New Types and New Approaches to OI  
Chicago, IL  
April 27-29, 2008

A two-day meeting, April 27-29, 2008, brought together more than 70 clinicians, clinical investigators, geneticists, biochemists, biologists, and molecular biologists to discuss the rapidly emerging data concerning the newly recognized recessively inherited forms of osteogenesis imperfecta (OI).

The prevalence of OI is about 1/10,000 which translates to 30,000 individuals with this disorder in the United States. The majority of individuals have a relatively mild form of the condition and endure a few to dozens of fractures throughout their lifetimes. For the remainder, the condition entails varying degrees of disability that range from the need for assists while walking, to the use of motorized wheelchairs or carts throughout their lifetimes. The incidence of OI is a little higher due to pregnancies with infants that die in the perinatal period from pulmonary insufficiency as a result of marked underdevelopment and fracture of bones of the chest. More than 90% of individuals with OI have mutations in one of the two genes (COL1A1 and COL1A2) that encode the chains of type I collagen and these mutations are either inherited in a dominant fashion through several generations or arise de novo in the first affected infant in a family. Unexpected recurrence of the OI phenotype in families in which affected siblings are born to unaffected parents was first attributed to recessive inheritance but careful molecular genetic studies identified parental mosaicism as the source of recurrence in both severe and milder forms of OI. Nonetheless, the idea that recessive forms of OI existed persisted. In rare instances, mutations in type I collagen genes were identified in both parents of children with more apparent forms of OI (DePaepe, Ghent), in consanguineous families, but this proved to be an uncommon example of recessive forms of OI. Indeed, the very first of the mutations in type I collagen genes in a family with OI was identified in a child born to a consanguineous couple in Germany (Pope, London). Such mutations have proved to be rare in subsequent studies.

Clues to the basis of recessively inherited forms of OI emerged from studies in animals and, very recently, in the identification of two genes (CRTAP, LEPRE1) with mutations in humans. CRTAP was identified as a candidate following its cloning from chick as a protein expressed in cartilage and the creation of a knockout mouse with a dramatic bone phenotype similar to that seen in moderate to severe OI (Morello, Baylor). Taking advantage of studies in a First Nations Canadian family that linked a recessively inherited form of OI (Glorieux, Montreal Shriners Hospital) designated as OI type VII and characterized by rhizomelic shortening and fractures with coxa vara, to the region found to contain the CRTAP gene, a mutation was identified in that family and in another with a lethal form of OI (Morello). In both, the extent of hydroxylation of the prolyl residue at position 986 of the triple helix of the \( \alpha_1(I) \) chains of type I collagen was reduced (OI type VII) or absent (severe lethal form). At the time of mutation characterization, the CRTAP protein had been recognized to be part of a 3-protein complex that included the product of the LEPRE1 gene (McCarthy, LSU Shreveport), prolyl 3-hydroxylase 1, and CYPB, prolyl cis-trans isomerase (Vranka, Portland Shriners Hospital). The recognition that these proteins interacted suggested that mutations in all three genes might result in forms of OI. Analysis of additional families identified more than 10 with mutations in the CRTAP gene,
almost all in infants that survived only days to weeks, but with two that had hypormorphic alleles and survived longer (Marini, NIH and Baldridge, Baylor, Malfait, Ghent). Mutation analysis of the \textit{LEPRE1} gene provided more than twice the number of families with mutations and also led to the recognition of population specific alleles (Marini, NIH and Baldridge, Baylor). Perhaps most striking was the identification of a unique splice site alteration in the \textit{LEPRE1} gene among African Americans and West Africans (Marini and Baldridge). Analysis of samples from anonymous African Americans in the Washington/Baltimore/Philadelphia area determined that the frequency of the mutant allele was about 1/200 (Marini) while analysis of a different population on the West Coast of the US suggested a frequency about twice that rate. These findings suggested that non-African admixture could lead to significantly lower frequencies and analysis of West African DNA samples from Ghana identified groups with frequencies of the mutant allele of 2-3%. The frequency of the severe forms of OI is unknown in that region but the high carrier frequency suggestion a birth frequency of about 1/20,000. It is not clear if heterozygote advantage or simply genetic drift fueled the high allele frequency. Several other mutant alleles have been identified in some populations, including the Irish Travelers. Mice that have no expressed \textit{Lepre1} have a mild bone phenotype and no real evidence of OI (Vranka). The reason for the difference is unclear.

The understanding of the pathogenesis of this form of OI is still at the beginning. In severe forms of OI, premature termination codons lead to loss of mRNA from one of the genes but analysis of both CRTAP and P3H1 in cells indicates that there is reciprocal loss so that both proteins are not apparent (Marini, Baldridge, and Pyott, University of Washington). The mRNA levels are not modified, but the proteins could not be restored by treatment with proteosome inhibitors, suggesting that the site of loss or degradation may be lysosomal or in the ER itself (Pyott). The reciprocal loss of proteins is consistent with the difficulty in distinguishing the phenotypes that result from mutations in the two genes, and, indeed, distinguishing them from those with mutations in type I collagen genes (Krakow).

