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Osteogenesis Imperfecta: Causes, Symptoms and Treatment

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ABSTRACT

Osteogenesis imperfecta is a genetic disorder that causes harm to the bones. It causes the bones to break from little or no apparent trauma. The disease results in a weak or brittle skeletal system, the condition of which can affect quality of life and basic function. It is characterized by fragile and easily broken bones which are responsible for other health problems. The severity ranges over 19 main types, being either inconvenient or life-threatening. However, it has 4 main types, with Type I, II, III and IV being the most common, with the symptoms varying over the different types. The disease causes a variety of different problems regardless of its severity and can affect all genetic groups with equal frequency, occurring in 1 in 20,000 individuals. OI has a wide variety of causes and therefore a large possibility of treatments. Caused by a genetic defect to the COL1A1 and COL1A2 gene, gene therapy and other bone cycle altering treatments are likely therapy for the currently uncurable disease.

THE CAUSE

Osteogenesis Imperfecta occurs due to a single dominant mutation to the COL1A1 or the COL1A2 gene present in the fibroblasts of the body's cells. These genes provide information for making proteins used to create larger molecules known as collagen. Collagen is the most common protein the bone, skin, and other tissue. It provides strength and structure to the body and connective tissue. These genetic deformities cause both quantitative and qualitative mutations to the collagen produced. OI can also be caused by recessive mutations to the CRTAP gene present in the body, although this is less common and results in Type III OI. This affects osteoblasts of the body. Mutations of COL1A1 or COL1A2 gene lead to I, II, III types of Osteogeneses Imperfecta, each caused by either a mutation to the form or amount of collagen produced.

Mutations in the COL1A1 gene are more likely to occur than mutations in the COL1A2 gene, occurring in an autosomal dominance pattern. This is responsible for most forms of OI, especially the more severe cases such as Type II. The recessive autosomal form of the diseases, Type III occurs due to mutations apart from the COL1A1 and the COL1A2 gene. However, it is also possible that the disease is not inherited but rather newly mutated in the infant. New mutations are responsible for 20-35% of cases.

GENES AND THEIR MUTATIONS IN DETAIL:

There can be over 100 different mutations leading to Osteogenesis Imperfecta, either to the CRTAP, COL1A1 or COL1A2 gene. However, COL1A1 and COL1A2 are most commonly mutated. The COL1A1 gene and the COL1A2 gene are both responsible for producing type 1 form of collagen which is a fibrilous collagen present in the connective tissue, bone, cornea, dermis, and tendons. This makes it integral to the structure of these parts of the body. COL1A1 codes for the pro-alpha-1 chains of type 1 collage whereas COL1A2 codes for the pro-alpha-2 chains of type 1 collagen. Mutations in the COL1A2 gene are not as detrimental to the health of those affected as compared to mutations occurring in the COL1A1 gene. Due

to these mutations, various problems occur during collagen production. Firstly, enough collagen is not produced. Secondly, the collagen produced can have the incorrect amino acids. Lastly, the mutations produced can alter the protein chain. These all result in deformities in either the quantity or the form of collagen produced.

COLLAGEN PRODUCTION AND MUTATION:

Collagen is protein produced by fibroblasts of the body. It provides strength and structure to various layers and organs of the body. It is present in the skin, tendons, connective tissue, derma, bone, and cellular tissue. Collagen is a complex protein molecule made of amino acids through both intracellular and extracellular production methods. It is made of the amino acids, glycine-proline-X or glycine-X-hydroxyproline. It forms a triple helix from these three chains of amino acids. It tightly binds together in a firm configuration as a triple helix structure, which is what gives it the ability to withstand stress and pressure. The structure of collagen is the reason it is used in so many of the body's organs and structures. Without its structure, collagen is unable to provide strength or support to the body, which is what makes it amino acids and configuration of the same so integral to the function of collagen.

Collagen synthesis occurs both inside and outside the cell through a lengthy and complicated process. First genes for the pro-alpha-1 and pro-alpha-2 collagen chains are transcribed. This is done by RNA polymerase within the nucleus. After this, the mRNA travels into cytoplasm for translation. Here, the mRNA interacts with ribosomes to be translated, forming a polypeptide chain. Following this, the polypeptide chain moves to the Endoplasmic Reticulum for post-translational modification. A hydroxyl group is added to the polypeptide chain using the hydroxylase enzyme and vitamin C. Glycosylation occurs, where a carbohydrate is added to the hydroxyl group present on the polypeptide chain. Finally, the chain is folded, and the helix is formed. This forms procollagen, which is the preliminary form of collagen. This is ensued by the extracellular production of tropocollagen. Procollagen is formed within the cell by fibroblasts. Then, collagen peptidase enzymes form the tropocollagen. Lysyl oxidase, a copper-based enzyme produces the final step of the collagen pathway. This enzyme acts on lysine and hydroxylysine, producing aldehyde groups. These eventually undergo covalent bonding between the tropocollagen molecules. Through this process collagen is produced and used in the various organs and tissues of the body.

