# The EEC syndrome and SHFM: report of two cases and mutation analysis of p63 gene

Hacer Ergin[1], C. Nur Semerci[2], Y. Tuğrul Karakuş[1], Hans Scheffer[3], Şeniz Ergin[4], Uğur Koltuksuz[5], Rowdy Meijer[3], N. Lale Şatıroğlu-Tufan[2]

*Departments of [1]Pediatrics, [2]Medical Genetics, [4]Dermatology, and [5]Pediatric Surgery, Pamukkale University Faculty of Medicine, Denizli, Turkey, and [3]Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands*

**SUMMARY: Ergin H, Semerci CN, Karakuş YT, Scheffer H, Ergin Ş, Koltuksuz U, Meijer R, Şatıroğlu-Tufan NL. The EEC syndrome and SHFM: report of two cases and mutation analysis of p63 gene. Turk J Pediatr 2010; 52: 529-533.**

**The p63 gene is a transcription factor and a member of the p53 family. Heterozygote mutation of the p63 gene is suggested in a number of human syndromes including limb development and/or ectodermal dysplasia. The EEC syndrome, consisting of ectrodactyly (E), ectodermal dysplasia (E) and cleft lip (C) with or without cleft palate, is the prototype of these syndromes with the presence of heterozygote mutation in the p63 gene in most of the patients. Nonsyndromic split hand/foot malformation (SHFM) is one of the EEC-like syndromes, and the p63 gene mutation was reported in only a few patients. Five different loci have been mapped to date, but the etiology is yet to be explained in the rest of the patients. Here, we report two cases. Case 1, diagnosed with EEC syndrome, had type 2 urogenital sinus and a new heterozygous mutation of 934G>A (D312N) in exon 8 of the p63 gene. Case 2 was diagnosed as SHFM with no mutation in the p63 gene. Genotype and phenotype correlation of these two cases among the reported patients is discussed in this report.**

***Key words: EEC syndrome (ectrodactyly (E), ectodermal dysplasia (E) and cleft lip (C) with or without cleft palate), nonsyndromic split hand-foot malformation, p63 gene.***

Ectrodactyly - ectodermal dysplasia - cleft of the lip and palate (EEC) syndrome and split hand/foot malformation (SHFM) are members of a complex group of developmental syndromes. The central reduction of the hands and feet are the common phenotypic feature of these syndromes.

The EEC syndrome consists of ectrodactyly (E), ectodermal dysplasia (E) and cleft of the lip and palate (C) (OMIM 604292). The term EEC syndrome was first used by Rüdiger et al.[1] in 1970, although cases with similar anomalies had been reported previously. There are some further anomalies, such as genitourinary (GU) and nipple anomalies, deafness, choanal atresia, anorectal malformation, variable degrees of minor ear anomalies, hypothalamopituitary insufficiency, thymic hypoplasia, and mental retardation[2]. Rosselli and Gulienetti[3] were probably the first to describe structural anomalies of the GU system as an associated finding in a patient with ectodermal dysplasia. Preus and Fraser[4] suggested that structural anomalies of the kidney are an integral component of the EEC syndrome. The EEC syndrome is a model of the disturbance of apoptosis, because split hands and feet are caused by a failure of cell death between fingers and toes, while urogenital anomalies are due to abnormal regression of the Wolffian or Müllerian duct, and facial clefts are attributed to the abnormal elimination of excess cells during fusion of the archetypal palate[5]. At least three types of EEC syndromes and their respective gene loci have been identified. EEC syndrome type 1 is linked to 7q11.2-q21.3, type 2 to chromosome 19, and type 3 to 3q27 (p63) [6,7].

Split hand/foot malformation (SHFM) or ectrodactyly is characterized by aberrant development of the central digital rays with absence of fingers or toes, a deep median cleft and fusion of the remaining digits. SHFM occurs as an isolated anomaly or in combination with other abnormalities (syndromic ectrodactyly). Five loci have been mapped for nonsyndromic SHFM patients to date (SHFM1-5). Furthermore, Gurnett et al.[8] reported a new locus on chromosome 8q in a family.

