

# Relationship between postmenopausal osteoporosis and glucocorticoid receptor gene (NR3C1) polymorphism in a Turkish population

---

## Type

Research paper

---

## Keywords

polymorphism, osteoporosis, Glucocorticoid receptor gene (NR3C1)

---

## Abstract

### Introduction

Glucocorticoids (GCs) are used in the treatment of numerous diseases, and long-term use of GCs causes bone loss. GC receptor activation decreases Bone Mineral Density (BMD) by inhibiting and stimulating osteoblasts and osteoclasts, respectively. GC receptor gene polymorphisms are also 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 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 was observed between patients and controls according to genotype frequencies ( $p=0.01$ ). C and G allele frequencies of C/G polymorphism were 58.6% and 41.4% in patient group and 69.0% and 31.0% in control group ( $p=0.005$ ).

### Conclusions

The NR3C1 BclI gene C/G polymorphism could be one of the genetic factors causing osteoporosis.

**Title:**

Relationship between postmenopausal osteoporosis and glucocorticoid receptor gene (NR3C1) polymorphism in a Turkish population

**Short title:**

Postmenopausal osteoporosis and glucocorticoid receptor gene polymorphism

**Authors and affiliations:**

Bülent Çakmak, M.D. Assoc Prof

Department of Obstetrics and Gynecology, Niğde Ömer Halisdemir University, Niğde, Turkey

Serbülent Yiğit, M.D. Assoc Prof

Department of Veterinary Genetics, School of Veterinary Medicine, Ondokuz Mayıs University, Samsun, Turkey

Nevin Karakuş, M.D. Assoc Prof

Department of Medical Biology, School of Medicine, Gaziosmanpaşa University, Tokat, Turkey

Eren Yıldırım, M.D, Specialist

Department of Physical Therapy and Rehabilitation, Liv Hospital, Samsun, Turkey

Ahmet İnanır, M.D. Assoc Prof

Department of Physical Therapy and Rehabilitation, Privite Clinic, Ankara, Turkey

**Corresponding Author:**

Bülent Çakmak, Niğde Ömer Halisdemir Üniversitesi Eğitim ve Araştırma Hastanesi

Kadın Hastalıkları ve Doğum Kliniği, 51000, Merkez, Niğde, Turkey.

Email: [drbulentcakmak@hotmail.com](mailto:drbulentcakmak@hotmail.com)

Phone: +905335726978

Preprint

## ABSTRACT

**Introduction:** Glucocorticoids (GCs) are used in the treatment of numerous diseases, and long-term use of GCs causes bone loss. GC receptor activation decreases Bone Mineral Density (BMD) by inhibiting and stimulating osteoblasts and osteoclasts, respectively. GC receptor gene polymorphisms are also 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 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 was observed between patients and controls according to genotype frequencies ( $p=0.01$ ). C and G allele frequencies of C/G polymorphism were 58.6% and 41.4% in patient group and 69.0% and 31.0% in control group ( $p=0.005$ ).

**Conclusions:** The NR3C1 BclI gene C/G polymorphism could be one of the genetic factors causing osteoporosis.

**Keywords:** 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 osteoporosis prevalence increases with life expectancy, and the risk of fractures and associated mortality increases with age. The related factors with a decrease in Bone Mineral Density (BMD) are age, ethnicity, hormonal status, diet, lifestyle, drug use, and a variety 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 use of GCs causes 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). Gene polymorphisms of GC receptor are reportedly related to increased hypersensitivity to GCs (5,6). GC receptor gene polymorphisms are also related to increased susceptibility to GCs and, in women, the G allele is related to a reduced lumbar BMD. In a study of 800 Chinese patients, the GC receptor gene was found to modulate BMD (7). A number of GC receptor haplotypes have adverse effects. In a study of 112 subjects with congenital adrenal hyperplasia and primary adrenal insufficiency, the homozygous BclII GC receptor gene polymorphism was associated with increased bone resorption. In such cases, hydrocortisone should be used at lower doses because of GC hypersensitivity (8). We investigated the relationship between osteoporosis and 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, 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. **Sample size calculator was performed to determine the sample size in the study. The minimum sample size for this study was calculated as 172 (n = 86 for each group) using standard effect size ( $\alpha = 0.05$ ,  $1-\beta = 0.95$ , effect size 0.30).**

The subjects were postmenopausal women residing in central Turkey. The postmenopausal is specified as at least one 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 antiosteoporotics were also excluded to render the groups more homogenous.

