Diagnostic Yield of Sequencing Familial Hypercholesterolemia Genes in Patients with Severe Hypercholesterolemia

Amit V. Khera, MD, Hong-Hee Won, PhD, Gina M. Peloso, PhD, Kim S. Lawson, MS, Traci M. Bartz, MS, Xuan Deng, MSc, Elisabeth M. van Leeuwen, Pradeep Natarajan, MD, MMSc, Connor A. Emdin, HBSc, Alexander G. Bick, BS, Alanna C. Morrison, PhD, Jennifer A. Brody, BA, Namrata Gupta, PhD, Akihiro Nomura, MD, Thorsten Kessler, MD, Stefano Duga, PhD, Joshua C. Bis, PhD, Cornelia M. van Duijn, PhD, L. Adrienne Cupples, PhD, Bruce Psaty, MD, PhD, Daniel J. Rader, MD, John Danesh, FMedSci, Heribert Schunkert, MD, Ruth McPherson, MD, Martin Farrall, FRCPath, Hugh Watkins, MD, PhD, Eric Lander, PhD, James G. Wilson, MD, Adolfo Correa, MD, PhD, Eric Boerwinkle, PhD, Piera Angelica Merlini, MD, Diego Ardissino, MD, Danish Saleheen, MB, BS, PhD, Stacey Gabriel, PhD, Sekar Kathiresan, MD

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Amit V. Khera, MD*,[a,b] Hong-Hee Won, PhD*,[c] Gina M. Peloso, PhD*,[b,d] Kim S. Lawson, MS,[e] Traci M. Bartz, MS,[f] Xuan Deng, MSc,[d] Elisabeth M. van Leeuwen,[g] Pradeep Natarajan, MD, MMSc,[a,b] Connor A. Emdin, HBSc,[b] Alexander G. Bick, BS,[b] Alanna C. Morrison, PhD,[e] Jennifer A. Brody,[h] BA, Namrata Gupta, PhD,[b] Akihiro Nomura, MD,[b, i] Thorsten Kessler, MD,[j] Stefano Duga, PhD,[k] Joshua C. Bis, PhD, [h] Cornelia M. van Duijn, PhD,[g] L. Adrienne Cupples, PhD,[d] Bruce Psaty, MD, PhD,[h,l] Daniel J. Rader, MD,[m] John Danesh, FMedSci,[n] Heribert Schunkert,[j] MD, Ruth McPherson, MD,[o] Martin Farrall, FRCPath,[p] Hugh Watkins, MD,[p] PhD, Eric Lander, PhD,[b] James G. Wilson, MD,[q] Adolfo Correa, MD, PhD,[r] Eric Boerwinkle, PhD,[e] Piera Angelica Merlini, MD,[s] Diego Ardissino, MD,[t] Danish Saleheen, MB,BS,PhD,[u] Stacey Gabriel, PhD,[b] Sekar Kathiresan, MD[a,b]

* Drs. Amit V. Khera, Hong-Hee Won, and Gina M. Peloso contributed equally to this work

[a] Center for Human Genetic Research, Cardiovascular Research Center and Cardiology Division (Khera, Natarajan, Kathiresan), Massachusetts General Hospital, Harvard Medical School, Boston MA
[b] Program in Medical and Population Genetics, Broad Institute, Cambridge, MA
[c] Samsung Advanced Institute for Health Sciences and Technology (SAIHST), Sungkyunkwan University, Samsung Medical Center, Seoul, Korea
[d] Department of Biostatistics, Boston University School of Public Health
[e] Human Genetics Center and Institute of Molecular Medicine, University of Texas-Houston Health Science Center, Houston, TX
[f] Department of Biostatistics, University of Washington, Seattle, Washington;
[g] Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands
[h] Cardiovascular Health Research Unit, University of Washington
[i] Division of Cardiovascular Medicine, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan
[j] Deutsches Herzzentrum München, Technische Universität München, Deutsches Zentrum für Herz-Kreislauf-Forschung (DZHK); Munich Heart Alliance, München, Germany (Kessler, Schunkert);
[k] Department of Biomedical Sciences, Humanitas University, Via Manzoni 113, 20089 Rozzano, Milan, Italy; Humanitas Clinical and Research Center, Via Manzoni 56, 20089 Rozzano, Milan, Italy
[l] Departments of Medicine, Epidemiology, and Health Services, University of Washington
[m] Departments of Genetics, University of Pennsylvania, Philadelphia
[n] Public Health and Primary Care, University of Cambridge, Cambridge, Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK, and NIHR Blood and Transplant Research Unit in Donor Health and Genomics, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
[o] University of Ottawa Heart Institute, Ottawa, Canada
[p] Division of Cardiovascular Medicine, Radcliffe Department of Medicine and the Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom

