JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 72, NO. 6, 2018

PUBLISHED BY ELSEVIER

THE PRESENT AND FUTURE

JACC SCIENTIFIC EXPERT PANEL

Clinical Genetic Testing for Familial Hypercholesterolemia

JACC Scientific Expert Panel

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Convened by the Familial Hypercholesterolemia Foundation

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CME/MOC/ECME Editor Disclosure: *JACC* CME/MOC/ECME Editor Ragavendra R. Baliga, MD, FACC, has reported that he has no financial relationships or interests to disclose.

Author Disclosures: Ms. Sturm has served on scientific advisory boards for Clear Genetics and Genome Medical. Dr. Knowles has served as the chief medical advisor for the Familial Hypercholesterolemia Foundation; and has received funding from an American Heart Association National Innovative Research Award and the Doris Duke Charitable Trust. Dr. Gidding has served as a consultant for Regenxbio. Dr. Ahmad has performed research for the National Institutes of Health and Regeneron; has served as a speaker for Amgen, Sanofi, and Akcea; and has served on advisory boards for Sanofi and Akcea. Dr. Ballantyne has received grant/research support (all paid to institution, not individual) from Abbott Diagnostic, Amarin, Amgen, Esperion, Ionis, Novartis, Pfizer, Regeneron, Roche Diagnostics, and Sanofi-Synthelabo; and has served as a consultant for Abbott Diagnostics, Amarin, Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Esperion, Ionis, Matinas BioPharma Inc., Merck, Novartis, Novo Nordisk, Pfizer, Regeneron, Roche Diagnostics, and Sanofi-Synthelabo. Dr. Baum has served on scientific advisory boards, provided consulting, and performed clinical research for Amgen, Sanofi/Regeneron, Esperion, Akcea, AstraZeneca, Boehringer Ingelheim/Lilly, Novo Nordisk, and Gemphire; and has served as a speaker for Amgen, Boehringer Ingelheim/

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ISSN 0735-1097/$36.00 https://doi.org/10.1016/j.jacc.2018.05.044

Lilly, Novo Nordisk, and Aralez. Dr. Bourbon has received research funding from Praxis (Aegerion), Alexion, Regeneron, and Gendiag. Dr. Carrie has received honoraria from Amgen SAS and Alexion Pharma France SAS; and a grant from Alexion Pharma France SAS. Dr. Cuchel has received industry support for the performance of clinical trials from Akcea, Regeneron Pharmaceuticals, and Regenxbio; has served on the speakers bureau for Aegerion Pharmaceuticals; and has received federal funding from the National Heart, Lung, and Blood Institute (HL059407). Dr. de Ferranti has received royalties from UpToDate for topics related to pediatric lipid screening and treatment; and grant funding from the Pediatric Heart Network (no overlap) and the New England Congenital Cardiology Research Foundation (no overlap). Dr. Freiberger has received grants from the Ministry of Health of the Czech Republic (16-29084A and 15-28277A) (all rights reserved). Dr. Hovingh has served as an advisory board member and/or speaker for Pfizer, Regeneron, Sanofi, Amgen, and Aegerion (his institution receives the financial compensation for these services). Dr. Kastelein has provided consulting for Sanofi, Affiris, Akarna Therapeutics, Amgen, CSL Behring, Regeneron, Staten Biotech, Madrigal, The Medicines Company, Kowa, Lilly, Esperion, Gemphire, Ionis Pharmaceuticals, and Akcea Pharmaceuticals. Dr. Linton has performed research for Merck, Amgen, Sanofi, Regeneron, Genzyme, Ionis, the National Institutes of Health (HL116263), FH Foundation CASCADE; and has served as a consultant for Regenxbio. Dr. Mata has received honoraria for advisory boards and research grants from Amgen and Sanofi. Dr. Santos has received honoraria related to consulting, speaker activities, and research from Amgen, AstraZeneca, Akcea, Biolab, Esperion, Merck, Novo Nordisk, Pfizer, and Sanofi/Regeneron. Dr. Harada-Shiba has received grants from Japan Agency for Medical Research and Development, Japanese Ministry of Health, Labour and Welfare, The Japanese Circulation Society, Aegerion, Astellas Pharm, Takeda, MSD, and Kaneka Medics; and has received honoraria for talks from Astellas, Astellas Amgen, Sanofi, Takeda, MSD, Boeringer Ingelheim, Daiichi-Sankyo, Kaneka Medics, and Kowa. Dr. Sijbrands received a grant from Amgen for scientific research (not drug related). Dr. Yamashita has received research grants from Merck Sharp & Dohme, Bayer, Boehringer Ingelheim, Takeda, Ono, and Astellas; has received consulting fees from Skylight Biotec; and has served on the speakers bureau for Kowa, Merck Sharp & Dohme, Astellas, Astellas-Amgen Biopharma, and Sanofi. Ms. Wilemon has served as the chief executive officer for the Familial Hypercholesterolemia Foundation. Dr. Ledbetter has served as the chief scientific officer for Clear Genetics, Inc. Dr. Rader has served as the chief scientific advisor for the Familial Hypercholesterolemia Foundation. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Medium of Participation: Print (article only); online (article and quiz).

CME/MOC Term of Approval

Issue Date: August 7, 2018
Expiration Date: August 6, 2019

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JACC VOL. 72, NO. 6, 2018
AUGUST 7, 2018:662–80

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ABSTRACT

Although awareness of familial hypercholesterolemia (FH) is increasing, this common, potentially fatal, treatable condition remains underdiagnosed. Despite FH being a genetic disorder, genetic testing is rarely used. The Familial Hypercholesterolemia Foundation convened an international expert panel to assess the utility of FH genetic testing. The rationale includes the following: 1) facilitation of definitive diagnosis; 2) pathogenic variants indicate higher cardiovascular risk, which indicates the potential need for more aggressive lipid lowering; 3) increase in initiation of and adherence to therapy; and 4) cascade testing of at-risk relatives. The Expert Consensus Panel recommends that FH genetic testing become the standard of care for patients with definite or probable FH, as well as for their at-risk relatives. Testing should include the genes encoding the low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*), and proprotein convertase subtilisin/kexin 9 (*PCSK9*); other genes may also need to be considered for analysis based on patient phenotype. Expected outcomes include greater diagnoses, more effective cascade testing, initiation of therapies at earlier ages, and more accurate risk stratification. (J Am Coll Cardiol 2018;72:662–80) © 2018 by the American College of Cardiology Foundation.

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Manuscript received February 12, 2018; revised manuscript received May 11, 2018, accepted May 15, 2018.

This statement provides the rationale for genetic testing for familial hypercholesterolemia (FH) and recommendations for its utilization in the clinical setting. Although other comprehensive FH guidelines include recommendations related to FH genetic testing (1–4), no statement specifically dedicated to clinical genetic testing for FH exists, in contrast to other genetic cardiovascular conditions such as inherited cardiomyopathies and arrhythmias (5). Because FH is common yet underdiagnosed, it is expected that genetic testing will facilitate the diagnosis of FH, the initiation and intensity of recommended lipid-lowering therapy (LLT), and the identification of affected relatives, thus reducing the burden of cardiovascular disease in families with FH.

The Familial Hypercholesterolemia Foundation therefore convened an international expert panel consisting of cardiologists, lipidologists, endocrinologists, genetic epidemiologists, molecular pathologists, patient representatives, nurses, genetic counselors, and genetic testing experts to address this important gap. A subgroup (A.C.S., J.W.K., S.S.G., K.A.W., D.H.L., and D.J.R.) wrote the initial draft, which was then circulated to the entire authorship group for further critique and approval. Early versions were based on expert opinion; later versions before circulating included an evidence review using PubMed searches on terms that included familial hypercholesterolemia, genetic testing, and genetic counseling. Evidence grades were based on the American College of Cardiology/American Heart Association schema (6). The recommendations provided herein are based on data indicating the value of molecular genetic information in FH diagnosis, prognosis, risk stratification, therapy, and cascade testing. The analytic and clinical validity and clinical and personal utility of FH genetic testing are described.

