RESEARCH LETTER

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Recurrence of Frontometaphyseal Dysplasia in Two Sisters With a Mutation in *FLNA* and an Atypical Paternal Phenotype: Insights Into Genotype–Phenotype Correlation

Debora Bertola,[1]* Maria Rita Passos-Bueno,[2] Alexandre Pereira,[3] Chong Kim,[4] Tim Morgan,[5] and Stephen P. Robertson[6]

[1]Faculdade de Medicina da Universidade de São Paulo, Unidade de Genética do Instituto da Criança, São Paulo, Brazil

[2]University of São Paulo, Department of Biology, São Paulo, Brazil

[3]Instituto do Coração, Faculdade de Medicina da Universidade de São Paulo, Cardiology, São Paulo, Brazil

[4]Instituto da Criança, Pediatrics, São Paulo, Brazil

[5]University of Otago, Dunedin School of Medicine, Department of Women's and Children's Health, New Zealand

[6]University of Otago, Dunedin School of Medicine, Department of Women's and Children's Health, New Zealand

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TO THE EDITOR:

Mutations in *FLNA*, encoding the cytoskeletal protein filamin A, are responsible for a myriad of X-linked clinical conditions, including frontometaphyseal dysplasia (FMD; OMIM 305620), one of the otopalatodigital syndrome spectrum disorders. FMD is characterized by pronounced supraorbital hyperostosis, hypodontia, hearing loss, joint contractures especially at the interphalangeal and metacarpophalangeal joints, undermodelled long bones and campomelia. Males are usually more severely affected than females although the latter usually present some clinical and radiographic manifestations to indicate carrier status for the disorder [Robertson et al., 2003; Robertson, 2007]. Mutations in exons leading to substitutions in the N-terminal actin binding domain or in filamin repeats 1–16 cause FMD in both males and females. Substitutions or small in-frame deletions in repeats 17–24 lead to FMD in only females; these variants have not been observed in males, suggesting gain-of-function mutations in the C-terminal of the protein may not be tolerated in the hemizygote [Robertson, 2007]. Herein we describe two sisters with FMD who have a novel missense mutation in repeat 20 of the paternally-derived allele. The father, who clinically manifests pronounced cutaneous syndactyly of one hand and foot as his only clinical anomaly, is likely a gonosomal mosaic for the mutation.

Two sisters, aged 25 and 21 years, presented with gradually progressive supraorbital prominence first noted at seven years. They are the second and the fourth children of a sibship of four; their parents are nonconsanguineous. A cranioplasty for frontal bone recontouring was performed at 23 and 19 years, respectively, with satisfactory cosmetic results. Both affected women had hypodontia and the younger one had hearing loss. Neurodevelopment was normal. Assessment at the time of surgery identified the following findings: P1, the older sister, had a weight of 42.5 kg (<5th centile), height of 1.45 m (<5th centile) and OFC of 53.5 cm (25th centile); facial anomalies including prominent supraorbital region, apparent ocular hypertelorism, absent inferior central incisors, and micrognathia; and digital anomalies including widening of the proximal interphalangeal joints, deviated 3rd digit, bilateral 5th finger clinodactyly, flat feet, broad-valgus halluces, and elongated toes (Fig. 1A). P2, the younger sister, weighed 46 kg (5th–10th

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*Correspondence to:
Debora Bertola, Faculdade de Medicina da Universidade de São Paulo, Unidade de Genética do Instituto da Criança, Av. Dr. Enéas Carvalho de Aguiar, 647 São Paulo, São Paulo 05403.000, Brazil.
E-mail: debora.bertola@usp.br
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FIG. 1. Clinical and radiographic presentation. (A) P1, (B) P2, and (C) father. The photographs of P1 and P2 were taken after the cranioplasty. Note the deviated fingers and toes in (A) and complete cutaneous syndactyly between the 3rd and 4th digits of the left hand and the 2nd and 3rd toes in the left foot in C. Skeletal radiographs show scoliosis (A), undermodelling of the long bones and phalanges (B), bowing of the tibia (B). No skeletal abnormalities were observed in the father (C).

centile), had a height of 1.65 m (50th–75th centile), and an OFC of 54 cm (25th centile). She had a more prominent supraorbital region than her sister, as well as apparent ocular hypertelorism, downslanting palpebral fissures, absence of the permanent inferior incisors with retention of the deciduous teeth, retrognathia and a pointed chin. Her digital anomalies included widening of the interphalangeal joints, camptodactyly of the fourth digits, clinodactyly of the fifth fingers, spatulate thumbs; and bilateral hallux valgus, and elongated third toes (Fig. 1B). The physical examination of the mother was unremarkable. The father had complete cutaneous syndactyly between the second and third fingers of the left hand and the second and third toes of the left foot (Fig. 1C). Skeletal survey of the sisters revealed skull base sclerosis, absent frontal sinuses, frontal bone hyperostosis, undertubulated and bowed long bones, undertubulated phalanges, carpal bone fusion (scaphoid and trapezium), hypoplastic distal phalanges on the feet and scoliosis. The scoliosis was more severe in the older sister (Fig. 1A, 1B). These skeletal findings were not present in the father (Fig. 1C).

