Clinical report

Metaphyseal dysplasia with maxillary hypoplasia and brachydactyly in a Finnish woman: First confirmation of a duplication in RUNX2 as pathogenic variant

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ABSTRACT

Metaphyseal dysplasia with maxillary hypoplasia and brachydactyly (MDMHB) is an autosomal-dominant bone dysplasia that until now has only been reported in French Canadian individuals. We have recently identified an intragenic duplication in RUNX2, encompassing exons 3 to 5, as a cause of MDMHB in French Canadian families. Here we describe a 20-year-old Finnish woman who had typical clinical and radiological signs of MDMHB, the first reported individual with MDMHB who is not of French–Canadian origin. Copy number variant assays based on quantitative PCR of genomic DNA showed the presence of three copies within a part of RUNX2. Sequencing RUNX2 cDNA from the skin fibroblasts revealed a duplication of exons 3 to 5. The results demonstrated that the intronic breakpoints of the duplication differed from those previously found in the French Canadian family, but that the consequences on RUNX2 transcript were identical. These findings demonstrate that the MDMHB phenotype results from an intragenic duplication of RUNX2 exons 3 to 5 also outside of the community where the disorder was first identified.

1. Introduction

Metaphyseal dysplasia with maxillary hypoplasia and brachydactyly (MDMHB: MIM 156510) is a rare bone dysplasia that was first described in 1982 [Halal et al., 1982]. MDMHB is transmitted in an autosomal dominant fashion and is mainly characterized by flaring of the metaphyses, thin cortices of long-bone diaphyses, bilateral shortness of middle phalanges or metacarpals and maxillary hypoplasia. Mild short stature, enlargement of the medial halves of the clavicles, mild thickening of frontal and parietal skull bones, platyspondyly and osteoporosis can also be part of the presentation. Teeth appear yellow and small.

We recently studied a French–Canadian family where several members were affected by MDMHB and found that the disorder was caused by an intragenic duplication in RUNX2 [MIM 600211, NM_001024630.3] that encompassed exons 3 to 5 [Moffatt et al., 2013]. This duplication led to an in-frame addition to the RUNX2 sequence. Our functional studies showed that the variant resulted in a gain of function of RUNX2 [Moffatt et al., 2013].

The two published studies on MDMHB exclusively described French Canadian individuals from a geographically restricted area. MDMHB has thus not been reported in other ethnicities. In the present contribution we describe a 20-year-old Finnish woman with the clinical and radiological characteristics of MDMHB. She had an intragenic duplication in RUNX2 that was similar but not identical to the one that we had previously found in individuals of French Canadian origin.

2. Materials and methods

2.1. Subjects

The individual described here and her mother were followed at Helsinki University Central Hospital and at the Central Hospital of Central Finland Health Care District, Jyväskylä, Finland. Genetic investigations were performed at Shriners Hospital for Children...
in Montreal, Canada, after informed consent, with approval from the Institutional Review Board of McGill University.

2.2. Copy number variation assay

Quantitative PCR for copy number state was carried out using the Taqman probes indicated in the legend of Fig. 2 together with a control probe (ribonuclease P RNA component H1) according to the manufacturer’s protocol (Applied Biosystems, Foster City, CA).

2.3. Cell culture and cDNA sequencing

Human primary skin fibroblasts from a control and the proband were grown in αMEM supplemented with 10% fetal bovine serum, GlutaMAX (final concentration: 2 mM), 100 units/mL penicillin, 100 μg/ml streptomycin and 2 μg/ml amphotericin B. These cells were plated at 100,000 cells per well in 6-well tissue culture plates. Total RNA was extracted from confluent fibroblasts using the Trizol reagent (Invitrogen) and cDNA was obtained. Sequencing was performed using a 3130xl genetic analyzer (Applied Biosystems, Foster City, USA).