INTRODUCTION

FH is a genetic condition that results in premature atherosclerotic cardiovascular disease due to lifelong exposure to elevated low-density lipoprotein cholesterol (LDL-C) levels. FH is the most common genetic cause of cardiovascular disease, with an estimated prevalence of ~1:220 (7–10) and is even more common in certain ethnic groups and founder populations (11). If not identified and appropriately treated from an early age, untreated male subjects are at a 50% risk for a fatal or nonfatal coronary event by 50 years of age and untreated female subjects are at a 30% risk by 60 years of age (12,13). The EOMI (Early-Onset Myocardial Infarction) study within the US National Heart, Lung, and Blood Institute's Exome Sequencing Project found that ~2% of cases of early myocardial infarction (male subjects ≤50 years of age, female subjects ≤60 years of age) have a pathogenic variant in the main gene known to cause FH, the LDL receptor (*LDLR*) (14). Early diagnosis and medical management beginning in childhood with statins and other LLTs have the potential to reduce the incidence of atherosclerosis in patients with FH to that of individuals without FH (15–17).

FH encompasses a spectrum of clinical phenotypes, based in part on the range of pathogenic variants (**Figure 1**). Heterozygous FH (HeFH [also referred to as FH]) is usually caused by a single pathogenic variant in 1 of the 3 primary genes associated with FH: *LDLR* and the genes encoding apolipoprotein B (*APOB*) and proprotein convertase subtilisin/kexin 9 (*PCSK9*) (18). The pathogenic variants in *LDLR* are the most common. Homozygous FH (HoFH) is caused by biallelic pathogenic variants, generally in *LDLR* (19), with recent data suggesting a prevalence of molecularly defined HoFH to be ~1 in 200,000 to 300,000 persons (20). HoFH causes markedly premature atherosclerotic cardiovascular disease and, if untreated, early death. Additional genetic variation can also influence the LDL-C level in patients with an FH-causing variant, suggesting that the FH clinical phenotype, at least in some patients, is due to a large effect pathogenic variant in one of the main FH genes in combination with a polygenic component (21,22). Patients with the FH clinical phenotype (both very high LDL-C levels and positive family history) may have negative genetic test results for the 3 primary genes but still may have genetic variation contributing to high LDL-C levels.

A large proportion of patients with FH are undiagnosed, even when they have not only elevated LDL-C levels but premature coronary artery disease (CAD) and/or myocardial infarction (7,23). Current estimates show that >90% of 30 million individuals with FH worldwide and the >1 million in the United States are undiagnosed (24,25). Recent data from studies using electronic health record searches have shown suboptimal treatment of FH in addition to underdiagnosis, with at most one-half of patients treated adequately and up to one-third not treated at all (7,26).

Although genetic testing has the potential to improve diagnosis and provide prognostic data and accurate risk assessment, data from the CASCADE FH

ABBREVIATIONS AND ACRONYMS

ACMG = American College of Medical Genetics and Genomics

APOB = apolipoprotein B

CAD = coronary artery disease

DLCNC = Dutch Lipid Clinic Network Diagnostic Criteria

DNA = deoxyribonucleic acid

FH = familial hypercholesterolemia

HeFH = heterozygous familial hypercholesterolemia

HoFH = homozygous familial hypercholesterolemia

LDL-C = low-density lipoprotein cholesterol

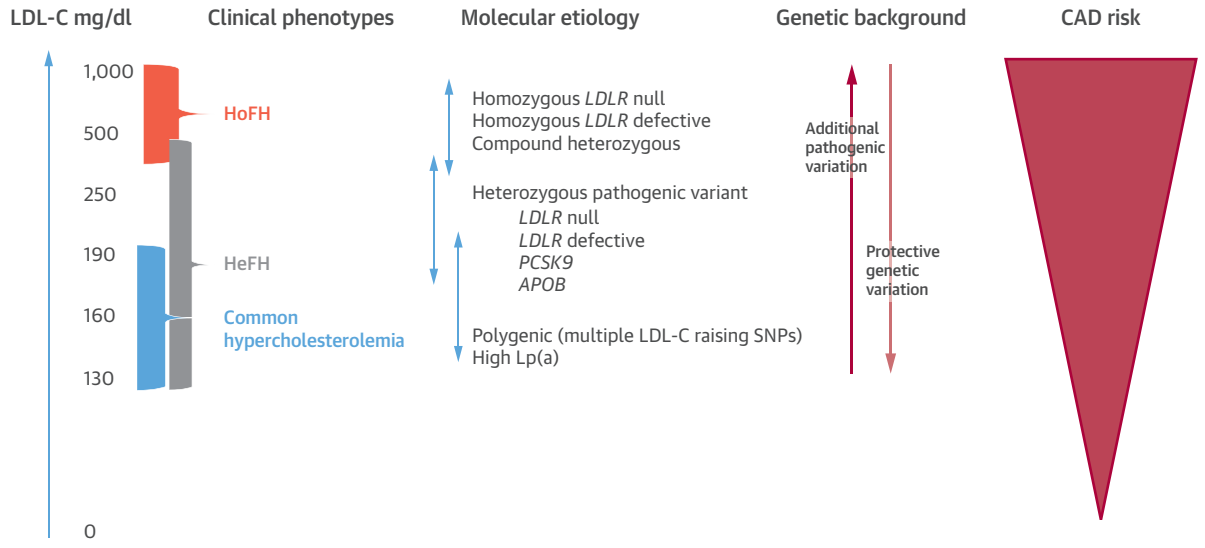
LDLR = low-density lipoprotein receptor

LLT = lipid-lowering therapy

NGS = next-generation sequencing

PCSK9 = proprotein convertase subtilisin/kexin 9

FIGURE 1 Phenotypic Spectrum of FH



Familial hypercholesterolemia (FH) includes multiple clinical phenotypes due to different underlying molecular etiologies and additional genetic background. Low-density lipoprotein cholesterol (LDL-C) level, number of mutations, and additional pathogenic and/or protective genetic variation determines coronary artery disease (CAD) risk level. *APOB* = gene encoding apolipoprotein B; HeFH = heterozygous familial hypercholesterolemia; HoFH = homozygous familial hypercholesterolemia; *LDLR* = gene encoding low-density lipoprotein receptor; Lp(a) = lipoprotein a; *PCSK9* = gene encoding proprotein convertase subtilisin/kexin 9; SNP = single nucleotide polymorphism.

(Cascade Screening for Awareness and Detection of FH) Registry indicate that FH genetic testing is underutilized for patients in the United States, with genetic testing reported in 3.9% of individuals in the registry with a clinical diagnosis (27). FH genetic testing has been performed more extensively and/or at the population level in the Netherlands, Norway, United Kingdom, Spain, Denmark, Belgium, Czech Republic, Slovakia, Iceland, Switzerland, Canada, Australia, New Zealand, and South Africa, among others (24,25,28).

THE RATIONALE FOR CLINICAL GENETIC TESTING FOR FH

GENETIC TESTING PROVIDES A DEFINITIVE MOLECULAR DIAGNOSIS OF FH. Historically, the diagnosis of FH has been based on several sets of clinical diagnostic criteria that include elevated LDL-C levels, clinical history of premature cardiovascular disease, family history of hypercholesterolemia and/or cardiovascular disease, physical examination findings (including tendon xanthomas and corneal arcus), and deoxyribonucleic acid (DNA) testing evidence of a pathogenic variant causative of FH. Although a diagnosis of FH can be made based on clinical findings alone, the Dutch Lipid Clinic Network Diagnostic Criteria (DLCNC) (29), the Simon Broome Register Diagnostic Criteria (30), and the criteria presented in the 2015 American Heart Association scientific statement on FH (18) all include genetic testing as a key approach to making the diagnosis of definite FH. The identification of a pathogenic variant, or variants, in *LDLR*, *APOB*, or *PCSK9* provides the highest number of points toward a definite diagnosis of FH in the DLCNC (29). Detection of a pathogenic variant has also been described as the "gold standard" for FH diagnosis secondary to variants affecting LDLR function (3,31).

