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Mutation-in-Brief

A novel missense variant of *FGD1* disrupts critical cysteine residues of the FYVE domain in Japanese siblings with Aarskog–Scott syndrome

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Highlight

- A novel missense variant of *FGD1*, p.C782R, was identified in two siblings with Aarskog–Scott syndrome.
- The results of this study emphasize the importance of the FYVE domain as a mutational region of FGD1 in Aarskog–Scott syndrome.

Key words: camptodactyly, FGD1, FYVE domain, short stature

Introduction

Aarskog–Scott syndrome (AAS), also known as faciodigitogenital dysplasia, is a rare X-linked recessive syndrome characterized by facial dysmorphic features, short stature, brachydactyly, and genital anomalies such as shawl scrotum and cryptorchidism (1). This syndrome is caused by pathological variants of *FGD1* (faciogenital dysplasia 1) that encode FGD1, which acts as a guanine nucleotide exchange factor by specifically catalyzing the guanosine diphosphate (GDP)–guanosine triphosphate (GTP) exchange of Rho GTPase Cdc42 (2). In a few patients with clinically diagnosed AAS, *FGD1* variants were not detected, suggesting clinical and genetic heterogeneity and possibly other genes responsible for AAS (1). Cdc42 plays a key role in the regulation of cytoskeletal restructuring, gene transcription, cellular morphology, extension, and cell adhesion for cellular migration (2). Cdc42 is activated by FGD1 via the exchange of GDP for GTP with Cdc42 (2). This signaling pathway, which includes Cdc42 and FGD1, is important for the normal development of the human body and is associated with the risk of cancer invasion (2).

FGD1 is a 961-residue protein composed of five functional domains: the N-terminal PRD (proline rich domain) (amino acids 7–320) that is important for negatively regulating the activity of the guanine exchange factor; the catalytic region, which is composed

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of two domains, DH (amino acids 376–559) and PH1 (amino acids 592–698); the FYVE domain (amino acids 732–788); and the C-terminal PH2 domain (amino acids 815–920) (2). The FYVE domain consists of a phosphatidyl-inositol-3-phosphate (PI3P)-binding region and a zinc-finger cysteine-rich region, which directs proteins into various cellular compartments by attaching to surface signaling molecules, such as PI3P (3). More than 50 pathological variants of *FGD1* have been described in AAS, demonstrating no hotspots or common gene variants, and most of the variants are private within families (1). In this report, we describe two siblings with AAS caused by a novel *FGD1* variant, p.C782R, which has been predicted to substitute arginine for cysteine at amino acid residue 782 in the FYVE domain of FGD.

Case Report

The patients were two Japanese brothers of short stature who visited our hospital. The elder brother is a 14-yr-old boy born to non-consanguineous Japanese parents with a birth weight of 2,668 g (−1.7 SD) and a height of 45.5 cm (−2.1 SD) at a gestational age of 40 wk and 1 d. He was short during middle childhood, endocrinologically evaluated as normal, and was followed up regularly by clinicians (**Fig. 1A**). The height and weight were 154.2 cm (−2.0 SD) and 43.0 kg (−1.3 SD), respectively, at the age of 14 yr and 10 mo. The pubertal stage was evaluated as genital stage 3, pubic hair was consistent with Tanner stage 4, and testicular volume was 8 mL, bilaterally.

The younger brother is an 8-yr-old boy whose birth weight and height were 3,006 g (−0.6 SD) and 45.5 cm (−2.1 SD), respectively, at a gestational age of 40 wk and 0 d. He was identified as short at the age of 8 yr, with normal endocrinological laboratory test results (**Fig. 1B**). The height and weight were 117.2 cm (−2.0 SD) and 26.0 kg (−0.3 SD), respectively, at the age of 8 yr and 6 mo. The pubertal stage was evaluated as genital stage 1, pubic hair was consistent with Tanner stage 1, and testicular volume was 2 mL, bilaterally.

