

Chromosome and p63 Gene Analysis of an Infant with Ectrodactyly-Split Hand and Foot Malformation

Osman DEMİRHAN¹

Deniz TAŞTEMİR¹

Nejat NARLI²

Süreyya SOYUPAK³

Kenan ÖZCAN²

Aim: Split hand-split foot malformation (SHFM) results from central ray reduction and presents as median clefts of the hands and feet, syndactyly, aplasia or hypoplasia of the phalanges, metacarpals and metatarsals, which are frequently associated with other phenotypic abnormalities. We aimed to investigate the genetic pathway of SHFM in a child.

Materials and methods: Cytogenetic and molecular genetic analysis was performed in a 10-day-old boy with split-syndactyly hand and flat-syndactyly foot.

Results: We found a complex chromosomal rearrangement including breaks in 4q12, fragility in the 9q11-13 band region and 9qh+. Cytogenetic results agree with the literature findings. The mutation analysis of the p63 gene revealed no mutation.

Conclusion: The phenotype of our patient may be due to variable expressivity and penetrance of the p63 gene and to other genetic factors, or the mutation can be located in the other 4 loci for SHFM. Additional minor modifying genes, which predispose to non-syndromic cleft palate, could also contribute to the expression of the cleft palate component of the EEC syndrome.

Key Words: Ectrodactyly, bilateral syndactyly, flat-syndactyly foot, split-syndactyly hand, p63 gene, cytogenetics

¹ Department of Medical Biology and Genetics, Faculty of Medicine, Çukurova University, Adana - TURKEY

² Department of Neonatology, Faculty of Medicine, Çukurova University, Adana - TURKEY

³ Department of Radiology, Faculty of Medicine, Çukurova University, Adana - TURKEY

Ektrodaktilli-Yarık El ve Ayak Malformasyonlu Bir Olgunun Kromozom ve p63 Gen Mutasyon Analizleri

Amaç: Yarık el- yarık ayak kusurlu oluşumu (SHFM), parmak oluşum merkezindeki duraksama sonucunda meydana gelir ve el ile ayaklarda medyan yarıklar, sindaktili ve diğer fenotipik anomaliler ile genellikle ilişkili bulunan falanj, metakarpus ve metatarsuslardaki aplazi veya hipoplazi olarak kendini göstermektedir. Bu çalışmada SHFM'li bir çocukta SHFM'in genetik mekanizmasının araştırılması amaçlandı.

Materyal ve metod: Bu çalışmada, baş ve işaret parmağı arasında sindaktilisi olan yarık el ile sindaktilisi olan düz taban ayağa sahip olan 10 günlük bir erkek çocukta sitogenetik ve moleküler genetik analizler uygulandı.

Bulgular: Hastamızda, 4q12'de kırılma, 9q11-13 band bölgesinde frajilite ve 9qh+ gibi çeşitli kromozomal yeniden düzenlenmeler saptandı. Sitogenetik bulgular literatür bulguları ile benzerlik gösterirken, p63'deki moleküler analiz ise herhangi bir mutasyonun olmadığını gösterdi.

Sonuç: Hastamızın fenotipi muhtemelen, p63 geninin değişken ekspresivitesi ve penetransı nedeniyle ve diğer genetik faktörler veya SHFM genindeki diğer dört bölgede lokalize olan mutasyonlar nedeniyle oluşmuş olabilir. Non-sendromik yarık damağa yatkınlık oluşturan diğer minor modifiye edici genler de EEC sendromunun yarık damak oluşumuna katkıda bulunabilir.

Anahtar Sözcükler: Ektrodaktili, bilateral sindaktili, sindaktillili düz taban ayak, sindaktillili yarık el, p63 geni, sitogenetik

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Correspondence

Osman DEMİRHAN

Department of Medical Biology and Genetics,
Faculty of Medicine,
Çukurova University
01330, Balcalı,
Adana - TURKEY

osdemir@cu.edu.tr

Introduction

Split hand-split foot malformation (SHFM) is a limb malformation involving the central rays of the autopod, presenting with syndactyly, and median clefts of the hands and feet, varying from mild degrees of clefting to almost absent limbs (1). Although the clinical expression of the SHFM syndrome is highly variable, it occurs in approximately 1 in 18,000 newborns. SHFM can also occur as a non-syndromic disorder, with genes for at least 5 forms localized to different chromosomes: on 7q21, Xq26, 10q24, 3q27

