A New SOX9 Gene Mutation in A Case of Campomelic Dysplasia

Kampomelik Displazili Bir Olguda Yeni SOX9 Geni Mutasyonu

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ABSTRACT Campomelic dysplasia (CD, OMIM 114290), an autosomal dominant multiple malformation syndrome with or without XY sex reversal, is characterized by skeletal and developmental abnormalities. CD is caused by heterozygous mutations in the transcription factor SOX9, which is involved in various steps of chondrogenesis and sex determination. We present a new missense mutation in the High Mobility Group (HMG) domain of SOX9 in a patient with typical features of CD. The proband, with a normal 46,XY karyotype and male phenotype, was found to be heterozygous for the de novo c.508C> T (p.P170S) mutation. Our report expands the mutation spectrum in this disorder, and provide valuable information for the importance of the Pro170 residue in SOX9.

Key Words: Campomelic dysplasia; SOX9 protein, human; inheritance patterns

ÖZET Kampomelik displazi (CD, OMIM 114290), otozomal dominant geçiş gösteren, XY cinsiyet dönüşümü ile birliktelik gösterebilen, iskelet sistemine ait ve gelişimsel anomaliler ile karakterize olan bir çoklu malformasyon sendromudur. CD, kıkırdak doku gelişimi ve cinsiyetin belirlenmesinde çeşitli basamaklarda görev alan transkripsiyon faktörünü kodlayan SOX9 geninde yer alan heterozigot mutasyonlarla oluşur. Biz, bu çalışma ile CD'nin tipik klinik bulgularına sahip olan bir hastada, SOX9 geninin Yüksek Mobilite Grup (HMG) bölgesinde yeni bir yanlış anlamlı mutasyon gösterdik. Normal 46,XY karyotip ve erkek fenotip bulgularına sahip olan proband, de novo oluşan c.508C> T (p.P170S) mutasyonu için heterozigot olarak belirlendi. Bu çalışma CD'ye neden olan mutasyon spektrumunu genişletmektedir, ayrıca SOX9 proteininin 170. pozisyonunda yer alan Proline aminoasidinin önemini vurgulayan değerli bilgiler sağlamaktadır.

Anahtar Kelimeler: Kampomelik displazi; SOX9; otozomal dominant kalıtım

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Campomelic dysplasia (CD, OMIM 114290) is an autosomal dominant multiple malformation syndrome with or without XY sex reversal.[1-4] CD is a rare disorder, the incidence of this syndrome is 0.5-1 per 100.000 live births.[3] CD is characterized by bowing of the femora and tibiae, club feet, pretibial skin dimples, hypoplastic scapulae, 11 pairs of ribs, lack of mineralization of thoracic pedicles, pelvis and spine malformations, and Robin sequence.[1,3,5-7] Death frequently occurs during the neonatal period as a result of respiratory insufficiencies, which has been attributed to Robin sequence, tracheobronchial cartilage defects, hypoplastic lungs with narrow airways, and a bell shaped small thorax.[1,5] An important aspect of CD

is XY sex reversal that occurs in about two thirds of karyotypic male patients.[1,5] Campomelia, the bending of the long bones, is absent in about 10% of the cases, referred to as the acampomelic form of CD.[1,5]

CD is due to heterozygous mutations in and around the SOX9 gene at 17q24.[2,5,8] SOX9, a transcription factor belonging to the SOX (SRY-related HMG box) gene family, has a critical role in chondrogenesis and sex determination.[9-13]

Here, we report a new missense mutation in the High Mobility Group (HMG) domain of SOX9 gene in a 46,XY male.

CASE REPORT

The propositus was the first child of a non-consanguineous, healthy couple. The father and mother were 35 and 30 years old, respectively. Family history was unremarkable. Pregnancy was uneventful. The child was delivered at term with a birth weight of 2730 g (3-10th centile). His weight, length, and head circumference were 3350 g (below the 3rd centile), 49.5 cm (below the 3rd centile), and 37 cm (25th centile), respectively at 1 and 1/2 months.

A flat facial profile with blue sclera, low set ears, and micrognathia with a U-shaped cleft palate, leading to the consideration of Robin sequence were noted during physical examination (Figure 1).

