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Original Article

Relationship between SP1 polymorphism and osteoporosis in β-thalassemia major patients

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Abstract ***Background***: β-Thalassemia is an autosomal recessive disease characterized by defective β-globin chain production. Osteoporosis is an important cause of morbidity in patients with β-thalassemia major. The pathogenesis of reduced bone mineral density (BMD) is multifactorial. A range of genetics factors have been implicated in other populations of patients with osteoporosis. Polymorphism at the Sp1 binding site of the *collagen type I A1* (*COLIA1*) gene is thought to be an important factor in the development of osteoporosis.

Methods: Alleles *S* and *s*, detected by presence of a G or T nucleotide, respectively in a regulatory site of the *COLIA1* gene were investigated in 37 β-thalassemia major patients with osteoporosis and 92 controls without osteoporosis or osteopenia using polymerase chain reaction–restriction fragment length polymorphism.

Results: Fifteen and nine β-thalassemia major patients displayed SS and Ss genotypes, respectively, whereas 13 were found to have an ss genotype. The mean BMD of the β-thalassemia major patients with ss genotype was similar to those with the Ss and SS genotypes. In the control group, 77 and 15 subjects had SS and Ss genotypes, respectively, with no ss genotype. Allelic and genotypic distribution in patients were significantly different from controls.

Conclusion: Determining base substitutions at the Sp1 binding site on the *COLIA1* gene in early years may be important in preventing osteoporosis in children with β-thalassemia major.

Key words β-thalassemia major, collagen type I A1, osteoporosis, polymerase chain reaction–restriction fragment length polymorphism, Sp1 polymorphism.

β-Thalassemia is an inherited disorder with an autosomal recessive mode of inheritance and constitutes one of the most serious health problems worldwide, as well as in Antalya.[1] It is well known that increased erythropoiesis in bone marrow in β-thalassemic patients results in expansion of marrow cavity and reduced bone mass.[2] Regular blood transfusions from infancy until adulthood in β-thalassemia major patients have facilitated transformation of severe bone deformities into less marked skeletal lesions, such as osteoporosis.[3]

Osteoporosis is a common disease characterized by reduced bone mass, microarchitectural deterioration of bone tissue, and increased risk of fragility fractures.[4,5] Genetic factors play an important role in the pathogenesis of osteoporosis, involving variation in several genes such as *collagen type I A1* (*COLIA1*), vitamin D, estrogen receptors and interleukin (IL)-6 that regulate bone mineral density (BMD) and bone geometry and quality.[6] One of the most important candidate genes for predisposition to osteoporosis is the *COLIA1* gene, which encodes the α-1(I) protein chain of type I collagen, the major protein of bone. The G-T substitution at base 1 of intron 1 at the binding site of the Sp1 transcription factor of the *COLIA1* gene is a putative marker for low BMD and osteoporotic fractures. Several studies demonstrated that Sp1 polymorphism has been associated with low BMD and an increased risk of osteoporotic fracture in β-thalassemic patients in different populations.[5,7]

Therefore, the aim of the present study was to examine the distribution of *COLIA1* polymorphism and its relationship with BMD at the lumbar spine and femur in 37 patients with β-thalassemia major compared to a control group in a Turkish population.

Methods

Patients

Blood samples were obtained from 37 transfusion-dependent patients (20 girls and 17 boys, mean age 13.5 ± 3.5 years) clinically diagnosed as having β-thalassemia major with osteoporosis at Thalassemia Center, Antalya State Hospital. All patients were treated with regular blood transfusions in order to maintain pre-transfused hemoglobin levels >9.5 g/dL, and with adequate chelation therapy using a combination of deferioxamine and deferiprone. None of them had any clinical and laboratory

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findings of endocrinopathy. BMD of the lumbar spine and femur was assessed on dual-energy X-ray absorptiometry (DPX-L Densitometer, Lunar, Madison, WI, USA) and provided as g/cm^2. Blood samples from 92 healthy individuals (49 women and 43 men, mean age of 25.4 ± 5.0 years) without osteoporosis or osteopenia were used as a control group. All patients and healthy individuals gave informed consent form to participate in the present study, approved by Akdeniz University Ethics Committee.

