A novel *FGD1* mutation in a family with Aarskog–Scott syndrome and predominant features of congenital joint contractures

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Abstract Mutations in *FGD1* cause Aarskog–Scott syndrome (AAS), an X-linked condition characterized by abnormal facial, skeletal, and genital development due to abnormal embryonic morphogenesis and skeletal formation. Here we report a novel *FGD1* mutation in a family with atypical features of AAS, specifically bilateral upper and lower limb congenital joint contractures and cardiac abnormalities. The male proband and his affected maternal uncle are hemizygous for the novel *FGD1* mutation p.Arg921X. This variant is the most carboxy-terminal *FGD1* mutation identified in a family with AAS and is predicted to truncate the FGD1 protein at the second to last amino acid of the carboxy-terminal pleckstrin homology (PH) domain. Our study emphasizes the importance of the 3′ peptide sequence in the structure and/or function of the FGD1 protein and further demonstrates the need to screen patients with X-linked congenital joint contractures for *FGD1* mutations.

INTRODUCTION

Aarskog–Scott syndrome (AAS or faciogenital dysplasia; MIM# 305400) is an X-linked recessive condition typically characterized by facial dysmorphology and skeletal and genital anomalies that result from mutations in *FGD1* (Pasteris et al. 1994). Males affected with AAS typically present with dysmorphic facial features, genital hypoplasia, short stature, and brachydactyly (Orrico et al. 2010); however, a broad spectrum of clinical heterogeneity has been reported in patients with a molecular diagnosis of AAS, including intellectual disability, unilateral focal polymicrogyria, and myopathy and distal arthropathy (Lebel et al. 2002; Bottani et al. 2007; Al-Semari et al. 2013). These atypical features may prohibit clinical recognition of AAS, leading to a failure to screen for *FGD1* mutations and a delayed diagnosis for patients and families (Orrico et al. 2010).

To date, 63 mutations in *FGD1* have been reported in AAS (Orrico et al. 2014; Pérez-Coria et al. 2015). The *FGD1* gene contains 18 exons that code for a specific guanine nucleotide exchange factor (GEF) for the Rho protein CDC42 that is critical for embryogenesis (Gorski et al. 2000). The 961-amino-acid FGD1 protein has five discrete domains, including a

proline-rich domain, an SH3-binding domain, a Rho GEF homology domain, two PH domains, and a cysteine-rich Zn^{2+}-finger domain. Missense, frameshift, nonsense, splice site, branch point, and large and small in-frame deletion mutations have been reported to cause AAS (Orrico et al. 2014). Interestingly, these mutations are randomly distributed throughout the protein's functional domains, indicating a loss-of-function mechanism of pathogenesis in AAS through the disruption of GEF catalytic activity and/or protein localization of FGD1 (Orrico et al. 2000, 2004, 2010; Schwartz et al. 2000; Bedoyan et al. 2009).

Here, we report the identification of a novel truncating mutation in *FGD1* via exome sequencing of genomic DNA from a family that presented with X-linked distal joint contractures, cardiac defects, and mild craniofacial and genital manifestations typical of AAS. This report illustrates the importance of screening boys with distal skeletal abnormalities and a family history suggesting X-linked inheritance for *FGD1* mutations.

RESULTS

Clinical Presentation and Family History

The proband (Individual IV-1) (see Fig. 2A) was observed to have bilateral clubfeet at 20 wk gestation via ultrasound. In early infancy, the Ponseti method was used to correct the clubfeet with serial casting, followed by bilateral Achilles tenotomy, and corrective shoes with a foot abduction orthosis (Ponseti et al. 2006). Additionally, it was noted that he had difficulty opening his hands because of flexion contractures of the fingers and was treated with hand splints and occupational therapy that improved his range of motion.

Upon physical exam, the proband was observed to have some mild craniofacial dysmorphic features including a space between the upper teeth (diastema), ankyloglossia, a round face, down-slanted palpebral fissures with ptosis of the left eyelid, a short, upturned nose with a well-formed philtrum, and small, low-set ears with overfolded helices (Fig. 1A). Additionally, an ophthalmologic exam revealed farsightedness and astigmatism bilaterally, a genital exam revealed a mild shawl scrotum and bilateral undescended testes, and a Still's murmur was appreciated on physical exam (data not shown).

