Bi-allelic loss of ERGIC1 causes relatively mild arthrogryposis

Caterina Marconi1 | Laure Lemmens1 | Frédéric Masclaux1 | Francesca Mattioli1 | Joël Fluss2 | Philippe Extermann3 | Purificacion Mendez4 | Russia Ha-Vinh Leuchter2 | Elissavet Stathaki1 | Sacha Laurent1 | Eva Hammar1 | Anne Vannier1 | Konstantinos Varvagiannis1 | Michel Guipponi1,5 | Frédérique Sloan-Bena1,5 | Jean-Louis Blouin1,5 | Marc Abramowicz1,5 | Siv Fokstuen1,5

1 Genetic Medicine division, Diagnostic Department, Hôpitaux Universitaires de Genève, Genève (CH), Switzerland
2 Pediatric Specialties division, Department of Women, Children and Adolescents, Hôpitaux Universitaires de Genève, Genève (CH), Switzerland
3 Prenatal Ultrasonography, Dianecho, Genève (CH), Switzerland
4 Obstetrics and Gynecology, Centre Médical Eaux-Vives, Genève (CH), Switzerland
5 Department of Genetic Medicine and Development, School of Medicine, University of Geneva, Genève (CH), Switzerland

Correspondence
Marc Abramowicz, Genetic Medicine division, Diagnostics Department, Hôpitaux Universitaires de Genève, Rue Gabrielle-Perret-Gentil 4, 1205 Genève, Switzerland. Email: marc.abramowicz@unige.ch

Funding information
Fondation Privée des HUG, Grant/Award Number: QS05-28

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 to date. All patients presented relatively mild non-syndromic arthrogryposis and achieved mobility. It is highly heterogeneous in terms of genetic causes with more than 400 genes reported so far.

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. Two homozygous missense variants have been reported to date. All patients presented relatively mild non-syndromic arthrogryposis and achieved

1 INTRODUCTION

Arthrogryposis is a descriptive clinical term that represents multiple non-progressive joint-contractures. 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.

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. Two homozygous missense variants have been reported to date. All patients presented relatively mild non-syndromic arthrogryposis and achieved
ability to walk with the help of orthopedic prosthesis and surgical interventions.\textsuperscript{4,6} The causative role of \textit{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 \textit{ERGIC1}, which co-segregated with relatively mild arthrogryposis in a consanguineous family. RNA analysis showed complete absence of \textit{ERGIC1} expression in the two affected siblings providing strong evidence for loss-of-function as the pathogenic mechanism.

2 \hspace{1cm} 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 QTX Human All Exon V5 kit (Agilent Technologies), and sequenced on an Illumina NextSeq500.\textsuperscript{8} SNV and InDels analysis was performed as previously described\textsuperscript{7} and focused on 227 candidate genes (Supplementary Table S1, Panel 1). Copy number variants (CNVs) were assessed using CoNIFER\textsuperscript{8} and XHMM.\textsuperscript{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\textsuperscript{8}. 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,\textsuperscript{10} Delly,\textsuperscript{11} and Manta,\textsuperscript{12} and annotated with AnnotSV.\textsuperscript{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.

\textit{ERGIC1} deletion was submitted to ClinVar (SCV001499869).

2.4 | Quantitative PCR on DNA

To confirm \textit{ERGIC1} deletion, quantitative real-time PCR was performed on peripheral blood DNA from each family member, using the qBiomarker SYBR\textsuperscript{8} 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 \textit{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 \textit{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, \textit{ACTNB} and \textit{GAPDH}. Relative quantification followed the DDCt method.\textsuperscript{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

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{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]}
\end{figure}
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.

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. 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.
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 S2). All four assays with congenital arthrogryposis. Complete and specific loss-of-function mutation of ERGIC1 were heterozygous (Figure 2D, Supplementary Figure S3). 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, 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

**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).

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).

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

| Feature                      | Lebenthal et al. 1970/Reinstein et al. 2017 (1 kindred, 7 families, 23 cases) | Pehlivan et al. 2019 (1 case) | 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 |
| **Involving joints**         | Elbows 78% (13 bilateral, 4 right, 1 left) Wrist 26% Hip 26% Knees 56% (10 bilateral, 2 right, 1 left) Ankles-feet 61% | Wrist, Finger, Ankle-Feet (Pes equinovarus) | Elbows, Fingers (adducted thumbs) Knees, Ankles-feet (Pes equinovarus) | Elbows, Fingers (adducted thumbs) Knees, Ankles-feet (Pes equinovarus) |
| **Other features**           | Hyperlordosis and R pelvic obliquity (n = 1), Left dorsal scoliosis (n = 1), Patellar hyperesthesia (n = 2), Aortic stenosis (n = 1), Cyanotic congenital heart disease (n = 1), Coarctation of aorta (n = 1), Aortic stenosis and bicuspid aorta (1) | Secundum atrial septal defect | Mild developmental delay, Unilateral cryptorchidism |

*Only 12 out of the 23 cases were tested for ERGIC1 mutation.

*Five cases of congenital heart/aortic valve diseases clustered in a single sibship, suggesting a different cause from ERGIC1 mutation.
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 assessment 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.

ACKNOWLEDGEMENTS

We kindly acknowledge Keith Harshman and the Health2030 Genome Center Sequencing Platform for performing the Genome Sequencing. A warm thanks to the family who agreed to this publication. This work was supported by the Fondation Privée des HUG (Genève, Switzerland) grant QS05-28 to Marc Abramowicz.

CONFLICT OF INTEREST

The authors have no competing interests to declare.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.14004.

DATA AVAILABILITY STATEMENT

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

ORCID

Caterina Marconi https://orcid.org/0000-0002-1210-3969
Marc Abramowicz https://orcid.org/0000-0003-0623-8768

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SUPPORTING INFORMATION

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

How to cite this article: Marconi C, Lemmens L, Mascia L, et al. Bi-allelic loss of ERGIC1 causes relatively mild arthrogryposis. Clinical Genetics. 2021;1–5. https://doi.org/10.1111/cge.14004