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Diagnostic Yield and Clinical Utility of Sequencing Familial Hypercholesterolemia Genes in Patients With Severe Hypercholesterolemia

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ABSTRACT

BACKGROUND Approximately 7% of American adults have severe hypercholesterolemia (untreated low-density lipoprotein [LDL] cholesterol ≥190 mg/dl), which may be due to familial hypercholesterolemia (FH). Lifelong LDL cholesterol elevations in FH mutation carriers may confer coronary artery disease (CAD) risk beyond that captured by a single LDL cholesterol measurement.

OBJECTIVES This study assessed the prevalence of an FH mutation among those with severe hypercholesterolemia and determined whether CAD risk varies according to mutation status beyond the observed LDL cholesterol level.

METHODS Three genes causative for FH (*LDLR*, *APOB*, and *PCSK9*) were sequenced in 26,025 participants from 7 case-control studies (5,540 CAD case subjects, 8,577 CAD-free control subjects) and 5 prospective cohort studies (11,908 participants). FH mutations included loss-of-function variants in *LDLR*, missense mutations in *LDLR* predicted to be damaging, and variants linked to FH in ClinVar, a clinical genetics database.

RESULTS Among 20,485 CAD-free control and prospective cohort participants, 1,386 (6.7%) had LDL cholesterol ≥190 mg/dl; of these, only 24 (1.7%) carried an FH mutation. Within any stratum of observed LDL cholesterol, risk of CAD was higher among FH mutation carriers than noncarriers. Compared with a reference group with LDL cholesterol <130 mg/dl and no mutation, participants with LDL cholesterol ≥190 mg/dl and no FH mutation had a 6-fold higher risk for CAD (odds ratio: 6.0; 95% confidence interval: 5.2 to 6.9), whereas those with both LDL cholesterol ≥190 mg/dl and an FH mutation demonstrated a 22-fold increased risk (odds ratio: 22.3; 95% confidence interval: 10.7 to 53.2). In an analysis of participants with serial lipid measurements over many years, FH mutation carriers had higher cumulative exposure to LDL cholesterol than noncarriers.

CONCLUSIONS Among participants with LDL cholesterol ≥190 mg/dl, gene sequencing identified an FH mutation in <2%. However, for any observed LDL cholesterol, FH mutation carriers had substantially increased risk for CAD. (J Am Coll Cardiol 2016;67:2578–89) © 2016 by the American College of Cardiology Foundation.

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Severe hypercholesterolemia, defined as having a low-density lipoprotein (LDL) cholesterol level ≥190 mg/dl, is a treatable risk factor for coronary artery disease (CAD) (1,2). Current treatment guidelines place particular emphasis on intensive life-style and pharmacological therapy in this population (3). One cause of severely elevated LDL cholesterol is familial hypercholesterolemia (FH), an autosomal dominant monogenic disorder linked to impaired hepatic clearance of LDL cholesterol particles (4). Patients with LDL cholesterol ≥190 mg/dl are often assumed to have FH, but this may not be the case. Large-scale gene sequencing provides an opportunity to clarify the diagnostic yield and clinical impact of identifying an FH mutation in severely hypercholesterolemic patients.

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Previous studies of the diagnostic yield of genetic testing in severe hypercholesterolemia have focused on subjects with clinically suspected FH and reported FH mutation prevalence has ranged from 20% to 80% (5–16). This variability is likely caused by differing ascertainment schemes utilizing family history, physical examination features, elevated LDL cholesterol at a young age, or referral to specialized clinics, each of

ABBREVIATIONS AND ACRONYMS

APOB = apolipoprotein B

CAD = coronary artery disease

CI = confidence interval

FH = familial hypercholesterolemia

LDL = low-density lipoprotein

LDLR = low-density lipoprotein receptor

OR = odds ratio

PCSK9 = proprotein convertase subtilisin/kexin type 9

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Adrienne Cupples, principal investigator for the Framingham Heart Study (FHS), and Bruce Psaty, principal investigator for the Cardiovascular Health Study (CHS). Sequencing was performed at the Baylor Genome Center (U54 HG003273). The ARIC Study is conducted as a collaborative study supported by NHLBI contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). The FHS is conducted and supported by the NHLBI in collaboration with Boston University (contract No. N01-HC-25195), and its contract with Affymetrix, Inc., for genome-wide genotyping services (contract N02-HL-6-4278), for quality control by FHS investigators using genotypes in the SNP Health Association Resource (SHARe) project. 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which may enrich for monogenic causes. In contrast, if ascertainment from the general population is solely on the basis of elevated LDL cholesterol, the extent to which FH mutations contribute to severe hypercholesterolemia is unknown. Such knowledge may inform the design and effectiveness of universal FH screening proposals (17,18).

Knowledge of FH mutation status could also provide CAD risk information beyond that from a single LDL cholesterol measurement (4,18). An FH mutation could lead to higher cumulative exposure to LDL cholesterol levels over a lifetime; as such, within any stratum of LDL cholesterol, the risk of CAD might be greater if the LDL elevation is due to a monogenic mutation versus other causes. The extent to which the presence of a causal FH mutation modulates CAD risk is uncertain.

We analyzed gene sequences of 3 FH genes (low-density lipoprotein receptor [*LDLR*], apolipoprotein B [*APOB*], and proprotein convertase subtilisin/kexin type 9 [*PCSK9*]) in 12 distinct cohorts, including 26,025 participants, to determine: 1) the diagnostic yield of gene sequencing to identify an FH mutation in severely hypercholesterolemic participants; and 2) the clinical impact of an FH mutation on CAD risk within any given stratum of LDL cholesterol levels.

METHODS

STUDY POPULATIONS. Whole-exome sequencing was performed in 7 previously described CAD case-control cohorts of the Myocardial Infarction Genetics Consortium (Online Table 1), including the Italian Atherosclerosis, Thrombosis, and Vascular Biology study (19), the ESP-EOMI (Exome Sequencing Project Early-Onset Myocardial Infarction) study (20), a nested case-control of the JHS (Jackson Heart Study) (21), the Munich Myocardial Infarction study (22), the Ottawa Heart Study (23), the PROCARDIS (Precocious Coronary Artery Disease) study (24), and PROMIS (Pakistan Risk of Myocardial Infarction Study) (25). The effect of lipid-lowering therapy in those reporting use at the time of lipid measurement was taken into account by dividing the measured total cholesterol and LDL cholesterol by 0.8 and 0.7, respectively, as implemented previously (26-28). Primary, severe LDL cholesterol elevation was defined as ≥190 mg/dl, in accordance with current cholesterol treatment guidelines (3).

