Deficiency for the ER-stress transducer OASIS causes severe recessive osteogenesis imperfecta in humans

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Abstract

Osteogenesis imperfecta (OI) is a clinically and genetically heterogeneous brittle bone disorder. Whereas dominant OI is mostly due to heterozygous mutations in either COL1A1 or COL1A2, encoding type I procollagen, recessive OI is caused by biallelic mutations in genes encoding proteins involved in type I procollagen processing or chaperoning. Hitherto, some OI cases remain molecularly unexplained. We detected a homozygous genomic deletion of CREB3L1 in a family with severe OI. CREB3L1 encodes OASIS, an endoplasmic reticulum-stress transducer that regulates type I procollagen expression during murine bone formation. This is the first report linking CREB3L1 to human recessive OI, thereby expanding the OI gene spectrum.

Keywords: Osteogenesis imperfecta, Type I collagen, OASIS, CREB3L1, Endoplasmic reticulum stress

Background

Osteogenesis imperfecta (OI) is a genetically heterogeneous brittle bone disorder with varying degrees of clinical severity, ranging from perinatal lethality to generalized osteopenia [1]. The predominant autosomal dominant forms display mutations in either COL1A1 or COL1A2, encoding the α1- and α2-chains of type I procollagen, while rarer autosomal recessive forms mostly result from defective endoplasmic reticulum (ER)-resident proteins involved in post-translational processing or chaperoning of these α(I)-chains [1,2]. Processing defects prevent normal collagen fibrillogenesis and on biochemical analysis often show perturbed modification of the collagen α-chain. Known defects include biallelic mutations in LEPRE1 [3-5], CRTAP [5,6], PPIB [7,8], BMP1 [9,10], and PLOD2 [11]. Mutations in chaperones (including Hsp47 (SERPINH1) and FKBP10) impair intracellular collagen trafficking with intracellular retention or aggregation of collagen molecules and show dilation of the ER on electron microscopy, resulting in OI or related phenotypes [12-14]. Finally, rare other defects linked to distinct mechanisms involve the transcription factor osterix (SP7) [15], pigment epithelium derived factor (SERPINF1) [16] and transmembrane protein 38B (TMEM38B) [17,18]. A recurrent mutation in a gene encoding the Interferon-inducible transmembrane protein 5 (IFITM5), which is involved in bone growth during prenatal murine development, was recently shown to cause autosomal (AD) dominant OI [19-21]. Recently, heterozygous and homozygous mutations in WNT1 (WNT1), which is a key signalling molecule in osteoblast function and bone development, were shown to underlie certain forms of AD early-onset osteoporosis and AR OI, which was in some patients associated with severe intellectual disability [22-26]. However, a small proportion of OI patients remain molecularly unexplained.

Findings

We describe a Turkish family (Figure 1A) with three sibs, two of whom were affected by severe OI (written informed consent of the family was obtained and the study was approved by the Ethics Committee of the Ghent University Hospital (Ghent, Belgium)). Consanguinity was not reported, but the parents originated from neighbouring villages. The first affected child (III:3) developed several fractures in utero and was small for
gestational age. His birth length was 40 cm (<P3). At the first day of life he was hospitalized for hyperbilirubinemia and O-bain-like deformities, soft calvarial bones and widely open fontanelles were noticed. He developed several fractures after birth and multiple fractures healed with extremity deformities. He also had a right inguinal hernia. X-rays showed beaded ribs, callus formation and multiple fractured tubular bones with an accordion-like broadened appearance. He was hospitalized several times due to recurrent constipation and pulmonary infections (bronchopneumonia). During this period, he developed abdominal distention and hepatomegaly, the latter due to cardiac insufficiency. No signs of T-cell dysfunction or other immune deficiencies have been noted. He died at 9 months of age. The second affected sib (III:4, Figure 1A) was a male foetus from a pregnancy that was medically terminated at 19 weeks of gestation. Post-mortem examination showed bowed extremities and pes equinovarus. C. X-rays of foetus III:4 revealed beaded ribs and multiple fractures of tubular bones.