The "classic" FH clinical presentation has changed over time due to statin treatment and potentially due to decreased saturated fat intake (32). For example, physical examination findings and family history of premature atherosclerotic cardiovascular disease are present in only a minority of molecularly defined FH patients, as evidenced in the SAFEHEART Registry (Spanish Familial Hypercholesterolemia Cohort Study) (33). Xanthomas were present with a frequency of <15%, and corneal arcus was present in ~30% (34). Similar results have been seen in the US-based CASCADE FH Registry (27). In addition, in a national FH screening program, only 8% of affected relatives had xanthomas and only 5% had xanthelasma at the time of genetic testing (35).

There are also limitations to the clinical sensitivity of a family history of cardiovascular disease, which is part of all published diagnostic criteria for FH. These limitations can be due to several reasons, including reduced penetrance (36), affected relatives receiving LLT (thereby "masking" the hypercholesterolemia and coronary heart disease phenotype), the reduced clinical sensitivity and/or specificity of self-reported family history (37), as well as the simple unavailability of reliable family history information (38). Only 41% of children with a molecularly confirmed FH diagnosis in a Slovenian national universal lipid screening program had a family history of cardiovascular disease (39). In the absence of molecular genetic testing, there are limitations to diagnosing FH in children, as the DLCNC are not valid in children; thus, the diagnosis relies on family history and serial fasting plasma LDL-C measurements (2,40). The Simon Broome diagnostic criteria can be applied to children <16 years of age, using lower total cholesterol and LDL-C cut points, in the setting of tendon xanthoma or positive family history (30).

Furthermore, recent large-scale DNA sequencing studies have illustrated the limitations of using specific LDL-C cut-points for the identification of those with pathogenic FH variants. Although LDL-C levels in those with FH-associated variants are increased overall, a wide spectrum of LDL-C levels is observed (**Figure 1**) (7,36). Khera et al. (36) reported the sequencing of *LDLR, APOB,* and *PCSK9* in >26,000 individuals from 7 case-control studies (cases were those with CAD, and controls were CAD-free) and 5 prospective cohort studies. Although the average LDL-C level was 190 mg/dl in those with an FH pathogenic variant, 55% of those with a pathogenic variant had LDL-C levels <190 mg/dl and 27% had an LDL-C level <130 mg/dl. Abul-Husn et al. (7) assessed the prevalence and clinical impact of FH-associated variants in >50,000 individuals who underwent whole exome sequencing. In this study, by retrospectively applying the DLCNC to electronic health record data, a probable or definite FH clinical diagnosis was present in just 24% of those with an FH variant, and a maximum LDL-C level ≥190 mg/dl was absent in 45% of those with an FH variant. In addition, Wald et al. (10) found that not all children with an FH mutation had hypercholesterolemia at ~12 months of age. Because individuals with FH-associated variants may not have LDL-C levels above certain thresholds, yet have an elevated risk for CAD, genetic testing has utility in identifying those with FH who are at increased risk and who likely would not otherwise be diagnosed.

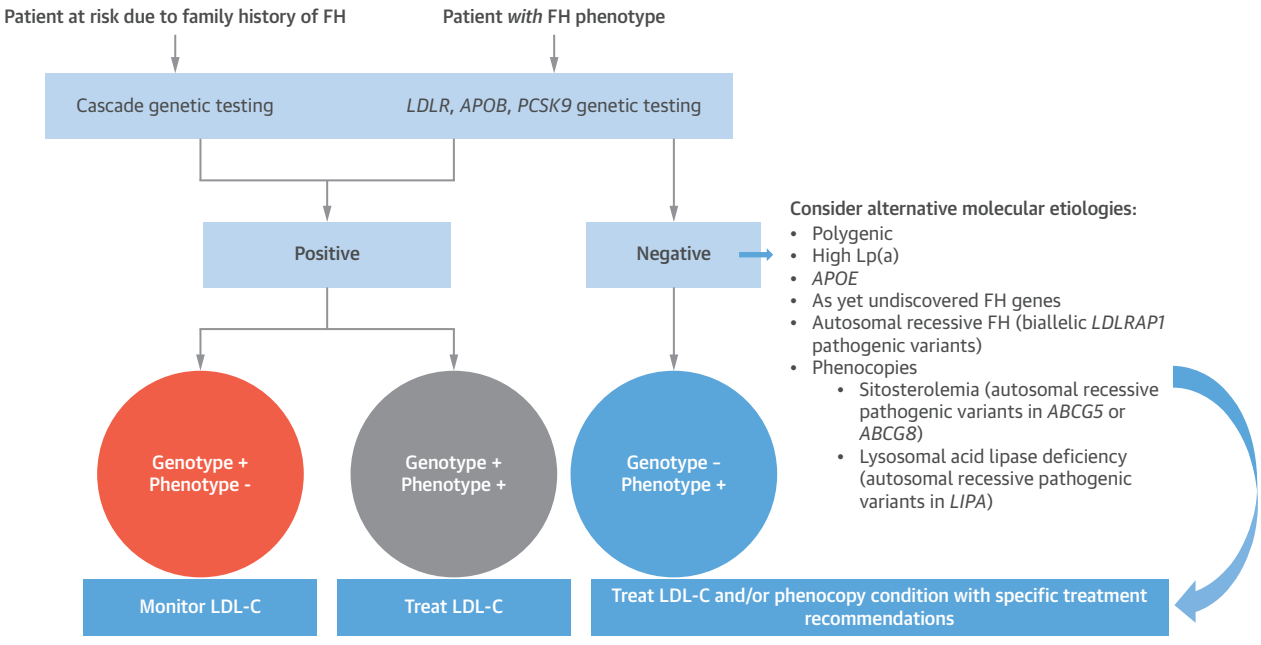
In addition to LDL-C threshold levels (i.e., ≥190 mg/dl) missing significant numbers of individuals with FH pathogenic variants, the ability to distinguish those with FH from those with elevated cholesterol levels due to other reasons is complicated by an overlap in LDL-C levels between individuals with and without an FH pathogenic variant (10,36,41). Discrimination based on LDL-C levels is best in youth (42), but because LDL-C rises with age, overlap increases between those with an FH pathogenic variant and those without (41,43,44). Genetic testing can help distinguish these 2 groups of individuals.

In sum, although there are several sets of FH diagnostic criteria, there is no international consensus on which set of criteria is superior. The use of diagnostic tools that rely on the presence of physical features, premature CAD, and family history limits diagnostic efficacy and the goal of identifying all patients with FH because although these tools have higher specificity, they have lower sensitivity. Diagnostic accuracy is key; however, to best identify and subsequently treat the spectrum of patients with FH (inclusive of those with an identifiable pathogenic variant or variants [genotype positive], those without [phenotype positive, genotype negative], and those who do not undergo genetic testing), both genotype-positive and phenotype-positive definitions of FH should be used (**Figure 2**). The American Heart Association scientific statement on FH presents a clinical classification of FH focused on hypercholesterolemia in the proband (or index case) and the presence of a positive family history of hypercholesterolemia or premature CAD (phenotypic FH), as well as the presence of genetic mutation information (genotype-positive FH) (18).

All of these factors, together with a general lack of awareness, contribute to the very low rate of formal FH diagnosis in both the United States and worldwide (25). Genetic testing aids FH diagnosis by identifying those with pathogenic variants who do not meet diagnostic criteria based on lipid levels, clinical and physical features, and/or family history. Thus, one rationale for FH genetic testing is to facilitate the diagnosis of FH in those who may not have otherwise been diagnosed with FH.

GENETIC TESTING PROVIDES PROGNOSTIC AND RISK STRATIFICATION INFORMATION. FH genetic testing provides prognostic information and the ability to perform refined risk stratification. Within the Myocardial Infarction Genetics Consortium case-control cohort populations, the risk for CAD was higher in FH pathogenic variant carriers compared with noncarriers at any LDL-C value (**Figure 3**) (36).

