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Familial Hypercholesterolemia and Treatments

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Familial Hypercholesterolemia and Treatments

Elevated serum cholesterol concentrations, defined as greater than 240 mg/dL, affect more than 25 percent of Americans and can lead to early coronary heart disease (Taylor et al., 2017). One cause of elevated cholesterol concentrations is a genetic disorder called familial hypercholesterolemia (FH). FH is dominantly inherited and typically identified by high concentrations of LDL in blood. FH is the most common genetic cardiovascular disease affecting approximately 14-34 million people worldwide (Nordestgaard et al., 2013).

FH is a general term used to describe mutated genes that lead to elevated plasma LDL concentrations. The three major genes known to play a role in plasma LDL regulation and FH are low density lipoprotein receptor (LDLR), apolipoprotein B (APOB), and proprotein convertase subtilisin/kexin type 9 (PCSK9) (Sturm, 2018). Mutations in any of these three genes can lead to high plasma LDL, but severity can differ based on the number of dominant alleles inherited. Heterozygous FH (HeFH) individuals tend to have total cholesterol concentrations between 310-580 mg/dL and homozygous FH (HoFH) individuals have cholesterol concentrations between 460-1160 mg/dL. Some may also be double heterozygotes meaning there are heterozygous mutations in two of the genes causing an intermediate cholesterol concentration between HeFH and HoFH (Nordestgaard et al., 2013). HeFH affects one in every 200 people and increases the risk of a coronary event in males by 50% by the age of 50 and 30% by the age of 60 for females. HoFH is predominantly found in the LDLR gene and affects one in every 200,000-300,000 people. HoFH leads to greater atherosclerosis and cardiovascular disease than HeFH and if left untreated, can lead to early death (Sturm, 2018).

As stated previously, a significant component of LDL is cholesterol. Cholesterol is a major component of cell membranes, precursor of steroid hormones such as cortisol, androgens, and estrogen, and is converted to bile acids by the liver to aid in digestion and absorption of fats. Cholesterol is synthesized in cells via a biochemical pathway. Acetyl-CoA is converted to hydroxymethylglutaryl-CoA (HMG-CoA) and then to mevalonate by HMG-CoA reductase. Mevalonate is converted to isoprene through a few more reactions and then six isoprene molecules are condensed to form squalene. From squalene, more than twenty steps occur to produce cholesterol. The HMG-CoA reductase reaction is the rate limiting step of cholesterol biosynthesis, and is therefore a major target to treat elevated cholesterol concentrations. HMG-CoA reductase can be controlled through competitive inhibition, allosteric effects, and covalent modification such as phosphorylation, but the major regulator for HMG-CoA reductase is a feedback mechanism that controls the amount of the enzyme present in cells (Voet et al., 2016, pp. 706-711).

It is important to maintain adequate concentrations of cholesterol in the body to serve its essential functions but too much becomes extremely dangerous over time. High concentrations of LDL can cause lipids to deposit in the walls of blood vessels triggering an inflammatory response. The endothelial cells of the vessel will help to recruit white blood cells that delve into the walls of the vessel and take up lipids which causes the formation of foam cells. This damage to the walls of the vessel causes the formation of plaques which consist of cholesterol and dead macrophages. Smooth muscle surrounds these plaques and may calcify causing hardening of the artery. The plaques may become large enough to block the flow of blood through the artery, but typically the plaque will rupture, or a piece will break off causing a blood clot. This blood clot may then lead to myocardial infarction or stroke (Voet et al., 2016, pp. 713).

The LDLR gene codes for a receptor protein primarily found in hepatocytes. It is responsible for the uptake of LDL into cells for the use of cholesterol (Goldstein and Brown, 2009.) As of 2013, there have been more than 1200 mutations identified in the LDLR gene that affect all the functional domains. LDLR mutations account for about ninety percent of all HeFH mutations (Nordestgaard et al., 2013).

APOB is another gene involved in the regulation of plasma LDL. B-100 is a large protein product of this gene that binds to LDL. Arg3500Gln is a mutation that changes the binding between an arginine and tryptophan residue which lowers the binding affinity to the LDL receptor, resulting in elevated plasma LDL concentrations (Andersen et al., 2016). This is the only common mutation in the APOB gene and accounts for about five percent of HeFH mutations (Nordestgaard et al., 2013).

The final gene associated with FH is PCSK9 which is a 692 amino acid serine protease that is primarily found in hepatocytes but is also expressed in the intestine, kidneys, and central nervous system. At the membrane of hepatocytes, PCSK9 associates with LDLR causing the two proteins to be internalized in a vesicle and transported to the lysosome for degradation (Hess et al., 2018). This gene has over 20 known mutations which account for about one percent of HeFH mutations (Nordestgaard et al., 2013). These mutations are gain-of-function which cause a greater amount of LDL receptors to be degraded than normal. A low number of LDL receptors results in less LDL being removed from the blood and ultimately elevated plasma LDL concentrations.

