Ectrodactyly, Ectodermal Dysplasia and Cleft Lip/Palate Syndrome (EEC) in Patients with TP63 Variants

Original Article

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ABSTRACT

Background: Ectrodactyly, ectodermal dysplasia, cleft lip/palate syndrome (EEC) is a limb malformation in the form of Split hand/split foot malformations (SHFM). Tumor Protein P63 (TP63) is an important gene that has a role in tissue development and apoptosis. Different mutations in the TP63 gene can cause variable clinical disorders, such as EEC3 syndrome and SHFM4.

Objective: We report two Egyptian families with SHFM that are consistent with EEC syndrome. The molecular investigation was performed in the proband of each family using Next Generation Sequencing.

Results: Two variants were detected in exons 5 and 7 of TP63 gene. Family segregation of the identified variants has been confirmed by Sanger sequencing. The two affected patients have different variants in two different exons that lead to clinical variability.

Conclusion: This study is the first to identify the genetic variants involved in the EEC syndrome. Gene variants may lead to phenotypic variability among differently affected patients with similar TP63 gene variants..

Key Words: EEC syndrome, Limb malformations, SHSF, *TP63* gene.

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INTRODUCTION

Split hand/split foot malformation (SHFM) is a congenital limb malformation described as a deep central cleft within the hands and feet, missing digits associated with syndactyly between the remaining digits. It is either isolated or associated with other manifestations as part of a syndrome e.g. EEC syndrome (**Temtamy and McKusick, 1978**).

Ectrodactyly, ectodermal dysplasia, cleft lip/palate syndrome (EEC) (OMIM: 129900, 604292) is a rare pleiotropic condition. The cardinal clinical features of EEC are limb malformations in the form of SHFM, ectodermal dysplasia in which the primary defect involves two or more tissues that originate from embryonic ectoderm including dental anomalies, onychodysplasia, dry skin, or tricho-dysplasia with inflammation in eye structures **(Gorlin *et al.,* 1990; Itin and Fistarol, 2004**). In addition, oral cleft lip/palate emphasizes as a feature of EEC (**Childs *et al.,* 2020**). Other clinical manifestations that may be associated with the classical clinical triad includes eye abnormalities that involves the nasolacrimal duct, telecanthus, trichiasis, sparse eyelashes, blepharitis, corneal scars, erosions, blepharospasm, dry eye and photophobia. Urogenital system abnormalities were also reported with EEC syndrome (**Wawrzycki *et al.,* 2019**).

EEC syndrome was identified in 1804 by Eckholdt and Martens (**South *et al.,* 2002**). The first documentation describing the cardinal clinical features of the syndrome as a single clinical entity was in 1970 (**Rudiger *et al.,* 1970**). The incidence of EEC was estimated at 1/90.000 populations (**Bharati *et al.,* 2020**). EEC syndrome is inherited in an autosomal dominant manner with variable clinical expression and incomplete penetrance (**Brunner *et al.,* 2002**). Both sporadic and familial cases were reported (**Barrow *et al.,* 2002**).

Mutations in the *TP63* gene represent the most common cause in all typical EEC cases (**Brunner *et al.,* 2002**). It is responsible for about 10-16 % of isolated SHFM cases, and 98% of EEC cases in which limb malformations show up as a part of the clinical phenotype presentation

(**van Bokhoven *et al.,* 2001; Ray *et al.,* 2004**). Tumor protein p63 (*TP63*) is part of the p53 family, which is a homolog to TP53 and TP73. *TP63* has a significant role in the regulation of epithelial, limb, and craniofacial development (**Celli *et al.,* 1999**). The gene locus was found on chromosome 3q28 (**Alves *et al.,* 2015**). *TP63* consists of 2 promoters, fifteen exons, and alternative splicing sites, which express 6 different isoforms (**Abbas *et al.,* 2018**). More than 120 mutations were found within the *TP63* gene and were correlated with many different syndromes such as limb-mammary syndrome, SHFM4, orofacial cleft-8, EEC3 syndrome, AEC syndrome (ankyloblepharon-ectodermal defects-cleft lip/palate), and Rapp-Hodgkin syndrome (**Umair and Hayat, 2019**). The impact of particular mutations or the position of mutated residues in specific domains on *TP63*-related pathology stays difficult (**Wawrzycki *et al.,* 2019**).

This study reports two new Egyptian cases with EEC syndrome, discussing their clinical and molecular findings.

PATIENTS AND METHODS:

The study included four patients from two Egyptian families with EEC syndrome recruited from the Limb Malformations and Skeletal Dysplasia Clinic (LMSDC), National Research Centre (NRC). Participants in the study or their parents signed informed written consents according to the guidelines of The Medical Research Ethics Committee of the National Research Centre.

