Cumhuriyet Tıp Derg 2010; 32: 61-66
Cumhuriyet Med J 2010; 32: 61-66

The distribution of FV Leiden mutation in patients with obstructive sleep apnea

Obstruktif uyku apneli hastalarda FV Leiden mutasyonun dağılımı

Taha T. Bekçi, Nadir Koçak, Filiz Özen, Turgut Teke

Clinics of Chest Disease (T. T. Bekçi, MD, Specialist) and Genetic (N. Koçak, MD, Specialist), Konya Education and Research Hospital, TR- 42090 Konya; Clinic of Genetic (F. Özen, MD, Specialist), Göztepe Education and Research Hospital, TR-34722 İstanbul; Department of Chest Disease (T.Teke), Selçuk University School of Medicine, TR-42080 Konya

Abstract

Aim. We aimed to investigate whether this polymorphism has any effects on the development of the differences in the progression of the OSAS patients. **Methods**. The patient group was selected from patients admitted to the our Sleep Laboratory. Total 122 subjects, 44 OSAS patients (only), 32 OSAS patients with cardio vascular diseases (CVD) and 46 controls, were enrolled in the study. The control group consisted of healthy volunteers who had no symptoms of OSAS and scored 0 on the Epworth sleepiness scale. In order to determine gene mutations, DNA isolation was performed from peripheral blood samples. **Results**. While mean weight and BMI were significantly different, no significant differences were found between patient and control groups in terms of FV Leiden allele distributions. **Conclusion**. FV Leiden mutation does not affect progression in patients with OSAS

Keywords: Obstructive sleep apnea, polymorphism, mutation

Özet

Amaç. Bu çalışmada OSAS hastalarının progresyonunda farklılıklar oluşmasında FV Leiden polimorfizminin herhangi bir etkisi olup olmadığını araştırmayı amaçladık. **Yöntem.** Hasta grubu uyku laboratuvarımıza başvuran hastalardan seçildi. Toplam 122 kişi (44 OSAS hastası (yalnızca), 32 kardiyovasküler hastalığı (KVH) olan OSAS hastası ve 46 kontrol grubu) çalışmamıza katıldı. Kontrol grubu OSAS semptomları bulunmayan, Epworth uykusuzluk skalası 0 olan sağlıklı gönülülerden oluşturuldu. Gen mutasyonlarını belirlemek için DNA izolasyonu periferik kandan yapıldı. **Bulgular**. BMI ve ağırlık ortalamaları önemli oranda farklıyken, FV Leiden allel dağılımları açısından hasta ve kontrol grupları arasında önemli bir farklılık bulunamadı. **Sonuç**. FV Leiden mutasyonu OSAS hastalarında progresyonu etkilememektedir

Anahtar sözcükler: Obstruktif uyku apnesi, polimorfizm, mutasyon

Geliş Tarihi/Received: December 9,2009; **Kabul Tarihi/Accepted**: December 21,2009

Corresponding address:

Dr. Nadir Koçak, Tıbbi Genetik Kliniği, Konya Eğitim ve Araştırma Hastanesi., TR- 42090 Konya. Email: nadirkocak@yahoo.com

Introduction

Obstructive sleep apnea syndrome (OSAS) is characterized by attacks of hypopnea and apnea during sleep. These attacks occur repeatedly throughout sleep and result in severe hypoxemia and hypercapnia [1].

Cardiovascular diseases (CVDs), such as hypertension, coronary artery disease, atherosclerosis, and stroke, are frequently encountered in patients with OSAS. In such

patients, the hypoxemic attacks observed during sleep are considered as the basis for the rise in the incidence of CVDs [2, 3]. Moreover, obesity, high blood pressure, and advanced age, which are common in OSAS patients, also contribute to the development of CVDs [4, 5]. Although the exact mechanism is not known, chronic intermittent hypoxia has been reported to trigger the vasoconstrictive, proinflammatory and procoagulant mechanisms in the cardiovascular system [6, 7]. Among these mechanisms, particular attention should be paid to the procoagulant factors, including factor V Leiden (FVL), one of the important glycoproteins in the coagulation cascade, due to the crucial role in the pathogenesis of CVDs.