We report here two newborn cases. Case 1 had EEC syndrome associated with a new heterozygous mutation of 934G>A (D312N) in exon 8 of the p63 gene, and Case 2 was diagnosed as isolated SHFM associated with congenital heart defect (CHD) without mutation of the p63 gene.

## Case Reports

### *Case 1*

A newborn girl was delivered spontaneously after 39 weeks of gestation. She was the second child of a 31-year-old mother and 36-year-old father. Her non-consanguineous parents and the elder sister were healthy and normal. Pregnancy and delivery were unremarkable. There was no maternal diabetes, oligohydramnios or other conditions that could be associated with malformation. No spontaneous abortions were reported. Ultrasonography was not performed during pregnancy. She was referred to our hospital due to hand-feet and face anomalies. Birth weight was 2000 g (<3rd centile), length 43 cm (<3rd centile), and occipitofrontal head circumference 30.5 cm (<3rd centile). She was a symmetric small for gestational age (SGA) baby. There was no scalp hair except on the occipital area. The skin of the scalp, forehead, face and body was dry, thin and scaly. Eyebrows and eyelashes were absent. Ocular examination was normal. Complete cleft palate and bilateral cleft lip were observed. Nipples were not seen bilaterally (Fig. 1a). Both arms and legs were normal except typical lobster-claw hands and feet abnormality (Figs. 1c-1f). The nails were dystrophic at one of the digits of the hand and feet (Figs. 1b, 1c). GU examination showed anteriorly placed anus. Routine laboratory tests, hearing screening test and echocardiography showed no abnormalities. Urinary ultrasonography and voiding cystourethrography revealed left pelvicaliceal dilation and type 2 urogenital sinus, respectively. Chromosome analysis on peripheral blood revealed normal female (46,XX) karyotype. As a result of clinical and laboratory findings, the case was diagnosed with EEC syndrome.

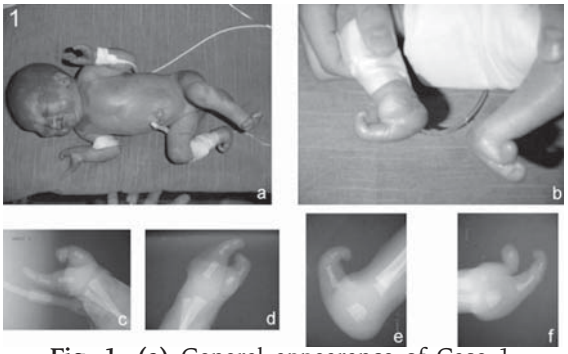


**Fig. 1. (a)** General appearance of Case 1. **(b)** Severely affected feet of patient with EEC syndrome. **(c, d)** X-ray of right and left hands. **(e, f)** X-ray of right and left feet.

She was operated for repair of cleft lip on the 6th day and was discharged from the hospital on the 34th day. It was learned that she died at home due to febrile illness at the age of 4.5 months.

### *Case 2*

A 28-day-old boy was referred to our clinic because of features consistent with lobster-claw malformation. He was born at term (3800 g) to healthy, non-consanguineous parents after an uncomplicated pregnancy. It was learned that prenatal ultrasonography was not performed. His parents and the elder sister were healthy and normal. The mother had a history of two previous spontaneous abortions at the fourth and eighth weeks. His weight was 3500 g (<3rd centile), length 53 cm (<3rd centile), and occipitofrontal head circumference 38 cm (<3rd centile). Physical examination was normal except for bilateral inguinal hernia and ectrodactyly of the upper and lower extremities (Figs. 2a, 2b). No ectodermal dysplasia, cleft lip or palate, or urogenital abnormalities were detected. Echocardiography showed secundum type atrial septal defect (ASD). Chromosome analysis performed on peripheral blood showed normal male (46,XY) karyotype. On radiological

examination, 2nd, 3rd and 4th metacarpal bones and phalanx aplasia and hypoplastic 1st and 5th distal phalanx were demonstrated on the hands (Figs. 2c, 2d). Third and 4th phalanx aplasia on the feet was noted (Figs. 2e, 2f). The radius and ulna were normal. Thus, the patient was diagnosed with SHFM.

