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SHORT REPORT

CLINICAL GENETICS WILEY

Bi-allelic loss of *ERGIC1* causes relatively mild arthrogryposis

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Abstract

Arthrogryposis describes the presence of multiple joint-contractures. Clinical severity of this phenotype is variable, and more than 400 causative genes have been proposed. Among these, *ERGIC1* is a recently reported candidate encoding a putative transmembrane protein of the ER-Golgi interface. Two homozygous missense variants have been reported in patients with relatively mild non-syndromic arthrogryposis. In a consanguineous family with two affected siblings presenting congenital arthrogryposis and some facial dysmorphism we performed prenatal array-CGH, postnatal targeted exome and genome sequencing. Genome sequencing identified a homozygous 22.6 Kb deletion encompassing the promoter and first exon of *ERGIC1*. mRNA quantification showed the complete absence of *ERGIC1* expression in the two affected siblings and a decrease in heterozygous parents. Our observations validate the pathogenic role of *ERGIC1* in congenital arthrogryposis and demonstrate that complete loss of function causes a relatively mild phenotype. These findings will contribute to improve genetic counseling of *ERGIC1* mutations.

KEYWORDS
arthrogryposis, *ERGIC1*, loss of function mutation, whole genome sequencing

1 | INTRODUCTION

Arthrogryposis is a descriptive clinical term that represents multiple non-progressive joint-contractures.[1] Because of its prenatal onset, it is often discovered during fetal ultrasound scan. Clinical severity depends on the affected joints, and on associated features in syndromic forms. Several etiologies have been described for arthrogryposis, including neurological disorders, muscle diseases or skeletal conditions that hamper prenatal mobility. It is highly heterogeneous in terms of genetic causes with more than 400 genes reported so far.[2]

ERGIC1 is a recently described candidate gene encoding a putative transmembrane protein of the Endoplasmic Reticulum-Golgi Intermediate Compartment (ERGIC), a tubulovesicular membrane cluster that serves in protein sorting and trafficking.[3] Two homozygous missense variants have been reported to date.[4,5] All patients presented relatively mild non-syndromic arthrogryposis and achieved

ability to walk with the help of orthopedic prosthesis and surgical interventions.[4,6] The causative role of *ERGIC1* remains however uncertain and the pathogenic mechanism has not been definitely proven.

We here report a homozygous deletion of the promoter and first exon of *ERGIC1*, which co-segregated with relatively mild arthrogryposis in a consanguineous family. RNA analysis showed complete absence of *ERGIC1* expression in the two affected siblings providing strong evidence for loss-of-function as the pathogenic mechanism.

2 | MATERIALS AND METHODS

2.1 | Array-CGH

Prenatal array-CGH analysis was carried out in the proband using Human Genome CGH Microarray Kit G3 44 (Agilent Technologies, Santa Clara, CA, USA) and Agilent Genomic Workbench Software.

2.2 | Exome sequencing for targeted analysis

A library was prepared from the proband's peripheral blood DNA using the SureSelect QXT Human All Exon V5 kit (Agilent Technologies), and sequenced on an Illumina NextSeq500 ® (Illumina, San Diego, CA, USA). SNV and InDels analysis was performed as previously described[7] and focused on 227 candidate genes (Supplementary Table S1, Panel 1). Copy number variants (CNVs) were assessed using CoNIFER[8] and XHMM.[9]

2.3 | Genome sequencing for targeted analysis

A library was prepared from the proband's peripheral blood DNA using the Illumina TruSeq DNA PCR-free sequencing kit, and sequenced on an Illumina NovaSeq6000®.

SNV and InDels analysis was performed as for exome data focusing on 355 candidate genes (Supplementary Table S1, Panel 2). CNVs were called from aligned reads using the software CNVnator,[10] Delly,[11] and Manta,[12] and annotated with AnnotSV.[13] CNVs were selected based on the following criteria: MAF <0.02 in gnomAD-SV-2.1; MAF < 0.10 in an internal dataset ($n = 38$); affecting a transcript sequence over at least 1 bp.