FIGURE 2 Different Categories of Patients May Undergo FH Genetic Testing



Individuals at risk due to family history as well as individuals with an FH phenotype may undergo FH genetic testing. The results of this testing can result in 3 categories of individuals: 1) genotype positive, phenotype negative; 2) genotype positive, phenotype positive; and 3) genotype negative, phenotype positive. In some cases, alternative molecular etiologies should be explored. Abbreviations as in Figure 1.

Compared with a reference group with LDL-C levels <130 mg/dl and no pathogenic variant, individuals with LDL-C levels ≥190 mg/dl and no FH pathogenic variant had a 6-fold higher risk for CAD, whereas those with LDL-C levels ≥190 mg/dl and an FH pathogenic variant exhibited a 22-fold increased risk for CAD. The presence of an FH pathogenic variant increases CAD risk >3-fold at the same LDL-C level, presumably related to greater lifelong exposure to elevated LDL-C levels. Even for those with LDL-C levels <190 mg/dl, and <130 mg/dl, CAD risk is higher in those with an FH pathogenic variant compared with those without.

Similar findings from Japan confirm that knowledge of FH pathogenic variant status allows for the identification of individuals at the highest CAD risk by contributing additional risk information beyond that predicted by clinical data alone, including LDL-C levels (45). Female subjects >18 years of age with a confirmed *LDLR* pathogenic variant have been shown to have an increased risk of premature CAD compared with women without a documented FH variant, even after adjustment for lipid levels and traditional CAD risk factors (hazard ratio: 2.53) (46). Furthermore, among patients with severe hypercholesterolemia and a family history of early cardiovascular disease, those with genetically confirmed FH had a higher prevalence of coronary artery calcification and positive exercise stress test results (47).

The specific type of pathogenic variant and its severity (i.e., LDLR-defective vs. receptor-null) is associated with the degree of hypercholesterolemia and the risk for CAD development, including premature CAD risk; *LDLR* null variants are the most severe (7,36), and non-null *LDLR* variants, as well as *APOB* and *PCSK9* pathogenic variants, generally having a milder phenotype (7). Pathogenic variant type has also been shown to be an independent predictor of attainment of LDL-C treatment goals (34). In addition, coronary and carotid atherosclerosis severity has been shown to be higher in those with monogenic FH compared with those with an elevated LDL-C level due to a polygenic etiology (48). These findings reinforce the utility of genetic testing in the provision of cardiovascular risk information beyond that provided by LDL-C level alone.

In addition, FH genetic testing has been shown to have a positive effect on the initiation of LLT, adherence to therapy, and LDL-C reduction (35,44). Even in patients with FH who were already receiving LLT, significant effects on plasma LDL-C levels were observed after confirmation by genetic testing (49). In Norway,

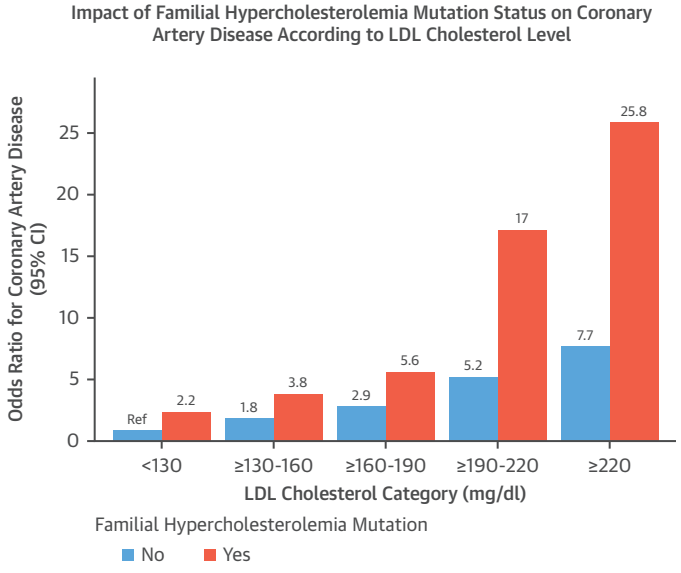
the proportion receiving LLT increased from 53% at the time of genetic testing to 89%, with a 21% reduction in total serum cholesterol 6 months after testing (50).

Thus, a further rationale for FH genetic testing is that detection of a pathogenic variant indicates higher cardiovascular risk and the need for more aggressive LDL-C reduction, and that a positive genetic test result increases initiation and adherence to LLT.

GENETIC TESTING FACILITATES FAMILY-BASED CASCADE TESTING FOR FH. Because FH is an autosomal dominant disorder, screening the at-risk relatives of a patient with FH ("cascade testing") can be highly effective in identifying additional individuals with FH who require treatment (44,51). Cascade testing of relatives of people with FH has been given the Tier 1 classification by the U.S. Centers for Disease Control and Prevention Office of Public Health Genomics (52), meaning clinical practice guidelines based on systematic review supports the testing. Cascade testing using DNA analysis is also recommended in the U.K. National Institute for Health and Care Excellence clinical guidelines (1) and the European Atherosclerosis Society consensus statement on FH (25). Cascade testing has been shown to be a cost-effective method for the identification of new patients with FH (53–56) and also a cost-effective means of preventing coronary heart disease, myocardial infarction, and death (57–59). Cascade testing can also reduce the average age at which relatives with FH are diagnosed compared with the age of diagnosis for index patients (60). In the United States, cascade testing for FH is not currently systematically performed (38).

Cascade testing can be performed by using analysis of LDL-C levels alone, but this approach has sensitivity and specificity issues (41). LDL-C levels in FH and non-FH relatives overlap considerably, especially in adults. A substantial number of relatives who inherit the causal pathogenic variant have some degree of "reduced penetrance" and LDL-C levels that, although usually elevated, would not qualify them for a clinical diagnosis of FH (61). In some cases, individuals with genetically proven FH also carry genetic variation associated with lower LDL-C levels (62,63). If only LDL-C levels are used for cascade screening, and are below a pre-defined threshold, the screening cascade is at risk of stopping at family members who carry the causal pathogenic variant. DNA testing, however, yields unambiguous cascade testing results for at-risk relatives. Moreover, knowledge of the pathogenic variant in the family increases the number of patients with FH identified per family. This concept is supported by findings from the Czech national database: in families with a known causal pathogenic variant, the number of patients with FH per family is on average 1.77, whereas in families without this information it is 1.18 (28).

FIGURE 3 FH Genetic Testing Provides Prognostic Information and the Ability to Perform Refined Risk Stratification



The risk for CAD is higher in FH pathogenic variant carriers compared to noncarriers at any LDL-C value. Reproduced with permission from Khera et al. (36). Abbreviations as in Figure 1.

Identification of a pathogenic variant, or variants, in the FH proband allows for targeted, site-specific cascade genetic testing in at-risk relatives, with very high sensitivity and specificity. This approach can provide unambiguous results for relatives with and without FH. When the FH proband is a child, this method allows for reverse cascade testing and identification of the affected parent (10), or parents, when affected children have 2 pathogenic variants; affected siblings can also be identified. For those with FH, recommended medical management can be initiated, and it has been well documented that identifying affected relatives by using cascade genetic testing has significant therapeutic consequences, as reviewed by Leren et al. (35). Specifically, in the Netherlands, the proportion of adult affected relatives receiving LLT increased from 39% at the time of genetic testing to 93% 1 year after, and in affected but previously untreated adult relatives, a 23% reduction in total serum cholesterol level was observed 1 year after testing (44). Subsequently, a 30% reduction in mean baseline LDL-C level was observed. Cascade genetic testing also identifies those relatives who did not inherit the

familial pathogenic variant and therefore are highly unlikely to have FH (unless inherited from the unrelated parent). This outcome is of high personal utility, as relatives who test negative will be relieved by the knowledge of being unaffected and having no risk to pass the familial pathogenic variant to their offspring.

Published FH clinical guidelines already state that if a pathogenic variant has been identified in an index patient, the variant should be used to identify affected relatives (1,3,25).

