The effection of gene variation in Familial Hypercholesterolemia

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Abstract. Familial hypercholesterolemia (FH) is a genetic disorder characterized by significantly elevated levels of low-density lipoprotein cholesterol (LDL-C) in the blood, which increases the risk of atherosclerotic cardiovascular diseases (ASCVD). It is usually accompanied by tendinitis xanthomatosis and a family history of early-onset coronary heart disease. Mutations in LDLR, PCSK9, and APOB are the main pathogenic basis of FH. Although there have been many studies on FH in recent years, there has been no clear summary of the mutation types in the population, thus making it impossible to conduct targeted screening and medication for different FH patients. This review conducts an in-depth analysis of the distribution of mutations in the LDLR, PCSK9, and APOB genes, the impact of LDLR, PCSK9, and APOB mutations on FH, and summarizes the main mutation types of these three genes in different populations. This can further summarize the genetic basis of FH and provide a strong basis for the subsequent development of treatment strategies targeting specific mutation types.

Keywords: LDLR; PCSK9; APOB; familial hypercholesterolemia; founder effect.

1. Introduction

Familial hypercholesterolemia is a common autosomal dominant genetic disorder, with a global prevalence of approximately 1:313, but the prevalence estimates in 90% of countries are missing [1]. Its main characteristic is the abnormally elevated level of low-density lipoprotein cholesterol in the blood, which increases the risk of atherosclerotic cardiovascular diseases, resulting in over 19 million deaths each year [2,3]. FH is usually caused by mutations in genes such as low-density lipoprotein receptor (LDLR), proprotein convertase subtilisin/kexin type 9 (PCSK9), or apolipoprotein B (APOB). Currently, the diagnosis of FH is insufficient, and in most countries, only less than 10% of FH patients are diagnosed [4]. Through cascade screening of family members, the use of PCSK9 inhibitors, small interfering RNA (siRNA), and statin drugs, etc., can effectively diagnose and treat FH patients.

In recent years, with the development of high-throughput sequencing technology and the deepening of genetic research, people have gained a more comprehensive understanding of the mutation spectrum of FH-related genes. This review aims to conduct a comprehensive analysis of the mutations of LDLR, PCSK9 and APOB genes in FH patients, summarize the main distribution types of mutations in LDLR, PCSK9 and APOB genes among different populations and their effects on FH by comparison, analyze and clarify the mechanisms by which these three mutations cause FH, and hopes to summarize the genetic characteristics of FH, providing a scientific basis for subsequent precise medical treatment of FH.

2. The impact of the founder's effect on FH

The founder effect refers to the phenomenon where, in a small initial population, due to the randomness of genetic variation, certain gene frequencies are distributed unevenly throughout the entire population. The founder effect leads to a reduction in genetic diversity and the solidification of genetic traits, allowing specific genes to expand within the population and become dominant characteristics. Due to the founder effect, in some races and geographical groups, there are more common pathogenic variations specific to this region. For example, in a global study, the p.Cys681*

variation accounted for 81.5% of FH patients in Lebanon, and five unique variations accounted for 76% of the FH population in French-Canadian individuals. The p.Gly219del is also a specific variation found in German Jews, Finns, and South African Dutch people [5]. In certain populations with the founder effect (such as South Africa, French-Canadian), the prevalence of FH increases to 1/70-150. Higher prevalence of FH in certain populations also leads to a higher incidence of premature myocardial infarction in these countries [6].

However, the founder effect is more pronounced in certain regions, while it does not exist in other populations. For instance, in a study of FH patients in the Czech Republic, over 180 types of LDLR gene mutations were identified in the Czech Republic, and 6 new ADH (autosomal dominant FH, i.e., FH) mutations were discovered each year on average. Therefore, in some countries such as the Czech Republic, there are no specific variations that account for a majority of cases [7].

