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A Novel *ECEL1* Variant Associated with a Congenital Contracture Disorder

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ABSTRACT

A consanguineous Pakistani family with three affected siblings was investigated to determine the genetic diagnosis of an inherited contracture disorder. Whole-exome sequencing was performed for four participants. Variants were filtered based on homozygosity in the three patients and heterozygosity in the obligate carrier (mother), predicted effect of variants on the encoded protein, and their frequencies in public databases. Sanger sequencing was performed to explore the segregation of the variant with the phenotype. All patients had congenital limb contractures. These included camptodactyly of hands and feet, ptosis, adducted thumb and clubfoot morphology. A novel homozygous missense variant in *ECEL1* c.2051A>G, p.(Tyr684Cys) was identified in all three patients. The variant was absent from the DNA of 500 ethnically matched control samples as well as from all public databases. In conclusion, this study reports a family with clinical features of distal arthrogryposis type 5D and extends the genotype spectrum of the disorder.

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SN and KL designed the study. HM, NB, HMJ, TB, FH, MW and AH collected samples and performed clinical analysis. HM and SN collected and analyzed the data. HM and SN wrote and finalized manuscript.

Key words
Distal arthrogryposis, type 5D, *ECEL1*, Exome sequencing, Pakistan

INTRODUCTION

Arthrogryposis multiplex congenita (AMC) is a heterogeneous disorder involving contractures of the distal parts of the limbs which affect joint mobility. More than ten subtypes of DA have been identified on the basis of phenotypic features. Most DA subtypes are inherited as autosomal dominant disorders, and are caused by pathogenic variants in genes encoding contractile proteins of myofibers (Bamshad *et al*., 2009). Distal arthrogryposis type 5D (DA5D) (OMIM# 615065) is a subtype of DA inherited as an autosomal recessive disorder. It is characterized by severe camptodactyly of the hands, adducted thumbs and wrists, mild camptodactyly of the toes, clubfoot and/or a calcaneovalgus deformity, extension contractures of the knee, unilateral ptosis or ptosis that is more severe on one side. A round-shaped face, arched eyebrows, a bulbous, upturned nose, and micrognathia are also characteristic (Dieterich *et al*., 2013). Mutations in *ECEL1* encoding endothelin converting enzyme like 1 have been identified in DA5D families (Dieterich *et al*., 2013; McMillin *et al*., 2013). Here, we report a family presenting characteristic features of DA5D with a novel homozygous missense variant in *ECEL1*.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Institutional Review Board of School of Biological Sciences, University of the Punjab, Lahore, Pakistan. All participants provided written informed consent.

Subjects

Family RDHR-01 had four affected male individuals in two consanguineous marriages (Fig. 1a). Three individuals (IV:1, IV:2, IV:3) were videotaped according to a standardized video protocol. The videos were evaluated by movement disorder specialists (N.B., T.B., A.M.).

Molecular analyses

Blood samples were collected from all available family members and genomic DNA was extracted by a

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standard protocol. Exome sequencing was performed for four individuals of the family at Centogene AG (Rostock, Germany). NimblegenSeqCap EZ Human Exome Library v2.0 was used for exome capturing, enrichment and sequencing was performed on an Illumina HiSeq 2000 machine with a medium coverage of 100X. Data were analyzed and filtered to remove those variants with minor allele frequencies equal to or greater than 0.01 in the public databases such as gnomAD and 1000 genomes and with a high number of homozygotes or hemizygotes (>20). All exonic and splice site variants were prioritized if they were present in the data of the three affected siblings in the homozygous state and heterozygous in that of the unaffected parent.

Sanger sequencing was performed on samples for the available family members to confirm the segregation of the candidate variants. DNA from 200 ethnically matched controls was sequenced to determine the frequency of the variant in the Pakistani population together with the exome data from 300 unrelated in-house ethnically matched individuals.

In silico predictions

We performed *in silico* analysis by using online prediction tools such as PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), PROVEAN (http://provean.jcvi.org/protein_batch_submit.php?species=human), SIFT (https://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html) and FATHMM (http://fathmm.biocompute.org.uk/inherited.html) among others in order to predict the pathogenicity of the identified variants. Moreover, HOPE (https://www3.cmbi.umcn.nl/hope/) was used to predict the effect of mutant amino acid residue on the protein.

