INTRODUCTION

Classical Osteogenesis Imperfecta is caused by mutations in the genes that encode type I collagen, COL1A1 and COL1A2. Affected individuals have growth deficiency and susceptibility to fracture from minimal trauma. The Brtl mouse, a model for moderately severe OI, has a point mutation in one Col1a1 allele that results in a G349C substitution in half of its type I collagen α1(I) chains. Brtl mice recapitulate the molecular, biochemical and phenotypic features of type IV OI, including osteoporosis with decreased trabecular number and cortical cross-section [1]. Brtl has a smaller femur than wild-type mice throughout the growing period, with decreased moment of inertia, predicting fracture at a lighter load [2]. However, 4-point bending tests show no significant difference in breaking strength We previously demonstrated that post-pubertal improvement in Brtl femoral strength and stiffness occur through changes in bone material properties. Raman spectroscopy demonstrates increased mineral to matrix and decreased carbonate to phosphate ratios in Brtl bone. Decreased carbonate-phosphate ratios are associated with a decreased susceptibility to fracture. These results suggest that changes in the mineral phase may contribute to the improved material properties of Brtl bone.

METHODS

To investigate the role of the mineral component in OI bone pathology, we analyzed bone mineralization in Brtl femurs using density distribution (BMDD) and FTIR and quantitated expression of genes involved in matrix mineralization. BMDD parameters were measured by quantitative backscattered electron imaging (qBEI) [3]. Different grey levels correspond to different calcium contents, because bone matrix is not homogeneously mineralized; the different calcium levels can be assembled into a histogram. For this study, two parameters were evaluated: CaPeak – which indicates the most frequently occurring calcium concentration, and CaWidth – the full width at half maximum of the distribution, which describes the variation in mineralization density. Normal healthy bone has heterogeneous mineral content, which reflects both older and newer bone. FTIR imaging was performed to determine bone mineral and matrix compositional properties at ~7μm resolution [4]. Osteoblasts from whole femurs and newborn calvaria were analyzed for mineral deposition and expression of genes involved in matrix mineralization.

RESULTS

Brtl mouse femurs show BMDD changes characteristic of OI, with significantly higher matrix mineralization (CaPeak) of both cancellous metaphyseal and mid-diaphyseal cortical bone than WT littersmates. Brtl cortical bone mineral heterogeneity (CaWidth) decreased at 6 vs 2 months (~ -8%). At 6 months, Brtl CaWidth was lower (~ -11%) than WT femurs. (Fig 1) Consistent with BMDD, FTIR on 2-month Brtl femurs yielded a trend toward increased mineral/matrix index in trabecular bone. Furthermore, alizarin red staining detected increased mineral deposition by Brtl vs WT cultured osteoblasts ± BMP2 treatment. (Fig 2) During differentiation of newborn calvarial osteoblasts in culture, Brtl Dmp1 and Phex transcripts, which act to stimulate mineralization, were increased vs WT cells after 5 days, and reached over 2x WT levels. Transcripts for Sost, which has an inhibitory role in normal mineralization, and Bmp2, a stimulator of osteoblast development, were decreased over 2-fold in Brtl vs WT calvarial osteoblasts during in vitro differentiation. RNA from whole femurs of Brtl vs WT 2-month mice reflects primarily the osteocyte component of bone cells. Measurement of transcripts of late osteoblastic and osteocyte markers by real time RT-PCR revealed significant increases of Dmp1 (1.8x) and Phex (1.3x) expression (Fig 3). On Osteogenesis PCI arrays, Bmp2 transcripts were decreased 2.4x, while Sost expression was unchanged in Brtl vs WT femurs (Fig 3). Finally, we addressed the question of whether the changes in gene expression were due to inherent cellular defects or resulted from interaction of cells with defective matrix. Preliminary cell-matrix exchange experiments support a contribution of matrix composition to altered Brtl mineralization, because expression of Dmp1, Phex, Sost and Bmp2 is altered in both WT and Brtl cells grown on Brtl matrix.

SIGNIFICANCE

This investigation changes our concept of the mineralization defect in OI from a simply passive process to one with both active and passive components. Because the mineral component is crucial to bone strength in the Brtl OI mouse, understanding the mechanism of OI mineralization may provide insights for enhancing the strength of OI bone.

REFERENCES