His feet at most recent exam have reduced creases on the plantar surfaces and residual metatarsus adductus (Fig. 1B). The individual's hands showed a single palmar crease on the right and a transitional crease on the left with mild flexion contractures of the fingers, brachydactyly, and bilateral clinodactyly (Fig. 1C).

There was a strong family history of craniofacial abnormalities, hand contractures, and clubfeet that segregated in an X-linked pattern (Fig. 2A). The proband has two maternal half uncles with similar features, including craniofacial dysmorphisms and hand contractures (Individuals III-4 and III-5 in Fig. 2A). One uncle also had bilateral clubfeet (III-4 in Fig. 2A), whereas the other uncle has a ventricular septal defect (III-5) (Fig. 2A). In addition, multiple affected male relatives were reported in at least two earlier generations, and two individuals were diagnosed with supraventricular tachycardia in addition to the observed skeletal abnormalities (Table 1). Taken together, this family represented a multigenerational pedigree with a prominent X-linked skeletal syndrome characterized by distal joint contractures and craniofacial abnormalities with the occurrence of cardiac abnormalities in multiple affected individuals.

Genomic Analysis

Because of the X-linked inheritance pattern, our analysis focused on variants identified on the X Chromosome. To identify variants of interest, whole-exome sequencing was performed on DNA from Individuals III-4 and IV-1. Initial bioinformatic analysis identified three putative nonsynonymous single-nucleotide variants common between the two DNA

Figure 1. Phenotypic characteristics of the proband include (*A*) mild craniofacial dysmorphisms including round face, down-slanted palpebral fissures, ptosis of the left eyelid, short, upturned nose, and small, low-set ears, (*B*) bilateral metatarsus adductus (partially corrected clubfeet), and (*C*) mild flexion contractures of the fingers, brachydactyly, and clinodactyly.

samples evaluated. Sanger sequencing determined that only one variant was indeed present in both affected individuals and ruled out the other two variants as sequencing errors. Both affected individuals were found to be hemizygous for a nonsense mutation in exon 18 of *FGD1* (c.2761 C>T; Table 2; Fig. 2B) (forward 5′-CTGAAGACAGAGCATATGC-3′, reverse 5′-CAAGTATTGACTGAGCTGG-3′). This mutation results in a substitution of a stop codon for an arginine at amino acid residue 921 (p.Arg921X) and is predicted to result in a 40-amino-acid truncation of the gene product (Fig. 2C). Targeted Sanger sequencing revealed that the mutation is carried by both the proband's mother and maternal grandmother (Fig. 2A; Individual II-7 and III-3, respectively). No additional family members were available for segregation studies. Identification of the *FGD1* p.Arg921X mutation provided a molecular diagnosis of AAS in this family.

