American Journal of Men's Health
2018, Vol. 12(6) 2152–2156

DOI: 10.1177/1557988318801158
journals.sagepub.com/home/jmh
SAGE

The Effect of PAI-1 Gene Variants and PAI-1 Plasma Levels on Development of Thrombophilia in Patients With Klinefelter Syndrome

Burcin Erkal[1], Aysel Kalayci Yigin[1], Sukru Palanduz[2], Selcuk Dasdemir[1], and Mehmet Seven[1]

Abstract

Klinefelter syndrome (KS) is a common sex chromosome-related abnormality seen among men. KS negatively affects spermatogenesis and testosterone production. It increases the risk of thrombosis but its molecular mechanism has not been well described yet. Elevated PAI-1 is a risk factor for thrombosis. The rs1799889 polymorphism located in the promoter region of the PAI-1 gene was detected in patients with deep venous thrombosis. In this study, the PAI-1 gene variant and its plasma levels in KS patients were examined. Forty-one KS patients (47, XXY) and 50 age-matched healthy controls participated. DNA was isolated from peripheral blood and a real-time PCR method was used to detect known SNPs in the PAI-1 gene. In addition, PAI-1 plasma levels were measured by using ELISA method. There was no significant difference between PAI-1 gene polymorphisms of KS patients and controls ($p > .05$). The significant difference was observed in PAI-1 plasma levels between two groups (high PAI-1 plasma level in KS patients compared to controls). The patients' group mean was 55.13 and control group mean in PAI-1 level was 29.89 ng/ml ($p = .020$). Clinical features related to thromboembolism especially varicose veins were detected in KS patients frequently ($p = .04$). These results suggest that thromboembolism related to clinical features is seen more frequently in cases with KS, but it may not be dependent only on the PAI-1 gene polymorphism structure.

Keywords

Klinefelter syndrome, physiological and endocrine disorders, PAI-1 polymorphism, thrombosis, varicose vein

Received May 23, 2018; revised July 15, 2018; accepted August 13, 2018

Klinefelter syndrome (KS) is a disease with chromosomal abnormality detected only in men. KS as a sex chromosomal abnormality is a common genetic disease and can be seen in every 1 in 500–1000 births throughout all ethnic groups. According to karyotype analyses, these patients have an extra one or more X chromosomes. Eighty percent of these patients have 47, XXY karyotype; others have 46, XY/XXY mosaicism, chromosomal aneuploidies, and structurally damaged X chromosome (Grabski, Pusch, & Schirren, 1979; Lanfranco, Kamischke, Zitzmann, & Nieschlag, 2004).

Clinodactyly and other malformations like undescended testes and cleft palate may manifest at higher frequencies in patients with extra X chromosomes. Besides, KS patients have risks of several complications such as epilepsy, abdominal obesity, taurodontism, osteoporosis, cancer, systemic lupus erythematosus, and heart disease. In addition, these patients suffer from vascular diseases, including varicose veins, thromboembolism, and pulmonary embolism during their life span (Nieschlag & Behre, 2004; Paduch, Fine, Bolyakov, & Kiper, 2008; Zöller, Ji, Sundquist, & Sundquist, 2016).

Many studies have demonstrated that PAI-1 gene polymorphisms may have an important role in the formation of

[1]Department of Medical Genetics, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, İstanbul, Turkey
[2]Department of Internal Diseases, Istanbul University, School of Medicine, İstanbul, Turkey

Corresponding Author:
Mehmet Seven, MD, İstanbul University-Cerrahpaşa, Cerrahpaşa School of Medicine, Department of Medical Genetics, 34098 İstanbul, Turkey.
Email: mimseven@istanbul.edu.tr

thrombosis. PAI-1 gene has a role in the fibrinolytic system and is in a balance with tissue plasminogen activator (t-PA) in the coagulation process. PAI-1 gene is a member of the serpin family (Bajzar, 2000). The increased PAI-1 level is a risk factor for thrombosis. 4G/4G, 4G/5G, 5G/5G polymorphisms (rs1799889) are located in the promoter region of PAI-1 gene. Shanmugam, Tsagaris, and Attinger (2012) demonstrated that low testosterone increases the PAI-1 level and elevated PAI-1 activity is associated with the pathogenesis of ulceration, thromboembolism, and so forth. In addition, increased PAI-1 activity affects fibrinolysis negatively in KS and this inverse relationship supports the beneficial role of androgen therapy in KS patients with venous leg ulcers (Gattringer, Scheurecker, Höpfl, & Müller, 2010). In the present study, the frequency of PAI-1 gene 4G/5G polymorphism in KS patients was investigated.