The dual-energy X-ray absorptiometry was used to measure the total femur, femoral neck, and BMD lumbar spine (L2–L4) values in subjects. Kg/cm<sup>2</sup> was used as the unit of BMD values and transformed to osteoporosis T-scores, which correspond to standard deviations (SDs) higher or lower than the bone's mean mass for normal young adults of the same gender. The World Health Organization defines osteoporosis as a BMD > 2.5 SD (T-score) below the young adults' mean value or peak bone mass (9). According to BMD scores, subjects were classified as osteoporosis and healthy control groups.

Patients with demographic characteristics such as age, weight, gender, BMI, and systemic diseases similar to the osteoporosis group 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 BclII gene C/G polymorphism. PCR was conducted in a 25  $\mu$ L reaction mixture having 2.5  $\mu$ L of 10 $\times$  PCR buffer, 100 ng of genomic DNA, 10 pM of each primer, 1 unit of Taq DNA polymerase, and 200  $\mu$ M dNTPs. PCR was performed employing these primers: 5 $\phi$ -AAA TTG AAG CTT AAC AAT TTT GGC-3 $\phi$  and 5 $\phi$ -GCA GTG AAC AGT GTA CCA GAC C-3 $\phi$ . An initial melting step of 5 min at 94 $^{\circ}$ C, followed by 30 cycles of 1 min at 94 $^{\circ}$ C, 1 min 45 s at 60 $^{\circ}$ C, and 1 min 30 s at 72 $^{\circ}$ C and a final elongation step of 5 min at 72 $^{\circ}$ C was also performed. The PCR products were resolved in 2% agarose gels and stained with ethidium bromide. In heterozygous samples, in the lack of the 287 bp intron, 16 of the NR3C1 BclII gene PCR yielded a 190 bp product (C allele) and a 477 bp product (G allele) in the presence of intron 16. Ambiguous results performed the PCR test again.

SPSS software (ver. 20.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Results are presented as means  $\pm$  SD. The Hardy–Weinberg equilibrium of the patients and controls genotype distributions was evaluated by the  $\chi^2$  test. The  $\chi^2$  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  $\chi^2$  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 as significant.

## Results

The demographic and basic medical characteristics of the osteoporosis and control groups are listed in Table 1. Both groups had similar demographic characteristics, such as age, menopause age and duration, BMI, osteoporosis family history, smoking, and calcium intake. 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 2. The frequencies of the CC, CG, and GG genotypes were 34.5%, 48.3%, 17.2% in the osteoporosis group, 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% CI 0.46–0.86; Table 2). Therefore, the G allele of the C/G polymorphism was found to be related to osteoporosis.

STRING database annotates the functional interactions among the proteins in a cell. Analyzing the NR3C1 protein with STRING database, predicted the functional partners of the protein with high confidence was found as follows: EP300, NCOA1, HSP90AA1, FKBP4, CREBBP, NCOA2, NCOR1, FKBP55, PPARGC1A. The interaction network of these proteins is shown in Figure 1.

## Discussion

The association between NR3C1 C/G polymorphism and osteoporosis were 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 degree of receptor stimulation, were higher in patients with this polymorphism. The osteoporosis frequency 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 closely related to BMD in a Chinese population (7). Indeed, the single nucleotide polymorphism (SNP) rs1866388 haploid frequency was higher in Chinese men with a high BMD. In both genders, the SNP rs1866388 and rs2918419 haploid distributions differed between individuals with extremely low and extremely high BMD. Therefore, the GC receptor gene was shown to be involved in BMD regulation in a gender-dependent manner (7). The present study is the first to show a relationship between GC receptor gene polymorphism and osteoporosis in a Turkish population.

Feldman et al. reported that 11beta-hydroxysteroid dehydrogenase type 1 enzyme SNP rs4844880 polymorphism was associated with an increased BMD (as shown 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, ER $\alpha$  gene PvuII polymorphism was closely related to 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 homogenous group of patients.

## **Conclusion**

Several genes are implicated in the etiology of osteoporosis. The GG polymorphism of the GC receptor gene may be a postmenopausal osteoporosis risk factor. However, this finding needs to be confirmed in other populations.