GENETIC TESTING ALLOWS FOR PRECISION DURING GENETIC COUNSELING. FH genetic testing should be accompanied by pre- and post-test genetic counseling (2,18). Genetic testing implications and considerations for individuals who may have FH, which should be discussed during pre-test genetic counseling, are outlined in **Table 1**. Genetic testing in the FH proband affords the ability to provide precise and accurate recurrence risk information during genetic counseling and informs the correct approach to family cascade genetic testing. Genetic testing provides discrimination, at the molecular genetic level, between individuals with HeFH, compound heterozygous FH, double heterozygous FH, HoFH, autosomal recessive FH, and those patients without an identifiable pathogenic variant but with the FH phenotype. The recurrence risks to relatives and implications for family planning differ among these scenarios. For example, in cases in which genetic test results identify probands who are double heterozygotes (e.g., pathogenic variants in both *LDLR* and *APOB*), this finding affects the recurrence risk to relatives and the recommended approaches to cascade testing. Specifically, for probands whose genetic testing diagnoses them as compound or double heterozygotes or homozygotes, parents of the proband should undergo known familial variant testing to determine which variant was maternally inherited and which was paternally inherited and/or whether one of the variants is de novo, which although rare, is possible (64); thus, all maternal and paternal relatives with FH can next be identified by testing for the appropriate variant on each side of the family. Known familial variant testing for both variants identified in the proband is recommended for siblings of the proband and for children of the proband. In addition, without genetic testing, these FH probands with 2 mutations may be misclassified as having severe HeFH, and this misclassification could have negative consequences for the proper identification of all at-risk relatives if it is not known that both sides of the family are at risk due to the presence of 2 mutations in the proband (65). Risks to relatives will also differ for those patients diagnosed with FH "phenocopies" and for those with polygenic etiologies.

GENETIC TESTING HAS IMPLICATIONS FOR THERAPEUTIC CHOICES IN FH. It is anticipated that the impact of genetic testing on the clinical management of FH will increase (66). Particularly in patients with severe HeFH or HoFH, molecular genetic test results may influence therapeutic choices. For example, lomitapide and mipomersen are approved only for HoFH (67), although HoFH can be diagnosed clinically and does not require genetic testing per se.

PCSK9 inhibitors are specifically approved for FH, in which clinical diagnosis can suffice for prescribing but molecular diagnosis can confirm FH, especially in borderline cases. Patients with FH due to gain-of-function *PCSK9* mutations are remarkably responsive to PCSK9 inhibition (68,69). In addition, in individuals with HoFH and 2 *LDLR* null alleles (i.e., without LDLRs on the liver surface), PCSK9 inhibitors had no effect on LDL-C level (66), but if at least 1 allele had residual LDLR activity, PCSK9 inhibitors lowered LDL-C levels by ~35% (70).

Studies comparing the efficacy of different agents in the setting of specific pathogenic variants will need to be performed. It must be emphasized that because not all patients with phenotypic FH have identifiable pathogenic variants, these medications should not be denied to patients with the clinical diagnosis of FH in whom detectable pathogenic variants cannot be detected.

GENETIC TESTING HAS VALUE TO THE PEDIATRIC PATIENT POPULATION WITH FH. According to the American Society of Human Genetics position statement on points to consider for genetic testing in children and adolescents, genetic testing in childhood is appropriate when there is a clinical intervention in childhood (71). In HeFH, statin treatment should be initiated from as early as 8 to 10 years of age, and interventions to promote a healthful lifestyle can begin even earlier. For children with HoFH, aggressive treatment is required at the time of diagnosis (40). Serious adverse events have not been reported with childhood statin treatment, including no reports of negative effects on growth and development (72). If left untreated, children with FH will be at higher risk of coronary events as adults because of the cumulative burden of elevated LDL-C levels, with many experiencing their first cardiovascular event at a young age. Children with FH who start a statin have statistically lower event rates than their affected parents (16). Depending on the age of initiating statin therapy, the cumulative LDL-C burden can be lowered to an extent that the LDL-C burden in the patient may be

TABLE 1 Genetic Testing Implications and Considerations for Individuals Who May Have FH

Benefits of genetic testing
- May establish or confirm a formal, definite diagnosis of FH.
- Provides prognostic information and the ability to perform refined risk stratification because the detection of a pathogenic variant indicates higher cardiovascular risk.
- Positive genetic test results have been shown to increase initiation of lipid-lowering therapy, adherence to therapy, and reductions in LDL-C levels.
- Earlier detection provides the opportunity for earlier treatment and lifestyle modifications.
- When genetic testing in the proband is informative, it leads to cascade genetic testing in at-risk family members with high sensitivity and specificity.
- May exclude FH in at-risk family members who did not inherit the pathogenic variant(s).
- Genetic testing provides discrimination, at the molecular genetic level, between individuals with HeFH, compound heterozygous FH, double heterozygous FH, HoFH, autosomal recessive FH, and those patients without an identifiable pathogenic variant but with the FH phenotype. The recurrence risks to relatives and implications for family planning differ among these scenarios.
- Genetic testing allows for the potential identification of FH "phenocopies" that may require specific therapies and have different inheritance patterns than FH.
- Enhances personal utility.
  - May provide additional motivation for individuals to remain adherent to prescribed medications.
  - Provides an explanation for failure of diet and exercise management to control elevated lipid levels.
  - Provides a helpful explanation for family history of premature heart disease and difficult-to-treat LDL-C levels.

Limitations of genetic testing
- FH genetic testing is not completely sensitive or specific.
  - Not all patients with a clinical diagnosis of FH will have an identifiable pathogenic variant(s).
  - Some patients will have a variant of uncertain significance identified, which may be reclassified as pathogenic or benign over time as more information is gained.

Potential risks of genetic testing
- Genetic discrimination.
  - In the United States, the federal Genetic Information Nondiscrimination Act of 2008 (GINA) prohibits discrimination by health insurers and employers based on genetic information. "Genetic information," as defined by GINA, includes an individual's family medical history, the results of individual's or family member's genetic tests, and the fact that an individual or individual's family member sought or received genetic services.
  - In the United States, the federal Americans with Disabilities Act and the Affordable Care Act provide important protection and fill critical gaps in GINA. The Americans with Disabilities Act protects employees whose genetic conditions are symptomatic or "manifest." The Affordable Care Act prohibits discrimination in coverage and benefits based on health condition, whether already symptomatic or a predisposition and regardless of etiology.
  - Other countries may have similar laws and/or protections against misuse of genetic information.
  - Some gaps in protection against disadvantaging individuals based on genetic information remain because life, disability, and long-term care insurance discrimination are not covered under current US laws.

Familial implications of genetic testing
- Genetic testing results may affect family dynamics and relationships.
  - Cascade testing: FH probands should receive a recommendation to warn at-risk relatives about their risk for FH.
  - Privacy: individuals with FH may experience difficulty in communicating their genetic testing results to at-risk relatives, and may experience a loss of privacy in doing so.
  - Parental guilt: parents may experience feelings of guilt related to passing their pathogenic variant(s) to children; in this situation, it may be helpful to emphasize the benefits provided by this information in children because early and sufficient lipid-lowering therapy will effectively reduce the risk of heart disease to that of the general population.
  - Survival guilt: individuals in the family who test negative for the familial pathogenic variant may experience feelings of guilt; however, it is important to explain that early and sufficient lipid-lowering therapy in family members with the familial pathogenic variant will effectively reduce the risk of heart disease to that of those without the pathogenic variant.

Cost of genetic testing
- Individuals may want to undergo genetic testing, but the cost and/or lack of insurance coverage may limit ability to obtain testing.

FH = familial hypercholesterolemia; HeFH = heterozygous FH; HoFH = homozygous FH; LDL-C = low-density lipoprotein cholesterol.

comparable to a nonaffected individual (73). Comparisons of cardiovascular outcomes between statin and pre-stain eras show maximum benefit in younger individuals (15,74). By identifying children with FH early, lifestyle changes can be introduced, which can affect both elevated LDL-C levels and acquisition of other risk factors such as tobacco use (40).