Materials and Methods

Study Cohort

The study groups were composed of 41 KS patients and 50 fertile and healthy males as control group. These KS patients were admitted to Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, Department of Medical Genetics and Istanbul University Medical Faculty, Department of Medical Genetics between 2009 and 2016 to the infertility outpatient clinic. This study was approved by the Cerrahpasa Medical Faculty ethics committee. These patients were asked for their history of abnormal hormonal level and any other medical treatments. Patients were examined whether they had a stroke, circulatory disease, vasculitis, varicose, leg ulcer, diabetes, high tension, thrombosis, thromboembolism, hormone treatment, autoimmune disease, and hepatitis B and C infections. All patients had 47,XXY genotype. Those patients who had 46 XY/47XXY mosaicism and other chromosomal abnormalities were excluded.

The control group was selected randomly from age-matched healthy males. In the selection process, the following imperative criteria were considered: men have at least 1 child. Individuals with symptoms as deep vein thrombosis, autoimmune disease, hepatitis B and C infections, and individuals under hormonal treatment and so forth were excluded from the study as well.

Methods

At the study onset, as a total of two Ethylenediaminetetraacetic acid (EDTA) (anticoagulated tubes) tubes, 2 milliliters of whole blood sample was collected with the standard venipuncture technique. One of these tubes was taken for the isolation of DNA by using genomic DNA purification mini kit (E.Z.N.A.® Omega Kit, Georgia); the other one was used to obtain plasma, which is used to determine PAI-1 levels. Real-time PCR technique (FLUORINE; PAI-1 4G-5G QLP 3.0 Real-Time PCR Kit) was applied to assess PAI-1 gene polymorphisms (4G/4G, 4G/5G, 5G/5G) in patients with KS and control group. PCR was performed with the following components: Tris-Cl, KCl, (NH4)2SO4, 8 mM MgCl2, dNTP, 0.6 µM each primer, 10 ng/µl genomic DNA, and 0.05 U/µl *HotStarTaq DNA* polymerase. The temperature protocol for the PCR was adjusted to the following: the sample mixture was heated to 95°C and held for 15 min to fully denature the template, then 15 s at 95°C, followed by a series of 45 cycles 1 min at 62°C annealing temperature, which was followed by a final at 22°C.

Moreover, Enzyme-linked immunosorbent assay (ELISA) method was carried out to determine PAI-1 plasma levels.

All of these data were analyzed using independent sample *t* test, Mann–Whitney test, and chi-square tests in the SPSS software package.

Results

Patients and control groups were matched in terms of their ages; 41 KS patients (age range: 19–42; mean age: 30.80 ± 5.69 years) and 50 healthy males as control group (age range: 25–38; mean age: 32.68 ± 3.26 years). Table 1 demonstrated all parameters related to the aim of this study. In addition, the average of BMI index range: 16.3–36.3 (mean BMI 25.35 ± 5.13 kg/m^2), follicle-stimulating hormone (FSH) range: 1.65–103.7 mlU/ml (mean of FSH levels 42.84 ± 21.13), and testosterone range 0.36–5.63 ng/ml; (mean of FSH levels 2.34 ± 1.28) in KS patients. There was no male who had testosterone treatment in KS patients and also approximately %50 of them was smoking and %25 of them was alcohol consumer.

The PAI-1 gene polymorphisms (4G/4G; mutant polymorphism, 4G/5G; heterozygote polymorphism, 5G/5G; wild-type polymorphism) of patients with KS and control group are shown in Table 1. The distribution of PAI-1 gene polymorphisms was in accordance with the Hardy-Weinberg equilibrium (patients $p = .1$, controls $p = .157$).

There were no statistically significant differences between two types of polymorphisms (4G/4G; mutant, 5G/5G; wild type) and PAI-1 plasma levels. But, PAI-1 plasma levels of heterozygote polymorphism carriers (4G/5G) were identified to be statistically significant compared to heterozygote 4G/5G polymorphism carriers among controls (Patient mean = 67.01; control mean = 28.60; $p = .033$). In general, without considering polymorphic groups, the PAI-1 plasma levels of patients were detected higher than the control group ($p = .020$). Clinical symptoms related to thromboembolism especially the