[q] Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi
[r] Jackson Heart Study, Department of Medicine, University of Mississippi Medical Center
[s] Ospedale Niguarda, Milano Italy
[t] Division of Cardiology, Azienda Ospedaliero-Universitaria di Parma, University of Parma Parma, Italy; ASTC - Associazione Per Lo Studio Della Trombosi In Cardiologia, Pavia Italy (Ardissino)
[u] Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania

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Corresponding Author:
Sekar Kathiresan, MD
Cardiovascular Research Center & Center for Human Genetics
Massachusetts General Hospital
185 Cambridge Street, CPZN 5.252
Boston, MA 02114
Telephone: 617 643 6120
Fax: 8779915996
Email: skathiresan1@mgh.harvard.edu

Abstract:
Background: About 7% of US adults have severe hypercholesterolemia (untreated LDL cholesterol ≥190 mg/dl). Such high LDL levels may be due to familial hypercholesterolemia (FH), a condition caused by a single mutation in any of three genes. Lifelong elevations in LDL cholesterol in FH mutation carriers may confer CAD risk beyond that captured by a single LDL cholesterol measurement.
Objectives: Assess the prevalence of a FH mutation among those with severe hypercholesterolemia and determine whether CAD risk varies according to mutation status beyond the observed LDL cholesterol.
Methods: Three genes causative for FH (*LDLR*, *APOB*, *PCSK9*) were sequenced in 26,025 participants from 7 case-control studies (5,540 CAD cases, 8,577 CAD-free controls) 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 8,577 CAD-free control participants, 430 had LDL cholesterol ≥190 mg/dl; of these, only eight (1.9%) carried a FH mutation. Similarly, among 11,908 participants from 5 prospective cohorts, 956 had LDL cholesterol ≥190 mg/dl and of these, only 16 (1.7%) carried a FH mutation. Within any stratum of observed LDL cholesterol, risk of CAD was higher among FH mutation carriers when compared with non-carriers. When compared to a reference group with LDL cholesterol <130 mg/dl and no mutation, participants with LDL cholesterol ≥190 mg/dl and no FH mutation had six-fold higher risk for CAD (OR 6.0; 95%CI 5.2–6.9) whereas those with LDL cholesterol ≥190 mg/dl as well as a FH mutation demonstrated twenty-two fold increased risk (OR 22.3; 95%CI 10.7–53.2).
Conclusions: Among individuals with LDL cholesterol ≥190 mg/dl, gene sequencing identified a FH mutation in <2%. However, for any given observed LDL cholesterol, FH mutation carriers are at substantially increased risk for CAD.

Clinical trial: ??? Please query authors.
Keywords: familial hypercholesterolemia, low-density lipoprotein cholesterol, gene sequencing, coronary artery disease, genetics

Abbreviations:
APOB = apolipoprotein B
CAD = coronary artery disease
FH = familial hypercholesterolemia
HDL = high-density lipoprotein
LDL = low-density lipoprotein
LDLR = low-density lipoprotein receptor
PCSK9 = proprotein convertase subtilisin/kexin type 9

Introduction

Primary, severe hypercholesterolemia, defined as having a low-density lipoprotein (LDL) cholesterol ≥ 190 mg/dl, is a treatable risk factor for coronary artery disease (CAD) (1,2); current treatment guidelines place particular emphasis on intensive lifestyle and pharmacologic therapy in this population (3). One etiology of severely elevated LDL cholesterol is familial hypercholesterolemia (FH), an autosomal dominant monogenic disorder linked to impaired hepatic clearance of LDL cholesterol particles (4). It is often assumed that individuals with LDL cholesterol ≥ 190 mg/dl 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 a FH mutation in severely hypercholesterolemic patients.

