A novel 110-bp insertion in a patient with homocysteinuria

Homosistinürili bir hastada ilk kez tanımlanan 110 BÇ'lik insersiyon

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To the Editor,

Homocysteinuria is an inherited recessive disorder caused by cystathionine beta-synthase (CBS) deficiency that has a wide spectrum of clinical manifestations, including ocular lens dislocation, skeletal disproportion, osteoporosis, vascular thrombosis, and central nervous system dysfunction [1]. The CBS enzyme catalyzes the synthesis of cystathionine from homocysteine and serine in the methionine pathway. This results in accumulation of homocysteine and methionine in plasma, leading to excretion of excessive urinary homocysteine. CBS deficiency has a worldwide incidence of 1/344,000 live births (range: 1/58,000-1/1000 live births) [2].

Human CBS is located in the cytoplasm of cells and is composed of 4 identical 63-kDa subunits. The CBS gene is located on the long (q) arm of chromosome 21 at position 22.3, encodes a protein consisting of 551 amino acids, and has 16 exons [3]. Herein we report a patient with homocysteinuria that was a carrier of compound heterozygote mutations in the CBS gene, 1 of which is novel, and a discussion of the clinical and molecular findings.

A 14-year-old boy was referred to our hospital for evaluation of ocular symptoms that began approximately 1 year earlier. He was the first born of non-consanguineous parents that had lost 1 fetus. Family history of neurologic and thromboembolic disease was negative. Pregnancy and delivery were uneventful. The proband had neuromotor developmental delay. At the age of 10 years he had a clonic seizure of the left side. He had a marfanoid face and neurological examination showed left-sided central facial palsy, and mild left hemiparesis. Complete blood count results were normal. Cranial CT showed an area of low attenuation in the left frontoparietal region consistent with infarction. Prothrombotic work-up results were as follows: serum methionine level: 4.7 mg / dL (normal: 0.09-0.6 mg/dL); free homocysteine concentration: 50 mmol/L (normal: <12.5 mmol/L); urine homocysteine concentration: 360 mmol/L (normal: 5-15 mmol/L). The patient was diagnosed as homocysteinuria, and hydroxy cobalamin injections, vitamin B6, acetyl-salicylic acid, and a low-methionine diet were started and maintained.

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Ophthalmological examination showed bilateral lens subluxation, bilateral cataracts, and secondary glaucoma. The patient underwent cataract extraction in both eyes via phacoemulsification. Both eyes were treated with topical anti-glaucoma drops. Additionally, the right eye underwent diode laser cyclophotocoagulation with normalization of intraocular pressure without any drops at the 1-year follow-up. Intraocular pressure in the left eye remained normal with topical anti-glaucoma drops.

Written consent was provided by the patient's parents. Following DNA extraction, all the exons of the CBS gene were screened via sequencing. Exon 8 of the CBS gene was amplified using 2 primer sets (F: 5' CTGAACATTTAGGTCATTACC 3'; R: 5' TTT CACACGTTTTCCCTGC 3') under standard PCR conditions, with an annealing temperature of 57°C. PCR showed a 598-bp amplified product, which was cleaned using a DNA purification kit (Metis, Turkey). Then, the sample was sequenced using a DNA sequencer (Beckman Coulter DNA sequencer, USA). Direct PCR analysis and sequencing showed a 110-bp insertion at exon 8 in the CBS gene (Figures 1 and 2). A novel 110-bp insertion starting at nt 855 up to 965 ending with a new amino acid formation was determined. In exon 8 the serine amino acid coded by TCC was altered to tryptophan (TGG) due to the 110-bp insertion. Additionally, a missense mutation at exon 8 in the CBS gene was also noted, which caused a T-C transition at base pair 833, resulting in an amino acid change from isoleucine to threonine (Figure 3). 833 T>C (p.I1278T) was previously described (HGMD_CM990350).

