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# Novel fibronectin mutations and expansion of the phenotype in spondylometaphyseal dysplasia with "corner fractures"

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ABSTRACT

Heterozygous pathogenic variants in the *FN1* gene, encoding fibronectin (FN), have recently been shown to be associated with a skeletal disorder in some individuals affected by spondylometaphyseal dysplasia with "corner fractures" (SMD-CF). The most striking feature characterizing SMD-CF is irregularly shaped metaphyses giving the appearance of "corner fractures". An array of secondary features, including developmental coxa vara, ovoid vertebral bodies and severe scoliosis, may also be present. FN is an important extracellular matrix component for bone and cartilage development. Here we report five patients affected by this subtype of SMD-CF caused by five novel *FN1* missense mutations: p.Cys123Tyr, p.Cys169Tyr, p.Cys213Tyr, p.Cys231Trp and p.Cys258Tyr. All individuals shared a substitution of a cysteine residue, disrupting disulfide bonds in the FN type-I assembly domains located in the N-terminal assembly region. The abnormal metaphyseal ossification and "corner fracture" appearances were the most remarkable clinical feature in these patients. In addition, generalized skeletal fragility with low-trauma bilateral femoral fractures was identified in one patient. Interestingly, the distal femoral changes in this patient healed with skeletal maturation. Our report expands the phenotypic and genetic spectrum of the *FN1*-related SMD-CF and emphasizes the importance of FN in bone formation and possibly also in the maintenance of bone strength.

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## 1. Introduction

Metaphyseal ossification irregularities, so called corner fractures, are a typical feature of a subtype of autosomal dominant spondylometaphyseal dysplasia (SMD), SMD with "corner fractures" (SMD-CF) [MIM: #184255], which was first described as a separate entity in 1990 [1]. Previously, *COL2A1* mutations were reported in three individuals [2,3]. Only recently, SMD-CF has been linked to mutations in the *FN1* gene, encoding fibronectin (FN) [4,5]. FN is a ubiquitous glycoprotein in the extracellular matrix (ECM) with multiple structural and regulatory functions [6,7]. FN forms an insoluble and highly organized matrix that connects cells and other matrix proteins such as collagens and glycosaminoglycans to the ECM [8–10]. Through integrin binding, FN mediates cellular interactions facilitating migration, proliferation and differentiation [6,11,12]. In the skeleton, FN is important for mesenchymal stromal cells' (MSCs) condensation, migration, proliferation and differentiation, and thereby crucial for cartilage development and bone formation [12–16].

SMDs comprise a group of genetically and phenotypically heterogeneous bone dysplasias that affect the growth plates and the spine. Although most of the clinical features overlap with other types of bone dysplasias, SMD-CF presents with some characteristic features. These include developmental coxa vara, metaphyseal irregularities, scoliosis, and abnormal ossification at the growth plate and secondary ossification sites, which give rise to a "corner-fracture" like phenotype [1,3,4,17]. Only eleven patients in eight families with *FN1* mutation-related SMD-CF have been described thus far [4,5] and therefore the complete genetic and phenotypic spectrum of the disease remains to be more thoroughly characterized.

As part of our ongoing research on genetic determinants of skeletal dysplasias, we identified five new patients with SMD-CF caused by five novel missense mutations in *FN1*. Here we present the clinical, radiographic and genetic findings for these patients. These cases further demonstrate the presence of corner fractures as an important diagnostic feature for *FN1*-related SMD, but also underscore the variable severity of the disease, which may lead to diagnostic challenges. Further, the presence of femoral fractures and osteopenia in one patient suggests that FN may not only be important in the development of skeletal ECM, but might also play a role in bone strength and fracture resistance.

## 2. Subjects and methods

### 2.1. Subjects, editorial policies and ethical considerations

One patient with an unknown form of skeletal dysplasia was recruited at the Children's Hospital, Helsinki University Hospital, Finland. Four other patients with a similar phenotype were recruited through the International Skeletal Dysplasia Society and the GeneDx genetic testing company.

Ethical approvals for the study were obtained from the Institutional Ethics Boards and complied with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. A written informed consent to be part of the current research study and for editorial purposes was obtained from each participant or his/her legal guardian before sample collection. Clinical and radiographic evaluations of all study participants were carried out as part of their routine clinical care.

### 2.2. Methods

#### 2.2.1. Clinical and imaging data

Clinical data were collected retrospectively from hospital records. Anthropometric data were compared with appropriate population-based reference values to calculate Z-scores. Radiographs and other imaging results were collected and reviewed for fractures and other characteristics.

#### 2.2.2. Next generation sequencing (NGS)

At the onset of this study, *FN1* had not been described as a potential candidate gene for SMD. Therefore, in four out of the five patients, we used either whole-exome sequencing (WES) or whole-genome sequencing (WGS). Genomic DNA was extracted from peripheral blood using standard procedures.

For Patient #1, WGS was carried out at the Science for Life Laboratory (SciLifeLab) in Stockholm, Sweden. Library preparation was made using the Illumina TruSeq PCR-free method. Clustering was performed by 'cBot' and the libraries were sequenced as 2 × 150 bp paired-end reads on the HiSeqX instrument (Illumina) with an average coverage of 30×. Reads were mapped to the human genome assembly GRCh37/hg19 with Burrows-Wheeler Aligner (BWA-MEM) [18,19]. Data processing and variant calling were performed according to Genome Analysis Toolkit (GATK) best practices [20]. Finally, variants were annotated using Variant Effector Predictor (VEP) [21], data were analyzed using GEMINI [22] and candidate variants were manually assessed using Integrative Genomics Viewer (IGV).