Research has shown favorable parental attitudes toward genetic testing in children, and testing can be accomplished via readily accessible sample types, including saliva and buccal swabs. The great majority of parents (87%) from FH families in the Netherlands want their children to undergo genetic testing (75). Data on the acceptability of pediatric FH genetic testing in the United States are not currently available. In circumstances in which FH is suspected but parental testing cannot be accomplished, genetic testing should be conducted, especially if a parent died of coronary heart disease and even if the child has only moderate hypercholesterolemia (40).

PERSONAL UTILITY OF FH GENETIC TESTING AND PSYCHOSOCIAL IMPLICATIONS. Data suggest that a DNA-based diagnosis of FH seems to have minimal adverse psychological impact (76,77), and genetic testing for FH is not perceived as anxiety provoking (78). Interviews with individuals with a clinical diagnosis of FH who underwent genetic testing found that it was regarded as useful; it confirmed for them

that they have a genetic disorder, provided an etiology for their clinical diagnosis, and offered the ability for younger family members to access genetic testing and, thus, timely treatment (78). In addition, interviews have shown that receiving a molecular diagnosis of FH could provide reassurance to patients that diet and lifestyle factors were not the primary cause of their condition (77). Compared with individuals at increased risk for cardiovascular disease with no DNA testing information, individuals diagnosed with FH through DNA testing had higher perceived efficacy of medication (79). Furthermore, research conducted to date has shown that children identified as FH pathogenic variant carriers generally cope well (80,81).

GENETIC TESTING YIELD, METHODS, AND ACCESS

Of the 3 primary genes in which pathogenic variants cause FH (*LDLR*, *APOB*, and *PCSK9*), mutations in *LDLR* are the most common: >90% of reported FH-causing variants are in *LDLR,* with 5% to 10% in *APOB* and <1% in *PCSK9* (82-84). More than 2,000 unique variants have been reported in association with FH, with ~1,000 of these having enough evidence to be considered pathogenic or likely pathogenic when applying American College of Medical Genetics and Genomics (ACMG) guidelines for variant classification (85). *LDLR* pathogenic and likely pathogenic variants include nonsense, missense, and a few synonymous variants; variants in the promoter and canonical splice sequences; and small insertions and deletions and large DNA rearrangements (86,87). Analysis of *LDLR* for structural variants should also be routinely performed because up to 10% of pathogenic variants in *LDLR* are large rearrangements (88-90). Most *APOB* pathogenic variants causing FH are missense variants in the region of the apolipoprotein B-100 protein that binds to the LDLR, resulting in a ligand-defective apolipoprotein B protein that binds poorly to the LDLR; this condition is sometimes called familial defective apolipoprotein B (91). In the European population, p.Arg3527Gln in *APOB* (previously referred to as p.Arg3500Gln) is the most predominant and is identified in ~6% to 10% of all FH cases (92), but other pathogenic variants in this region can also cause FH (93). Although *APOB* variants located outside this region have been reported, pathogenicity has been difficult to establish (94-98). Gain-of-function pathogenic variants in *PCSK9* cause FH by increasing the ability of the PCSK9 protein to promote degradation of LDLRs, leading to reduced numbers of receptors on the cell surface. Multiple *PCSK9* gain of function variants have been reported to date (69,99), with p.Asp374Tyr being the most frequently reported in FH (100).

The yield of FH genetic testing depends on the pretest probability of FH, as determined by clinical diagnostic criteria, and by other clinical factors, such as premature CAD and/or extreme hypercholesterolemia, in the absence of known secondary causes. For those designated according to clinical diagnostic criteria as "definite" FH, a pathogenic variant in 1 of the 3 known FH-causing genes can be identified in ~60% to 80%; in "possible" FH, the yield is lower (~21% to 44%) (83,101-103). In cohorts of pediatric patients in which there is a strong clinical index of suspicion for FH, results of genetic testing have revealed clinical sensitivities ranging from ~60% to 95% (39,104). Therefore, a negative genetic test result in a patient with an FH phenotype as defined by using clinical criteria does not exclude a diagnosis of FH. Negative genetic test results may be due to technical limitations and/or the presence of mutations in yet-to-be identified genes. FH should be diagnosed clinically (definite/probable/possible diagnosis based on the common clinical criteria) in the presence of negative genetic test results (these patients are phenotype positive, genotype negative) if the patient has severe hypercholesterolemia and a family history of hypercholesterolemia and/or premature CAD, as cardiovascular risk according to the FH phenotypic definition remains high (105).

The prevalence of FH pathogenic variants in adults with LDL-C levels ≥190 mg/dl and no additional clinical or family history data is ~2% (7,36). Therefore, not every patient with LDL-C levels ≥190 mg/dl should be considered to have FH. However, the prevalence of genetically confirmed FH in patients with acute coronary syndrome who are ≤65 years of age and with LDL-C levels ≥160 mg/dl is ~9% (101); the prevalence in unselected adults with a maximum electronic health record-documented level of LDL-C ≥250 mg/dl is ~13% in a US-based cohort (7); and the prevalence in suspected FH index patients in Brazil with LDL-C levels ≥230 mg/dl is ~50% (102). In the Danish general population, the most optimal threshold for LDL-C to discriminate between carriers and noncarriers of FH pathogenic variants was 170 mg/dl, with the highest yield of carriers being 13% at LDL-C levels >230 mg/dl (106). The likelihood of detecting a pathogenic variant is proportional to the absolute LDL-C level (107). These collective data illustrate the utility of using specific, isolated clinical features, such as acute coronary syndrome and extreme hypercholesterolemia, to select individuals who have a reasonably high likelihood of having a

pathogenic variant causative of FH. Recently, a model to predict the presence of an FH-causing mutation in individual patients was developed and validated in a Dutch population (108). It is important to note that the LDL-C cutpoints used to offer or consider FH genetic testing may differ among countries (109), as well as between individuals of different races and ethnic backgrounds.

At a minimum, genetic testing for patients with suspected FH should include analysis of *LDLR*, the region of *APOB* encoding the LDLR ligand, and *PCSK9*. Importantly, an FH diagnosis is not excluded if genetic testing does not detect a pathogenic variant in one of these genes, as the FH phenotype may be due to undetected pathogenic variants, variants in other genes, and/or variants in as-yet-undiscovered genes (3). Pathogenic variants in other genes can cause an FH phenotype. There is an autosomal recessive form of hypercholesterolemia caused by biallelic pathogenic variants in *LDLRAP1* encoding the LDLR adaptor protein 1 (110). Confirming a diagnosis of autosomal recessive hypercholesterolemia allows accurate diagnosis of this recessive form as well as the provision of accurate recurrence risk information to relatives. Pathogenic variants in *LDLRAP1* should be considered if a patient with severe hypercholesterolemia has no detectable variant in *LDLR, APOB,* or *PCSK9* and the family history represents possible autosomal recessive inheritance.

There are also other potential etiologies for an FH phenotype. A pathogenic variant in the gene encoding apolipoprotein E, *APOE,* (p.Leu167del), reportedly causes an autosomal dominant phenotype, including premature myocardial infarction, tendinous xanthomas, xanthelasmas, and elevated LDL-C levels (111). Other Mendelian lipid conditions have phenotypic overlap with FH and should be considered (112,113). Sitosterolemia (caused by autosomal recessive pathogenic variants in *ABCG5* or *ABCG8*) (114) can present with xanthomas and hypercholesterolemia. Lysosomal acid lipase deficiency, caused by autosomal recessive pathogenic variants in *LIPA*, can also present with elevated LDL-C levels, often accompanied by fatty liver disease (113,115). Genetic testing for these conditions in the appropriate patients may have direct therapeutic implications.

A polygenic etiology may explain the FH clinical phenotype in a majority of individuals in whom no pathogenic variant is found in the 3 main genes, as they may have a greater than average number of relatively common LDL-C-raising single nucleotide polymorphisms (i.e., high "LDL-C level raising gene score") (22,107,116). High lipoprotein(a) concentrations are also common in patients with phenotypic FH with no identifiable pathogenic variant (117), with one study suggesting that one-quarter of patients with clinical FH acquired the diagnosis due to high lipoprotein(a) concentrations (118).

