Bone 92 (2016) 145–149

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Case Report

Bone structure in two adult subjects with impaired minor spliceosome function resulting from *RNU4ATAC* mutations causing microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1)☆,☆☆

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ARTICLE INFO

Article history:
Received 5 January 2016
Revised 22 August 2016
Accepted 30 August 2016
Available online 31 August 2016

Keywords:
MOPD1
Taybi-Linder syndrome
RNU4ATAC
Osteodysplasia
HR-pQCT
Minor spliceosome

ABSTRACT

Microcephalic osteodysplastic primordial dwarfism type 1 (MOPD1), or Taybi-Linder syndrome is characterized by distinctive skeletal dysplasia, severe intrauterine and postnatal growth retardation, microcephaly, dysmorphic features, and neurological malformations. It is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the *RNU4ATAC* gene resulting in impaired function of the minor spliceosome. Here, we present the first report on bone morphology, bone density and bone microstructure in two adult MOPD1 patients and applied radiographs, dual energy X-ray absorptiometry, high-resolution peripheral quantitative computed tomography and biochemical evaluation.

The MOPD1 patients presented with short stature, low BMI but normal macroscopic bone configuration. Bone mineral density was low. Compared to Danish reference data, total bone area, cortical bone area, cortical thickness, total bone density, cortical bone density, trabecular bone density and trabecular bone volume per tissue volume (BV/TV) were all low. These findings may correlate to the short stature and low body weight of the MOPD1 patients. Our findings suggest that minor spliceosome malfunction may be associated with altered bone modelling.

1. Introduction

Microcephalic Osteodysplastic Primordial Dwarfism type I (MODP1), or Taybi-Linder syndrome [1,2], presents with distinctive skeletal dysplasia, severe intrauterine and postnatal growth retardation, microcephaly, central nervous system abnormalities, cataract, facial dysmorphism, sparse thin hair and dry skin [3]. MOPD1 is caused by biallelic mutations in the *RNU4ATAC* gene encoding the small nuclear RNA (snRNA) U4atac. The patients often die in early childhood. At present, approximately 42 children with MOPD1 and ten different *RNU4ATAC* mutations have been reported [4]. Radiological findings in infants with MOPD1 include dysplasia of the osseous skeleton with cleft vertebral arches, horizontal acetabula and short and bowed long bones [5]. However, the MOPD1 phenotype of adults, including bone phenotype, is previously unreported.

Two splicing mechanisms are present in eukaryotic cells. While the majority of introns, U2-type introns, are removed by the major spliceosome, the human genome contains around 800 U12-type introns, spliced by the slower minor spliceosome [6]. The U12 introns are found in 563 genes, typically containing only a single U12-type intron, surrounded by U2-type introns [7] and the resulting gene products are involved in a broad variety of cellular functions as they are found, according to the U12 Intron Database [8].

The pathophysiological background of MOPD1 is impaired function of the minor spliceosome as the *RNU4ATAC* gene encodes the snRNA U4atac, one of five snRNAs constituting the minor spliceosome [9]. Functional assays show that mutations in *RNU4ATAC* reduce U12 dependent splicing activity by >90% [10].

In our clinic, two adult siblings were diagnosed with MOPD1. Their short height prompted us to study the bone morphology and microarchitecture in this rare condition in order to explore the role of minor spliceosome function in relation to bone structure. We applied dual energy X-ray absorptiometry (DXA) scans and high-resolution peripheral quantitative computed tomography (HR-QCT) and identified abnormal bone microstructure. Biochemical analyses were performed in order to exclude common conditions affecting bone metabolism.

☆ The authors declare no conflict of interest.
☆☆ The work is not supported by any grants.
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http://dx.doi.org/10.1016/j.bone.2016.08.023

2. Patients and methods

2.1. Patient material

The two siblings, age 17 and 24 years, diagnosed with MOPD1 are the second and third child of healthy non-consanguineous Caucasian parents with an otherwise unremarkable family history. The family includes a 29 year old, unaffected sister. Both patients presented with pre- and postnatal growth retardation (-4SD), microcephaly, developmental delay, cataract, hearing loss and dysmorphic features and they did not report of any previous fractures. The siblings are the first subjects with MOPD1 reported to have survived into adult life [11].

The study was approved by The Ethic Committee, Region of Southern Denmark (Project ID: S-20130058) and participants gave signed informed consent.

2.2. Mutation analysis

Genomic DNA from the patients and the parents were analysed at the Institute of Genetics & Molecular Medicine, University of Edinburgh, UK [11]. The *RNU4ATAC* gene was screened by bidirectional Sanger sequencing and analyses were performed using Mutation Surveyor (Softgenetics Inc.). The findings were validated by bidirectional Sanger sequencing at the Department of Clinical Genetics, Odense University Hospital, using SeqMan Pro v.12.0, DNA Star.

2.3. Bone parameters and body composition

Radiographs of radius, ulna, tibia and fibula, and proximal femur were obtained in two projections. Areal bone mineral density (aBMD) was measured at the lumbar spine (L1–L4), total hip and the femoral neck using DXA (Hologic Discovery, Waltham, Massachusetts, USA). *Z*-scores and T-scores were calculated using the reference range provided by the manufacturer and the Third National Health and Nutrition Examination Survey reference [12]. Body fat per cents were evaluated by whole body scans.

