Short Report

Identification of three novel ECEL1 mutations in three families with distal arthrogryposis type 5D


Arthrogryposis refers to congenital contracture in at least two different body parts. When distal joints are primarily involved, the term distal arthrogryposis (DA) is used. The recognition of clinically distinct subtypes of DA has proven very useful in mapping the disease genes for this genetically heterogeneous condition. DA5D is characterized by ocular involvement usually in the form of ptosis and incomitant strabismus, but extraocular manifestations have also been reported. In a multiplex consanguineous family with DA5D, we combined autozygosity mapping and exome sequencing to identify a novel mutation in ECEL1. This was followed by targeted sequencing of this gene in another two extended consanguineous family with the same phenotype, which revealed two additional novel homozygous mutations. Our results support the recent identification of mutations in ECEL1 as a disease gene in DA5D and expand the clinical and allelic spectrum of this condition.


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Arthrogryposis is a term used to describe congenital limited joint mobility of at least two different body parts. The developmental nature of this malformation typically manifests with significant pathological changes in the affected joints and its surrounding tissues, including the muscles (1). Several classifications have been proposed to address the remarkable clinical heterogeneity and insure that more homogeneous subgroups are defined to improve our understanding of their natural history and etiology (2). Distal arthrogryposis (DA), as the name implies, primarily involves the distal joints of the upper and lower extremities. Again, several clinically distinct subgroups have been defined and such classification has
Identification of three novel ECEL1 mutations in three families

Human subjects

Three families with clinical features consistent with DA5 were recruited using an institutional review board (IRB)-approved protocol after signing a written informed consent. Blood was drawn in ethylenediaminetetraacetic acid (EDTA) tubes for DNA extraction and subsequent analysis.

Linkage and autozygome analysis

We performed genome-wide genotyping using the Axiom GeneChip platforms (Affymetrix, Santa Clara, CA) on Family 1. Resulting genotyping data were analyzed with the EASYLINKAGE software package v.5.08, assuming fully penetrant autosomal-recessive inheritance and a disease allele frequency of 0.0001, with a consanguinity loop. Identification of the full set of genomic regions that are identical by descent per individual (autozygome) was performed with AutoSNPa, using the genotypes obtained from the Axiom GeneChip as described before (10).

Exome sequencing

Exome capture was performed using the TruSeqExome Enrichment kit (Illumina, San Diego, CA) following the manufacturer’s protocol. Samples were prepared as an Illumina sequencing library, and in the second step, the sequencing libraries were enriched for the desired target using the IlluminaExome Enrichment protocol. The captured libraries were sequenced using IlluminaHiSeq 2000 Sequencer. The readings were mapped against UCSC hg19 (http://genome.ucsc.edu/) by BWA (http://bio-bwa.sourceforge.net/). The single-nucleotide polymorphisms (SNPs) and Indels were detected by SAMTOOLS (http://samtools.sourceforge.net/). Only homozygous novel coding variants were considered in the candidate gene analysis.

Results

Clinical report

Family 1 comprises four siblings born to first cousin healthy parents from Saudi Arabia (Fig. 1). The index (Patient 1, F1_IV:3) is an 11-year-old Saudi girl born at term after normal pregnancy. Growth parameters at birth were not available. After birth, she was noted to have flexion deformities involving the hands. She had poor weight and height gain and developed progressive scoliosis. She had normal cognition and performed well in school. On examination at the age of 9, her weight was 16.1 kg (−4.4 SD), height was 114 cm (−3.6 SD), and occipitofrontal circumference was 49.5 cm (25th centile). No facial dysmorphism was noted. Examination of the heart, lungs, abdomen, and central nervous system (CNS) was unremarkable. She had a completely normal ophthalmological examination. She had limited extension of the fingers and toes bilaterally with flexion deformity but she was able to hold the pen and write normally. She was able to extend the knees normally but could not flex them beyond 90°. There was thoracolumbar scoliosis with chest deformity. Spinal X-rays showed S-shaped scoliosis of thoracolumbar spine with the upper component to the right side measuring 27° between T1 and T8. The lower component of the thoracolumbar scoliosis measured 25° between the T9 and L4.