DISCUSSION

Here we describe a novel *FGD1* mutation in a large pedigree with X-linked distal joint contractures, cardiac abnormalities, and craniofacial and genital abnormalities consistent with a molecular and clinical diagnosis of AAS. AAS is typically diagnosed based on clinical
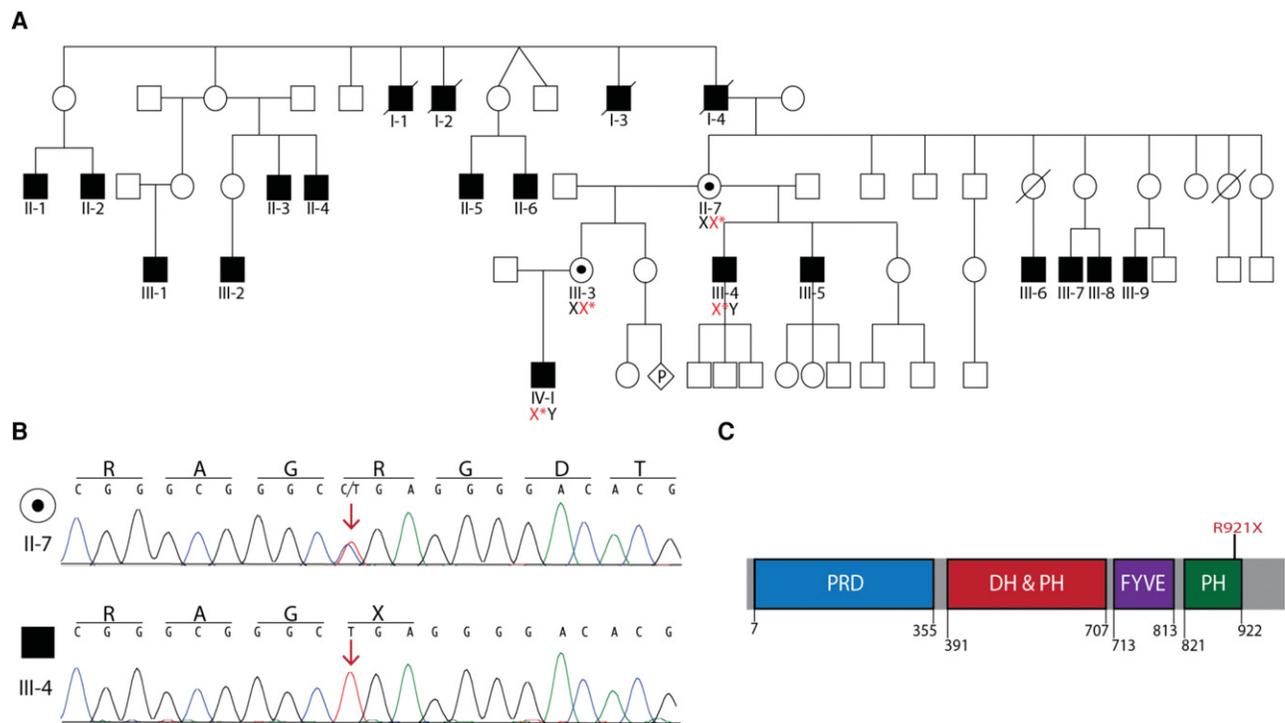
Figure 2. Identification of a *FGD1* mutation that segregates with phenotypic characteristics of Aarskog–Scott syndrome (AAS). (*A*) Pedigree of the affected family. DNA samples from the proband (IV-1) and his maternal uncle (III-4) were used for whole-exome sequencing. Sanger sequencing of Individuals IV-1 and III-4 confirmed the presence of the *FGD1* mutation c.2761 C>T (red X*) in affected individuals and determined carrier status for Individuals II-7 and III-3. Empty symbols indicate unaffected individuals, filled symbols indicate affected individuals, and black dots indicate unaffected mutation carriers. Diamond-shaped symbol with a "P" represents unknown sex of a fetus in utero and slashes through symbols indicate deceased individuals. (*B*) Representative traces from Sanger sequencing confirming the presence of the c.2761 C>T mutation in an unaffected carrier female (II-7) and an affected male (III-4). Red arrow marks the affected nucleotide. The single-letter abbreviations for the nucleotides and corresponding amino acid codes are provided along the *top* of each trace. (*C*) Protein cartoon illustrates the known FGD1 functional domains in blue (proline-rich domain; PRD), red (DBL homology domain and pleckstrin homology domain; DH and PH), purple (FYVE zinc finger domain; FYVE), and green (pleckstrin homology domain; PH). The numbers indicate the amino acid positions for each domain. The position of the FGD1 R921X affected residue is indicated in red.

features, specifically the presence of craniofacial and genital abnormalities in conjunction with short stature and brachydactyly. However, in cases where the presenting phenotypic characteristics are atypical of AAS, such as the congenital joint contractures and clubfeet described in this report, a diagnosis of AAS should be considered and molecular techniques should be used for proper diagnosis.

