Brief Report
Further Evidence for *FGF16* Truncating Mutations as the Cause of X-Linked Recessive Fusion of Metacarpals 4 / 5

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Background: Metacarpal 4–5 fusion (MF4; MIM#309630) is a rare congenital malformation of the hand characterized by the partial or complete fusion of the fourth and fifth metacarpals. The anomaly occurs as an isolated trait or part of a genetic syndrome. Recently, we have identified *FGF16* nonsense mutations as the underlying cause of isolated X-linked recessive MF4. **Methods:** In this report, we provide a detailed clinical description of a sporadic male patient showing MF4 in whom we performed Sanger sequencing of the entire coding sequence of *FGF16*. **Results:** In addition to MF4 symptoms, the patient presented with generalized joint laxity and hypermobility. *FGF16* sequencing detected a novel truncating mutation (c.474_477del; p.E158DfsX25) in exon 3 of the gene. A heterozygous mutation was found in a clinically and radiologically unaffected mother of the proband. **Conclusion:** Our finding confirms that truncating mutations of *FGF16* are causative for X-linked recessive metacarpal 4–5 fusion. Importantly, the mutation detected in this study was located in last exon of the gene (exon 3), like the only two *FGF16* disease-causing variants identified to date. Thus, all *FGF16* mutations known to give rise to this rare skeletal hand malformation are C-terminal and most probably do not result in a nonsense mediated decay. Additionally, our proband showed mild symptoms of a connective tissue disorder, as some other patients previously reported to have X-linked MF4. Therefore, we suggest that impaired *FGF16* function may also be responsible for connective tissue symptoms in MF4 patients.

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Key words: metacarpal 4–5 fusion; metacarpal synostosis; X-linked inheritance; *FGF16*; truncating mutation; connective tissue; MF4

Introduction

Metacarpal 4–5 fusion (MF4; MIM#309630) is a relatively rare congenital malformation of the hand skeleton characterized by the partial or complete synostosis of the fourth and fifth metacarpals. Clinical manifestation of this anomaly includes ulnar deviation and clinodactyly of the fifth and in some cases additionally fourth fingers, shortened fifth metacarpals, and reduced mobility of the ulnar digits. MF4 can either be an isolated malformation or can occur as part of a genetic disorder, for example, Kallmann syndrome (Jamsheer et al., 2013). In most of the reported families, isolated MF4 was inherited in an X-linked recessive manner (Orel, 1928; Habighorst and Albers, 1965; Holmes et al., 1972; Hooper and Lamb, 1983; Annerén and Amilon, 1994; Lonardo et al., 2004), although pedigrees typical of autosomal dominant inheritance have been described (Temtamy and MsKusick, 1978).

Syndactyly type V (SD5; MIM#186300) is an isolated limb malformation showing partial clinical overlap with MF4, in which metacarpal but additionally metatarsal synostosis coexists with other hand and foot abnormalities. SD5 is extremely rare and have been reported only in a single large Han Chinese family, in which it resulted from a *HOXD13* missense mutation (Zhao et al., 2007). Apart from metacarpal and metatarsal bone fusion, the family members with SD5 presented with various additional hand and foot deformities, such as ulnar deviation of fingers 2 to 5, lobster claw-like or Y-shaped fingers 4 and 5, shortening of metacarpals 4 and 5, deviation of metatarsals and toes, cutaneous syndactyly of toes 2 and 3 or 3 and 4 (Zhao et al., 2007).

Recently, by applying whole exome analysis, we demonstrated *FGF16* nonsense mutations to be associated with X-linked isolated metacarpal bone 4-5 fusion in two unrelated patients of different ethnicity (Jamsheer et al., 2013). In this study, we report on a novel case showing MF4 caused by a novel truncating variant in *FGF16*.

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Materials and Methods

PATIENT

We investigated a sporadic index case of Polish ethnicity, who presented with isolated metacarpal bone 4-5 fusion. Additionally, we studied both healthy parents of the proband. The Institutional Review Board at Poznan University of Medical Sciences approved the study. Venous blood samples were collected from all above mentioned participants of this study. Written informed consent was obtained before genetic testing from all patients or their legal guardians. Furthermore, parents of the affected individual gave written consent for presentation of his photographs for publication.

MOLECULAR TESTING

Proband was subject to mutational screening of the entire coding sequence of *FGF16* (MIM *300827; GenBank NM_003868). Primers for amplification and sequencing of all three exons of human *FGF16* and their flanking intronic regions were described elsewhere (Jamsheer et al., 2013). Additionally, we performed *HOXD13* sequencing in the proband, because this gene is involved in the pathogenesis of a similar limb phenotype, namely SD5. Sanger sequencing was done with dye terminator chemistry (ABI Prism DigDye v3.1) and run on automated sequencer Applied Biosystems Prism 3700 DNA Analyzer. Because exon 1 was not annotated in UCSC and Ensemble databases, DNA sequence numbering was based on annotated chromosome X genomic scaffold (NW_004078123.1). Translated protein sequence was identical with the RefSeq entry NP_003859. Finally, we analyzed *FGF16* sequence in the proband's mother.

