Advances in Molecular Biotechnology for the Diagnosis and Treatment of Familial Hypercholesterolemia (Postprint)

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Abstract

Familial hypercholesterolemia is an inherited lipid metabolism disorder characterized by markedly elevated low-density lipoprotein cholesterol levels in patients, thereby increasing the risk of atherosclerotic cardiovascular disease and imposing a substantial burden on individuals, families, and society. Advances in molecular biotechnology are vital for the screening, diagnosis, and treatment of familial hypercholesterolemia. This article systematically reviews how genetic testing technologies, particularly the development of next-generation sequencing, have enhanced screening efficiency and diagnostic accuracy for familial hypercholesterolemia, while concurrently introducing numerous variants of uncertain significance. In contrast to pharmacotherapy, gene editing or transgenic technologies can rectify molecular defects in patients with familial hypercholesterolemia, holding promise for curative treatment at the molecular level. However, clinical studies have demonstrated that these therapeutic modalities are associated with adverse effects such as hepatic injury, and long-term follow-up remains necessary to establish their efficacy. Therefore, this review summarizes the latest advances in molecular biotechnologies—including genetic testing and gene therapy—for the diagnosis and treatment of familial hypercholesterolemia, aiming to provide novel perspectives for future research on this disease.

Full Text

1. Literature Search Strategy

We conducted computerized searches of PubMed, Web of Science, and other databases from inception through April 2024. Keywords included "Familial Hypercholesterolemia"and related terms such as "genetic testing,""screening,""Next-Generation Sequencing," "CRISPR/Cas9," "gene editing," "atherosclerosis," and

"coronary heart disease." Inclusion criteria comprised studies on Type II hypercholesterolemia characterized by elevated LDL-C (including both heterozygous and homozygous FH), while excluding other inherited lipid disorders such as familial dysbetalipoproteinemia, familial lipoprotein lipase deficiency, Tangier disease, and familial LCAT deficiency. Studies addressing screening, genetic diagnosis, and treatment were included. Exclusion criteria comprised irrelevant studies, those with unavailable full text, and articles with questionable content. A total of 69 references were included.

2. FH Genetic Screening

Early identification of FH relies on effective screening strategies. Current approaches include universal screening, systematic screening, targeted screening, and opportunistic screening. Among these, cascade screening of first-degree relatives of confirmed FH patients is the most efficient method for identifying potential cases. Given that FH is more readily detectable in childhood, particularly in homozygous patients, researchers have proposed a "child-parent screening" approach: lipid levels are measured during 12-month vaccination visits, and if abnormalities are detected, next-generation sequencing (NGS) is used to confirm FH variants. Positive results trigger screening of parents and first-degree relatives. A UK pilot program has demonstrated the cost-effectiveness of this method, though official approval remains pending.

FH genetic screening can be categorized into targeted testing (e.g., gene panels) and whole-genome testing (e.g., NGS). Targeted testing offers lower cost and faster turnaround but only detects known mutations. Whole-genome testing, while more expensive, can identify rare mutations in genes such as PCSK9 and LDL receptor adaptor protein 1 (LDLRAP1). Studies show that whole-exome sequencing identifies pathogenic mutations in approximately 27% of suspected FH patients, whereas targeted testing detects mutations in only 8%, resulting in a high false-negative rate. BENEDEK et al. found that Swedish FH patients exhibit specific high-frequency mutations, with LDLR mutations accounting for 96% of all variants, primarily c.2311+1_{2312}-1(2514)del (FH Helsinki) and c.259T>G. They therefore recommend initial targeted screening for suspected FH patients in Sweden, with NGS used for further clarification when needed. In contrast, ZHANG et al.' s survey of Chinese patients revealed no predominant high-frequency mutations: LDLR mutations accounted for only 37%, ATP-binding cassette subfamily G member 5/8 mutations for 7% each, and lipoprotein lipase and lipase C mutations for 3% each, with digenic mutations present in 7%. Consequently, targeted screening is not recommended for China. Meanwhile, BRANDON et al.' s US study found that genetic testing alone identifies 3.7 cases per 1,000 adults, while the Dutch Lipid Clinic Network (DLCN) criteria alone identifies 3.8 cases per 1,000. However, combining DLCN criteria with genetic testing increases detection to 6.6 cases per 1,000, substantially improving screening efficiency. Based on China' s epidemiological characteristics, we recommend using NGS combined with clinical diagnostic

criteria such as DLCN for FH screening to optimize cost-effectiveness, while piloting the "child-parent screening" method to evaluate its socio-economic value in the Chinese context.

