JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 6, NO. 11, 2021

EDITORIAL COMMENT

Genetic Testing in Familial Hypercholesterolemia

Strengthening the Tools, Reinforcing Efforts, and Diagnosis*

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Fighting familial hypercholesterolemia (FH) is mobilizing more and more health care professionals and scientists worldwide to better diagnose, treat, and prevent cardiovascular complications of the disease. Genetic research has repeatedly broadened the horizon and leads to effective treatments that significantly lower cholesterol to reach the recommendation levels.

FH is 1 of the most frequent genetic disorders with a prevalence of 1:200 to 1:250 for heterozygous FH. This prevalence is higher in several regions of the world due to founder effects. Despite the important risk of premature atherosclerosis and cardiovascular complications, FH is largely underdiagnosed, with no more than 10% of FH patients aware of their genetic disease. Thus, increasing awareness, improving diagnosis and prevention, providing counseling to a larger number of patients, and adapting treatment could lower the burden of the disease, reduce its cardiovascular events, and save lives (1-3).

Autosomal dominant FH is mainly caused by mutations in 4 genes: the gene encoding the low-density lipoprotein (LDL) receptor and the *APOB* gene encoding apolipoprotein B, the LDL receptor's ligand. The third gene *PCSK9* encodes proprotein convertase subtilisin kexin 9, and the fourth is *APOE*. We identified the latter 2 by a pedigree-based genetic approach, and, in the case of *PCSK9*, this led to the new therapeutic class of anti-PCSK9 antibodies decreasing LDL cholesterol (LDL-C) to unprecedentedly low levels. *LDLR*, *APOB*, and *PCSK9* have been included by the American College of Medical Genetics and Genomics (ACMG) among the 59 "medically actionable" genes. Genetic testing in FH is recommended worldwide and notably by the International and European Societies of Atherosclerosis through their joint FH Studies Collaboration (that includes over 56 countries), the European Society of Cardiology and American College of Cardiology, and institutions in the United Kingdom (the National Institute for Health and Care Excellence) and the United States (the Centers for Disease Control and Prevention and the FH Foundation) (3).

Genetic testing in FH aims at providing precise molecular diagnosis and important prognostic information and improving atherosclerosis risk stratification. This guides optimal management, prevention, and recruitment of affected relatives through cascade screening and family studies. It helps in rapidly implementing adequate treatment and offering optimal disease management knowing that an informative genetic test may boost patient motivation and compliance (1,2) but also underscores the existence of a lifelong cholesterol burden, requiring effective and sustained cholesterol lowering. Furthermore, genetic testing has the potential to properly tailor treatment and confirm which patients will need more aggressive LDL-C lowering (4), such as compound heterozygotes

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

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

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2021.10.004

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 6, NO. 11, 2021
NOVEMBER 2021:831–833

(2). Indeed, homozygous patients for null alleles will not profit from any PCSK9 monoclonal antibody, whereas evolocumab causes a significant LDL-C reduction (by 30.9%) in patients with *LDLR* gene mutations in both alleles of which at least 1 is defective (2,3). It is noteworthy that compared with patients with polygenic hypercholesterolemia or of unknown genetic cause, monogenic FH patients display reduced responsiveness to standard LDL-C lowering treatments, increased preclinical coronary atherosclerosis, and increased risk of atherosclerotic-related events (3).

DNA sequencing tools have become more powerful, rapid, and available at affordable prices. Next-generation sequencing with a gene panel specific for FH-associated genes as well as polymorphisms (used to establish the polygenic risk score) help detect both rare and common DNA variations underlying clinical hypercholesterolemia with a single laboratory method (4). The use of whole exome and genome sequencing is also increasing for FH diagnosis. These technical developments improve turnaround time for diagnosis, reduce cost, and increase chances in identifying the genetic cause of FH, whether monogenic, oligogenic, or polygenic. It should be noted that Sanger sequencing, the historic gold standard method, is still useful for confirmation and family cascade screening (4) once the pathogenic variant (ie, the mutation) is known.

As the molecular methods have improved, the rate of data generated has increased and the interpretation of nucleotide events (missense, nonsense, and copy number variation) has become a bottleneck. Indeed, it is not always easy to interpret the clinical significance of genetic variants and to prove that novel *LDLR* sequence variations are pathogenic, especially for synonymous and missense variants (1). Furthermore, to standardize the interpretation of sequencing data, the Association for Clinical Genetic Science issued criteria for classifying variants in 2013, which were followed in 2015 by the ACMG/Association for Molecular Pathology (AMP) guidelines that aim to implement a very rigorous variant classification strategy valid for all genetic diseases, including FH. The guidelines include the use of computational analysis and modeling algorithms implemented in bioinformatics prediction tools (1,2).