The FVL mutation is currently known to be associated with an increased risk for recurrent venous, and probably, arterial thrombosis. Recurrent thrombosis is observed in both heterozygous and homozygous subjects for FVL at a high rate. The risk for thrombosis has been reported to be increased 5-10-fold in heterozygous subjects for FVL, whereas 50-100-fold in homozygous subjects for FVL [5, 8]. The 80-kb FVL gene is localized on chromosome 1q21-25 and consists of 24 introns and 25 exons, varying in size from 72-2820 bp. Transcription of the gene results in a 6,8-kb mRNA [9]. The G1691A mutation on exon 10 of the FVL gene leads to the substitution of guanine at position 1691 to adenine. This exchange expresses itself in the amino acid chain of the FVL molecule and leads to the replacement of arginine by glutamine at position 506 (FVR506Q). While this mutation is called FV R506Q or FV G1691A, the mutant allele is called FV: Q506 or FVL [10].

Activated protein C (APC) inactivates the FVa protein by cleaving the chain at arginine at positions 679, 506 and 306, and regulates the coagulation. However, the FVL mutation leads to termination of the cleavage at position 506. The mutant FV can be inactivated by cleavage at position 306; however, this process is approximately 10-fold slower. Thus, the FV molecule becomes resistant against proteolytic inactivation, so-called activated protein C resistance (APRC; 5). APCR causes a delay in the inactivation of activated FV and consequently leads to excessive coagulation.

It is obvious that the inclusion of other pathologic factors to the pre-existing procoagulant, pro-inflammatory, and vasoactive bases in the patients with OSAS would negatively affect the progression of the disease. The fact that the FVL mutations are associated with CVDs, myocardial infarction (MI), deep vein thrombosis, and arterial thrombosis gives prominence to this mutation [8, 11, 12]. Considering the above-mentioned points, we aimed to determine the distribution of the FVL polymorphism, which is among the risk factors for CVD in patients with OSAS.

Methods

One hundred twenty-two subjects, including 76 patients with OSAS and 46 healthy controls, were included in the study. The patient group consisted of patients who were admitted to Konya Training and Research Hospital Sleep Laboratory and were diagnosed with OSAS. The patient group was allocated to two groups including OSA patients with and without cardiovascular morbidities [OSA-CVD (n=32) and OSA-only (n=44), respectively]. There were 25 patients with a history of MI, 3 patients with a history of cerebrovascular accidents, and 6 patients with a history of peripheral vascular disease in the OSA-CVD group. The control group consisted of healthy volunteers who had no symptoms of OSAS and scored 0 on the Epworth sleepiness scale. The body mass index (BMI) of all subjects was calculated based on height and body weight measurements. Informed consent was obtained from all subjects before their inclusion in the study.

Polysomnography

At least one full-night polysomnography (PSG) was performed on all patients. Electroencephalography (EEG), submental electromyography (EMG), leg EMG, electrooculography (EOG), and electrocardiography (ECG) recordings were obtained; air-flow was measured using a nasal cannula (NC), oxygen saturation (SaO2) was measured

using a pulse oximeter, and chest and abdominal respiratory movements were monitored. A reduction in oxygen saturation to ≤ 4 or the occurrence of symptoms of physiologic awakening, following at least a 30% reduction in air flow for a minimum of 10 sec, was considered as hypopnea. Individuals with an apnea hypopnea index (AHI) >5 were diagnosed as OSAS and included in the study.

Determination of Gene Mutations

Genomic DNA was isolated from peripheral blood samples using a QIAamp DNA Blood Mini Kit (catalog number 51306; Qiagen, Australia). A genomic polymerase chain reaction (PCR) of a 267-base pair (bp) fragment in exon 10 of the factor V gene was performed using the following primers: the forward primer, 5'-TGCCCAGTGCTTAACAAGACCA-3', and the reverse primer, 5'-TGTTATCACACTGGTGCTAA-3' [13, 14]. Amplification was carried out in 10 µl of reaction mixture, which contained 10 mM Tris–HCl (pH 8,3), 50 nM KCl, 1,5 mM MgCl2, 0,01% gelatin, 50 ng of template DNA, 200 µM of each dNTP, 200 nM of each primer, and 0,2 IU of Taq DNA polymerase (Boehringer Mannheim, Germany). The PCR was performed using a thermal cycler (Corbett Research, Sydney, Australia) under the following conditions: denaturation for 1 min at 94°C; then 35 cycles for 40 s at 94°C, 40 s at 55°C, and 40 s at 72°C. A portion of the PCR products was digested with Mnl I and electrophoresed on 4% agarose gel in Tris-acetate-EDTA buffer. The fragments were stained with ethidium bromide and visualized using ultraviolet light. Normal alleles with a guanine at nucleotide 1691 yielded 163, 67, and 37 bp fragments, while FVL alleles (substitution of the guanine to adenine) yielded 200 and 67 bp fragments.

