluation of the hypercoagulable states. ***Clin Chest Med*** 1995; 16: 375-387.
34. Thomas DP, Roberts HR. Hypercoagulability in venous and arterial thrombosis. ***Ann Intern Med*** 1997; 126: 638-644.
35. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. ***Blood*** 1996; 87: 3531-3544.
36. Coller BS, Owen J, Jesty J, Horowitz D, Reitman MJ, Spear J, et al. Deficiency of plasma protein C, protein S or antithrombin III and arterial thrombosis. ***Arteriosclerosis*** 1987; 7: 456-462.
37. Ogasawara N, Kijima Y, Ike S, Nakagawa Y, Takagi T, Hata T, et al. Hereditary protein S deficiency with a history of recurrent myocardial infarction. ***Circ J*** 2003; 67: 166-168.
38. Tiong I, Alkotob ML, Ghaffari S. Protein C deficiency manifesting as an acute myocardial infarction and ischaemic stroke. ***Heart*** 2003; 89: E7.