LDLR, APOB, and PCSK9 Increasing The Risk of Hypercholesterolemia Among Asian Populations

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Familial hypercholesterolemia (FH) is an inherited condition characterized by high levels of low-density lipoprotein cholesterol in the blood and caused by genetic changes that affect many members in a family across generations (Kamar et al., 2021). Many genes have been determined to involve in cholesterol metabolism, and mutations in these genes that result in changes in protein function may cause the disease or increase the risk of FH (Kamar et al., 2021; Lye et al., 2013). With advancements in genetic testing, many gene mutations have been identified; interestingly, most mutations occur in *Low Density Lipoprotein Receptor* (*LDLR*) gene, *Apolipoprotein B* (*APOB*) gene, and *Proprotein Convertase Subtilisin/Kexin type 9* (*PCSK9*) gene (Kamar et al., 2021). *LDLR* gene encodes LDLR protein, which is a receptor on cell membrane responsible for the uptake of cholesterol into cells (Kamar et al., 2021). *APOB* gene provides genetic information necessary for making APOB proteins, a component of lipoprotein particles transporting cholesterol in the blood (Barale et al., 2021). *PCSK9* gene encodes PCSK9, a protein that regulates the level of LDRL expression on cell surface (Barale et al., 2021). Mutations in these genes cause changes in functions of the encoded proteins that impair cholesterol uptake and result in an increase in cholesterol level in the blood (Kamar et al., 2021). These gene mutations are mostly studied on Western populations, such as Europeans and Americans, while information about genetic profile of FH in Asian populations is scarce (Han et al., 2015). This lack of knowledge prevents Asian patients with FH from being diagnosed early and treated effectively. Mutations in three genes *LDLR*, *APOB*, and *PCSK9* may increase the risk of FH in Asian populations by their molecular impacts on cholesterol metabolism, and the identification of these gene mutations can aid in early diagnoses and effective treatment of FH in Asian populations.

FH is caused by a disruption in cholesterol distribution and transport (Kamar et al., 2021). Cholesterol plays crucial roles in maintaining membrane integrity and fluidity and transmitting signals across the cell membrane. Although cells can synthesize cholesterol, enterocytes of the small intestine and hepatocytes of the liver are the most important cells in cholesterol absorption and synthesis (Kamar et al., 2021). Cholesterol is delivered to cells by lipoprotein particles that have a hydrophobic core surrounded by a layer of hydrophilic membrane (Barale et al., 2021). APOB proteins and phospholipid are the main components of the membrane, and the percentage apolipoprotein in the membrane, which is called density, is used to name different types of lipoproteins (Fernández-Higuero et al., 2015). The two lipoproteins that primarily transport cholesterol in the body are very-low-density-lipoprotein (VLDL) and high-density-lipoprotein (HDL) (Barale et al., 2021). Being contained in the core of these lipoproteins, cholesterol is transported between the liver and other tissues by VLDL and HDL (Barale et al., 2021). For cholesterol to be brought into cells, VLDLs must pick up cholesterol ester from HDLs to become LDLs because the cell membrane has LDLR receptors that only bind LDLs and enable receptor-mediated endocytosis (Kamar et al., 2021). After internalization, cholesterol is released for cells to use, while LDLR can be degraded in endosomes or recycled to express on the cell membrane. The degradation of LDLR requires its binding to PCSK9 on the cell surface (Malo et al., 2020). Therefore, three proteins APOB, LDLR, and PSCK9 are critical for cholesterol transport and internalization. Mutations in any of the three genes *LDLR*, *APOB*, and *PCSK9* can disrupt the functions of the three proteins and reduce cholesterol uptake at cells (Kamar et al., 2021). Due to the impairment in cholesterol internalization that results from the mutations in any of the three genes *LDLR*, *APOB*, and *PCSK9*, the level of cholesterol in the blood increases, leading to hypercholesterolemia, and

because of the inheritance of the mutations, FH can affect multiple members in a family across back-to-back generations (Kamar et al., 2021).

