

A Novel Variant in the Endothelin-Converting Enzyme-Like 1 (*ECEL1*) Gene in an Emirati Child

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Significance of the Study

- This is a case of an Emirati child with developmental delay, multiple congenital abnormalities, and dysmorphic features due to a novel homozygous mutation in the *ECEL1* gene.

Keywords

Distal arthrogryposis type 5D · Novel mutation · Arab ethnicity · Consanguinity

Abstract

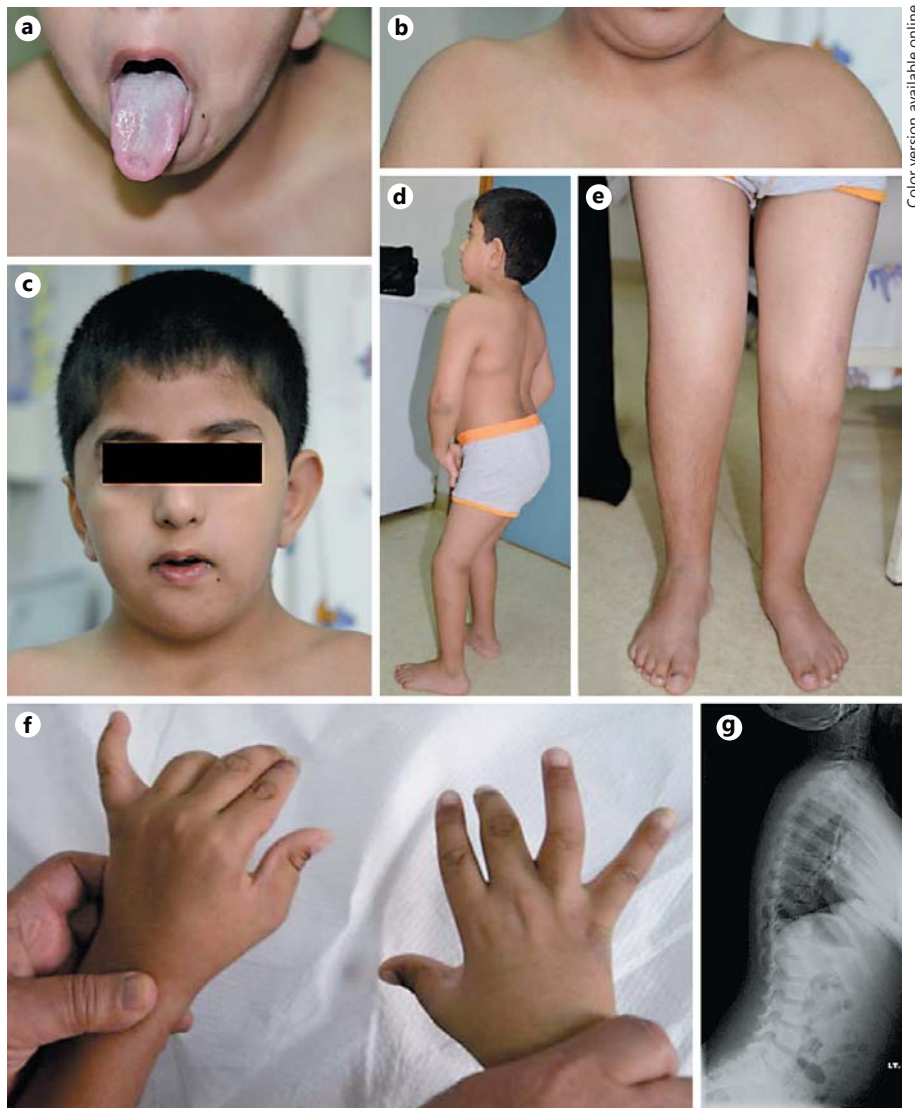
Objective: The aim of this work was to report a case of an Emirati child who presented with developmental delay and multiple congenital abnormalities that are consistent with distal arthrogryposis type 5D. **Clinical Presentation and Intervention:** The clinical presentation comprised contractures of the shoulders, elbows, and knees in addition to camptodactyly and neck pterygium. The facial dysmorphic features noted include ptosis and microretrognathia. Importantly, left orchidopexy was also observed and corrected surgically. Whole exome sequencing revealed that the patient is homozygous for the novel c.1184+1G>T variant in endothelin-converting enzyme-like 1 (*ECEL1*). **Conclusion:** This is a case of a novel homozygous splice site mutation in the

ECEL1 gene in a child with a phenotype consistent with distal arthrogryposis type 5D. The child was born to consanguineous Emirati parents heterozygous for the novel *ECEL1* mutation.

