Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.9412-22
Intern Med 61: 2829-2830, 2022
http://internmed.jp

【 EDITORIAL 】

Multiple Ligation-dependent Probe Amplification Along with Whole Exome Sequencing Should be Required for the Diagnosis of Structural Heterozygous Familial Hypercholesteremia

Masao Saotome and Yuichiro Maekawa

Key words: familial hypercholesteremia (FH), genetic test, multiple ligation-dependent probe amplification, whole exome sequencing

(Intern Med 61: 2829-2830, 2022)
(DOI: 10.2169/internalmedicine.9412-22)

Familial hypercholesterolemia (FH) is an autosomal-dominant hereditary lipid disorder, which can eventually cause the premature coronary artery disease (CAD). Recently, FH has been recognized as relatively common disease due to its prevalence (1 in 250-300 general population) (1), and the estimated number of FH patients in Japan is estimated to be approximately 300,000. Although the guidelines list several available diagnostic tools for FH, FH is undetected in most cases. Thus, the identification of undetected FH is important for preventing premature CAD and sudden cardiac death.

The diagnosis of FH can be based on either in clinical criteria or genetic testing. In the guidelines provided by the Japan Atherosclerosis Society and Asian Pacific Society of Atherosclerosis and Vascular Diseases, patients who meet two or more of the criteria listed below should be diagnosed with FH (2).

- Hyper-low density lipoprotein cholesterol (LDL)-cholesterolemia (untreated LDL-cholesterol ≥180 mg/dL)
- Tendon xanthoma (thickening of tendons on the dorsal side of hands, elbows, knees, or Achilles' tendon hypertrophy) or xanthoma tuberosum
- Family history of FH or premature CAD (within the patient's second-degree relatives)

Although it is not essential, the positive detection of known pathogenic mutations (with hyper-LDL-cholesterolemia) can afford a definitive diagnosis of FH (2, 3). In addition, when a proband is diagnosed with FH via a genetic testing, it provides a definite diagnosis of FH in the family who exhibits hyper-LDL-cholesterolemia. Thus, when physicians diagnose an index FH patient, cascade screening of the family may provide an opportunity to conduct genetic testing to detect FH.

Genetic testing to detect FH is performed using either pathogenic variants in the LDL receptor (*LDLR*) gene, Apolipoprotein B (*ApoB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*), or whole-gene sequencing. Causative genetic mutations can be detected in approximately 60-80% of cases. In genetically-defined FH cases, the majority of patients exhibit mutations in the LDL receptor (*LDLR*; 79%) gene, and others mutations in Apolipoprotein B (*ApoB*; 5%), proprotein convertase subtilisin/kexin type 9 (*PCSK9*; <1%), and some accessory genes (4). Meanwhile some FH patients (approximately 10%) have a genetic background of structural polygenic variations (4, 5).

In contrast with heterozygous FH (HeFH), homozygous FH (HoFH), which has causative pathogenic gene mutations in both alleles, is rare (approximately 1 in 1 million in the general population) and exhibits severe clinical features (2). However, there is a phenotype of "severe HeFH," which is associated with severe clinical feature, similarly to HoFH. Thus, when physicians encounter very severe hypercholesterolemia, a clear definitive diagnosis is required to differentiate between HoFH and HeFH, as most HoFM patients require prompt induction of more advanced LDL-cholesterol lowering therapies, including LDL-apheresis and/or microsomal triglyceride transfer protein inhibitor.

Okada et al. presented a case of "severe HeFH" in which the patient exhibited severe clinical features of FH, including supravalvular aortic stenosis. Although her causative mutations could not be identified by whole exome sequencing (WES) using next generation sequencing (NGS), the multiples ligation-dependent probe amplification (MLPA) technique disclosed structural polygenic variations in *LDLR* (6).

Division of Cardiology, Internal Medicine III Hamamatsu University School of Medicine, Japan
Received for publication January 19, 2022; Accepted for publication January 20, 2022
Correspondence to Dr. Masao Saotome, msaotome@hama-med.ac.jp

A comprehensive whole genome analysis has been used in genetic testing for FH (7); however, NGS may have critical limitations regarding the assessment of genomic structural variations of FH. Indeed, it is technically possible to analyze genomic structural variations by NGS with consideration of the depth coverage; however, hundreds of control samples would be needed (8). Thus, at the present time, MLPA may be better for detecting genomic structural variations of FH (5, 8).

In conclusion, Okada et al. recommended the performance of MLPA along with WES for the detection of structural genetic variations of severe HeFH (6). For successful treatment of FH, it is important for physicians to understand the characteristics of both the genetic etiology and genetic testing.

The authors state that they have no Conflict of Interest (COI).

References

1. Mabuchi H, Nohara A, Noguchi T, et al. Molecular genetic epidemiology of homozygous familial hypercholesterolemia in the Hokuriku district of Japan. Atherosclerosis **214**: 404-407, 2011.

2. Harada-Shiba M, Arai H, Ishigaki Y, et al. Guidelines for diagnosis and treatment of familial hypercholesterolemia 2017. J Atheroscler Thromb **25**: 751-770, 2018.

3. McGowan MP, Hosseini Dehkordi SH, Moriarty PM, Duell PB. Diagnosis and treatment of heterozygous familial hypercholesterolemia. J Am Heart Assoc **8**: e013225, 2019.

4. Migliara G, Baccolini V, Rosso A, et al. Familial hypercholesterolemia: a systematic review of guidelines on genetic testing and patient management. Front Public Health **5**: 252, 2017.

5. Wang J, Ban MR, Hegele RA. Multiplex ligation-dependent probe amplification of LDLR enhances molecular diagnosis of familial hypercholesterolemia. J Lipid Res **46**: 366-372, 2005.

6. Okada H, Tada H, Nomura A, et al. Whole exome sequenching insufficient for a definitive diagnosis of a patient with compound heterozygous familial hypercholesterolemia. Intern Med **61**: 2883-2889, 2022.

7. Iacocca MA, Wang J, Dron JS, et al. Use of next-generation sequencing to detect LDLR gene copy number variation in familial hypercholesterolemia. J Lipid Res **58**: 2202-2209, 2017.

8. Mu W, Li B, Wu S, et al. Detection of structural variation using target captured next-generation sequencing data for genetic diagnostic testing. Genet Med **21**: 1603-1610, 2019.