3.1 Genetic Testing for FH Diagnosis

Pathogenic mutations in LDLR, APOB, PCSK9, apolipoprotein E (APOE), and LDLRAP1 are the primary causes of FH . LDLR mutations predominantly involve single-base changes clustered in exons 4, 2-8, and 14, likely due to their encoding of critical protein domains. LDLR gene rearrangements account for approximately 10% of FH variants and are classified as copy number variations. Certain intronic variants, such as c.2140+103G>T and c.2141-218G>A, can also affect splicing or transcription, leading to FH. APOB mutations are concentrated in exon 26, particularly near codon 3500, with over 1,100 variants identified–90% base substitutions, 8% deletions, and 2% insertions. Recent discoveries of variants such as p.(Arg50Trp), p.(Arg3527Gln), and p.(Arg3527Trp) may impair APOB binding to LDLR, elevating LDL-C levels. PCSK9 mutations represent a smaller proportion of FH cases, primarily involving single nucleotide variations. The ClinVar database records approximately 1,000 PCSK9 variants, with only 15 classified as pathogenic or likely pathogenic, reflecting the complexity of determining pathogenicity for PCSK9 variants. Certain APOE mutations are associated with FH phenotypes, such as p.(Arg163Cys) and p.(Leu167del). Other APOE variants have been identified in clinically suspected FH patients but remain unclassified due to lack of functional analysis and family studies. LDLRAP1 mutations cause autosomal recessive FH, with approximately 100 variants recorded, 34 of which are considered pathogenic, mostly insertions or deletions. Compared to other genes, LDLRAP1 mutation carriers exhibit lower ASCVD event rates and later onset.

3.2 Variants of Unknown Significance

As more countries establish FH molecular diagnostic laboratories and commercial testing services, the number of identified variants has increased substantially. However, detecting a mutation in an FH patient does not confirm its pathogenicity. In 2015, the American College of Medical Genetics and Genomics published guidelines classifying variants into five categories–benign, likely benign, variant of unknown significance (VUS), likely pathogenic, and pathogenic –based on multiple data types including population data, computational predictions, functional data, and co-segregation evidence. The UK Association for Clinical Genomic Science classifies most LDLR variants as pathogenic, with a minority remaining VUS. The ClinGen Familial Hypercholesterolemia Variant Curation Expert Panel has developed specific interpretation rules for LDLR variants, clarifying the importance of cysteine residues and functional domains and establishing evaluation criteria such as carrier frequency in healthy versus patient populations and familial co-segregation evidence. These standards help unify pathogenicity assessment of novel variants. Additionally, various bioinfor-

matics analysis methods based on NGS data can streamline this process.

3.3 Identification of Beneficial Mutations Through Genetic Testing

BJORNSSON et al. recently identified a gain-of-function LDLR mutation in an Icelandic pedigree. This mutation involves a 2.5 kb deletion at the 3' untranslated region terminus, removing a microRNA target site that normally suppresses LDLR expression. Carriers exhibited 1.79-fold higher LDLR protein levels and 74% lower LDL-C compared to non-carriers. MENG et al. discovered two PCSK9 loss-of-function mutations (E144K and C378W) in Chinese Uyghur populations that inhibit PCSK9 autocleavage or endoplasmic reticulum release, effectively reducing PCSK9 expression. Genetic testing of patients with extremely low lipid levels, combined with functional studies, can thus provide deeper insights into protein function and identify novel therapeutic targets for lipid intervention.