The index has a 19-year-old brother (Patient 2, F1_IV:1) with growth retardation, severe camptodactyly of the hands and feet, bilateral congenital ptosis, incontinent strabismus (elevation deficiency OD, adduction deficiency OS), moderate myopia but no scoliosis. Another 17-year-old sister (Patient 3, F1_IV:2) has growth retardation, camptodactyly of the hands and feet, and severe progressive right thoracic scoliosis (measuring 70°). She died because of pulmonary insufficiency secondary to the thoracic deformity. Additionally, she had congenital right ptosis, exotropia in upgaze and lacrimal duct obstruction. A younger sister (Patient 4, F1_IV:4, 6 years old) has growth retardation, camptodactyly of the hands and feet, and right hip dislocation but no scoliosis. She also had right ptosis and right incomitant strabismus (esotropia with abduction defect) (Fig. 1a–e).
Family 2 comprises one male and one female members belonging to two branches of an extended consanguineous family from Upper Egypt (Fig. 1). The index (Patient 5, F2_V:4) is a 3 year 5 month old boy. His pregnancy was notable for reduced fetal movement. Congenital anomalies in the form of ptosis and abnormal extremities were apparent after birth. While cognitive development was normal, his motor milestones were delayed. Ptosis was operated twice with unsatisfactory results. His weight was 14 kg (−1 SD), head circumference 48.7 cm (−1.3 SD) and length 90 cm (−2 SD). He had long face, high forehead, left eye ptosis, prominent nasal bridge, bulbous nasal tip, mild retrognathia and low set ears (Fig. 1g–i). There was no ophthalmoplegia or strabismus. Skeletal anomalies included bilateral camptodactyly of the hands, adducted thumbs, bilateral mild adducted wrist, limited hip extension, knee contractures, and mild calcaneovalgus deformity. Neurological examination showed reduced motor power, hypotonia, absent deep tendon reflexes and normal fundus. Investigations included brain CT, echocardiography, abdominal ultrasound, electromyography (EMG) and nerve conduction studies (NCS) in both upper and lower limbs and serum creatine phosphokinase, all of which revealed normal results. His IQ was normal at 100. Skeletal survey showed bilateral hip dislocation, calcaneovalgus deformity, and camptodactyly of hands.

The cousin (Patient 6, F2_V:3) is a 2.5-year-old girl whose pregnancy was complicated by reduced fetal movements and abnormal fetal position. Parents realized the close similarity to her cousin with distinct features, ptosis and skeletal deformities. In addition, she had club feet and dislocated hips that were managed conservatively and surgically, respectively. There was a history of tendon release of the index fingers at the age of 2 years and of surgery to correct ptosis three times unsuccessfully. On examination, head circumference was 46.5 cm (−0.7 SD), weight was 11.5 kg (−1 SD) and length was 84 cm (−1.5 SD). She had a long face, synophrys, left ptosis without ophthalmoplegia or strabismus, upturned nose with rounded tip, prominent philtrum pillars, open mouth, V-shaped upper lip, mild retrognathia and low set ears (Fig. 1f). The upper limbs showed bilateral adducted thumb, camptodactyly, adducted wrist with limitation of dorsiflexion. The lower limbs showed internal rotation of the left uncorrected hip dislocation, extension contracture of both knees, mild bilateral talipes equinovarus. Neurological evaluation revealed hypotonia with limitation of assessment of motor power and reflexes. Brain computed tomography (CT), EMG, NCS, abdominal ultrasound, echocardiography, and creatine kinase (CK) were normal. Skeletal survey revealed bilateral camptodactyly, corrected right hip and dislocated left hip.

Family 3 comprises three siblings born to first cousin Saudi parents. The index (Patient 7, F3_IV:1) is a 26-year-old Saudi female who was born at term via C-section because of breach presentation. She was noted
Identification of three novel *ECEL1* mutations in three families

Fig. 2. Mapping of *ECEL1* as a disease gene in DA5. (a) Linkage analysis shows a significant peak that spans *ECEL1*. (b) Autozygosity mapping in Family 1 shows a single autozygous interval that is exclusively shared by the affected members and spans *ECEL1*. (c) Filtration scheme of the exome variants in the index in Family 1. (d) DNA sequence chromatograms showing the novel mutation identified in this study and the multi-sequence alignment of *ECEL1* orthologs showing the conservation of the arginine at position 404. (e) Cartoon of *ECEL1* summarizing the location of the three mutations reported in this study and those reported by others in the course of this study.

to have arthrogryposis multiplex (Fig. 1j,k), absence of normal knee prominence and bilateral hip dislocation but no facial dysmorphism. The skeletal survey showed severe bilateral hip dysplasia. EMG was normal.

