Hypercholesterolemia: So much cholesterol, so many causes

Student group names kept anonymous
Department of Biology, Lake Forest College, Lake Forest, IL 60045, USA

Hypercholesterolemia is a disease characterized by excess cholesterol in the blood. Often there are no apparent symptoms, with a blood test being the only way to detect high cholesterol. Hypercholesterolemia has several causes, the most common being a diet that includes many foods containing saturated fats and cholesterol. Obesity and underlying diseases such as diabetes mellitus and liver dysfunction, as well as mutant genes affecting cholesterol metabolism also cause Hypercholesterolemia. The latter forms are known as Familial Hypercholesterolemia (FH), and have been well studied and are the focus of this review. With FH, the physical symptoms are severe, involving visible accumulations of cholesterol throughout the body. Autosomal dominant mutations affect low-density lipoprotein receptors (LDLR). Mutant apolipoprotein, mutant adaptor protein, and mutant ABC transporters, which causes a sister disease *Sitosterolemia*, also lead to FH. Experiments over the past fifteen years have illuminated both the molecular basis of this disease as well as the biology underlying endocytosis. Currently a change in diet, surgery to remove cholesterol deposits, and several cholesterol-lowering drugs are the methods used to treat FH. Possible treatments in the future include gene therapy using adenoviruses.

Introduction

The common symptom for all types of Hypercholesterolemia is an increased level of low-density lipoprotein (LDL) in the blood.[1] The only way to detect this increase is through a blood test. With FH, however, symptoms include xanthomas and atheromas, physically noticeable fatty deposits in tendons and arteries, respectively.[2] These tumor-like accumulations have been described since before the year 1900.[3] It was observed that certain families tended to have the disease.[4] Experiments done in the 1940s and 1950s demonstrated that the disease was genetic.[3] Although FH is caused by many different genetic mutations, all are autosomal dominant. Heterozygotes merely exhibit symptoms of Hypercholesterolemia, while most homozygotes die from heart disease in childhood.[5]

Common Treatments and Cures

While the visible symptoms and phenotypes appear to be the same, the disease is much more complex beneath the surface. The majority of the types of Hypercholesterolemia are caused by familial genetic alterations, but a small portion of cases are caused by sporadic mutations or are diet-induced. When intake of cholesterol into the body is too high, and uptake of cholesterol into the hepatic cells is too low, the result can be a build up in the blood stream, resulting in Hypercholesterolemia.

The Familial case of FH are passed down from generation to generation. Nine different mutations code for defective alleles: the null allele mutation; the transport-defective allele mutation; the binding-defective allele mutation; the internalization-defective allele mutation; the recycling-defective allele mutation; the protein-mistargeting allele mutation; the promoter-defective allele mutation; the ApoB100-defective allele mutation; and the excretion-defective allele mutation.

The null allele mutation causes the Low-Density Lipoprotein Receptor (LDLR) to never be produced. If it is synthesized in a cell that expresses the null allele, the receptor will be manufactured incorrectly and sent immediately to the lysosome to be degraded. No LDLR reaches the surface in a cell that expresses the null allele.

Another allele, the transport-defective allele causes an inhibited pathway of the LDLR from the Endoplasmic Reticulum (ER) to the

Trans-Golgi Network (TGN) restriction proper direction to the appropriate part of the cell. The LDLR is produced in a cell that expresses the transport-defective allele, but it is of no use because it never reaches a functional destination.

A binding-defective mutation causes the cell to produce an LDLR that cannot bind to LDL. The protein reaches the surface of the cell, but cannot bind with its cargo because of the change in conformation of the protein due to the mutation.

The internalization-defective allele mutation does not allow the LDLR bound to its cargo to be endocytosed. The LDL remains bound to the LDLR on the surface of the hepatic cell, never reaching its lysosome destination in the interior of the cell.

