JACC: ASIA VOL. 1, NO. 1, 2021

EDITORIAL COMMENT

The Ongoing Efforts Toward a Definite Diagnosis of Familial Hypercholesterolemia*

Haojie Yu, PhD[a,b,c]

Familial hypercholesterolemia (FH) is one of the most common genetic disorders in humans and is characterized by substantially elevated levels of low-density-lipoprotein cholesterol (LDL-C) in the blood (1). Because of the lifelong exposure to increased LDL-C levels, individuals with FH have significantly higher risk for premature atherosclerotic cardiovascular disease (ASCVD) compared with the general population (1,2). Molecular genetic research in the past 4 decades has discovered several causal genes of FH, the mutations in which all result in reduced efficacy of the liver to remove LDL particles from the circulation (3,4). Pathological mutations in the gene encoding the LDL receptor (*LDLR*), which plays a crucial role in the clearance of atherogenic LDL particles, account for most cases of FH (60% to 80%) (1,3,4). Mutations in additional genes, including *APOB* (encoding apolipoprotein B100, which is the LDLR ligand on the LDL particle) and *PCSK9* (encoding proprotein convertase subtilisin/kexin type 9, which binds to LDLR and promotes its degradation), are further identified to account for 5% to 10% and ~1% of cases of FH, respectively (4). Moreover, rare mutations in *APOE* (encoding apolipoprotein E) and *STAP1* (encoding signal-transducing adaptor protein 1) have also been found in FH patients. While mutations in the above-mentioned 5 genes (*LDLR*, *APOB*, *PCSK9*, *APOE*, and *STAP1*) act in autosomal dominant pattern, in which one copy of a mutant allele leads to the disease phenotype (heterozygous FH), 2 mutant alleles in *LDLRAP1* (encoding LDL receptor adaptor protein 1), *LIPA* (encoding lysosomal acid lipase/cholesteryl ester hydrolase), *ABCG5* (encoding adenosine triphosphate-binding cassette subfamily G member 5) and *ABCG8* act recessively to produce a severe hypercholesterolemia phenotype in patients (1,4). In addition, many patients who meet clinical criteria of hypercholesterolemia do not have monogenic mutations in the known FH genes, and instead the disease phenotype results polygenetically, with many small-effect alleles of single-nucleotide polymorphisms cumulatively raising the LDL-C levels in those patients (1,4,5).

The incidence of heterozygous FH (HeFH) in the general population is estimated to be 1:311 from a recent meta-analysis of 7,297,363 individuals across 42 studies (>60% from European populations) (6). Moreover, HeFH patients have about 18-fold increased risk of ASCVD compared with general population (6). Despite the high prevalence and the presence of potent LDL-C-lowering drugs, such as statins, ezetimibe, and PCSK9 inhibitors, FH remains severely underdiagnosed and undertreated. In general, FH can be diagnosed based on clinical criteria, such as elevated LDL-C levels, physical stigmata, and family history of premature ASCVD, or genetic testing of pathogenic variants in 3 main FH genes, including *LDLR*, *APOB*, and *PCSK9*. With more widely used next-generation sequencing in clinical practice, genetic testing of all first-degree relatives of FH patients (cascade screening) has been demonstrated to be a cost-effective approach to identify additional patients of FH (1,4,7,8).

In this issue of *JACC: Asia*, Cao et al. (9) performed genetic testing of 169 Chinese individuals (124 index cases and 45 relatives) with clinical definite or

*Editorials published in *JACC: Asia* reflect the views of the authors and do not necessarily represent the views of *JACC: Asia* or the American College of Cardiology.

From the [a]Precision Medicine Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [b]Cardiovascular Disease Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; and the [c]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

The author attests they are in compliance with human studies committees and animal welfare regulations of the author's institution and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2772-3747 https://doi.org/10.1016/j.jacasi.2021.04.005

probable FH and found that 62.1% of the patients had monogenic mutations in *LDLR*, *APOB*, or *PCSK9*. The FH monogenic detection rate in this cohort was further increased by 6.5% when 6 additional FH minor genes, including *LDLRAP1*, *LIPA*, *STAP1*, *ABCG5*, *AGCG8*, and *APOE*, and large-scale copy number variants were included in the analysis, suggesting that expanding FH genes in the genetic testing would improve the definite diagnosis of FH in clinical practice. Broadly consistent with previous studies, they found that mutations identified in *LDLR* accounted for 68.8% of the FH-associated variants in the study cohort. In addition, 35% of the discovered variants in FH genes were novel and without any functional annotation, thus highlighting the necessity of further functional validation of those variants of uncertain significance (VUSs) to be used to guide FH diagnosis in the future.