The prevalence of an FH mutation in participants with LDL cholesterol >190 mg/dl was additionally determined in 11,908 participants from 5 prospective cohort studies derived from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium (29), ARIC (Atherosclerosis Risk in Communities), Cardiovascular Health Study, FHS (Framingham Heart Study), Rotterdam Baseline Study, and Erasmus Rucphen Family Study (Online Table 2).

To determine the cumulative exposure to LDL cholesterol according to FH mutation status, publically available data from the National Center for Biotechnology Information dbGAP database were analyzed, which included 5,727 ARIC cohort participants and 2,714 FHS Offspring Study participants.

GENE SEQUENCING. CAD case-control whole-exome sequencing was performed at the Broad Institute (Cambridge, Massachusetts) as described previously

(BroadGO) and RC2 HL102926 (SeattleGO). Exome sequencing in ATVB, PROCARDIS, Ottawa, PROMIS, Munich Study, and the Jackson Heart Study was supported by 5U54HG003067 (to Drs. Lander and Gabriel). The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the NHLBI; the National Institutes of Health; or the U.S. Department of Health and Human Services. The sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication. Dr. Khera is supported by an American College of Cardiology/Merck Fellowship award and has received consulting fees from Merck and Amarin Corporation. Dr. Peloso is supported by National Heart, Lung, and Blood Institute award K01HL125751. Dr. Kessler is supported by a Deutsches Zentrum für Herz-Kreislauf-Forschung rotation grant. Dr. Psaty has served on a data safety and monitoring board for a clinical trial funded by Zoll LifeCor; and on a steering committee for the Yale Open Data Access project, funded by Johnson & Johnson. Dr. Rader has received consulting fees from Aegerion Pharmaceuticals, Alnylam Pharmaceuticals, Eli Lilly and Company, Pfizer, Sanofi, and Novartis; is an inventor on a patent related to lomitapide that is owned by the University of Pennsylvania and licensed to Aegerion Pharmaceuticals; and is a cofounder of Vascular Strategies and Staten Biotechnology. Dr. Ardissino has received speaker fees from AstraZeneca, Boehringer Ingelheim, Johnson & Johnson, Bayer, Daiichi-Sankyo, GlaxoSmithKline, Eli Lilly and Company, Boston Scientific, Bristol-Myers Squibb, Menarini Group, Novartis, and Sanofi; and research grants from GlaxoSmithKline, Eli Lilly and Company, Pfizer, and Novartis. Dr. Saleheen has received grants from Pfizer and the National Institutes of Health. Dr. Kathiresan is supported by a research scholar award from the Massachusetts General Hospital, the Donovan Family Foundation, and R01 HL127564; has received grants from Bayer Healthcare, Aegerion Pharmaceuticals, and Regeneron Pharmaceuticals; consulting fees from Merck, Novartis, Sanofi, AstraZeneca, Alnylam Pharmaceuticals, Leerink Partners, Noble Insights, Quest Diagnostics, Genomics PLC, and Eli Lilly and Company; and holds equity in San Therapeutics and Catabasis Pharmaceuticals. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Khera, Won, and Peloso contributed equally to this work.

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(20). Population-based cohort sequencing was performed at the Baylor College of Medicine (Houston, Texas) for the ARIC, CHS, and FHS cohorts and at Erasmus Medical Center (Rotterdam, Netherlands) for the Rotterdam Baseline Study and Erasmus Rucphen Family Study cohorts. Additional sequencing methodology details are available in the Online Appendix.

GENETIC VARIANT ANNOTATION. Three classes of genetic variants were aggregated with respect to association with FH: 1) loss-of-function variants in *LDLR*: single-base changes that introduce a stop codon, leading to premature truncation of a protein (nonsense), insertions or deletions (indels) of deoxyribonucleic acid (DNA) that scramble protein translation beyond the variant site (frameshift), or point mutations at sites of pre-messenger ribonucleic acid splicing that alter the splicing process (splice-site); 2) missense variants in *LDLR* predicted to be deleterious by each of 5 in silico prediction algorithms (LRT score, MutationTaster, PolyPhen-2 HumDiv, PolyPhen-2 HumVar, and Sorting Intolerant From Tolerant [SIFT]), as described previously (20,30); and 3) variants in *LDLR*, *APOB*, or *PCSK9* annotated as "pathogenic" or "likely pathogenic" in ClinVar, a publicly available archive of genetic variations associated with clinical phenotypes (31). Additional sensitivity analyses aggregated all rare (allele frequency <0.01) missense mutations in *LDLR*; exon 26 of *APOB,* which encodes key components of apolipoprotein B binding to the LDL receptor and harbors the majority of *APOB* variants linked to FH (32); and those that produce a change in *PCSK9* at an amino acid associated with FH in ClinVar. Rare synonymous variants at these same locations were included as a negative control. Software used to annotate observed variants included Variant Effect Predictor (version 77) (33) and the associated LOFTEE plugin (34), as well as the dbNSFP database (version 3.0b1) (35).

LONGITUDINAL ANALYSIS OF LDL CHOLESTEROL EXPOSURE. Individuals with an FH mutation and LDL cholesterol ≥130 mg/dl were identified in the ARIC and FHS Offspring Study cohorts. LDL cholesterol values were adjusted in those who reported lipid-lowering therapy by dividing measured values by 0.7. Mean LDL cholesterol exposure was calculated as cumulative exposure, determined via area under the curve analysis, divided by length of follow-up. Twenty-seven FH mutation carriers met the inclusion criteria described previously and were matched 1:1 to a mutation-negative control according to age (within 10 years), sex, statin use at time of last visit, and similar LDL cholesterol at last visit (within 10 mg/dl). A match was available in 25 of 27 participants (93%). Mean LDL cholesterol exposure was compared among those with and without FH mutation using a paired Student *t* test.