However, the brothers showed facial features such as hypertelorism, short nose, long philtrum, and Widow's peak; genital dysmorphism: shawl scrotum; and digital features such as camptodactyly (**Figs. 1C and D**) and interdigital webbing (**Fig. 1C**). The development of the older and younger brothers was normal, with intelligence quotients of 90 and 86, respectively, according to the Wechsler Intelligence Scale for Children–Forth Edition (WISC−IV). However, working memory and processing speed indices, as per WISC−IV, of the older and younger brothers were lower than normal at 76 and 76 and 79 and 76, respectively. The clinical characteristics of the brothers included features recognized in previously reported cases of AAS, suggesting congenital syndromes, including AAS (1).

The heights of their father (II2), mother (II3), maternal uncle (II4), and maternal grandmother (I2) were 179 cm (+1.4 SD), 147 cm (−2.1 SD), 155 cm (−2.7 SD), and 145 cm (−2.4 SD), respectively (**Fig. 1E**). Both short stature and camptodactyly were observed in the mother, maternal uncle, and maternal grandmother.

Genetic Analysis

The patients were recruited through the "Initiative on Rare and Undiagnosed Diseases" project (4). The study protocol was approved by the Ethics Committee of the Akita University Graduate School of Medicine, Akita, Japan. After obtaining informed consent from the parents of the patients, the genomic DNA of the patients and their parents was extracted from peripheral blood leukocytes by using the standard phenol extraction protocol. All exons were captured using the SureSelect XT Human All Exon V6 kit (Agilent Technologies, USA), and exome analyses were performed using the NovaSeq 6000 platform (Illumina,USA). Bioinformatic analysis was performed as described previously (4). Briefly, the sequence reads were mapped to the human reference genome (GRCh37) according to the Burrows-Wheeler Aligner and Genome Analysis Tool Kit best-practice guidelines, as packaged in the integrated analysis suite variant tools. The variants were annotated using SnpEff (4).

A hemizygous missense variant, NM_004463.2 c.2344T>C, p.(Cys782Arg), in *FDG1* derived from the mother was identified in the brothers with whole-exome sequencing (**Fig. 2A**). This extremely rare variant was confirmed using Sanger sequencing; c.2344T>C has not been identified in any of the 8.3 K-person cohort of normal Japanese individuals or the gnomAD database. This variant was absent from pathogenic variant databases such as ClinVar and HGMD. The pathogenicity of the variant was predicted as follows: Polyphen2 HumDiv/Var predicted the variant as probably damaging; MutationTaster (v2021) predicted the variant as deleterious; and the combined annotation-dependent depletion score was 25.4, which is highly suggestive of a deleterious effect. Overall, the c.2344T>C allele was scored as "likely pathogenic" (PM1, PM2, and PP3) according to the American College of Medical Genetics and Genomics standards and guidelines for the interpretation of sequence variants (5). Trioexome analysis revealed no pathogenic *de novo*, compound heterozygous, or homozygous variants consistent with the patients' symptoms.

Discussion

To date, more than 52 different pathogenic variants of *FGD1* have been reported in AAS across all genes (https://www.hgmd.cf.ac.uk/ac/index.php) (1). In the *FGD1*-pathogenic variants, 19 missense variants are responsible for AAS. All of them are exclusively located in specific regions of the functional domains in FDG1: p.S205I and p.P312L on the PRD domain (amino acids 7–320); p.E380A and p.R391G on the DH domain (amino