ERGIC1 deletion was submitted to ClinVar (SCV001499869).

2.4 | Quantitative PCR on DNA

To confirm *ERGIC1* deletion, quantitative real-time PCR was performed on peripheral blood DNA from each family member, using the qBiomarker SYBR® ROX green Mastermix (Qiagen, Hilden, Germany).

Two assays were designed within the deleted region and one reference assay (Mref, VPH000-0000000A). Data analysis was performed using the Qiagen GeneGlobe tool https://geneglobe.qiagen.com/ch/analyze/.

2.5 | Sanger sequencing

PCR and Sanger sequencing were carried out on peripheral blood DNA from each family member to confirm candidate SNVs and to determine *ERGIC1* deletion breakpoints.

2.6 | Quantitative PCR on cDNA

Quantitative real-time PCR was performed on cDNA from oligoDT reverse transcription of peripheral blood total RNA of the proband, the affected sister, the parents and an unrelated control, used as reference. Two experiments were carried out in triplicate using the TaqPath qPCR Master Mix, CG (Thermo Fisher Scientific, Waltham, MA, US). Four *ERGIC1* regions were tested, corresponding to junctions of exons 1–2, 3–4, 4–5 and 7–8 of the canonical transcript (ENST00000393784.3), together with one region of each of the three housekeeping genes 18S, *ACTNB* and *GAPDH*. Relative quantification followed the DDCt method.[14]

Informed consents were obtained for all patients in this study. The study followed the Helsinki Declaration principles.

3 | RESULTS

The proband was a consanguineous male born to first cousins once removed. Prenatal ultrasound scanning at 20 weeks revealed bilateral flexed arms and permanent incomplete extension of the right elbow, bilateral adducted thumbs, flexed lower limbs and bilateral malposition of the feet with normal quantity of amniotic fluid. Prenatal array-CGH was unremarkable. He was born at term by caesarian section because of

FIGURE 1 Proband (top panel) and affected sister (bottom panel) at birth showing bilateral flexion contractures of the elbows and knees, malposition of the feet, and minor dysmorphism [Colour figure can be viewed at wileyonlinelibrary.com]

