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## Broken Bones and Token Genomes: A Look at Type I Osteogenesis Imperfecta

Osteogenesis Imperfecta, or OI for short, is a genetic disease that receives little attention in the mainstream media even though OI's genetic defect causes brittle bones and a lifetime of worry that every minor injury could lead to hospitalization and bone fractures. However, in its most common and mildest form, Type I OI is manageable with a variable of treatments though no cure was classically available. Due to recent advances in science, however, there is hope to be had that personal medicine will finally replace diet and orthopedic surgery as methods of treatment and management, potentially leading to a cure.

Type I OI is usually caused by "mutations that introduce either premature-termination codon for translation or aberrant RNA splicing and that thereby reduce the expression of the COL1A1 [collagen-I] gene."[1] Similar mutations can arise in the COL1A2 gene, a gene that codes for an α chain that trimerizes with COL1A1's α chains to form triple coil of collagen. Although a COL1A2 mutation is rarer, either kind can render a particular allele functionally null, thereby leading to haploinsufficiency.[2] Several specific mutations have been implicated to lead to a Type I OI phenotype but no studies have had a large enough sample size to give a statistical analysis for the overall distribution of mutations. Although the distribution of particular mutations is lacking, it is known that the Type I OI phenotype has an autosomal dominant pattern of inheritance for the collagen-I alleles.[2] Thus, this disease has trans-generational implications because a child born to an Type I OI parent has a 50% of having the disease.

It is important to note here that anyone with Type I OI will be heterozygous for the relevant mutations. Collagen-I is so abundant in the human body that a homozygous COL1A1/2 mutations would likely lead to spontaneous abortion of the zygote. The collagen-I haploinsufficiency in the heterozygotes is itself particularly serious because collagen-I is found in the bones, skin, tendons, and the connective tissues that supports and connects the tissues to each other.[2] Therefore mutations in its gene lead to the body-wide effects seen in Type I OI patients. Somewhat fortunate, having half the wildtype amount of collagen only creates mild symptoms, the worst being a diathesis for bone fractures; the mutations present in other forms of OI code for poorer quality collagen, leading to more severe symptoms that can result in perinatal death and severe bone deformity.[3]

Beyond the mild bone fragility already mentioned, Type I OI's lack of a sufficient quantity of collagen often leads to blue sclerae (white part of the eye) because the underlying veins will show through the thinner sclerae.[4] In addition, slight spinal curvature, average or slightly shorter-than-average stature, muscle and joint weakness, decreased skin elasticity and distensibility, smaller craniofacial measurements, hearing loss in late adulthood, and social stigma are common symptoms of the disease which affects all sexes and ethnic/racial groups similarly.[2,3] Since Type I OI has no visible phenotypic effects beyond bone fractures and blue sclerea which may sometimes not be readily visible, OI is sometimes undiagnosed until adulthood when hearing loss and a history of bone fractures elucidate a proper diagnosis. This is especially true of sporadic cases where there is no family history of the disease, a surprisingly common precipitating factor that has been significantly correlated to increased paternal age, though the effect over the whole population is negligible.[5] For OI in general, the rate of sporadic mutation is 35% of all cases, but no data is available on Type I OI.[3] It is these sporadic cases that help to increase the incidence rate of Type I OI to 1 in 20,000-25,000 people.[6]

Classically, there is not much doctors, or even medicine, could due for Type I OI patients. Before genetic or biochemical tests were available, diagnosis was made simply based on the symptoms, which vary greatly among individuals and can be confused with secondary osteoporosis and accidental injury.[2,3] Skin or blood samples were used but they were not always definitive. Fetal testing involved the use of ultrasounds but they were likewise not conclusive for OI because only rarely would they detect fetal bone bowing or fracture.[7] Even the blue sclerea were not always diagnostically helpful as it is normal for newborns to display blue sclerea up to 12 months and sometimes a few months longer.[7] Indeed, it was not until 2006 that guidelines for genetic diagnosis were published even though the tests themselves had been used since the 90's.[8]

Biochemical testing involves culturing skin fibroblasts and testing to see if less type I collagen is produced relative to the normal amount. Labeling the collagenous proteins, passing them through polyacrylamide gel electrophoresis, and then analyzing the autoradiographs allows for accurate measurements of the number of the collagen chains present in the sample. This approach has a sensitivity of almost 90%.[8] Genetic testing using PCR in cultured fibroblasts can reveal whether the coding regions for the two collages coding genes contain the mutations that cause Type I OI. This test is also 90% accurate at detecting true positives.[8] Unfortunately, biochemical analysis was not, up until recently, available for fetuses. As a 2002 described it, "the reduced synthesis of type I collages chains typically seen in individuals with OI type I cannot [currently] be detected reliably" using chorionic villus sampling.[8]

Unfortunately, there is no cure for Type I OI. The usual treatment regimen includes physical therapy, careful exercise, and a diet rich in minerals to strengthen bones. Orthopedic surgery may be required to strengthen broken bones with implanted rods. Specifically in terms of medications, treatment focuses on strengthening bones to protect against damage instead of increasing collagen production. The medications most widely used to treat OI are

bisphosphanates, and much research has focused specifically on pamidronate (aminohydroxypropylidene bisphosphonate), a drug capable of inhibiting (via apoptosis) the osteoclast cells that reabsorb bony tissue, thereby leading to increased bone density.[2] Several studies have shown that pamidronate therapy also leads to lower incidence of bone fracture, vertebral remodeling, and, surprisingly, continued bone mass growth even after treatment was withheld for two years.[2] However, this last benefit is cause for concern as bisphosphonates accumulate in the bones and, by inhibiting the osteoclasts that lead to homeostatic bone remodeling, significantly reduce the remodeling that occurs in treated patients.[9] Presumably, this could have serious consequences if old bone is not removed, especially during adolescent development, but long-term studies have not been done on this question.[2]