For Patient #3, WES was performed at CEGH-CEL-Universidade de São Paulo. Using genomic DNA from the proband, the exome was captured using the Illumina TrueSeq kit, and sequencing was performed on an Illumina HiSeq 2500. Reads were aligned to the GRCh37/hg19 assembly of the human genome with the BWA-MEM aligner. Variant calling included single nucleotide variants (SNVs), small insertions and deletions (InDels) and was performed with GATK. The resulting data (in variant call format – VCF) were annotated with ANNOVAR [23]. Sanger sequencing confirmed the variant in the proband and in her affected mother.

For Patients #4 and #5, trio WES was conducted at GeneDx. Using genomic DNA from the proband and parents, the exonic regions and flanking splice junctions of the genome were captured using the Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA). Massively parallel NGS sequencing was done on an Illumina system with 100 bp or greater paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool. Additional sequencing technology and variant interpretation protocol has been previously described [24]. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

#### 2.2.3. PCR and Sanger sequencing

PCR followed by Sanger sequencing according to standard methods was used for targeted gene sequencing in one patient and as a validation method for the WGS and WES findings in the other patients.

For Patient #2, Sanger sequencing of exons 1–7 of *FN1* was performed from the patient's genomic DNA because of phenotypic suspicion, using the primers detailed in Table S1. Sanger sequencing was performed at the McGill University and Genome Quebec Innovation Centre. The reads were mapped to human *FN1* and analyzed using Geneious version R8. Once the mutation was identified, genomic DNA from the parents was sequenced for the same exon to demonstrate the *de novo* nature of the mutation.

## 3. Results

### 3.1. Clinical and radiographic findings

Based on radiographic evaluation all five patients featured metaphyseal irregularities and "corner fracture" appearances, predominantly in proximal and distal tibias, but also in femurs (Fig. 1). Four of the patients displayed developmental coxa vara, while all five had genu valgum knee abnormalities (Table 1). Scoliosis was seen in three of the five patients, and abnormal vertebral bodies was detected in three (Fig. S1). None of the patients had chest abnormalities, and renal function was normal in all five (Table 1). Detailed clinical

information is summarized for each patient in Table 1, and some additional clinical information is presented patient-wise below.

#### 3.1.1. Patient #1

This 12.5 year-old boy is the second child of non-consanguineous healthy Finnish parents (Table 1, Patient #1). He was born after an uneventful pregnancy and delivery, with normal birth measurements. His four siblings are healthy. Development and growth were normal during his first year of life. He learned to walk at 14 months and developed a waddling gate. He was first evaluated for suspected skeletal disease at 1.8 years of age. Radiographs showed metaphyseal irregularities in the long bones and ribs, and coxa vara, while hands, feet and spine were normal (Fig. S1). The most prominent metaphyseal changes along with "corner fractures" were seen in the distal tibiae and fibulae. He has undergone several surgical procedures to correct skeletal deformities, including the severe coxa vara (Table 1). Cognitive development, hearing and vision are normal, and abdominal and heart ultrasounds have been normal. Notably, this patient sustained bilateral femoral fractures at the age of 3.5 years through low trauma mechanisms (Fig. 2B–C). Following conservative treatment fractures healed well without further complications. Lumbar spine DXA measurements at 9.6 years of age showed low bone mineral density (BMD) (Z-score −2.3). Since 10 years of age he suffers from hip and knee pain and receives physiotherapy. At latest appointment he walked independently.

#### 3.1.2. Patient #2

This 6.8 years old boy is the first-born to non-consanguineous Caucasian parents with unremarkable family history (Table 1, Patient #2). Short long bones were detected at 20 weeks gestation. He was born at 28 weeks of gestation following spontaneous labour, delivered by C-section for fetal distress. Concerns with short stature were raised at 1.5 years of age. His height was 78 cm (−4.1 SD) and he was disproportionate with a normal sitting height. His head circumference was on the 25th percentile. Facial features included flat profile, prominent eyes and pointed chin. He had genu varum and walked with a waddling gait. He was reviewed in the Genetic Clinic at 3, 4 and 6 years of age, respectively. The suspicion of SMD-CF was raised at 6 years based on radiographic findings, including widespread metaphyseal irregularities and "corner fractures" appearance in the long bones, and vertebral endplate irregularity with anterior sloping (Fig. S2). He complained of occasional upper leg and knee pain after prolonged exertion. Genu varum and waddling gait have become less prominent with age.

#### 3.1.3. Patient #3

The proband is a 22 year-old female, the only living child of non-consanguineous Brazilian parents (Table 1, Patient #3); the mother, also affected, had two previous miscarriages. She was born preterm at 31 weeks. Developmental milestones and cognition were normal. She developed bowed legs, progressive difficulty in walking and stunted growth. She was first evaluated at 6 years of age, when her height was 105 cm (< 5th centile). She displayed rhizomelic shortening of upper limbs and bowing of lower limbs, short humeri, coxa vara, irregular metaphyses with corner fractures, and abnormal dorsal vertebral bodies. At the age of 19 years her height was 127 cm, showing mild kyphoscoliosis with irregular dorsal vertebral bodies, narrowing of the intervertebral spaces, short humeri, coxa vara and short femoral neck (Fig. S3).

The affected mother has short stature and abnormal gait since early childhood. She developed progressive, painful joint limitations and kyphoscoliosis, requiring spinal surgery in adulthood. At the last evaluation she was wheelchair bound. Her final height is 125 cm. Skeletal survey at 59 years showed short humeri, coxa vara, osteoarthrosis in the large and interphalangeal joints, spine involvement with flattened vertebral bodies and irregularities of C1–C2 (Fig. S3).

