European Journal of Human Genetics (2000) 8, 869–874

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ARTICLE

Two novel mutations confirm FGD1 is responsible for the Aarskog syndrome

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The Aarskog syndrome or facio-genital dysplasia (FGDY, MIM No. 305400) is an X-linked condition characterized by short stature, macrocephaly, facial, genital and skeletal anomalies. It is caused by mutation of the *FGD1* gene mapped to the Xp11.21 region. To date, only one point mutation has been reported in an affected family, consisting of the insertion of an additional guanine residue at nucleotide 2122 of exon 7, which causes premature translational termination. We now report the finding of two novel *FGD1* mutations, a missense mutation in a family of Italian origin and a deletion of 3 exons in a sporadic case from Germany. These mutations confirm the role of *FGD1* as the gene responsible for the Aarskog syndrome. *European Journal of Human Genetics* (2000) **8**, 869–874.

Keywords: Aarskog syndrome; FGD1; mutation; FGDY

Introduction

Aarskog syndrome (FGDY) is a multiple congenital anomalies/dysplasias syndrome.[1,2] Minor anomalies and/or malformations usually affect the midline and the urogenital system (hypertelorism, umbilical hernia, shawl scrotum, hypospadias, undescended testes), while dysplastic changes involve the skeleton, resulting in vertebral and phalangeal defects and shortness of stature. The syndrome was first described at the Third Conference (1970) on the Clinical Delineation of Birth Defects by Aarskog[3] and Scott[4] in two different families with multiple affected males. Aarskog suggested the condition was X-linked, noting that female carriers might exhibit mild short stature. The *FGDY* gene had been mapped to the pericentromeric region of the X chromosome by the observation of an X;8 translocation in an affected mother and son.[5] Porteus *et al*[6] and Stevenson *et al*[7] linked the Aarskog gene to Xp11.3–Xq13. Glover *et al*[8] precisely assigned the breakpoint to Xp11.21 on re-evaluation of the chromosomes in 1993. This localization allowed the cloning of *FGD1*, the gene responsible for the syndrome.[9] *FGD1*, which encodes a 961 amino acid zinc finger protein with strong homology to the Rho/Rac guanine nucleotide exchange factors (GEF), was shown to be interrupted by the t(X;8) breakpoint. Until now, only one point mutation had been reported in an affected family, consisting of an insertion of an additional guanine residue at nucleotide 2122 of exon 7, which causes premature translational termination.[9]

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Received 25 April 2000; revised 15 June 2000; accepted 12 July 2000

Methods

Patients

In family K8345, two maternally related male cousins, FC and IT (see III-1, III-3, respectively, Figure 3A), were referred because of short stature and peculiar face at the age of 7 and 1 8/12 years, respectively. The mothers, who are sisters, are also short, whilst a brother of theirs appears to be phenotypically normal. FC is the only child, and the mother's second pregnancy ended in early spontaneous abortion. IT has an older brother who is normal. The second pregnancy of the mother ended in the late spontaneous abortion of a pair of twins, one male and one female. The third pregnancy ended at 8 months with the stillbirth of a male fetus. The propositi,

in addition to being short, had relative macrocephaly and a strikingly similar face with ocular hypertelorism, downslanting palpebral fissures, ptosis of the upper eyelids, flat philtrum, bow shape of the upper lip and pointed chin (Figure 1). They also had brachydactyly, typical hyperextensibility of the proximal and incomplete extensibility of the distal interphalangeal joints, and transverse palmar crease. Also noted were umbilical (FC) and inguinal (IT) hernia, dorsal groove of the penis (FC) and shawl scrotum (IT). Psychomotor development was normal in both.

Patient MK (CMS0843) was referred for genetic evaluation at age 3 years because of short stature. His height was 91 cm (–2.6 SD) and his head circumference was 47 cm (–2.8 SD). He had a widow's peak, hypertelorism, downslanting palpebral fissures, ptosis of the upper eyelids and a short nose with anteverted nares (Figure 2A). Also present were brachydactyly and cutaneous syndactyly of both hands (Figure 2B), and a shawl scrotum. At age 5 years 11 months bone age was retarded and dissociated, corresponding to 2 to 4 years. By age 9 he had developed myopia and at age 11 he was operated on because of an inguinal hernia and cryptorchidism. The patient has had normal psychomotor development. There was no family history of Aarskog syndrome.