Though mutations in any of the three genes *LDLR*, *APOB*, and *PCSK9* can cause FH, most mutations observed on Asian populations are in the *LDLR* gene (Kamar et al., 2021). Indeed, in a study conducted on Korean patients with FH, Han and colleagues used whole-exome sequencing to detect mutations in the three genes *LDLR*, *APOB*, and *PCSK9* and found that 82.6% of patients had mutations in the *LDLR* gene (2015). Missense mutations were the most common (10/17 *LDLR* gene mutations), followed by frameshift deletion (2/17) and splice site mutations that occurred in exons of the *LDLR* gene (2/17) (Han et al., 2015). Similarly, on Asian Indian patients, Setia and colleagues utilized Sanger sequencing to detect point mutations in the three genes *LDLR*, *APOB*, and *PCSK9* and then used multiplex-ligation-dependent probe amplification technique to find large deletions/duplications in the gene *LDLR*, and they found that 33/38 mutations were in the *LDLR* gene (2020). Missense was also the most common type of *LDLR* gene mutations found on these Asian Indian patients (17/33 *LDLR* gene mutations) (Setia et al., 2020). The mutations reported in the two studies dispersed on all 18 exons of the *LDLR* gene, and due to causing amino acid replacements, these mutations led to the dysfunction of the resultant LDLR proteins (Han et al., 2015; Setia et al., 2020). Nevertheless, mutations did not exclusively occur in exons of the *LDLR* gene. Setia and colleagues reported six cases with a point mutation in intron 10 and one case with a deletion mutation in intron 12 that disrupted RNA splicing (2020). These intron mutations disrupted the normal RNA splicing and led to the addition of a nonfunctional segment or the loss of a functional domain in the LDLR protein (Setia et al., 2020). In short, most mutations associated with FH are found in the *LDLR* gene whose exons and introns can undergo mutations that decrease the expression of LDLR on cell

surface or diminish their affinity for LDL particles; consequently, cholesterol is accumulated in the blood and promote the development of FH (Kamar et al., 2021).

While mutations in the *LDLR* gene are most observed in FH cases in Korea and India, single nucleotide polymorphisms (SNP) associated with an increased risk of FH are more common in the *APOB* gene in Malaysian patients with FH (Lye et al., 2013). In a study examining sequence polymorphisms to gain information about genetic profile of FH on Malaysian population, Lye and colleagues found 11 SNPs associated with a high risk of FH, seven of which were in the *APOB* gene (2013). Lye and colleagues also observed that two of the seven SNPs in the *APOB* gene were in exons (2013). Then, in a systemic review analyzing interactions of two or more genes in the pathogenesis of FH, Kamar and colleagues considered SNP variants in exons of the *APOB* gene as missense mutations because they resulted in amino acid replacements in APOB proteins (Kamar et al., 2021). Missense is the major type of mutations in the *APOB* gene not only in Malaysian patients but also in Indian cases. Of 33 mutations reported in the study by Setia and colleagues, there were only three mutations found in *APOB* gene, but all of them were missense (2020). One similarity between the mutations found in the two populations is that they occurred in exons that do not encode the APOB protein domains responsible for binding LDLR receptors (Lye et al., 2013; Setia et al., 2020). Despite of not affecting the binding domains of the APOB protein, the mutations could cause damaging effects on the resultant APOB proteins, which was proved by Fernández-Higuero and colleagues in their study (2015). Fernández-Higuero and colleagues examined the structure of two APOB protein variants, p.(Arg1164Thr) and p.(Gln4494del), that were extracted from patients with FH; then, they analyzed the influence of structure on the function of the protein variants (2015). In this study, although the protein variant p.(Arg1164Thr) was encoded by a mutant *APOB* gene

caused by a missense mutation in a gene region not encoding the ligand binding domains, the mutation still caused changes in the secondary structure of the resultant APOB protein, a reduction in LDL particle size, and a decrease in LDLR receptor recognition, and hence an impairment in cholesterol uptake (Fernández-Higuero et al., 2015). In other words, though mutations observed on Asian populations do not affect ligand binding domains of APOB protein, they cause the mutant proteins to lose their function and make LDL particles unrecognized by LDLRs; as a result, cholesterol in the blood is not internalized into cells but accumulated in the blood, increasing the risk of FH (Fernández-Higuero et al., 2015; Setia et al., 2020).