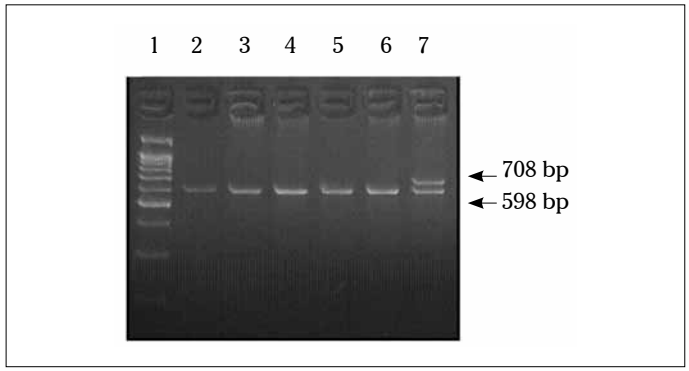

Figure 1. Agarose Gel Electrophoretic Analysis of the 110-bp Heterozygous Insertion at Exon 8 in the CBS Gene. Band 1 is the DNA Marker (ØX174 HaeIII, Fermentas), and Bands 2-6 are DNA Samples for the Control. Band 7 is The Patient's PCR

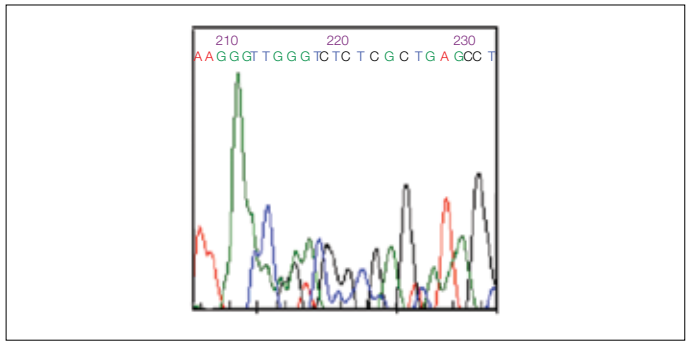

Figure 2. Starting Point of the 110-bp Insertion at Exon 8 in the CBS Gene

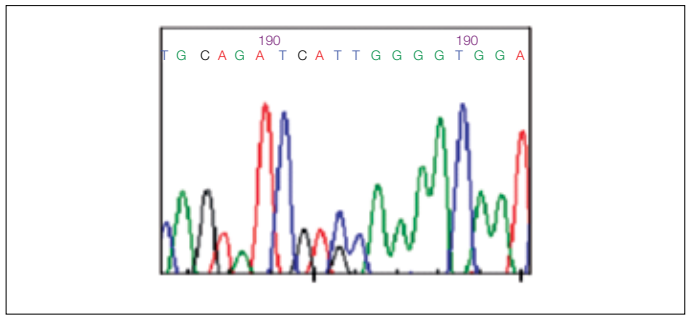

Figure 3. CBS Gene 833 T>C Transition at Exon 8

Discussion

We described a 14-year-old patient with homocysteinuria due to 2 mutations in the CBS gene in a compound heterozygous state. The novel 110-bp insertion mutation at the intron-exon junction of the 5' end of exon 8 affected the splicing site. An 844ins68 polymorphism was previously reported at this localization [4]. Previous research has shown that 844ins68 insertion in the CBS gene has a heterogeneous distribution in human populations. The effect of 844ins68 on homocysteine metabolism and its thrombotic effect remain speculative. The frequency of this polymorphism is 5.9% in the Turkish population [5]. The novel 110-bp insertion observed in the presented case shows that this region of the CBS gene may be a hot spot for mutations; the observed mutation and the 844ins68 variant do not overlap. In conclusion, 110-bp insertion plays an important role in homocysteine-related diseases.

Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

References

1. Kaur M, Kabra M, Das GP, Suri M, Verma IC. Clinical and biochemical studies in homocystinuria. Indian Pediatr 1995;32:1067-75.
2. Kraus JP, Janosík M, Kozich V, Mandell R, Shih V, Sperandeo MP, Sebastio G, de Franchis R, Andria G, Kluijtmans LA, Blom H, Boers GH, Gordon RB, Kamoun P, Tsai MY, Kruger WD, Koch HG, Ohura T, Gaustadnes M. Cystathionine Beta-Synthase Mutations In Homocystinuria. Hum Mutat 1999;13:362-75. [CrossRef]
3. Münke M, Kraus JP, Ohura T, Francke U. The gene for cystathionine beta synthase (CBS) maps to the subtelomeric region on human chromosome 21q and to proximal mouse chromosome 17. Am J Hum Genet 1988;42:550-9.
4. Tsai MY, Bignell M, Schwichtenberg K, Hanson NQ. High prevalence of a mutation in the cystathionine beta-Synthase gene. Am J Hum Genet 1996;59;1262-7.
5. Akar N, Akar E, Mısırlıoğlu M, Avcu F, Yalçın A, Cin Ş. Search For Genetic Factors Favoring Thrombosis in Turkish Population. Thromb Res 1998;92;79-82. [CrossRef]