Previous studies of the diagnostic yield of genetic testing in severe hypercholesterolemia have focused on individuals with clinically-suspected FH and in these samples, a FH mutation prevalence ranging from 20 to 80% has been reported (5-16). This variability is likely due to differing ascertainment schemes based on family history, physical exam features, elevated LDL cholesterol at a young age, or referral to specialized clinics, each of which may enrich for monogenic etiologies. In contrast, if ascertainment from the general population is based solely on elevated LDL cholesterol, the extent to which FH mutations contribute to severe hypercholesterolemia is unknown. Such knowledge may inform design and effectiveness of universal FH screening proposals (17,18).

Knowledge of FH mutation status may also provide CAD risk information beyond that of a single LDL cholesterol measurement (4,18). A FH mutation leads to cumulative exposure to higher LDL cholesterol levels over a lifetime and as such, within any stratum of LDL cholesterol, the risk of CAD may be greater if the LDL elevation is due to a monogenic mutation

versus other causes. The extent to which CAD risk is modulated by the presence of a causal FH mutation is uncertain.

We analyzed gene sequences of three FH genes, low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*), in twelve distinct cohorts including >26,000 participants to determine: 1) the diagnostic yield of gene sequencing to identify a FH mutation in severely hypercholesterolemic individuals; and 2) the clinical impact of a FH mutation with regard to CAD risk within any given stratum of LDL cholesterol levels.

Methods

Study Populations

Whole exome sequencing was performed in seven previously described CAD case-control cohorts of the Myocardial Infarction Genetics Consortium (**Online Table 1**). Studies included the Italian Atherosclerosis Thrombosis and Vascular Biology study (19), the Exome Sequencing Project Early-Onset Myocardial Infarction (ESP-EOMI) study (20), a nested case-control of the Jackson Heart Study (JHS) (15), the Munich Myocardial Infarction study (22), the Ottawa Heart Study (23), the Precocious Coronary Artery Disease (PROCARDIS) study (24), and the Pakistan Risk of Myocardial Infarction Study (PROMIS) (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 has been 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 a FH mutation in individuals with a LDL cholesterol > 190 mg/dl was additionally determined in 11,908 participants from five prospective cohort studies derived from

the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (29). Atherosclerosis Risk in Communities Study (ARIC), Cardiovascular Health Study, Framingham Heart Study (FHS), Rotterdam Baseline Study, and Erasmus Rucphen Family Study (**Online Table 2**).

In order 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 was analyzed. These data included 5,727 ARIC cohort participants and 2,714 FHS Offspring Study participants.

Gene Sequencing

The CAD case-control whole exome sequencing was performed as previously described at the Broad Institute (Cambridge, MA) (20). The 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 RS and ERF cohorts respectively. Additional sequencing methodology details available in **Supplementary Methods**.

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 DNA that scramble the protein translation beyond the variant site (frameshift), or point mutations at sites of pre-mRNA splicing that alter the splicing process (splice-site); 2) missense variants in *LDLR* predicted to be deleterious by *each* of five *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 publically 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 harbor 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 associated LOFTEE plugin (34), and the dbNSFP database (version 3.0b1) (35).

Longitudinal Analysis of LDL Cholesterol Exposure

Individuals with a FH mutation and LDL cholesterol ≥ 130 mg/dl were identified in ARIC and FHS Offspring Study cohorts. LDL cholesterol values were adjusted in those reporting lipid-lowering therapy by dividing measured value by 0.7. Mean LDL cholesterol exposure was calculated as cumulative exposure, determined via an area under the curve analysis, divided by length of follow-up. 27 FH mutation carriers met the above inclusion criteria and were matched 1:1 to a mutation negative control according to age (within 10 years), gender, 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 (93%) individuals. Mean LDL cholesterol exposure was compared among those with and without FH mutation using a paired t-test.

Statistical Analysis

The impact of aggregations of genetic variants on levels of LDL cholesterol was assessed using linear regression with adjustment for age, age^2, gender, cohort, and the first five principal components of ancestry. Odds ratios for CAD were calculated using logistic regression with

adjustment for gender, cohort, and the first five principal components of ancestry. In analyses conducted on ordinal strata of LDL cholesterol, individuals with LDL cholesterol <130 mg/dl and no mutation linked to FH served as the reference group.