### *p63 Mutation Analysis*

Intron-specific primers suitable for amplification of exons 1 to 15 and exon 3' of the p63 gene were designed: 8F: 5'- caagtgcttttgggtccatt -3'; 8R: 5'- cctggagtccacttcaatgc -3'. The amplification mix (per amplicon per sample) consisted of 1 $\mu$l genomic DNA (100 ng/$\mu$l), 2.50 $\mu$l 10x polymerase chain reaction (PCR) Gold Buffer (Applied Biosystems, Foster City, CA, USA), 2.00 $\mu$l $MgCl_2$ (25 mM, AB), 0.5 $\mu$l dNTPs (8.33 mM/dNTP), 1 $\mu$l forward and reverse primers (100 ng/$\mu$l), 0.2 AmpliTaq DNA polymerase Gold (5 U/$\mu$l, AB), and 17.80 $\mu$l distilled milliQ water. The reaction volume per sample was 25 $\mu$l. The following parameters were used for PCR: 1 min at 94ºC, 1 min at 58ºC, and 1 min at 72ºC for 35 cycles. PCR products were fractionated by electrophoresis in 1% agarose gels and visualized by ethidium bromide staining. PCR products were purified using a Multiscreen $PCR_{\mu 96}$ filter plate (Millipore). Sequencing of these fragments was done with the BigDye Terminator chemistry (Applied Biosystems). Electrophoresis and analysis were performed on an ABI 3730 (Applied Biosystems).

## Discussion

Case 1 fulfilled the criteria of completely expressed sporadic EEC syndrome with ectrodactyly (split hand and foot anomalies), ectodermal dysplasia (anomalies of skin, hair, nails, mammary glands, sweat glands), clefting (complete cleft lip-palate) and some further anomalies such as anteriorly placed anus and type 2 urogenital sinus. Symptoms in the other inherited syndromes with p63 mutations are overlapping, but each of these syndromes has its own characteristic combination of phenotypic features. Clinically, the EEC patient was differentiated from the other syndromes exhibiting both ectodermal dysplasia and orofacial clefting such as Rapp–Hodgkin syndrome (OMIM 129400), ankyloblepharon - ectodermal defects - cleft lip/palate (AEC) or Hay Wells syndrome (OMIM 106260) because of normal limbs[9-11]. This patient was also differentiated from limb mammary syndrome (LMS, OMIM 603543) with the presence of ectodermal dysplasia and cleft lip in addition to cleft palate[12]. Acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome (OMIM 103285) was not taken into consideration with the presence of orofacial clefting in our patient[13].

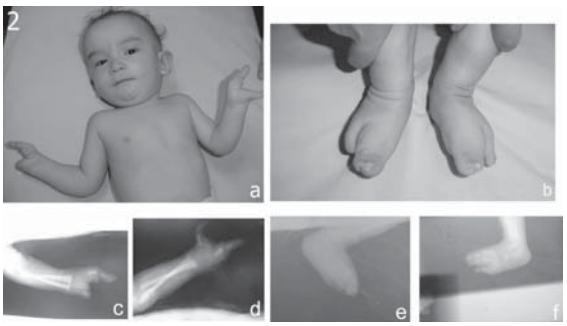


**Fig. 2. (a)** Face and hands of Case 2 with SHFM. **(b)** Feet of Case 2. **(c, d)** X-ray of right and left hands. **(e, f)** X-ray of right and left feet.