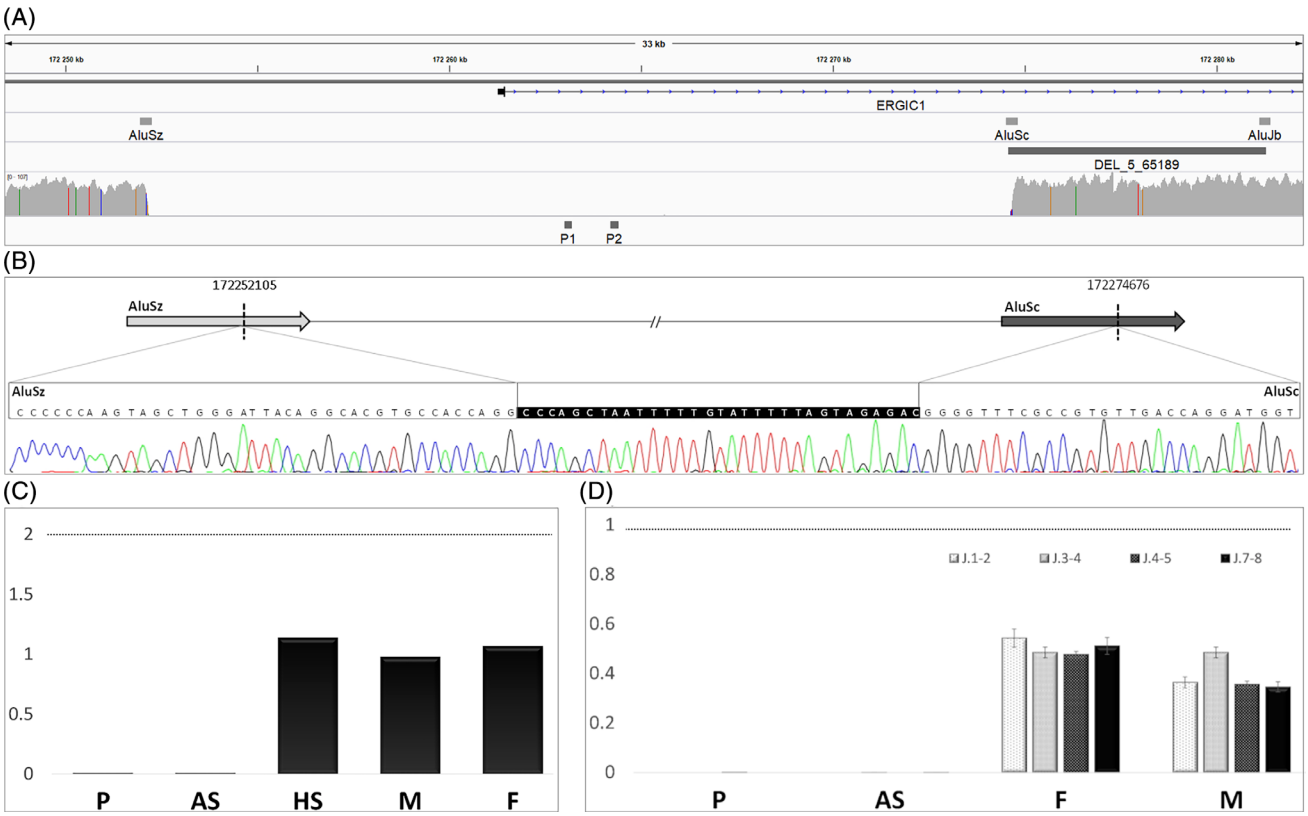

FIGURE 2 A homozygous 22.6 kb deletion abolishes expression of ERGIC1. (A) Genomic localization of the deletion. IGV representation from top to bottom: chromosome 5 positions; *ERGIC1* proximal promoter region, first exon and part of first intron; *Alu* elements at deletion boundaries; deletion DEL_5_65189 reported in gnomAD dataset which shares one breakpoint at *AluSc* with reported proband; sequencing coverage in reported proband amounting to 100X at the boundaries and decreasing to zero within the deleted region (alignment details in Supplementary Figure S1); qPCR assays at loci P1) chr5:172263001–172 263 200 and P2) chr5:172264201–172 264 400 for copy number evaluation; (B) Rearrangement of the *Alu* elements (top) and Sanger sequencing of the breakpoint (bottom), showing part of the resulting chimeric *Alu* and the 31 bp microhomology (black box). (C) *ERGIC1* Copy Number. qPCR on genomic DNA was performed in triplicates at P1 and P2 loci (results shown for P1. Similar results obtained for P2 are not shown). The dotted line indicates the expected level for 2 copies. The proband (P) and affected sister (AS) display no detectable copies of the region, while the father (F), mother (M) and healthy sister (HS) have one copy, consistent with heterozygosity. (D) *ERGIC1* mRNA relative expression. Average results of N = 2 qPCR experiments performed in triplicates on cDNA at four *ERGIC1* mRNA segments, corresponding to junctions (J) of exons 1–2, 3–4, 4–5 and 7–8. One *GAPDH* mRNA segment was used as reference. The dotted line indicates expression level of the control normalized to 1. Error bars correspond to standard deviation. The father and mother both show reduced expression with respect to the control, while the proband and the affected sister present a virtually complete lack of mRNA expression. Similar results were obtained using *18S* and *ACTNB* as reference (Supplementary Figure S2) [Colour figure can be viewed at wileyonlinelibrary.com]

breech position, with good postnatal adaptation. At birth, the prenatally observed multiple joint contractures were confirmed and no organ malformations were found apart from unilateral cryptorchidism. Some minor facial dysmorphism was noted (plagiocephaly, large ears, microretrognathia) (Figure 1). Dysmorphism waned in early childhood. With the help of orthopedic splints and surgical interventions he walked at the age of 2 years. At the age of six he could run, jump and ride a bike. IQ was between 61 and 74 (CI 95%), which corresponds to percentile 1, and subscales scores were homogeneous. The nonverbal index was between 68 and 84 (CI 95%), which correspond to the fourth percentile.

