Basic research

Rheumatology

Association between postmenopausal osteoporosis and the glucocorticoid receptor gene (*NR3C1*) BclI C/G polymorphism in a Turkish population

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Abstract

Introduction: Glucocorticoids (GCs) are used in the treatment of numerous diseases, and long-term GC use can cause bone loss. GC receptor activation can decrease bone mineral density (BMD) by inhibiting osteoblast activity and stimulating and osteoclast activity. GC receptor gene polymorphisms have also been associated with increased susceptibility to GCs. The purpose of this study was to assess the relationship between osteoporosis and glucocorticoid receptor gene (*NR3C1*) polymorphism in a Turkish population.
Material and methods: The study group consisted of 232 unrelated patients with osteoporosis and 150 unrelated healthy controls. All participants, patients and healthy controls, were of Turkish origin, from the central region of Turkey. Genomic DNA was isolated from whole venous blood samples using a commercial DNA isolation kit. The *NR3C1* BclI gene C/G polymorphism was analyzed by polymerase chain reaction.
Results: The frequencies of CC, CG, and GG genotypes in the patients were 34.5%, 48.3%, and 17.2% and in the controls were 48.0%, 42.0%, and 10.0%. A statistically significant difference in genotype frequencies was observed between patients and controls (p = 0.01). The C and G allele frequencies were 58.6% and 41.4%, respectively, in the patient group and 69.0% and 31.0%, respectively, in the control group (p = 0.005).
Conclusions: The *NR3C1* BclI gene C/G polymorphism may be associated with osteoporosis in the studied Turkish population.

Key words: glucocorticoid receptor gene (*NR3C1*), osteoporosis, polymorphism.

Introduction

Osteoporosis is a disease identified by a decrease in bone mass and changes in its microarchitectural structure, such that the bone becomes more fragile [1]. The prevalence of osteoporosis increases with life expectancy, and the risk of fractures and associated mortality increases with age. Factors associated with a decrease in bone mineral density (BMD) include age, ethnicity, hormonal status, diet, lifestyle, drug use, and a va-

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riety of diseases [2]. Genetic factors may also play a role in osteoporosis.

Glucocorticoids (GCs) are used in the treatment of numerous diseases, and long-term GC use can cause bone loss. Approximately 50% of patients who use GCs for > 6 months experience reduced BMD and osteoporosis [3]. GC receptor activation decreases BMD by inhibiting and stimulating osteoblasts and osteoclasts, respectively [4]. GC receptor gene polymorphisms are reportedly associated with increased hypersensitivity to GCs [5, 6]. GC receptor gene polymorphisms are also associated with increased susceptibility to GCs and, in women, the G allele is associated with reduced lumbar BMD. In a study of 800 Chinese patients, the GC receptor gene was found to be associated with BMD [7]. Several GC receptor haplotypes have been associated with adverse effects. In a study of 112 subjects with congenital adrenal hyperplasia and primary adrenal insufficiency, the homozygous BclI GC receptor gene polymorphism was associated with increased bone resorption. In such cases, lower doses of hydrocortisone may be appropriate because of increased sensitivity to GC [8]. We investigated the relationship between osteoporosis and the GC (NR3C1 BclI) receptor gene polymorphism.

Material and methods

This study involved 232 osteoporosis patients as the study group and 150 healthy patients as the control group. The patients were selected from a university hospital in Turkey. This study was approved by the institutional Ethics Committee (approval no. 13-KAEK-74), and written approval and informed consent were obtained from each participant. A sample size calculation was performed to determine the required sample size for the study. The minimum sample size was calculated as 172 (n = 86 for each group), assuming a standard effect size of 0.30, $\alpha = 0.05$, and $1\text{-}\beta = 0.95$.

The subjects were postmenopausal women residing in central Turkey; postmenopausal was defined as at least 1 year after the end of menstruation. Patients with systemic diseases such as parathyroid disorders, Crohn's disease, hyperthyroidism, chronic inflammation, malignancy, premature or surgical menopause, and patients taking drugs that affect bone metabolism were excluded from the study. Patients receiving hormone replacement therapy (estrogens/progesterone) or anti-osteoporosis medications were also excluded to ensure greater homogeneity between the groups.

Dual-energy X-ray absorptiometry was used to measure the total femur, femoral neck, and BMD lumbar spine (L2–L4) values in subjects. BMD was expressed in kg/cm^2, and T-scores were calculated as the number of standard deviations (SDs) above or below the mean BMD of healthy young adults of the same sex. The World Health Organization defines osteoporosis as a T-score ≤ –2.5 SD [9]. According to BMD T-scores, subjects were classified into the osteoporosis and healthy control groups.

Patients with demographic characteristics similar to those of the osteoporosis group, such as age, weight, gender, and BMI, with no systemic diseases and a T-score > –2.5 SD were included in the healthy control group.