The \textit{LEPRE1} gene encodes a protein that has tetratricopeptide motifs and is homologous to the prolyl 4 hydroxylases. CRTAP is homologous, but shorter and has lost the hydroxylation domain. The CYPB protein is unrelated to the other two but is well characterized as a prolyl cis-trans isomerase and is essential (Bachinger). These three proteins form a complex in a 1:1:1 ratio, although there also is an abundance of free CYPB. In the absence of either CRTAP or P3H1 protein, the 3-hydroxylation of the single prolyl residue does not occur and the thermal stability of the collagen is slightly higher, compatible with an increase in other forms of post-translational modification. The amount of type I procollagen produced may be higher but that remains unsettled (Morello, Marini). The structure of the trimeric collagen peptide domain differs such that the 3-hydroxyproline protrudes more into the local space than the unhydroxylated residue and could interact with other macromolecules, including decorin which is known to bind in that region (Bachinger). Attempts to express CRTAP and P3H1 separately have been compromised by their tendency to aggregate and precipitate (Bachinger). Thus crystal structure studies that identify the nature and location of interaction may have to await analysis of the complex. Of note is that a missense mutation in CRTAP (p.Leu67Pro, which occurs in the remnants of one of the interacting domains) almost completely ablates stability of both proteins, providing some evidence of a site of interaction (Pyott).

Two recessive mouse models have been relatively well studied. In the \textit{oim/oim} strain a mutation near the 3’end of the coding domain of the mouse \textit{Col1a2} gene results in a frame shift and loss of ability of the pro\alpha2(I) chains to interact with pro\alpha1(I) chains. As a consequence,
cells produce trimers that contain only proα1(I) chains (Phillips, University of Missouri). Like the human counterpart, who died at age 18 from complications unrelated to his moderately severe OI (Pope), they have an OI phenotype, in contrast to people in whom nonsense or frameshift mutations in the COL1A2 gene result in an EDS-like clinical picture. This suggests that the protein degradative machinery could influence the manner in which genotype is reflected in phenotype. Thus, in other forms of OI such as those related to mutations in CRTAP or LEPRE1, the degradation of the reciprocal proteins could influence the phenotype (Marini). Other mutations in the Col1a1 gene in mice can activate the unfolded protein responses and contribute to apoptosis of osteocytic cells (Lisse, NIH) and could point to a pathway that might help understand pathogenesis. Two other homozygous mouse models of OI-related phenotypes point to additional considerations for pathogenicity. The fro/fro mouse, which is homozygous for a mutation that inactivates the Smpd3 gene, which encodes a sphingomyelin phosphodiesterase, has a significant and variable OI-like phenotype (Poirier, University of Georgia). The relationship between the mutation and the phenotype has not yet been worked out but could involve defects in collagen transport. Homozygosity for a missense mutation in the Col1a1 gene that substitutes a cysteine for a triple helical glycine has the unexpected effect of resulting in a milder phenotype than in the heterozygotes (Forlino, University of Pavia and NIH) and is in contrast to a human example in which homozygosity for a COL1A2 mutation in humans that leads to substitution of serine for glycine in the triple helical domain is far more severe than in the heterozygotes (DePaepe). This could reflect the special nature of cysteine and its ability to produce local stability through interchain crosslinks when in the proα1(I) chains. Other recessive OI-like clinical conditions have been identified and in some mutations have been identified. Most notably, these include osteoporosis-pseudoglioma syndrome (OPPG) that results from recessive mutations in the LRP5 gene (dominant mutation result in increased bone mass), and Bruck syndrome of OI and contractures that appear to result from mutations in a lysyl hydroxylase gene that encodes only the enzyme that alters the hydroxylation of telopeptide lysyl residues involved in crosslink formation, and juvenile Paget disease that results from mutations in the osteoprotegrin receptor gene (Warman, Boston Children's Hospital).

Therapeutic intervention in children or adults with different forms of OI has not yet reached a consensus approach. Bisphosphonates have become the most commonly used drugs, with the hope that decreased bone resorption would favor long term maintenance of normal rather than abnormal bone. There is relatively little knowledge of use of these drugs in infants with recessive forms of mutation outside the First Nations group with the original OI type VII phenotype. In them there was a drop in the N-terminal propeptide, similar to what was seen in other children with OI and increase in BMD in the lumbar spine with increased cortical thickness in iliac crest biopsies but no change in ultimate height or fracture incidence (Rauch, Montreal Shriners Hospital). In one infant among the Irish Travelers, treatment with bisphosphonates was associated with survival beyond infancy (Baldridge). Surgical intervention is not easy and probably best reserved at this point for anatomic deformities that are truly disabling from a functional perspective (Smith, Chicago Shriners Hospital). The use of other types of therapy, including stem cell infusion, seem distant hopes at this point (Niyibizi, Penn State; Horwitz, CHOP) although infusion of a stabilized and modified component of the prolyl 3-hydroxylation pathway would be a consideration (Whyte, Washington University) for some of the recessive forms. The role of medications that might influence the neural control of bone mass cannot be excluded (Karsenty, Columbia University).
This meeting provided the first opportunity to synthesize our understanding of recessively inherited forms of OI, to examine the range of clinical presentation, to understand the roles of the enzymatic systems involved, and to examine how insights gained from both animal and human studies might lead to more effective treatments of recessive form of OI and of the more common dominantly inherited forms.

**Plans for publication of the conference results**

The meeting’s outcomes will be disseminated through the OI Foundation quarterly newsletter, *Breakthrough* (dist. 4,000), and on the OI Foundation website, www.oif.org (more than 240,000 visitors annually), and through targeted distribution of the full meeting summary to participants and interested parties.