When the proband was 5 years old a sister was born presenting the same features at the 20 weeks prenatal ultrasound scan, and similar multiple joint contractures and minor dysmorphism at birth (Figure 1). She sat at 7 months and did not walk yet at the age of 18 months, but made progress with the orthopedic splints. On the cognitive level she is slightly behind her age. Between the two children a female sibling was born who did not present arthrogryposis nor facial dysmorphism, but had unilateral renal agenesis.

We performed exome sequencing in the proband and targeted 227 genes associated with arthrogryposis (Supplementary Table S1, Panel 1). A homozygous missense variant was identified in *COL6A3* (c.5968C > T; p.Arg1990Trp) initially classified as of Unknown clinical Significance (VUS), according to the ACMG guidelines.[15] Segregation analysis showed that the affected sister was heterozygous, allowing to exclude this variant as the cause of the phenotype in this family (Supplementary Table S2). No CNVs were detected in the 227 candidate genes.

TABLE 1 Clinical features of *ERGIC1*-related arthrogryposis in previous reports and in the present one

	Lebenthal et al.1970/Reinstein et al. 2017 (1 kindred, 7 families, 23 cases[a])[4,6]	Pehlivan et al. 2019 (1 case)[5]	This report - proband	This report - affected sister
ERGIC1 mutation	p.Val98Glu (NM_001031711 c.293 T>A)	p.Gly261Asp (NM_001031711; c.782G>A)	NC_000005.9: g.172252136_172274628del	NC_000005.9: g.172252136_172274628del
Dysmorphic features	Not reported	Microretrognathia, low-set ears	Microretrognatia, plagiocephaly, large ears	Microretrognatia, plagiocephaly, large ears
Involved joints	Elbows 78% (13 bilateral, 4 right, 1 left) Wrists 26% Hip 26% Knees 56% (10 bilateral, 2 right, 1 left) Ankles-feet 61%	WristsFingersAnkles-feet (Pes equinovarus)	ElbowsFingers (adducted thumbs) KneesAnkles-feet (Pes equinovarus)	ElbowsFingers (adducted thumbs)KneesAnkles-feet (Pes equinovarus)
Other features	Hyperlordosis and R pelvic obliquity ($n = 1$), Left dorsal scoliosis ($n = 1$), Marked mental retardation ($n = 1$), Patent ductus arteriosus ($n = 2$), Aortic stenosis[b] ($n = 1$), Cyanotic congenital heart disease[b] ($n = 1$), Coarctation of aorta[b] ($n = 1$), aortic stenosis and bicuspid aorta[b] (1)	Secundum atrial septal defect	Mild developmental delayUnilateral cryptorchidism	

[a]Only 12 out of the 23 cases were tested for *ERGIC1* mutation.
[b]Five cases of congenital heart/aortic valve diseases clustered in a single sibship, suggesting a different cause from *ERGIC1* mutation.

We then resorted to genome sequencing that was carried out in the proband. We looked for homozygous SNVs and InDels in an updated panel of 355 arthrogryposis genes (Supplementary Table S1, panel 2), and for homozygous genic CNVs on a genome-wide scale.

SNVs and Indels analysis revealed only one rare homozygous missense variant in the *MYOM2* gene (c.2282A > G; p.Asn761Ser). A causal role for this variant was excluded by segregation analysis showing that the affected sister was homozygous for the reference allele (Supplementary Table S2).