Analyses were performed using R version 3.2.2 software (The R Project for Statistical Computing, Vienna, Austria). Figures were creating using 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 controls and 5,540 CAD cases (**Online Table 3**). The study population included 10,421 (74%) males with mean age 53 years (SD 14). Proportions of self-identified race were 47%, 46%, and 7% for white, South Asian, and black, respectively. 47% of study participants had a history of hypertension, 27% had a history of diabetes, 34% were current smokers, and 14% were on lipid-lowering medications.

Sequencing identified 86 variants linked to FH on the basis of leading to loss of function in *LDLR*, missense mutations in *LDLR* predicted to be damaging by each of five computer prediction algorithms, or 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**).

164 individuals harbored a mutation linked to FH, including 48 CAD-free controls (0.6%; 95%CI 0.4 – 0.7%) and 116 CAD cases (2.1%; 95%CI 1.7 – 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 one 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*.

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 a FH mutation increased across categories of LDL cholesterol levels ($P < 0.001$) (**Online Figure 2)**. Of 8,577 control participants, 430 participants (5% of control sample) had LDL cholesterol ≥ 190 mg/dl and of these 430, only 8 carried a FH mutation (1.9%; 95%CI 0.9 – 3.8%) (**Table 1 & Central Illustration**).

This prevalence estimate was replicated in 11,908 participants from five prospective cohort studies of the CHARGE consortium; 956 (8%) had a LDL cholesterol >190 mg/dl and of these, 16 (1.7%; 95%CI 1.0 – 2.8%) harbored a FH mutation. Across the twelve studies combined (n=20,485), 1386 (7%) displayed LDL cholesterol ≥ 190 mg/dl and of these, 24 (1.7%) carried a 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 a FH mutation was associated with a 50 mg/dl (95%CI 44– 57) increase in LDL cholesterol and a 3.8 fold (95%CI 2.6 – 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 a FH mutation and 73/164 (45%) had a 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**). 44 of 164 (27%) mutation carriers had an observed LDL cholesterol less than 130 mg/dl; reflecting

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 lead to a change in amino acid sequence, had no effect on either parameter (**Figure 1).** Beyond LDL cholesterol levels, a FH mutation was associated with a nominally significant reduction in high-density lipoprotein cholesterol (-1.9 mg/dl; 95%CI -3.7 – -0.1; p = 0.04) but no association with circulating triglycerides (p = 0.36).

Within the Myocardial Infarction Genetics Consortium case-control cohort populations, those with a FH mutation were at substantially higher risk compared to those without a mutation (**Table 2**, p-value for difference = 0.001). For participants with both LDL cholesterol ≥ 190 mg/dl and a FH mutation, the odds of coronary artery disease were increased twenty-two fold (OR 22.3; CI 10.7 – 53.2) when compared to those with LDL cholesterol < 130 mg/dl and no mutation. For participants with LDL cholesterol ≥ 190 mg/dl and no FH mutation, odds of coronary artery disease were increased six-fold (OR 6.0; CI 5.2 – 6.9) compared to 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 towards higher risk in those with a FH mutation within each stratum (**Central Illustration; Supplementary Table 6)**. The impact of a FH mutation was similar across strata of LDL cholesterol levels (p-interaction = 0.51). Within the subgroup of participants with a 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 difference was noted despite similar observed LDL cholesterol levels in this stratum (mean LDL cholesterol in those with

mutation=205 mg/dl versus mean LDL cholesterol in those without a FH mutation = 203 mg/dl; p-value for difference = 0.22).

Cumulative LDL Cholesterol Exposure According to FH Mutation Status

For any given observed LDL cholesterol, those harboring a mutation might have a higher average LDL cholesterol exposure over their lifetime compared to those who do not harbor a mutation and this could explain a higher CAD risk among mutation carriers. We tested this hypothesis using two prospective cohort studies – ARIC and the FHS Offspring Study – where sequencing data and serial measurements of LDL cholesterol were available. We identified 25 individuals with a FH mutation and LDL cholesterol ≥ 130 mg/dl. Mean LDL cholesterol at time of last study visit was 185 mg/dl. As compared to matched non-carriers with similar LDL cholesterol at the last visit, individuals with a FH mutation had a 17 mg/dl (95%CI 5 – 29; 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 of those with severe hypercholesterolemia, only a small fraction (<2%) also carried a FH mutation. However, within any stratum of LDL cholesterol, those who carried a FH mutation were at substantially higher risk for CAD compared to those who did not. This increased CAD risk among mutation carriers was at least partially explained by a greater cumulative exposure to LDL cholesterol over a lifetime.