Table 1. All Observed Parameters and *P* Values of Patients With KS and Controls

Variables		Patients (±*SD*)	Controls (±*SD*)	*P* value
Age		(*N* = 41) 30.804 ± 5.69	(*N* = 50) 32.680 ± 3.2	.052
Polymorphism	4G/4G (mut)	10 (24.3%)	15 (30%)	.46
	4G/5G (het)	16 (39%)	20 (40%)	.8
	5G/5G (wild)	15 (36.5%)	15 (30%)	.32
Allele	4G	36	50	.41
	5G	46	50	
PAI-1 plasma level	Total	*N* = 40 (55.13 ± 60.25)	*N* = 50 (29.89 ± 32.19)	.020*
	4G/4G (mut)	10 (30.43 ± 10.9)	15 (28.11 ± 21.83)	.73
	4G/5G (het)	16 (67.01 ± 63)	20 (28.60 ± 23.74)	.033*
	5G/5G (wild)	14 (59.2 ± 74.6)	15 (33.41 ± 48.6)	.28
Varicose veins	Present	17/38	6/29	.040*
	Absent	21/38	23/29	

Note. Mut = Mutant allele; het = heterozygote; wild = wild type allele.
*shows the statistically significant parts

presence of varicose veins were found more frequent in patients compared to control group ($p = .040$).

Discussion

KS has a tendency toward hypercoagulability because of hormonal imbalance and one or more inherited thrombophilic factors (Kang et al., 2012; Niemann, 2013). In several studies, prevalence of venous thrombosis, thromboembolism (especially pulmonary thromboembolism), and leg ulcers was seen to be increased in patients with KS because of androgen deficiency. According to Campbell and Price's study (Campbell, 1981) where 412 KS patients were examined, the prevalence of deep vein thrombosis and pulmonary embolism was observed to be increased in cases who are 1–20 years old. Winkler (1996) reported the reason for this disease is androgen imbalance which in turn affects hemostasis negatively.

Hypogonadism increases PAI-1 plasma level in this way high PAI-1 plasma level causes to decrease fibrinolytic activity. The formation of thrombosis or thromboembolism does not depend on only hormonal imbalance but also depends on hereditary thromboembolic factors (Campbell, 1981; Winkler, 1996). It is thought that the polymorphic structure formed in the 675 pair in the PAI-1 gene promoter region may play an important role in the formation of thrombophilia. The polymorphic structure seen in the PAI-1 gene; 4G/4G, 4G/5G, and 5G/5G. The 5G allele has been reported to have an additional protein binding site. In the 4G allele, this region is not present. It is understood that the elevated level of PAI-1 is positively correlated with the 4G allelic polymorphism structure. Thus, the idea that the additional protein binding site in the 5G allele is involved in a repressor protein is gaining in importance (Cooper, Whinna, Jackson, Boyd, & Church, 1995; Francis, 2002).

This study investigated that the polymorphism in the promoter region at 675 base pair of PAI-1 gene could have an important role in the formation of thrombophilia. The 5G allele of PAI-1 gene was identified to have an additional linkage protein region in contrast to the 4G allele. Increased PAI-1 levels and 4G allele polymorphisms were seen to be positively correlated. In this regard, the 5G allele can bind E2F transcription repressor of PAI-1 and 4G allele does not. 4G allele polymorphism may explanation for increased PAI-1 plasma levels in patients with KS (Rérolle et al., 2008). This study also compared both PAI-1 gene polymorphisms 4G/4G, 4G/5G, 5G/5G and PAI-1 plasma level in KS and control group. The risk of thromboembolism increases with the age, which was why we composed a cohort that would include young individuals within a patient group and control group. The clinical symptoms related to thromboembolism (especially varicose vein) were searched in the patients with KS and the control group who were included in this study.

PAI-1 polymorphisms and the increased plasma levels of PAI-1 may trigger the formation of thromboembolism in patients with KS due to hypoandrogenism (Kang et al., 2012; Shanmugam et al., 2012). In this study, heterozygote polymorphism (4G/5G) PAI-1 gene was identified to be statistically significant as compared to PAI-1 plasma levels ($p = .033$). The appearance of high PAI-1

plasma levels in patients with KS who have 4G/5G polymorphism suggests that thromboembolism formation may be more common in these individuals. It was determined that individuals with 4G/5G polymorphism in the patient group had a significant effect on clinical features related to thromboembolism rather than the control group.

