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Genetic Mutation Explains Form of Brittle Bone Disease

A newly identified gene mutation helps explain a subset of cases of osteogenesis imperfecta (OI), or brittle bone disease, whose origin had until now remained mysterious. Identifying the new mutation is important because children with the disorder, whose bones break easily, are sometimes mistaken as victims of child abuse—particularly those who do not carry the genetic mutation known to cause most cases of brittle bone disease.

Most cases of brittle bone disease are known to be caused by a structural change in a particular collagen protein. The new mutation—responsible for up to 15 percent of cases—acts differently and prevents collagen proteins from being properly modified after they are produced. The finding may also offer clues to the causes of as-yet-undescribed connective tissue diseases that may affect other parts of the body and gives insight into the basic mechanism of collagen formation, said the researchers.

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— **Brendan Lee**

In an article published in the October 20, 2006, issue of the journal *Cell*, Howard Hughes Medical Institute investigator Brendan Lee and his colleagues reported how mutations in a gene called cartilage-associated protein (*CRTAP*) can affect bone formation. Lee and his colleagues at the Baylor College of Medicine collaborated on the studies with researchers from Istituto Nazionale per la Ricerca sul Cancro in Italy, Shriners Hospital for Children in Canada, McGill University, Oregon Health & Science University, the University of Washington, and the University of Rochester Medical Center.

The long protein fibrils that make up collagen serve as a principal support for skin, tendon, bone, cartilage and connective tissue. Immediately after the collagen protein is manufactured by cells, it undergoes several biochemical modifications to transform it into functional fibers. One of the least understood of these modifications is the addition of a molecular unit called a hydroxyl group to the collagen protein. This modification, known as prolyl

3-hydroxylation, occurs at only one position along the thousand-unit collagen protein fiber.

In previous studies, Lee and his colleagues had discovered that a protein known as *CRTAP* associates with the type of collagen that helps give structure to bone. To learn more about *CRTAP*'s role, Lee and his colleagues produced mice that lacked the *CRTAP* gene.

They found that without *CRTAP*, mice developed deformed and brittle bones similar to those of patients with brittle bone disease. In further studies, they found that the *CRTAP* protein interacts with the enzyme responsible for prolyl 3-hydroxylation of collagen and is required for this process to occur. Without *CRTAP*, they found, collagen structure in the mice was abnormal, Lee said.

Reasoning that the same mutation might cause brittle bone disease in humans, the researchers next analyzed *CRTAP* in two families who had brittle bone disease, but lacked the pattern of inheritance commonly associated with it. Most forms of brittle bone disease arise from mutations in the genes for the most abundant bone protein, called type I collagen. These mutations are spontaneous and dominant, not inherited from parents. The two families that Lee and his colleagues focused on, in contrast, showed a recessive form of the disease that was inherited. Other researchers had mapped this defect to the chromosomal region that contains the *CRTAP* gene.

By studying these families, Lee and his colleagues found that partial loss of function of the *CRTAP* protein caused mild brittle bone disease, and profound loss called a more severe form of the disease. This was the clincher, said Lee. We had produced a mouse model of osteoporosis stemming from clear and novel biochemical activity associated with the mutant *CRTAP* protein. And we had shown that there is an effect on the collagen fibers. And now we had shown that there is a clinical spectrum of brittle bone disease associated with differential loss of function of the protein.

Lee said that the new mechanism of brittle bone disease will have important diagnostic implications. In child abuse cases, a frequent question is whether the children have brittle bone disease that would cause frequent bone fractures, said Lee. Up until now, the only known genetic cause of brittle bone disease has been structural mutations in type I collagen. Now, we have explained a percentage of cases of brittle bone disease—perhaps as high as fifteen percent—that had all the biochemical hallmarks of the disorder but did not have type I collagen mutations. So this adds a new dimension in terms of DNA testing; clinical geneticists and pediatricians will have a new genetic test to determine whether children have this form of OI.

The finding opens up a whole new field in bone biology, because this has been an unappreciated mechanism of collagen modification, said Lee. Since prolyl 3-hydroxylation occurs in collagen throughout the body—including the heart, blood vessels and kidneys—defects in this process affecting these other tissues could be the cause of as-yet-undescribed connective tissue diseases, he said.

In further studies, Lee and his colleagues are exploring the role of prolyl 3-hydroxylation in such disorders. Also, they are using the new findings to understand the role of the process in collagen formation.