These results permit several conclusions. First, FH mutations explain only a small fraction of severe hypercholesterolemia in the population. Previous reports have noted a

substantially higher rate of mutation detection in those with clinically-suspected FH, as ascertained on the basis of features (e.g. family history, physical exam, or severe hypercholesterolemia at a young age) that enrich for a monogenic etiology (5-16). Here, we address a scientific question – what fraction of severely hypercholesterolemic individuals carry a mutation in any of three high LDL genes – that is distinct from these earlier, seminal reports. When participants are ascertained solely on the basis of a single elevated LDL cholesterol level, we identify a FH mutation in fewer than 2% of severely hypercholesterolemic individuals. These sequencing results are broadly consistent with those of a recent study of 98,098 individuals from the Copenhagen General Population Study in which genotyping of the four most common FH mutations was used to extrapolate overall FH mutation prevalence. In this Danish study, of 5,332 individuals with LDL cholesterol ≥ 5 mmol/l (193 mg/dl), fewer than 5% were predicted to harbor a FH mutation (28).

If not a monogenic mutation in the three FH genes, what might be the cause of elevated LDL cholesterol in the remaining >95% of participants with severe hypercholesterolemia? Possibilities include polygenic hypercholesterolemia, lifestyle factors, and/or a combination of these. For example, individuals in the top quartile of a polygenic LDL cholesterol gene score comprised of 95 common variants were 13 fold more likely to have high LDL cholesterol (37). Similarly, individuals in the top decile of a LDL cholesterol gene score comprised of 12 common variants were 4.2 fold more likely to have a LDL ≥ 190 mg/dl in the UK Whitehall II study (38). Future genetics studies may identify additional causal variants, genes beyond those considered in this study, or large-effect regulatory variants that underlie severe hypercholesterolemia. Other non-genetic explanations for severe elevations in LDL cholesterol include secondary causes (e.g.

hypothyroidism or nephrotic syndrome), lifestyle factors such as dietary fat, or some combination of these.

Second, within any stratum of a single observed LDL cholesterol, CAD risk was higher in those with a FH mutation when compared to those without, reinforcing the potential utility of genetic testing. We analyzed 25 matched pairs of individuals with similarly elevated LDL cholesterol levels at time of ascertainment and found a higher cumulative exposure to LDL cholesterol in those with a FH mutation. These data support the hypothesis that a 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 may reflect a time-limited exposure related to a current environmental perturbation or a value that is more likely to regress towards the mean in the future. Future studies may identify additional metabolic parameters, such as increased lipoprotein(a) levels (40), that also contribute to the excess CAD risk noted in those with a 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 two features – LDL cholesterol threshold and mutation definition – impact FH prevalence estimates (**Table 3**). An approach that includes all individuals with untreated LDL cholesterol ≥ 190 mg/dl (i.e., without a FH mutation requirement) would combine non-genetic and genetic causes and classify about 7% of the US adult population as having FH. An alternative possibility is to withhold an LDL cholesterol threshold and require only a stringent mutation definition; in such an analysis of 20,485 participants, we identified a FH mutation in 97 individuals (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 a FH mutation is accompanied by an elevated LDL cholesterol, FH prevalence in our study declines (1 in 301 with LDL threshold ≥130 mg/dl and 1 in 853 with LDL threshold ≥ 190 mg/dl).