#### 3.1.4. Patient #4

This 14 year-old boy was born by SVD at 35 weeks of gestation to Vietnamese-American parents from an uncomplicated pregnancy (Table 1, Patient #4). He was seen in Genetics first time at 4 years of age for congenital coxa vara. At 4.5 years he displayed short stature and abnormal gait, radiological evidence of metaphyseal irregularities and sclerosis of all long and phalangeal bones, and possible corner fractures of proximal humeri and tibiae (Fig. S4). He underwent bilateral proximal femoral valgus osteotomy to correct the coxa vara. This patient has a history of additional clinical features. From infancy, he has had vomiting episodes lasting a few days, decreasing in frequency, with so far non-revealing work-up. At 4 years of age he was diagnosed with high myopia, and at 9 years, borderline elevated intraocular pressures. At 10 years of age he received a transfusion for microcytic anaemia (haematocrit: 22.9%) due to iron deficiency, which was managed without further episodes of anaemia. Other blood biochemistry has been normal. At 11 years serum intact parathyroid hormone (PTH) was normal while serum N-terminal telopeptide was elevated (66.3 nM BCE/L, normal 5.4–24.2). At age 14, osteocalcin was slightly elevated (54 ng/ml (normal 3–40)) and N-terminal telopeptide was still elevated (43 nM BCE/L (normal 5.4–24.2)), while bone-specific alkaline phosphatase (82 μg/L (normal 78–170)) and beta-CrossLaps: 1299 pg/ml (normal 240–1734) [25] were normal. He was also found to be hypertensive (blood pressure 150/90 mm Hg) and has responded well to treatment. Renal function was normal.

#### 3.1.5. Patient #5

This 32 year-old female presented to the prenatal diagnosis clinic at 23 weeks gestation due to fetal risk associated with a family history of autosomal dominant short stature attributed to familial metaphyseal dysplasia (Table 1, Patient #5). Fetal evaluation was considered normal and the patient was referred to Medical Genetics to elucidate the etiology of her clinical findings. Medical history indicated that the patient was born at 28 weeks of gestation after a pregnancy complicated by prenatal diagnosis of bone abnormalities suggestive of skeletal dysplasia. Findings included vertebral abnormalities and lower extremity shortening. At birth, coxa vara was diagnosed along with lumbosacral vertebral fusions. Family history includes similar findings for the father, sister and paternal aunt, suggesting an autosomal dominant pattern of segregation. Current physical examination shows normal craniofacial shape but asymmetry of the ears with posterior rotation, micrognathia, a high arched palate, short distal phalanges, and normal trunk length with rhizomelic shortening of the legs. The patient is currently healthy with no additional complications.

### 3.2. Genetic findings

In order to identify the genetic cause of unknown skeletal dysplasia in Patient #1, we carried out WGS for the index patient, his healthy parents and his three siblings (two brothers and one sister), who were also free from the disease. After applying the following filtering criteria; 1) MAF < 0.001 in GnomAD [26] and SweGen [27] databases, 2) autosomal recessive/compound heterozygous inheritance pattern or *de novo* variant, and 3) impact severity different than low according to GEMINI, we ended up with only one candidate variant: a novel missense heterozygous mutation, c.638G > A (p.Cys213Tyr) in *FN1* (reference transcript: NM_212482.2; ClinVar submission ID: SUB4019866) (Table 2, Fig. 3). This variant was only identified in the index patient and thus determined to be a *de novo* variant. At the time of genetic investigations in this family, *FN1* mutations had not been reported in patients with SMD-CF. In the other patients we used either exome sequencing (Patients #3, #4 and #5) or, after discovery of *FN1* mutations in SMD-CF, targeted Sanger sequencing of *FN1* (Patient #2). We identified four novel heterozygous single-nucleotide mutations, c.368G > A (p.Cys123Tyr), c.506G > A (p.Cys169Tyr), c.693C > G (p.Cys231Trp) and c.773G > A (p.Cys258Tyr) in *FN1* (ClinVar submission ID:

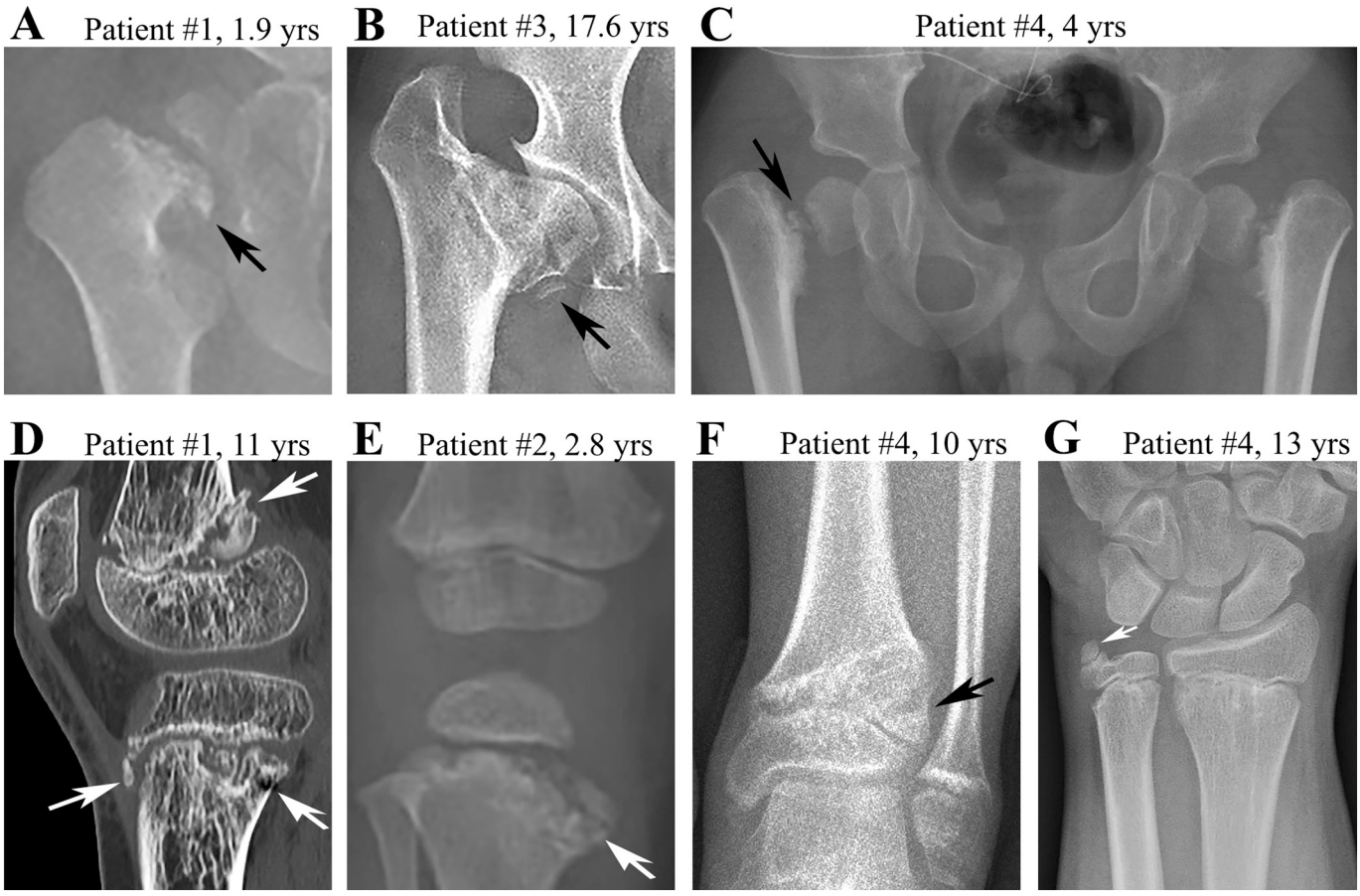


**Fig. 1.** Corner fractures.
Radiographs show "corner fracture" appearances (arrows) in Patients #1–4 at different ages, in hips (A–C), knees (D–E), ankle (F) and wrist (G). Metaphyseal irregularities are also visible.

SUB4019866) (Table 2, Fig. 3). All mutations were *de novo* except for Patient #3 where the mother is affected and Patient #5 where the father is affected, carrying the corresponding mutation (Table 2). Since the clinical phenotypes were consistent with the clinical diagnosis of SMD-CF caused by *FN1* mutations we classified these variants as disease-causing in our patients. Moreover, all the identified mutations affected highly conserved nucleotides and they were predicted to be deleterious according to several in silico bioinformatics tools (Table 2). All these mutations substitute cysteine residues (p.Cys123Tyr, p.Cys169Tyr, p.Cys213Tyr, p.Cys231Trp and p.Cys258Tyr) in FN type-I domains (Fig. 3). At the protein level, the mutations were predicted to destroy disulfide bridges that normally form between these affected cysteine residues and another cysteine residue within the same FN type-I domain (UniProt-database, http://www.uniprot.org) (Fig. 3B). The loss of a stabilizing intra-domain disulfide bond could result in structural changes or instability within the type-1 protein domain, hence disrupt normal protein function.

## 4. Discussion

FN plays a major role in the ECM and numerous in vitro studies, supported by some animal models, have clearly demonstrated the critical role of FN for MSCs' differentiation in osteogenesis [13–16] as well as chondrogenesis [12,14]. Still, the importance of FN as a structural and functional component of the mature bone matrix and in maintaining a strong skeleton in humans is less well understood. Only recently, mutations in *FN1* were confirmed as the cause of SMD-CF by identifying six heterozygous missense mutations and one three base-pair deletion in *FN1* [4,5] in affected patients.

In this report we identified five novel *FN1* mutations in patients with SMD-CF. All the patients show peculiar metaphyseal changes and "corner fractures". The "corner fractures", visible on radiographs as lucent areas in the proximal and/or distal metaphyses of tibias and/or femurs, represented an important clinical feature in all these patients. This is in line with the previous report on *FN1* mutations in patients with SMD [4]. "Corner fractures" are triangular or curvilinear metaphyseal ossification centers, usually in the proximal and distal femurs and in the distal tibias, radiuses and ulnas, resembling microfractures. Metaphyseal changes usually present as irregular margins with irregular sclerosis in the adjacent metaphyseal spongiosa. Metaphyseal and vertebral changes appear after the first year and persist throughout adolescence but are no longer visible in the adult [28]. This fits well with the location, timing, and frequency of "corner fractures" seen in our patients. In most patients, the metaphyseal changes and "corner fractures" were visible already in early radiographs (< 3 years of age).