Statistical Analysis

Data were analyzed using the SPSS 15.0 package program (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to test whether the sample is normally distributed, and a one-way ANOVA test was used for homogeneity of data. One-way ANOVA and Tukey tests were used for parametric comparisons, while an independent chi-square test was used for non-parametric comparisons. A p value <0,05 was considered statistically significant.

Results

There were no significant differences between patient groups and controls in terms of age (42,3±13,1, 41,9±8,2 and 45,4±7,4, respectively), whereas there was a significant difference in terms of body weight (72,3±8,1, 80,2±10 and 82,8±8,7, respectively) and BMI values (25,9±3,1, 28,7±2,4 and 28,9±4,6, respectively; p<0,05). However, no such difference was observed between patient groups (p>0,05). Moreover, patient groups were similar in terms of AHI values (36,3±17,1 and 42,1±19,5, respectively; Table 1).

Table 1. Demographic and Clinical Data of Study Groups*.

	Controls (Mean±SD)	OSAS-Only (Mean±SD)	OSAS-CVD (Mean±SD)	P value*
Age,(yr)	42,2 ± 13,1	41,9 ± 8,1	45,3 ± 7,3	0,3
Weight, (kg)	72,3 ± 8.1	80,2 ± 10	82,8 ± 8,7	<0,01
BMI, kg/m2	25,9 ± 3,1	28,7 ± 2,4	28,9 ± 4,6	<0,01
AHI, events/h		36,3 ± 17,1	42,1 ± 19,5	0,5

*Data are presented as mean (SD), or mean. **One-way-ANOVA and Tukey analysis, OSAS= obstructive sleep apnea syndrome, AHI= apnea hypopnea index, BMI= body mass index, OSAS-only = OSAS patients without any cardiovascular morbidity, OSAS-CVD= OSAS patients with Cardiovascular disease

No significant difference was observed between groups in terms of the FV A1691G polymorphism (p>0,05). While there were 43 (93,5%) subjects with AA genotype and 3 (6,5%) subjects with AG genotype in the control group, there were 39 (92,9%) subjects with AA and 3 (7,1%) subjects with AG genotype in the OSA-only group, and 27 (79,4%) subjects with AA, 6 (17,6%) subjects with AG, and 1 (2,9%) subject with GG

genotype in the OSA-CVD group. While A allele was present in 9 (96,7%), 81 (96,4%) and 60 (88,2%) subjects, G allele was present in 3 (3,2%), 3 (3,5%) and 8 (11,7%) subjects in control, OSA-only and OSA-CVD groups, respectively (Table 2).

Table 2. Genotype and Allele Frequencies of Factor V Leiden Polymorphism in Controls and OSAS patients.

	Controls n (%)	OSAS-only n (%)	OSAS-VCD n (%)
Factor V Leiden*Genotype			
1691AA	43 (% 93,5)	39 (% 92,9)	27 (% 79,4)
1691AG	3 (% 6,5)	3 (% 7,1)	6 (% 17,6)
1691GG	0 (% 0)	0 (% 0)	1 (% 2,9)
Factor V Leiden Allel			
A	89 (% 96,7)	81 (% 96,4)	60 (% 88,2)
G	3 (% 3,2)	3 (% 3,5)	8 (% 11,7)

*Chi-square analysis ($p > 0,05$). OSAS= obstructive sleep apnea syndrome, OSAS-only = OSAS patients without any cardiovascular morbidity, OSAS-CVD= OSAS patients with Cardiovascular disease.

Discussion

The hypoxia attacks that are characteristic for the patients with OSAS cause an increase in reactive oxygen species, which lead to endothelial dysfunction by generating oxidative stress on the vascular endothelium [15, 16]. Consequently, as the response to cholinergic stimulation, such as acetylcholine, decreases the vasoconstrictive response to angiotensin II increases [17, 18]. The vascular endothelial dysfunction also leads to an increase in potent, long-acting vasoconstrictors, such as endothelin I [19].

