CLINICAL SCIENCE

Relationship between plasminogen activator inhibitor type-1 (PAI-1) gene polymorphisms and osteoporosis in Turkish women

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OBJECTIVE: The development of osteoporosis is associated with several risk factors, such as genetic structures that affect bone turnover and bone mass. The impact of genetic structures on osteoporosis is not known. Plasminogen activator inhibitor type-1 regulates the bone matrix and bone balance. This study assessed the correlation between plasminogen activator inhibitor type-1 gene 4G/5G polymorphisms and osteoporosis in a population of Turkish women.

METHODS: A total of 195 postmenopausal female patients who were diagnosed with osteoporosis (Group I) based on bone mineral density measurements via dual-energy x-ray absorptiometry and 90 females with no osteoporosis (Group II) were included in this study. Correlations between PAI-1 gene 4G/5G polymorphisms and osteoporosis were investigated through the identification of PAI-1 gene 4G/5G polymorphism genotypes using the polymerase chain reaction.

RESULTS: No significant differences in the genotype and allele frequency of 4G/5G plasminogen activator inhibitor type-1 polymorphisms were observed between the two groups, and both groups exhibited the most frequently observed 4G5G genotype.

CONCLUSION: No correlation between the development of osteoporosis in the female Turkish population and 4G/5G plasminogen activator inhibitor type-1 gene polymorphisms was observed.

KEYWORDS: Osteoporosis; Polymorphism; Plasminogen Activator Inhibitor Type-1 (PAI-1) Gene; Bone Mineral Density; Turkish Women.

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INTRODUCTION

Osteoporosis is a systemic skeletal disease that is characterized by an increase in bone fragility due to a decrease in bone mass and the deterioration of bone microarchitecture (1). This disease is especially prevalent in the elderly population, and it is a significant public health issue that reduces patient functioning and quality of life. An improved understanding of the risk factors for osteoporosis is important for the diagnosis, maintenance, and treatment of this significant disease (2,3).

No potential conflict of interest was reported.

Several epidemiological and clinical studies have demonstrated the importance of genetics in osteoporosis pathogenesis (4-6). Genetic factors affect bone turnover and can result in the reduction of bone mass to ~50-80% (5-7). Gene polymorphisms may contribute to osteoporosis and impact bone mineral density (4-8).

Plasminogen activator inhibitor-1 (PAI-1) is a 50-kDa, single-chain glycoprotein in the serine protease inhibitor family (9). A plasminogen activation system (PAS) that was initially identified in the fibrinolytic system, and its fundamental inhibitor, PAI-1, regulate the bone matrix and alter bone balance (10). PAI-1 primarily inhibits tissue-type (tPa) and urokinase (uPa) plasminogen activators (11) and reduces extracellular matrix destruction by decreasing the plasmin-mediated activation of matrix metalloproteinases (MMPs) (12).

Genetic factors predominantly determine plasma PAI-1 levels (13). The human PAI-1 gene contains various

polymorphic loci in approximately 12.22 kb on chromosome 7q22. The 4G/5G insertion/deletion is the most investigated polymorphism, which is 675 base pairs (bp) upstream of the transcriptional start site. This polymorphism regulates the expression of the PAI-1 gene (9,13,14).

The correlation of the PAI-1 4G/5G insertion/deletion polymorphism with several diseases, such as coronary artery disease, asthma, hypertension, stroke, obesity, rheumatoid arthritis, and osteoarthritis, has been investigated previously (15-21). However, the contribution of PAI-1 insertion/deletion variations (4G/5G) to osteoporosis has not been investigated in the Turkish population. This study investigated the correlation between PAI-1 gene polymorphisms and osteoporosis in Turkish females.

MATERIALS AND METHODS

Subjects

Postmenopausal females who were admitted to the Osteoporosis Clinic of the Physical Medicine and Rehabilitation Department of Eskisehir Osmangazi University (Turkey) were informed of the study, and patients who opted for inclusion in the study were evaluated. Patients who were diagnosed with parathyroid, thyroid, liver, and rheumatological diseases that affect bone metabolism; patients with a history of malignancy or surgically induced menopause; and patients who used drugs affecting bone metabolism (e.g., corticosteroids, anticonvulsants, and heparin) during the clinical and laboratory assessments were excluded from the study. Erythrocyte sedimentation rate, complete blood count, serum alkaline phosphatase, calcium, phosphorous, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, gamma-glutamyl transpeptidase, blood urea nitrogen, creatinine, glucose, uric acid, albumin, total protein, urine calcium/creatinine, thyroid-stimulating hormone, parathyroid hormone, cortisol and vitamin D levels were measured prior to the study. A total of 285 patients satisfied the study criteria and were included in the study. The age, height, weight, and body mass index (BMI) of the participants were evaluated. All participants underwent dual-energy x-ray absorptiometry (DEXA) evaluations, and 195 postmenopausal females were diagnosed with osteoporosis based on this assessment (Group I). Ninety patients without osteoporosis were included in the control group (Group II). All participants provided informed consent in compliance with the study protocol (#2009/229), which was approved by the Ethics Committee of the Medical Faculty of Eskisehir Osmangazi University (Turkey).