Accurate variant interpretation is of paramount importance in the application of clinical genetic testing. The *LDLR* variant database (119) includes variant classification information based on the 2013 published guidelines from the Association for Clinical Genetic Science (120). In this recently updated *LDLR* variant database, 7% of variants are currently classified as variants of unknown significance (87). However, a study that applied the published 2015 standards and guidelines for the interpretation of variants from the ACMG and the Association for Molecular Pathology (121), with specific FH assumptions, to potential FH variants found that ~47% were classified as variants of uncertain significance, mainly due to insufficient evidence, including lack of functional studies for non-null (i.e., missense) alleles (85). ClinVar, housed at the National Center for Biotechnology Information, is a freely available database that also includes interpretations of the clinical significance of variants (122). A main goal of the FH Expert Panel (123) of the Clinical Genome Resource (ClinGen) (124) is to improve FH variant classification by performing ongoing revisions and specifications to the ACMG/Association for Molecular Pathology guidelines to make them more robust for the accurate interpretation of variants in the FH genes and to provide expert level classifications of FH variants deposited in ClinVar.

Genetic testing for FH is available via multiple clinical genetic testing laboratories in the United States and worldwide (82). Costs continue to decrease over time, due in part to the use of next-generation sequencing (NGS) technologies (32,82). Most laboratories offer NGS panels, including full gene sequencing of *LDLR, APOB,* and *PCSK9,* as well as deletion/duplication analysis of *LDLR*. FH genetic testing in the index case is offered by several of these laboratories for an out-of-pocket cost of <$500 (32). Site-specific genetic testing for the known pathogenic variant(s) in at-risk relatives (cascade genetic testing) is performed at a considerably lower cost. Larger, more inclusive, lipid disorder NGS panels are also available that provide evaluation of not only the main FH genes but also the genes causing conditions with phenotypic overlap. Up-to-date information on tests and what genes they include, costs, testing methods, laboratory certification, and sample requirements can be located by searching publicly available databases, including the Genetic Testing Registry (125). Of note, because of the clinical severity

TABLE 2 Recommendations and Considerations for Genetic Testing for FH

A. Proband (index case)
Genetic testing for FH **should be offered** to individuals of any age in whom a strong clinical index of suspicion for FH exists based on examination of the patient's clinical and/or family histories. This index of suspicion includes the following: 1. Children with persistent* LDL-C levels ≥160 mg/dl or adults with persistent* LDL-C levels ≥190 mg/dl without an apparent secondary cause of hypercholesterolemia† and with at least 1 first-degree relative similarly affected or with premature CAD‡ or where family history is not available (e.g., adoption) 2. Children with persistent* LDL-C levels ≥190 mg/dl or adults with persistent* LDL-C levels ≥250 mg/dl without an apparent secondary cause of hypercholesterolemia,† even in the absence of a positive family history Evidence Grade: Class of Recommendation IIa, Strength of Evidence B-NR
Genetic testing for FH **may be considered** in the following clinical scenarios: 1. Children with persistent* LDL-C levels ≥160 mg/dl (without an apparent secondary cause of hypercholesterolemia†) with an LDL-C level ≥190 mg/dl in at least 1 parent or a family history of hypercholesterolemia and premature CAD‡ 2. Adults with no pre-treatment LDL-C levels available but with a personal history of premature CAD‡ and family history of both hypercholesterolemia and premature CAD‡ 3. Adults with persistent* LDL-C levels ≥160 mg/dl (without an apparent secondary cause of hypercholesterolemia†) in the setting of a family history of hypercholesterolemia and either a personal history or a family history of premature CAD‡ Evidence Grade: Class of Recommendation IIb, Strength of Evidence C-EO
B. At-risk relatives
1. Cascade genetic testing for the specific variant(s) identified in the FH proband (known familial variant testing) should be offered to all first-degree relatives. If first-degree relatives are unavailable, or do not wish to undergo testing, known familial variant testing should be offered to second-degree relatives. Cascade genetic testing should commence throughout the entire extended family until all at-risk individuals have been tested and all known relatives with FH have been identified Evidence Grade: Class of Recommendation I, Strength of Evidence B-R

If LDL-C values are unavailable, total cholesterol values ≥320, 260, and 230 mg/dl (corresponding to LDL-C levels ≥250, 190, and 160 mg/dl, respectively) could be used. *Two or more measurements, including assessment after intensive lifestyle modification. †Hypothyroidism, diabetes, renal disease, nephrotic syndrome, liver disease, medications. ‡Premature coronary artery disease (CAD) = male subjects ≤55 years of age, female subjects ≤65 years of age; adapted from the American Heart Association phenotype definition of HeFH. Other abbreviations as in Table 1.

of FH and the ability to prevent its complications when appropriately diagnosed and managed, the ACMG has included the 3 main FH genes in its published recommendations for reporting of secondary findings in clinical exome and genome sequencing (126).

RECOMMENDATIONS FOR FH GENETIC TESTING

Our recommendations and considerations for genetic testing for FH are summarized in Table 2. This summary includes recommendations for probands, or index cases, and cascade testing for at-risk relatives. Genetic testing for patients with suspected FH should, at a minimum, include analysis of *LDLR, APOB,* and *PCSK9*. This analysis should include for *LDLR* and *PCSK9* sequencing of all exons and exon/intron boundaries, as well as *LDLR* deletion/duplication analysis, and for *APOB* the exons encoding the LDLR ligand-binding region.

Evidence evaluation supporting the evidence grades in Table 2 has been presented in earlier sections of this paper. In brief, genetic testing of index cases is supported by the following: 1) the presence of increased risk in individuals with pathogenic variants associated with FH; 2) the availability of effective treatment to lower LDL-C levels; 3) current yields published in the literature estimated from these testing criteria; 4) enhancement of cascade testing with genetic testing; and 5) the likelihood of improved medication compliance in the presence of a genetic diagnosis. In those in whom genetic testing is recommended or may be considered, there is a sufficient likelihood of a positive genetic test result. Cascade testing is considered a grade I recommendation because of extensive epidemiological and cost analyses with supporting data regarding the value of earlier recognition of FH and the demonstrated value of statin therapy in randomized trials.

Larger, more inclusive, lipid disorder NGS panels are also available that provide evaluation of not only the main FH genes but also the genes causing conditions with phenotypic overlap previously described. These expanded panels should be considered to improve the diagnosis of patients with these "phenocopy" conditions that may require specific therapies, and they should include the following genes: *LDLR, APOB, PCSK9, LDLRAP1, LIPA, ABCG5, ABCG8,* and *APOE*.

FH genetic testing should be accompanied by pre- and post-test genetic counseling so patients can be presented with the benefits, limitations, potential risks, and familial implications of genetic testing (Table 1). The genetic counseling process for patients with FH, including specific recommendations for pre- and post-test genetic counseling, family dynamics, privacy, and potential for genetic discrimination and the laws that address this potential (127), can be found elsewhere (2,18,128,129). The genetic counseling process for patients with FH is summarized in Table 3. In addition, the Central Illustration displays

TABLE 3 The Genetic Counseling Process for Patients With Familial Hypercholesterolemia

Collection of ≥3 generation family medical history information (pedigree), with special attention to "red flags" for FH • "Red flags" for FH in the pedigree include hypercholesterolemia; premature CAD (onset in men before age 55 years and women before age 65 years) including angina pectoris and myocardial infarction; sudden cardiac death; physical features of FH (e.g., xanthomas, corneal arcus) • Because patients' self-reported family history information can have both reduced sensitivity and specificity, it is important to collect medical records, autopsy reports, and death certificates when possible so that diagnoses can be confirmed • In some cases, it may not be until clinical screening commences through the family that FH can be diagnosed • Family history is not static, but changes over time, and should therefore be updated periodically
Performance of risk assessment utilizing medical and family history information
Discussion of mode of inheritance and recurrence risk to family members
Facilitation of genetic testing • Pre- and post-test genetic counseling • Disclosure and documentation of genetic testing results
Facilitation of family-based care
Cascade testing
Discussion of screening, prevention, and medical management options in conjunction with managing physician
Discussion of reproductive options
Provision of written documentation of medical, genetic, and counseling information to referring health care providers and patients, including "Dear Family Member Letters"
Provision of psychosocial counseling and anticipatory guidance
Provision of education and resources from national organizations and advocacy groups
Discussion of available research study options • For example, enrolling FH patients into the CASCADE™ FH Registry
Discussion of the availability of DNA banking, when applicable

Reproduced with permission from Sturm (129).