STATISTICAL ANALYSIS. The impact of aggregations of genetic variants on levels of LDL cholesterol was assessed with linear regression, with adjustments for age, age squared, sex, cohort, and the first 5 principal components of ancestry. Odds ratios (ORs) for CAD were calculated by use of logistic regression with adjustment for sex, cohort, and the first 5 principal components of ancestry. In analyses conducted on ordinal strata of LDL cholesterol, participants with LDL cholesterol <130 mg/dl and no mutation linked to FH served as the reference group.

Analyses were performed with R version 3.2.2 software (R Project for Statistical Computing, Vienna, Austria). Figures were created with the ggplot2 package within R (36).

RESULTS

Within the Myocardial Infarction Genetics Consortium CAD case-control cohorts, a total of 14,117 participants with both LDL cholesterol level and sequence data for FH genes were available for analysis: 8,577 CAD-free control subjects and 5,540 CAD case subjects (Online Table 3). The study population included 10,421 men (74% of participants) with a mean age of 53 ± 14 years. Proportions of self-identified race were 47%, 46%, and 7% for white, South Asian, and black, respectively. Forty-seven percent of study participants had a history of hypertension, 27% had a history of diabetes mellitus, 34% were current smokers, and 14% were taking lipid-lowering medications.

Sequencing identified 86 variants linked to FH because they led to loss of function in *LDLR*, were missense mutations in *LDLR* predicted to be damaging by each of 5 computer prediction algorithms, or were a variant in *LDLR*, *APOB*, or *PCSK9* previously linked to FH in the ClinVar genetics database. These included 13 premature stop ("nonsense") codons, 6 splice acceptor/donor variants, 4 frameshift mutations, and 63 missense mutations (Online Table 4).

Mutations linked to FH were found in 164 participants, including 48 CAD-free control subjects (OR: 0.6%; 95% confidence interval [CI]: 0.4% to 0.7%) and 116 CAD case subjects (OR: 2.1%; 95% CI: 1.7% to 2.5%) (Online Table 5). The mutation was located in *LDLR* for 141 participants (86%), in *APOB* for 22 (13%), and in *PCSK9* for 1 (0.6%) (Online Table 4). Only 1 homozygote (or compound heterozygote) participant was identified; a 33-year-old patient with LDL cholesterol of 539 mg/dl and CAD was homozygous for a p.Q33* premature stop codon in *LDLR*.

TABLE 1 Prevalence of an FH Mutation Among Participants With Severe Hypercholesterolemia (LDL Cholesterol ≥190 mg/dl) in CAD-Free Control Subjects and Population-Based Cohort Studies

	LDL Cholesterol ≥190 mg/dl (% of Cohort)	FH Mutation (% Participants With LDL Cholesterol ≥190 mg/dl)
Control subjects of the MIGen Consortium		
Atherosclerosis, Thrombosis, and Vascular Biology Italian Study (N = 1,050)	44 (4.0)	1 (2.3)
Exome Sequencing Project; Early-Onset Myocardial Infarction (N = 1,213)	160 (13.0)	3 (1.9)
Jackson Heart Study (N = 599)	26 (4.0)	1 (3.8)
Munich Myocardial Infarction Study (N = 272)	15 (6.0)	0 (0.0)
Ottawa Heart Study (N = 889)	59 (7.0)	0 (0.0)
Precocious Coronary Artery Disease (N = 870)	36 (4.0)	1 (2.8)
Pakistani Risk of Myocardial Infarction Study (N = 3,684)	90 (2.0)	2 (2.2)
Total (N = 8,577)	430 (5.0)	8 (1.9)
CHARGE Consortium		
Atherosclerosis Risk in Communities Study (N = 7,959)	657 (8.0)	12 (1.8)
Cardiovascular Health Study (N = 732)	47 (4.0)	1 (2.1)
Framingham Heart Study (N = 1,175)	38 (5.0)	2 (5.3)
Rotterdam Baseline Study (N = 794)	99 (12.0)	0 (0.0)
Erasmus Rucphen Family Study (N = 1,248)	115 (9.0)	1 (0.9)
Total (N = 11,908)	956 (8.0)	16 (1.7)
Combined MIGen Controls + CHARGE (N = 20,485)	1,386 (7.0)	24 (1.7)

Values are n (%).

CAD = coronary artery disease; CHARGE = Cohorts for Heart and Aging Research in Genomic Epidemiology; FH = familial hypercholesterolemia; LDL = low-density lipoprotein; MIGen = Myocardial Infarction Genetics.

DIAGNOSTIC YIELD OF GENE SEQUENCING IN SEVERE HYPERCHOLESTEROLEMIA. Among 8,577 CAD-free control participants from the Myocardial Infarction Genetics Consortium cohorts, LDL cholesterol approximated a normal distribution (Online Figure 1). The prevalence of an FH mutation increased across categories of LDL cholesterol levels ($p < 0.001$) (Online Figure 2). Of 8,577 control participants, 430 (5% of control sample) had LDL cholesterol ≥190 mg/dl, and only 8 of these carried an FH mutation (OR: 1.9%; 95% CI: 0.9% to 3.8%) (Table 1, Central Illustration).

This prevalence estimate was replicated in 11,908 participants from 5 prospective cohort studies of the CHARGE consortium: 956 (8%) had LDL cholesterol >190 mg/dl, and of these, 16 (OR: 1.7%; 95% CI: 1.0% to 2.8%) harbored an FH mutation. Across the 12 studies (n = 20,485), 1,386 participants (7%) displayed LDL cholesterol ≥190 mg/dl, of whom 24 (1.7%) carried an FH mutation (Table 1).