Patient 8 (F3_IV:3) is the 21-year-old sister. She was diagnosed with arthrogryposis multiplex at birth. She underwent bilateral hip dislocation repair at the age of 4 months. On examination, she had arthrogryposis, left eye ptosis, limited movement of the shoulders, elbows, and wrists joints. She had absence of the normal knee prominence. EMG was normal.

Patient 9 (F3_IV:4) is the 19-year-old brother. He was diagnosed with mild arthrogryposis, bilateral hip dislocation, and cleft palate at birth; the latter was repaired at the age of 2. On examination, he had normal height, facial asymmetry, micrognathia, and absence of the normal knee joints prominence.

Identification of a linkage interval on 2q37.1

Linkage analysis revealed a single peak with logarithm of odds (LOD) score of 3.4 in Family 1 (Fig. 2a). Similarly, autozygome analysis revealed a single run of homozygosity that is exclusively shared between the affected members of Family 1 (Fig. 2b). This interval (chr2:227,657,254-237,805,659; GRCh37/hg19) contains 123 genes.

Identification of *ECEL1* mutations in three families with DA5

We filtered the exome variants obtained on the index in Family 1 by only focusing on those that are within the coordinates of the critical linkage interval ($n = 266$). Of those variants, we only considered those that are homozygous and coding/splicing and are absent in dbSNP132 and local 250 Saudi exomes. This left us with a single in-frame duplication in *ECEL1* (NM_004826.2: c.1221_1223dup) (Fig. 2c). This mutation segregated fully with the phenotype within Family 1. On the basis of this result, we sought another two families with the same phenotype (Family 2 and Family 3). Targeted sequencing of *ECEL1* revealed a truncating homozygous mutation (NM_004826.2: c.1057dupC) in Family 2 and a missense homozygous mutation (NM_004826.2: c.1210C>T; p.Arg404Cys) in Family 3 (Fig. 2d). The amino acid at position 404 is highly conserved (Fig. 2d). However, by this time two groups had reported their identification of recessive
Table 1. Clinical features observed in the study patients

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>F1_IV:1</th>
<th>F1_IV:2</th>
<th>F1_IV:3</th>
<th>F1_IV:4</th>
<th>F2_V:3</th>
<th>F2_V:4</th>
<th>F3_IV:1</th>
<th>F3_IV:3</th>
<th>F3_IV:4</th>
<th>Dietterich et al.</th>
<th>McMillian et al.</th>
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<tr>
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<td>6</td>
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<td>F</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>NA</td>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>?</td>
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<td>Camptodactyly</td>
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<td>−</td>
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LDO, lacrimal duct obstruction.

Mutations in \textit{ECEL1} as causal of DA5D (8, 9). Thus, our identification of \textit{ECEL1} as a disease gene is no longer novel but is in line with these reports.

**Discussion**

\textit{ECEL1} encodes a neuronal endopeptidase and deficiency of its mouse ortholog adversely affects the interface between nerves and muscles leading to poor contractility (11). Surprisingly, knockout mice die shortly after birth due to respiratory insufficiency owing to the involvement of respiratory muscles, a phenomenon that is distinct from the human phenotype but consistent with the notion that \textit{ECEL1} is necessary for normal muscle contractility (12). The mutations reported by Dieterich et al. and McMillin et al. are both missense and truncating in nature with no clear phenotype/genotype correlation (8, 9). Similarly, we show that the phenotype of the three families reported here is very similar despite the apparent difference in severity between the two mutation classes (in-frame one amino acid duplication and one amino acid substitution vs truncation of the extracellular domain and the zinc binding/activation site of the protein). In fact, we show some variability even within the same family as in the index in Family 1 who completely lacks ocular manifestations (Table 1).

In addition to expanding the allelic heterogeneity, the families we report here expand the spectrum of associated ocular phenotype. Mcmillin et al. noted most \textit{ECEL1} mutation-positive individuals had ptosis, none had incomitant strabismus, and none had structural ocular abnormalities (8). Dieterich et al. noted most affected individuals had ptosis, one had incomitant strabismus (Duane syndrome), and none had structural ocular abnormalities (9). In Family 1, three of four affected individuals had incomitant strabismus. This confirms incomitant strabismus is a recurrent feature rather than a coincidence. We also show that individuals with \textit{ECEL1} may completely lack ocular involvement as evident by the lack of this feature in a number of affected members in the study families.

To conclude, mutations in \textit{ECEL1} represent a recurrent cause of DA5 and the diagnosis should be considered even in the absence of ocular features or short stature.

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**References**