Detailed family history, pedigree construction, history of exposure to environmental factors during pregnancy, clinical examination of different body systems with special emphasis on the skeleton and limbs were performed. Radiological examination of the affected limb(s) were done. Other Investigations such as; chromosomal analysis, abdominal ultrasound, echocardiography, fundus examination, and hearing assessment were carried out whenever indicated.

Molecular Studies:

Genomic DNA was extracted from venous blood collected on EDTA tubes for the patients, affected sibling and their parents. PAXgene DNA blood extraction kit (Qiagen, Germany) has been used according to the manufacturer's protocol. Samples were measured by fluorometer using a DeNovix kit (Life sciences technologies, USA) to determine the concentration of the double-stranded DNA, then subjected to library preparation, sequencing, and data analysis. *TP63* gene was sequenced in patients by miSeq Illumina Next Generation Sequencer (NGS), by using in-house customized panel of inheritable genetic disorders related genes. Data was annotated and genomic variants were reported on the instrument. Detected variants were interpreted and reported.

Sanger sequencing was performed using ABI 3500 Genetic Analyzer (Applied Biosystems) for confirmation. Segregation analysis was done for the proband and available family members. PCR is performed using intron specific primers, targeting exons 5 and 7 of *TP63* gene. Primers sequences are designed by Primer-3 and OligoAnalyzer tools.

RESULTS

Patient 1: A six-year-old female, offspring of non-consanguineous parents. she was delivered at full term by normal vaginal delivery after an uneventful pregnancy. Her father and younger sister were similarly affected. Clinical examination revealed sparse hair, tented sparse eyebrows, prominent nasal root, micrognathia, low set ears, and a scar of an operated cleft upper lip. The upper limbs showed bilateral absent 2nd fingers and syndactyly between 4th and 5th fingers, while the lower limbs showed absent 2nd and 3rd toes with syndactyly between 4th and 5th toes. Radiological examination of both hands revealed transverse position of proximal phalanx of 2nd finger in the right hand with absent middle and terminal phalanges of 2nd finger in both hands. X-ray of both feet showed absent phalanges of 2nd and 3rd toes. Chest, abdomen, genital, and neurological, examinations were normal (Figure 1).

Sequencing of *TP63* gene using NGS customized panel showed heterozygous missense mutation (c.605A>G; p.Tyr202Cys) in exon 5 of *TP63* gene of the patient, her sister and her father while the mother showed wild-type sequence (Figure 2).

Patient 2: A two-month-old male descended from non-consanguineous parents. He was delivered at full term, by cesarean section with normal birth weight. There was family history of distant relatives with delayed motor and mental milestones and another family member with blindness. Clinical examination revealed sparse hair, sparse eyebrows, high forehead, epicanthic folds, and without oral clefting. The upper and lower limbs showed bilateral SHFM. Absence of 2nd and 3rd fingers in both hands, right foot with absent 2nd and 3rd toes and left foot with absent 2nd toe and hypoplastic nails in both feet were noted. X-ray of both hands showed absent proximal, middle and terminal phalanges of 3rd and 4th fingers. X-ray of right foot revealed absence of 3rd toe and absent middle and terminal phalnges of 2nd toe with hypoplastic phalanges of other toes, while the X-ray of the left foot showed hypoplastic phalanges of all toes with absence of middle and terminal phalanges of 2nd toe (Figure 3).

Sequencing of *TP63* gene using NGS customized panel showed heterozygous missense mutation (c.931A>G; p.Ser311Gly) in exon 7 of *TP63* gene of the patient, while his parents showed wild-type sequences (Figure 4).