The recycling-defective mutation codes for a protein that does not react in the acidic environment of the endosome. This lack of reaction prohibits the separation of the LDLR from the LDL. While the LDL and the LDLR normally have two separate fates within the cell, the LDL is sent to the lysosome and the LDLR is sent back to the cell surface, cells with the recycling-defective allele mutation send the LDL and the LDLR to the lysosome to be destroyed. In this case, the LDLR is not recycled.

The protein-mistargeting allele sends the LDLR to the wrong surface of the cell. The functional segment of the LDLR is unaffected and would carry out the normal metabolic procedure except that the LDLR never comes in contact with the LDL and therefore they cannot bind.

A mutation that causes the promoter-defective allele is a single-base substitution in the promoter segment for the LDLR gene; this results in a lack of promotion of the LDLR. This therefore means that less is protein is formed, decreasing the uptake of LDL from the blood and increasing the amount of unwanted cholesterol.

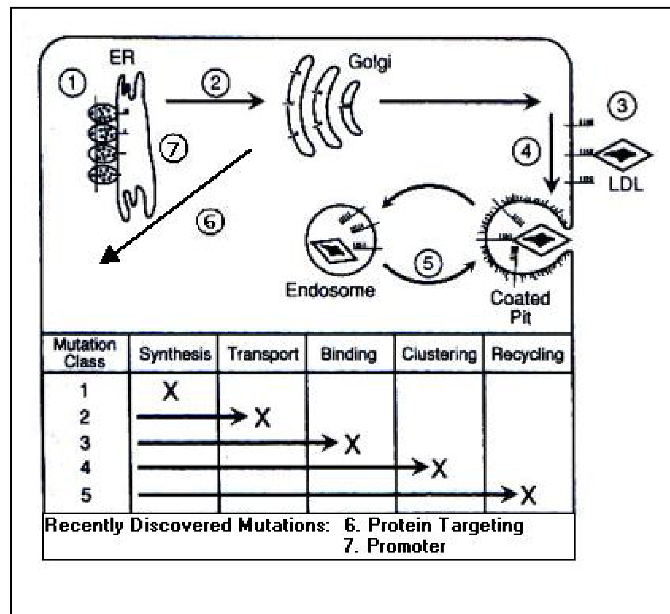

Figure 1. Several Autosomal Dominant FH Mutation Classes Affecting LDLR. Various problems occur during protein synthesis, from the initial transcription in the nucleus to the transport of the proteins to the cell surface. (Figure adapted from Goldstein et al.)[3]

The ApoB100-defective allele mutation is a change in the genetic structure of the Apolipoprotein B100 (ApoB100). ApoB100 is the protein that cholesterol in the body binds to in order to become soluble in the bloodstream and able to be transported throughout the body. There is nothing wrong with how the protein binds to the cholesterol, but the protein does not properly bind to the LDLR, which is the part of the LDL that binds to the LDLR. If binding to the LDLR does not occur, than endocytosis is repressed and cholesterol (LDL) remains in the blood stream.

The Major Players: LDL and LDLR

Cholesterol by itself is insoluble in the blood. The process of binding to an apolipoprotein makes cholesterol a low-density lipoprotein (LDL) that is soluble and able to travel freely throughout the body via the bloodstream. In the liver, there are many hepatic cells with a multitude of low-density lipoprotein receptors that are able to bind to LDL. The key to this endocytosis process is the cell-surface receptor that binds to LDL by interacting with its ApoB100 component[3]. Human fibroblasts are able to produce approximately 20,000 to 50,000 LDLR per cell according to the cholesterol requirements of the particular cell[3].

The receptor is first synthesized in the rough ER and then sent to the TNG[2]. The TGN subsequently targets the LDLR to the cell surface, where they gather in clathrin-coated pits. Clathrin coated pits are specialized regions of the plasma membrane that are lined on the cytoplasmic surface by a protein called clathrin[6].