(TP63), and 2q31 loci (OMIM 183600, 313350, 600095, 605289, 60678). Variable expressivity is common in EEC syndrome families (2). Autosomal recessive and X-linked forms occur more rarely, and other cases of SHFM are caused by chromosomal deletions and duplications. Sharland et al. (1991) reported a boy with tetramelic ectrodactyly who had a complex chromosomal rearrangement resulting from breaks in 5q11.2, 5q34, 7q21.2, 7q31.3 and 9q22.1 chromosomal regions (3). Ignatius et al. (1996) also reported a complex 6-break chromosomal rearrangement in an adult male with tetramelic ectrodactyly (4). Mutation analysis has demonstrated that the EEC3 gene is the locus for tumor protein 63 (5). Mutations in the p63 gene localized on chromosome 3q27 were identified in families with isolated SHFM (1) and in individuals affected by the EEC syndrome (3). Ectrodactyly is a distal limb malformation that can occur isolatedly or as part of complex genetic malformation syndromes (6). Recently, it has been demonstrated that p63 is a major locus for

syndromic (EEC, LMS, and ADULT syndromes) or isolated (SHFM) autosomal dominant ectrodactyly (7).

In this article, we report the clinical features and genetic findings (using cytogenetic screening and molecular analysis) of a 10-day-old Turkish boy with SHFM.

Case Report

The proband was first seen when 10 days old. He was born at full-term following a normal pregnancy and delivery with a birth weight of 3300 g. Both of his parents were first degree cousins and were healthy. His mother was 22 years old and his father 24 years old. At birth, it was noted that both hands were split between the third and fourth fingers, and each had 4 fingers; the first and second fingers were bilaterally syndactylous (Figure 1). Both feet were flat, and almost completely syndactylous between the third and fourth toes of the left

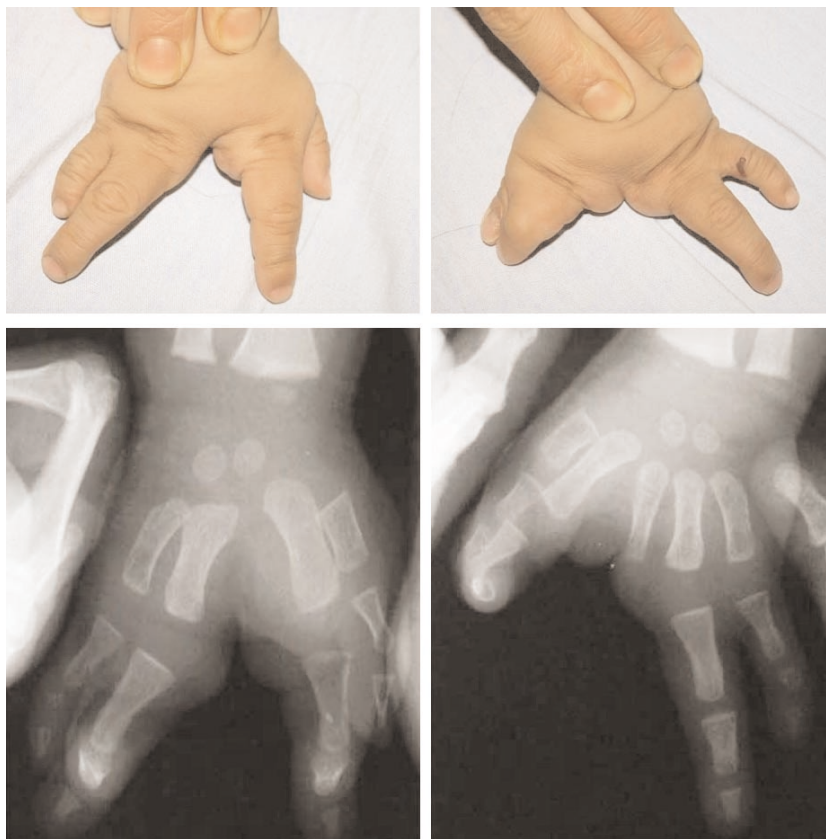


Figure 1. Clinical expressions and radiographs of the hands in the affected boy.

foot. The nails were normal (Figure 2). Investigations including echocardiography, abdominal ultrasonography, electrocardiography, telecardiography, all body scans, and visual observations were normal. No other abnormality was noted (Figure 3).