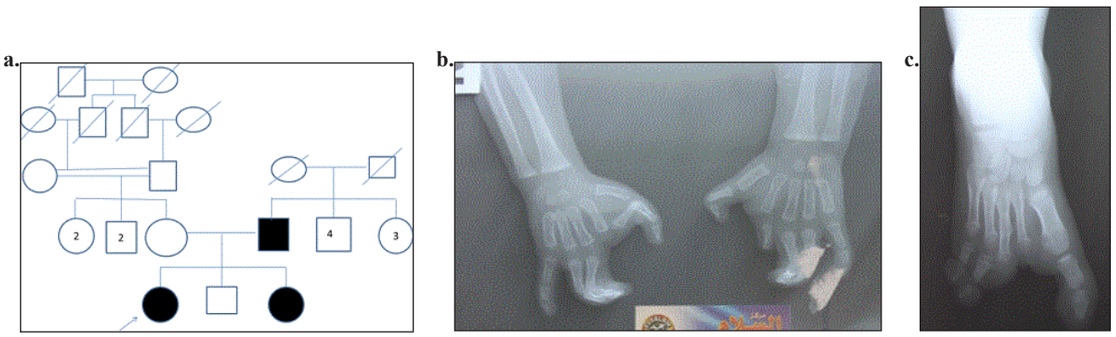

Figure 1: (a) Family pedigree of (Patient 1) showing –ve parental consanguinity and similarly affected sister and father. (b) X-ray of both hands showing transverse position of proximal phalanx of finger II in the right hand with absent middle and terminal phalanges of finger II in both hands. (c) Right split foot with absent phalanges of 2nd and 3rd toes.

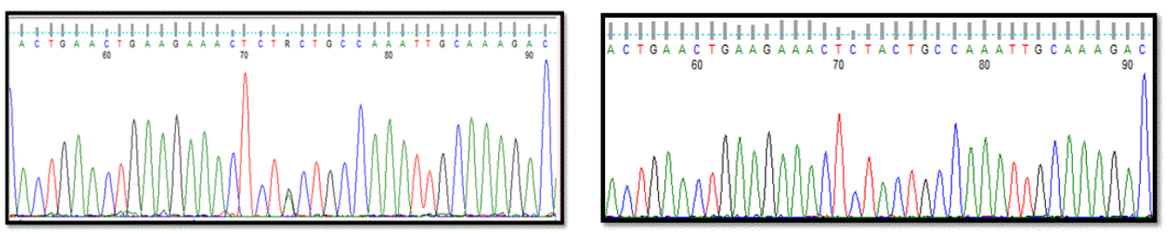

Figure 2: Sequence chromatogram of exon 5 of TP63 gene in Family (1) showing (a) Heterozygous mutation c.605A>G of Patient 1. (b) Wild-type sequence in the mother. The black arrow indicates the site of the mutation.

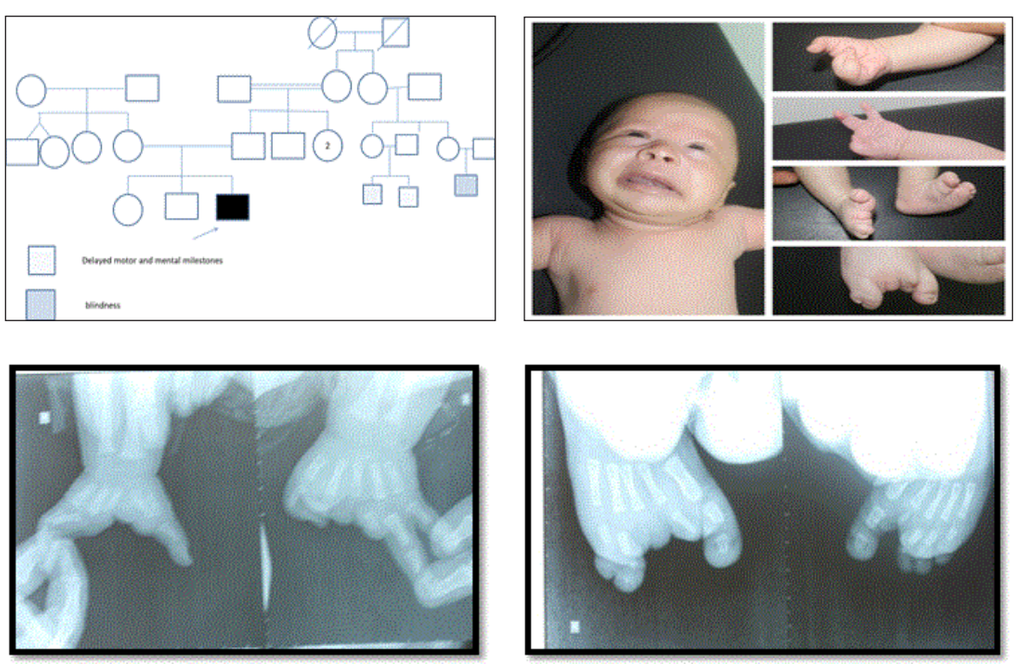

Figure 3: (a) Family pedigree of (Patient 2) showing –ve parental consanguinity and other distant family members with delayed motor and mental milestones and blindness. (b) Face of Patient 1 showing sparse hair and eye brows, both hands with absent 2nd and 3rd fingers, right foot with absent 2nd and 3rd toes and left foot with absent 2nd toe and hypoplastic nails in both feet. (c) X-ray both hands showing axial defect with absent proximal, middle and terminal phalanges of fingers III and IV. (d) X-ray both feet with total absence of 3rd toe and absent middle and terminal phalnges of 2nd toe with hypoplastic phalanges of other toes in right foot. The left foot shows hypoplastic phalanges of all toes with absence of middle and terminal phalanges of 2nd toe.