Even before these criteria, shared databases of variants were built, either gene specific or all-encompassing such as ClinVar. This National Center for Biotechnology Information-funded resource has now become the principal database for archiving significant variants for numerous Mendelian diseases (1). It allows for a complete approach with the presentation of patient and molecular data from different resources (published studies or directly submitted by investigators through the dedicated platforms), as well as a variety of related tools to aid in variant interpretation. FH-associated mutations in ClinVar have been classified using a variety of criteria. These include the general ACMG/AMP guidelines (2015), specified guidelines adhering to the ACMG/AMP framework, and a number of independent methods. However, there is a lack of a standardized approach that is challenging to avoid different interpretations of similar variants (1). To date, more than 3600 *LDLR* gene variants are found in ClinVar. With the increasing need to establish genetic testing on a large scale to improve FH diagnosis and prevention, it has become crucial to develop and share a specifically adapted tool for the analysis of molecular results. This would guarantee a homogenous and standardized interpretation and classification of clinically relevant variants for FH (1). Beyond these tools, additional hard criteria can greatly help in evaluating the pathogenicity of an identified variant. These are family-related and functional analyses. In the first instance, familial segregation is investigated as well as genotype-phenotype correlation and the absence or very low frequency of phenotype-free individuals carrying the variation. Functional analysis is essential to confirm the pathogenicity of a variant by studying the impact of the variant at the transcript and/or protein level (RNA size and level, protein size, amount, and functionality) (4). These analyses generally remain the gold standard for conclusive pathogenic variant identification.

In practice, several tools are widely used, each of them having some particular characteristic, strength, and weakness. A new tool named "MLb-LDLr" has been proposed by Larrea-Sebal et al (5) in this issue of *JACC Basic to Translational Science*, which was designed specifically for the identification of variants in the *LDLR* gene. The MLb-LDLr is a very relevant, specific, and selective tool taking into consideration not only several general parameters dealing with the nature of the change but also others more focused on the LDL receptor structure (different functional domains of the protein). It offers a step forward in the study of the in silico pathogenicity of a variant and gains in being incorporated into other existing analysis platforms and score calculating tools to develop a disease-specific database highly accurate for the exploration of the pathogenicity of the *LDLR* gene variants as well as those of *APOB* or *PCSK9*. Furthermore, it is a scalable database that can be enriched by

new variants submitted to ClinVar. One concern is that it is limited to the study of missense variants (5) and should be further developed to allow the analyses of splicing variants and copy number variations that are common in the *LDLR* gene and frequently pathogenic in FH.

However, because neither this new in silico tool nor already known tools can be 100% accurate, there is a need to foster the procurement of highly accurate and complete data. Therefore, an effort should be undertaken to gather maximum information for new as well as already published variants, especially clinical data, family information and segregation (when possible), and functional or protein investigation. This will be very helpful in better characterizing variants in the *LDLR* gene with "conflicting interpretations of pathogenicity" in ClinVar or of uncertain significance (1). A huge effort is being made for FH registries and guidelines, but the scientific community should also be encouraged and helped in deciphering all the variants. This could be achieved by providing funds to academia and research or clinical laboratories (without omitting low-income countries) to help enhance FH family recruitment and perform functional studies. Further incentive is needed to recognize this huge body of work performed by laboratories. This could be done through special issues of journals or journals that would consider for publications such basic but very important detailed data that are still lacking despite being clinically very relevant.

An effort to continue sharing more detailed results and precise data is essential to allow the full benefit of the new genomic tools to be available to patients worldwide (1,4). The continued development and scaling of prediction tools such as the MLb-LDLr, together with FH-dedicated databases (integrating variant frequencies from populations of various geographic origins and genotype-phenotype correlations), are crucial. This will be important and strengthen the commitment of consortium and expert committees or geneticists with long-standing expertise in FH in providing a better and more accurate diagnosis along with effective tools for proper and efficient result interpretation in order to improve patient care and fight the disease.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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REFERENCES

1. Iacocca MA, Chora JR, Carrié A, et al. ClinVar database of global familial hypercholesterolemia-associated DNA variants. *Hum Mutat*. 2018;39(11):1631–1640.

2. Brown EE, Sturm AC, Cuchel M, Braun LT, et al. Genetic testing in dyslipidemia: a scientific statement from the National Lipid Association. *J Clin Lipidol*. 2020;14(4):398–413.

3. Sturm AC, Knowles JW, Gidding SS, et al. Clinical genetic testing for familial hypercholesterolemia: JACC Scientific Expert Panel. *J Am Coll Cardiol*. 2018 7;72(6):662–680.

4. Berberich AJ, Hegele RA. The role of genetic testing in dyslipidaemia. *Pathology*. 2019;51(2):184–192.

5. Larrea-Sebal A, Benito-Vicente A, Fernandez-Higuero J, et al. MLb-LDLr: a machine learning model for predicting the pathogenicity of *LDLr* missense variants. *J Am Coll Cardiol Basic Trans Science*. 2021;6(11):815–827.

KEY WORDS bioinformatic tools, genetic testing, familial hypercholesterolemia, *LDLr*, MLb-LDLr