Mindful of the current state of affairs regarding Type I OI, it seems several original, government-mediated recommendations are in order:

(1) *Introduce genetic screening for all types of OI during pregnancy if possible, but until the technology is widely available, newborn screening OI should be the requisite norm.*

(2) *Invest in research for collagen-increasing medication.*

(3) *Alternative and novel strategies such as transplantation of allogenic bone marrow-derived mesenchymal cells should be assessed for therapeutic potential.*

It should first be recognized that part of the difficulty of having an OI diagnosis is that unless there is reason to test for the defective genes, a test will usually not be done until several bone fractures have already occurred. Fractures can easily occur during the early years when the child is just learning to walk and is exploring the world, constantly falling and tripping over household objects. Moreover, OI fractures may be confused with child abuse. Given that a child is 24 times more likely to have bruises from child abuse than from OI, a misdiagnosis for either OI or child abuse endangers the health, safety, and development of the child.[8]

However, these problems could be offset by mandating newborn screening for OI. It would empower a parent with the information necessary to ensure that the childhood environment was structured so that the child would not suffer unnecessary fractures. Although the disease is heterogeneous with respect to its mutations, they are still confined to two genes for Type I and two others for the remaining seven types of OI. Sequencing of the relevant genes by targeting the 5' UTR or any other early (yet constant) upstream region of the coding sequence would enable doctors to see if the strand were whole or mutated. A variety of DNA microchips could accomplish this if the child's DNA could be obtained. A more direct, non-invasive, and effective method than counting collagen would be to harness the power of a new high-throughput shotgun sequencing technology that analyzes fetal cell-free DNA in the mother's bloodstream to look in the child's genome for the relevant mutations.[10] If this technology becomes more widely available and less expensive, a diagnosis of OI could be made much months or even years before the first signs of the disease appear in bone fractures.

This would be of greatest benefit to those parents whose child was a victim of sporadic OI, and given the fact that sporadic cases tend to be higher that normal for OI, and that children are developmentally sensitive both *in utero* and *ex utero*, early diet can be directed toward increasing bone strength as a result of the screening. Moreover, a pregnant mother with Type I OI tends to have increased bone pain as a result of the pregnancy while more serious types of OI can have a greater impact on the mother and lead to anemia and premature delivery.[3] Regardless of which type of OI the mother has, the child's developmental plasticity allows for an increase in any kind of bone-strengthening agent, whether it be Vitamin D, calcium, or a synthetic substance yet to be discovered, and this is one period where significant effort should be undertaken to improve the strength of the child's bones.

Second, it is important to recognize that Type I OI is not just a disease of the bones; that is only part of a constellation of systems affected and each deserves attention, even if brittle bones are the most serious consequence of OI. As of now, there is no medication, at least not that has been used for OI, that seeks to induce an increase in collagen I expression. Unlike other types of OI where the collagen is mutated, Type I is simply a case of insufficient quantity so any increase is sure to have a body-wide positive effect so long as it does not increase collagen to dangerously high levels. Animal models should not be hard to conduct as many different mutations can lead to the Type I OI phenotype, and there is clearly a moral and financial incentive to begin the search for a cure. Naturally, the effects of increased collagen production in humans would have to be monitored exceedingly carefully because any mistake would have body-wide effects. Nevertheless, the potential to alleviate muscle weakness, short stature, low bone density, hearing loss, and social stigma exists, and given the great benefits someone can expect to receive from just increased collagen production warrant at the very least a few studies to see whether collagen expression can be accomplished successfully.

Third, novel strategies to combat OI should be undertaken and assessed for therapeutic potential. In a second 2002 study, bone-marrow derived mesenchymal cells were given to children with severe (not Type I) OI who had undergone a bone marrow transplantation to see if it would stimulate growth. Though the study contained only 6 patients, "Over the first 6 months after MSC [Marrow stromal cells] therapy, each patient had a striking increase in growth velocity, ranging from 60% to 94% of the predicted median."[11] The study also shows that the engrafted MSC had the potential to "differentiate to osteoblasts capable of extending the clinical benefits of BMT [bone marrow transplant.]" However, the results were not curative and the study's sample size was extremely small. An expanded study would need to correct for these two factors if further research were to be done based on the success of the MSC study. The question

of what other novel therapies could gain ground on finding a cure to OI warrants further investigation.

With renewed hope and evidence that improved tests and novel strategies can bring us one step closet to ending OI, research must be pushed forward. For a disease that affects so many of the body's systems, Type I OI certainly deserves more attention from the medical community, and that is not to forgot that is it the mildest form of a disease that can, in its worst incarnation, lead to perinatal death. Type I OI is not even a question of the typical mutation ravaging the body, but rather, of a sufficient lack of protein that should be relative easy to offset with the proper therapies and drugs. However, it is only with further research and increased scientific effort that such a day will come when management exceeds the bounds of physical therapy and orthopedic surgery and OI becomes one more genetic disease for which we have a cure.

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