Regarding collagen production, there are three main types of mutation that can occur. The first occurs when sections of the COL1A1 and the COL1A2 gene are missing. This means that the collagen produced is unaffected, but the amount produced is less than what is required. This means that the body doesn't not receive enough collagen to support the structure or give it strength, weaking the organs and tissues of the body. Similar to cement, insufficient collagen results in insufficient structure in the body.
Secondly, some mutations alter the sequence of amino acids needed, replacing glycine in the sequence of amino acids. This subsequently affects the bonding of collagen fibril. The inter-chain hydrogen bonding is lost, which gives the wrong structure. Lastly, some mutations alter the end of the protein chain. While the amino acids may be correct, glycine residues that are incorrectly bonded lead to bulky and unstructured helix of collagen. When the amino acids are incorrect, the structure produced is wrong meaning that the collagen's tightly bound helix structure can no longer withstand stress. Similarly, when the helix is bulky

due to glycine residues it is unable to form its firm configuration and therefore withhold pressure. With the wrong structure, the primary function of collagen is lost, and it can no longer perform as intended for the body.

SYMPTOMS

TYPE I

Type I is the mildest form of OI and is not life-threatening. It is characterized by fractures in the early childhood and adolescence often from minor trauma. Fractures occur less commonly in adulthood. Affected usually have discolored sclera and around 50% develop hearing loss in adulthood. They also have normal height unlike most with the disease. It is caused when insufficient collagen is produced the body. Around 50% of all cases are Type I

TYPE II

Type II is the severest form of OI. Infants that are born with this form of OI usually die after birth. They are born with bent or crumpled bones. They have fractured ribs and misshapen lungs and internal organs due to damage to their ribcage. They also have bowed or short limbs and very soft skull bones. They have discolored sclera and scoliosis or misshapen spinal cords. Apart from this Type II also results in dentinogenisis imperfecta, joint deformities, hearing loss, respiratory problems, and issues with mobility. Most infants die after birth due to respiratory problems due to their malformed ribs and other life-threatening problems. It is caused by a defect in the collagen produced by the body's cells.

TYPE III

Type III is relatively severe. Soft fragile bones may begin to fracture before birth. This includes rib fractures which cause life threatening breathing problems and other bone abnormalities. These bone abnormalities affect the body's ability to perform basic function such as walking or standing up straight. The limbs are also deformed or shorter than normal. The skull and face shape are likely to be abnormal, as well as chest and spine. There are also problems with breathing and swallowing. The symptoms tend to vary over each case. Type III OI is caused by a defect in the collagen produced by the body's cells.

TYPE IV

Type IV ranges from normal to severe. It includes malformed limbs and fractures later in life. Fractures are not present immediately after birth but may be present after basic function such as walking or crawling. The limbs are bowed and affected are typically short in stature. Type IV varies per case. It is caused by a defect in the collagen produced by the body

Other symptoms of Osteogenesis Imperfecta including the ones described above include progressive hearing loss, discoloration of the sclera, dermatogenesis imperfecta, scoliosis and other spinal problems, loose joints, bone abnormalities and short stature. Since collagen is present in the sclera, the skin, the bones and connective tissue, an irregular amount or structure of collagen subsequently affects the rest of these organs.

DIAGNOSTICS:

Diagnostics for the disease range widely. The first is laboratory-based testing for the disease, either biochemically or through DNA-based sequencing of COL1A1/COL1A2 genes. Biochemical testing for OI involves checking for structural abnormalities in the helical region of type 1 collagen. DNA-based sequencing involves checking for mutations and abnormalities in the sequence of the genes of COL1A1 and COL1A2. Apart from this, skin biopsies can be used to check for the presence of abnormal or insufficient collagen. Another physical method that is used is x-rays to check for frequent hairline fractures, codfish vertebrae, and Wormian bones. Finally, previous family history and DNA analysis of the same can help predict ahead of time if the child is likely to inherit the disease.

TREATMENTS

Due to Osteogenesis Imperfecta's wide variety of disease-causing possible mutations, it also leads to a variety of possible treatments. Due to OI's rarity the current treatments are not exhaustive or large in number. However, this being said, Osteogenesis Imperfecta causes a large range of issues for not only the musculoskeletal system but for the patient as a whole. Since most bone related disorders have similar causes, the treatments for osteogenesis imperfecta can later be used to investigate and treat other bone diseases. In the following treatments, I hypothesize possible treatments for OI based on current experimentation and research, including newer treatments that employ biotechnology's latest advancements.