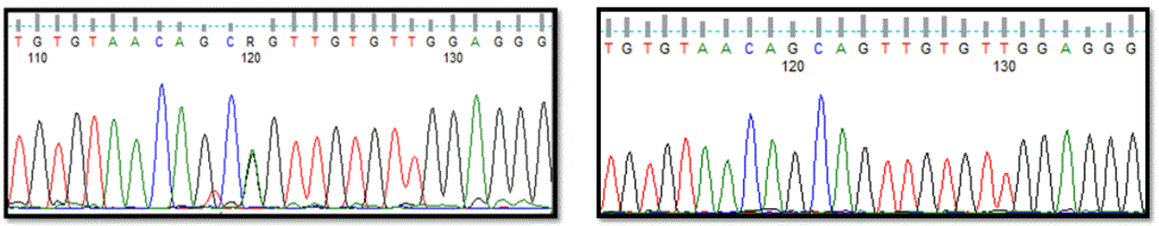

Figure 4: Sequence chromatogram of exon 7 of TP63 gene in family (2) showing (a) Heterozygous mutation c.931A>G in patient 2. (b) wild type sequence of his mother. The black arrow indicates the site of the mutation.

DISCUSSION

Ectrodactyly, ectodermal dysplasia, cleft lip/palate (EEC) syndrome is principally characterized by a set of three cardinal symptoms; ectodermal dysplasia, ectrodactyly, and cleft lip/palate (**Bharati *et al.,* 2020**). Phenotypic variability in EEC syndrome was reported as no single feature involved in the three cardinal signs proved to be mandatory for the diagnosis (**Bigatà *et al.,* 2003; Wei *et al.,* 2012**).

The EEC syndrome follows an autosomal dominant pattern of inheritance with variable clinical expressivity and incomplete penetrance (**Brunner *et al.,* 2002**). Many sporadic cases of EEC syndrome were reported in the literature including Egyptian families (**Ashour and El-Badry, 2004**). Patient 1 in this study has an affected father and sister, while patient 2 was sporadic.

Wawrzycki *et al.,* (2019) stated that ectrodactyly occurs in 68% (43% also had syndactyly) of EEC syndrome. Of more than 230 declared cases only 16% did not manifest with SHFM, while 77% had ectodermal dysplasia, and oral clefts were reported in 68% (**Augello *et al.,* 2015**). The two reported patients in this study presented with SHFM in addition to ectodermal manifestations and cleft lip in patient 1, that are consistent with EEC syndrome.

Basso *et al.,* (2018) indicated that ectrodactyly in EEC syndrome may not be an obligate feature. **Bharati *et al.,* (2020)** proposed that low birth weight and polysyndactyly could be signs of EEC without ectrodactyly but in this study, the two cases had normal birth weight and no polysyndactyly.

Ectodermal dysplasia includes a variable group of inherited disorders including dental abnormalities, nail dystrophy, dry skin, or sparse hair, and lacrimal duct obstruction (**Gorlin *et al.,* 1990; Glorio *et al.,* 2003**). In this study, both patients have sparse hair and eyebrows, and patient 2 has hypoplastic nails which are manifestations of ectodermal dysplasia.

The representative collection of symptoms of EEC, involving skin, hair, and nail, lacrimal duct obstruction, dental and skeletal malformations, genitourinary abnormalities, and nasal dryness are likely because of hindered structure or function of mucous and salivary glands within the ectodermal dysplasia setting (**Itin and Fistarol, 2004; Wawrzycki *et al.,* 2019**). Hypopigmentation and hyperkeratosis of the skin were formerly recorded in EEC cases (**Knaudt *et al.,* 2012**). **Buss *et al.,* (1995)** and **Ashour and El- Badry, (2004)** concluded that hair and teeth affection was universal in all their patients and stated that hypohidrosis was not the main sign of the syndrome which agrees with our findings as normal sweating was observed in our cases.

Oral clefting in EEC syndrome may involve the lip with or without the palate or cleft palate only (**Marwaha and Nanda, 2012; Bharati *et al.,* 2020**). Sporadic cases of ectrodactyly and ectodermal dysplasia without clefting were documented as a part of the variable expressivity in some EEC families (**Buss *et al.,* 1995; Lourencone *et al.,* 2018**). Patient (1) had a corrected cleft upper lip that is part of the syndrome while patient (2) has no cleft lip or palate.