SSCP analysis

Exons 2, 3, 5–17 and the promoter region were amplified as individual units except for exons 7 and 8 which were amplified as one amplicon of 428 bp. Primers (Table 1) were designed based on the FGDY intron/exon sequences deposited in GenBank (Accession Nos. U11690, L48693–L48709). All primers were synthesized and labeled with fluorescein amidite (FluorePrime, Amersham Pharmacia Biotech, Piscataway, NJ, USA) on a Beckman Oligo 1000 DNA synthesizer and desalted through sephadex G-25 (NAP-10 columns, Amersham Pharmacia Biotech, Piscataway, NJ, USA). After amplification, 1 μl of a 1:5 dilution of the PCR product was added to a 0.3 μl each of two sizers flanking the amplicon and 2 μl of loading dye (100% dionized formamide, 5 mg/ml Dextran Blue 2000). SSCP analysis was conducted using an Automated Laser Fluorescent Sequencer (Amersham Pharmacia Biotech, Piscataway, NJ, USA) under the following conditions: 1500 V, 43 mA, 25 W at 20°C with a sampling interval of 1.25 s. The gel matrix was 0.5 X MDE (FMC).

Restriction endonuclease fingerprinting (REF)

As the PCR products for exons 1, 4, and 18 are 10396 bp, 588 bp, and 956 bp, respectively, these were analyzed using REF.[10,11] After amplification, 8 μl of the PCR product was digested with 4 U of each enzyme in separate reactions. For exon 1, *Vma*I, *Hinf*I and *Bam*HI + *Sac*II were used to generate fragments. For exon 4, *Aci*I, *Msp*I, *Alu*I, *Ban*I + *Hinf*I and *Ban*I + *Hinc*II were used. For exon 18, *Dra*I + *Nla*III, *Dra*I + *Ban*II and *Alu*I + *Ban*I were used. All digestions contained 0.4 U of calf intestinal alkaline phosphatase, (Roche Molecular Biochemicals, Indianapolis, IN, USA). After overnight digestion, these tubes were heated to 96°C for 10 min and 5 μl of each digestion were mixed together and heated a second time at 96°C for 10 min to insure inactivation of the

