Makings of a brittle bone: Unexpected lessons from a low protein diet study of a mouse OI model

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Abstract

Glycine substitutions in type I collagen appear to cause osteogenesis imperfecta (OI) by disrupting folding of the triple helix, the structure of which requires Gly in every third position. It is less clear, however, whether the resulting bone malformations and fragility are caused by effects of intracellular accumulation of misfolded collagen on differentiation and function of osteoblasts, effects of secreted misfolded collagen on the function of bone matrix, or both. Here we describe a study originally conceived for testing how reducing intracellular accumulation of misfolded collagen would affect mice with a Gly610 to Cys substitution in the triple helical region of the α2(I) chain. To stimulate degradation of misfolded collagen by autophagy, we utilized a low protein diet. The diet had beneficial effects on osteoblast differentiation and bone matrix mineralization, but also affected bone modeling and suppressed overall animal growth. Our more important observations, however, were not related to the diet. They revealed how altered osteoblast function and deficient bone formation by each cell caused by the G610C mutation combined with increased osteoblastogenesis might make the bone more brittle, all of which are common OI features. In G610C mice, increased bone formation surface compensated for reduced mineral apposition rate, resulting in normal cortical area and thickness at the cost of altering cortical modeling process, retaining woven bone, and reducing the ability of bone to absorb energy through plastic deformation. Reduced collagen and increased mineral density in extracellular matrix of lamellar bone compounded the problem, further reducing bone toughness. The latter observations might have particularly important implications for understanding OI pathophysiology and designing more effective therapeutic interventions.

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Introduction

Osteogenesis imperfecta (OI), also known as the “brittle bone disease”, is a heterogeneous group of heritable bone disorders characterized by abnormal formation, deformities and fragility of bones [1]. Similar to other rare skeletal diseases, OI studies identify molecules and pathways essential for bone development in general and might provide valuable insights for treatment of more common pathologies [2]. Many novel OI mutations in different genes have been discovered in recent years [1,3,4]. Yet, most OI patients have autosomal dominant mutations in the genes coding for α1(I) and α2(I) chains of type I collagen, which is the main building block of organic bone matrix. Furthermore, most cases of severe OI result from substitutions for an obligatory Gly in the sequence of repeating Gly–X–Y triplets of type I collagen triple helix (X and Y are variable amino acids in this sequence).

Hundreds of substitutions for Gly have been described (http://www.le.ac.uk/ge/collagen/), but it is still unclear what makes some of them more severe than others and why the same substitution might result in mild, severe or lethal outcome in different patients [1,5]. It is unclear whether Gly

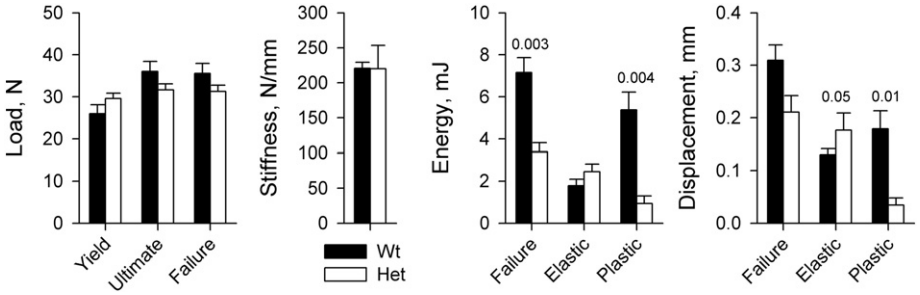

Fig. 1. Key mechanical parameters of right femurs from Wt (N = 5) and Het (N = 5) RP diet animals in 4-point bending tests. Yield, failure, and ultimate loads represent the applied force at the beginning of non-elastic (non-linear) displacement, at bone failure, and at maximum applied force prior to failure, respectively. Stiffness is the slope of the load vs. displacement curve within the elastic (linear) region. Energies are the areas under the load vs displacement curve within the linear (Elastic) and post-yield (Plastic) regions as well as overall (Failure), which represent the work of overcoming elastic, plastic and total resistance of bone to the applied forces. Hereafter, the error bars show the standard error of the mean value (SE) and the numbers above the error bars show statistically significant ($p < 0.05$) and borderline ($p < 0.1$) p-values. The p-values show the more conservative of two estimates: (i) two-way ANOVA (presumes equal variances) and (ii) two-tail heteroscedastic Student's t-test (presumes unequal variances). Full results of the statistical analysis are presented in Supplementary Table S1.

substitutions cause OI by altering differentiation and function of bone-producing cells (osteoblasts) that synthesize collagen, by affecting collagen function in bone matrix outside the cells, or by doing both [1]. It is also unclear how the underlying molecular processes affect the amount, geometry and density of bone as well as why OI bones are brittle.

To test the role of osteoblast malfunction in OI, we conducted a pilot study of a low protein (LP) diet effect on heterozygous Col1a2$^{tm1Mcbr/J}$ (G610C) mice. This mouse model has a Gly610 to Cys substitution in the triple helical region of the α2(I) chain of type I collagen, resulting in moderate OI [6]. It is commercially available from Jackson Laboratories and has been utilized in several OI studies [6–10]. G610C mice mimic the mutation and OI phenotype found in a large group of related patients from an Old Order Amish community in Lancaster County, PA. As expected for most Gly substitutions [11], the G610C mutation disrupts folding of the procollagen precursor of type I collagen and causes accumulation of misfolded procollagen in the Endoplasmic Reticulum (ER), resulting in cell stress and osteoblast malfunction [7]. LP diet was selected as a simple approach to activating autophagy [12] with an expected additional effect of reducing overall procollagen synthesis (and therefore misfolding) by osteoblasts. Since earlier studies suggested that autophagy might play a key role in degradation of misfolded procollagen in OI [7,13,14], our objective was to see how an LP diet would affect the bone material before initiating more complex studies of more precise autophagy targeting. We combined multiple approaches to analyzing bone material properties in order to distinguish different LP diet effects on bone synthesis, structure and mineralization. We found closer to normal mineralization of bone matrix in G610C mice, although other diet effects reduced the net bone strength. Most surprisingly, we observed a long-lasting beneficial effect of the LP diet on bone marrow stromal cells from the treated G610C animals after the cells were cultured in vitro at normal conditions without nutrient deprivation. Based on the observed LP diet effects, we are now conducting a more refined study of autophagy manipulation with genetic tools.

In the present paper, however, we focus primarily on our other, unanticipated findings that might have more general and important implications for understanding mechanisms of bone fragility in OI irrespective of the dietary treatment. Specifically, we discuss how the mutation disrupts the bone modeling process, affects matrix composition and mineralization, and reduces bone toughness.

Results

LP diet treatment

At 8 weeks of age, 5 wild type (Wt) and 5 heterozygous G610C (Het) male animals were put on 18% protein (regular protein, RP) diet. At the same time, 5 Wt and 5 Het male animals were placed on 8% protein (low protein, LP) diet. The latter diet was supplemented with Met, Phe, Tyr, Trp, and carbohydrates and had the same, 3.8 kcal/g energy content. At the start of the diets, the animals were injected with calcein, a fluorescent dye which binds to mineralized bone surfaces. The animals were given a second calcein injection at 11 weeks of age and a third calcein injection at 16 weeks of age. They were sacrificed at 17 weeks of age while remaining on the 18% and 8% protein diets.