Current Treatments:

- Bisphosphonates
- Antibodies

Possible Treatments:

- Dietary Changes
- Calcitonin

Gene Therapy

- SP7
- RUNX2
- FGF15
- CRISPR and AAV

BISPHOSPHONATES

Bisphosphonates are chemical medication made from a dual phosphonate group. It can be taken both intravenously and orally. Bisphosphonates work to prevent the loss of bone density. They essentially work by limiting osteoclast activity. Osteoclasts are cells present in the bone that work to remove bone cells and therefore maintain the balance of the bone tissue. However, in cases where the bone density is already low, osteoclasts only further weaken the bone structure. Therefore, by limiting osteoclast activity, the amount of bone lost also reduces and bone density can be maintained at a somewhat normal level. Bisphosphates are

effective because they control bone mineralization and prevent bone resorption. Bisphosphonates have a hydroxyl group that binds to calcium present in bone allowing for high affinity to bone. This gives them a high local concentration throughout the body, making the treatment long-lasting and efficient.

ANTIBODIES AGAINST RANKL

The RANKL gene encodes for a protein which a member of the tumor necrosis cytokine factor. This protein is a ligand for osteoprotegerin and works in favor for osteoclast activation. Osteoprotegerin is an antiresorptive cytokine and a mechanism for immunosuppressant osteopenia. It is a member of the tumor necrosis factor receptor superfamily and plays an important role the regulation of bone resorption. During this treatment, certain synthetically produced antibodies will mute the RANKL gene. This will prevent osteoclasts from functioning and osteoprotegerin from being produced. Therefore, bone resorption is reduced, and the bone density is maintained.

ANTIBODIES AGAINST SCLEROSTIN

Sclerostin is an enzyme produced in osteocytes. This enzyme inhibits bone formation and maintains the bone cycle and balance of the tissue. It works similarly to osteoprotegerin. It inhibits osteoblasts and stimulates osteoclasts. Osteoblasts are bone producing cells that work to produce more bone tissue. Antibodies against sclerostin protein prevent it from being produced. This in turn allows osteoblasts to function and prevents osteoclasts from removing healthy bone tissue quickly. This way, more bone is produced, and bone density is maintained.

RECOMBINANT DNA TECHNOLOGY

Calcitonin is a hormone produced by the thyroid gland. This hormone inhibits osteoclasts. On the same lines as the previous two therapeutics, by increasing calcitonin levels in the body, osteoclast function is reduced. This means less bone is removed from the body and bone resorption is reduced. One of the main benefits of the calcitonin treatments is that increasing these levels in the body do not seem to have any negative side effects after conducting clinical trials. This means that the hormone levels can be increased in the body to a sustainable amount to controllably alter the bone cycle according to the bone tissue of those affected. Treatment can be done using recombinant DNA technology and chemical peptide synthesis. Using recombinant DNA technology, scientists are able to create new DNA sequences that do not exist under normal or environmental conditions. These can then be placed in the host cell where they produce the intended protein, also known as the recombinant protein. This protein production can be controlled to produce a larger amount of the protein that is needed for bodily function. Chemical peptide synthesis is process by which amino acids are linked together to form their intended protein or polypeptide chain. In this case, the enzyme calcitonin is chemically engineered and produce using artificial chemical peptide synthesis.

Recombinant DNA synthesis can also be used to create missing segments of the COL1A1 and COL1A2 gene. This is common in Type I OI, and by adding correct sequences to the gene, the adequate amount of

collagen can be produced. This can then be completed and attached using the Sanger Sequencing process. This is relatively inexpensive.

DIETARY CHANGES

Collagen is primarily produced by vitamin C and amino acids. By increasing the amount of vitamin C and protein in the body, and taking collagen supplements, the collagen produced can increase. Secondly, vitamin B, B12, and folic acid should also be increased. This is so that homocysteine, a harmful amino acid can be broken down. Homocysteine or HCY decreases and degrades bone quality. Making antibodies against HCY or adding more vitamins to the diet can ensure bone quality is not reduced. These supplements can be used for mild cases of OI such as Type I to ensure bone quality and collagen quality is not entirely compromised.

SP7

The Sp7 codes for the protein osterix which is also known as the OSX protein. The protein is produced by the osteocytes and is used as a transcription factor for osteoblast stimulation. OSX is essential for osteoblast stimulation, production, and differentiation. Transfer of osterix into the organism inhibits tumor cell growth and osteolysis, which is the disappearance of bone tissue. In order to increase the expression of the Sp7 gene and therefore protein production in the body, bacterial plasmids can be used.