With regard to defining a FH mutation, all schema agree on the inclusion of loss of function alleles in *LDLR* but 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 five 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

Our data did not permit stratifying individuals by family history or physical exam features, as suggested by the Dutch Lipid Clinic Network and Simon Broome criteria (41,42). Secondly, we accounted for an estimated 30% reduction in LDL cholesterol in those on lipid-lowering therapy as has been previously implemented (26-28). This approach may imperfectly estimate untreated LDL cholesterol given heterogeneity in drug selection, dosing, response, and variability across baseline LDL cholesterol levels or mutation status. However, a sensitivity analysis limited to Myocardial Infarction Genetics Consortium cohort participants not on lipid-

lowering therapy similarly noted a pronounced difference in risk among severely hypercholesterolemic individuals when stratified by mutation status (**Online Table 8**). Third, structural and copy number genetic variation are inadequately captured by current exome sequencing techniques and as such, some FH mutations may have been missed. Fourth, our approach to annotating missense variants using prediction algorithms and the ClinVar database may have led to misclassification in some cases. Additional studies that implement large-scale functional screens of identified variants or pool phenotypes across additional studies may provide additional refinement of pathogenicity annotations. Lastly, FH mutation prevalence was determined in CAD-free controls and population-based cohorts. These individuals survived to middle-age and few had clinically manifest CAD, raising the possibility of survivorship or selection bias. Our case-control population was enriched for individuals with premature CAD; effect estimates of mutations on coronary risk may be different in patients with later disease onset.

Conclusions

Genetic sequencing identified a FH mutation in only a small proportion of severely hypercholesterolemic individuals. For any given observed LDL cholesterol level, risk for CAD is substantially higher in carriers of a FH mutation versus non-carriers, 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 individuals who carry a FH mutation may represent one such opportunity.

CLINICAL PERSPECTIVES

Competency in Medical Knowledge: Sequencing of three genes causing familial hypercholesterolemia identifies a mutation in only a small fraction of severely hypercholesterolemic individuals.

Translational Outlook: Additional research is needed to determine the relative contributions of other genetic variants and lifestyle factors and evaluate the clinical utility of genetic testing in patients with severe hypercholesterolemia.

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Figure Legends

Central Illustration: Sequencing Familial Hypercholesterolemia Genes in Severe Hypercholesterolemia: Prevalence and Impact

A. Prevalence of a FH mutation amongst severely hypercholesterolemic individuals. B. Risk of coronary artery disease across LDL cholesterol and familial hypercholesterolemia mutation status categories. Odds ratios for CAD were calculated via logistic regression with adjustment for gender, cohort, and principal components of ancestry relative to a reference category of LDL cholesterol <130 mg/dl without a familial hypercholesterolemia (FH) mutation. Counts of CAD-free controls vs. CAD cases in each category are provided in **Supplementary Table 6**. P-value for mutation carriers vs. noncarriers across strata of LDL cholesterol < 0.0001. P-interaction between LDL cholesterol category and mutation status = 0.51

Figure 1. Impact of Familial Hypercholesterolemia, Rare Missense, and Rare Synonymous Mutations on LDL Cholesterol and Coronary Artery Disease.

For each class of variants, the number of individuals within the 14,117 participants of the Myocardial Infarction Genetics Consortium case-control studies and % of CAD cases and CAD-free controls is provided. Number of individuals within each mutation category sum to more than the overall familial hypercholesterolemia mutation numbers due to overlap across variant classification. Increase in LDL cholesterol values determined via linear regression with adjustment for age, age^2, gender, cohort, and principal components of ancestry. Odds ratios for CAD were calculated via logistic regression with adjustment for gender, cohort, and principal components of ancestry.

Figure 2. LDL Cholesterol Values According to Familial Hypercholesterolemia Mutation Status.

The distribution of low-density lipoprotein (LDL) cholesterol values according to familial hypercholesterolemia (FH mutation status) among the Myocardial Infarction Genetics Consortium studies is displayed. LDL cholesterol values were higher in FH mutation carriers (N = 164) as compared to noncarriers (N=13,954), $p < 0.001$.

Figure 3. **Cumulative LDL cholesterol Exposure in Familial Hypercholesterolemia Mutation Carriers Compared on Non-carriers Matched on LDL cholesterol at Ascertainment**

Hypercholesterolemic [low-density lipoprotein (LDL) cholesterol ≥ 130 mg/dl] carriers of a familial hypercholesterolemia (FH) mutation were identified in the Atherosclerosis Risk in Communities (ARIC) and Framingham Heart Study (FHS) Offspring cohorts and matched 1:1 to a FH mutation non-carriers according to age, gender, 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 t-test demonstrated higher cumulative exposure to LDL cholesterol in FH mutation carriers versus non-carriers.