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Introduction

Arthrogryposis multiplex congenita is used to describe multiple congenital joint contractures that can be caused by a myriad of etiologies [1]. In fact, arthrogryposis is a clinical finding in hundreds of different disorders as opposed to a specific diagnosis. One major subcategory of arthrogryposis multiplex congenita is distal arthrogryposis (DA), in which congenital joint contractures mainly affect distal extremities without primary neurological and/or muscle disease [2]. Certain clinical criteria have been compiled to delineate DA. For the upper extremities



Color version available online

Fig. 1. Clinical features of the patient with homozygous *ECEL1* and *CHRNG* mutations. These features include puckering of the tongue (a) and pterygium between the chin and sternum (b), as well as facial dysmorphic features including plagiocephaly and bitemporal narrowing (c). The patient had mild bilateral shoulder abduction, mild limitation in elbow extension (d), limited knee flexion, left calcaneovalgus (e), and camptodactyly with limited extension of the metacarpophalangeal joints (f). g An X-ray shows the scoliotic posture with a straightening of the dorsolumbar spine.

these are camptodactyly, hypoplastic and/or absent flexion creases, overriding fingers, and ulnar deviation of the wrist, and for the lower extremities, talipes equinovarus, calcaneovalgus deformities, and vertical talus. In the absence of family history, individuals must have at least 2 affected body parts in order to be diagnosed with DA. There is considerable genetic as well as clinical heterogeneity among DA cases, and numerous loci have been implicated in the causality of the latter conditions; examples include *MYH2*, *MYH3*, *MYH8*, *MYBPC1*, *TPM2*, *TPM3*, *TNNI2*, *TNNT3*, *ACTA1*, and *NEB*. Autosomal recessive DA type 5D (DA5D) is one such condition and it is caused by mutations in genes coding for endothelin-converting enzyme-like 1 (*ECEL1*).

ECEL1 encodes a member of the M13 family of endopeptidases. These are zinc metalloproteases and type II integral membrane proteins that play a very important role in regulating the activity of neuropeptides and peptide hormones [3]. Studies on *ECEL1* in mice have shown that it has a vital role in the terminal branching of motor neurons to the endplate of skeletal muscles. The *ECEL1* mutations are linked to DA5D cases and these are characterized by deformities in the upper and lower limbs (camptodactyly, calcaneovalgus feet, and extension contractures of the knee). Certain facial features are linked to this condition, including unilateral ptosis, bulbous nose, and micrognathia [3]. Mere dependence on these clinical features is often misleading because there is a significant

overlap between the abovementioned symptoms and those of other conditions such as multiple pterygium syndrome (OMIM 265000) caused by mutations in the *CHRNA3* gene [4]. We here report the case of an Emirati child who presented with developmental delay and multiple congenital abnormalities that are consistent with DA5D.

Case Report

The patient was a 5-year-old male, born to healthy first cousin parents, once removed, who was referred to the genetic clinic for evaluation of developmental delay, dysmorphic features, and multiple congenital abnormalities. No family history of such symptoms exists for the patient. A clinical examination revealed an active and alert boy who walked with extended knees and on the medial side of 1 foot. His height at 119 cm was between the 25th and 50th centile, while his weight of 27.1 kg was at the 97th centile. He was intellectually normal, although his motor skills were slightly delayed.

The facial dysmorphic features included plagiocephaly, bitemporal narrowing, prominent eyes with downslanting palpebral fissures, ptosis, which was more pronounced on the left side, long eyelashes, a broad nasal bridge, microretrognathia, a short, slightly webbed neck with limited head movement, and puckering of the tongue (Fig. 1a–c). Musculoskeletal examination revealed mild bilateral shoulder abduction, mild limitation in elbow extension, limited knee flexion, and left calcaneovalgus (Fig. 1d, e). Camptodactyly was noticed, more on the right than the left, along with limited extension of the metacarpophalangeal joints (Fig. 1f). The thumbs were slightly abducted bilaterally. The calf muscles were atrophic. Radiological examination of the pelvis showed developmental dysplasia of the right hip with abnormal left femoral epiphysis. In addition, a pelvic ultrasound scan revealed an undescended left testis with evidence of left encysted hydrocele of the cord. A scoliotic posture with a straightening of the dorsolumbar spine was seen in the spinal X-ray (Fig. 1g). Mild hypertrophy of the adenoids was noted on X-ray of the postnasal space. Other systems were normal.

The patient underwent surgical correction for the hip dislocation and hyperextension of both knees along with a left orchidopexy. Peripheral blood samples were collected from the proband and his parents. Thereafter, DNA was extracted from blood samples according to standard protocols. Amplicon library construction, exome capture, sequencing, and standard data analysis for the affected child and parents were performed by Sengenics (Kuala Lumpur, Malaysia). The SureSelect Human All Exon V5 exome capture kit was used for library preparation and the samples were sequenced at a minimum of $\times 100$ mean standard coverage using the Illumina HiSeq 2,500 platform. The resulting number of variants was 94,907. These variants were filtered according to quality, frequency, genomic position, consequences on encoded proteins, pathogenicity, and previously reported associations with the phenotype. Findings from whole exome sequencing were confirmed by Sanger sequencing in the trio. Reference sequence identifiers for wild-type *ECEL1* were obtained from GenBank (NCBI reference sequences: NG_034065.1).