4. FH Gene Therapy

Gene therapy aims to correct genetic molecular defects and restore normal physiological function, theoretically offering the potential to cure FH at the molecular level. Current approaches include: (1) transgenic technology using adeno-associated virus (AAV) vectors, already FDA-approved for inherited retinal dystrophy and spinal muscular atrophy. Preclinical studies in LDLR knockout mice demonstrated that AAV-8-mediated gene therapy could restore LDLR protein expression, reduce LDL-C, and reverse atherosclerotic plaque progression. However, a Phase I/II trial in HoFH patients (NCT02651675) showed no significant LDL-C reduction and dose-dependent transaminase elevation, likely due to T-cell mediated autoimmunity against the viral vector. (2) CRISPR/Cas gene editing technology, which precisely modifies DNA sequences and has shown efficacy in treating amyloidosis and sickle cell disease. Preclinical studies targeting various cholesterol metabolism pathways are underway. Recent non-human primate studies using lipid nanoparticles (LNPs) to deliver CRISPR demonstrated 83% reduction in plasma PCSK9 and 69% reduction in LDL-C, with effects lasting 476 days post-administration. The primary adverse effect was transient liver enzyme elevation without pathological changes, suggesting clinical feasibility. A Phase I human trial (NCT05398029) has been initiated.

Vaccine research targeting cholesterol metabolism is in early development, with expected effects similar to monoclonal antibodies but potentially more durable due to continuous antibody production. Two PCSK9-targeting vaccines have completed Phase I safety testing; one candidate (AT04A) showed 7.2% LDL-C reduction at 90 weeks, with fatigue, headache, and myalgia as the most common systemic adverse events. Angiopoietin-like protein 3 (ANGPTL3) inhibits lipoprotein lipase and endothelial lipase, regulating lipid metabolism independently of LDLR, making it theoretically suitable for FH treatment. The fully human monoclonal antibody evinacumab, which inhibits ANGPTL3, has demonstrated clear lipid-lowering efficacy and good safety in clinical trials for HoFH

and HeFH. ANGPTL3-targeting vaccines are also in preclinical development, showing preliminary efficacy in reducing atherosclerosis in FH mouse models, though further safety and efficacy studies are needed.

5. Summary and Outlook

Familial hypercholesterolemia, a relatively common inherited lipid metabolism disorder, has seen its diagnosis and treatment advance alongside evolving molecular biotechnology. Key contributions include: (1) **Screening optimization**: Cascade screening is widely adopted globally. The recently proposed "child-parent screening" combines universal and reverse screening advantages, facilitating early FH detection in children while identifying carrier parents, demonstrating favorable cost-effectiveness in theory and preliminary practice. Given China' s epidemiological characteristics with diverse mutations, NGS combined with clinical criteria like DLCN is recommended to improve screening efficiency, while piloting child-parent screening to validate its local value. (2) **Genetic testing optimization**: NGS technology continues to improve diagnostic yields. International guidelines help classify VUS, and bioinformatics methods may streamline analysis. Genetic testing of patients with extremely low lipid levels can uncover novel therapeutic targets. (3) **Gene therapy**: The feasibility of gene editing and transgenic approaches has been validated in animal models, with several Phase I/II trials underway to establish efficacy and safety in humans, offering hope for curative treatment.

Current limitations remain. First, child-parent screening awaits ethical and governmental approval and requires validation across diverse populations. Second, the relatively recent adoption and high cost of NGS mean most studies still use Sanger sequencing of limited gene regions, potentially underestimating prevalence. Wider NGS implementation and cost reduction will enable whole-exome or whole-genome sequencing for greater accuracy. Finally, while AAV and CRISPR-based gene therapies show promise in preclinical studies, preliminary results indicate hepatotoxicity and other adverse effects, necessitating long-term follow-up to clarify treatment-related complications, lipid-lowering efficacy, and prognostic impact.

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Note: Figure translations are in progress. See original paper for figures.