Table 1. Prevalence of a Familial Hypercholesterolemia Mutation Among Participants with Severe Hypercholesterolemia (LDL Cholesterol ≥ 190 mg/dl)

	N with LDL Cholesterol ≥ 190 mg/dl (% of Cohort)	**N with FH Mutation (% of Individuals with LDL Cholesterol ≥ 190)**
Controls of the Myocardial Infarction Genetics (MIGen) Consortium		
Atherosclerosis, Thrombosis and Vascular Biology Italian Study (N = 1,050)	44 (4%)	1 (2.3%)
Exome Sequencing Project; Early-Onset Myocardial Infarction (N = 1,213)	160 (13%)	3 (1.9%)
Jackson Heart Study (N = 599)	26 (4%)	1 (3.8%)
Munich Myocardial Infarction Study (N = 272)	15 (6%)	0 (0%)
Ottowa Heart Study (N = 889)	59 (7%)	0 (0%)
Precocious Coronary Artery Disease (N = 870)	36 (4%)	1 (2.8%)
Pakistani Risk of Myocardial Infarction Study (N = 3,684)	90 (2%)	2 (2.2%)
Total (N = 8,577)	*430 (5%)*	*8 (1.9%)*
Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium		
Atherosclerosis Risk in Communities Study (N = 7,959)	657 (8%)	12 (1.8%)
Cardiovascular Health Study (n = 732)	47 (4%)	1 (2.1%)
Framingham Heart Study (N = 1,175)	38 (5%)	2 (5.3%)
Rotterdam Baseline Study (N = 794)	99 (12%)	0 (0%)
Erasmus Rucphen Family Study (N = 1,248)	115 (9%)	1 (0.9%)
Total (N = 11,908)	*956 (8%)*	*16 (1.7%)*
Combined MIGen Controls + CHARGE (N = 20,485)	**1386 (7%)**	**24 (1.7%)**

Table 2. Risk of Coronary Artery Disease in those with Elevated LDL cholesterol (≥190 mg/dl) According to Familial Hypercholesterolemia Mutation Status.

	N (N CAD-free Controls / N CAD Case)	OR for CAD (95%CI) P-value*	P-value (FH Mutation + vs. –)[y]	LDL Cholesterol-Adjusted OR for CAD (95%CI) P-value*	P-value (FH Mutation + vs. –)[y]
LDL Cholesterol ≥ 190 mg/dl					
Familial Hypercholesterolemia Mutation –	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
Familial Hypercholesterolemia Mutation +	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 Familial Hypercholesterolemia Mutation –	7,485 (5,175 / 2,310)	Reference		Reference	

Odds ratios (OR) for coronary artery disease (CAD) calculated via logistic regression with adjustment for gender, cohort, and principal components of ancestry relative to a reference category of LDL cholesterol <130 mg/dl without a familial hypercholesterolemia (FH) mutation. Odds ratio values with and without additional adjustment for observed LDL cholesterol, expressed as a continuous variable, are provided.

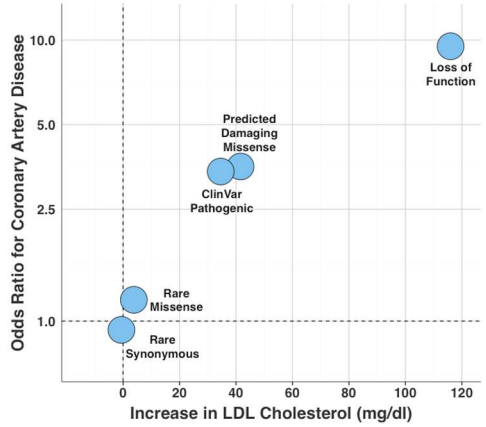
[*] P-value for difference in OR compared to reference category.