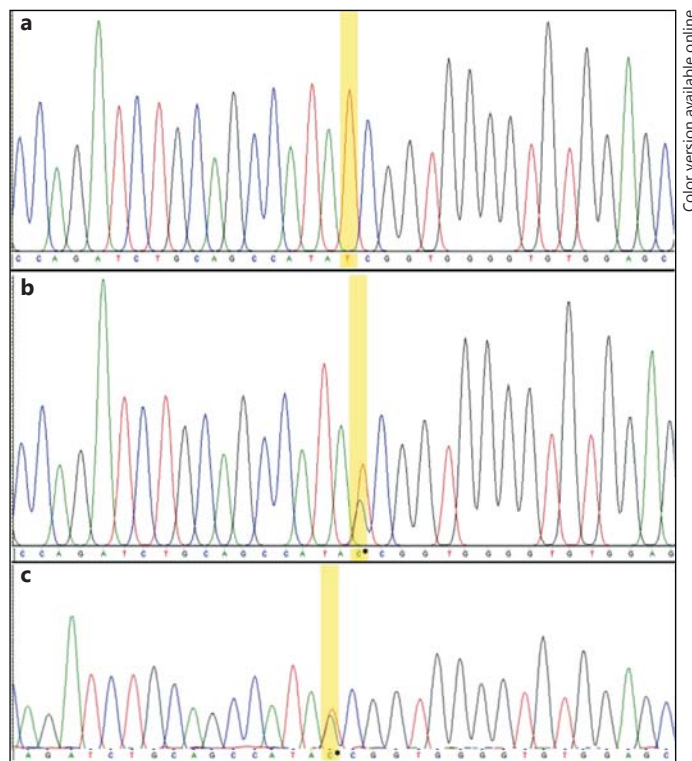


Fig. 2. Sequence chromatograms showing the novel *ECEL1* variant in a homozygous state in the patient (a), and in a heterozygous state in each of the parents (b, c).

The patient harbored a novel single nucleotide variant, c.1184 +1G>T (GenBank accession No.: KU870924), which occurs at the genomic position chr2:233349181 (assembly GRCh37/hg19) at the beginning of intron 6 in *ECEL1* (Fig. 2). This variant was found in a homozygous state in the patient while both parents were heterozygous for it. The variant is predicted to break the WT donor site, which will probably disrupt normal splicing as per prediction algorithms in the Human Splicing Finder and CADD. The variant was not reported in the Exome Aggregation Consortium, Exome Sequencing Project, or in the 1000 Genomes browser. It was also not found in the GalaxC™ Allele Frequency Database, which contains >2.5 million unique Middle Eastern pathogenic mutations and variants.

Discussion

In this case, the patient harbored a homozygous variant that is located at a splice donor in the *ECEL1* gene, hence it might affect proper splicing, as was predicted by 2 R package splicing prediction tools: ADA and Random Forest [5]. The latter findings were corroborated by both Human Splicing Finder and CADD, which yielded a

PHRED scaled score of 24.8. Numerous mutations in the *ECEL1* gene have been reported recently, and, with the spectrum of clinical features of the associated condition, DA5D is becoming more established with each one [6–8]. Many of the characteristic features of DA5D are present in this reported case.

In addition to the *ECEL1* variant, the patient was found to harbor a homozygous variant in the *CHRNA7* gene: c.195+29C>T. It is unlikely that the latter intronic variant is involved in the overall phenotype of the patient as indicated by the results of various in silico prediction tools. According to the prediction made by CADD, the *CHRNA7* variant had no significant functional consequences, while certain prediction algorithms of Human Splicing Finder suggested that the variant may create an intronic ESE site with potential alteration of splicing (<http://www.umd.be/HSF3/>). Even though certain symptoms, such as pterygia and cryptorchidism, are more associated with *CHRNA7* mutations, they are not exclusive to the Escobar type of multiple pterygium syndrome and have occasionally been reported with *ECEL1* mutations [9]. The importance of having functional *ECEL1* and *CHRNA7* for normal formation of the neuromuscular junction is much clearer than the exact mechanisms underlying the phenotypes caused

by their mutations. Shedding light on more cases of such mutations will undoubtedly help elucidate the abovementioned mechanisms, especially if in silico studies are supported by functional ones.

Conclusion

This is a case of a novel homozygous splice site mutation in the *ECEL1* gene in a child with a phenotype consistent with DA5D. The child was born to consanguineous Emirati parents heterozygous for the novel *ECEL1* mutation.

Statement of Ethics

This study was approved by the Institutional Ethics Review Board and conducted with informed written consent from the parents.

Disclosure Statement

The authors have no conflicts of interest to declare.

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