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Prevalence of Thrombophilic Mutations in Ischemic Stroke Patients in Isparta, Turkey

Türkiye'nin Isparta İlinde Yaşayan İskemik İnme Geçirmiş Hastalar Arasında Trombofilik Mutasyonların Görülme Sıklığı

Nilgün Erten[1], Serpil Demirci[2], Recep Sütçü[3]

[1]Giresun University Faculty of Medicine, Department of Neurology, Giresun, Turkey
[2]Süleyman Demirel University Faculty of Medicine, Department of Neurology, Isparta, Turkey
[3]Katip Çelebi University Faculty of Medicine, Department of Biochemistry, İzmir, Turkey

Summary

Objective: The present study aimed to investigate whether the frequency of factor V, methylenetetrahydrofolate reductase (MTHFR), prothrombin, β-fibrinogen gene mutations, and human platelet alloantigens (HPA), plasminogen activator inhibitor 1 (PAI1), apolipoprotein E (APOE), and angiotensin converting enzyme (ACE) gene polymorphisms in stroke patients is higher than that in normal individuals.

Materials and Methods: Two hundred twelve patients with cerebral infarction and 238 individuals of similar age and gender with no history of stroke were included. Demographics and risk factors for cerebrovascular disease of all individuals were determined. Biochemical parameters were analyzed in serum, and electrocardiography was performed. Factor V, MTHFR, prothrombin, β-fibrinogen mutations and HPA, PAI, APOE, and ACE polymorphisms were investigated. Data were analyzed with SPSS 15.0 software using descriptive statistics, chi-square, independent two-group t-test, and logistic regression tests. Statistically significant differences in independent variables were further analyzed by logistic regression.

Results: HPA, PAI, APO, and ACE polymorphism frequency was not significantly different between the stroke and control groups. Factor V H1299R, factor V Leiden, and β fibrinogen -455GA mutation frequencies were significantly higher in the stroke than the control group in the chi-square test, but not in logistic regression analysis.

Conclusion: Stroke etiopathogenesis is multifactorial, and prothrombin gene mutations increase the impact of existing risk factors when other risk factors are considered. (Turkish Journal of Neurology 2015; 21:42-8)

Key Words: Stroke, prothrombine gene mutations, risk factors

Özet

Amaç: Dünyanın farklı bölgelerinde kalıtsal trombofilik risk faktörlerinin görülme sıklığı değişiklik göstermektedir. Bu çalışma ile bölgemizde iskemik inme geçirmiş hastalarda faktör V, metilentetrahidrofolat redüktaz (MTHFR), protrombin, β-Fibrinojen gen mutasyonları ve human platelet alloantigens (HPA), plazminojen aktivatör inhibitör 1 (PAİ), Apolipoprotein E (APO-E), Anjiyotensin dönüştürücü enzim (ADE) gen polimorfizmlerinin görülme sıklıklarının kontrol grubuna göre anlamlı olup olmadığının araştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmamıza kliniğimizde takip edilen serebral enfarkt geçirmiş 212 hasta ve inme geçirmemiş yaş ve cinsiyet özellikleri hasta grubuna benzer 238 birey alındı. Tüm katılımcıların demografik verileri ve beyin damar hastalıkları için risk faktörleri sorgulandı. Serumlarında bazı biyokimyasal parametrelere bakıldı ve elektrokardiyografileri çektirildi. Faktör V, MTHFR, protrombin, β-Fibrinojen gen mutasyonları ve HPA, PAİ, APO-E, ADE gen polimorfizmlerine bakıldı. İnme ve kontrol grubunda karşılaştırılan bağımsız değişkenlerden istatistiksel olarak anlamlı bulunanlar lojistik regresyon analizine alındı.