After the LDLR binds to LDL, the coated pits internalize and the clathrin coat disintegrates. Multiple endocytic vesicles fuse to form endosomes where the pH begins to fall below 6.5[3]. At this acidity, the LDL dissociates from the receptor while the LDLR returns to the cell surface[6]. LDL that is dissociated from the receptor is delivered to the lysosome where hydrolytic enzymes degrade LDL for metabolic use[6].

What Actually Goes Wrong?

On a molecular level, Familial Hypercholesterolemia is caused by a number of different mutations affecting the LDLR gene. The particular gene is located on the short arm of chromosome 19 and is composed of 18 exons that span 45 kb[3]. Numerous classes of mutations of the LDL receptor have been identified based on phenotypic behavior of the mutant gene[3].

In the past years many hypotheses have been offered to determine the defects in the LDLR gene that lead to FH symptoms. For example, scientists tested whether a mutation failed to produce a proper functioning LDLR in class 1.[7] Class 2 mutations tested the hypothesis that the LDLR failed to travel from the ER to the TGN correctly, while class 3 hypothesized the appropriate binding capabilities between LDL and LDLR.[8,9] Scientists also investigated whether internalization of bound LDL to LDLR occurred, denoting class 4 mutations.[10] Hypotheses of recycling defective alleles as well as LDLR mistargeting also have been tested mutations.[11,12] The Promoter of the LDLR gene was analyzed to see whether proper amounts of LDLR were being produced[9]. A final experiment tested the binding capability of ApoB100 on the LDL to the LDLR[10].

Class 1: The Null Allele

The expression of the Null allele results in the absence of LDLR synthesis. If the LDLR is made, it is made incorrectly and immediately sent to the lysosome to be destroyed. Scientists studying a null allele mutation in a French Canadian population discovered that a deletion of the promoter and first exon of the code for LDLR had occurred.[13] Because of these mutations, afflicted cells produced no LDLR. This study was a breakthrough in genomic DNA diagnosis. This advance allowed People with Hypercholesterolemia in this ethnic group to be tested for this deletion.

Class 2: Transport-Defective Alleles

In healthy cells, proteins made in ribosomes are sent from the ER to the TGN, while cells that express the transport-defective allele cannot complete this transport. This is because the ER has a mechanism through which it prevents defective proteins from being sent to the TGN and subsequently to its standard destination.[15] When defective proteins are detected they will remain in the ER in an effort to either repair or recycle them.

There are two subgroups of Class 2 mutations.[14] Class 2A mutations prevent the damaged or mutated LDLR from leaving the ER in an attempt to repair the affected protein.

Class 2B mutations release some of the receptors to the cell surface, but this number is significantly reduced compared to normal. However, the amount is unimportant because the receptors are not functional.

Class 3: Binding-Defective Alleles

The class 3 mutation is similar to class 2B, where the receptors reach the cell surface but are defective in ligand binding. However, class 3 mutations are categorized in that they still are able to perform intracellular transport. This is mostly due to a result of in-frame rearrangements in the cystein-rich repeats of the ligand-binding domain of the LDLR or the adjacent growth factor repeats of the Epidermal Growth Factor (EGF) precursor domain.[3] Figure 2 illustrates the domains on the LDLR.

Deletions of ligand binding repeats have different effects on the ability of the receptor to bind LDL and b-VLDL (very low-density lipoprotein). *FH-Paris* has a deletion of exon 5, resulting in LDLR binding with b-VLDL but not with LDL.[15]

Other studies show that replacement of an amino acid also produces a LDLR dysfunction. *FH-Puerto Rico* is also characterized by failed receptor binding of LDL, but binding to b-VLDL with normal affinity due to a replacement in a sequence in repeats 4 and 5.[16]

FH French Canadian-3 also has a replacement, but the substitution in repeat 5 results in reduced amounts of both LDL and VLDL binding.[16] These two mutations are examples that prove only a single repeat can result in detrimental effects of LDLR binding.