3. LDLR

The LDLR protein is a cell surface receptor that can recognize and bind to a lipoprotein mainly responsible for transporting cholesterol - low-density lipoprotein (LDL). Under normal conditions, LDLR is expressed on the cell membranes of the liver and other cells, binding and initiating the process by which LDL particles enter the cell from the extracellular fluid and are directed to lysosomes for destruction. In the LDLR gene, various types of mutations have been discovered, including missense mutations, nonsense mutations, and splice site variations. Among them, missense mutations account for a large proportion. For example, in FH patients in the Czech Republic, 56% of the LDLR gene mutation types are missense mutations [7]. The FH caused by LDLR gene mutations is mostly autosomal dominant inheritance. The pathogenic variations in the LDLR gene are the main cause of familial hypercholesterolemia, which also leads to about 2% of early myocardial infarction (MI) [8]. The effects of pathogenic variants in the LDLR gene mainly include a reduction in the number of cell surface receptors or the presence of functionally impaired receptors [5]. These mutations may result in reduced expression or impaired function of the LDLR protein, affecting the uptake and metabolism of LDL-C, thereby causing familial hypercholesterolemia.

The p.Val429Met mutation in LDLR is a severe form of mutation related to FH, and carriers of this gene mutation have a significantly higher relative risk of developing CVD [9]. Additionally, another rare variation of the LDLR gene is p.Arg248Trp, which also leads to impaired LDLR function and affects LDL uptake. In a study in the Czech Republic, through PCR sequencing, it was concluded that although there was no founder effect in the LDLR mutations of the FH patient population in the Czech Republic, p.Gly592Glu, p.Asp266Glu, p.Cys209Tyr, and p.Arg416Trp were some of the more common LDLR mutations. Moreover, mutations in exon 4 of LDLR were also found more frequently in FH patients, and these mutations often led to an early-onset coronary heart disease in the FH patients of the Czech Republic [7]. In a study in Taiwan, among 443 FH mutation patients, the LDLR mutation p.Cys329Tyr was found in 51 cases (11.5%); p.His583Tyr was found in 48 cases (10.8%). These two mutations were the most common in Taiwan's LDLR mutations. In this investigation, a mutation p.Glu380Ala was also discovered, which is a missense mutation in the coding region of exon 8 of the LDLR gene. After polyphen-2 and SIFT analysis, it was predicted to be a benign mutation and does not cause adverse symptoms and is tolerable [10].

4. PCSK9

The PCSK9 protein is an enzyme encoded by the PCSK9 gene. When functioning normally, it mainly plays a role in cholesterol homeostasis. In the distribution of mutation types of the PCSK9 gene mutations which lead to familial hypercholesterolemia, missense mutations also account for the majority. Additionally, there are some splicing site variations, which may affect the expression and function of the PCSK9 protein. The protein encoded by the PCSK9 gene participates in regulating the quantity of LDLR. Mutations in the PCSK9 gene will cause LDLR to be transported to lysosomes

for degradation, reducing the level of LDLR, decreasing its uptake of LDL-C, raising the level of LDL-C in the blood, and leading to familial hypercholesterolemia. Moreover, mutations in the PCSK9 gene may accelerate atherosclerosis by enhancing inflammation and activating signaling pathways[11]. In Iran, the common variant of PCSK9 is p.Glu32Lys [12], and this variant is also the most common pathogenic PCSK9 variant in the Japanese FH population [13]. In the UK, the common PCSK9 variant is p.Asp374Tyr [14]. Iran and Japan belong to Asian countries, while the UK is located in Europe. Therefore, this review infers that the main mutation types of FH patients in regions that are relatively close may be more similar, while the mutation types of FH in countries that are geographically distant may differ significantly.

In recent years, with the development of sequencing technology, people's understanding of the molecular basis of FH has been continuously increasing. Up to now, the LDLR gene accounts for the vast majority of known pathogenic mutations (90-95%), APOB accounts for 5-10%, and PCSK9 accounts for less than 3% [15]. Even some studies in certain countries suggest that PCSK9 mutations should not be regarded as one of the main factors causing FH. For example, in a study in the Czech Republic, 1296 FH patients were diagnosed, among which 432 patients carried APOB gene mutations, 864 patients carried LDLR gene mutations, and no PCSK9 functional gain-of-function mutations causing FH were found in these patients [7]. This result is comparable to some other studies published in European countries. A multicenter study in China detected 148 cases of FH mutations, but only 4 cases (2.70%) of the indicated cases were identified with PCSK9 gene variations. Moreover, so far, no pathogenic mutations of PCSK9 have been detected in Taiwan. Therefore, some researchers in Taiwan believe that LDLR and APOB mutations are the main genetic causes of FH, while PCSK9 mutations are not [10].