Address for Correspondence/Yazışma Adresi: Nilgün Erten MD, Giresun University Faculty of Medicine, Department of Neurology, Giresun, Turkey
Phone: +90 454 310 10 00 E-mail: nderten@hotmail.com
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Bulgular: Çalışmamızın sonucunda inme ve kontrol grupları arasında HPA, PAİ, APO, ADE gen polimorfizmlerinin görülme sıklıkları arasında anlamlı bir fark görülmezken sadece faktör V H1299R, F V Leiden ve β Fibrinojen -455GA mutasyonlarının görülme oranları inme geçirmiş hasta grubunda Ki-kare testine göre anlamlı yükseklikte bulundu. Ancak lojistik regresyon analizine göre ise bu mutasyonların istatistiksel olarak anlamlılıkları kayboldu.

Sonuç: Çalışmamızdan elde edilen bulgular ışığında inme etiyopatogenezinin multifaktöriyel olduğunu ve protrombin gen mutasyonlarının ancak diğer risk faktörleri ile birlikte olduklarında bu faktörlerin neden olabilecekleri riski arttırabilecekleri düşünülmektedir. (Türk Nöroloji Dergisi 2015; 21:42-8)

Anahtar Kelimeler: İnme, protrombin gen mutasyonları, risk faktörleri

Introduction

Stroke, the third most common cause of death worldwide, is a multifactorial disease, and its incidence increases with age (1). Most cryptogenic strokes are due to hypercoagulability, responsible for 1% and 2-7% of all strokes in adults and young individuals, respectively (2). Factor V, prothrombin G20210A, methylenetetrahydrofolate reductase (MTHFR), and fibrinogen mutation, and plasminogen activator inhibitor 1 (PAI1), human platelet alloantigens (HPA), and angiotensin converting enzyme (ACE) polymorphisms cause primary hypercoagulability.

Active factor V (FVa) is the component of the prothrombinase complex that synthesizes thrombin. Active protein C degrades factor Va and thereby exhibits anticoagulant effects (3). The factor V Leiden (FVL) mutation prevents inactivation of factor Va by active protein C and increases predisposition to thrombosis (4,5). The factor V R2 variant is expressed at lower levels than normal factor V and causes resistance to active protein C (6). MTHFR enzyme deficiency due functional polymorphisms contributes to hyperhomocysteinemia, a prominent risk factor for stroke (7,8,9,10,11). The prothrombin G20210A mutation leads to a 30% increase in serum prothrombin levels compared to those in normal controls (12,13,14). The β-fibrinogen -455GA mutation is associated with high plasma fibrinogen levels, and individuals carrying this mutation have higher fibrinogen levels than individuals carrying the β-fibrinogen -455 GG and β-fibrinogen -455 GA mutations (15).

PAI-1 inactivates plasminogen, and defects in PAI-1 lead to severe bleeding after surgery and trauma, whereas high PAI-1 levels preclude plasminogen activation and lead to a tendency for thrombosis (16). The most frequent PAI1 polymorphisms are a guanine insertion (5G) and deletion (4G) at position 675 of the PAI1 promoter. The PAI1 4G/4G genotype leads to 25% higher PAI-1 levels than the PAI1 5G/5G genotype (17). Activated thrombocytes aggregate via glycoprotein (GP) 2b/3a receptors and fibrinogen to form the primary hemostatic plug (18). GP 3a, 1b, 2b and 1a, also named human platelet alloantigens (HPA)-1, -2, -3, and -5, respectively, are the major GPs in adhesion and aggregation (19,20). ACE converts angiotensin 1 to 2, leading to vascular hypertrophy and vasoconstriction, and reduces bradykinin, which exhibits vasodilator functions (21). ACE polymorphisms are defined by the presence (I allele) or absence (D allele) of a repetitive sequence in intron 16 (22,23). Homozygosity for the D allele causes 56% higher ACE activity than homozygosity for the I allele (24). A moderate increase in ACE activity is observed in the ACE I/D genotype (25). Genetic factors determine atherosclerosis development by regulating lipoprotein levels via apolipoproteins, such as apolipoprotein E (APOE), a ligand for low-density lipoprotein (LDL) receptors (26,27,28). The APOE allelic variants are ε2, ε3, ε4, and combinations of these alleles lead to the production of the isomorphic proteins E2, E3, and E4 (29). The presence of APOE4 leads to higher plasma LDL concentrations than the presence of APOE2, whereas the presence of both APOE2 and APOE4 leads to higher triglyceride concentrations than the presence of APOE3 (30,31).