DNA analysis

The DNA was isolated from peripheral blood samples of β-thalassemia major patients and control group by conventional salting-out methods as described previously.[8] A 850 bp fragment including the intronic polymorphic region of the Sp1 binding site in the *COLIA1* gene was amplified on polymerase chain reaction (PCR) using forward (5′-AGC AGG TCC CCT TGG AAC CG-3′) and reverse (5′-AC TCT GTC CGC TTG TCC CGC-3′) primers. The expected size of the specific PCR product was 850 bp, which was digested overnight at 37°C with *Bal*I restriction enzyme (Fermentas GmbH, St. Leon-Rot, Germany). Digested products were visualized in 3% NuSieve agarose gel (FMC BioProducts, Rockland, ME, USA) using UV transilluminator (Hero Lab, Wiesloch, Germany). The *s* allele did not have the *Bal*I restriction site, while the *S* allele was digested into 600 bp and 250 bp fragments by the same enzyme compared to molecular marker (Fermantas). β-Globin Strip Assay Kit (Vienna Lab, Vienna, Austria) based on reverse dot blot hybridization was used for detection of the 22 most common β-thalassemia mutations and sickle cell anemia mutation in the Mediterranean region.

Statistical analysis

The χ^2 test was used to compare allele and genotype frequencies between β-thalassemia major patients and controls. The Mann–Whitney *U*-test was used to compare BMD scores between genotypes in β-thalassemia major patients.

Results

Thirty-seven β-thalassemia major patients with osteoporosis and 92 healthy controls were studied using PCR-restriction fragment length polymorphism (PCR-RFLP) to detect the Sp1 polymorphism in *COLIA1* gene. The different alleles were assessed according to the digestion pattern detected with the restriction enzyme *BalI*, a recognition site of which is present within the 850 bp-amplified region of the *COLIA1* gene. The *s* allele is said to be present if no digestion is observed, whereas an *S* allele is indicated in the case of digestion, producing two different-sized fragments (250 bp and 600 bp). The distribution of *COLIA1* genotypes and allele frequencies in the β-thalassemia major patients and controls is given in Table 1. While the allelic frequencies in control group were 91.8% for S and 8.2% for s, frequencies of S and s alleles were found to be 52.7% and 47.3%, respectively, in the β-thalassemia major patients. Patients with β-thalassemia major had higher allelic frequencies for the s allele and ss genotype. Also, the allelic and genotype distributions were significantly different between osteoporotic β-thalassemia major patients and controls (Table 1, $P < 0.001$).

The BMD (expressed as *Z*-scores) of β-thalassemia major patients were in the range of −1.35 and −3.71. No significant differences in lumbar or femoral BMD scores between genotypes were observed in the osteoporotic β-thalassemia major patients (Fig. 1). We did not observe any significant relationship between the genotypes obtained and the type of β-thalassemic mutations as a result of Sp1 polymorphism. Also, there were no data on bone fractures for the thalassemic patients.

Discussion

Even though all of genes responsible for regulation of bone mass are non-identified, multiple factors such as polymorphisms in estrogen receptors, vitamin D receptor, *COL1A,* and *COL1A2* genes are believed to play a role in bone formation.[9] Type I collagen, a protein encoded by *COLIA1* and *COLIA2* genes, is the major protein of the bone matrix, at 90%.[10] The first intron of the *COL1A1* gene has previously been shown to be of importance in the regulation of collagen transcription. Recent studies have verified the association between polymorphism for the binding site of the transcription factor Sp1 in the first intron of the *COL1A1* gene, and bone mass in several populations.[4,11–13]

Little information is available concerning the factors responsible for the development of osteoporosis, characterized by reduced bone mass, in β-thalassemia major. Several studies have shown that heterozygotes at the polymorphic Sp1 site (Ss) had significantly lower BMD than SS homozygotes, and bone mass was even lower in ss homozygotes in patients with β-thalassemia major.[5,7] We analyzed the osteoporosis of the lumbar spine and femur at the presence of the Sp1 polymorphism. Of the present 37 β-thalassemia major patients, nine had the Ss (G/T) allele while 13 had the ss (T/T) alleles, and the remaining 15 had the SS (G/G) allele (Table 1). When the genotypes and BMD scores were compared in 37 β-thalassemia major patients with osteoporosis, there was no significant correlation between osteoporosis and genotype (Fig. 1).

In the present study population we observed Ss and SS genotypes in 24.3% and 40.5% of β-thalassemic patients, and in 16% and 84% of the control group, respectively. The frequency of the

Table 1 Genotype and allele frequencies

	Alleles, *n* (%)		Genotype, *n* (%)		
	S	s	SS	Ss	ss
Controls ($n = 92$)	169 (91.8)	15 (8.2)	77 (84)	15 (16)	—
β-Thalassemia major patients ($n = 37$)	39 (52.7)	35 (47.3)	15 (40.5)	9 (24.3)	13 (35.2)