Novel clinical features reported in the current cohort include bilateral femoral fractures in Patient #1. The fractures occurred after low impact trauma, in the distal femurs close to the areas with prominent metaphyseal changes. The fractures healed well, and the patient has not sustained additional fractures. In addition to the bilateral femoral fractures, Patient #1 presented with rather severe bone changes in his distal femurs. Surprisingly, some of these bone changes reversed spontaneously and at later follow-up the metaphyseal areas in the femurs have normalized. This finding is in line with what has been observed in the patient recently reported by Cadoff et al., who featured bilateral corner fractures in both femurs and wrist that resolved over time [5]. A similar clinical scenario is typical for metaphyseal anadysplasia, a disease that is characterized by severe metaphyseal changes

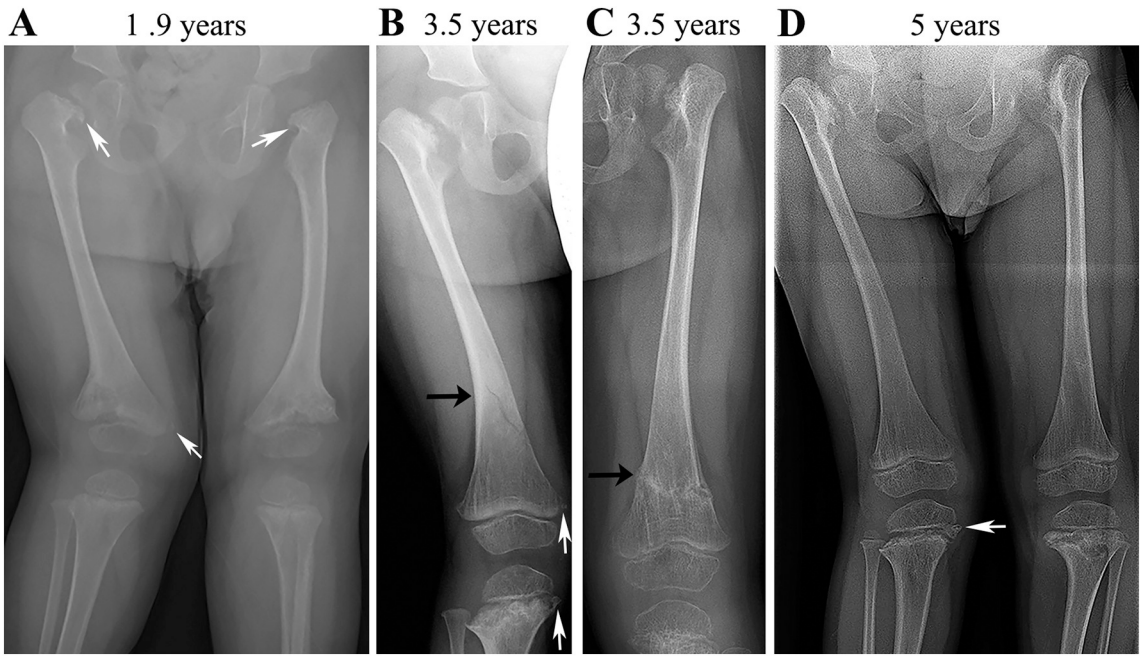


**Fig. 2.** Femoral fractures and metaphyseal changes in Patient #1.
A) Abnormal proximal and distal femoral and proximal tibial and fibular metaphyses at 1.9 years of age. The femoral necks are short, in varus position and corner fractures can be seen in both hips and in the right knee (white arrows). B–C) Bilateral distal femoral fractures (black arrows) at 3.5 years of age; both femurs have osteopenic appearance. Corner fractures are visible in the right distal femur and proximal tibia (white arrows). The distal femoral metaphyses are more regular than in A). D) Spontaneous regression of distal femoral metaphyseal changes by 5 years of age. In contrast, the metaphyseal changes at proximal tibias and the knee valgus deformity progress with age (B–D).

during growth, which resolve spontaneously upon skeletal maturation [29,30]. Interestingly, both these diseases have a defective ECM in common. In fact metaphyseal anadysplasia is caused by mutations in *MMP9* and *MMP13*, encoding the corresponding matrix metalloproteinases (MMPs) [29]. These MMPs are important for bone remodeling, repair and regeneration through their regulation of ECM turnover [31]. FN is one of the substrates for MMPs, and remodeling of FN in ECM is regulated by MMP-mediated cleavage [32], and regulation of integrins [33], which are the major FN receptors [33]. It is possible that some cysteine substitutions in FN type-I domains render the protein more susceptible to cleavage by MMP9 and MMP13. Enhanced proteolytic susceptibility has been demonstrated for cysteine mutations involved in disulfide bonds in fibrillin-1 leading to Marfan syndrome [34].

As a key ECM component, FN regulates the assembly of the collagen I matrix and the composition and stability of the ECM [6], which also affects the degree of matrix mineralization [35,36]. One of the earliest steps in ECM formation is the assembly of FN into fibrils, which form a network for deposition of other ECM components. Interestingly, all the novel mutations in this report are located within the N-terminal assembly region of FN, and all were found to substitute cysteine residues with a hydrophobic amino acid (either tyrosine or tryptophan), which most likely destabilizes the structure of the respective FN type-I assembly domains by disrupting intra-domain disulfide bridges (Fig. 3) [4]. Five out of seven previously reported mutations describe similar cysteine substitutions that affect disulfide bonds in the FN type-I domains (Fig. 3) [4,5]. Only one missense mutation, p.Tyr240Asp, affects an amino acid other than cysteine in the FN type-I domain 5 (Fig. 3) [4]. This N-terminal region in FN containing the mutated type-I domains works as a separate functional unit that in addition to initiating the fibril assembly, also binds to ECM, other proteins and to other regions in FN [8,10]. Only one patient with SMD-CF harbors a small in-frame *FN1* deletion that resides outside this region [4]. This three base-pair deletion removes threonine 809 in the FN type-III domain 2 (Fig. 3A). Like the FN type-I domains, this domain also enhances conformational changes to promote FN assembly [10] and since the patient's phenotype is similar to other patients with missense mutations, the underlying disease mechanism is likely to be the same.