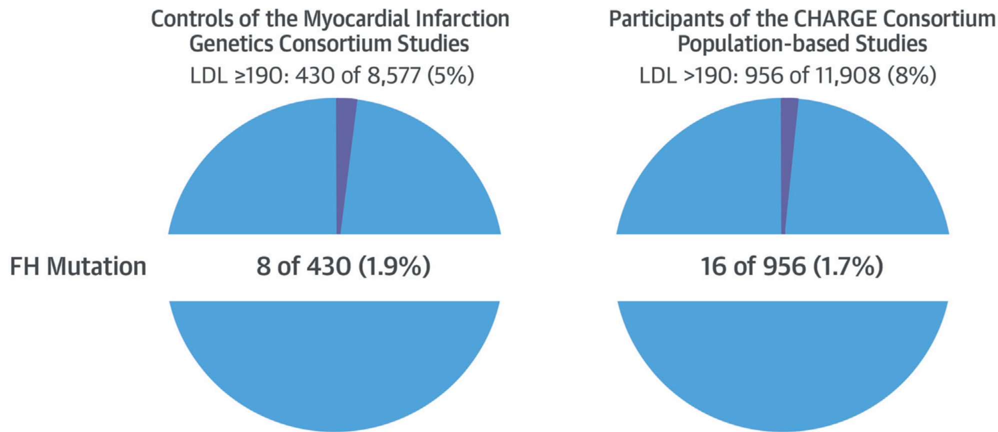
CLINICAL IMPACT OF FH MUTATION IDENTIFICATION ON CAD RISK. In the Myocardial Infarction Genetics Consortium case-control studies, the presence of an FH mutation was associated with a 50 mg/dl (95% CI: 44 to 57 mg/dl) increase in LDL cholesterol and a 3.8-fold (95% CI: 2.6 to 5.4) increase in odds of CAD. These effects were most pronounced in those with loss-of-function mutations in *LDLR* (Figure 1). Average LDL cholesterol was 190 mg/dl in those with an FH mutation, and 73 of 164 mutation carriers (45%) had LDL cholesterol ≥190 mg/dl. However, despite the observed large effect on average levels, a wide spectrum of circulating LDL cholesterol concentrations was noted in those who were mutation positive (Figure 2). Forty-four of 164 (27%) mutation carriers had an observed LDL cholesterol level <130 mg/dl, which reflects incomplete penetrance. An aggregation of all rare missense mutations had a modest impact on both LDL cholesterol and CAD risk. As expected, synonymous mutations, which do not change the amino acid sequence, had no effect on either parameter (Figure 1). FH mutations were also associated with a nominally significant reduction in high-density lipoprotein cholesterol (−1.9 mg/dl; 95% CI: −3.7 to −0.1; $p = 0.04$) but not with circulating triglycerides ($p = 0.36$).

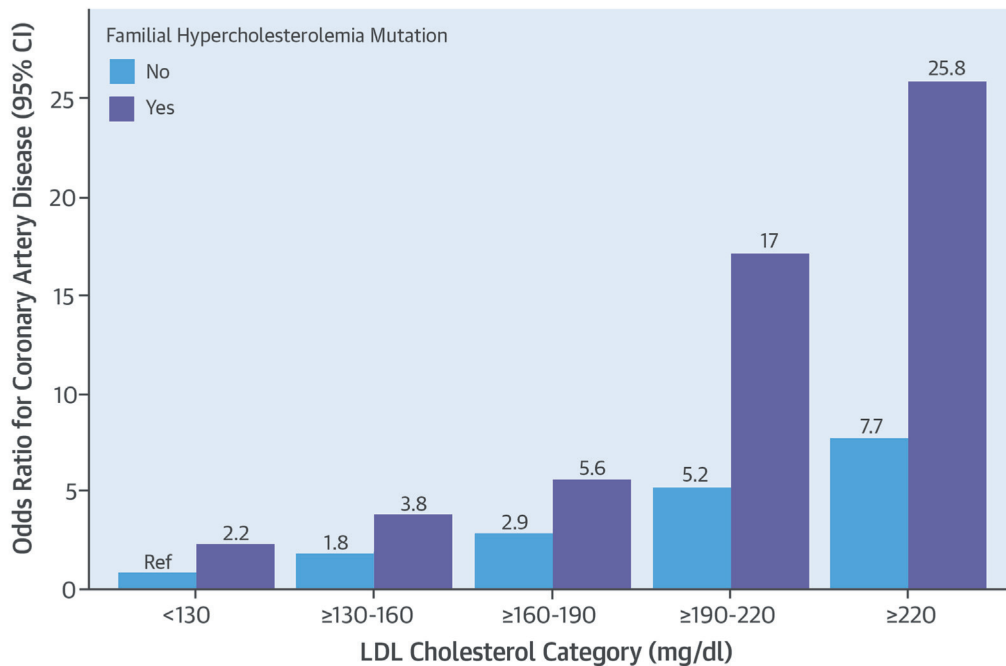
Within the Myocardial Infarction Genetics Consortium case-control cohort populations, those with an FH mutation were at higher risk of CAD than those without a mutation (Table 2) (p value for difference = 0.001). For participants with both LDL cholesterol ≥190 mg/dl and an FH mutation, the odds of CAD were increased 22-fold (OR: 22.3; 95% CI: 10.7 to 53.2) compared with those with LDL cholesterol <130 mg/dl and no mutation. For participants with LDL cholesterol ≥190 mg/dl and no FH mutation, odds of CAD were increased 6-fold (OR: 6.0; 95% CI: 5.2 to 6.9) compared with the same reference group. This difference persisted after additional adjustment for measured LDL cholesterol level ($p = 0.02$).

Separation of the population into clinically relevant categories of LDL cholesterol levels demonstrated trends toward higher risk in those with an FH mutation within each stratum (Central Illustration, Online Table 6). The impact of an FH mutation was similar across strata of LDL cholesterol levels (p value for interaction = 0.51). Within the subgroup of participants with LDL cholesterol in the ≥190 to 220 mg/dl range, those with a mutation had 17-fold increased CAD risk versus 5-fold for those without a mutation. This was despite similar observed LDL cholesterol levels in this stratum (mean LDL cholesterol 205 mg/dl in those with an FH mutation versus 203 mg/dl in those without; p value for difference = 0.22).