The effect of prothrombin gene mutations in the etiopathogenesis of cerebrovascular events is not clear. This study aimed to compare the frequency of prothrombin gene mutations in ischemic stroke patients and controls.

Materials and Methods

This study included 212 ischemic stroke patients followed up at Research and Training Hospital Neurology Clinic, Süleyman Demirel University, between 2007 and 2009, and 238 individuals of similar age and same gender, but without a history of stroke. Ethical approval was obtained from the Institutional Ethics Committee of Süleyman Demirel University (14.01.2009, #7). All participants were asked to fill out a volunteer consent form.

The age, smoking habits, alcohol consumption, and diet of all participants were recorded. Dietary habits were defined as (a) primarily red meat consumption or (b) a Mediterranean diet (fruit, vegetable, and white meat consumption). Participants were questioned for a history of hypertension, diabetes, cardiac valve disease, and arrhythmia. Biochemical profiles of all patients were recorded. The distribution of ischemic stroke patients according to the "Trial of Org 10172 in Acute Stroke Treatment" (TOAST) was determined (Table 1).

Three milliliters of venous blood was collected in EDTA-containing tubes to analyze factor V, MTHFR, prothrombin, and beta-fibrinogen mutations and HPA, PAI1, APOE, and ACE polymorphisms. Genomic DNA sequences were amplified by multiplex polymerase chain reaction, and mutations were analyzed by reverse in situ hybridization with "Cardiovascular Disease Strip Assay" (Vienna Lab, Austria).

Table 1. Distribution of patients with ischemic stroke according to "Trial of Org 10172 in Acute Stroke Treatment" criteria

Type of stroke	n (%)
Cause undetermined	108 (50.9)
Lacunar infarction	59 (27.8)
Cardioembolism	26 (12.3)
Large artery atherosclerosis	19 (9.0)
Due to other causes	-
Total	212 (100.0)

Data have been presented as counts, percentages, mean, standard deviation, and minimum and maximum values. Data were analyzed with SPSS 15.0 software by using descriptive statistics, chi-square, independent samples t-test, and logistic regression test. Statistically significant differences were further analyzed by a logistic regression test. P<0.05 was considered statistically significant.

Results

The age of the stroke patients ranged between 17 and 96 years 102 patients were female and 110 male. The age of the controls ranged between 28 and 90 years; 130 patients were female and 108 male. Gender distribution did not significantly differ between the stroke and the control groups (p=0.142). The mean age was higher in the stroke than the control group (p=0.002). Mean systolic and diastolic blood pressures were higher in stroke patients than in controls (p=0.041 and p=0.501, respectively). The incidence of individuals adhering to a Mediterranean diet was lower in the stroke than in the control group (p=0.016). The incidence of arrhythmia, cardiac failure, and atrial fibrillation and of other diseases such as cardiac valve and coronary artery disease in stroke patients was higher than in controls (p<0.001 and p<0.001, respectively). There were more male individuals and the incidence of smoking, low alcohol consumption, and diabetes, and lipid levels were higher in the stroke than in the control group (p>0.05). The mean glucose, blood urea nitrogen (BUN), homocysteine, and free T4 levels in stroke patients were higher than those in the control group (p<0.05), whereas the mean total and high density lipoprotein (HDL) cholesterol, free T3, and thyroid stimulating hormone (TSH) levels in stroke patients were lower than those in controls (p<0.05, Table 2).

Among the independent variables tested in the established model, the effects of HDL cholesterol, T3 and T4 thyroid hormones, and arrhythmia were statistically significant (p<0.05). According to the odds ratio, one unit decrease in HDL cholesterol level increased stroke risk by 0.9-fold. One unit decrease in T3 and T4 caused a 0.5- and 6.4-fold increase in stroke risk, respectively. The stroke risk for patients exhibiting arrhythmia was 5.9-fold higher than that for patients who did not have arrhythmia. Conversely, the effects of other independent variables on the model were not significant (Table 3, 4, and 5).