Results

CLINICAL REPORT

Index case was the second child of healthy nonconsanguineous couple. He was born at 37 weeks of gestation by Caesarian section after uneventful pregnancy with following birth parameters: weight 4550 g, length 63 cm, Apgar score 10. After birth, bilateral congenital hand malformation comprising ulnar deviation of fingers 4 and 5 was noted. Developmental milestones, including independent sitting (at 6 months of age), independent walking, and onset of expressive speech (both at 12 months of age), were achieved on time. Hand radiographs showed partial fusion of the fourth and fifth metacarpal bones as well as shortening of fifth metacarpals (Fig. 1A). Additionally, easy bruisable skin was observed. When referred to us at the age of 5 years, the proband had normal body measurements: weight 19.5 kg (between 3rd and 10th percentile), height 115 cm (between 10th and 25th percentile), and head circumference 52.5 cm (between 3rd and 10th percentile). Bilateral abnormal positioning with ulnar deviation of fourth and fifth fingers as well as shortening of fifth rays of the hands were conspicuous (Fig. 1B). In feet, bilateral sandal gaps and slight syndactyly of toes 2 and 3 were observed. In addition, the boy presented with slender upper and lower extremities (Fig. 1C), pectus carinatum, as well as generalized joint laxity and hyperextensibility (7 points in Beighton score), with no other congenital anomalies noted. His intellectual development was normal. Echocardiogram performed at the age of 5 years showed no cardiac abnormalities. Family history of the proband was noncontributory, as his two parents and sister were clinically and radiologically normal.

RESULTS OF MOLECULAR SCREENING

To confirm the clinical diagnosis of MF4 at a molecular level, we sequenced *FGF16* in our proband. In addition, we screened *HOXD13* to exclude SD5, a similar phenotype that could potentially account for the observed limb anomaly. The result of *HOXD13* sequence analysis was normal. Upon *FGF16* sequencing, we detected a frameshift mutation at position c.474_477del (p.E158DfsX25) in exon 3 of the gene (NM_003868) (Fig. 2A). The mutation results in substitution of 25 amino acids and introduces a premature stop codon in the last exon of *FGF16*. Next, we performed parental studies and showed that clinically and radiologically unaffected mother was a heterozygous carrier for the frameshift variant.

Discussion

Metacarpal 4-5 fusion is a rare congenital malformation of the hand usually inherited in an X-linked recessive manner. Recently, we identified X-linked recessive nonsense mutations in the last exon of the *FGF16* gene as the cause of isolated MF4 in two unrelated sporadic male cases (Jamsheer et al., 2013). In this report, we present another patient with typical metacarpal synostosis 4-5, in whom we identified a novel frameshift truncating mutation also in the last exon of *FGF16*.

FGF16 is a member of the fibroblast growth factor family (FGFs), which in vertebrates comprises 22 proteins (Itoh and Ornitz, 2004). The gene belongs to the FGF-9 subfamily (consisting of FGF9, FGF16, and FGF20), has an open reading frame of 621 bp and encodes for a protein composed of 207 amino acids (Miyamoto et al., 1993). The FGF16 protein is built of an FGF domain containing a heparin binding site (glycine box) and the receptor interaction sites (Fig. 2B). FGFs function as crucial signaling factors during embryonic development, cell growth, and limb bud organization and outgrowth (Martin, 1998; Mariani et al., 2008). One of the most critical developmental role of *Fgf16* demonstrated in mouse studies was its involvement in cardiac morphogenesis. *Fgf16* showed expression in fetal mouse endocardium and epicardium at day 10.5 and 12.5 in a unique but mostly overlapping pattern with other molecules from its FGF subfamily, namely *Fgf9* and *Fgf20* (Lavine et al., 2005). FGF16 protein secreted from endocardial and epicardial layers was proposed to