Scientists have shown that normal binding of LDL, but not VLDL, is maximized when there is at least one cystein-rich repeat in the EGF domain.[17] In *FH Osaka-2,* the entire EFG precursor domain is deleted, and the

receptor is then unable to bind LDL, but will bind VLDL.[18]

An insertion within the ligand-binding domain may also cause a class 3 phenotype. *FH-St. Louis* has duplication of exons 2-8 and makes a longer LDLR.[15] The results is that LDLR is transported normally to the surface, but there is reduced amounts of LDL binding.[1]

Class 4: Internalization-Defective Alleles

Although very rare, class 4 mutations occur when the receptors are distributed evenly over the cell surface and are not arranged in coated pits. A singles LDLR cannot carry bound LDL into the cell.[19] This provides evidence to scientists that cell-surface receptors must cluster in clathrin-coated pits to internalize.[9] Like class 2, class 4 is separated into two subgroups. Class 4A deals with a mutation on the cytoplasmic domain alone, while class 4B involves a mutation on the cytoplasmic domain together with the adjacent membrane-spanning region.[3]

Two examples of class 4 mutations are *FH-Bahrain* and *FH-Paris-3*. Both have premature stop codons that shorten the protein and alter the ability to internalize ligand into the cell.[20] Another Class 4 mutation is the *J.D.* allele that has a single base pair change from cysteine to tyrosine in amino acid residue 807. Later experiments show that position 807 must be occupied by an aromatic amino acid, such as tyrosine, phenylalanine, or tryptophan for endocytosis to occur normally.[21] This position is part of a tetrameric sequence, NPVY, which is a vital sequence for correct internalization.[21]

Class 4B alleles produce shortened receptors that lack the membrane spanning domain as well as the cytoplasmic tail.[3] Although most molecules are secreted from the cell, 10 percent remain near the cell membrane so that they bind LDL but cannot internalize it. Some examples of this deletion mutation are *FH Rochester, FH Osaka-1,* and *FH Helsinki.*[3] In all of these mutations, the deletion occurs from intron 15 to the noncoding region of exon 18, but all have different end points.[3] During the translation of intron 15 into mRNA, an abnormal C-terminal sequence is formed.[22] The NPxY sequence is missing and is unable to internalize.[22]

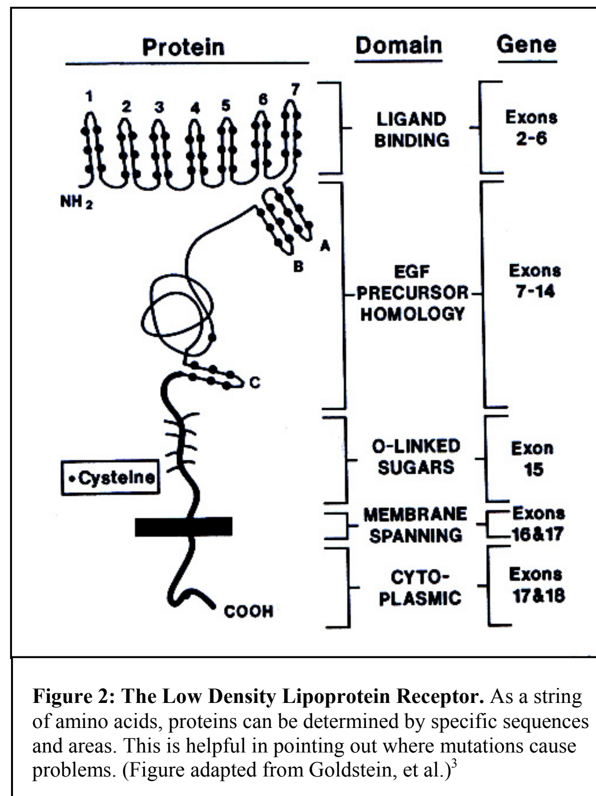

Figure 2: The Low Density Lipoprotein Receptor. As a string of amino acids, proteins can be determined by specific sequences and areas. This is helpful in pointing out where mutations cause problems. (Figure adapted from Goldstein, et al.)[3]