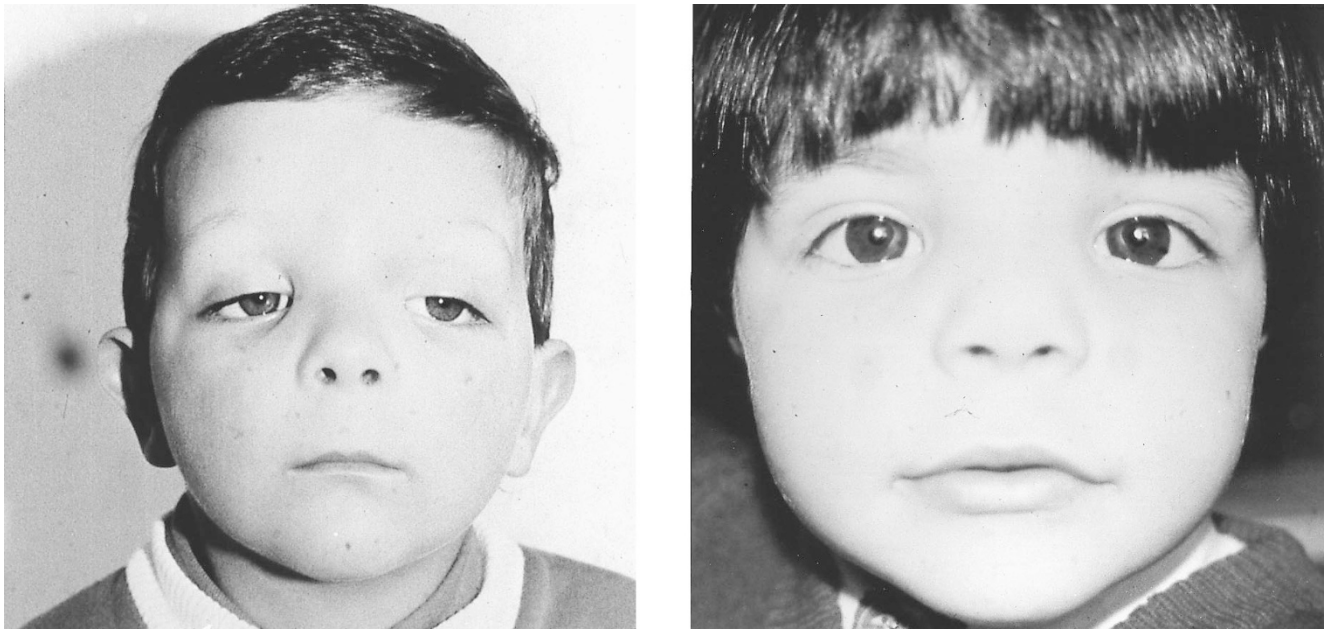


Figure 1 Facial appearance of the two probands in K8345 with Aarskog syndrome, left, person III-1, right, person III-3.

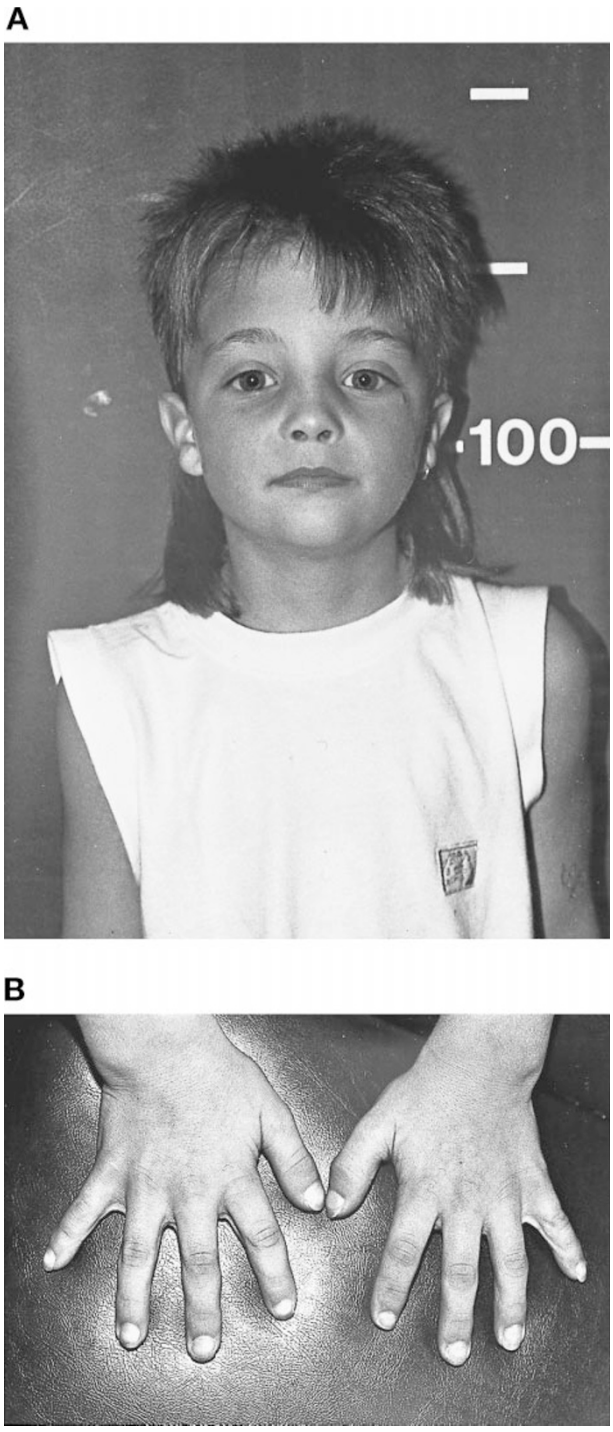


Figure 2 **A** Facial appearance and **B** hands of patient MK.

phosphatase. Approximately 11 ng (1.1 μl) of the digested fragments were 5' end-labeled and electrophoresis was performed as described previously.[11]

Sequencing

The exon 7-8 PCR product was sequenced on the ALF using fluorescein labeled primers and a cycle sequencing kit (Promega, Madison, WI, USA). The gel conditions were as follows: 6% polyacrylamide at 45°C, 1.25 s sampling time. The gel was run for 10 h.