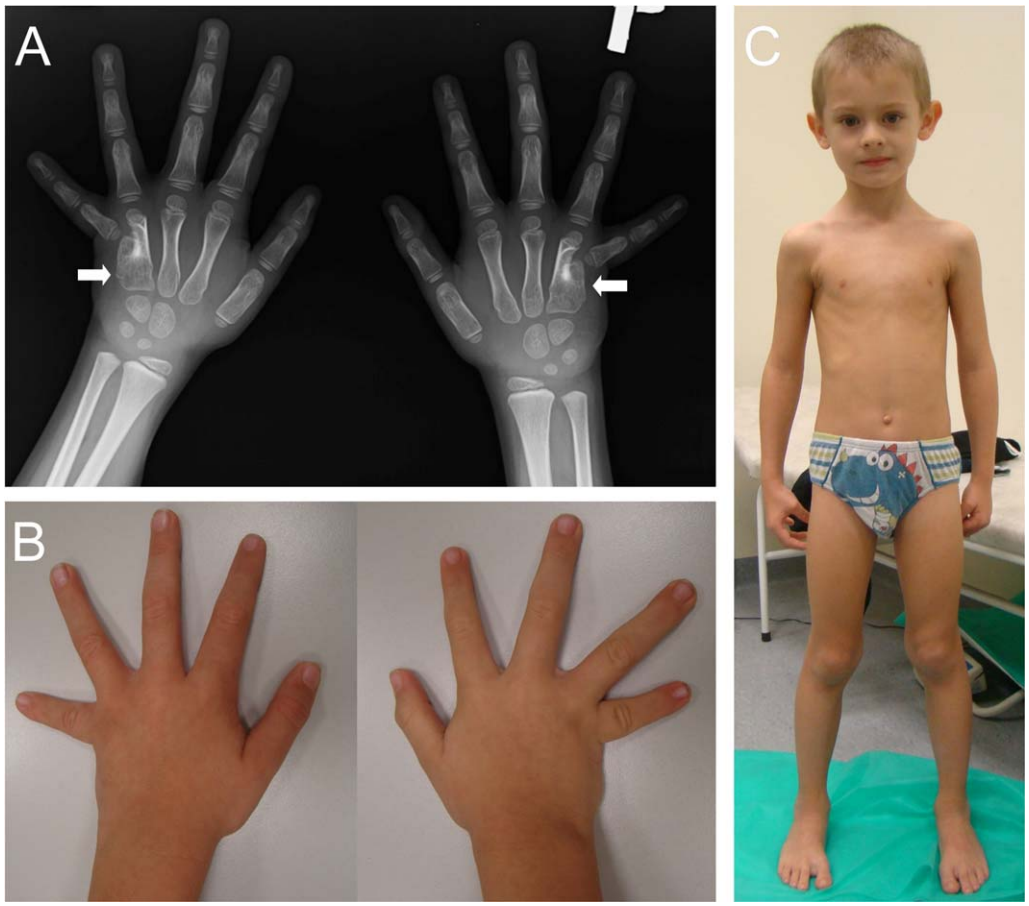

FIGURE 1. *Upper limb malformation observed in our proband.* **(A)** *Radiograph of the hands showing bilateral partial fusion of metacarpals 4 and 5 and bilateral shortening of fifth metacarpals.* **(B)** *Ulnar deviation and shortening of fifth fingers seen upon clinical assessment.* **(C)** *Frontal view of the proband showing his slender built.*

promote the growth of embryonic cardiomyocytes thereby directly contributing to heart formation (Lu et al., 2008). Importantly, *Fgf16*-deficient male mice generated by null mutation of the gene located on the murine chromosome X, presented with severe heart hypoplasia, including myocardial thinning and dilatation of cardiac chambers, and died around embryonic day 11.5 (between day 10.5 and 12.5). No limb abnormalities have been observed by day 12.5 in *Fgf16*-lacking murine male embryos; however, some of them manifested additional congenital malformations at day 11.5, such as abnormal mandible and vascular ruptures resulting in hemorrhage to the thoracic and ventral cavities of the body (Lu et al., 2008). On the contrary, female heterozygous mice were viable, healthy, and showed no overt structural defects.

The mutation identified in this study (p.E158DfsX25) as well as both previously reported *FGF16* mutations (p.R179X and p.S157X) introduce premature termination codons in the last exon of *FGF16* (Jamsheer et al., 2013). Due to their position in the last exon, all the transcripts are most probably stable and unlikely to be degraded in the process of nonsense-mediated decay. Consequently, truncated forms of FGF16 protein that lack 29 to 51 C-terminal amino acids are likely to be expressed. Based on the predicted sequences, the mutation reported here as well as both previously identified *FGF16* nonsense variants result in similar truncations of the protein, which loses some of the amino acids responsible for receptor binding and a significant portion of heparan sulphate binding site (Fig. 2B). As FGFs activate FGF receptors (FGFR) in an heparan sulphate-dependent manner, lack of heparan sulphate-binding domain in mutated FGF16 is expected to markedly reduce strength and/or specificity of FGF–FGFR interaction (Rapraeger et al., 1991; Yayon et al., 1991; Plotnikov et al., 2000). The variant described here, therefore, supports our previous hypothesis that MF4 causing mutations most probably lead to the impairment of the FGF16 interaction with the corresponding receptor (for