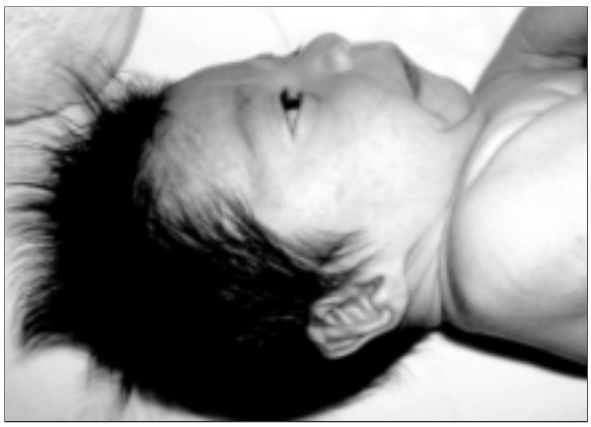

FIGURE 1: Photograph of the patient showing flat facial profile, micrognathia, and low set ears.

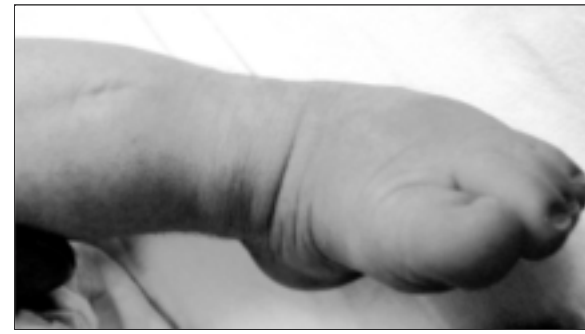

FIGURE 2: Pretibial skin dimples and clubfeet of the patient.

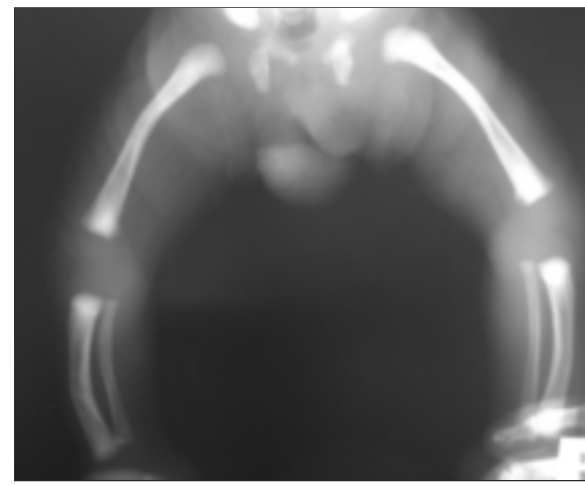

FIGURE 3: Radiograph of lower extremities showing bowed femora and tibiae.

There was bilateral bowing of the legs with an overlying skin dimple, and talipes equinovarus (Figure 2). His radiological findings included hypoplastic scapula, small chest, 11 pairs of ribs, and bowed femora and tibiae (Figure 3, 4). The external genitalia were normal male.

Karyotype, obtained from peripheral blood lymphocytes, was 46,XY. The patient could not pass neonatal hearing screening using otoacoustic emissions. Further investigations with brainstem auditory evoked response confirmed the presence of severe mixed hearing loss. An echocardiography was reported to be normal.

After obtaining appropriate consents, mutations in the SOX9 gene was screened using direct DNA sequencing. The primers for the 3 coding exons and intron exon boundaries were amplified

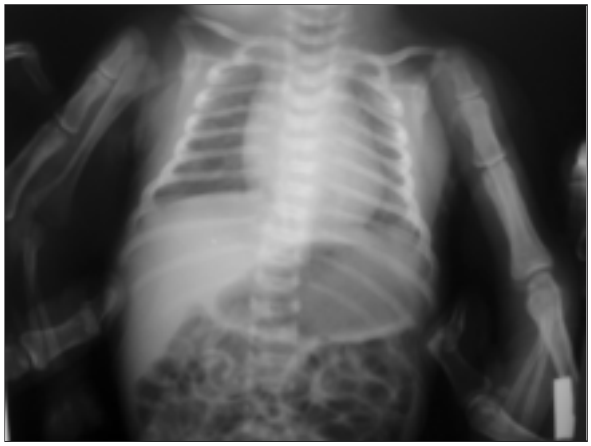

FIGURE 4: Radiograph of thorax revealing 11 pairs of ribs and chest narrowing.

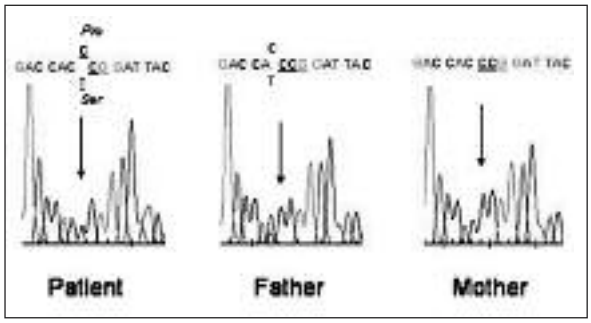

FIGURE 5: Sequence analysis of the SOX9 gene. The figure shows the c.508C> T (p.P170S) heterozygous mutation in the patient and the heterozygous c.507C> T (p.H169H) SNP in the father. The mother and father do not carry the c.508C >T mutation.

with PCR. The proband was found to be heterozygous for a missense mutation, c.508C> T (p.P170S). The parents did not have this alteration proving its de novo nature (Figure 5).