A HR-pQCT system (XtremeCT, Scanco Medical AG, Brüttisellen, Switzerland) was used to assess bone geometry, volumetric bone mineral density (vBMD) and microarchitecture of the non-dominant distal radius and tibia. Measures of total, cortical and trabecular microarchitecture were computed using the standard manufacture software [13]. Standardized algorithms were used to separate the bone into cortical and trabecular compartments, calculate trabecular bone volume per tissue volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular spacing (Tb.Sp) as previously described [14]. Lastly, images were used as input in a finite element analysis (FEA) using software provided by the manufacturer (μFE element analysis solver v.1.15, Scanco Medical, Brüttisellen, Switzerland) in order to estimate bone strength [15]. The manufacturer phantom was scanned daily for quality control (QRM, Möhrendorf, Germany).

2.4. Biochemical evaluation

Blood samples were collected at 8 am (not fasting) and biochemical evaluation, including measurements of bone turnover markers Procollagen Type 1 N-Terminal Propeptide. (PNP1) and collagen type 1 cross-linked C-telopeptide (CTX), was performed with automated techniques in an accredited laboratory including use of liquid chromatography-mass spectrometry (LC-MS) technique, Architect c16000 (Abbott Diagnostics) and Cobas 4800 (Roche Molecular Diagnostics).

2.5. Candidate genes with U12 dependent splicing activity

Phenolyzer (http://phenolyzer.usc.edu/) and Phevor [16] software were applied to search for disease associated candidate genes among the 563 genes predicted to be affected by malfunction of the minor spliceosome. The software tools use phenotype terms to weight genes by the chance of being associated with the specified phenotype. The following terms were used for input: postnatal growth retardation; growth retardation; dwarfism; dwarfism microcephalic osteodysplastic primordial dwarfism; microcephaly; intrauterine growth retardation; skeletal dysplasias; short stature; insulin like growth factor I deficiency (IGF-1).

3. Results

The MOPD1 patients were shown to be compound heterozygous for a n.40C > T nucleotide substitution and a 85 base tandem duplication (n.17_101dup) in *RNU4ATAC* (NR_023343.1) which results in an insertion of a 85 base pair long sequence in position n.101. The n.40C > T mutation is extremely rare in the background population (ExAC minor allele frequency $< 1 \times 10^{-4}$) and predicted to disrupt the 5′ stem I loop of the snRNA U4atac as the n.40C is one of four bases stabilizing this essential loop [11]. The other mutation, a novel 85 base pair insertion in position n.101, is also predicted to have a major impact on conformation by destroying the 3′ stem I loop (*in silico* predictions made by Protein Data Bank 3SIU and PyMol v.1.7 software). The parents were each heterozygous carriers for one of these mutations confirming a cis-configuration of the mutations in the patients. The father was carrier of the 85 base pair long tandem duplication at n.101 and the mother was carrier of the n.40C > T mutation.

The two cases with MOPD1, female and male, age 24 and 17 years, respectively, presented with short stature of 142 and 143 cm and body mass index of 18.3 and 16.1 (kg/m^2), for the female and male patient, respectively (Table 1). The MOPD1 patients had fat percent of 36.3% and 20.8% and a lean body mass of 23.07 kg and 25.92 kg for the female and male patient, respectively. The height and weight of the parents were within the normal range.

Radiographs of radius, ulna, femur and tibia showed normal bone morphology including normal metaphyses (Supplementary Figs. 1–4). Results of the bone DXA scans are summarized in Table 2. The MOPD1 patients had low total bone mineral density, and Z-scores varied between −2.0 and −3.3 SD and −3.3 and −3.7 SD in the female and male patient, respectively. The father, age 50, also had a low bone mineral density with T-scores of −2.0 SD in the lumbar spine, −2.8 SD in the femoral neck and −2.4 in the hip. The mother, age 46, had normal bone mineral density. HR-pQCT results from scans of radius and tibia revealed that both MOPD1 patients had low values of cortical bone area, cortical thickness, total bone density, cortical bone density, trabecular bone density and trabecular bone volume per tissue volume (BV/TV) compared to age- and gender matched normal material [17,18] (Table 3). Estimated bone strength in both tibia and radius showed significantly lower failure load in cases compared to age- and sex matched normal values. For the female MOPD1 patient the estimated failure load in radius and tibia were 2377 and 5166 N compared to a mean of 3993 and 10,923 N, respectively, in the normal population [17]. For the male MOPD1 patient, the estimated failure load in radius and tibia were 1879 and 6307 N compared to a mean of 3009 N and 7957 N, respectively, in the normal population [18].

Biochemical evaluations are shown in Table 4. Normal levels of parathyroid hormone and thyroid stimulating hormone were seen in all family members. The male patient had a high level of follicle stimulating

Table 1
Clinical findings.

	Female patient	Male patient	Mother	Father
Gender	F	M	F	M
Age (years)	24	17	46	50
Height (cm)	142	143	169	180
Weight (kg)	37.6	33.0	69.8	66.0
BMI (kg/m^2)	18.3	16.1	24.1	20.3