Previously, Bottani et al. (2007) and Al-Semari et al. (2013) described three individuals from two families with a molecular diagnosis of AAS who presented with atypical arthropathic features (Bottani et al. 2007; Al-Semari et al. 2013). The case presented here reinforces the need to strongly consider screening for *FGD1* mutations and additional phenotypic characteristics of AAS in male patients presenting with either X-linked or de novo distal joint contractures or clubfeet. Careful evaluations for more subtle features of AAS and targeted molecular testing in these cases would prevent unnecessary clinical and diagnostic testing for families, and identification of novel *FGD1* mutations may improve the understanding of the clinical and molecular spectrum of AAS. Similar to what was observed in members of

Table 1. Phenotypes reported for affected individuals

Individual	Facial gestalt[a]	Hand contractures	Clubfeet	Cardiac abnormalities	Adult height
I-1	Yes	Yes	NA	NA	NA
I-2	Yes	Yes	NA	NA	NA
I-3	Yes	Yes	NA	NA	NA
I-4	Yes	Yes	NA	NA	NA
II-1	Yes	Yes	NA	NA	NA
II-2	Yes	Yes	NA	NA	NA
II-3	Yes	Yes	NA	SVT	NA
II-4	Yes	Yes	NA	NA	NA
II-5	Yes	Yes	NA	NA	NA
II-6	Yes	Yes	NA	NA	NA
III-1	Yes	Yes	NA	NA	NA
III-2	Yes	Yes	NA	SVT	NA
III-4	Yes	Yes	Yes	No	5′8″
III-5	Yes	Yes	No	VSD	5′9″
III-6	Yes	Yes	NA	NA	5′8″
III-7	Yes	Yes	NA	NA	5′8″
III-8	Yes	Yes	NA	NA	5′8″
III-9	Yes	Yes	NA	NA	5′8″
IV-1	Yes	Yes	Yes	Murmur	-

NA, information not available; SVT, supraventricular tachycardia; VSD, ventricular septal defect.
[a]Includes widow's peak, round face, ptosis, down-slanting palpebral fissures, hypertelorism, and anteverted nares. Specific features are not available for each individual except as noted in the text.

the family reported here, previous reports have shown the occurrence of cardiac abnormalities in patients with *FGD1* mutations, specifically ventricular septal defect (Fernandez et al. 1994; Nouraei et al. 2005; Orrico et al. 2007), which was identified in Individual III-4 (Fig. 2A) in this study. Additionally, supraventricular tachycardia (SVT) was observed in two individuals, which to our knowledge, has not been previously associated with AAS; however, we cannot confirm that the SVT is a component of AAS in this family and not an unrelated abnormality. Still, this further illustrates the need for careful cardiac evaluations in patients with *FGD1* mutations.

Identification of novel *FGD1* mutations has led to important insights into FGD1 function and the mechanism of AAS pathogenesis. Mutations in *FGD1* have been identified in all known functional domains of the FGD1 protein, indicating a loss-of-function mechanism. A complete gene deletion as well as multiple nucleotide insertions that are predicted to result in premature translation termination have been identified in the *FGD1* gene in

Table 2. *FGD1* (NC_000023.11)

Chr: position GRCh37 (hg19)	HGVS cDNA	HGVS protein reference	HGVS protein	Variant type	Predicted effect	Genotype
X: 54472667	c.2761C>T	NP_004454.2	p.Arg921X	Nonsense	Aarskog–Scott syndrome	Hemizygous

HGVS, Human Genome Variation Society.

