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Short Report

Expanding the phenotypic spectrum of *ECEL1*-related congenital contracture syndromes

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Using a combination of homozygosity mapping and whole-exome sequencing (WES), we identified a novel missense c.1819G>A mutation (G607S) in the endothelin-converting enzyme-like 1 (*ECEL1*) gene in a consanguineous pedigree of Turkish origin presenting with a syndrome of camptodactyly, scoliosis, limited knee flexion, significant refractive errors and ophthalmoplegia. *ECEL1* mutations were recently reported to cause recessive forms of distal arthrogryposis. This report expands on the molecular basis and the phenotypic spectrum of *ECEL1*-associated congenital contracture syndromes.

Conflict of interest

The authors declare no conflict of interest.

S. Shaaban [a,b,c,d,e,f], F. Duzcan[g], C. Yildirim[h], W.-M. Chan[a,c,i], C. Andrews[a,b,e,i], N. A. Akarsu[j] and E. C. Engle[a,k,l,b,c,d,e,m,i,n]

[a]Department of Neurology, [b]F.B. Kirby Neurobiology Center, [c]Program in Genomics, [d]Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA, USA, [e]Department of Neurology, Harvard Medical School, Boston, MA, USA, [f]Dubai Harvard Foundation for Medical Research, Boston, MA, USA, [g]Department of Medical Genetics, [h]Department of Ophthalmology, Faculty of Medicine, Pamukkale University, Denizli, Turkey, [i]Howard Hughes Medical Institute, Chevy Chase, MD, USA, [j]Department of Medical Genetics, Faculty of Medicine, Hacettepe University, Ankara, Turkey, [k]Department of Medicine (Genetics), [l]Department of Ophthalmology, Boston Children's Hospital, Boston, MA, USA, [m]Department of Ophthalmology, Harvard Medical School, Boston, MA, USA, and [n]Broad Institute of MIT and Harvard, Cambridge, MA, USA

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Corresponding authors:
Elizabeth C. Engle, CLS14075, Boston Children's Hospital, 300 Longwood Ave, Boston, MA 02115, USA.
Tel.: +1 617 919 4030;
fax: +1 617 919 2769;
e-mail:
elizabeth.engle@childrens.harvard.edu
and
Nurten A. Akarsu, Department of Medical Genetics, Faculty of Medicine, Hacettepe University, Ankara 06100, Turkey.
Tel.: +90 312 305 2559;
fax: +90 312 426 8592;

e-mail: nakarsu@hacettepe.edu.tr

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Arthrogryposis multiplex congenita (AMC; OMIM 108110), is a heterogeneous group of syndromes characterized by congenital contractures of more than two joints in multiple parts of the body (1). AMC affects both sexes equally with an incidence of 1 in 3000 live births, and both genetic and environmental factors are implicated in its etiology (2–4). When contractures are non-progressive and affect mainly the distal joints (i.e. hands and feet) with limited involvement of proximal joints, the disorder is known as distal arthrogryposis (DA) (5, 6). Recently, recessive mutations in the endothelin-converting enzyme-like 1 (*ECEL1*) gene were reported in patients with distinct forms of DA (Table 1) (7, 8).

We studied a Turkish consanguineous pedigree with two affected siblings presenting with a unique AMC syndrome of bilateral camptodactyly, scoliosis, limited knee flexion, pes cavus, ophthalmoplegia, and myopic astigmatism with high cylinder component. The findings in the affected individuals did not meet the major criteria for the diagnosis of DA, and had previously been reported as the second of three families classified with the MIM syndrome 'camptodactyly, myopia, and fibrosis of the medial rectus muscle of eye' (OMIM 602612) (9). We now report that homozygosity mapping and whole-exome sequencing (WES) revealed a novel homozygous missense mutation in *ECEL1* that segregated appropriately within the family. The two siblings differ from the recently reported patients harboring *ECEL1* mutations because of their significant ophthalmoplegia, refractive errors, and less pronounced contractures of feet or ankles.

Subjects and methods

Patients

Members of a consanguineous Turkish family (pedigree UP, Fig. 1a) with two affected children (IV:2, IV:3) were enrolled in a genetic study of strabismus approved by Boston Children's Hospital and Pamukkale University Institutional Review Boards. Informed consent was obtained from participating family members. Investigations were conducted in accordance with the principles of the Declaration of Helsinki. IV:2 and IV:3 underwent ophthalmic, neuromuscular, nerve conduction and electromyography (EMG) studies at the age of 30 and 28, respectively. A previous report described the phenotypes of IV:2 and IV:3 when they were 13 and 11 years old (9). At that time, participating family members underwent ophthalmic, neuromuscular and orthopedic examinations, IV:2 and IV:3 underwent body X-rays, brain computerized tomography (CT) and brain and orbital magnetic resonance imaging (MRI), and medial rectus muscle biopsies, and IV:2 underwent electroretinography (ERG).