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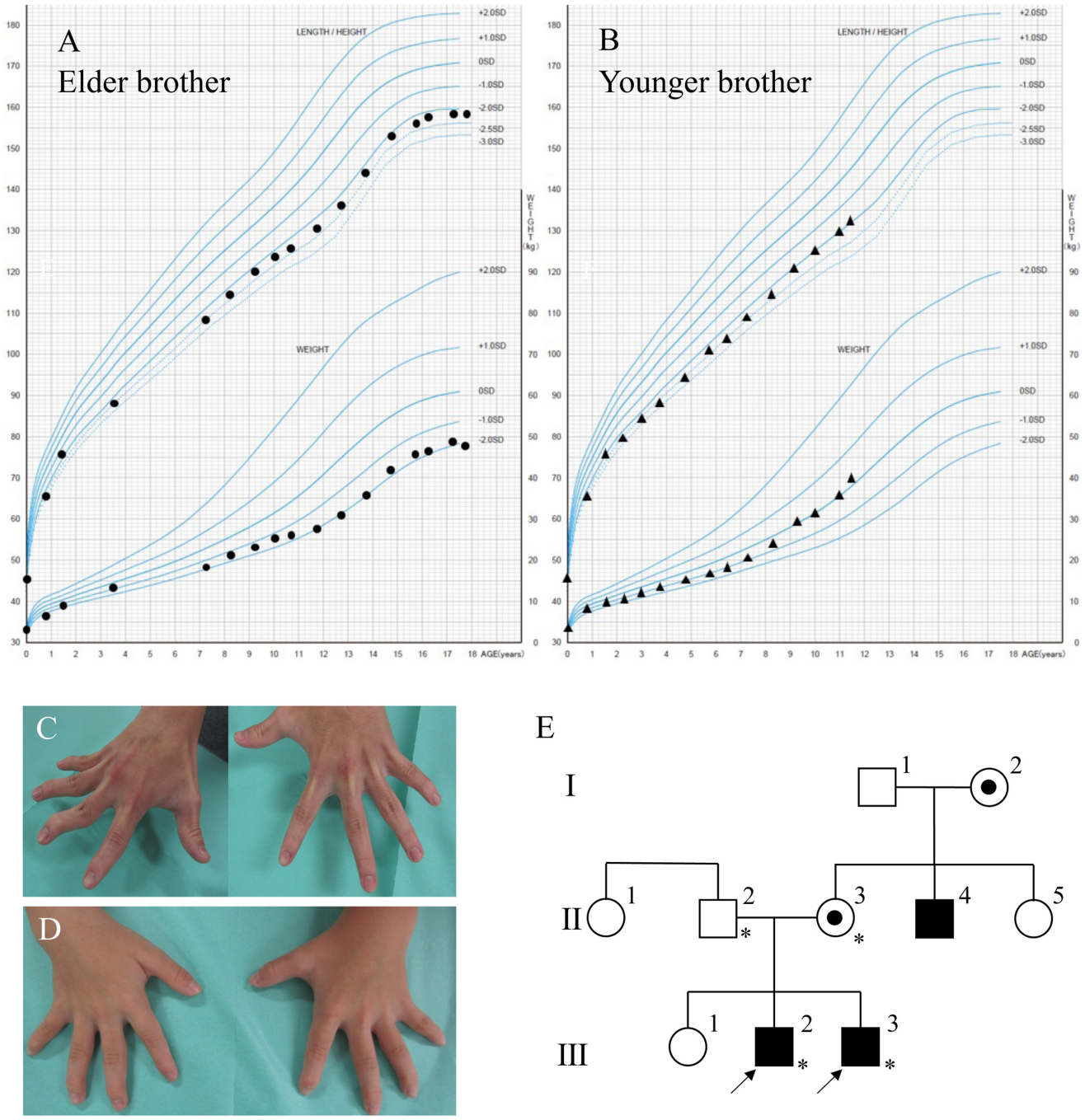

Fig. 1. A: Growth curve of the older brother. B: Growth curve of the younger brother. C: Camptodactyly and interdigital webbing in the older brother. D: Camptodactyly in the younger brother. E: Pedigree of the Aarskog–Scott syndrome (AAS) family. Black and white symbols show affected and unaffected individuals, respectively; central dot symbols indicate carrier females. Molecular analyses were performed for the individuals highlighted with asterisks.

acids 376–559); p.R402Q, p.R402W, p.R408Q, p.N424D, p.R443H, p.R443L, p.M466V, p.P516A, p.R522H, p.S558W, and p.R610Q on the PH1 domain (amino acids 592–698); p.P742S, p.K748E, p.C753G, and p.G757R on the FYVE domain (amino acids 732–788) (**Fig. 2B**). p.C782R, which was identified in both patients in this study, was located in the FYVE domain.