RESULTS

Clinical data

The proband, IV:1, now aged 30 years in family RDHR-01 was reported to have upper and lower limbs contractures at birth. The two affected siblings (IV:2, aged 28 years and IV:3, aged 26 years) were reported to have similar clinical features at birth as exibited by the proband (Table I). All affected individuals had ptosis. Individual IV:1 exhibited unilateral ptosis which was more severe on the right side (Fig. 1b). Mild bilateral ptosis were observed in individuals IV:2 and IV:3. Bulbous nose and arched eyebrows were observed in individual IV:2. The posture deformity of the hands associated with curved fingers, and adducted thumbs of both hands were present in all affected individuals. The thumbs of the hands were curved inside medially (Fig. 1c). The lower limbs abnormalities were associated with problems in knee joints; as the joints were not flexible, the right leg could not be bent and remained straight. The feet exhibited *pes cavus*. The posture deformity of feet in individual IV:2 resembles clubfoot deformity in which the foot rotated towards the other foot (Fig. 1d).

Table I. Clinical features of family RDHR-01.

Patients ID	IV:1	IV:2	IV:3	Symptoms in all 59 patients*
Sex	M	M	M	Not reported
Onset	At birth	At birth	At birth	At birth
Age at examination (yrs)	30	28	26	Varied
Ptosis	+	+	+	44 (74%)
Arched eye brows	-	+	+	10 (17%)
Bulbous nose	+	+	+	22 (32%)
Hands and/or fingers contracture	+	+	+	59 (100%)
Adducted thumbs	+	+	+	30 (51%)
Foot and or toe contracture	-	+	+	50(85%)
Club foot	-	+	+	16 (27%)
Pes cavus	-	+	+	7 (12%)

M, male; yrs, years; +, present; -, absent; *(Barnett *et al*., 2014; Bayram *et al*., 2016; Dieterich *et al*., 2013; Hamzeh *et al*., 2017; McMillin *et al*., 2013; Patil *et al*., 2014; Rai *et al*., 2018; Shaaban *et al*., 2014; Shaheen *et al*., 2014; Stattin *et al*., 2018; Ullmann *et al*., 2018; Umair *et al*., 2019).

Table II. Segregating variants in family RDHR-01.

Position*	Gene	Transcript	cDNA change	Protein change	GERP-RS	CADD	REVEL	ClinPred	gnomAD	**Predic
chr2:233345805T>C	*ECEL1*	NM_004826.2	c.2051A>G	p.Tyr684Cys	5.81	25.9	0.928	0.999	0	D, D, D, D, D
chr6:43030695C>T	*KLC4*	NM_201523.1	c.353C>T	p.Ser118Leu	5.58	28.7	0.16	0.621	0.00003899	D, P, D,N,T
chr6:43153265C>T	*CUL9*	NM_015089.2	c.667C>T	p.Arg223Cys	2.57	24.9	0.142	0.522	0.00002387	B, P, D D,T
chrX:39923684C>T	*BCOR*	NM_001123385.1	c.3407G>A	p.Arg1136His	5.63	27.1	0.178	NA	0.00001639	D, P, D, N,T

*, position according to the human genome build GRCh37/hg19; GERP-RS, genomic evolutionary rate profiling-rejected substitutions; CADD, combined annotation dependent depletion; REVEL, rare exome variant ensemble learner; ClinPred, (https://sites.google.com/site/clinpred/); ExAC, exome aggregation consortium; ,**, predictions according to PolyPhen2; MutationTaster, PROVEAN, SIFT and FATHMM, respectively; D, deleterious or damaging, P, pathogenic; N, neutral; T, tolerated; B, benign; NA, not available.