Class 5: Recycling-Defective Alleles

The recycling defective mutation is a very specific deletion in the gene that codes for LDLR in hepatic cells. The EGF Precursor Homology domain in the LDLR protein, adjacent to the ligand-binding domain, has three repeats that contain a great deal of cysteine. When an LDLR has this EGF Precursor Homology domain deleted, the protein does not release the LDL when inside the endosome. "Intercellular Dissociation that happens inside the endosome is governed by protein pumps within the wall of the endosome. These pumps lower the pH of the environment inside the endosome.[10] The three cystein-rich growth factor areas in LDLR are responsible for the acceleration of the acid-dependant ligand binding dissociation and thereby facilitate receptor recycling. In the mutated LDLR, the acidic environment of the endosome no longer separates the LDLR from the LDL, as it does for normal proteins.[10] LDLR is one example of a protein from a class of recycling receptors that bind to ligands at the surface of a cell, endocytose them in clathrin-coated pits, separate from the ligands in the endosome and are returned to the cell surface. [10]

In a mutated cell, the LDLR is not sent back to the cell surface, and is therefore not recycled. It is sent to the lysosome where it is quickly broken down and destroyed. The three

repeats composed mainly of cystein are necessary for the receptor to be repeatedly recycled without degradation.[23]

A New Mutation: FH-Turku

The FH–Turku mutation is a single base pair substitution of glycine to aspartic acid of the 34th amino acid.[11] The 34th amino acid is found in the signaling protein on the carboxy-terminus end of the protein and is extremely important in determining where in the cell the protein is sent. If it is sent to the apical surface, the LDLR will be useless in binding with LDL. The apical surface is the surface adjacent to another hepatic cell and does not come in contact with LDL because LDL travels through the blood stream. If the protein is sent to the basolateral surface, the surface adjacent to the bloodstream, the LDLR will bind to the LDL and continue the process of removing cholesterol from the body.

A unique characteristic of this allele is that only the gene that codes for the signal peptide is mutated. The functional (binding) part of the receptor is unchanged and would operate correctly if it were given the opportunity. Because the LDLR never comes in contact with LDL, the receptor never gets an occasion to work.

Another New Mutation: Promoter-Defective Allele

The promoter defective allele mutation is the single base substitution of Cytosine to Thiamine on nucleotide –43 (counting backwards from the core promoter) in the LDLR Sp1 proximal promoter site.[12] DNA promoters have more than one binding site. The proximal Sp1 (repeat 3) binding site has the highest affinity for binding.[12]

The mutation changes the way the transcription factor interacts with the Sp1 promoter and this results in transcription factor not being able to bind to this portion of the DNA. Because the transcription factor cannot bind to the DNA to synthesize it, the LDLR, while still being produced in minuscule amounts, is not produced in enough quantity to be affective (to avoid Hypercholesterolemia).

In cells that express the promoter-defective allele, only one twentieth of the normal amount of LDLR is made. Virtually no LDLR production means no LDL uptake from the blood stream, which, therefore means that the cholesterol is still in the blood.[12] "The C to T substitution at nucleotide –43 described here appears to be the first naturally occurring promoter mutation associated with a disorder of lipid metabolism."[12]

More Than Just an LDLR Problem

Cholesterol requires the binding of a protein to be soluble in the bloodstream to be transported throughout the body. It is this protein, Apolipoprotein B100 (ApoB100), which binds to the LDLR during LDL ligand binding. When this protein is mutated, it will not bind to the LDLR, again unable to remove LDL from the blood stream. In this mutation, the LDLR is unaffected.