## **Acknowledgments**

None.

## **Disclosure**

Authors have no interest to disclose.

## References

1. Genant HK, Cooper C, Poor G, et al. Interim report and recommendations of the World Health Organization Task-Force for Osteoporosis. *Osteoporos Int* 1999; 10: 259-64.
2. Lane NE. Epidemiology, etiology, and diagnosis of osteoporosis. *Am J Obstet Gynecol* 2006; 194(2 Suppl): S3–S11.
3. Epstein S, Inzerillo AM, Caminis J, Zaidi M. Disorders associated with acute rapid and severe bone loss. *J Bone Miner Res* 2003; 18: 2083-94.
4. Wright AP, Zilliacus J, McEwan IJ, et al. Structure and function of the glucocorticoid receptor. *J Steroid Biochem Mol Biol* 1993; 47: 11-9.
5. Huizenga NA, Koper JW, De Lange P, et al. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. *J Clin Endocrinol Metab* 1998; 83: 144-51.
6. van Rossum EF, Koper JW, van den Beld AW, et al. Identification of the BclI polymorphism in the glucocorticoid receptor gene: Association with sensitivity to glucocorticoids in vivo and body mass index. *Clin Endocrinol (Oxf)* 2003; 59: 585-92.
7. Peng YM, Lei SF, Guo Y, et al. Sex-Specific association of the glucocorticoid receptor gene with extreme BMD. *J Bone Miner Res* 2008; 23: 247-52.
8. Koetz KR, van Rossum EF, Ventz M, Diederich S, Quinkler M. BclI polymorphism of the glucocorticoid receptor gene is associated with increased bone resorption in

- patients on glucocorticoid replacement therapy. *Clin Endocrinol (Oxf)* 2013; 78: 831-7.
9. Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO study group. *Osteoporos Int* 1994; 4: 368-81.
  10. Morrison NA, Yeoman R, Kelly PJ, Eisman JA. Contribution of trans-acting factors alleles to normal physiological variability: Vitamin D receptor gene polymorphisms and circulating osteocalcin. *Proc Natl Acad Sci* 1992; 89: 6665-9.
  11. Smith EP, Boyod J, Frank GR, et al. Estrogen resistance caused by a mutation in the estrogen receptor gene in a man. *New Engl J Med* 1994; 331: 1056–61.
  12. Zmuda MJ, Cauley JA, Kuller LH, et al. Androgen receptor CAG repeat length is associated with increased hip bone loss and vertebral fracture risk among older men, in: *Proceedings of the ASBMR 22nd Annual Meeting*. *J Bone Miner Res* 2001; 15: 491.
  13. Canalis E. Mechanisms of glucocorticoid action on bone: implications to glucocorticoid-induced osteoporosis. *J Clin Endocrinol Metab* 1996; 81: 3441–7.
  14. Centrella M, McCarthy TL, Canalis E. Glucocorticoid regulation of transforming growth factor 1 activity and binding in osteoblast-enriched cultures from fetal rat bone. *Mol Cell Biol* 1991;11: 4490–6.
  15. Cooper MS, Hewison M, Stewart PM. Glucocorticoid activity, inactivity and the osteoblast. *J Endocrinol* 1999; 163: 159–64.

16. Oursler MJ, Riggs BL, Conover CA. Glucocorticoid-induced activation of latent transforming growth factor by normal human osteoblast-like cells. *Endocrinology* 1993; 133: 2187–96.
17. Stromstedt PE, Poellinger L, Gustafsson JA, Carlstedt-Duke J. The glucocorticoid receptor binds to a sequence overlapping the TATA box of the human osteocalcin promoter: a potential mechanism for negative regulation. *Mol Cell Biol* 1991; 11: 3379–83.
18. Cakmak B, Inanir A, Karakus N, Ates O, Yigit S. Association between the ACE gene I/D polymorphism and osteoporosis in a Turkish population. *Z Rheumatol* 2015; 74: 346–50.
19. Feldman K, Szappanos A, Butz H, et al. The rs4844880 polymorphism in the promoter region of the HSD11B1 gene associates with bone mineral density in healthy and postmenopausal osteoporotic women. *Steroids* 2012; 7: 1345–51.
20. Erdogan MO, Yıldız H, Artan S, et al. Association of estrogen receptor alpha and collagen type I alpha 1 gene polymorphisms with bone mineral density in postmenopausal women. *Osteoporos Int* 2011; 22: 1219-25.