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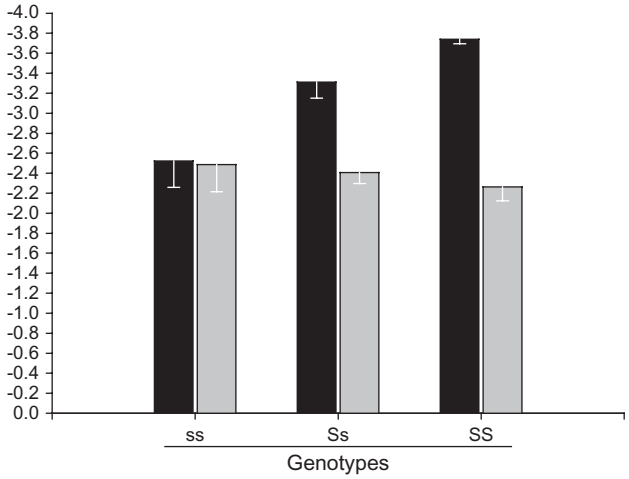

Fig. 1 Relationship between (■) lumbar and (▨) femoral bone mineral density and *collagen type I A1* (*COLIA1*) genotypes in patients with β-thalassemia major.

ss genotype was 35.2% in thalassemic patients, while it was not detected in the control group (Table 1). When allelic frequency was compared between β-thalassemia major patients and controls we found the s allele to be higher in the β-thalassemia major patients. These results show that there was a significant correlation between allelic and genotypic distribution of Sp1 polymorphism. Also, we compared β-globin gene mutations and genotypic distribution of Sp1 polymorphism. IVSI.110 mutation was found to be the most common mutation, but a correlation was not found between β-globin gene mutation types and allelic distribution of Sp1 polymorphism.

The present results indicate that osteoporosis has a strong genetic component related with Sp1 polymorphism in children and young adults who had β-thalassemia major with severe osteoporosis. As a result, mutation at the Sp1 binding site on the *COLIA1* gene should be detected early in order to initiate preventative therapy before fractures occur in children with β-thalassemia major. In addition to regular screening, preventive intervention (regular transfusion, chelation etc.) and early management of possible endocrine complications are important in order to secure normal bone health in children with β-thalassemia major. Genetic counseling should be given to families with a β-thalassemia major child, in order to prevent osteoporosis.

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References

1 Keser I, Sanlioglu AD, Manguoglu E *et al*. Molecular analysis of beta-thalassemia and sickle cell anemia in Antalya. *Acta Haematol*. 2004; **111**: 205–10.
2 Vaskaridou E, Kyrtsonis MC, Terpos E *et al*. Bone resorption is increased in young adults with thalassaemia major. *Br. J. Haematol*. 2002; **112**: 36–41.
3 Wonke B. Annotation: bone disease in Thalassemia major. *Br. J. Haematol*. 1998; **103**: 897–901.
4 Grant SF, Reid DM, Blake G *et al*. Reduced bone density osteoporosis associated with a polymorphic sp1 binding site in the collagen type a1 gene. *Nat. Genet*. 1996; **14**: 203–5.
5 Perrotta S, Cappellini MD, Bertoldo F *et al*. Osteoporosis in β-thalassemia major patients: Analysis of the genetic background. *Br. J. Haematol*. 2000; **111**: 461–6.
6 Ralston SH. Genetic control of susceptibility to osteoporosis. *J. Clin. Endocrinol. Metab*. 2002; **86**: 2460–66.
7 Wonke B, Jensen C, Hanslip JJ *et al*. Genetic and acquired predisposing factors and treatment of osteoporosis in thalassaemia major. *J. Pediatr. Endocrinol. Metab*. 1998; **3**: 795–801.
8 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988; **16**: 12–15.
9 Eisman JA. Genetics of osteoporosis. *Endocr. Rev*. 1999; **20**: 788–804.
10 Qureshi AM, Herd RJ, Blake GM *et al*. COLIA1 Sp1 polymorphism predicts response of femoral neck bone density to cyclical etidronate therapy. *Calcif. Tissue Int*. 2002; **70**: 158–63.
11 Uitterlinden AG, Burger H, Huang Q *et al*. Relation of alleles of the collagen type I alpha 1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. *N. Engl. J. Med*. 1998; **338**: 1016–21.
12 Alvares K, Siddiqui F, Malone J, Veis A. Assembly of the type 1 procollagen molecule: Selectivity of the interactions between the alpha 1(I)- and alpha 2(I)-carboxyl propeptides. *Biochemistry* 1999; **38**: 5401–11.
13 Bernad M, Martinez ME, Escalona M *et al*. Polymorphism in the type I collagen (COLIA1) gene and risk of fractures in postmenopausal women. *Bone* 2002; **30**: 223–8.

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