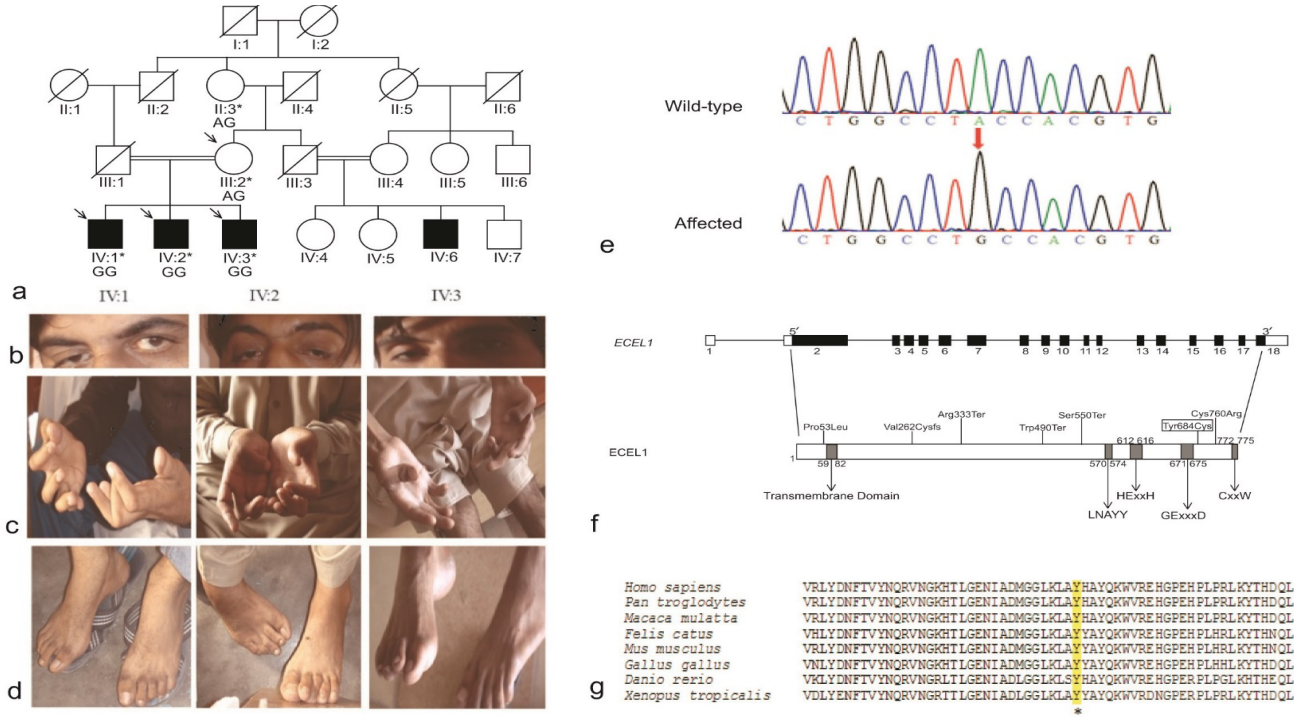

Fig. 1. Pedigree of family RDHR-01 and genotypes for the *ECEL1* variant segregating with the phenotype.
(**a**) RDHR-01 pedigree. *Individuals who participated in this study. Arrows denote individuals for whom whole-exome sequencing was performed. The genotypes for *ECEL1* (c. 2051A>G) p.(Tyr684Cys) variant are indicated below the individual symbols of all participants. Clinical images of affected individuals IV:1, IV:2 and IV:3. (**b**) Unilateral ptosis severe on right side in individual IV:1 and mild bilateral ptosis in others. (**c**) curved fingers and adducted thumb of hands. (**d**) mild camptodactyly of toes in individual IV:1, clubfoot deformity in individual IV:2 and high arched of feet in individual IV:3. (**e**) Electropherogram of *ECEL1* sequence analyses. The site of mutation is depicted by an arrow. (**f**) Schematic representation of *ECEL1* (NM_004826.2), black boxes represent translated exons and plain boxes denote 5′ and 3′ untranslated regions. Introns are depicted by horizontal lines. The variant p. Tyr684Cys identified in family RDHR-01 is boxed. LNAYY motif is involved in orientation of substrate peptide bond. HExxH is a zinc biding motif. GExxxD is a Zinc coordinating motif. CxxW is the conserved carboxy terminal sequence of metalloprotease. (**g**) Conservation of ECEL1 residue p. Tyr684 from eight species of vertebrates. The residue p. Tyr684 is highlighted and marked with an asterisk. (Coloured figure may be observed in the online issue of the journal).

Molecular data

Only four different missense variants fulfilled our filtering criteria applied to the exome data analysis (Table II). All these variants segregated with the phenotype and were absent from the DNA of the ethnically matched controls (1000 chromosomes). The novel missense variant in *ECEL1*, c.2051A>G, p.(Tyr684Cys) (NM_004826.2) best explained the disorder in the family based on phenotypic overlap with patients described with other pathogenic variants in this gene, complete conservation of the amino acid among different vertebrates orthologues (Fig. 1g), the highest pathogenicity scores (Table II). In addition, the variant was not present in any public database.