Beside the vascular and sympathetic nervous systems, the coagulation cascade is also affected in patients with OSAS. The total serum fibrinogen and blood viscosity are increased in such patients [20]. Furthermore, platelet activity and aggregation are increased in those patients [21]. Decreased fibrinolytic activity observed in patients with OSAS has been suggested to be associated with an increase in the level of plasminogen activator inhibitor (PAI) [22]. Beside increased thrombin-antithrombin III levels, increased coagulation factors such as XXa and VIIa was also observed in patients with OSAS [23].

While the prevalence of FVL mutation is 3%-7% in the normal population, it varies from 30% to 60% among patients with a history of a venous thromboembolism (VTE). In the majority of papers, it has been stated that the phenotype of APC resistance and the FV: Q506 allele increase the risk for venous thrombosis. The clinical findings vary according to the genotype [23, 24]. Although the risk for VTE is 3-8-fold higher in heterozygous subjects than normal subjects, this rate is 30-140-fold higher in homozygous subjects. Zöller et al. [25] have reported that 8% of normal subjects, 20% of heterozygous subjects for FVL, and 40% of homozygous subjects for FVL experience venous thrombosis before 33 years of age. The clinical signs of hereditary APCR are similar to those of protein C, protein S, and antithrombin deficiencies. The risk for thrombosis is about 60%-70% in families with hereditary APCR in the presence of negative environmental conditions [5, 25, 26].

In studies on various risk factors FVL mutation was determined to increase the incidence of arterial thrombosis and MI together with age, diabetes mellitus, cigarette smoking and/or alcohol consumption, hypertension, obesity, and hypercholesterolemia [24]. The risk for venous thrombosis, peripheral vascular diseases, paralysis, recurrent miscarriage, pulmonary embolism, and MI is increased in subjects with a FVL mutation. Therefore, the screening of subjects at high risk for thrombophilia and CVDs is of crucial importance [29]. Rosendal [5, 30] determined that the FVL mutation was frequent among

patients who experienced MI before 50 years of age, and reported that the risk for MI was 30-fold greater in young female cigarette smokers with a FVL mutation, even in the absence of other risk factors.

In the current study, although no statistical difference was observed between the OSA patients with and without cardiovascular morbidities in terms of the FVL mutation, we suggest that the FVL mutation, which, alone, possesses a remarkable risk for CVD, would possess a greater risk in the presence of OSAS that predisposes to CVD. The absence of health problems in whole subjects with FVL mutation despite of its autosomal dominant pattern of inheritance has been suggested to be associated with the decrease in penetrance [27, 28]. In R506Q studies, it was emphasized that the fact that the decrease in the penetrance could conceal the risk for coagulation [24].

Therefore, the absence of a statistically significant relationship in the etiologic research concerning diseases, such as OSAS involving multifactorial processes and pathologies, does not require these etiologies to be ignored. Beside factors, such as the extensiveness of the related disease, the rate of diagnosis and possibility of coexistence with other diseases, the extensiveness of the mutation, penetration rate, effects of the heterozygous conditions and the relationship with other diseases should be considered during evaluations. A non-etiologic factor should not be considered as a factor which does not possess a risk.

In conclusion, although no statistical difference was observed between the normal subjects and patients with OSAS in terms of the distribution of the FVL mutation, the distribution of this mutation might show variations in larger patient populations. Furthermore, it should be considered that such a mutation may have an influence on triggering of the procoagulant mechanisms in OSAS.

References

1. Kato M, Adachi T, Koshino Y, Somers VK. Obstructive sleep apnea and cardiovascular disease. Circ J 2009 ; 73:1363-70.
2. Lavie L. Sleep-disordered breathing and cerebrovascular disease: a mechanistic approach. Neurol Clin 2005; 23:1059-75.
3. Suzuki YJ, Jain V, Park AM, Day RM. Oxidative stress and oxidant signaling in obstructive sleep apnea and associated cardiovascular diseases. Free Radic Biol Med 2006;40:1683-92.
4. Peker Y, Kraiczi H, Hedner J, Löth S, Johansson A, Bende M. An independent association between obstructive sleep apnoea and coronary artery disease. Eur Respir J 1999; 14: 179-84.
5. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 1995; 85: 1504-8.
6. Akinnusi ME, Paasch LL, Szarpa KR, Wallace PK, El Solh AA. Impact of nasal continuous positive airway pressure therapy on markers of platelet activation in patients with obstructive sleep apnea. Respiration 2009; 77: 25-31.
7. Schulz R. The vascular micromilieu in obstructive sleep apnoea. Eur Respir J 2005; 25: 780-2.
8. März W, Seydewitz H, Winkelmann B, Chen M, Nauck M, Witt I. Mutation in coagulation factor V associated with resistance to activated protein C in patients with coronary artery disease. Lancet 1995; 345: 526.
9. Rees DC. The population genetics of factor V Leiden (Arg506Gln). Br J Haematol 1996; 95: 579-86.
10. Ornstein DL, Cushman M. Cardiology patient page. Factor V Leiden. Circulation 2003;107: 94-7.
11. Araújo F, Santos A, Araújo V, Henriques I, Monteiro F, Meireles E, Moreira I, David D, Maciel MJ, Cunha-Ribeiro LM. Genetic risk factors in acute coronary disease.