individuals with AAS (Orrico et al. 2004; Bedoyan et al. 2009), indicating that complete loss of *FGD1* in hemizygous individuals leads to the AAS phenotype. The mutation described here (p.Arg921X) resides 181 nucleotides into the final exon of *FGD1*. Therefore, the transcript is not predicted to undergo nonsense-mediated decay (NMD) and thus would be expected to produce a truncated protein (Amrani et al. 2006); however, it is important to note that NMD has been reported when a nonsense mutation occurred in the last exon of the Type X collagen gene *COL10A1* (Chan et al. 1998). Thus protein studies would be needed to ensure the corresponding protein product of the p.Arg921X *FGD1* mutation is indeed present. FGD1 contains five domains: a proline-rich, SH3-binding domain (residues 7–330); a Rho GEF (DH or DBL) homology domain; a PH domain; a cysteine-rich Zn^{2+} finger (FYVE) domain; and a second PH domain (Fig. 2C). Functional studies of the FGD1 protein indicated that the amino terminus of FGD1 is responsible for localization to the subcortical actin cytoskeleton and Golgi complex, whereas the carboxy-terminal PH domain interacts with phosphorylated phosphatidylinositol (PtdIns) molecules in the plasma membrane (Estrada et al. 2001). Although the carboxy-terminal 40 amino acids of FGD1 are not part of a known functional motif, their exclusion from the protein likely results in a detrimental effect on FGD1 function, as evidenced by the phenotype seen in this family. Thus, it will be important to evaluate how the carboxy-terminal 40-amino-acid residues affect protein folding and/or the function of FGD1. It should be noted that there is one nonsense mutation (p.Glu938X) listed in the ExAC database for *FGD1* that would affect the carboxy-terminal domain of FGD1. This variant was identified as a single allele and is noted in the ExAC database to be a low-confidence variant because of coverage in <80% of sequenced individuals; thus, it is difficult to exclude sequencing errors for the p.Glu938X variant. Additionally, there is no phenotypic data available for the individual carrying p.Glu938X *FGD1*, including gender or evaluation for subtle AAS characteristics. Thus, the pathogenicity of this allele cannot be determined at this time. However, if p.Glu938X is a nonpathogenic allele, it would serve as an excellent control for studies on the carboxy-terminal region of FGD1.

In conclusion, we report a novel *FGD1* mutation, p.Arg921X, in a family with clinical manifestations of AAS and predominant congenital contractures. This study provides a strong rationale for screening male patients with distal skeletal abnormalities for *FGD1* mutations.

METHODS

Sample Collection and Whole-Exome Sequencing

DNA was extracted from whole blood using the Wizard Genomic DNA Purification Kit per the manufacturer's instructions (Promega). DNA samples from Individuals III-4 and IV-1 (Fig. 2A) were subjected to whole-exome sequencing to identify variants in common between these two individuals. Genomic DNA was sheared using a Covaris ultrasonicator to 400 bp. The libraries were prepared using the NEBNext DNA Library Prep Master Mix kit and gel size selected for 450-bp fragments. A 250-ng library for each sample was pooled and exome captured with NimbleGen SeqCap EZ System version 3. Paired-end 100-nt Illumina sequencing was performed (Illumina HiSeq 2000). FastQC was used to assess the quality of the data. Pair end reads were aligned to the human genome build GRCh37 (hg19) using Burrows–Wheeler alignment (BWA). To remove duplicates, the resultant mapped reads were processed by Picard. Variants were called using the following GATK (https://www.broadinstitute.org/gatk/) modules: local realignment by "RealignerTarget-Creator" and "IndelRealigner"; base quality recalibration by "BaseRecalibrator" and "PrintReads"; variant calling by "UnifiedGenotyper"; and variant quality recalibration by "VariantRecalibrator" and "ApplyRecalibration."

Table 3. Sequencing results

Patient	10× Coverage	Mean coverage	Yield (Gb)	>Q30 (%)	Mean Q	Filtered variants	FGD1 mean exon coverage	Variant coverage
III-4	97.8	82	11.3	83.9	33.64	52,632	40.11	35
IV-I	97.8	89	11.2	83.8	33.69	52,813	46.49	46

Variants that passed GATK variant quality score recalibration (VQSR) assessment were output as the final set of variants (Table 3). The detected variants were matched to detected variants in dbSNP database and further annotated by SnpEff.

ADDITIONAL INFORMATION

Data Deposition and Access

Whole-exome sequencing data is not publicly available because patient consent could not be obtained. The variant has been submitted to ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/) under accession number SCV000265836.

Ethics Statement

Written informed consent was obtained from all study participants in accordance with approved protocols from the Institutional Review Board of the University of Michigan.

Author Contributions

L.B.G., A.A., and C.E.K. conceived and designed the experiments. L.B.G. performed DNA preparation, mutation validation, and data analysis. L.B.G. drafted the manuscript. C.E.K. and F.A.F. provided the clinical data. C.E.K., A.A., and F.A.F. critically reviewed the manuscript.

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