Cytogenetic and Molecular Genetics Analysis

Karyotype analysis was performed on peripheral blood samples cultured for 72 h in RPMI-1640 normal

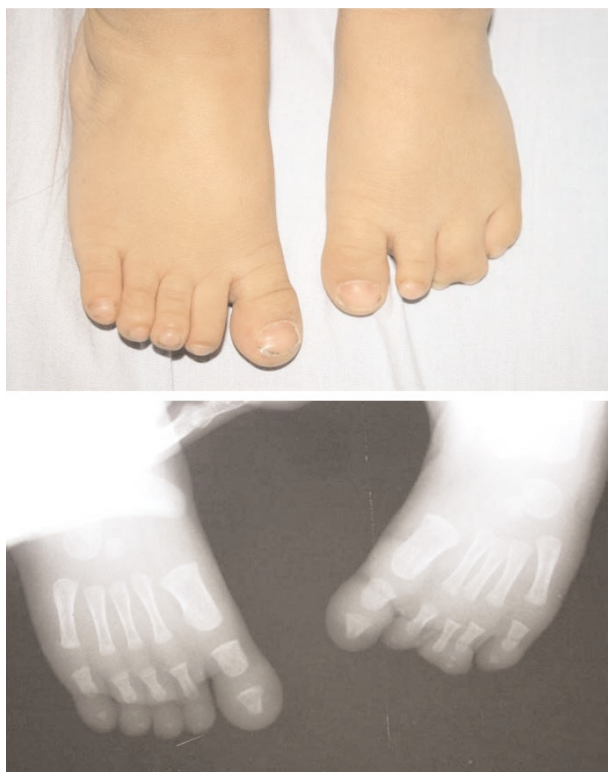


Figure 2. Clinical expressions and radiographs of the feet of the boy.



Figure 3. Patient at the age 14 months demonstrating facial features.

medium by standard techniques in our genetic laboratory. GTG-banding chromosome analysis in the patient revealed fragility in the 9q11-13 region in a metaphase, breaks on chromosome 4q12 in 2 metaphases, and constitutive heterochromatin of the 9qh+ in all cells, and his parents had normal chromosomes in all cells. Molecular genetic analyses were performed at the Institute for Medical Genetics, Charité, Berlin, Germany. Genomic DNA was extracted from blood lymphocytes using the commercial GenoPrep™ DNA Isolation Kit. All exons of p63 were amplified from genomic DNA with the primers designed by van Bokhoven et al. (8). PCR reactions were performed in 25 μ l containing 100 ng of genomic DNA, 20 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTPs, 100 ng of each primer, and 1 unit of Taq polymerase. The PCR conditions included an initial denaturation for 15 min at 96 °C, followed by 35 cycles of 94 °C for 1 min, 55 °C for 2 min, 72 °C for 1 min, and a final extension for 10 min at 72 °C. The analysis of PCR products was performed on 2% agarose gels. The sequencing reaction was performed with a 4-dye-terminator cycle sequencing ready reaction kit (ABI Big Dye Terminator Cycle Sequencing Kit (ABI, Weiterstadt, Germany) according to the manufacturer's instructions. Sequence products were purified through thin columns (Sephadex G-501; Princtown Separations, Adelphia, NJ, USA) and resolved in a sequencer ABI Prism 3100 Genetic Analyzer (PE Biosystems, USA). We performed mutation screening in all exons of p63 in the affected boy and his parents. The mutation analysis of the p63 gene revealed no mutation.