Results

We screened the 18 exons and the promoter region of the *FGD1* gene which were analyzed either by SSCP (promoter, exons 2, 3, 5–17) or restriction endonuclease fingerprinting (exons 1, 4, 18). An altered SSCP pattern was observed for the PCR product containing both exons 7 and 8 in patient III-1 of family K8345 (data not shown). Sequence analysis showed a G to A alteration (G2296A) causing an arginine to be replaced with an histidine (R522H) (Figure 3B). The G to A change eliminates an *Aci*I site in the normal sequence. The lack of the *Aci*I site segregated with the Aarskog syndrome in the family (Figure 3A) and its absence was not detected in 100 normal X chromosomes in the Italian population. Thus, it is highly unlikely that the alteration is a normal polymorphism.

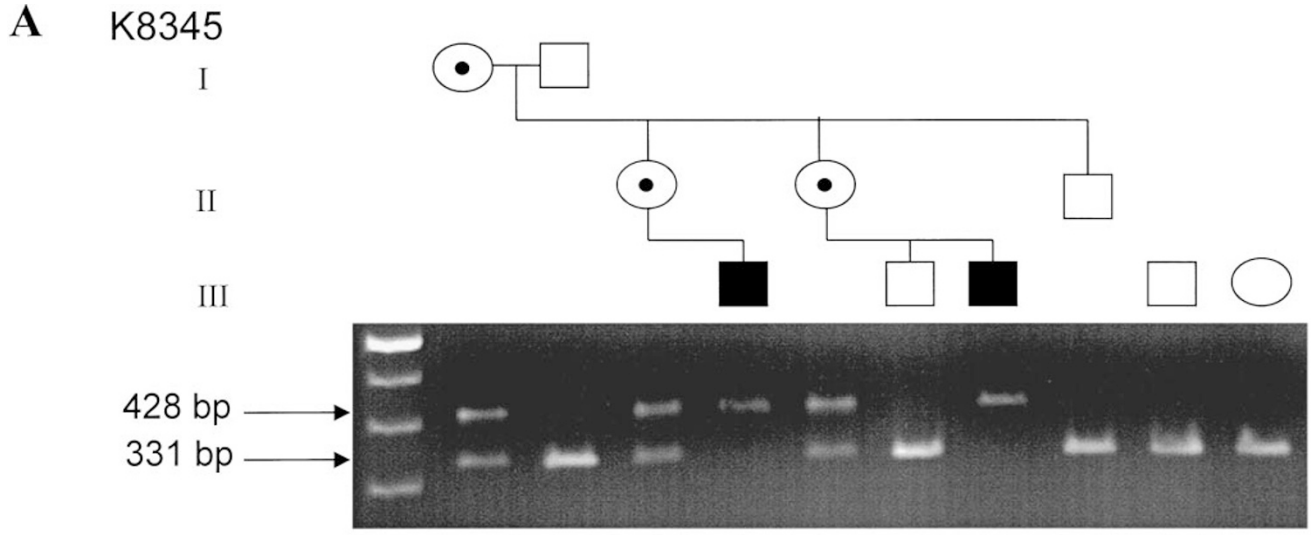
Exon analysis of patient CMS0843 demonstrated a lack of amplification of exons 9–12 (Figure 4). The exact extent of the deletion has yet to be determined.

Discussion

We now report the finding of two mutations of *FGD1* that cause Aarskog syndrome. *FGD1* was first described by Pasteris *et al*.[9] The authors proposed the gene was involved in the Aarskog syndrome based on two observations. First, it was directly disrupted by a t(X;8) breakpoint present in a mother and her affected son.[5,8,9] Second, analysis of a small family with two affected male siblings revealed an insertion of an additional guanine residue at position 2122 in exon 7.[9] This frameshift was likely to result in a premature stop codon to appear at residue 469. However, since the original report of Pasteris *et al*,[9] no other mutations in *FGD1* have been reported. We have identified two additional mutations in *FGD1*. An Italian family with FGDY was found to have an R522H mutation in the third SCR (structural conserved region) of GEF domain of *FGD1*. The arginine at codon 522 is highly conserved, being present in three other Rho/Rac GEF proteins. Two other members of the Rho/Rac GEF family have a lysine in place of the arginine.[9] The bulkier histidine with its ring side chain probably alters the conformation of the GEF domain, thereby adversely affecting the protein's ability to efficiently activate Rho/Rac proteins by exchanging bound GDP for free GTP.[12] We consider this sufficient evidence to support the conclusion that the observed mutation is responsible for the Aarskog syndrome in the present family. This conclusion is further supported by the absence of this mutation in 100 Italian control X chromosomes.