DISCUSSION

Our patient's clinical and radiographic findings are clearly consistent with the diagnosis of CD. CD is usually lethal in the first year of life, however it is reported that a small ratio of patients with CD survive. The great majority of patients die in the neonatal period as a consequence of respiratory insufficiency. In a previous study, a survival rate of 5% and 10% was reported.[5] Mansour et al described the phenotypes of five survivors of CD, with an age range of 7 to 20 years.[14] Recurrent apnea, chest infections and stridor are the main clinical problems in the neonatal period.[14] The complications in the later years include conductive hearing loss, developmental delay, dental caries and myopia.[14] Severe and progressive kyphoscoliosis, talipes equinovarus, congenital subluxation or dislocation of the hips, dislocation of the radial heads are the orthopaedic complications of the disease.[14] Some survivors have been reported with normal intelligence whereas the majority of the patients have mild to moderate developmental delay.[14]

CD with XY sex reversal has been shown to be caused by heterozygous loss-of-function mutations in the SOX9 gene, indicating haploinsufficiency for SOX9 being responsible for both CD and XY reversal phenotypes.[2,8] Consistent with the CD phenotype, SOX9 is expressed throughout chondrogenesis and in the developing testis.[11-13] Additionally, studies have identified the collagen genes Col2a1 and Col11a2 and the testicular Sf1 and Amh genes as direct targets for SOX9.[15-19] SOX9 belongs to the SOX family of transcription factors, which contains a high mobility group (HMG) DNA binding domain. SOX9 also has a transactivating domain at the C-terminus of the protein as well as

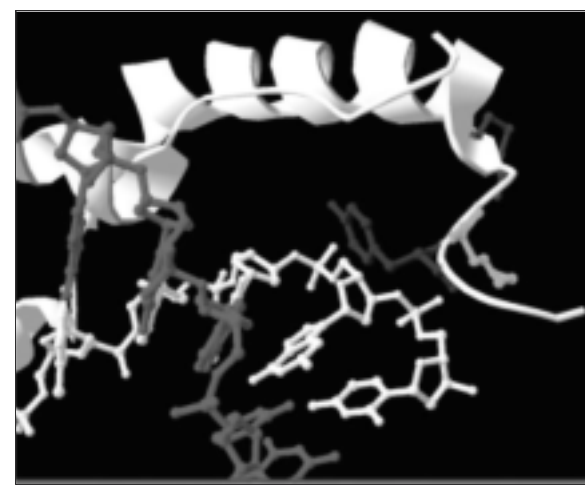

FIGURE 6: Rendered three dimensional image of the C-terminal of the HMG domain of SOX9. Kinking effect of Pro70 residue brings Lys73 and Tyr74 residues closer to the DNA molecule. Previously created 3D model of the HMG domain of SOX9 was obtained from RCSB Protein Databank (http://www.rcsb.org/pdb/home/home.do) as a pdb file (1SX9) and the image was rendered with DeepView/Swiss-PdbViewer version 3.7 program.

a dimerization domain localized in a conserved region preceding the HMG domain enabling SOX9 to dimerize on target sites.[9,10,20,21]

Among different types of mutations that result in CD described so far, no clear genotype/phenotype correlation is evident indicating variable expressivity and incomplete penetrance.[2,6,8,9]

We identified a previously unreported missense mutation in the HMG domain of SOX9. In a previous study by Meyer et al., the p.P170R mutation affecting the highly conserved proline residue (Pro70 in the HMG domain), as that was observed in our patient, was defined.[6] It was shown in in-vitro studies that while the wild-type SOX9 HMG domain binds the oligonucleotide containing the high affinity binding site for murine and human SRY, the P170R mutant SOX9 HMG-domain displayed reduced DNA binding. The patient carrying the mutation lived one month.[6] In vitro studies as well as in silico modeling of the HMG domain of SOX9 provided evidence for a very important role of Pro70 residue. It has been shown that its kinking effect on the protein brings the C-terminal of the HMG domain closer to DNA molecule, especially at residues 73 and 74 (Figure 6). Thus, substitution of this important residue with serine (P170S) in our patient, and arginine (P170R) in a previously reported child results in disruption of DNA-HMG domain interaction.

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