Bacterial plasmids carrying the gene will produce more of the required protein for the intended effect of increasing bone density. For gene expression, protein production will be done outside of the organism. This means that the intended gene, Sp7 will be inserted to the plasmid and then the protein produced will be placed into the affected. This is because proteins are more specific and targeted than activators or expression. Activators or expression within the organism can often lead to overexpression or misdirected pathways. Instead, the Sp7 gene will be inserted into the plasmid of the E. coli strain JM110. This strain is used to the presence of the dam gene. The dam gene is necessary for preparing unmethylated DNA. It is also necessary for certain restriction enzymes such as Xbal, which is one of the restriction enzymes necessary for this specific gene. Other restriction enzymes necessary are given below

- DraI (2936)
- ApaLI(2708)
- StuI(2168)
- HindII(1912)
- XbaI(1606)
- Fspl(1292)
- Spel(586)

RUNX2

The RUNX2 gene encodes for a nuclear protein called the runt-related transcription factor. It is used for osteoblast stimulation and differentiation. By increasing run-related transcription factor, the amount of bone

produced will increase. Similarly, to the Sp7 gene, the protein will be produced outside of the organism and then be inserted into the affected. This is to prevent overexpression and control the amount of protein produced, reducing chances of overstimulation as well. The bacterial plasmid to be used is E. coli, strain BL21(DE3). This is used for general protein expression and is a common strain of E. coli used for bacterial plasmid protein production. It has a dcm gene that is important for certain restriction enzymes that are methylation sensitive. The restriction enzymes needed are given below

- FspI(143)
- PvuII(262)
- NotI(280)
- Kpnl(445)
- SacI(503)
- HindIII(697)
- AatII(934)
- ApaI(1105)
- AgeI(1388)
- EcorI(1521)

FGF15

FGF15 is a gene used in fibroblast stimulation. Fibroblasts are cells that produce collagen in the body. FGF15 codes for a protein known as the FGF growth protein which stimulates the fibroblasts. By increasing the expression of FGF15 more growth protein can be produced. This will in turn increase the amount of collagen produced by the fibroblasts. FGF15 can be inserted into bacterial plasmids and the protein for the same can be produced outside of the organism. This is especially important for the growth protein, as uncontrolled expression can lead to overstimulation which is difficult to manage. External protein production also allows for patient specified treatment. The bacterial plasmid to be used is E. coli, strain BL21(DE3). This is used for general protein expression and is the same used for the RUNX2 gene. It has a dcm gene that is important for certain restriction enzymes that are methylation sensitive. The restriction enzymes needed are given below

- EcorI(262)
- Kpnl(321)
- EcorV(724)
- SaII(1867)
- AatII(305)
- BamHI(594)
- StuI(1712)

CRISPR

CRISPR Cas9 is a powerful DNA engineering technology that allows scientists to cut and replace incorrect DNA sequences with artificially engineered correct sequences. CRISPR Cas9 can be used to edit genes inexpensively and easily. Is uses the Cas9portein which is a protein in bacteria that helps it defend against

viruses. The Cas9 protein can be easily programmed to find and bind to almost any desired target. Once the Cas9 protein finds and binds to the intended DNA target it can cut the DNA segment that is mutated or incorrect. This can then be replaced with correctly engineered DNA sequences Here, the COL1A1 and COL1A2 gene can be correctly replaced according to the patient's specific mutation. This therapy can also work for the CRTAP recessive mutation in Type III OI. Plasmid pBA439 is to be used for mammalian CRISPR sequencing

AAV

Adeno associated virus gene therapy is delivery system for therapeutic genetic material. AAV does not result in human disease and is easily controlled. The viral DNA is replaced with new DNA and becomes a precisely coded vector for the recombinant DNA. The AAV vector is used to deliver the normal copies of the gene to the right tissues and organs in the body. In this case, the correct versions of the COL1A1 and COL1A2 gene will be delivered. The corrections to change will be case specific since there are over 100 possible mutations that can occur during this disease. The correct recombinant DNA will be inserted into the AAV and be delivered to bone tissue to strengthen bone tissue for correct collagen synthesis. The plasmid to be used is the pAAV-CamKll-ArchT-GFP which is used for mammalian cells.

CONCLUSION

Osteogenesis Imperfecta presents us with many different opportunities for treatment. Its multitudes of complications also avail a vast number of possible treatments and cures. Although a considerably small amount of the population is affected by this disorder, its effects on everyday life and young infancy is reason to be carefully considered. With genetic engineering, protein synthesis, recombinant DNA technology, dietary changes, Bacterial plasmids and other current treatments it is possible to eradicate this disease from the population and prevent its inheritance for future generation, bettering quality of life for those affected by such bone-related diseases.

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