Since the clinical and radiological findings in these two sisters were compatible with the diagnosis of FMD, *FLNA* analysis was performed by DHPLC followed by Sanger sequencing [Robertson et al., 2006a]. Both sisters were heterozygous for a novel mutation, c.6611C>T in exon 41, that predicts the substitution p.Pro2204Leu within filamin repeat 20. This variant was considered pathogenic because it was predicted to be deleterious by in silico analysis (Mutation Taster, SIFT, Polyphen 2) and was not present in 200 controls from the Brazilian population, the EVS6500 (evs.gs.washington.edu) and 1000 Genomes Sequence dataset (www.1000genomes.org). The mutation c.6611C>T was not detected in DNA extracted from blood leukocytes from either parent (Fig. 2A). Sequencing of exon 41 in DNA extracted from

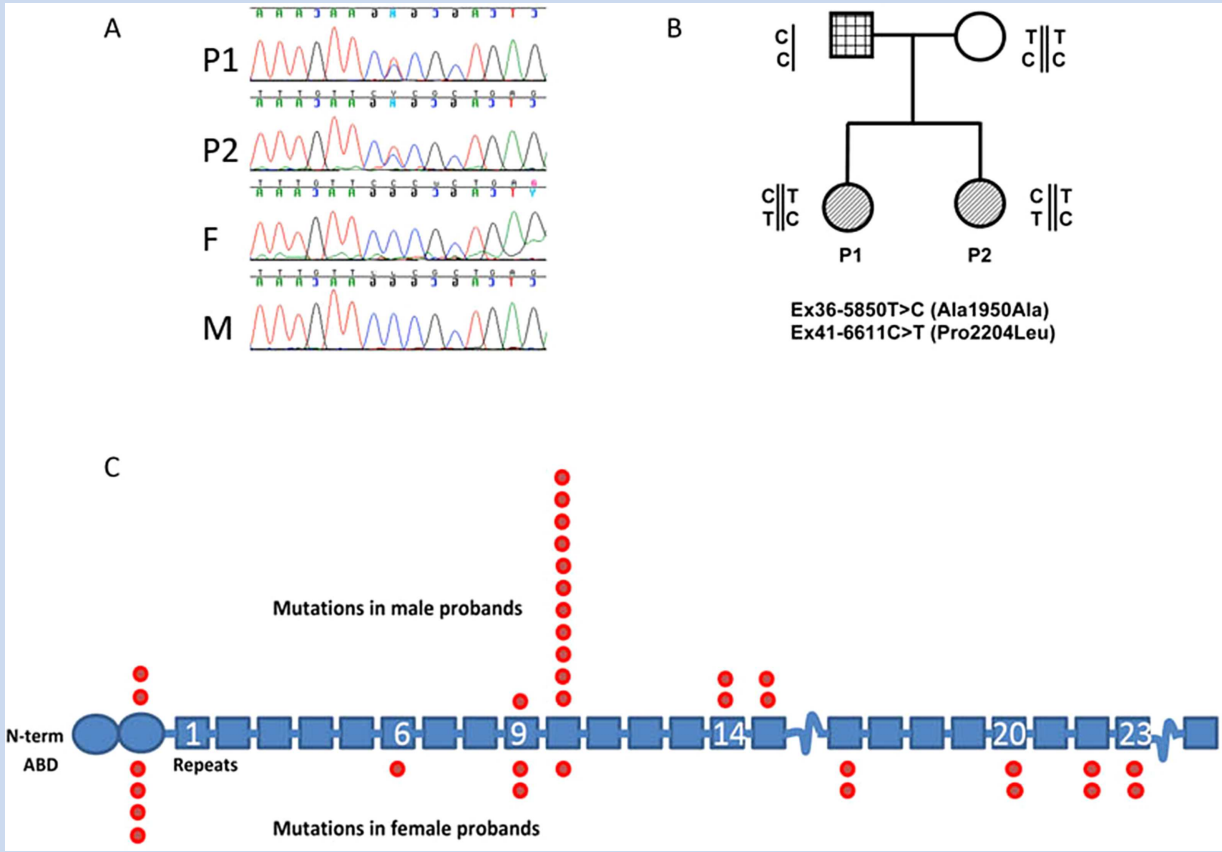

FIG. 2. (A) Chromatograms demonstrating heterozygosity for the c.5850T>C mutation in P1 and P2 and its absence in the mother (M) and father (F). (B) Pedigree of the family with haplotypes constructed using the mutation and rs2070825 genotypes. Vertical hatching indicates syndactyly. Oblique hatching indicates the FMD phenotype. (C) Distribution of substitutions and small deletions/insertions (circles) in the FLNA protein leading to FMD in males (above) and females (below). ABD (actin binding domain). Some filamin repeats are numbered.

the father's skin, hair and eyebrows showed no evidence for the c.6611C>T mutation. Familial relationships were confirmed with high probability ($P < 0.001$) by the examination of segregation of four unlinked fully informative polymorphic microsatellite markers.

Since the mutation was observed in two siblings but was absent in leukocyte DNA in both parents, gonadal mosaicism was suspected. Both sisters were shown to be heterozygous for an SNP located in exon 36 (c.5850T>C; p.Ala1950Ala; rs2070825). The father was hemizygous for the C allele and the mother homozygous for the T allele at the SNP site. To resolve whether the mutation had arisen on the maternal or paternal allele, a PCR-cloning strategy was devised to determine phase. A genomic fragment (1.65 kb) extending from exon 36 to exon 41 was amplified from DNA obtained from one of the affected sisters and cloned into the pGEM-T vector (Promega, WI). Clones ($n = 30$) were individually sequenced to determine the allele present at the mutation site and at rs2070825. The results indicated that the C allele was in cis with the mutation in 14/16 clones and the T allele at the SNP was in cis with the wild-type C allele at the mutation site in 12 out of 14 clones. The discordant clones likely represent in vitro template switching during PCR amplification. These data indicate that the mutation has arisen on the paternally-derived allele ($P < 0.001$ two-tailed Fisher's exact *t*-test; Fig. 2B). The mutation destroys the single recognition site for the restriction enzyme *Msp*A1I present in the exon 41 PCR amplicon. Genomic DNA (500 ng) extracted from peripheral blood obtained from the father was subject to digestion with *Msp*A1I overnight and PCR performed using primers that flank exon 41. No product was obtainable indicating that, if the mutant allele is present in this cell population, it is present at a very low level.

The two sisters reported here show a typical phenotype that is at the severe end of the spectrum for females with X-linked FMD [Robertson, 2007]. While the breadth of phenotypic severity in females with FMD and mutations in *FLNA* is relatively narrow, the range of severity in males is much broader, stretching from multiple congenital anomalies incompatible with life to survivable phenotypes with normal neurodevelopment and stature. Identifying genotype-phenotype correlations would therefore be of much clinical utility especially for females with FMD presenting as isolated cases in their families. The female presentation can be much milder than the male counterpart, moderated by, amongst other factors, the degree of X-inactivation skewing [Robertson

et al., 2001]. To date our laboratory and others [Zenker et al., 2004; Giuliano et al., 2005; Zenker et al., 2006] have characterized 34 *FLNA* mutations causing FMD (Fig 2C). Of the 28 probands with mutations affecting the actin binding domain or filamin repeats 1–16, 18 (66%) were male, whereas of the six mutations affecting domains 17–24, all six were female with no proband having an affected male relative with FMD (Fig 2C). The family described here with a predicted substitution in filamin repeat 20 further supports the idea that mutations in this region lead to a more pronounced phenotype in females and that this region of the gene has a relative paucity of mutations leading to FMD in males. The father of these two affected sisters described here has a unilateral syndactylous phenotype as an obligate, presumptively mosaic, carrier for the c.5850T>C mutation. Previously somatic and gonadal mosaicism has been described in females with an OPD spectrum disorder [Robertson et al., 2006b], but this man is the first described male gonadal mosaic for a *FLNA* mutation. His phenotype suggests he is likely to be a gonosomal mosaic for this mutation although the inability to detect the mutation in any of the sampled tissues stops short of definitive proof. Of note however, is that complete cutaneous syndactyly is not a feature of FMD. This sign may reflect a mild manifestation of mosaicism for a mutation that would otherwise be embryonic lethal in the germline.

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