With the declining costs for clinical next-generation sequencing, it is expected that genetic testing will be applied more widely for the diagnosis of FH, which concomitantly will lead to an increased identification rate of new rare variants in FH patients. However, great challenges remain for interpretation of VUSs and further translation for patient diagnosis. Taking the *LDLR* gene for example, among more than 1,800 FH-associated variants deposited in the Leiden Open Variation Database, fewer than 15% of them have functional evidence of pathogenicity; and even after variants classification with the use of The American College of Medical Genetics criteria, ~42% of the *LDLR* variants remain scored as VUSs, which largely limits their applications for clinical diagnosis (10). As such, great efforts should be made to establish functional assays that allow examination of the pathogenicity of FH VUSs in a systematic manner. Such functional interpretation of VUSs, in turn, would work together with family segregation data to achieve a better diagnosis of FH.

Given the fact that the rate of VUS discovery in FH patients currently far outweighs our ability to accurately characterize the functional impact of those variants, cell-based, quantitative, and high-throughput functional assays are urgently needed to fill in this interpretative gap. To validate LDLR variants uncovered in FH patients, a high-content automated microscopy-based LDL uptake assay was established, with which 70 coding variants were systematically examined and the receptor activity relative to wild-type LDLR was quantified in Hela-Kyoto cells (11). Compared with the array-based validation platform, development of an LDL uptake screening assay that allows for validation of many pooled LDLR variants at the same time might be helpful to further increase the throughput of LDLR VUS characterization. It is important to note that the functional impact of variants in other FH genes are more difficult to study in vitro compared with the variants in *LDLR*, so robust functional assays to test variants in those genes also should be developed in the future.

The study by Cao et al. (9) has successfully demonstrated the improvement of FH diagnosis by expanding the gene spectrum in clinical genetic testing, as well as emphasized the importance of functional profiling of VUS for a definite patient diagnosis. As a life-threatening genetic disease, FH is very much underdiagnosed. To address the root causes leading to poor diagnosis, awareness should be raised not only in the medical community and health care system, but also, and more importantly, in the general public. More widely used cascade screening or population-wide genetic screening will increasingly identify patients with FH variants, which, together with cost-effective and high-throughput functional validation assays, will ultimately promote early diagnosis of FH and prevention of premature ASCVD.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Dr. Yu is supported by a start-up grant from National University of Singapore.

ADDRESS FOR CORRESPONDENCE: Dr. Haojie Yu, National University of Singapore, 14 Medical Drive, MD6, #09-01P, Singapore 117599. E-mail: bchhaoy@nus.edu.sg.

REFERENCES

1. Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, Wierzbicki AS. Familial hypercholesterolaemia. Nat Rev Dis Primers 2017;3:17093.

2. Boren J, Chapman MJ, Krauss RM, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease: pathophysiological, genetic, and therapeutic insights: a consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 2020;41:2313–30.

3. Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol 2009;29:431–8.

4. Berberich AJ, Hegele RA. The complex molecular genetics of familial hypercholesterolaemia. Nat Rev Cardiol 2019;16:9–20.

5. Trinder M, Francis GA, Brunham LR. Association of monogenic vs. polygenic hypercholesterolemia with risk of atherosclerotic cardiovascular disease. JAMA Cardiol 2020;5:390–9.

6. Hu P, Dharmayat KI, Stevens CAT, et al. Prevalence of familial hypercholesterolemia among the general population and patients with atherosclerotic cardiovascular disease: a systematic review and meta-analysis. Circulation 2020;141:1742–59.

7. Louter L, Defesche J, Roeters van Lennep J. Cascade screening for familial hypercholesterolemia: practical consequences. Atheroscler Suppl 2017;30:77–85.

8. Sturm AC, Knowles JW, Gidding SS, et al. Clinical genetic testing for familial hypercholesterolemia: JACC scientific expert panel. J Am Coll Cardiol 2018;72:662–80.

9. Cao Y-X, Sun D, Liu H-H, et al. Improvement of definite diagnosis of familial hypercholesterolemia using an expanding genetic analysis. JACC: Asia 2021;1:82–9.

10. Bourbon M, Alves AC, Sijbrands EJ. Low-density lipoprotein receptor mutational analysis in diagnosis of familial hypercholesterolemia. Curr Opin Lipidol 2017;28:120–9.

11. Thormaehlen AS, Schuberth C, Won HH, et al. Systematic cell-based phenotyping of missense alleles empowers rare variant association studies: a case for LDLR and myocardial infarction. PLoS Genet 2015;11:e1004855.

KEY WORDS familial hypercholesterolemia, functional validation, genetic testing