A DNA isolation kit (Sigma-Aldrich, Taufkirchen, Germany) was used to isolate the genomic DNA from whole venous blood samples. Polymerase chain reaction (PCR) was used to analyze the *NR3C1* BclI gene C/G polymorphism. PCR was conducted in a 25 µl reaction mixture containing 2.5 µl of 10 × PCR buffer, 100 ng of genomic DNA, 10 pM of each primer, 1 unit of Taq DNA polymerase, and 200 µM dNTPs. PCR was performed employing the following primers: 5′-AAA TTG AAG CTT AAC AAT TTT GGC-3¢ and 5¢-GCA GTG AAC AGT GTA CCA GAC C-3′. An initial melting step of 5 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min 45 s at 60°C, and 1 min 30 s at 72°C and a final elongation step of 5 min at 72°C was also performed. The PCR products were resolved in 2% agarose gels and stained with ethidium bromide. In heterozygous samples, the absence of the 287-bp intron in *NR3C1* BclI gene PCR products yielded a 190-bp product corresponding to the C allele, whereas the presence of intron 16 yielded a 477-bp product corresponding to the G allele. Samples yielding ambiguous results were reanalyzed by PCR.

Statistical analysis

Statistical analyses were performed using SPSS Statistics (version 20.0; IBM Corp., Armonk, NY, USA). Results are presented as means ± SD. The Hardy-Weinberg equilibrium of the patients and controls genotype distributions was evaluated using the χ^2 test. The χ^2 test or variance analysis was also used to analyze the relationships between the C/G polymorphism and the patients' clinical and demographic features. Categorical variables were also evaluated using the χ^2 test. 95% confidence intervals (CIs) and odds ratios (ORs) were determined to estimate the risk. All p-values were two-tailed, and $p < 0.05$ was considered significant.

Results

The demographic and basic medical characteristics of the osteoporosis and control groups are presented in Table I. Both groups had similar demographic and clinical characteristics, including age, menopause age and duration, BMI, osteoporosis family history, smoking, and calcium intake.

Table I. Demographic and basic medical characteristics of the osteoporosis and control groups

Characteristic	Osteoporosis *n* = 232	Control *n* = 150	*P*-value
Age [years]	57.9 ±6.5	58.0 ±9.3	0.830
Age at menopause [years]	46.3 ±3.8	46.4 ±3.5	0.834
Years since menopause	3.7 ±2.5	4.0 ±2.3	0.318
BMI [kg/m^2]	30.8 ±4.0	31.2 ±4.4	0.382
T-score			
Femoral neck	–2.7 ±0.5	–0.0 ±0.8	0.000
Total femoral	–1.5 ±0.9	0.0 ±0.8	0.000
Lumbar spine	–0.7 ±0.9	0.3 ±1.0	0.000
Family history, *n* (%)	18 (7.8)	9 (6.0)	0.548
Fractures, *n* (%)			
Vertebral fracture	6 (2.6)	–	0.08
Hip fracture	–	–	–
All	18 (7.8)	–	0.000
Calcium supplementation, *n* (%)	72 (31.0)	43 (28.7)	0.649
Ever smoking, *n* (%)	9 (3.9)	8 (5.3)	0.613

Data were analyzed by Student's t-test and χ^2 test. BMI – body mass index.

Table II. Distribution of the *NR3C1* gene C/G polymorphism in the osteoporosis and control groups

NR3C1 (C/G)	Osteoporosis *n* = 232 (%)	Control *n* = 150 (%)	*P*-value	OR (95% CI)
Genotype				
CC	80 (34.5)	72 (48.0)	0.01	
CG	112 (48.3)	63 (42.0)		
GG	40 (17.2)	15 (10.0)		
Allele				
C	272 (58.6)	207 (69.0)	0.005	0.63 (0.46–0.86)
G	192 (41.4)	93 (31.0)		

Data were analyzed by χ^2 test.

The BMD T score was lower, whereas the bone fracture rate was higher, in the osteoporosis group ($p < 0.05$).

The *NR3C1* gene C/G polymorphism distributions of the osteoporosis and control groups are shown in Table II. The frequencies of the CC, CG, and GG genotypes were 34.5%, 48.3%, 17.2% in the osteoporosis group, and 48.0%, 42.0%, and 10.0% in the control group, respectively ($p = 0.01$). The frequency of the C/G polymorphism C and G alleles was 58.6% and 41.1% in the osteoporosis group and 69.0% and 31.0% in the control group ($p = 0.005$). Allele frequency also differed significantly between the osteoporosis and control groups ($p = 0.005$, OR = 0.63, 95% Cl: 0.46–0.86; Table II). These findings suggest that the G allele of the C/G polymorphism may be associated with osteoporosis.

The STRING database is used to annotate functional associations among proteins in cells. Analysis of the NR3C1 protein with the STRING database identified the following predicted functional partners of the protein with high confidence: EP300, NCOA1, HSP90AA1, FKBP4, CREBBP, NCOA2, NCOR1, FKBP55, and PPARGC1A. The interaction network of these proteins is shown in Figure 1.

Discussion

The association between NR3C1 C/G polymorphism and osteoporosis was investigated. The GG polymorphism frequency was higher in the osteoporosis group than the control group. Also, the G allele frequency was significantly higher in the osteoporosis group than in the control group.