Nevertheless, this review holds that the PCSK9 mutation can still be regarded as an important factor contributing to the FH mutation. This is because the excessive expression or activation of the PCSK9 protein can induce rapid endocytosis and degradation of LDLR, thereby significantly increasing the LDL-C level in the blood. Moreover, the developed inhibitors targeting the PCSK9 protein are now also used as an important treatment method for FH. Therefore, this review concludes that although the PCSK9 mutation is not the predominant prevalent mutation, it is still one of the important factors causing FH.

5. APOB

The APOB protein is the main structural protein of low-density lipoprotein (LDL) and is crucial for the formation and function of LDL particles. The distribution of mutation types of the APOB gene is different from that of LDLR and PCSK9. Although missense mutations are still common, their proportion is relatively lower than that of LDLR and PCSK9. Instead, the proportions of splicing site variations and nonsense mutations are higher, which may be related to the functional characteristics of the APOB gene. The APOB protein is the main component of LDL, and APOB mutations affect the synthesis of apolipoprotein B-100, which may lead to changes in the structure and function of LDL particles, affect their binding to LDLR, and cause abnormal metabolism of LDL-C. For example, p.Arg3527Gln is the earliest identified APOB mutation, which significantly reduces its affinity for LDLR. Studies have shown that the highly conserved receptor-binding site is stabilized by Arg3527, and the substitution of Arg3527 by Gln will damage receptor recognition.

Due to the abnormal structure of APOB, the metabolism of LDL particles is impaired, thereby increasing the symptoms of cardiovascular diseases, which is known as familial defective apolipoprote B100 (FDB). The most common mutation causing FDB is p.Arg3527Gln. The clinical and lipid phenotypes of FDB patients overlap with those of patients carrying LDLR mutations with the FH phenotype, but on average, they have milder manifestations than FH caused by LDLR mutations. In Poland, the most common mutation observed in APOB gene-deficient patients is p.Arg3527Gln, and they typically exhibit symptoms of tendon-like xanthomas [16]. In the Czech Republic, the most common mutation in the APOB gene detected by PCR-RFLP (restriction fragment

length polymorphism) methods is also p.Arg3527Gln [7]. Both Poland and the Czech Republic are located in central Europe, which precisely confirms the "founder effect" and the influence of adjacent geographical effects. In Iran, the common variant of homozygous APOB is p.Ser130Ter, while the main mutation type of heterozygous APOB is p.Arg3527Trp [12]. In Taiwan, 443 FH mutation patients were tested using a customized mass spectrometry-based genotyping method, and the most common mutation of APOB was also p.Arg3527Trp [10], which is consistent with the main mutation type of heterozygous APOB in Iran, which is also located in Asia.

6. Conclusion

By analyzing and summarizing the mutation types of LDLR, PCSK9 and APOB, as well as the mechanisms by which mutations cause FH, and the main mutation types of LDLR, PCSK9 and APOB that cause FH in different regional populations, this study found that the main mutation types of these three genes that cause FH are all missense mutations. Moreover, the main mutation types of populations in geographically close countries are similar, while populations in geographically distant regions have differences in the mutation types of LDLR, PCSK9 and APOB genes. This may be related to genetic background, racial differences, and specific geographical and environmental factors. Additionally, the frequency and types of gene mutations in different regions may be affected by the "founder effect", that is, certain mutations occur more frequently in specific populations, which may be related to genetic isolation in the region's history and specific genetic drift. Although no clear evidence was found in some regions' studies, the "founder effect" has a significant impact on the mutation types of genes causing FH in many regions. Therefore, this review hypothesizes that the "founder effect" has an important influence on the mutation types of genes causing FH in different regions. Through analyzing the mutation types of LDLR, PCSK9 and APOB genes and regional differences, this review summarizes the main mutation types of LDLR, PCSK9 and APOB in different populations, providing new insights into the genetic basis of familial hypercholesterolemia. These summaries also lay the foundation for future research on the development of diagnostic and treatment strategies for FH patients in specific mutation types and different regions.

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