Structural anomalies of the GU tract were reported to be a hallmark of the EEC syndrome[14]. Various GU anomalies have been reported, including nephrogenic cyst, renal dysplasia, unilateral or bilateral renal agenesis, duplication of kidney, duplication of urinary collecting system, ureteric occlusion, ureterocele, bladder neck contracture, unilateral hydronephrosis, bilateral hydronephrosis, and hypospadia[2-4,14-16]. The incidence of the reported GU anomalies varied according to the methods of study. Küster et al.[17] found in his review of 165 case reports that 8.3% of EEC patients had associated GU anomalies. A review of 230 cases from English, German, French, Italian, and Dutch publications found that 23% of EEC patients had urogenital abnormalities[18], while in the study of Rollnick and Hoo[15], 8 patients of 13 EEC cases had GU involvement. Nardi et al.[14], by using intravenous pyelogram, voiding ureterocystography and sonographic examination, found structural GU anomalies in 52% of the 25 patients studied. Our case presented anteriorly placed anus and type 2 urogenital sinus detected by intravenous pyelogram. Cases of EEC syndrome with anteriorly placed anus were reported previously[12,19]; however, to our knowledge, this is the first reported EEC case with type 2 urogenital sinus.

The inheritance of EEC syndrome is autosomal dominant with incomplete penetrance and variable expression. Sporadic cases also occur[20]. A review of 230 cases from different publications identified 116 cases as familial and 114 as sporadic[18]. The phenotypic features of these cases include ectrodactyly (84%), ectodermal dysplasia (77%), cleft lip and/or palate (68%), lacrimal tract abnormalities (59%), urogenital abnormalities (23%), and conductive hearing loss (14%). Roelfsema and Cobben[18] found that sporadic cases are affected more severely than familial cases. Case 1, whose parents did not have any anomalies pertaining to the EEC syndrome, fulfilled the three cardinal signs of completely expressed sporadic EEC syndrome together with some additional anomalies.

Celli et al.[6] reported that heterozygous mutations in the p63 gene on 3q27 are the major cause of the EEC syndrome. Almost all of these mutations reported to date were characterized by amino acid substitutions in the DNA-binding domain. Transversion at position 934G>A in exon 8 of the p63 gene resulted in D312N in our patient. To our knowledge, this amino acid substitution in the DNA-binding domain was not reported previously in the patients with EEC syndrome, although R304Q, R304W, C306R and D312G mutations were reported on exon 8 previously[5,6,21,22]. p63 mutations are suggested to make a modest contribution to nonsyndromic SHFM[6,12]. Van Bokhoven et al.[12] detected p63 mutations in 40 of 43 individuals who had EEC syndrome, but in only 4 of 45 unrelated SHFM patients. These investigators reported that the proportion of SHFM patients with p63 gene mutations was low, and the majority of SHFM cases are likely to be due to mutations in other genes. Elliot et al.[23] performed an epidemiologic study for identification and phenotypic analysis of a mapped SHFM patient. They found that while CHDs were associated in 10% of SFHM1 (chromosome 7q21.3-q22.1) and 47% of SHFM5 (chromosome 2q31) patients, CHDs were not found in SHFM2 (Xq26) and SHFM4 (3q27- p63 gene), even in patients mapped to chromosome 8. Case 2 had secundum type ASD with no mutation in the p63 gene. Therefore, Case 2 should be studied for mapping to the other loci, especially chromosome 7q and 2q, for SHFM.

In conclusion, we have demonstrated a novel mutation in the p63 gene in a patient with EEC syndrome who had type 2 urogenital sinus, and we reported a patient with SHFM associated with CHD who had no mutation in the p63 gene. It has been suggested that different modifier genes may explain the pathogenesis of a number of the abnormal limb developments and/or ectodermal dysplasia in this syndrome because of the clinical and genomic variability.