Deafness, lacrimal duct abnormalities, urogenital anomalies, developmental delay, risk of malignancy, and occasional intellectual disability are other reported features of EEC syndrome (**Obel *et al.,* 1993; Glorio *et al.,* 2003; Dhar and Bora, 2014**). In the current study, the patients did not have hearing affection, lacrimal duct defects, or genitourinary abnormalities.

EEC cases usually complain of recurrent infections in the eyes, skin, upper respiratory tract, and urogenital system. However, all patients possessed normal immunoglobulin and complement levels and normal WBCS function. The recurrent infections did not rise by an immunological defect (**León-Mateos *et al.,* 2008**). Our patients did not report recurrent infections in different systems.

TP63 is an important gene that has a role in tissue development and apoptosis. Different mutations in the *TP63* gene can cause variable clinical disorders, such as EEC3 syndrome and SHFM4 (**Enriquez *et al.,* 2016**). Most EEC cases induced by *TP63* gene missense mutations are in the core DNA binding domain (DBD); consequently,

affecting the normal protein translation (**Jin *et al.,* 2019**). Furthermore, 10% only of isolated SHFM cases are harboring mutations in *TP63* gene, which are also detected in the DBD (**Van Bokhoven *et al.,* 2011**).

The remarkable specificity of mutations' distribution in the *TP63* gene could point to a particular pathogenic mechanism. In EEC and SHSF syndromes mutations were related to DBD, whereas mutations in Sterile Alpha Motif (SAM) domain were accompanied with ankyloblepharon, ectodermal dysplasia, clefting (AEC) syndrome (**Brunner *et al.,* 2002**). The cardinal symptoms of EEC which include (split- hand/foot, ectodermal dysplasia, and cleft lip/palate) represent the main differences between it and SHFM4 which has only limb malformations (**Roelfsema and Cobben, 1996; Jindal *et al.,* 2009**). The genotype-phenotype correlation between the two disorders related to the structure and function of DBD is still vague (**Wei *et al.,* 2012**).

To the best of our knowledge, few Egyptian patients has been reported in the literature, clinically diagnosed with EEC syndrome (**Adel and Tarek, 2004; Metwalley and Fargalley, 2012; Elhamouly and Dowidar, 2019**). Whereas, the current study is the first to identify the genetic variants involved in the EEC syndrome.

Patients (1 and 2) in this study have different mutations in different exons that lead to clinical variability. Patient (1) manifested by cleft upper lip while patient (2) did not show cleft lip or palate. In patient (1), heterozygous missense mutation in DBD was detected. The mutation (c.605A>G; p.Tyr202Cys) in exon 5 of *TP63* gene was reported once in a patient with EEC syndrome (**van Bokhoven *et al.,* 2002**). The clinical manifestations of the previously reported patient included sparse hair, lacrimal duct stenosis, dystrophic nail, thin skin, cleft palate, syndactyly, hypohydrosis, and nipple hypoplasia. Functional analyses were performed, which showed reduced *TP63* protein degradation (**Rinne *et al.,* 2006; Browne *et al.,* 2011**). In patient (2), heterozygous missense (c.931A>G; p.Ser311Gly) mutation was detected, which was reported once in a case of syndromic ectrodactyly consistent with ADULT syndrome, the patient with ADULT syndrome harbors another mutation in CNGB3 gene, explaining the ocular involvement (**Hizem *et al.,* 2024**). However, this amino acid site Ser311 has been reported by **Di Iorio** and colleagues and by **Celli** and colleagues in patients with EEC syndrome. The patients had heterozygous missense variants p.Ser311Thr and p.Ser311Asn respectively (**Celli *et al.,* 1999; Di Iorio *et al.,* 2012**). Ectodermal dysplasia, ectrodactyly and syndactyly are manifestations of both EEC and ADULT syndromes, they can be differentiated by the occurrence of mammary gland hypoplasia and the absence of orofacial clefting in ADULT syndrome only (**Rinne *et al.,* 2006**). Despite the absence of orofacial clefting in patient 2, the other clinical manifestations are consistent with EEC syndrome. The genotype/phenotype correlation assumed incomplete penetrance, in addition to other gene variants may lead to phenotypic variability among differently affected patients with similar *TP63* gene variants.

CONCLUSION

This study is the first to identify the genetic variants involved in the EEC syndrome. Gene variants may lead to phenotypic variability among differently affected patients with similar TP63 gene variants.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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