Biochemical (pro)collagen SDS-PAGE analysis was performed on the medium and cellular fractions of cultured skin fibroblasts of foetus III:4. No obvious quantitative or qualitative abnormalities of 14C-labelled type I procollagen (data not shown) and mature secreted and intracellular type I collagen (Figure 2A) were detected. Subsequently, all known OI genes (COL1A1, COL1A2, BMP1, LEPRE1, CRTAP, PPIB, PLOD2, SERPINH1, FKBPI0, SP7, SERPINF1, TMEM38B, IFITM5 and WNT1) were sequenced by direct Sanger sequencing (ABI3730XL automated sequencer, Applied Biosystems), but no causal mutation(s) were detected.

We selected the CREB3L1 gene [GenBank:NM_052854.2], encoding the ER-stress transducer OASIS (Old Astrocyte Specifically Induced Substance), as an excellent candidate gene based on the observation that OASIS−/− mice were born with severe osteopenia and spontaneous fractures.
Figure 2 (See legend on next page.)
through activating OASIS was shown to be crucial for bone formation [29], reminiscent of severe human OI. In those mice, DGKZ (arr11p11.2(46268141−46359490)x0, Figure 2B) [30,31]. Whereas the arr11p11.2(46268141−46359490)x0 homozygous deletion was not reported before, heterozygous deletions or gains of this genomic region are described in the Decipher database [32] and the Database of Genomic Variants [33] but encompassing large genomic regions comprising multiple genes (6 to 86 genes and/or multiple chromosomal abnormalities) which, in some cases, are associated with intellectual disability. Both parents and the healthy sister were heterozygous for the deletion (data not shown). DGKZ encodes diacylglycerol kinase zeta, an ubiquitously expressed enzyme that is most abundantly present in the brain, thymus and skeletal muscle [34] and which has a regulatory role in T-cell receptor signalling and T-cell activation [35]. Two different isoforms (DGKζ1 in immune cells and DGKζ2 in other cells) are known, in which exon 1 is either present or absent and which have a tissue- and developmental stage-specific expression [28]. Hitherto, no known function in bone formation has been ascribed to DGKζ and thus a possible contributing role to (the severity of) the bone phenotype of patient III:3 and foetus III:4 cannot completely be excluded. Expression analysis by real time-quantitative PCR (RT-qPCR) on total RNA isolated from dermal fibroblasts of foetus III:4 confirmed complete absence of the CREB3L1 transcript. In order to investigate the expression of the two DGKζ isoforms (DGKζ1 and DGKζ2), two different primer pairs were designed, of which one was specific for exon 1 that is only present in the DGKζ1 isoform. RT-qPCR experiments revealed no amplification for the primer pair specific for exon 1 in cultured dermal fibroblasts, suggesting that the DGKζ1 isoform is not expressed in these cells. For the second primer pair normal DGKζ2 expression was observed, which implies normal expression of the DGKζ2 isoform in cultured human dermal fibroblasts (Figure 2C). RT-qPCR analysis of the ER-stress markers BiP, CHOP and the spliced form of XBP1 showed levels comparable to controls, even after stimulation of confluent fibroblasts for 4 hours with the ER-stress inducers Tunicamycin (Tu, 10 μg/ml, Sigma-Aldrich) and Thapsigargin (Th, 1 μM, Sigma-Aldrich) (Figure 2C). This is in accordance to the observations in OASIS−/− mice. The expression level of CREB3L1 was unchanged in control fibroblasts after treatment with Tu and Th (Figure 2C), suggesting that OASIS does not play a major role in the ER-stress pathways previously linked to disease pathogenesis [1]. Additionally, our finding that type I (pro)collagen production is normal in human dermal fibroblasts (Figure 2A) confirms that OASIS has a tissue-specific effect on type I (pro)collagen production [29].
Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
Conceived and designed the experiments: SS, PC. Identified and recruited human subjects, obtained ethical approvals, coordinated collection of samples, and provided clinical information: FM, BC, HK, ADP. Performed the experiments: SS, SD, AD, WS. Analyzed the data: SS, PC. Wrote the paper: SS, FM, BC, ADP, PC. All authors have read and approved the final manuscript.

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