Previous in vitro studies testing two cysteine substitutions, p.Cys87Phe and p.Cys260Gly, and the non-cysteine substitution p.Tyr240Asp in HEK293 cells showed that the mutated FN is retained within the cells instead of being secreted [4]. More recently, studies on skin fibroblasts of the patient with a p.Cys97Trp mutation showed that the mutated FN is preferentially retained in the endoplasmic reticulum instead of being secreted thus leading to a reduced production of FN matrix compared to control fibroblasts [5]. Furthermore, this patient also shows decreased levels of plasma FN. Retained secretion of fibronectin leading to reduced amount of FN in the ECM is a possible explanation to the observed skeletal changes seen in our patients with *FN1* mutations. However, functional studies using osteogenic cells or animal models are needed to better understand the underlying molecular mechanisms and explain why disulfide bridges between cysteine residues within the FN type-I domains are susceptible to mutations in the *FN1* gene, as the majority of mutations reported so far are located within this area.

Although *FN1* mutations have also been identified in patients with glomerulopathy with fibronectin deposits [MIM: #601894] [37–39], none of our patients had impaired renal function. In fact, the mutations previously identified in glomerulopathy with fibronectin deposits cluster on the FN type-III domains [26–29] whereas mutations causing SMD-CF seem to affect the FN type-I domains. This indicates that the location of the mutation within the FN protein is crucial for the manifestation of either one or the other disease.

Borderline-high intraocular pressure and hypertension beginning in early childhood were identified in Patient #4. Regarding potential for glaucoma, FN seems important for aqueous humour outflow [40]. Hypertension was previously noted only during the pregnancy of a previously reported patient [4]. In Patient #4, hypertension had an onset in the early childhood and has persisted. FN is an important factor for vascular elasticity and its expression is increased in hypertension [41], which suggests that abnormal FN might be responsible for increased

**Table 1**
Skeletal manifestations and clinical features of the five index patients with FN1 mutations.

| | Patient #1 | Patient #2 | Patient #3 | Patient #4 | Patient #5 |
|---|---|---|---|---|---|
| **Sex** | Male | Male | Female | Male | Female |
| **Ethnicity** | Finnish | Caucasian | African-Caucasian | Vietnamese-American | Caucasian |
| **Duration of pregnancy** | 38 weeks | 28 weeks, SROM, LSCS fetal distress | 31 weeks, PPROM, LSCS | Term | Term |
| **Birth measurements**[a] | | | | | |
| **Birth weight (g)** | 3230 g (-0.5 SD) | 1210 g (0.60 SD) | 1550 g (0.2 SD) | 2730 g (-1.3 SD) | NA |
| **Birth height (cm)** | 50 cm (-0.6 SD) | | 40 cm (0.1 SD) | 48.3 cm (-0.8 SD) | |
| **Head circumference (cm)** | 33 cm (-1.7 SD) | | 30 cm (1.4 SD) | | |
| **Age at last assessment** | 12.5 y | 6.8 y | 19.3 y | 13.8 y | 32 y |
| **Height (cm)**[a] | 138.3 cm (-2.4 SD) | 101 cm (-3.8 SD) | 127.3 cm (-5.6 SD) | 148.5 cm (-1.6 SD) | 141.1 cm (-3.4 SD) |
| **Weight (kg)**[a] | 42.7 kg (+3.2 SD) | 17 kg (-2.21 SD) | 48.9 kg (-1.3 SD) | 59.9 kg (+0.8 SD) | 57.1 kg (-0.05 SD) |
| **Head circ. (cm)**[a] | 56.3 cm (+1.0 SD) | 51 cm (-0.68 SD) | 55 cm (+0.6 SD) | 57 cm (+1.6 SD) | 56 cm (+1.5 SD) |
| **Short stature** | Yes | Yes | Yes | Yes | Yes |
| **Coxa vara** | Yes | No | Yes | Yes | Yes |
| **Irregular metaphyses** | Yes | Yes | Yes | Yes | Yes |
| **"Corner fractures"** | Yes<br>Proximal and distal femurs, proximal tibias present at 1.9 y | Yes | Yes<br>In proximal tibia starting at 3 y | Yes<br>Distal tibia since 3 y, proximal tibia and humeri since 4 y | NA |
| **Knee anomalies** | Genu valgum (more severe in the right knee) | Genu varum | Genu valgum (mild) | Genu valgum (mild, resolved) | Genu valgum |
| **Asymmetric bone involvement** | No | NA | Yes (right knee and left ankle more affected) | Yes | Yes |
| **Ovoid or abnormal vertebral bodies** | No | Yes,<br>Biconvex vertebral endplates;<br>Anterior sloping | No | Yes | Yes |
| **Scoliosis** | Yes (mild) | No | Yes (mild) | No | Yes |
| **Chest anomalies** | No | No | No | No | NA |
| **Dental complications** | Orthodontic treatment by dental braces | NA | NA | NA | NA |
| **Surgeries** | Bilateral femoral osteotomies for coxa vara (at 5.5 y); Posterior C1-C2 fusion for atlantoaxial instability (at 6.5 y); Three surgeries to correct progressive valgus deformity of left tibia (at 8.0, 10.5 and 11.5 y) | No | No | Bilateral femoral osteotomies for coxa vara (age 4 y) | Lumbar fusion due to abnormal vertebrae |
| **Renal function** | Normal | Normal | Normal | Normal | Normal |
| **Main complaints** | Pain in hips and knees since age 10.5 y | NA | Knees and lumbar pain; asymmetric legs | NA | NA |
| **Additional features** | NA | Retinopathy of prematurity, now normal vision and hearing | Pre-auricular skin tag | Hypertension; borderline high intraocular pressure | High arched palate; shortening of the legs; short distal phalanges |