The FYVE domain is a 57-residue sequence of a short motif composed of two double-stranded antiparallel sheets and a C-terminal α-helix in the presence of the RRHHCR motif, which provides specificity for PI3P as a PI3P code reader (2, 3) (**Fig. 2C**). The FYVE domain has a zinc-binding motif reminiscent of RING fingers, consisting of two pairs of four cysteine residues, cys-736/cys-739/cys-761/cys-764 and cys-753/cys-756/cys-782/cys-785, and the two zinc ions held tightly by four cysteines in a tetrahedral arrangement to provide essential structural stabilization (2, 3). p.C782R, which was identified in the patients, disrupts one of the preserved residues of the FYVE domain or preserved

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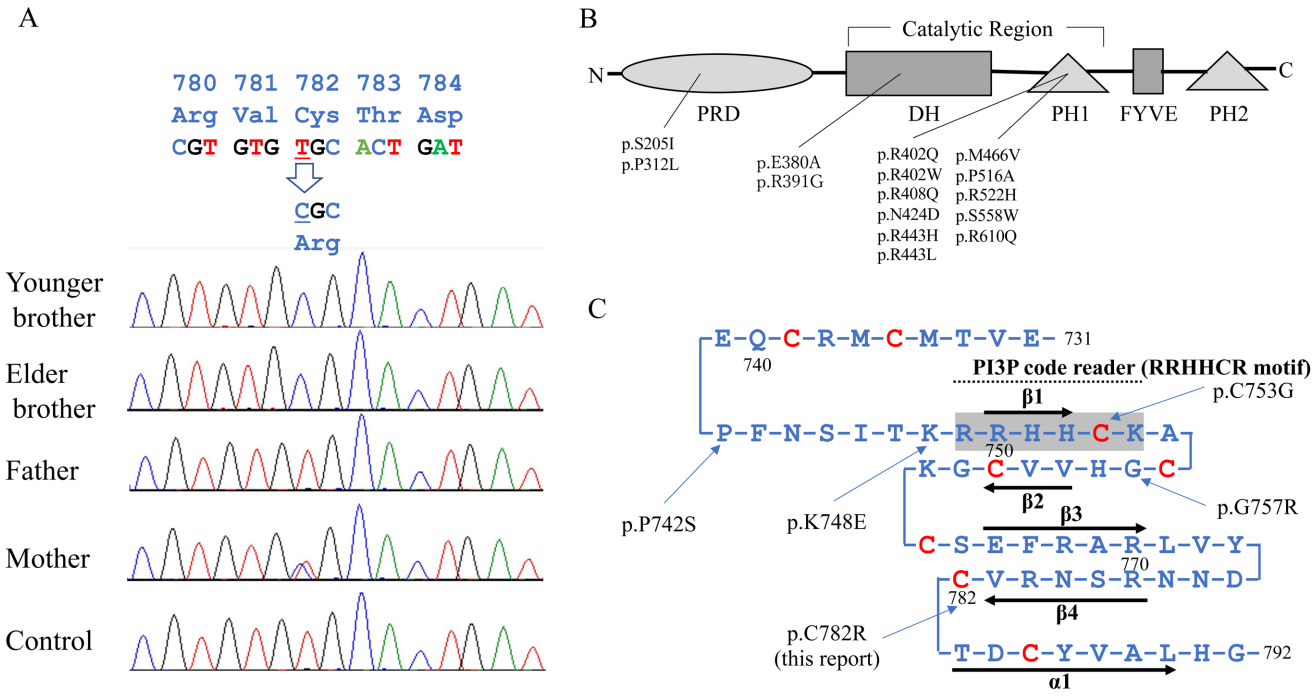

Fig. 2. A: A novel missense variant, p. C782R, was identified in the brothers as hemizygous and the mother as heterozygous; however, no variant was identified in the father. B: Fifteen reported missense variants in each functional domain in FGD1. C: Four reported missense variants and a novel p.C782R variant are located at the predicted amino acid residues of the FYVE domain of FDG1.

cysteine residues consisting of a zinc-binding motif, suggesting the molecular etiology of this novel variant. Regarding the phenotype related to p.C782R, the female carriers showed certain features of AAS, short stature, and camptodactyly, which were consistent with those of reported cases of other pathological variants (1).

In conclusion, we identified a novel missense variant of *FGD1* (c.2344T>C), p.C782R, associated with AAS. The results of this study emphasize the importance of the FYVE domain as a mutational region of FGD1 in patients with AAS.

Conflict of interests: The authors declare no competing interests.

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