## REFERENCES

1. Rüdiger RA, Haase W, Passarge E. Association of ectrodactyly, ectodermal dysplasia and cleft lip-palate: the EEC syndrome. Am J Dis Child 1970; 120: 160-163.
2. Chuangsuwanich T, Sunsaneevithayakul P, Muangsomboon K, Limwongse C. Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome presenting with a large nephrogenic cyst, severe oligohydramnios and hydrops fetalis: a case report and review of the literature. Prenat Diagn 2005; 25: 210-215.
3. Rosselli D, Gulienetti R. Ectodermal dysplasia. Br J Plast Surg 1961; 14: 190-204.
4. Preus M, Fraser FC. The lobster claw defect with ectodermal defects, cleft lip-palate, tear duct anomaly, and renal anomalies. Clin Genet 1973; 4: 369-375.
5. Akahoshi K, Sakazume S, Kosaki K, Ohashi H, Fukushima Y. EEC syndrome type 3 with a heterozygous germline mutation in the p63 gene and B cell lymphoma. Am J Med Genet 2003; 120A: 370-373.
6. Celli J, Duijf P, Hamel BC, et al. Heterozygous germline mutations in the p53 homolog p63 are the cause of EEC syndrome. Cell 1999; 99: 143–153.
7. Ray AK, Marazita ML, Pathak R, et al. TP63 mutation and clefting modifier genes in an EEC family. Clin Genet 2004; 66: 217-222.
8. Gurnett CA, Dobbs MB, Nordsieck EJ, et al. Evidence for an additional locus for split hand/foot malformation in chromosome region 8q21.11-q22.3. Am J Med Genet A 2006; 140: 1744-1748.
9. Rapp HS, Hodgkin WE. Anhidrotic ectodermal dysplasia: autosomal dominant inheritance with palate and lip anomalies. J Med Genet 1968; 5: 269-272.
10. Hay RC, Wells RS. The syndrome of ankyloblepharon, ectodermal defects and cleft lip and palate: an autosomal dominant condition. Br J Dermatol 1976; 94: 277-289.
11. Ogur G, Yüksel M. Association of syndactyly, ectodermal dysplasia, and cleft lip and palate: report of two sibs from Turkey. J Med Genet 1988; 25: 37-40.
12. Van Bokhoven H, Hamel BC, Bamshad M, et al. p63 gene mutations in EEC syndrome, Limb-Mammary syndrome and isolated split hand-split foot malformation suggest a genotype-phenotype correlation. Am J Hum Genet 2001; 69: 481-492.

13. Rinne T, Spadoni E, Kjaer KW, et al. Delineation of the ADULT syndrome phenotype due to arginine 298 mutations of the p63 gene. Eur J Hum Genet 2006; 14: 904-910.

14. Nardi AC, Ferreira U, Netto Junior NR, et al. Urinary tract involvement in EEC syndrome: a clinical study in 25 Brazilian patients. Am J Med Genet 1992; 44: 803-806.

15. Rollnick BR, Hoo JJ. Genitourinary anomalies are a component manifestation in the ectodermal dysplasia, ectrodactyly, cleft lip/palate (EEC) syndrome. Am J Med Genet 1988; 29: 131-136.

16. Balci S, Engiz O, Okten G, et al. A 19-year follow-up of a patient with type 3 ectrodactyly-ectodermal dysplasia-clefting syndrome who developed non-Hodgkin lymphoma. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108: 91-95.

17. Küster W, Majewski F, Meinecke P. EEC syndrome without ectrodactyly? Clin Genet 1985; 28: 130-135.

18. Roelfsema NM, Cobben JM. The EEC syndrome: a literature study. Clin Dysmorphol 1996; 5: 115–127.

19. Maclean K, Holme SA, Gilmour E, et al. EEC syndrome, Arg227Gln TP63 mutation and micturition difficulties: is there a genotype-phenotype correlation? Am J Med Genet 2007; 143A: 1114-1119.

20. Penchaszadeh V, De Negrotti T. Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome: dominant inheritance and variable expression. J Med Genet 1976; 13: 281-284.

21. Barrow LL, Van Bokhoven H, Daack–Hirsch S, et al. Analysis of the p63 gene in classical EEC syndrome, related syndromes, and non-syndromic orofacial clefts. J Med Genet 2002; 39: 559-566.

22. Wessagowit V, Mellerio JE, Pembroke AC, McGrath JA. Heterozygous germline missense mutation in the p63 gene underlying EEC syndrome. Clin Exp Dermatol 2000; 25: 441-443.

23. Elliott AM, Evans JA. The association of split hand foot malformation (SHFM) and congenital heart defects. Birth Defects Res A Clin Mol Teratol 2008; 82: 425-434.