Bone mineral density

The participants underwent DEXA scanning using a Hologic QDR 4500 W system (Hologic, Inc., Bedford, USA) to assess bone mineral density (BMD), and the lumbar spine (vertebrae L1-L4) and hip (femur neck) were evaluated. Patients with a mean bone density below 2.5 SD were diagnosed with osteoporosis, as recommended by the World Health Organization (WHO).

Sample collection and determination of PAI-1 genotypes

Genomic DNA isolation was performed using the salt-extraction method in 10 ml of peripheral blood that was collected in EDTA tubes for the analysis of 4G/5G polymorphisms in the promoter region of PAI-1. The obtained genomic DNA was maintained at 4℃. The PAI-1 polymorphism gene region was amplified in a thermal cycler (Sacem Life Technologies, Peltier-based Thermal Cycler SCM 96G, Turkey) using 25 µl of a PCR mixture containing 0.5 µl DNA, 10X PCR Buffer (Biolabs, New England), 0.2 mmol/L dNTPs (Sigma, Germany), 1.25 U Taq polymerase (Biolabs, New England), 50 pmol of 4G- or 5G-specific primer, 50 pmol of downstream primer, and 2.5 pmol of upstream primer. The following primers (Metabion, Germany) were used: 5′-GTC TGG ACA CGT GGG GG-3′ for the 5G allele, 5′-GTC TGG ACA CGT GGG GA-3′ for the 4G allele, 5′-TGC AGC CAG CCA CGT GAT TGT CTA G-3′ for the downstream primer, and 5′-AAG CTT TTA CCA TGG TAA CCC CTG GT-3′ for the upstream primer (positive control). The PCR mixture was subjected to 35 cycles for 60 sec at 94℃, 30 sec at 54℃, and 40 sec at 72℃ following the initial denaturation for 3 min at 94℃. These PCR products were processed in 2% agarose gel and analyzed under UV light (Labwork, Cambridge, United Kingdom). The 4G and 5G alleles were defined according to a 139-bp DNA fragment of the general downstream primer that was produced during the PCR process. Samples that produced a 139-bp band from the 4G primer and that did not produce a 139-bp band from the 5G primer were considered a homozygous 4G genotype. Samples that produced a 139-bp band from the 5G primer but that did not produce a 139-bp band from the 4G primer were considered a homozygous 5G genotype. Samples that produced a 139-bp band from both primers were considered a heterozygous 4G5G genotype.

Statistical analysis

The data were evaluated using SPSS Version 20 software (IBM Corp. Armonk, New York, USA). The continuous variables were not normally distributed based on the Shapiro-Wilk test for normality. The Mann-Whitney U test was implemented for the comparison of the two groups. Medians (quartiles) are provided as descriptive statistics. The Pearson chi-square test was conducted for categorical variables. N and % values are provided. A $p<0.05$ was considered statistically significant.

RESULTS

This study investigated the effect of the PAI-1 gene 4G/5G polymorphisms on the development of osteoporosis in Turkish women. The study groups are listed in Table 1. No significant differences in the genotype and allele frequency of the 4G/5G PAI-1 polymorphism were observed between the groups ($p=0.619$ and $p=0.361$, respectively). However, the most frequent genotype, 4G5G, was observed in both groups. The 4G5G genotype was 39.49% in Group I and 42.22% in Group II. The 4G and 5G allele frequencies ranged from 47.4 - 52.6% in Group I and 43.3 - 56.7% in Group II.

DISCUSSION

Osteoporosis is characterized by low bone mass, an increase in bone fragility, deterioration in bone microarchitecture, and an increase in the risk of fracture (1). Some metabolic changes, such as those that occur due to a lack of estrogen, immobilization, metabolic acidosis, hyperparathyroidism, and systemic and local inflammatory diseases, affect the osteoclast count and activity associated with bone

Table 1 - Age, body mass index, femoral neck and lumbar vertebrae T-score averages and 4G/5G PAI-1 gene distribution in both groups.