CUMULATIVE LDL CHOLESTEROL EXPOSURE ACCORDING TO FH MUTATION STATUS. For any given observed LDL cholesterol level, those harboring a mutation might have a higher average lifetime LDL

CENTRAL ILLUSTRATION Sequencing Familial Hypercholesterolemia Genes in Severe Hypercholesterolemia: Prevalence and Impact





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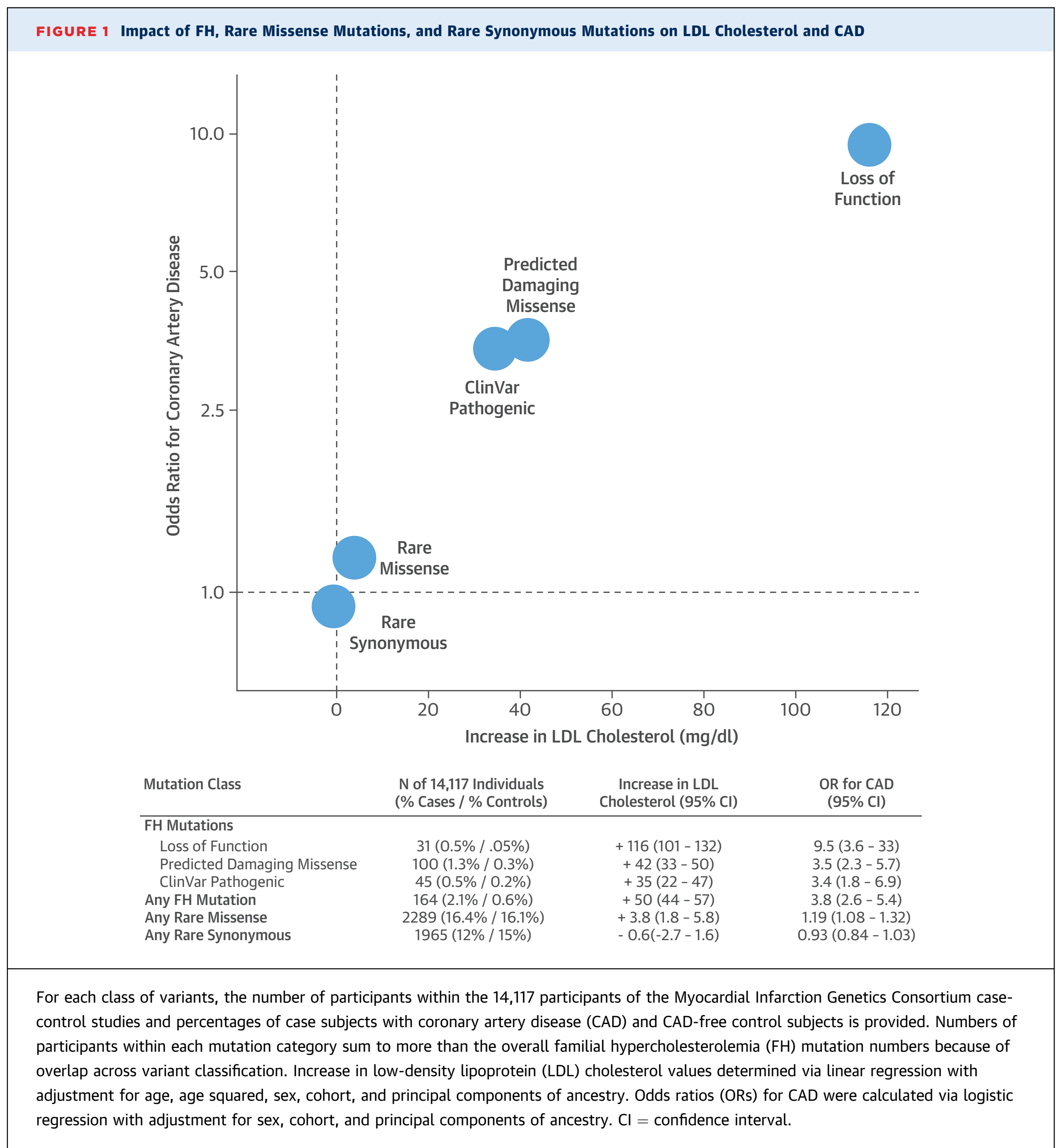
(A) Prevalence of a familial hypercholesterolemia (FH) mutation among severely hypercholesterolemic participants. **(B)** Risk of coronary artery disease (CAD) across low-density lipoprotein (LDL) cholesterol and FH mutation status categories. Odds ratios for CAD were calculated via logistic regression with adjustment for sex, cohort, and principal components of ancestry relative to a reference category of LDL cholesterol <130 mg/dl without an FH mutation. Counts of CAD-free control subjects versus CAD case subjects in each category are provided in Online Table 6. The p value for mutation carriers versus noncarriers across strata of LDL cholesterol was <0.0001. The p-interaction between LDL cholesterol category and mutation status was 0.51.

FIGURE 1 **Impact of FH, Rare Missense Mutations, and Rare Synonymous Mutations on LDL Cholesterol and CAD**



Mutation Class	N of 14,117 Individuals (% Cases / % Controls)	Increase in LDL Cholesterol (95% CI)	OR for CAD (95% CI)
FH Mutations			
Loss of Function	31 (0.5% / .05%)	+ 116 (101 - 132)	9.5 (3.6 - 33)
Predicted Damaging Missense	100 (1.3% / 0.3%)	+ 42 (33 - 50)	3.5 (2.3 - 5.7)
ClinVar Pathogenic	45 (0.5% / 0.2%)	+ 35 (22 - 47)	3.4 (1.8 - 6.9)
Any FH Mutation	164 (2.1% / 0.6%)	+ 50 (44 - 57)	3.8 (2.6 - 5.4)
Any Rare Missense	2289 (16.4% / 16.1%)	+ 3.8 (1.8 - 5.8)	1.19 (1.08 - 1.32)
Any Rare Synonymous	1965 (12% / 15%)	- 0.6(-2.7 - 1.6)	0.93 (0.84 - 1.03)

For each class of variants, the number of participants within the 14,117 participants of the Myocardial Infarction Genetics Consortium case-control studies and percentages of case subjects with coronary artery disease (CAD) and CAD-free control subjects is provided. Numbers of participants within each mutation category sum to more than the overall familial hypercholesterolemia (FH) mutation numbers because of overlap across variant classification. Increase in low-density lipoprotein (LDL) cholesterol values determined via linear regression with adjustment for age, age squared, sex, cohort, and principal components of ancestry. Odds ratios (ORs) for CAD were calculated via logistic regression with adjustment for sex, cohort, and principal components of ancestry. CI = confidence interval.

cholesterol exposure than those who do not harbor a mutation; this could explain the higher CAD risk among mutation carriers. We tested this hypothesis using 2 prospective cohort studies, ARIC and the FHS Offspring Study, in which sequencing data and serial measurements of LDL cholesterol were available. We identified 25 participants with an FH mutation and LDL cholesterol ≥130 mg/dl. Mean LDL cholesterol at time of last study visit was 185 mg/dl. Compared with matched noncarriers with similar LDL cholesterol at the last visit, participants with an FH mutation had a 17 mg/dl (95% CI: 5 to 29 mg/dl; $p = 0.007$) higher average LDL cholesterol exposure in the years preceding the last visit (**Figure 3**, Online Table 7).