CAD = coronary artery disease; CASCADE FH = Cascade Screening for Awareness and Detection of FH; FH = familial hypercholesterolemia.

the genetic testing process in an index patient and family, and includes the recommended pathways for patients who do and do not choose to undergo genetic testing, as well as how to utilize positive, negative, and uncertain genetic test results. For cascade genetic testing, if a relative is either critically placed in the pedigree and does not consent to genetic testing, or if a relative is deceased and therefore cannot undergo genetic testing, the cascade should continue to the critically placed relative's and/or deceased individual's first-degree relatives so additional at-risk relatives can determine whether they are at risk for FH or not.

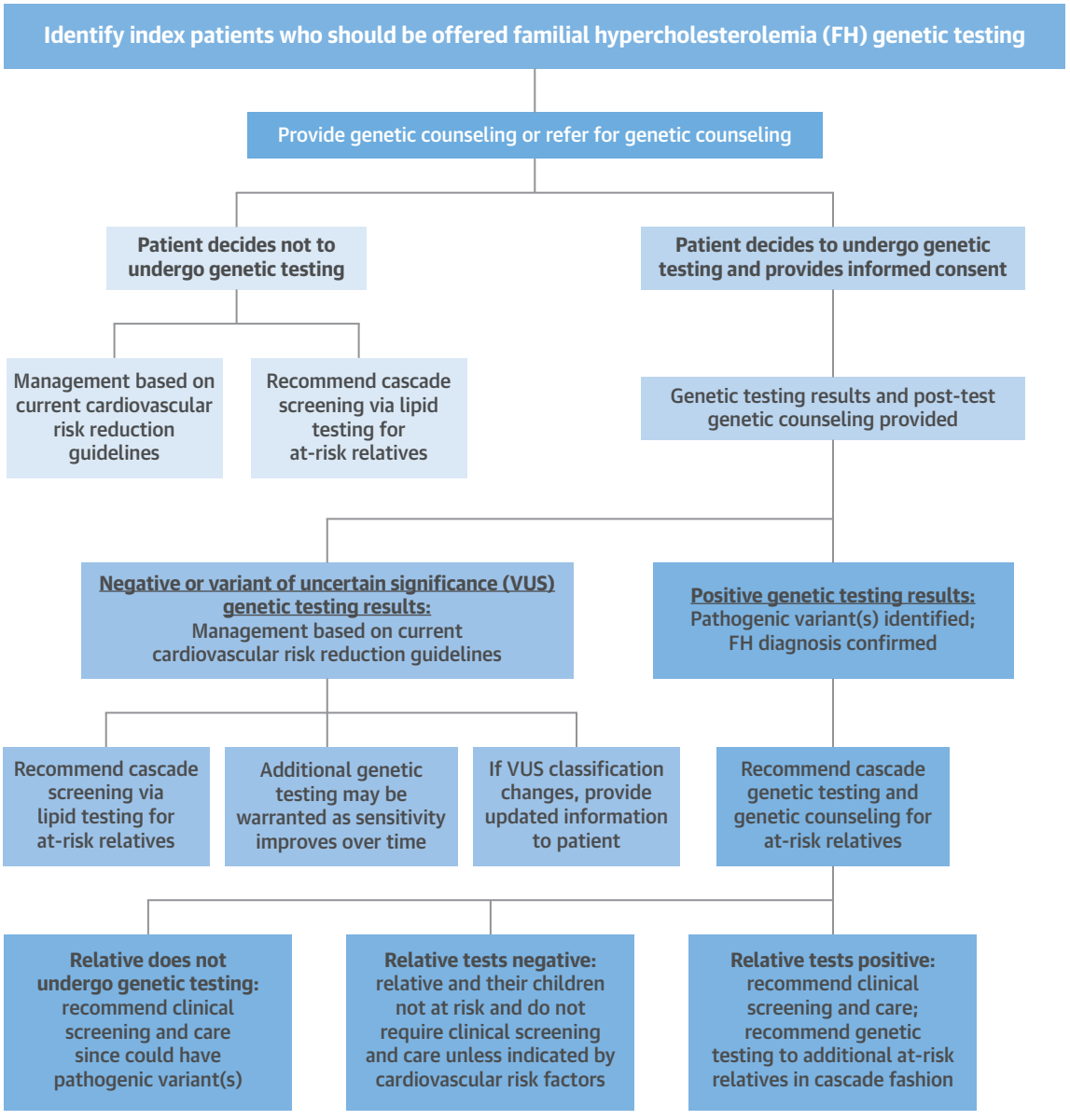
CONCLUSIONS AND EVIDENCE GAPS

Clinical genetic testing for patients with a clinical suspicion or diagnosis of FH has been underutilized. With costs of next-generation DNA sequencing continuing to fall, genetic testing for FH has become more accessible. Recent research suggests that by incorporating FH genetic testing as the standard of care for patients and their relatives with definite, probable, or possible FH, diagnosis of FH will improve, relatives of index cases will be identified, and initiation of recommended LLT can commence at younger ages, leading to improved clinical outcomes. Individuals will be able to receive more accurate prognostic and risk stratification information that has personal utility.

Some evidence and knowledge gaps exist in the application of FH genetic testing. Importantly, both phenotype-based and genotype-based definitions of FH should continue to be used, and clinical variability in patient presentation should be acknowledged. Positive genetic test results for LDLR mutations causing HeFH span the phenotypic spectrum from normal to extreme LDL-C levels, whereas patients with molecularly defined HoFH (2 mutations) may have LDL-C ranges close to those previously defined as HeFH (18). Conversely, high-risk patients may meet criteria for a clinical diagnosis of FH (high LDL-C level and positive family history of hypercholesterolemia and premature CAD) but may have negative FH genetic test results potentially due to other, undefined genetic causes of high LDL-C levels. Future research must further refine and expand genetic testing options for patients with hypercholesterolemia, including those with the phenotype due to polygenic risk factors and other metabolic pathways.

Understanding the value of genetic testing for precision medicine in lipid treatment is currently being studied. Having the capability to guide pharmacological therapies and improving our understanding of gene-gene and gene-environment interactions may affect patient outcomes. Further research is needed to evaluate how information from genetic testing can improve medication adherence and outcomes for patients with FH. Cost-benefit analyses of the potential role genetic testing plays in

CENTRAL ILLUSTRATION **The Genetic Testing Process in an Index Patient (Proband) and Family**



Sturm, A.C. et al. J Am Coll Cardiol. 2018;72(6):662-80.

Pre-test and post-test genetic counseling should be provided to all individuals undergoing genetic testing. Independent of genetic test results, all patients should be managed based on current cardiovascular risk reduction guidelines.

improving earlier recognition of coronary heart disease risk and improving life expectancy are required. In addition, patient acceptability of genetic testing requires additional research to understand and address potential fears surrounding genetic discrimination.

Most importantly, genetic testing provides a window of opportunity whereby we can identify those individuals at significantly higher risk than the general population for CAD at a given LDL-C level. Family screening based on genetic results can be implemented to identify and treat those individuals with

unrecognized FH. Early recognition of FH leading to guideline-based therapy will alter the natural history of this highly morbid genetic condition.

ACKNOWLEDGMENTS The authors gratefully acknowledge the Familial Hypercholesterolemia Foundation for convening the 2 in-person meetings of the authors designed to initiate and finalize the content and recommendations within this paper. Individuals at the Familial Hypercholesterolemia Foundation who provided administrative and graphic design support were David Zuzick and Susan Seim.

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KEY WORDS cascade testing, consensus statement, familial hypercholesterolemia, genetic counseling, genetic testing