3. Results

3.1. Clinical features

The patient was evaluated at 20 years of age. She was born after 42 weeks of uncomplicated gestation with a birth weight of 3580 g and a birth length of 54 cm (both measures between the 50th and 75th percentile). She had normal psychomotor development and is a high-school graduate. Height was 153 cm (3rd percentile). She complained of frequent bone pain, especially affecting the knees. Facial features were notable for micrognathia, beaked nose, and thin lips. She had one fracture in the left 5th metacarpal, after significant trauma.

Radiographs revealed a thick cranial vault (Fig. 1). Brachydactyly was present bilaterally and there was metaphyseal flaring at multiple locations. Clavicles were very wide. The shape of the vertebrae was irregular, and there was a compression fracture of lumbar vertebra 2. Bones appeared generally osteoporotic on radiographs. Bone densitometry using dual-energy X-ray absorptiometry confirmed bone density in the osteoporotic range at the L1 to L4 lumbar spine (T-score: −2.5), proximal femur (T-score: −2.7), and whole body (T-score: −4.5).

Fig. 1. A. Pedigree of the patient’s family. B. Lateral view of the skull demonstrating a thick cranial vault as well as maxillary and mandibular hypoplasia. C. Hands with brachydactyly of the 3rd and 5th right metacarpals and the 5th left metacarpal. C to F. Metaphyseal modeling defects are visible at the hand (C), distal femur and proximal tibia (D), the distal tibia (E), and the foot (F). G. Clavicles are very wide bilaterally. The outline of the clavicles is highlighted by arrows. H. Antero-posterior view of the lower thoracic and lumbar spine, showing a mild curvature. I. Orthopantomography at 12 years of age. Roots of the permanent teeth are short and thin. Resorption of the lower second primary molars is deficient so the second premolars are not able to erupt although their roots are almost completely developed. J. Orthopantomography at 18 years of age. Two upper lateral incisors were lost at the age of 14 years and upper canines have erupted to replace them. Roots of all four lower incisors are totally resorbed.
As to dental status, teeth were small and fragile. From 7 to 16 years the patient had undergone dental treatment because primary tooth resorption was deficient and the permanent teeth erupted exceptionally slowly. Several primary teeth had to be extracted to ensure eruption of permanent teeth. There was hypomineralization of both decidual and permanent teeth, leading to a diagnosis of amelogenesis imperfecta. The orthopantomography did not reveal congenitally missing teeth (Fig. 1), even though upper lateral incisors were lost at age 14, as roots were short and thin.

The maxilla was hypoplastic both vertically and anteroposteriorly, the mandible was retrognathic. At the age of 15 years, the patient underwent orthodontic treatment with upper jaw fixed appliances to stimulate upper canine eruption in order to replace upper lateral incisors.

The mother of the index patient was 46 years old with normal psychomotor function. Height was 155 cm. Clinically, metacarpal length appeared normal, but no skeletal X-rays were available. She had oligodontia with five congenitally missing teeth. Teeth were small with brownish enamel and short roots. There was an eruption disturbance. The mandible was progenic and the maxilla was retrognathic and had been corrected surgically. She also had undergone orthodontic treatment.

3.2. Laboratory results

Given the marked phenotypic resemblance between the present index patient and the previously reported individuals with MDMHB, we used the previously described copy number assays based on quantitative PCR to assess the patient’s genomic DNA for the presence of the intragenic RUNX2 duplication (Fig. 2A) [Moffatt et al., 2013]. This confirmed the presence of an intragenic duplication in RUNX2, as three copies were found for two loci within intron 5 of RUNX2 (marked as 4 and 5 in Fig. 2A and B), but not for the other tested sites. These results differed from