DISCUSSION

Among 20,485 multiethnic participants from 12 studies, we found that 1,386 (7%) had severe hypercholesterolemia (LDL cholesterol ≥190 mg/dl), and only a small fraction (<2%) of those also carried an FH mutation. However, within any stratum of LDL cholesterol, those who carried an FH mutation were at substantially higher risk for CAD than those who

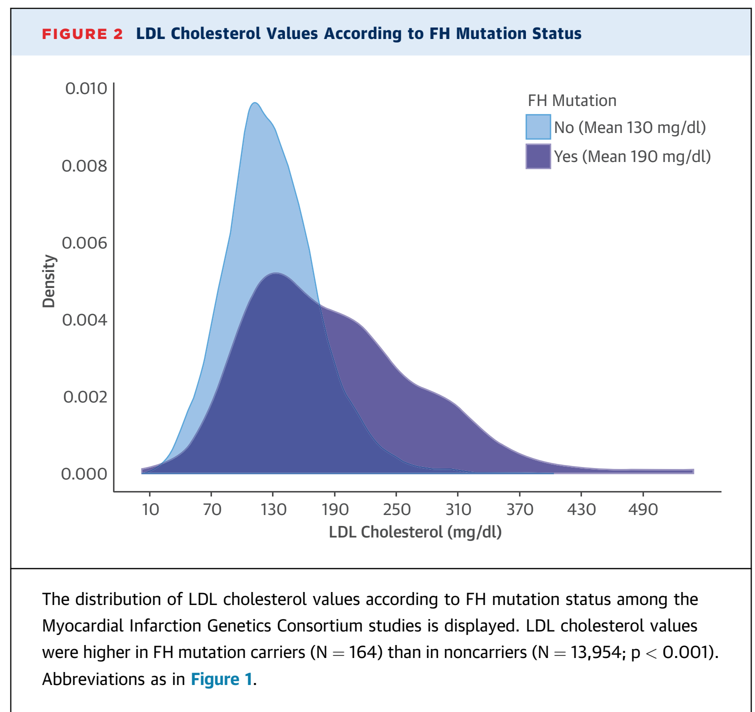
did not. This increased CAD risk among mutation carriers was explained at least in part by a greater cumulative lifetime exposure to LDL cholesterol.

These results permit several conclusions. First, FH mutations explain only a small fraction of severe hypercholesterolemia in the population. Previous reports noted a substantially higher rate of mutation detection in those with clinically suspected FH, ascertained on the basis of features (e.g., family history, physical examination, or severe hypercholesterolemia at a young age) that enrich for a monogenic origin (5-16). Here, we address a scientific question (what fraction of severely hypercholesterolemic subjects carry a mutation in any of 3 genes causal for FH?) that is distinct from these earlier seminal reports. When participants were ascertained solely on the basis of a single elevated LDL cholesterol level, we identified an FH mutation in fewer than 2% of severely hypercholesterolemic subjects. These sequencing results are broadly consistent with those of a recent study of 98,098 subjects from the Copenhagen General Population Study in which genotyping of the 4 most common FH mutations was used to extrapolate overall FH mutation prevalence. In that Danish study, of 5,332 subjects with LDL cholesterol ≥5 mmol/l (193 mg/dl), fewer than 5% were predicted to harbor an FH mutation (28).

If not a monogenic mutation in the 3 FH genes, what might be the cause of elevated LDL cholesterol in the remaining >95% of participants with severe hypercholesterolemia? Possibilities include polygenic hypercholesterolemia, life-style factors, or a combination of these. For example, subjects in the top quartile of a polygenic LDL cholesterol gene score composed of 95 common variants were 13-fold more likely to have high LDL cholesterol (37). Similarly, subjects in the top decile of a LDL cholesterol gene score composed of 12 common variants were 4.2-fold more likely to have LDL ≥190 mg/dl in the U.K. Whitehall II study (38). Future genetic studies might identify additional causal variants, genes beyond those considered in this study, or large-effect regulatory variants that underlie severe hypercholesterolemia. Other nongenetic explanations for severe LDL cholesterol elevations include secondary causes (e.g., hypothyroidism or nephrotic syndrome), life-style factors such as dietary fat, or some combination of these.

Second, within any stratum of a single observed LDL cholesterol level, CAD risk was higher in those with an FH mutation than in those without. This novel finding reinforces the potential utility of genetic testing to provide risk information beyond the LDL cholesterol level. We analyzed 25 matched

FIGURE 2 **LDL Cholesterol Values According to FH Mutation Status**



The distribution of LDL cholesterol values according to FH mutation status among the Myocardial Infarction Genetics Consortium studies is displayed. LDL cholesterol values were higher in FH mutation carriers (N = 164) than in noncarriers (N = 13,954; $p < 0.001$). Abbreviations as in Figure 1.

pairs of participants with similarly elevated LDL cholesterol levels at the time of ascertainment and found a higher cumulative exposure to LDL cholesterol in those with an FH mutation. These data support the hypothesis that an FH mutation, present since birth, increases CAD risk via lifelong exposure to high LDL cholesterol (39). By contrast, an isolated elevation in LDL cholesterol in those without a genetic predisposition might reflect a time-limited exposure related to a current environmental perturbation or a value that is more likely to regress toward the mean in the future. Future studies might identify additional metabolic parameters, such as increased lipoprotein(a) levels (40), that also contribute to the excess CAD risk noted in those with an FH mutation.