This genetic disorder is known as Familial Defective Apolipoprotein B100 (FDB). The mutation occurs on the 3500th nucleotide and substitutes R for Q. Arginine at the 3500th nucleotide is a positively charged nucleotide that is necessary for normal receptor binding.[18] Arginine 3500 and Tryptophan 4369 need each other in order for ApoB100 to maintain the correct shape required for normal receptor binding of LDL.[18] This mutation changes the conformation of the ApoB100 near the receptor-binding site.
This is a unique kind of genetic disorder because all of the known ligand-defective mutations in ApoB100 cause defective receptor binding.[18]

Beyond Autosomal Dominant

So far, FH has been described as a dominant trait, meaning heterozygotes express the disease, and homozygotes generally die before they reproduce. However, two genetic disorders that lead to Hypercholesterolemia are autosomal recessive: Sitosterolemia and Autosomal Recessive Hypercholesterolemia (ARH.)

Sitosterolemia: All This Cholesterol and Nowhere to Go

One genetic cause of hypercholesterolemia is an autosomal recessive disease called Sitosterolemia. Sitosterolemia is characterized by the body's hyperabsorption of cholesterol and the inability to excrete cholesterol out of the body. This is caused by two mutated transporters that when normal, regulate the intake and excretion of cholesterol.[24]

ARH: The Putative Adaptor Protein

Autosomal recessive Hypercholesterolemia is almost as severe as homozygous autosomal dominant as far as the severity of the symptoms, cholesterol deposits and heart disease showing up at a very young

age. With this disease, however, blood/LDL levels are normal. An adaptor protein, a putative protein in the cell helps mediate intracellular transport.[25] Without this protein, it seems that LDLR fails to function normally.[25] More studies need to be done in order to determine and clarify the exact role of these intracellular proteins.

4 Genetic Ways to Get Hypercholesterolemia

The first described genetic Hypercholesterolemia involved a mutation in LDLR. To date, seven mutations of LDLR have been discovered on various alleles in affected populations around the world. Additionally, two other diseases were also known to cause Hypercholesterolemia.[5] The first, Sitosterolemia, an autosomal recessive mutation, was discovered in the late 70s. Sitosterolemia involves the failure of the body to excrete cholesterol, therefore, causing a buildup, in this case, not in the blood. The most recent genetic cause for hypercholesterolemia is ARH, which affects adaptor proteins.[5] The mutations in ARH involve the cytoplasmic tails of the receptors. This new biological model adds another mechanism by which FH is expressed.

Things Still Unknown About Hypercholesterolemia

Most gaps in knowledge about FH have to do with the location of the mutations in the actual gene that causes the disease. Once found, these defective alleles could one day be changed through gene therapy and the use of adenoviruses. There is also the gap in knowledge as to why these mutations persist over time. It seems that FH is such a disadvantageous trait, yet 1 in 500 people in most countries are genotypically heterozygous.[3] Is there an evolutionary advantage for some individuals to have this trait?

Treatments for FH: Past, Present, and Future

Familial Hypercholesterolemia has numerous amounts of mutations continuously being discovered. Mutations are most likely to occur on the LDLR gene, and in some way inhibit the proper degradation of LDL. The newly discovered promoter mutation inhibits the correct amount of LDLR synthesized, which is a mutation not directly within the LDLR gene, but directly before it.

In the future, scientists will most likely continue to discover new mutations, the area of the chromosome, they are located in, and what the affect is. Any small mutation may cause a detrimental effect on the efficiency of LDLR.

Drugs currently prescribed for FH patients include cholestyramine, lovastatin, or niacin in addition to exercise.[26] The use of adenoviruses in gene therapy is a hopeful prospect for the treatment of this disease, as scientists have had some success in treating mice with FH.[27]

Conclusion

Familial Hypercholesterolemia is a classic disease that has been extensively studied for several decades. Mutations are still being discovered and tested to help scientists classify all types of mutations that can cause FH. Solving the problem of FH may require genetic engineering techniques or other drugs to further reduce LDLR levels in the blood plasma.

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