Discussion

The impact of prothrombin mutations in venous thromboembolisms is clear, and current research focuses on their potential role in arterial stroke. However, epidemiological studies investigating the correlation of prothrombin mutations and ischemic stroke are controversial.

Several studies have reported that FVL mutation is not an important risk factor for ischemic stroke (32,33,34,35,36). In contrast, the present study demonstrated a higher frequency of homozygous and heterozygous FVL mutations in stroke patients than in controls. The frequency of this mutation in stroke patients was higher than that reported in previous studies (33,34,35,36,37,38,39). Notably, the frequency of the FVL mutation in healthy individuals was similar to that of the general society. Furthermore, factor V mutations might increase stroke risk in young patients presenting with stroke originating from a cardiac embolism (40). The FVL mutation might slightly increase the risk of arterial thrombotic events compared to that of venous thromboembolism, and the effect is more pronounced in people younger than 55 and in women (41). The reported higher FVL mutation frequency in ischemic stroke patients suggested its relevance in ischemic stroke development, although it did not increase risk in the presence of other risk factors.

Previous research indicates that factor V R2 mutation increases thrombosis risk although it is not a risk factor for cerebrovascular diseases (42,43). However, the number of studies was limited. In the present study, the frequency of factor V R2 mutation was significantly higher in ischemic stroke patients than in controls, suggesting an important role for this mutation in ischemic stroke development. However, in the presence of other risk factors, the factor V R2 mutation does not pose a significant risk.

The relationship between the MTHFR C677T mutation and ischemic stroke remains unclear (44). MTHFR mutations cause a slightly higher risk of arterial thrombotic events than of venous thromboembolism (41,45). In accordance with previous findings, our study indicates that the MTHFR C677T mutation is frequent in healthy individuals and that there is no association between this mutation and stroke (46,47). Several studies reported that the MTHFR C677T mutation is not a risk factor for ischemic stroke (44,46,48). Conversely, this mutation is associated with stroke in Asian populations (45,49). Similarly, a report on young patients who experienced a stroke originating from a cardiac embolism indicated that the MTHFR gene mutation increases stroke risk (40). We did not observe an association of the MTHFR A1298C and C677T mutations with stroke, confirming a study addressing prevalence of the A1298C mutation in a healthy population in Turkey, and suggesting that MTHFR mutations are not a risk for ischemic stroke (47).

The role of the prothrombin G20210A mutation in ischemic stroke is controversial (34,40,41,48,50,51,52,53). The present study shows that the prothrombin G20210A mutation is not associated with stroke and is not important in the case of ischemic stroke.

Although previous studies demonstrated that the β-fibrinogen-455 GA mutation increases ischemic stroke risk, the findings are controversial (54,55,56,57). Although our results indicate that the β-fibrinogen -455 GA mutation is associated with ischemic stroke, this mutation did not pose significant risk in the presence of other risk factors.

PAI1 polymorphisms are a risk factor for ischemic stroke, whereas the PAI1 4G/4G polymorphism might protect from ischemic stroke (58,59,60,61,62,63). Venous thromboembolism is more frequent than ischemic stroke in young individuals, and reduced fibrinolytic capacity due to elevated PAI-1 levels predisposes to venous thromboembolism but not to ischemic stroke (64).

The significance of HPA polymorphisms in stroke development is controversial. HPA polymorphisms are associated with stroke; heterozygosity for the GP 3a A2 allele is a risk factor for atherosclerotic stroke in young individuals, and the HPA3 b/b polymorphism and homozygosity for the HPA1b polymorphism are associated with ischemic stroke (65,66,67). However, HPA

polymorphisms are not associated with hemorrhagic stroke, and the GP 3a A2 allele is not a risk for myocardial infarction, stroke, or venous thrombosis (67,68). Previous work indicated that the GP 3a polymorphism is a moderate risk factor for large vein atherosclerosis-induced stroke and revealed that heterozygosity for the GP 3a A2 allele is an independent risk factor (69). Furthermore, GP 1a (HPA5) is correlated weakly with ischemic stroke (70). In the present study, the frequency of the GP 3a A2 polymorphism did not differ between stroke patients and controls, indicating that the GP 3a A2 polymorphism is not a risk factor for ischemic stroke.