Heart disease has remained the leading cause of death for over twenty years worldwide, with nearly nine million deaths in 2019 (Pan American Health Organization, 2020). LDL concentrations are one of the greatest factors contributing to heart disease, yet

hypercholesterolemia remains vastly underdiagnosed. This is the case because high cholesterol has no symptoms and must be detected through a blood test. Accumulation of lipids in the skin and tendons called tendon xanthomas may present as a symptom, but this usually only occurs with more severe hypercholesterolemia (Voet et al., 2016, pp. 713). Although being vastly underdiagnosed, there have been several significant developments in medications and treatments to lower plasma LDL concentrations.

Statins were first developed in the 1980's and are now some of the most common drugs prescribed in the United States. Some of the statin medications include atorvastatin, rosuvastatin, and lovastatin. HMG-CoA reductase is the enzyme responsible for converting HMG-CoA to mevalonate in the pathway of cholesterol synthesis. Statins work by competitive inhibition of HMG-CoA reductase to reduce cholesterol biosynthesis (Farnier and Davignon, 1998). Statins have a very small inhibitor constant, K_i, which indicates they have a great binding affinity for the enzyme and small doses of the medication are needed to be effective. These medications effectively interfere with enzyme activity because they have bulky hydrophobic regions that cause conformational changes in HMG-CoA reductase (Voet et al., 2016, pp. 712). Once HMG-CoA reductase is blocked, the end products of the mevalonate pathway decrease which causes activation of transcription factors called sterol regulatory element binding proteins. These transcription factors upregulate transcription of HMG-CoA reductase gene and LDLR gene. Upregulation of LDLR causes there to be more receptors present for removing LDL from the blood and thus lowering plasma LDL concentrations (Clendening et al., 2010).

Ezetimibe is another medication used to treat elevated cholesterol concentrations. It is typically used in addition to statins when statins alone cannot lower LDL to target concentrations. Ezetimibe inhibits a transporter in the intestinal lining and hepatocytes called

Niemann-Pick C1-like 1 (NPC1L1). NPC1L1 is responsible for the uptake of cholesterol from the diet in the intestine and reuptake of cholesterol from bile acids in hepatocytes. Ezetimibe causes decreased delivery of cholesterol to the liver which results in the upregulation of LDL receptors further reducing plasma LDL concentrations. Since less cholesterol is absorbed, greater amounts of cholesterol will be excreted in the stool. Treatment with ezetimibe lowered total cholesterol by 15 percent, LDL was lowered by 19.8 percent, fecal excretion of cholesterol increased 66.6 percent, and there was no effect on HDL or triglyceride concentrations (Lin et al., 2017). Ezetimibe appears to be a successful treatment in addition to statins to lower plasma LDL concentrations.

The mechanism of ezetimibe is unclear, but the shuttling of NPC1L1 in the cell is regulated by cholesterol. When cholesterol concentrations are high, NPC1L1 is transported to the plasma membrane, but when cholesterol concentrations are low, NPC1L1 is internalized to reduce uptake. It is proposed that NPC1L1 uptake of cholesterol occurs through vesicular endocytosis which requires microfilaments and clathrin/AP2 complex (Ge et al., 2008). In 2008, several ideas were proposed for the mechanism of ezetimibe inhibition. Ge et al. proposed that ezetimibe may competitively inhibit NPC1L1 by binding to its sterol sensing domain where cholesterol would usually bind. It may also inhibit the conformational change of NPC1L1 induced by cholesterol binding and does not directly compete for the same binding site. Finally, they proposed ezetimibe may interfere with distribution of cholesterol in the plasma membrane which limits the binding of cholesterol to NPC1L1 (Ge et al., 2008). More recent studies provide conflicting ideas on the mechanism of NPC1L1 and ezetimibe action involving vesicular endocytosis. Xie et al. says that NPC1L1 mediates the transport of cholesterol into enterocytes in the small intestine of mice through vesicular endocytosis and that ezetimibe blocks this step

(2021). Johnson and Pfeffer say ezetimibe does not alter the rate of NPC1L1 endocytosis in rat hepatocytes and that NPC1L1 does not require endocytosis for the uptake of cholesterol (2016). Ezetimibe is an effective drug for lowering plasma LDL concentrations although the mechanism of this transport protein and inhibitor requires further research.