Finally, these data contribute to ongoing discussion regarding how to define FH. Classically, FH refers to elevated LDL cholesterol caused by a single mutation in any of several genes segregating in an autosomal dominant manner. Alternate approaches to 2 features, LDL cholesterol threshold and mutation definition, affect FH prevalence estimates (**Table 3**). An approach that includes all participants with untreated LDL cholesterol ≥190 mg/dl (i.e., without an FH mutation requirement) would combine nongenetic and genetic causes and classify approximately 7% of the U.S. adult population as having FH. An alternative possibility is to withhold an LDL cholesterol threshold and require only a stringent mutation

TABLE 2 Risk of CAD in Those With Elevated LDL Cholesterol (≥190 mg/dl) According to FH Mutation Status in CAD Case-Control Studies

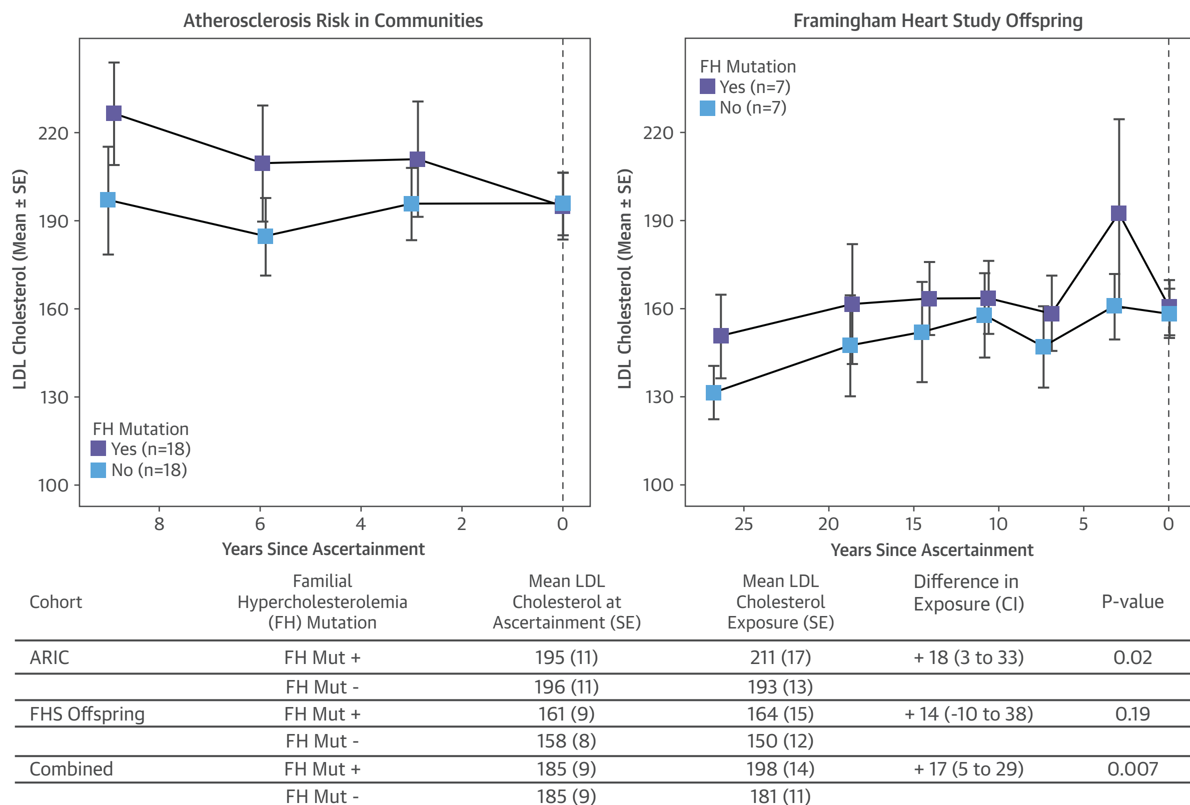
	Total N (CAD-Free Controls/ CAD Cases)	OR for CAD (95% CI)*	p Value (FH Mutation + vs. -)†	LDL Cholesterol-Adjusted OR for CAD (95% CI)*	p Value (FH Mutation + vs. -)†
LDL cholesterol ≥190 mg/dl					
FH mutation negative	1,264 (422/842)	6.0 (5.2–6.9) $p < 0.001$	0.001	1.6 (1.3–2.1) $p < 0.001$	0.02
FH mutation positive	73 (8/65)	22.3 (10.7–53.2) $p < 0.001$		4.2 (1.9–10.4) $p < 0.001$	
LDL cholesterol <130 mg/dl and FH mutation negative	7,485 (5,175/2,310)	Reference		Reference	

Values are N (n/n) unless otherwise indicated. OR for CAD was calculated via logistic regression with adjustment for sex, cohort, and principal components of ancestry relative to a reference category of LDL cholesterol <130 mg/dl without an FH mutation. OR values with and without additional adjustment for observed LDL cholesterol, expressed as a continuous variable, are provided. *p value for difference in OR compared with reference category. †p value for difference in OR between FH mutation positive and FH mutation negative among participants with LDL cholesterol ≥190 mg/dl.

CI = confidence interval; OR = odds ratio; other abbreviations as in Table 1.

definition; in such an analysis of 20,485 participants, we identified an FH mutation in 97 participants (1 in 211). This estimate is nearly identical to a population-based analysis in the Copenhagen General Population Study (1 in 217) (28). However, if one additionally requires that an FH mutation is accompanied by an elevated LDL cholesterol level, FH prevalence in our study declines (1 in 301 with an LDL

FIGURE 3 Cumulative LDL Cholesterol Exposure in FH Mutation Carriers Compared With Noncarriers Matched on Observed LDL Cholesterol at Ascertainment



Cohort	Familial Hypercholesterolemia (FH) Mutation	Mean LDL Cholesterol at Ascertainment (SE)	Mean LDL Cholesterol Exposure (SE)	Difference in Exposure (CI)	P-value
ARIC	FH Mut +	195 (11)	211 (17)	+ 18 (3 to 33)	0.02
	FH Mut -	196 (11)	193 (13)		
FHS Offspring	FH Mut +	161 (9)	164 (15)	+ 14 (-10 to 38)	0.19
	FH Mut -	158 (8)	150 (12)		
Combined	FH Mut +	185 (9)	198 (14)	+ 17 (5 to 29)	0.007
	FH Mut -	185 (9)	181 (11)		

Hypercholesterolemic (LDL cholesterol ≥130 mg/dl) carriers of an FH mutation were identified in ARIC (Atherosclerosis Risk in Communities) and FHS (Framingham Heart Study) Offspring cohorts and matched 1:1 to FH mutation noncarriers according to age, sex, statin use, and LDL cholesterol at time of ascertainment. Mean ± standard error (SE) LDL cholesterol values at each study visit are displayed in each cohort according to mutation status. A matched-pairs Student *t* test demonstrated higher cumulative exposure to LDL cholesterol in FH mutation carriers than in noncarriers. Abbreviations as in Figure 1.

threshold ≥130 mg/dl and 1 in 853 with an LDL threshold ≥190 mg/dl).