DNA sampling

DNA was extracted from the peripheral blood of participants using the Puregene kit (Qiagen, Valencia, CA).

Homozygosity mapping

Genotyping was performed using Human Mapping NspI 250K arrays (Affymetrix, Santa Clara, CA), according to the manufacturer's instructions with an average call rate of >95%. Genome-wide homozygosity analysis was performed, using the software VIGENOS (Visual Genome Studio, Hemosoft Inc., Ankara, Turkey) (10, 11).

WES and variant validation

Exome sequencing was conducted using 3 μg of genomic DNA from individual UP IV:2 and processed with the SureSelect Human All Exon Kit v.1 (Agilent Technologies, Santa Clara, CA) (12). Sequencing, alignment, variant calling, and annotation followed by filtering and ranking of high quality variants were conducted as previously described (13). Putative mutations and appropriate segregation were confirmed by Sanger sequencing.

Results

Clinical evaluation

When reported at 13 and 11 years of age, IV:2 and IV:3 were noted to have characteristic facial features (Table 1). They also had ophthalmoplegia with (IV:2) or without (IV:3) ptosis, compound myopic astigmatism, camptodactyly, scoliosis, limited knee flexion, and pes cavus with overlapping toes. They were not felt to meet diagnostic criteria for DA because they lacked significant foot contractures (9). Both sibs underwent strabismus surgery at the age of 10 and 8, respectively, with positive forced duction testing noted (Table S1, Supporting Information).

Recent clinical re-evaluation revealed normal cognition and motor development. On examination in primary gaze while fixing with the right eye, IV:2 had 20^{Δ} of exotropia and 5^{Δ} of hypotropia OS. Her right eye was ptotic with limited elevation in abduction and adduction and mild limitation of horizontal movements. Her left eye had no ptosis, but vertical movements were limited on upgaze, with marked adduction and moderate abduction deficits. Downshoot of the left eye occurred on

Table 1. Phenotypes of patients with endothelin-converting enzyme-like 1 (*ECEL1*) mutations

	Dieterich et al.	McMillin et al.	Current report
Inheritance	Recessive	Recessive	Recessive
Sex M/F	5/5	5/4	1/1
Eye			
Ptosis	7/10	8/9	1/2
Ophthalmoplegia	1/10	0/9	2/2
Facial involvement			
Arched eye brows	ND	(+)	(+)
Bulbous nose	ND	9/9	2/2
Small mouth	3/10	ND	2/2
Micrognathia	ND	8/9	2/2
Decreased facial expression	3/7	1/9	2/2
Neck			
Short neck	10/10	4/7	2/2
Webbed neck	ND	3/8	2/2
Neck contractures	ND	4/4	2/2
Upper limbs			
Hand and/or finger contractures	10/10	9/9	2/2
Wrist contractures	ND	9/9	2/2
Elbow contractures	3/7	5/5	0/2
Shoulder contractures	2/8	6/6	1/2
Lower limbs			
Foot and/or ankle contractures	9/10	9/9	0/2[a]
Knee contractures			
Limited extension	0/10	3/8	0/2
Limited flexion	10/10	5/8	2/2
Hip contractures	9/9	9/9	0/2
Spine			
Scoliosis	7/10	2/9	2/2
Hyperlordosis	9/9	ND	1/2
Decreased muscle mass	10/10	ND	2/2

M, male; F, female; ND, no data.
[a] Patients had bilateral pes cavus and overlapping toes.

attempted adduction (Fig. 2c). IV:3 had divergent strabismus fixus with both eyes frozen in extreme abduction with absent vertical and horizontal movements without ptosis (Fig. 2d). Apart from compound myopic astigmatism with high cylinder component (Table S1), their remaining ophthalmological examinations, including the optic nerve and retinal examination, were normal.

There had been no progression in skeletal deformities since the family was initially reported (Fig. 2e–i, Table 1, Table S1). Muscle strength was normal except for bilateral lower motor neuron facial paralysis in both siblings (Fig. 2a,b) and weakness of muscles of the right hand of IV:2 (2/5) and the left arm of IV:3 (2/5). Nerve conduction and EMG studies including median, ulnar, tibial and peroneal nerves revealed normal motor and sensory conduction velocities. No pathological findings were detected with needle EMG studies of thenar, hypothenar, gastrocnemius and quadriceps muscles.