Bile acid sequestrants are another treatment if statins and ezetimibe are unsuccessful at lowering LDL concentrations. Cholesterol is converted to bile acids through a process involving seventeen different enzymes in the liver. Approximately 500 mg of cholesterol is converted to bile acids each day. Bile acids are transported to the intestine to aid in emulsification and uptake of fat-soluble vitamins and lipids. About 95% of these bile acids are transported back to the liver to be reused. Bile acid synthesis is highly regulated so that when there are excess bile acids, synthesis is decreased, and when there is low supply, synthesis is increased. This regulation is important to ensure sufficient bile acids are released into the intestines for emulsification to occur. This tight regulation of bile acid synthesis is exploited as a drug strategy with bile acid sequestrants to ultimately lower plasma cholesterol concentrations (Russell, 2003).

Cholestyramine, colestipol, and colesevelam are bile acid sequestrants that currently have FDA approval to manage hypercholesterolemia (Lent-Schochet and Jialal, 2022). They are nondigestible and positively charged compounds that form a complex with bile acids. This causes the bile acids to be excreted in the feces rather than resorbed and transported to the liver. Decrease in return of bile acids to the liver causes the upregulation of bile acid synthesis, increasing the amount of cholesterol metabolized to form bile acids, and therefore, lowering plasma cholesterol concentrations. Colesevelam is also approved for glycemic control in type 2 diabetes mellitus patients because it helps to lower plasma glucose concentrations, reduce urinary glucose excretion, and lower glycosylated hemoglobin A_{1c} concentration (Staels et al.,

2010). These medications have proven to be another successful option to treat elevated cholesterol concentrations.

PCSK9 is the protease responsible for degrading LDL receptors and another target to lower plasma LDL concentrations. Alirocumab and evolocumab received FDA approval in 2015 for high cholesterol treatment when high doses of statins do not sufficiently lower LDL concentrations (Dayoub et al., 2021). PCSK9 inhibitors work by binding to the catalytic site of PCSK9 and inhibiting the binding of LDLR, therefore preventing its degradation. By preventing LDL receptor degradation, more LDL will be removed from the blood. These inhibitors have been found to reduce plasma LDL concentrations by about 65 percent in healthy individuals and 60-80 percent in those with hypercholesterolemia. This is the mechanism of the two current PCSK9 inhibitors with FDA approval. Another mechanism to inhibit PCSK9 uses small interfering RNA molecules that lead to mRNA degradation, and therefore, no translation of PCSK9. This mechanism underwent Phase 1 of clinical trials but was terminated for unknown reasons so there are currently no FDA approved PCSK9 inhibitors with this mechanism of action (Hess et al., 2018).

Although there is promising research on the effects of PCSK9 inhibitors on plasma LDL concentrations, they are not widely utilized. A study was conducted on 126,419 insured statin prescribed patients with history of atherosclerotic cardiovascular disease. Less than 1 percent of these patients were started on PCSK9 inhibitors after FDA approval and by mid-2019, only approximately 2 percent of the patients were on PCSK9 inhibitors. Clinical trials have shown PCSK9 inhibitors to be effective in improving cardiovascular risks, but there is still very low usage. The cost of the inhibitors and access to specialists' are leading causes of low PCSK9 inhibitor use. Two-thirds of insurers have limited PCSK9 prescribing to certain specialists. High

income individuals are more likely to be prescribed PCSK9 inhibitors due to cost and access to specialized prescribers, although these drugs have shown great promise in hypercholesterolemia treatment (Dayoub et al., 2021).

Lastly, another treatment to lower plasma LDL concentrations is lipoprotein apheresis. This treatment is typically for individuals with homozygous FH and compound heterozygous FH who have not had success with other treatments. It is much more rigorous than the other treatments and is reserved for severe cases of hypercholesterolemia. Two types of lipoprotein apheresis were performed on HoFH children with controlled diets and statin use (Gokay et al., 2016). Cascade filtration (CF) and double filtration plasmapheresis (DFPP) were used, and their efficiencies were compared. In CF, two systems for separation were used that ultimately removed LDL and other components of the blood that have large weights and diameters such as HDL. In DFPP, two filters were used to extract the LDL from the blood. Both techniques were effective in reducing plasma LDL concentrations, but there was less HDL reduction in DFPP than CF treatment. DFPP may be the better option for long term treatment because CF could cause decreases in large proteins such as immunoglobulins, albumin, and fibrinogen (Gokay et al., 2016).

Familial hypercholesterolemia is a great threat around the world today as it has no symptoms. These mutations typically are not lethal until after reproductive age so frequency may increase in the future and contribute to increased cardiovascular and stroke related deaths. It is crucial to increase public awareness regarding high cholesterol concentrations and encourage routine blood work to monitor cholesterol. Although there have been significant strides to improve recognition and treatment for hypercholesterolemia over the past few decades, there is still much work to be done. For individuals that are even diagnosed, many of these

treatments come with undesirable side effects, are ineffective, or too expensive. The underdiagnosis and undertreatment of FH must remain a large focus in research to reduce the millions of deaths from heart disease and strokes around the world.

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