Analysis of genic CNVs revealed a 22.6Kb homozygous deletion on chromosome 5 (NC_000005.9:g.172252136_172274628del) encompassing the first exon of *ERGIC1* (Figure 2A, Supplementary - Figure S1). The variant was detected by all exploited tools (Appendix S1). Quantitative PCR confirmed the homozygous deletion in the two affected children, while the healthy sister and the parents were heterozygous (Figure 2C). Of note, the deletion was below the resolution limit of the Agilent 44K microarray since no probes are located within the deleted region.

The deleted region was found to be flanked by two *Alu* elements (AluSc and AluSz, 86% similar) which are known to mediate genomic rearrangements (Figure 2A). We mapped the breakpoints at nucleotide-level within the resultant chimeric *Alu* and identified a 31 bp microhomology at the breakpoint junction (Figure 2B). The deletion is not reported in DGV nor in the gnomAD SV datasets (https://gnomad.broadinstitute.org/,[16]). Of note, one downstream heterozygous intronic deletion is reported twice in gnomAD (DEL_5_65189) that shares a breakpoint with the one described here and has the same 31 bp inverted-repeat segment at its 3′ boundary.

In order to demonstrate that the deletion abolished *ERGIC1* transcription, we used quantitative PCR assays designed to span four exon-exon junctions of the 10-exons canonical transcript (ENST00000393784.3) (Supplementary Figure S2). All four assays showed absence of *ERGIC1* expression in blood–derived RNA in the two affected children and an approximately 50% decrease in the heterozygous parents (Figure 2D, Supplementary Figure S3).

4 | DISCUSSION

We here describe the first case of a clearly documented bi-allelic loss-of-function mutation of *ERGIC1* in two consanguineous siblings with congenital arthrogryposis. Complete and specific absence of *ERGIC1* mRNA was demonstrated by RNA studies. Assays amplifying proximal and distal exon junctions allowed us to exclude transcription at an alternative, more distant start site that would produce a shorter protein by use of an in-frame ATG. Moreover, the analyzed exon junctions were representative of all six protein-coding isoforms described in Ensembl, thus allowing us to exclude the synthesis of alternative *ERGIC1* transcripts (Supplementary Figure S2).

These findings have important clinical implications. First, together with the two previous reports,[4,5] we establish *ERGIC1* as a definitive cause for congenital arthrogryposis and demonstrate the loss-of-function pathogenic mechanism. Second, our data show that the complete loss of *ERGIC1* expression is associated with a relatively mild arthrogryposis phenotype, similar to that observed in the patients with missense

mutations.[4,5] The common features are flexion contractures of the elbows and knees and talipes equinovarus without major associated organ malformations. Table 1 summarizes the clinical features of *ERGIC1*-mutated cases reported here and in previous works. The phenotype in our family was compatible with walking, running and jumping with the help of orthopedic interventions. Careful neurological examination of both siblings since infancy has never pointed towards a neuromuscular disorder. Therefore there was no a priori indication for additional muscular investigation, such as electromiography, muscle MRI or muscle biopsy. Developmental assessments revealed mainly motor delay consistent with arthrogryposis. At 6 years a general IQ test in the proband suggested mild cognitive delay. It is currently unclear whether intellectual deficiency is part of the *ERGIC1* phenotypic spectrum.

Genome sequencing showed a key advantage in the high resolution of breakpoint definition allowing prompt confirmation and segregation analysis. It recognized *Alu* repeats at the breakpoints consistent with an *Alu* recombination-mediated origin, with possible recurrence of this deletion in the population.

In summary, our data establish the pathogenic role of *ERGIC1* in congenital arthrogryposis, and correlate the complete loss of *ERGIC1* expression with a relatively mild arthrogryposis phenotype. These findings will be important for better understanding *ERGIC1* function and arthrogryposis mechanism, and for improved genetic counseling, especially in a prenatal setting.

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CONFLICT OF INTEREST

The authors have no competing interests to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data are openly available in the ClinVar repository (ID SCV001499869).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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