Unlike the mutations in the *LDLR* gene and *APOB* gene that result in the loss of function of the mutant proteins, the *PCSK9* gene must undergo gain-of-function mutations to increase the risk of FH (Malo et al., 2020). Without the binding of PCSK9, LDLR receptors can be recycled after internalization by adopting a closed conformation in endosomes that protects them from enzymatic degradation. However, when a PCSK9 protein binds to an LDLR receptor, the PCSK9-LDLR interaction prevents the LDLR from adopting the closed conformation (Malo et al., 2020). Furthermore, at low pH of endosomes, the affinity of the PCSK9 for LDLR is four-times higher than it is in neutral pH environment. Consequently, the LDLR bound by PCSK9 is targeted for enzymatic degradation (Malo et al., 2020). For its effective interaction with LDLR receptors, a PCSK9 protein uses its catalytic domain and a prodomain; nevertheless, mutations observed on Asian populations tend to affect these two domains (Malo et al., 2020). According to Han and colleagues, when using whole-exome sequencing to detect mutations in *PCSK9* gene on Korean patients with FH, two missense mutations in *PCSK9* gene were found (2015). These two mutations resulted in amino acid changes, p.(Glu32Lys) and p.(Arg215Cys), in PCSK9 proteins (Han et al., 2015). Since the amino acid change Glu32Lys occurred in the prodomain of

PCSK9, it was believed to increase the mutant protein's affinity for LDLRs and hence increase its activity (Barale et al., 2021). Meanwhile, the amino acid at the position 215 was in the catalytic domain of PCSK9 protein, and several gain-of-function mutations that occurred at or near this amino acid were reported (Malo et al., 2020). For instance, a missense mutation caused arginine at position 218 to be replaced by serine, and this replacement was proved to increase the stability of the mutant protein (Malo et al., 2020). Because serine and cysteine are similar in structure and properties, the amino acid change Arg215Cys may also increase the function of the mutant PCSK9 protein by enhancing its stability. As the mutant protein is more stable, the PCSK9-LDLR interaction becomes stronger, and LDLR is more likely to be degraded, instead of being recycled. Briefly, *PCSK9* gene mutations occur in regions that encode the catalytic domain and prodomain of PCSK9 protein and result in the increase in the activity and stability of mutant proteins; consequently, they enhance LDRL degradation, causing cells to express a lower level of LDLRs and diminish their cholesterol uptake, and thus FH is promoted (Malo et al., 2020).

By disrupting the normal cholesterol uptake, mutations in the three genes *LDLR, APOB,* and *PCSK9* can increase the risk of FH (Kamar et al., 2021). Missense mutations are most observed in the three genes on FH patients in Malaysia, Korea, and India (Han et al., 2015; Lye et al., 2013; Setia et al., 2020). While missense mutations in exons change the amino acid sequence of LDLR protein, intron mutations disrupt RNA splicing and result in the loss of a functional segment or the addition of a nonfunctional segment in the protein. These alterations impair the affinity of mutant LDLRs for LDL particles in the blood (Setia et al., 2020). Unlike LDLR mutations, SNP variants and missense mutations in the *APOB* gene do not affect the ligand binding domains of APOB protein; instead, they cause changes in the secondary structure of mutant APOB proteins, making LDL particles not recognized by LDLRs (Fernández-Higuero

et al., 2015; Setia et al., 2020). Changes in the *LDLR* and *APOB* genes are loss-of-function mutations; meanwhile, mutations in the *PCSK9* gene enhance the function of mutant PCSK9 proteins. Due to amino acid substitutions in the domains of mutant PCSK9 proteins responsible for PCSK9-LDLR interaction, these mutations increase the affinity of PCSK9 for LDLRs, making LDLRs become targets for enzymatic degradation (Han et al., 2015; Malo et al., 2020). These findings can be useful to propose diagnostic techniques detecting mutations in the three genes *LDLR*, *APOB*, and *PCSK9* in the early stage of FH on Asian populations; furthermore, this review contributes to formulating effective treatment, such as gene-transfer therapy for loss-of-function mutations causing FH. One limitation of this review is including only three studies conducted in Malaysia, Korea, and India; thus, its findings may not be applied to all Asian patients. A systematic review that includes a variety of studies on many Asian populations should be conducted to gain a thorough knowledge of the pathogenicity of FH.

Works Cited

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