Multiple studies have suggested that the ACE I/D polymorphism is not a risk factor for ischemic stroke, although the frequency of I/I and D/D polymorphisms differs between stroke patients according to sex (22,71,72). In agreement with these findings, we did not observe a difference in the frequency of the ACE I/I, I/D, and D/D polymorphisms between stroke patients and controls, confirming that this polymorphism is not a risk factor for ischemic stroke. However, homozygosity for the ACE D/D polymorphism confers a higher risk for stroke than the I/D and I/I genotypes (73,74).

The frequency of the APOE ε4 allele is higher in stroke patients than in controls (75,76,77). Conversely, other studies reported that the APOE ε4 allele does not increase ischemic stroke risk (78,79). No significant difference has been reported between hemorrhagic and ischemic stroke patients and controls for the frequency of the APOE, ε2/ε2, ε2/ε3, ε3/ε3, and ε3/ε4 polymorphisms (71). Data from the present study support the notion that APOE gene polymorphisms are not associated with stroke.

Table 2. Comparison of the data for the variables for stroke patients and controls

Variables	Ischemic stroke	Control	p
Age	65.80±14.41	61.82±13.22	0.002
Sex, female (%)	47.9	54.6	0.142
Mediterranean diet (%)	60.1	70.6	0.016
Smoking (%)	38.2	37.0	0.777
Alcohol (%)	13.9	17.2	0.312
Diabetes (%)	29.8	23.5	0.120
Hypertension (%)	60.5	42.9	0.000
Hyperlipidemia (%)	27.7	22.7	0.205
Cardiac issues (%)	39.5	18.5	0.000
Systolic blood pressure, mmHg	141.03±26.05	136.30±24.24	0.041
Diastolic blood pressure, mmHg	83.7±11.23	83.15±9.66	0.501
Cardiac Arrhythmia (%)	26.1	3.8	0.000
Glucose (mg/dl)	121.30±44.67	106.10±42.52	0.000
BUN (mg/dl)	22.09±13.08	18.68±12.10	0.003
Creatinine (mg/dl)	0.98±0.39	0.96±0.28	0.550
Total cholesterol (mg/dl)	181.78±48.39	193.42±48.96	0.009
HDL cholesterol (mg/dl)	40.35±12.21	48.70±14.46	0.000
LDL cholesterol (mg/dl)	113.72±37.21	115.92±41.73	0.544
VLDL cholesterol (mg/dl)	28.07±17.15	29.96±18.86	0.253
Triglyceride (mg/dl)	140.50±85.68	149.85±94.34	0.258
Vitamin B12 (pg/ml)	473.81±363.70	478.89±388.57	0.883
Homocysteine (µmol/l)	14.23±7.39	12.87±6.78	0.039
fT3 (pg/ml) (1.71-3.71)	2.48±1.01	2.78±0.60	0.000
fT4 (ng/dl) (0.71-1.47)	1.19±0.84	0.91±0.25	0.000
TSH (pg/ml) (0.35-4.94)	1.16±1.70	1.56±2.29	0.032

LDL: Low-density lipoprotein, HDL: High density lipoprotein, TSH: Thyroid stimulating hormone, BUN: Blood urea nitrogen

Table 3. Frequency of prothrombin mutations in stroke patients and controls

Mutation Type	Ischemic Stroke		Control	
	Heterozygous (%)	Homozygous (%)	Heterozygous (%)	Homozygous (%)
Factor -V (H1299R)**	43.7	-	20.6	0.4
B- Fibrinogen (-455G>A)**	39.9	5.9	43.7	1.3
MTHFR (A1298C)	26.5	10.9	41.2	16.4
Factor -XIII (V34L)	41.2	1.7	25.6	3.4
Factor -V Leiden (G1691A)**	13.9	1.3	8.8	-
MTHFR (C677T)	32.8	22.7	23.5	28.2
Prothrombin (G20210A)	6.3	-	4.2	-

**Chi-square test, $p<0.05$. MTHFR: Methylenetetrahydrofolate reductase

In conclusion, the results indicated that only factor V H1299R, FVL, and β-fibrinogen -455GA mutations are associated with stroke, and prothrombin gene mutations are stroke risk factors only when they occur in association with other factors.