Genetic analysis

We identified multiple regions of homozygosity shared only by the two affected siblings. The largest were 14 and 20 Mb on chromosomes 2 and 12, respectively, and contained hundreds of genes. Thus, we performed WES for individual IV:2 to identify causative variants. We obtained mean coverage of 86% at 10X resulting in ~18,000 variants. We investigated the novel homozygous variants within the shared regions of homozygosity. This analysis resulted in five variants, one of which was the missense change c.1819G>A in *ECEL1* gene, predicted to substitute a serine for glycine at the highly conserved residue 607 (p.G607S) which falls in the predicted α24 helix in ECEL1 lumenal domain (Fig. 1b–d). No other variant fell within a known DA gene. The variant was absent from 468 ethnically matched exomes, segregated appropriately within the family (Fig. 1a,b), and was predicted to be damaging by *in silico* analysis (14–16).

Discussion

ECEL1 mutations were recently reported by McMillin et al. and by Dieterich et al. in individuals with DA (7, 8). McMillin et al. identified *ECEL1* mutations in individuals with a phenotype of ptosis, distinct facial features, severe hand camptodactyly, toe and foot

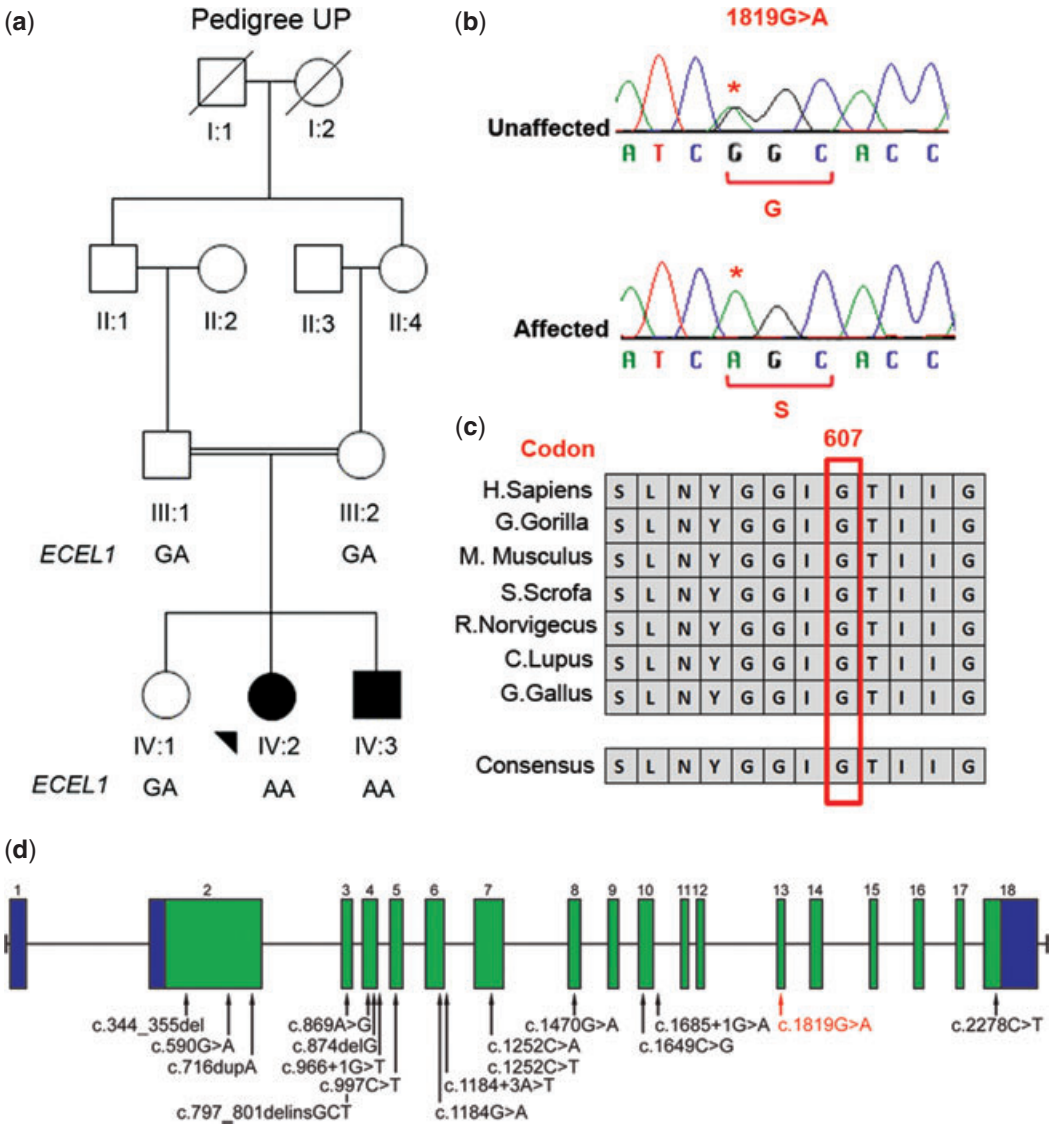

Fig. 1. Pedigree structure and mutation analysis. (**a**) Schematic of pedigree UP with genotypes of *ECEL1* c.1819G>A shown under participants. Squares, males; circles, females; filled symbol, affected. (**b**) Sanger sequencing chromatograms from an unaffected family member (heterozygous) (top), and from affected individual (homozygous) (bottom) for *ECEL1* c.1819G>A substitution. Wild-type and predicted amino acid substitutions are provided below each sequence. (**c**) Evolutionary conservation of ECEL1 glycine 607 in seven species. (**d**) Genomic structure and *ECEL1* mutations previously reported to cause DA indicated by black arrows (7, 8). *ECEL1* is composed of 18 exons that encode untranslated regions (UTRs) (blue) and protein-coding sequences (green). The red arrow indicates the c.1819G>A mutation.