Table 1 Primers and annealing temperatures for amplification of exons from the human *FGD1* gene

Exon	*Primer F*	*Primer R*	*Annealing temp (deg C)*	*Product size (Bp)*
Promoter	5' agccaagccctaggaacactgag 3'	5' gcagccgcagccacagc 3'	55	309
1	5' agagcggtagcagcgagaagtgag 3'	5' ccccaaaggccgaggtaagc 3'	55	1250
2	5' acacccatttttctcccttcc 3'	5' gtggctccctatccttctaaca 3'	55	325
3	5' agtgcccccaaagcccagctac 3'	5' ccaggctcccccttttccctagag 3'	55	228
4	5' ggaagggatagtcaggag 3'	5' gaagaatcaagcacaaaag 3'	55	588
5	5' gggcttgggtgagggttacgat 3'	5' ggccctatcactgcctccttgaaa 3'	55	223
6	5' ctgccctgcccgacaggtgtt 3'	5' tcggcaggcaggtggacagg 3'	55	197
7&8	5' ccacccagggaccgctat 3'	5' acactcatttgggcatccttgct 3'	50	418
9	5' tctctgctagtcccccatctga 3'	5' cctcctcgccccctaaca 3'	60	295
10	5' cgtgccttttgttccctgtctttt 3'	5' gggcatgacccacccacaat 3'	57	258
11	5' acatccccactaggccctctgc 3'	5' ttcctcccaacaccaatgc 3'	58	205
12	5' cctcaccatgccccttttctgc 3'	5' tctgggcctggaatgcctcag 3'	60	190
13	5' gggcctacctaaccaaacatctt 3'	5' caccctcaccttatacaccctcag 3'	55	321
14	5' acgaaggtgaggcaggggtaga 3'	5' ggtcaggtgggcatttggaagt 3'	58	238
15	5' cccctaccccccagcccaatc 3'	5' tcttcccttcagcataccaactcc 3'	60	194
16	5' aagtctgctgtgggagttgg 3'	5' tgtgggagagttcgtcagg 3'	55	275
17	5' ggtggcactggacaaatca 3'	5' ccaaggccaaggagaggt 3'	55	404
18	5' aggtggccccagctctgtcc 3'	5' ccccctgtttccctgtcctg 3'	60	903



B

Patient	Predicted protein sequence
8548	TGCAGCGCATCCCCCACTATGAGCTTCTTC L Q R I P **H** Y E L L
Control	TGCAGCGCATCCCCCGCTATGAGCTTCTTC L Q R I P **R** Y E L L

Figure 3 **A** Pedigree of family K8345. PCR product of exon 8 was digested with Aci I. The presence of a band of 331 bp represents normal X chromosome. The 428 bp band represents an X chromosome with the G2296A mutation **B** DNA sequence and predicted amino acid sequence for the normal and abnormal FGD1 exon 8. The base alteration is indicated by underlining and the resulting amino acid change (R522H) is indicated in bold.