With regard to defining an FH mutation, all schemata agree on the inclusion of loss-of-function alleles in *LDLR*, but they differ on how to handle missense mutations. For missense mutations, we applied a rigorous threshold, requiring that the mutation be designated as damaging by each of 5 computer prediction algorithms or be previously annotated as pathogenic in the ClinVar clinical genetics database. A key advantage of this approach is that it ensures that classification is both fully reproducible and generalizable to genes beyond those related to FH.

When routine genetic testing is not available, clinical scoring systems, such as the Dutch Lipid Clinical Network, Simon Broome, and MEDPED criteria, have been developed to approximate FH status (4). Ongoing collaborative efforts on how to optimally incorporate population-based genetic sequencing data into existing frameworks for the clinical diagnosis of FH will be critically important.

STUDY LIMITATIONS. First, our data did not permit us to stratify participants by family history or physical examination features, as suggested by the Dutch Lipid Clinic Network and Simon Broome criteria (41,42). Second, we accounted for an estimated 30% reduction in LDL cholesterol in those undergoing lipid-lowering therapy, as previously implemented (26–28). This approach might imperfectly estimate untreated LDL cholesterol, given heterogeneity in drug selection, dosing, and response and variability across baseline LDL cholesterol levels or mutation status. However, a sensitivity analysis limited to Myocardial Infarction Genetics Consortium cohort participants not undergoing lipid-lowering therapy similarly noted a pronounced difference in risk among severely hypercholesterolemic participants stratified by mutation status (Online Table 8). Third, current exome-sequencing techniques inadequately capture structural and copy-number genetic variation, and as such, some FH mutations might have been missed. Fourth, our approach to annotating missense variants using prediction algorithms and the ClinVar database might have led to misclassification in some cases. Additional studies that implement large-scale functional screens of identified variants or that pool phenotypes across additional studies could provide additional refinement of pathogenicity annotations. Lastly, FH mutation prevalence was determined in CAD-free control subjects and population-based cohorts. These participants survived to middle age, and few had clinically manifest CAD, which raises the possibility of survivorship or selection bias. Our case-control population was enriched for participants with premature CAD; effect estimates of mutations on coronary risk might be different in patients with later disease onset.

TABLE 3 Prevalence of FH According to Different LDL Cholesterol Thresholds and Mutation Classification Schemes

LDL Cholesterol Criteria	Mutation Criterion	Prevalence of FH
LDL cholesterol ≥190 mg/dl	No mutation required	1,386 of 20,485 (1 in 14)
No threshold requirement	•*LDLR* loss-of-function variant; or •*LDLR* predicted damaging rare missense variant; or •*LDLR*, *APOB*, *PCSK9* variant pathogenic in ClinVar	97 of 20,485 (1 in 211)
LDL cholesterol ≥190 mg/dl	•*LDLR* loss-of-function variant; or •any rare *LDLR* missense variant	80 of 20,485 (1 in 256)
LDL cholesterol ≥130 mg/dl	•*LDLR* loss-of-function variant: or •*LDLR* predicted damaging rare, missense variant; or •*LDLR*, *APOB*, *PCSK9* variant pathogenic in ClinVar	68 of 20,485 (1 in 301)
No threshold requirement	•*LDLR* loss-of-function variant; or •*LDLR* predicted damaging rare missense variant	60 of 20,485 (1 in 341)
LDL cholesterol ≥190 mg/dl	•*LDLR* loss-of-function variant; or •*LDLR* predicted damaging rare missense variant; or •*LDLR*, *APOB*, *PCSK9* variant pathogenic in ClinVar	24 of 20,485 (1 in 853)

For each classification scheme, the number of participants who met the criteria among a total of 20,485 participants (CAD-free control subjects of the Myocardial Infarction Genetics Consortium combined with CHARGE Consortium participants) is provided. Loss-of-function variants were defined as single-base changes that introduce a stop codon that leads to premature truncation of a protein (nonsense), insertions or deletions (indels) of DNA that scramble protein translation beyond the variant site (frameshift), or point mutations at sites of pre-messenger ribonucleic acid splicing that alter the splicing process (splice site). Predicted damaging variants refer to those *LDLR* predicted to be deleterious by each of 5 in silico prediction algorithms (LRT score, MutationTaster, PolyPhen-2 HumDiv, PolyPhen-2 HumVar, and Sorting Intolerant From Tolerant [SIFT]). Rare variants refers to those with minor allele frequency <1% in the sequenced population.

APOB = apolipoprotein B; LDLR = low-density lipoprotein receptor; PCSK9 = proprotein convertase subtilisin/kexin type 9; other abbreviations as in Tables 1 and 2.

CONCLUSIONS

Genetic sequencing identified an FH mutation in only a small proportion of severely hypercholesterolemic participants; however, for any given observed LDL cholesterol level, risk for CAD was substantially higher in FH mutation carriers than in noncarriers, which was likely related in large part to higher lifelong exposure to atherogenic LDL particles. A primary goal of precision medicine is to use molecular diagnostics to identify a small subset of the population at increased disease risk in which to deliver an intervention. Systematic efforts to identify and treat severely hypercholesterolemic patients who carry an FH mutation could represent one such opportunity.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: For any given observed LDL cholesterol level, carriers of a familial hypercholesterolemia mutation are at substantially increased risk of coronary disease compared with non-carriers, which is likely related to increased lifelong exposure to LDL cholesterol.

TRANSLATIONAL OUTLOOK: Additional research is needed to understand whether genetic testing can prove clinically useful in guiding the treatment of people with severe hypercholesterolemia to reduce risk of CAD.

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KEY WORDS coronary artery disease, gene sequencing, genetics, low-density lipoprotein cholesterol

APPENDIX For an expanded Methods section including references as well as supplemental tables and figures, please see the online version of this article.