contractures, and knee deformities (7). McMillin classified these families as DA5D, based on the presence of ptosis but not ophthalmoplegia (7). Similarly, Dieterich et al. identified *ECEL1* mutations in individuals with a syndrome of DA characterized by ptosis, tongue atrophy, camptodactyly, limited knee flexion, and feet deformities. Ocular motility was abnormal in only one patient with Duane syndrome (8). Five patients developed restrictive pulmonary disease (8).

The two siblings in this report share similar facial features with the reported patients, (Table 1 and Table S1), however, they differ because they have significant ophthalmoplegia, refractive errors, and severe camptodactyly involving the fingers while they lack major feet or ankle contractures and respiratory compromise (7, 8).

ECEL1 is a member of the neprilysin family of zinc metalloendopeptidases and is highly expressed in neurons within the central and peripheral nervous system in humans and rodents (8, 17–19). *Ecel1*$^{-/-}$ mice die soon after birth due to respiratory failure. They have a pathological decrease in final branching of nerve terminals to the diaphragm and skeletal muscles, and failure of formation of an adequate number of neuromuscular junctions (NMJs) (18, 20). While the molecular mechanism underlying this phenotype remains undefined, zinc metalloendopeptidases such as ADAMs and matrix metalloproteases may directly control axon growth by cleaving axon guidance receptors and ligands (21). Alternatively, ECEL1 could bind to factors required for the final axonal branching as seen with Meltrin β,