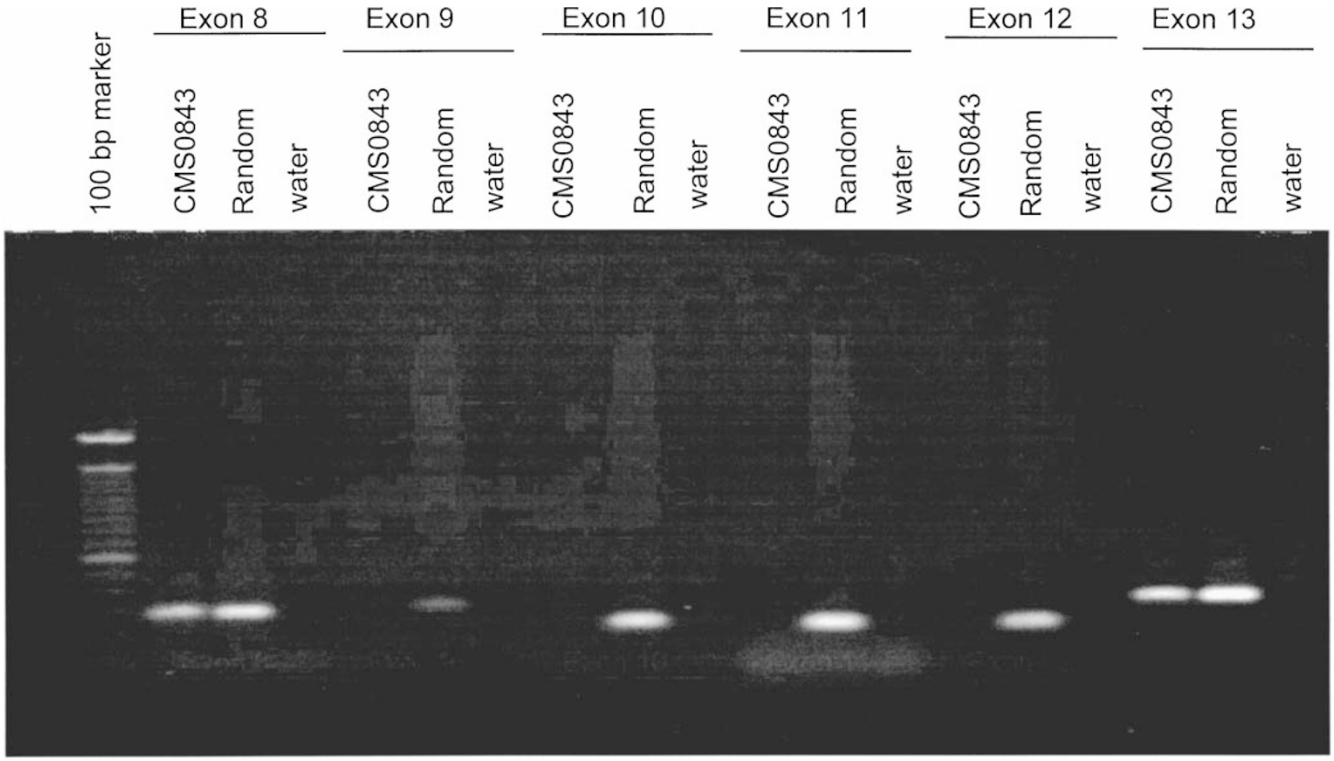


Figure 4 Exon 8–13 analysis of patient CMS0843. Primres for exons 8–13 were designed as described in Methods. Random lane is a normal male control. H_{20} = blank control containing all reaction reagents without genomic DNA. PCR products were run on a 2% agarose gel and visualized using Ethidium bromide.

The second mutation was found in a sporadic patient with clinical features consistent with a diagnosis of FGDY. He was found to have a genomic deletion involving exons 9 to 12 in the *FGD1* gene. Whilst the effect of the deletion at the transcript level has yet to be determined, at a minimum, the altered gene would lack the last 13 amino acids of the GEF domain (aa546–559), a portion of a PH1 domain (aa590–635) and a leucine zipper domain (aa636–659) if the deletion was in-frame. The absence of these critical segments would certainly adversely affect the function of *FGD1*.

Since the phenotype associated with the syndrome is quite consistent across many families and ethnic backgrounds, we had expected to find a common mutation. However, this has not been the case. The three mutations thus far reported, including the present ones, are unique to an individual family. Furthermore, we have been unable to detect similar alterations in *FGD1* in propositi from 25 families and 15 sporadic case. These include families utilized to establish linkage to Xp11.4[7] as well as the families originally reported by Aarskog[3] and Scott.[4]

This raises the possibility that some mechanism other than a point mutation or small deletion is responsible for the FGDY phenotype. Such an occurrence has not been uncommon in recent years. Friedreich ataxia is due to a GAA repeat expansion in intron 1 in the *X25* gene;[13] over 60% of Pelizaeus-Merzbacher disease (PMD) results from duplications of the PLP gene;[14,15] and 42% of severe hemophilia A results from an inversion involving intron 22 of Factor XIII.[16,17] All these mutations were not detected using standard mutational analyses of coding sequence genomic DNA. We are currently investigating whether any of these mechanisms are involved in producing the FGDY phenotype.

Acknowledgements

This research was supported in part by a grant from the South Carolina Department of Disabilities and Special Needs (SCDDSN) and a NICHD grant (HD26202) to CES.

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