![Fig. 2. Duplication in RUNX2. A. The genomic organization of the RUNX2 locus on chromosome 6. The locations analyzed by qPCR for copy number state are indicated by encircled numbers. The asterisks indicate loci that have a copy number state of three in the present patient. The DNA segment that is affected in French Canadian individuals with MDMHB is indicated [Moffatt et al., 2013]. The exact breakpoints of the duplication are not known in the present patient. B. The qPCR analysis in the patient shows copy number state three for locations 4 (Hs04907414_cn; location 45,412,249), and 5 (Hs06792104_cn; location 45,420,087), but copy number state two at locations 1 (Hs06157701_cn; location 45,272,961), 2 (Hs04922096_cn; location 45,292,671), 3 (Hs01529684_cn; location 45,332,959) and 6 (Hs00988269_cn; location 45,517,584). C. The cDNA sequence of wild type RUNX2 (upper panel) and RUNX2 with duplication of exons 3 to 5. The positions of the corresponding exons are shown by the numbers. The position of the primers used for PCR and sequencing of fibroblast cDNA is shown by arrows labeled as F and R. Only cDNA carrying the duplication will result in a PCR product. No PCR amplification occurs when primers anneal to the unmutilated cDNA (locations F’ and R’, in gray).]
those observed in French Canadian subjects with MDMHB, where a copy number state of three had been found for locus 3 (intron 2) and locus 4 (intron 5), but not for locus 5 (Fig. 2A).

We next evaluated cDNA derived from a skin fibroblast line from the patient. PCR amplification and sequencing using a forward primer in exon 4 and a reverse primer in exon 3 in the subject’s cDNA resulted in a PCR product in which the sequence of exon 5 was followed by the sequence of exon 3 (Fig. 2C). This proved that the duplication encompassed exons 3 to 5 and demonstrated the forward orientation of the duplication.

4. Discussion

Here we report on a Finnish woman who had characteristic features of MDMHB, with metaphyseal flaring, enlargement of the medial halves of the clavicles maxillary hypoplasia, brachydactyly and structural tooth defects. Like the previously described French—Canadian individuals with MDMHB, this woman had an intragenic RUNX2 duplication that included exons 3 to 5 [Moffatt et al., 2013].

The sequence analysis of RUNX2 cDNA from the proband’s skin fibroblasts showed that the duplication included exons 3, 4 and 5 and thus indicate that the duplication breakpoints were located in intron 2 and intron 5. We had made the same observations when analyzing RUNX2 cDNA from French Canadian individuals with MDMHB [Moffatt et al., 2013]. Nevertheless, the copy number variation analysis in genomic DNA demonstrated that the exact location of the intragenic RUNX2 duplication differed between the Finnish proband and the French Canadian family, as the intronic loci include in the duplication were not identical [Moffatt et al., 2013]. This establishes that the duplication has arisen independently in the Finnish proband and the French Canadian family with MDMHB, in accordance with the fact that there was no family relationship.

As the consequences of the intragenic RUNX2 duplication on the transcript level were identical between the Finnish proband and French Canadian individuals with MDMHB, the functional consequences of the two duplications will be similar. The sequence encoded by the three duplicated RUNX2 exons includes the functionally essential QA and runt domains of RUNX2 [Yoshida et al., 2002]. Our previous mechanistic studies suggest that this duplication leads to a gain of function [Moffatt et al., 2013]. The concept that this mutation leads to a gain of function in RUNX2 is in line with the observation that some characteristics of MDMHB are the opposite of what is commonly found in cleidocranial dysplasia (CCD), a condition that is caused by heterozygous loss of function mutations in RUNX2 [Hansen et al., 2011; Mundlos et al., 1997; Ott et al., 2010; Yoshida et al., 2002]. Whereas clavicles are hypoplastic or absent in CCD, MDMHB is associated with wide clavicles. The skull appears hypomineralized in CCD, whereas none of the participants of our previous study on MDMHB had brachydactyly [Moffatt et al., 2013]. Nevertheless, brachydactyly was noted as a feature in some of the individuals presented in the original description of MDMHB [Halal et al., 1982]. The mechanistic basis of brachydactyly in the presence of a gain of function mutation in RUNX2 is unclear at present.

In conclusion, we are reporting the first individual with MDMHB who is not of French Canadian origin. The findings demonstrate that the MDMHB phenotype results from an intragenic duplication of RUNX2 exons 3 to 5 also outside of the community where the disorder was first identified.

Conflict of interest statement

None of the authors declares a conflict of interest.

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