	Postmenopausal Osteoporosis Patients (Group I) Median (25-75%)	Healthy Controls (Group II) Median (25-75%)
Subjects (n)	195	90
Age (years)	61 (55-67)	59 (53-65)
BMI (kg/m^2)	26.37 (23.63-28,04)	24.54 (21.28-29.56)
Femoral neck T-score	-2.90 (-3.15-2,69)	1.00 (-0.99-1.33)
Lumbar vertebrae T-score	-2.88 (-3.14-2,66)	1.00 (-1.3-1.4)
Genotype		
4G4G	64 (32.82%)	32 (35.56%)
4G5G	77 (39.49%)	38 (42.22%)
5G5G	54 (27.69%)	20 (22.22%)
Allele frequency		
4G	205 (52.6%)	102 (56.7%)
5G	185 (47.4%)	78 (43.3%)

Age, 1-2: $p = 0.105$; BMI, 1-2: $p = 0.171$; Femoral neck T-score, 1-2: $p < 0.001$; Lumbar vertebrae T-score, group I - group II: $p < 0.001$; Genotype χ^2: 0.961 df = 2 $p = 0.619$; Allele frequency χ^2: 0.834, df = 1, $p = 0.361$.

turnover (22). Prostaglandins, insulin-like growth factors (IGFs), interleukins (IL-1, IL-6, and IL-11), tumor necrosis factor (TNF), and several local factors in bone, such as transforming growth factor (TGF), also contribute to the regulation of bone formation and resorption (22,23).

TGF-β is an anabolic factor that increases extracellular matrix production and the expression of various types of collagen and proteoglycans (24,25). TGFβ1 polymorphisms may be significantly relevant in BMD and the occurrence of fracture (24,26). PAI-1 is known to have a regulatory effect on matrix components, including TGF-β, matrix γ-carboxyglutamic acid (Gla) protein, and osteocalcin. Therefore, PAI-1 may affect bone matrix biology and significantly regulate bone remodeling (10). PAI-1 levels are regulated by a 4G/5G insertion/deletion polymorphism (13).

This study investigated the relationship of the 4G/5G polymorphism, which regulates PAI-1 as an inhibitor of the plasminogen activator system, with osteoporosis in Turkish women.

A relationship between the PAI-1 4G/5G gene polymorphism and diseases, such as coronary artery disease, hypertension, stroke, and obesity, has been reported previously, but this polymorphism is not related to asthma, rheumatoid arthritis, and osteoarthritis (15-21). Genetic variations occur in populations. Previous studies have investigated the PAI-1 4G/5G insertion/deletion polymorphism in the Turkish population, but its relationship with osteoporosis has not been investigated; this relationship was examined in our study for the first time.

No differences in the PAI-1 4G/5G genotype were observed between the postmenopausal osteoporotic patients and the healthy control group. The role of common variations of COLIA-1, TGFβ-1, and PAI-1 genes in early postmenopausal osteoporotic Caucasians and healthy women was previously investigated by Hubacek et al., who observed no significant difference in the PAI-1 4G/5G genotype between osteoporotic patients and the healthy control group, which is consistent with our study. However, the 4G4G genotype was more common in the osteoporotic patient group compared with the control group (27).

Our results suggest that the 4G/5G PAI-1 polymorphism cannot be used as a marker for the development of osteoporosis in Turkish women. However, this result may not be applicable to all populations when gene pools, lifestyles, and gene-environment interactions in various populations are considered. Therefore, multi-centered studies on different populations and in different gene regions in larger samples are required to establish the correlation between the 4G/5G PAI-1 polymorphism and osteoporosis.

AUTHOR CONTRIBUTIONS

Ozgen M was responsible for the study design, evaluation and collection of clinical data, manuscript writing, and critical review. Turgut Cosan D was responsible for the study design, molecular biological analysis, genetic counseling, manuscript writing, and critical review. Doganer F and Soyocak A contributed to the molecular biological analysis, genetic counseling, manuscript writing, and critical review. Gunes HV and Degirmenci I contributed to the genetic counseling, manuscript writing, and critical review. Armagan O contributed to the manuscript writing and critical review. Ogutler Ozkara G contributed to the evaluation and collection of clinical data. Sahin Mutlu F contributed to the statistical analysis.

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