y = years; NA = Not available; SROM = Spontaneous rupture of the membranes; LSCS = Low segment Caesarean section; PPROM = Preterm premature rupture of the membranes.
[a] measures given along with number of standard deviations (SD) compared with appropriate population-based reference values; NA = Not available.

**Table 2**
Genetic description of the FN1 mutations and bioinformatics prediction of their pathogenicity.

| | Patient #1 | Patient #2 | Patient #3 | Patient #4 | Patient #5 |
|---|---|---|---|---|---|
| **Mutation on DNA level**[†] | c.638G > A | c.368G > A | c.506G > A | c.693C > G | c.773G > A |
| **Mutation on protein level** | p.Cys213Tyr | p.Cys123Tyr | p.Cys169Tyr | p.Cys231Trp | p.Cys258Tyr |
| **Mutation effect** | Missense | Missense | Missense | Missense | Missense |
| **Evolutionary constraint GERP score**[‡] | 5.81 | 6.07 | 5.78 | 4.98 | 5.87 |
| **Pathogenicity classifiers** | | | | | |
| **CADD score (scaled C-score)** | 30 | 32 | 32 | 31 | 28.9 |
| **Polyphen** | Probably damaging | Probably damaging | Probably damaging | Probably damaging | Probably damaging |
| **SIFT** | Deleterious | Deleterious | Deleterious | Deleterious | Deleterious |
| **M-CAP score**[§] | 0.400 | 0.327 | 0.382 | 0.445 | 0.264 |
| **Mutation type (inherited/*de novo*)** | *De novo* | *De novo* | Inherited (from mother) | *De novo* | Inherited (from father) |
| **Detection method** | WGS | Sanger sequencing | WES | WES | WES |

WGS = whole genome sequencing; WES = whole exome sequencing.
[†] Reference sequence: NM_212482.2.
[‡] Genomic Evolutionary Rate Profiling (GERP) score.
[§] Mendelian Clinically Applicable Pathogenicity (M-CAP) score.