Discussion

In this report, we present a 10-day-old boy with both hands split, 4 fingers on each hand, and bilateral syndactyly between the first 2 fingers. Both feet were flat, and there was syndactyly between the third and fourth toes of the left foot with no detected mutation in p63. However, he had cytogenetically visible chromosome aberrations, which included a break on 4q12, fragility in the 9q11-13 band region, and 9qh+. No other abnormality was noted. It is well known that chromosomal abnormalities are usually combined with congenital anomalies. A previous work reported that chromosomal abnormalities are known to be important in the etiology of EEC syndrome (3). Sharland et al. reported the case of a boy with tetramelic ectrodactyly

who had an exceeding complex chromosomal rearrangement resulting from 5 breaks in the 5q11.2, 5q34, 7q21.2, 7q31.3, and 9q22.1 regions (3). Ignatius et al. also reported an adult male with tetramelic ectrodactyly and a complex 6 breaks chromosomal rearrangement, including a break at 7q21.3 (4). In other studies, Genuardi et al. found an apparently balanced translocation t(2;7) (q21.1;q22.1), in a patient with bilateral split-hand and right split-foot (9). Cobben et al. described a case of typical tetramelic split hand-foot malformation in association with a pericentric inversion of chromosome 7: 46,XY,inv(7)(p22q21.3) (10). The limbs and chromosomes of the parents were normal. A more recent study, by Ray et al., demonstrated maximal linkage to 2 regions on chromosomes 4q and 14 for the clefting phenotype in an EEC family (2). In the present study, a break at 4q and fragility at 9q also agree with the other studies. The constitutive heterochromatin of the 9qh+ was not observed in other studies. The constitutive heterochromatin of the 9qh+ region is considered a normal variation of human chromosome 9. The possible clinical effects of 9qh+ are not exactly known. Undoubtedly, further studies are necessary to understand the role of 4q12, 9q11-13 and 9qh+, and these regions may be new hot spots for SHFM.

Recent studies have provided important clues for identifying a genetic and molecular basis of EEC syndrome. Five SHFM loci have been genetically mapped on chromosomes 7q21, Xq26, 10q24, 3q27, and 2q31. For EEC syndrome, the candidate chromosomal regions are 7q21.3, 7q11, 9q21, and 19q. Other candidate regions include developmental genes implicated in the ectodermal/mesodermal interactive processes. We did not detect p63 mutation at q27 of chromosome 3 in the patient. SHFM can also occur as a non-syndromic disorder, with genes for at least 5 forms localized to different chromosomes (2). Variable expressivity is common in EEC syndrome families. Genetic heterogeneity also exists, as EEC has been mapped to more than one chromosomal location: EEC1 on chromosome 7q11.3-q21.1, EEC2 on chromosome 19, and EEC3 on 3q27 (5,11). However, a total of 12 heterozygous nucleotide

changes in p63 have been detected in EEC syndrome patients (5,12). Interestingly, genetic changes at both the EEC1 and EEC3 locations can also produce non-syndromic SHFM (11). SHFM occurs as an isolated anomaly and in combination with other abnormalities. It is often accompanied by syndactyly, and the adjacent fingers and toes may be hypoplastic or completely missing (13). Our patient had also bilateral syndactyly and 4 fingers, the first 2 fingers being bilateral syndactylous, and clefting, which was seen in both hands bilaterally.

The results of this study demonstrate that there is no correlation between the p63 gene and the clinical phenotypes in our patient. We have suggested different possibilities. The phenotype of our patient may be due to variable expressivity and penetrance of p63 or splice mutation. The observed variable expressivity and penetrance of p63 are probably due to other genetic factors. A reduction in the expression of this gene during embryogenesis may only explain the phenotype observed in our patient. Interestingly, it appears that some p63 isoforms can act as dominant-negative inhibitors of TP53 and p63 activation, suggesting a pathogenetic mechanism for the observed heterozygous mutations in patients with SHFM or EEC syndrome (14). However, the mutation in our patient can be located on the other 4 loci for SHFM that have been mapped: chromosome 7q21, Xq26, 10q24, and 2q31. Additional minor modifying genes, which predispose to non-syndromic cleft palate, could also contribute to the expression of the cleft palate component of the EEC in this patient. Once non-syndromic cleft palate susceptibility genes have been isolated from chromosomes 4q and/or 9q, further investigations of this and other large SHFM pedigrees will be needed to confirm or refute their involvement in the expression of EEC facial clefting.

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