Ethics Committee Approval: The study were approved by the Süleyman Demirel University of Local Ethics Committee (Ethics Board Decision; dated 14.01.2009 No. 7).

Informed Consent: Consent form was filled out by all participants.

Concept: Nilgün Erten, Serpil Demirci

Design: Nilgün Erten, Serpil Demirci

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Literature Search: Nilgün Erten

Table 4. Frequency of prothrombotic gene polymorphisms in stroke patients and controls

Polymorphism type	Phenotype	Groups	
		Ischemic stroke (%)	Control (%)
PAI1	4G/4G	22.3	25.2
	4G/5G	70.2	62.2
	5G/5G	7.6	12.6
HPA1	a/a	76.1	75.6
	a/b	22.7	22.7
	b/b	1.3	1.7
ACE	I/I	16.0	16.4
	I/D	53.4	51.3
	D/D	30.7	32.4
APOE	2/2	0.4	0.4
	2/3	9.7	12.2
	2/4	0.4	1.7
	3/3	73.1	68.9
	3/4	16.4	16.8

ACE: Angiotensin converting enzyme, APOE: Apolipoprotein E, PAI1: Plasminogen activator inhibitor 1, HPA: Human platelet alloantigens

Table 5. Results of logistic regression analysis for the patients

Variables	B	p	Odds ratio	95% Confidence interval (Cl)
Nutritional Status(1)	0.330	0.165	1.39	0.87-2.22
Hypertension(1)	0.459	0.052	1.58	0.99-2.52
Cardiac issues(1)	0.198	0.481	1.22	0.70-2.11
ECG Rhythm Disorder(1)	1.768	0.000	5.86	2.44-14.09
Age	-0.007	0.457	0.99	0.97-1.01
Glucose (mg/dl)	0.005	0.068	1.01	1.00-1.01
BUN (mg/dl)	-0.004	0.722	0.99	0.98-1.02
Total cholesterol (mg/dl)	0.001	0.749	1.00	0.99-1.01
HDL cholesterol (mg/dl)	-0.037	0.000	0.96	0.94-0.98
Homocysteine (µmol/l)	0.026	0.123	1.03	0.99-1.06
fT3 (pg/ml)	-0.604	0.000	0.55	0.41-0.74
fT4 (ng/dl)	1.865	0.000	6.45	2.84-14.65
TSH (pg/ml)	-0.030	0.604	0.97	0.86-1.08
Factor V (H1299R)		0.205		
Factor V (H1299R)(1)	0.450	0.075	1.57	0.96-2.58
Factor V (H1299R)(2)	-20.508	1.000	0.00	0.00-0.00
β-Fibrinogen (-455GA)		0.147		
β-Fibrinogen (-455GA)(1)	-0.263	0.252	0.77	0.49-1.20
β-Fibrinogen (-455GA)(2)	1.010	0.161	2.75	0.67-11.25
FVL (G1691A)		0.656		
FVL (G1691A)(1)	0.346	0.359	1.41	0.67-2.96
FVL (G1691A)(2)	21.981	0.999	0.00	0.00-0.00
Stroke (fixed)	0.125	0.900		

FVL: Factor V Leiden, HDL: High density lipoprotein, TSH: Thyroid stimulating hormone, BUN: Blood urea nitrogen

(1)Each unit represents an increase or heterozygous mutations. (2)Represents a homozygous mutation. Coefficients (B), significance levels, odds ratio, and 95% confidence interval in logistic regression analysis.

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