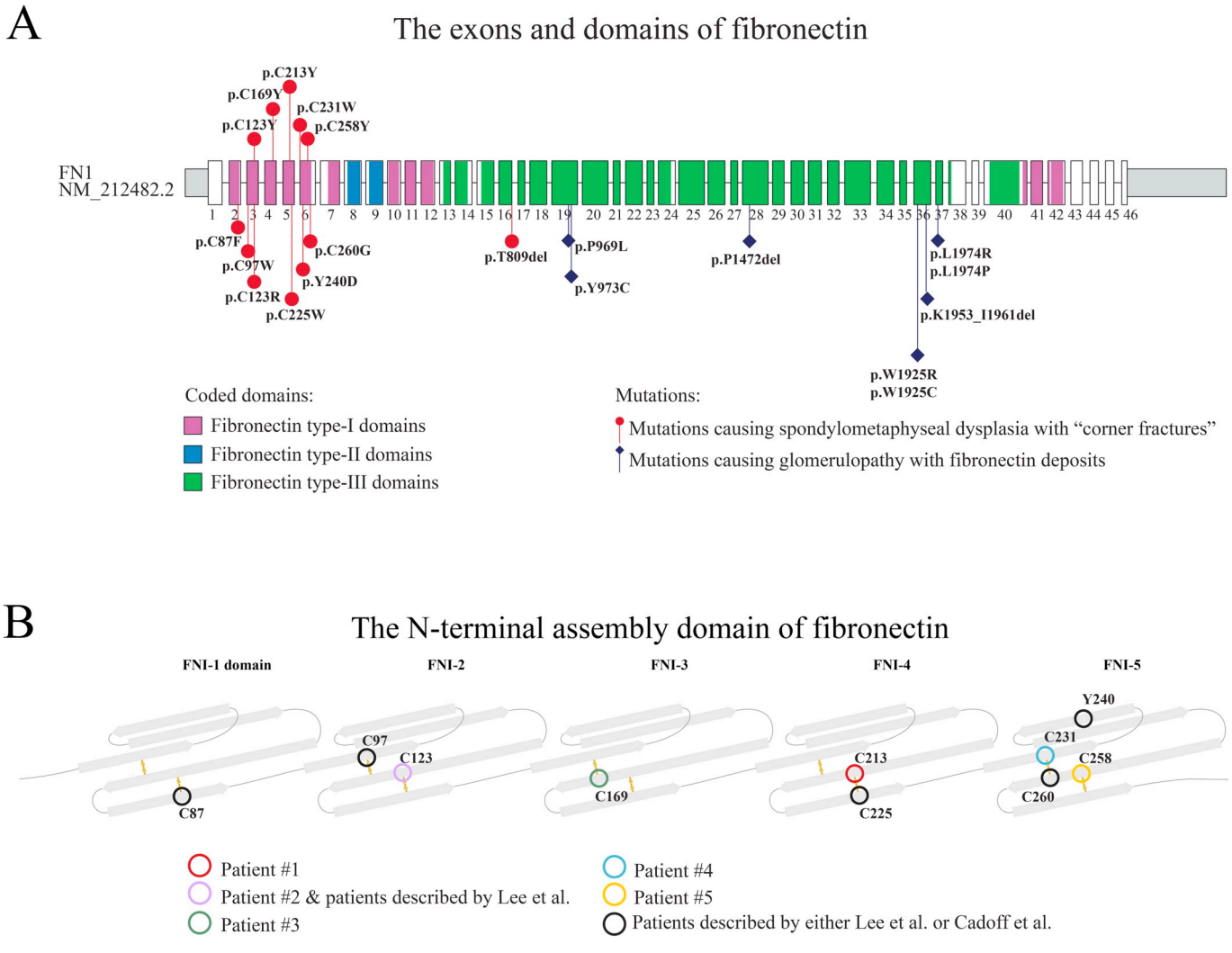


**Fig. 3.** Summary of all the currently identified mutations in the *FN1* gene.
A) Schematic illustration of the FN1 exons/domains and location of all currently identified mutations causing SMD-CF (red symbols) and glomerulopathy with fibronectin deposits (blue symbols), respectively. The five novel mutations described here are indicated above the scheme whereas all previously identified mutations appear below. Rectangles represent the exons (1–46) of *FN1* and are colour coded according to the codified domains. B) Locations of the five novel mutations identified in the current study, along with the previously reported mutations affecting the fibronectin type-I domains within the N-terminal FN assembly region. All these changes except one affect cysteine residues and disrupt intra-domain disulfide bonds.

vascular stiffness in this patient.

FN is crucial for ECM development in a range of tissues, as demonstrated by the embryonic lethality of FN knockout in mice [42]. Due to the undisputed stimulating effect on osteogenic and chondrogenic differentiation and tissue formation, FN has been highly utilized in tissue engineering applications [43–45]. However, the involvement of FN in maintaining bone strength is unclear. In our cohort, it is interesting to note that Patient #1 had a decreased lumbar BMD, while Patient #4 had increased serum N-terminal telopeptide, suggesting an increased bone turnover which might lead to reduced BMD. It is possible that *FN1* mutations disrupt normal ECM turnover affecting collagen expression or post-translational modification, leading to increased degradation of type I collagen. Another possibility is that *FN1* mutations limit proper mineral deposition. Using mouse models with conditional deletions of FN in the liver or osteoblasts [46], Bentmann et al. [46] demonstrated that plasma FN deposits in bone. Deletion in the liver led to decreased mineral and hydroxyapatite content in bone, decreased trabecular bone density, and increased collagen fiber diameter in bone. Deletion in osteoblasts did not affect the FN content in bone or new bone formation, but increased osteoblast numbers and osteoid volume, indicating an altered osteoblast function as the apposition rate was decreased and mineralization delayed. The increased collagen fiber size is interesting in view of the *COL2A1* mutations found in other individuals with SMD-CF. Crosslinking of FN is mediated by transglutaminases, a family of crosslinking enzymes. In a recent study, double knock-out of two transglutaminases (TG2 and FXIII-A) in mice resulted in altered bone cell differentiation, retention of FN in serum, lower FN content in bone, trabecular bone loss and decreased bone strength [47]. This further supports the role of functional FN in maintenance of trabecular bone quality. So far, evidence for an association between altered FN expression and decreased bone quality in humans is mainly derived from studies on increased osteoporosis and fracture risk in patients with severe liver diseases [48,49]. Detailed studies have demonstrated that an altered isoform of FN mediates bone loss in these patients [50,51].

Our study on metaphyseal bone changes and “corner fractures”, scoliosis, coxa vara, and knee abnormalities in patients with novel mutations in *FN1*, and even bilateral femoral fractures in one patient, together with previous reported *FN1* mutations in SMD [4,5], demonstrate the crucial role of FN for proper skeletal development and maintenance of bone strength. Data on BMD and bone turnover markers were available only for one patient in our cohort and should be investigated more carefully in future studies.

In summary, we describe here the phenotype of five new patients with SMD-CF, each of whom harbors a novel missense mutation in *FN1*. These findings expand the phenotypic and genetic spectrum of SMD with “corner fractures” and provide for further detailed functional studies for elucidating the role of FN in maintaining good bone quality.

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## Conflict of interest

The authors Kirsty McWalter, Heather McLaughlin, Rebecca Willaert and Amber Begtrup are employees of GeneDx, Inc., a wholly-owned subsidiary of OPKO Health, Inc. The other authors declare no conflict of interest.

## Authorship

Study design: AC, OM, PMC. Study conduct: AC, HV, NVB, PY, DRB, GLY, CAK, KW, LE, KMW, HML, RW, AB, JJA, DPR, OM, PMC. Data collection: AC, HV, NVB, PY, DRB, GLY, CAK, KW, LE, KMW, HML, RW, AB, JJA, DPR, OM, PMC. Data analysis: AC, HV, NVB, PY, DRB, GLY, CAK, KW, LE, KMW, HML, RW, AB, JJA, DPR, OM, PMC. Data interpretation: AC, HV, NVB, PY, DB, GY, CAK, KW, LE, KMW, HML, RW, AB, JJA, DPR, OM, PMC. Drafting manuscript: AC, HV, NVB, PY, DRB, GLY, CAK, KW, LE, KMW, HML, RW, AB, JJA, DPR, OM, PMC. Revision of manuscript content: all authors. Approval of final version of manuscript: all authors. Responsibility for the integrity of the data: AC, OM, PCM.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2018.12.020.

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