Results of in silico analyses predictions for ECEL1 p.(Tyr684Cys)

All online tools predicted the ECEL1 p.(Tyr684Cys) variant to be damaging or deleterious to the protein (Table II). The wild type amino acid Tyr is aromatic, partially hydrophobic and non-polar. In contrast, the mutant amino Cys is hydrophobic, polar, and sulphur containing. The Tyr684 residue is located within a peptidase M13 domain that is important for the activity of the protein as it contains the active site of the enzyme. The variant may disturb the domain structure and interaction between the domains, which could affect the function of the protein.

DISCUSSION

ECEL1 encodes endothelin converting enzyme like 1 (ECEL1), a member of neprilysin family of endopeptidases (peptidase family M13). Members of this family are zinc containing type II integral membrane proteins with a short cytosolic N-terminal tail and a long C-terminal extra cytosolic domain containing the catalytic site (Nagata *et al.*, 2016). The ECEL1 protein (O95672, UniProt https://

www.uniprot.org) consist of 775 amino acids with an N-terminal cytoplasmic domain (1-59 residues), a single putative membrane-spanning region (60-82 residues) and a large luminal C-terminal domain (83-775 residues) that contains a zinc-binding motif and the active site (Fig. 1f).

ECEL1 and distal arthrogryposis type 5D

Pathogenic variants in *ECEL1* were first identified as a cause for distal arthrogryposis type 5D in eleven families by two independent groups (Dieterich *et al*., 2013; McMillin *et al*., 2013). To date, 48 variants of *ECEL1* (Human Gene Mutation Database accessed March 2022) have been reported from different ethnic groups affected with distal arthrogryposis type 5D. *ECEL1* is highly expressed in the central and the peripheral nervous system in humans and rodents (Nagata *et al*., 2016). It plays an important role in the development of neuromuscular junctions and intramuscular axonal branching during fetal life both in mice and humans (Nagata *et al*., 2016). Mice deficient for the homologous murine gene, *Ecel1*, die immediately after birth due to respiratory failure. Knock-in mice with pathogenic variants show impaired axonal arborization of spinal motor nerves and axon guidance in abducens nerves (Nagata *et al*., 2017). Defects in terminal branching of motor neurons to the end plate of skeletal muscles result in poor formation of neuromuscular junction (Nagata *et al*., 2017). This may explain the pathogenesis in individuals with *ECEL1* variant and congenital contracture disorders.

The affected individuals in family RDHR-01 showed the characteristic clinical features of distal arthrogryposis type 5D. The 48 variants have been identified in a total of 59 individuals with distal arthrogryposis type 5D in several families (Barnett *et al*., 2014; Bayram *et al*., 2016; Dieterich *et al*., 2013; Dohrn *et al*., 2015; Hamzeh *et al*., 2017; McMillin *et al*., 2013; Patil *et al*., 2014; Rai *et al*., 2018; Shaaban *et al*., 2014; Shaheen *et al*., 2014; Stattin *et al*., 2018; Ullmann *et al*., 2018; Umair *et al*., 2019; Gowda *et al*., 2021; Huddar *et al*., 2021; Jin *et al*., 2020; Alesi *et al*., 2021). Pronounced unilateral ptosis more severe on the right side, adducted thumbs, and the limbs contracture presented by RDHR01 are consistent with features observed in reported patients of distal arthrogryposis, type 5D (Table I). Arched eye brows (17%) and Pes cavus (12%) are the less common phenotypes associated with previously reported ECEL1 variants (Table I). Both of these relatively rare phenotypes were observed in two of the three patients in our family.

Possible effects of missense variant ECEL1 p.(Tyr684Cys)

The missense variant identified in family RDHR-01 was located near the zinc coordination motif of ECEL1 (Fig. 1f) and therefore may affect the zinc binding ability of protein. Moreover, *in silico* analyses indicated that the identified variant may change protein configuration, thus adversely affecting protein activity. The allelic spectra of *ECEL1* variants is wide and no clear genotype-phenotype correlation has been identified as yet. The patients with truncating variants do not show more severe presentation than the patients with missense variants (Dieterich *et al*., 2013; Shaaban *et al*., 2014). The affected individuals in the family presented here manifest a severe phenotype due to a missense variant.

In conclusion, this report adds to the cases of distal arthrogryposis type 5D and expands the variant spectrum of *ECEL1*.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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