PAI-1 plasma levels of the patients' and control groups were determined in this study and according to obtained results, the mean of PAI-1 plasma level in KS was defined to be about two times higher than the control group (mean of patients: 55.13/controls: 29.89) when plasma levels were compared between the patients' and control group and the p value of the respective test was .02. When PAI-1 gene polymorphism with PAI-1 plasma levels were compared in both patients' and control groups, the PAI-1 plasma level was determined to be statistically significant in patients with 4G/5G polymorphism ($p = .033$). These results demonstrate that there is a correlation between PAI-1 plasma levels and heterozygote (4G/5G) polymorphism. This study also examined the patients' and the control group for the clinical features related to thromboembolism. Patients' and control groups were compared for PAI-1 polymorphism structures and clinical findings related to the formation of thromboembolism, and no statistically significant difference was seen (patients, $p = .55$; controls, $p = .99$). When patients and control group were compared, clinical findings related to thromboembolism (especially varicose vein) were defined to be more frequent in cases with KS. These results suggest that thromboembolism related to clinical features are seen more frequently in cases with KS, but it may not be dependent only on PAI-1 gene polymorphism. Also, the PAI-1 gene polymorphism suggests that it may also be associated with other mechanisms that are effective in the fibrinolysis and coagulation system as inhibitors or as catalysts. The number of patients might be one of the limitations of this study. By increasing the number of patients, the presence of the polymorphism could be correlated with the actual PAI-1 levels of KS patients.

In further studies, the other factors that are associated with the PAI-1 gene in both fibrinolysis and coagulation mechanism should be investigated separately by increasing the number of patients with KS and controls.

Acknowledgments

The authors would like to thank patients and their families for attending this study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by Scientific Research Project Coordination Unit of Istanbul University in Turkey. Project no: 58210.

Ethics approval

This study was approved by Cerrahpasa Faculty of Medicine ethics committee (approval no. 279743). All participants provided written informed consent prior to enrolment in the study.

ORCID iD

Mehmet Seven https://orcid.org/0000-0001-7878-2039

References

Bajzar, L. (2000). Thrombin activatable fibrinolysis inhibitor and an antifibrinolytic pathway. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *20*, 2511–2518.

Campbell, W. A., & Price, W. H. (1981). Venous thromboembolic disease in Klinefelter's syndrome. *Clinical Genetics*, *19*(4), 275–280.

Cooper, S. T., Whinna, H. C., Jackson, T. P., Boyd, J. M., & Church, F. C. (1995). Intermolecular interactions between protein C inhibitor and coagulation proteases. *Biochemistry*, *34*, 12991–12997.

Francis, C. W. (2002). Plasminogen activator inhibitor-1 levels and polymorphisms. *Archives of Pathology & Laboratory Medicine*, *126*(11), 1401–1404.

Gattringer, C., Scheurecker, C., Höpfl, R., & Müller, H. (2010). Association between venous leg ulcers and sex chromosome anomalies in men. *Acta Dermato-Venereologica*, *90*(6), 612–615.

Grabski, J., Pusch, H., & Schirren, C. (1979). Clinical, endocrinological, histological and chromosomal investigations on Klinefelter' s syndrome. *Andrologia*, *11*(3), 182–196.

Kang, B.-S., Cho, D.-K., Koh, W.-J., Yoo, S.-H., Won, K.-B., Cho, Y.-H., Hwang, E.-S., & Koh, J.-H. (2012). A case of severe pulmonary thromboembolism in a young male with Klinefelter syndrome. *Korean Circulation Journal*, *42*(8), 562–564.

Lanfranco, F., Kamischke, A., Zitzmann, M., & Nieschlag, E. (2004). Klinefelter's syndrome. *Lancet*, *364*(9430), 273–283.

Niemann, R. (2013). Klinefelter syndrome: The commonest form of hypogonadism, but often overlooked or untreated. *Cardiological Disorders*, *110*(40), 675.

Nieschlag, E., & Behre, H. M. (2004). Clinical uses of testosterone in hypogonadism and other conditions. In E. Nieschlag, H. M. Behre, & S. Nieschlag (Eds.), *Testosterone: Action, deficiency, substitution* (pp. 375–404). Cambridge: Cambridge University Press.

Paduch, D. A., Fine, R. G., Bolyakov, A., & Kiper, J. (2008). New concepts in Klinefelter syndrome. *Current Opinion in Urology*, *18*, 621–627.

Rérolle, J. P., Munteanu, E., Drouet, M., Szelag, J. C., Champtiaux, B., Yagoubi, F., … Le Meur, Y. (2008). PAI-1 donor polymorphism influences long-term kidney graft survival. *Nephrology Dialysis Transplantation*, *23*(10), 3325–3332.

Shanmugam, V. K., Tsagaris, K. C., & Attinger, C. E. (2012). Leg ulcers associated with Klinefelter's syndrome: A case report and review of the literature. *International Wound Journal*, *9*(1), 104–107.

Winkler, U. H. (1996). Effects of androgens on hemostasis. *Maturitas*, *24*(3), 147–155.

Zöller, B., Ji, J., Sundquist, J., & Sundquist, K. (2016). High risk of venous thromboembolism in Klinefelter syndrome. *Journal of the American Heart Association*, *5*(5), e003567.