Receptor gene polymorphism or mutation causes osteoporosis by increasing or decreasing receptor sensitivity [6, 10–12]. In this study, the GC receptor gene C/G polymorphism frequency was higher for the G/C genotype. Also, the G-allele frequency in the osteoporosis group, and the

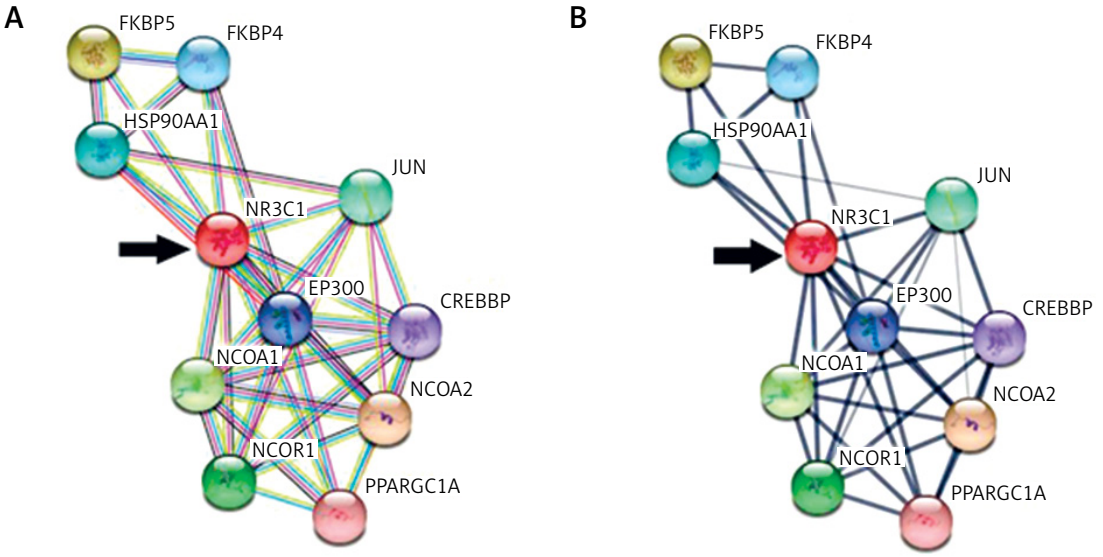

Figure 1. Protein-protein interaction (PPI) networks between *NR3C1* and other genes. **A** – NR3C1 (glucocorticoid receptor; isoform α-D3) association with EP300 (histone acetyltransferase p300), JUN (transcription factor AP-1), NCOA1 (nuclear receptor coactivator 1), HSP90AA1 (heat shock protein HSP 90-α), FKBP4 (peptidyl-prolyl cis-trans isomerase FKBP4), CREBBP (CREB-binding protein), NCOA2 (nuclear receptor coactivator 2), NCOR1 (nuclear receptor corepressor 1), FKBP5 (peptidyl-prolyl cis-trans isomerase FKBP5), PPARGC1A (peroxisome proliferator-activated receptor γ coactivator 1-α). **B** – Line thickness indicates the strength of data support

degree of receptor stimulation, were higher in patients with this polymorphism. The frequency of osteoporosis is higher in patients with high endogenous GC activity (such as those with Cushing syndrome) and in those taking steroids (exogenous GCs) [13–17]. The ACE gene I/D polymorphism was more frequent in our patients with osteoporosis because of increased ACE activity, likely because activation of the ACE system has a proinflammatory effect and osteoporosis is more likely to develop in the presence of inflammation [18].

GC receptor gene polymorphism was shown to be associated with BMD in a Chinese population [7]. Indeed, the single nucleotide polymorphism (SNP) rs1866388 haplotype frequency was higher in Chinese men with high BMD. In both sexes, the distributions of the rs1866388 and rs2918419 haplotypes differed between individuals with extremely low and extremely high BMD. Therefore, the GC receptor gene may be involved in BMD regulation in a sex-dependent manner [7]. The present study is, to our knowledge, the first to report an association between GC receptor gene polymorphism and osteoporosis in a Turkish population.

Feldman *et al*. reported that the 11β-hydroxysteroid dehydrogenase type 1 enzyme SNP rs4844880 polymorphism was associated with increased BMD (as indicated by the lumbar spine T- and Z-scores) in healthy women [19]. In a study of the estrogen receptor (ER), which is closely related to BMD and bone metabolism, *ERα* gene PvuII polymorphism was associated with the lumbar spine BMD of postmenopausal women [20].

There were some limitations in this study. First, the number of cases was small (< 1,000), and polymorphisms in other locations in the GC receptor gene were not investigated. However, this was the first Turkish study to evaluate the relationship between GC receptor gene polymorphism and postmenopausal osteoporosis in a homogeneous group of patients.

In conclusion, several genes have been implicated in the etiology of osteoporosis. The GG genotype of the GC receptor gene may be associated with an increased risk of postmenopausal osteoporosis. However, this finding needs to be confirmed in other populations and larger studies.

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Conflict of interest

The authors declare no conflict of interest.

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