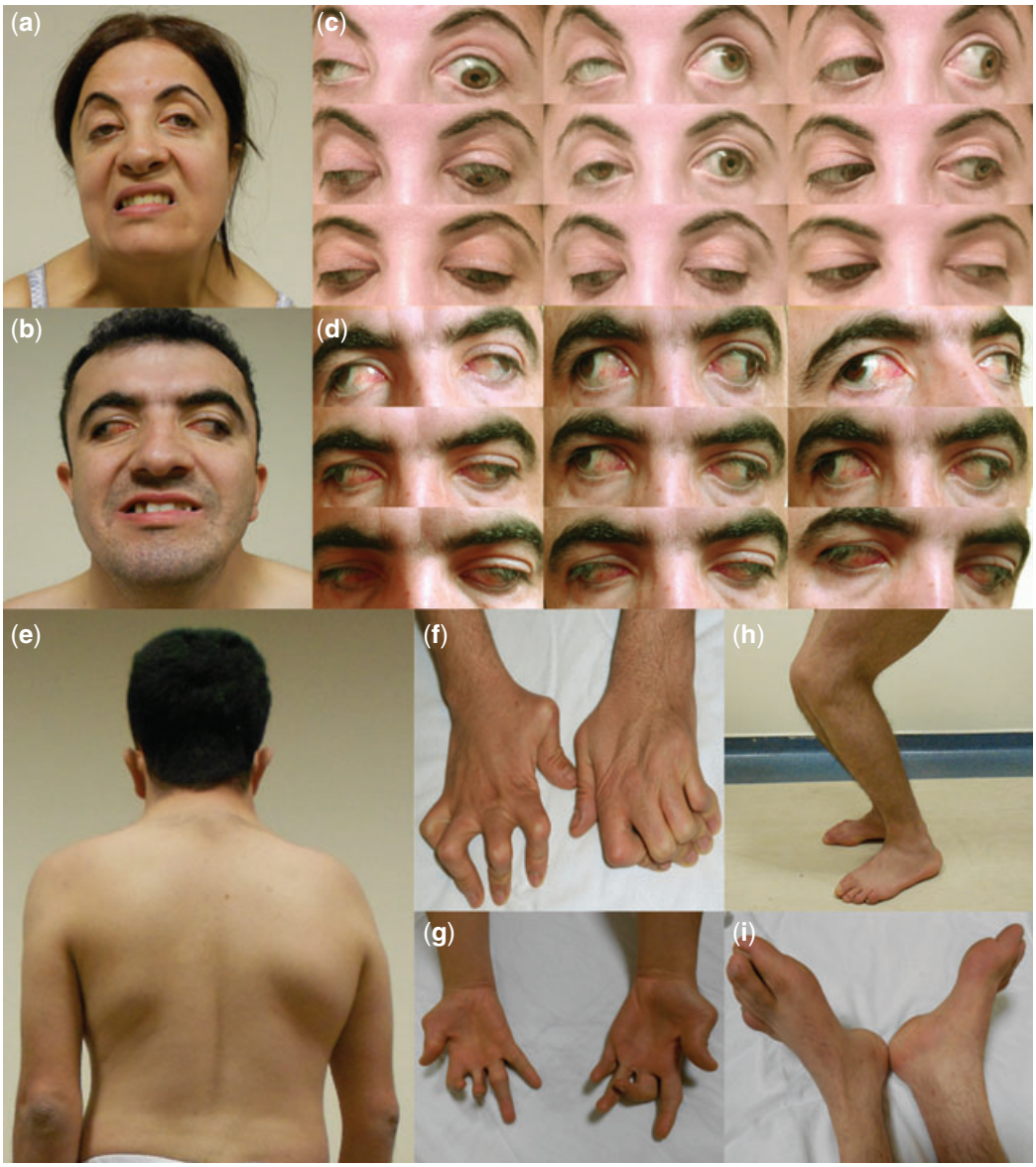

Fig. 2. Clinical phenotypes. Faces of UP IV:2 (**a**) and IV:3 (**b**) attempting to show their teeth; notice facial asymmetry with decreased expression. Ocular position and motility in nine directions of gaze in IV:2 (**c**) and IV:3 (**d**). IV:2 has ptosis OD and marked limitation of adduction and elevation OS. IV:3 has extreme exotropia and failure of eye movements in all directions. (**e**) Left thoracolumbar scoliosis in IV:3. (**f**) Bilateral metatarsal and interphalangeal extension and flexion contractures. (**g**) Prominent hypothenar and intrinsic hand muscle atrophy. (**h**) Limited knee flexion. (**i**) Bilateral pes cavus and overlapping toes.

a metallopeptidase that binds to EphA4 and regulates ephrinA4 signaling required for NMJ formation (22).

The significant congenital ophthalmoplegia reported here, together with the reports of expression of *ECEL1* in embryonic spinal and cranial motor neurons of rats (19), raises the possibility that *ECEL1* mutations can result in a new genetic form of the congenital cranial dysinnervation disorders (CCDDs). CCDDs are congenital neuromuscular diseases resulting from developmental errors in innervation of cranial musculature in humans (23), and a subset are caused by mutations in genes important for axon growth and guidance (24). Notably, the phenotype of the siblings in this report overlaps with the phenotype resulting from a subset of missense mutations in the neuronal-specific *TUBB3* gene (25).

Significant refractive errors have not been reported with *ECEL1* mutations (7, 8). *ECEL1* is, however, expressed in the developing retina (19), and may play a role in control of ocular growth and refraction (26, 27). Moreover, another form of AMC, Escobar syndrome (OMIM 609339), results from homozygous mutations in the cholinergic receptor nicotinic gamma gene, which was recently reported to also be associated with refractive errors (28, 29). These findings are intriguing given that retinal cholinergic signaling has been postulated as a mechanism through which retina affects refractive development (27, 30). Investigating

refraction in other *ECEL1*-mutation-positive patients might support an unrevealed role of *ECEL1* in ocular development and cholinergic signaling.

Supporting Information

The following Supporting information is available for this article:

Table S1. Clinical features of affected cases.

Additional Supporting information may be found in the online version of this article.

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