Haemostasis 1999; 29: 212-8.

12. Dutta TK, Venugopal V. Venous thromboembolism: the intricacies. J Postgrad Med 2009; 55: 55-64.
13. Gelhaus A, Urban B, Pirmez C. DNA extraction from urea-preserved blood or blood clots for use in PCR. Trends Genet 1995; 11:41.
14. Bertina RM, Koeleman BP, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64-7.
15. Lavie L. Obstructive sleep apnoea syndrome-an oxidative stress disorder. Sleep Med Rev 2003; 7: 35-51.
16. Schulz R, Mahmoudi S, Hattar K, Sıbelıus U, Olschewskı H, Meyer K, Seeger W, Grımmınger F. Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Impact of continuous positive airway pressure therapy. Am J Respir Crit Care Med 2000; 162: 566-70.
17. Carlson JT, Rångemark C, Hedner JA. Attenuated endothelium-dependent vascular relaxation in patients with sleep apnoea. J Hypertens 1996; 14: 577-84.
18. Kraiczi H, Hedner J, Peker Y, Carlson J. Increased vasoconstrictor sensitivity in obstructive sleep apnea. J Appl Physiol 2000; 89: 493-8.
19. Phillips BG, Narkiewicz K, Pesek CA, Haynes WG, Dyken ME, Somers VK. Effects of obstructive sleep apnea on endothelin-1 and blood pressure. J Hypertens 1999; 17: 61-6.
20. Chin K, Ohi M, Kita H, Noguchi T, Otsuka N, Tsuboi T, Mishima M, Kuno K. Effects of NCPAP therapy on fibrinogen levels in obstructive sleep apnea syndrome. Am J Respir Crit Care Med 1996; 153: 1972-6.
21. Bokinsky G, Miller M, Ault K, Husband P, Mitchell J. Spontaneous platelet activation and aggregation during obstructive sleep apnea and its response to therapy with nasal continuous positive airway pressure. A preliminary investigation. Chest 1995; 108: 625-30.
22. Rångemark C, Hedner JA, Carlson JT, Gleerup G, Winther K. Platelet function and fibrinolytic activity in hypertensive and normotensive sleep apnea patients. Sleep 1995; 18: 188-94.
23. Robinson GV, Pepperell JC, Segal HC, Davies RJ, Stradling JR. Circulating cardiovascular risk factors in obstructive sleep apnoea: data from randomised controlled trials. Thorax 2004; 59: 777-82.
24. Segev A, Ellis MH, Segev F, Friedman Z, Reshef T, Sparkes JD, Tetro J, Pauzner H, David D. High prevalence of thrombophilia among young patients with myocardial infarction and few conventional risk factors. Int J Cardiol 2005; 98: 421-4.
25. Zöller B, Svensson PJ, He X, Dahlbäck B. Identification of the same factor V gene mutation in 47 out of 50 thrombosis-prone families with inherited resistance to activated protein C. J Clin Invest 1994; 94: 2521-4.
26. Joseph L. Pathogenesis of thrombosis. In: Beutler E, Lichtman MA, Coller BS, Kipps TS (Eds) Williams Hematology. 5th ed. New York: McGraw-Hill Inc. 1995: 1525-31.
27. Koeleman BP, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. Blood 1994; 84:1031-5.
28. Zöller B, Dahlbäck B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. Lancet 1994; 343: 1536-8.
29. Griffin JH, Evatt B, Wideman C, Fernández JA. Anticoagulant protein C pathway defective in majority of thrombophilic patients. Blood 1993; 82: 1989-93.
30. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. Blood 1997; 90: 1747-50.