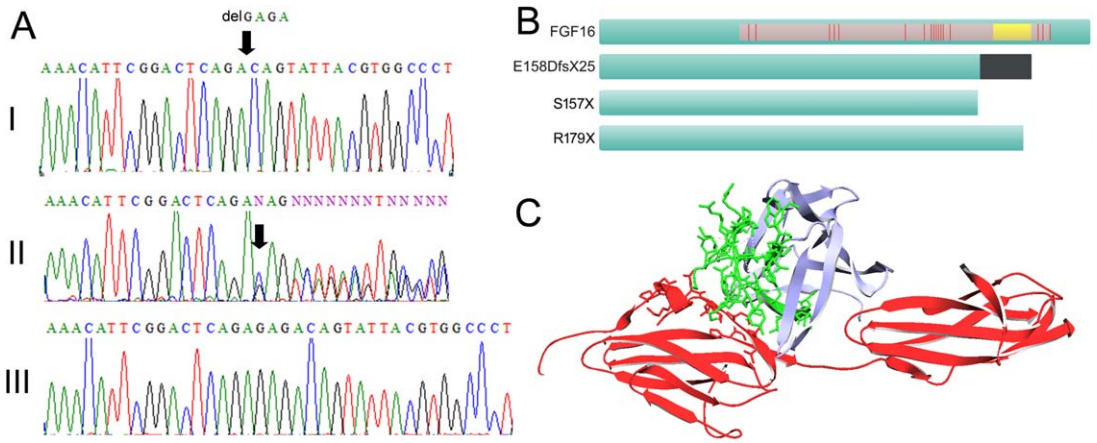

FIGURE 2. (A) *Electropherograms of the frameshift mutation detected in the FGF16 gene and a wild type sequence of an unaffected control individual. Mutation c.474_477del (p.E158DfsX25) in exon 3 was found in hemizygosity in proband (panel I) and in heterozygous state in his mother (panel II) but not in a wild-type male (panel III). Arrows indicate position of the mutations.* **(B)** *Schematic domain organization of wild type FGF16 protein (upper panel). The FGF domain is colored in gray, while the heparin binding site is shown in yellow. The amino acid residues responsible for the receptor binding are presented as red bars. The mutated variant of FGF16 protein expected to be expressed in our proband (p.E158DfsX25) as well as two previously described pathogenic forms of FGF16 (p.S157X and p.R179X) are juxtaposed below the wild type FGF16 protein. The amino acids overlapping with the wild type FGF16 sequence are shown in blue, whereas abnormally substituted amino acids due to frameshift mutation are colored in black.* **(C)** *Three-dimensional structure of a complex between FGF16 and FGF receptor (FGFR). FGFR is colored in red. The spared part of the E158DfsX25 FGF16 variant is colored in blue, whereas the lacking part, important for the interaction with FGFR, is highlighted in green. Interaction between FGF16 and FGFR was modeled on the basis of the structure of FGF1 in complex with the extracellular ligand binding domain of FGFR1 (PDB: 1EVT).*

schematic view of FGF16–FGFR interaction, see Fig. 2C). As mentioned above, *Fgf16* null mice show severe heart developmental defects. Congenital heart anomalies have been observed neither in our patient, nor in the two other previously described *FGF16*-deficient cases (Jamsheer et al., 2013). Thus, we postulate that C-terminally truncated FGF16 variants exert residual activity still sufficient for maintaining heart morphogenesis in humans, yet inadequate for the fine patterning of the hand skeleton. Alternatively, FGF16 is not so important for human heart development as for murine, and can be substituted during human embryogenesis by other FGFs.

In addition to MF4, our proband showed symptoms of a connective tissue disorder such as generalized joint laxity and hyperextensibility, easily bruisable skin, and pectus carinatum. Of interest, the MF4 case carrying the *FGF16* nonsense mutation (p.R179X) described by us previously also manifested slender build and unilateral inguinal hernia (Jamsheer et al., 2013). Furthermore, Lonardo et al. (2004) reported on a molecularly untested X-linked MF4 patient, who showed marfanoid habitus, scoliosis, and inguinal hernia. Based on these independent clinical observations, we hypothesize that *FGF16* truncating mutations give rise not only to MF4, but also to some of the connective tissue disorder symptoms. Therefore, we suggest that impaired FGF16 function may influence connective tissue properties in human MF4 patients, although further studies are needed to confirm this hypothesis.

To conclude, our report confirms that truncating mutations of *FGF16* are causative for X-linked recessive metacarpal 4-5 fusion. The mutation identified in this study was located in last exon of the gene (exon 3), as the two other previously reported *FGF16* disease-causing mutations. Thus, all *FGF16* mutations known to give rise to MF4 are located C-terminally and most probably do not result in a nonsense mediated decay. Additionally, our proband showed mild symptoms of a connective tissue disorder, as some of the other patients previously reported to have X-linked MF4. Therefore, we suggest that impaired FGF16 function may also be responsible for connective tissue symptoms in MF4 patients.

Acknowledgments

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