**Table 1.** Demographic and basic medical characteristics of the osteoporosis and control groups.

<b>Characteristic</b>	<b>Osteoporosis n = 232</b>	<b>Control n = 150</b>	<b><i>p</i></b>
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 <sup>2</sup> )	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, no. (%)	18 (7.8)	9 (6.0)	0.548
Fractures, no. (%)			
Vertebral fracture	6 (2.6)	-	0.08
Hip fracture	-	-	-
All	18 (7.8)	-	0.000
Calcium supplementation, no. (%)	72 (31.0)	43 (28.7)	0.649
Ever smoking, no. (%)	9 (3.9)	8 (5.3)	0.613

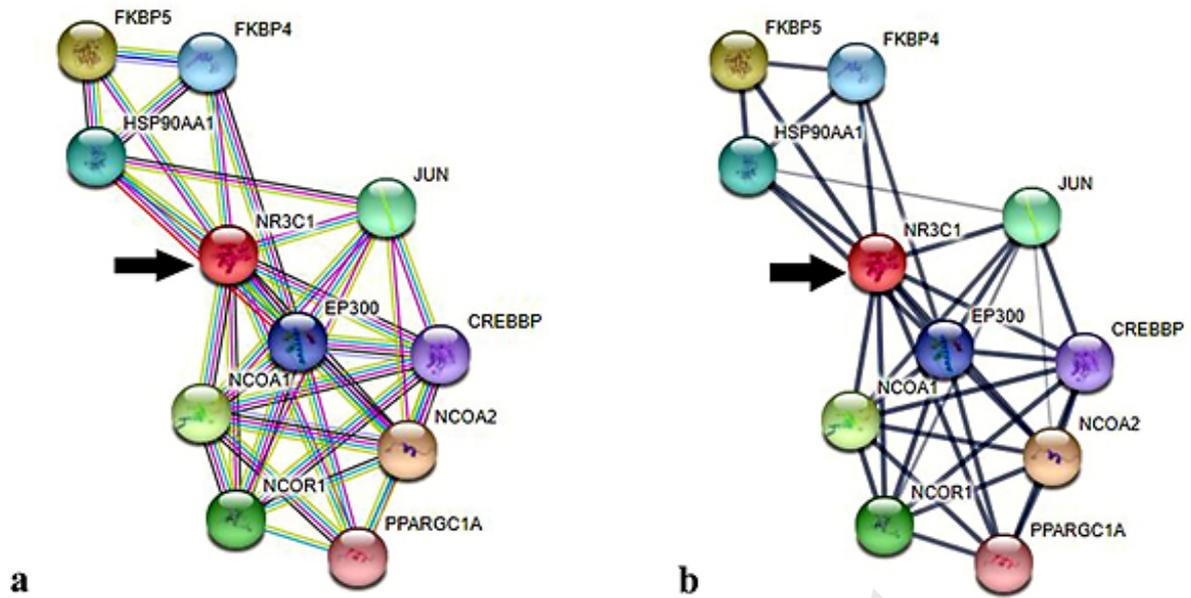
Data were analyzed by Student's *t*-test and  $\chi^2$  test. BMI, body mass index.

**Table 2.** Distribution of the NR3C1 gene C/G polymorphism in the osteoporosis and control groups.

NR3C1 (C/G)	Osteoporosis n = 232 (%)	Control n = 150 (%)	<i>p</i>	OR (95% CI)
<u>Genotype</u>				
CC	80 (34.5)	72 (48.0)	0.01	
CG	112 (48.3)	63 (42.0)		
GG	40 (17.2)	15 (10.0)		
<u>Allele</u>				
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  $\chi^2$  test.

Preprint



Protein-protein interaction (PPI) networks between NR3C1 and other genes. Figure 1. a) NR3C1 (Glucocorticoid receptor; Isoform Alpha-D3) association with EP300 (Histone acetyltransferase p300), JUN (Transcription factor AP-1), NCOA1 (Nuclear receptor coactivator 1), HSP90AA1 (Heat shock protein HSP 90-alpha), 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 gamma coactivator 1-alpha) b) Line thickness indicates the strength of data support.