[y] P-value for difference in OR between FH Mutation + vs. FH Mutation – among participants with LDL cholesterol (≥190 mg/dl)

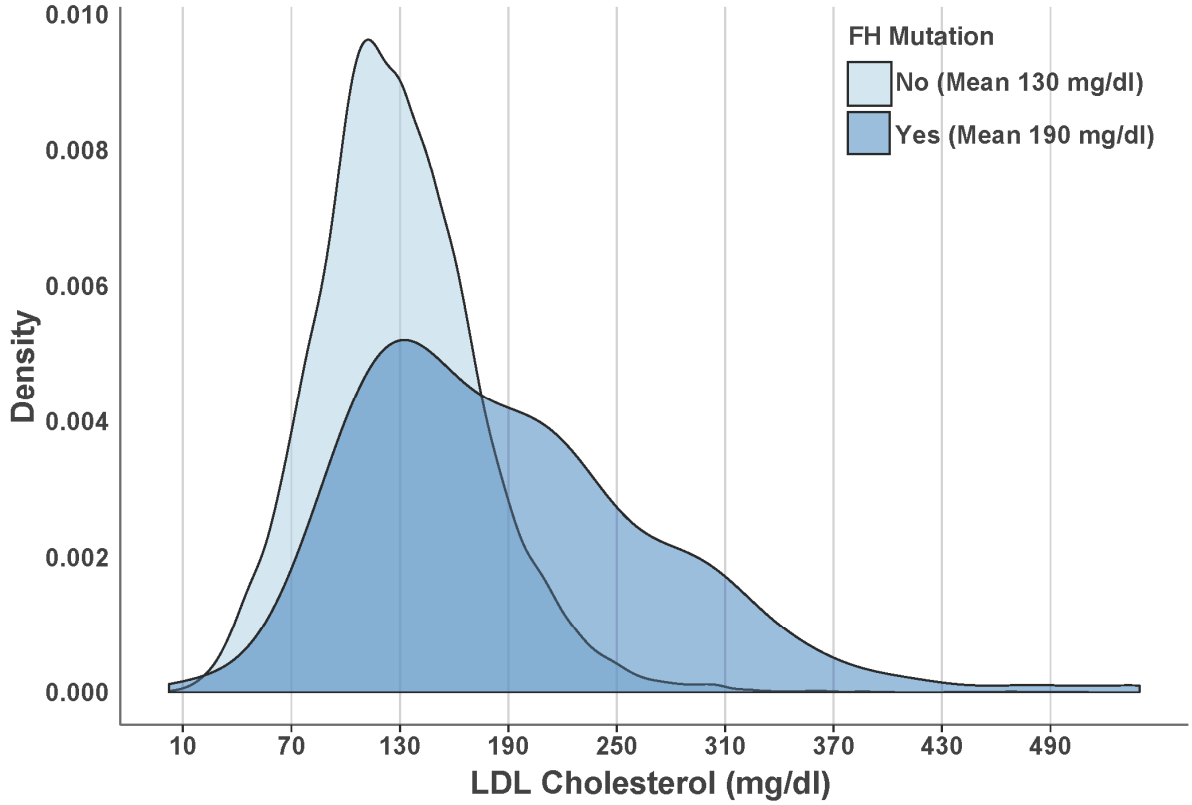
Table 3. Prevalence of Familial Hypercholesterolemia According to Different LDL Cholesterol Thresholds and Mutation Classification Schemes.

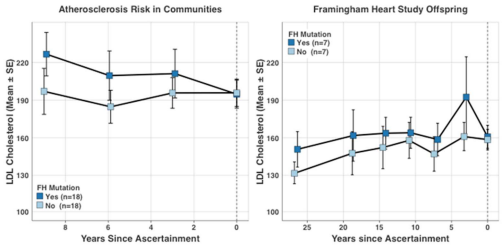
LDL Cholesterol Criteria	**Mutation Criterion**	**Prevalence of Familial Hypercholesterolemia**
LDL Cholesterol ≥ 190 mg/dl	No mutation required	1,386 / 20,485 (**1 in 14**)
No threshold requirement	**LDLR* loss of function variant; or **LDLR* predicted damaging rare missense variant; or **LDLR*, *APOB*, *PCSK9* variant pathogenic in ClinVar	97 / 20,485 (**1 in 211**)
LDL Cholesterol ≥ 190 mg/dl	**LDLR* loss of function variant; or *Any rare *LDLR* missense variant	80 / 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 / 20,485 (**1 in 301**)
No threshold requirement	**LDLR* loss of function variant; or **LDLR* predicted damaging rare *m*issense variant	60 / 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 / 20,485 (**1 in 853**)

For each classification scheme, we provide the number who meet the criteria out of a total 20,485 participants (CAD-free controls of the Myocardial Infarction Genetics Consortium combined with CHARGE Consortium participants). Loss of function variants defined as single base changes that introduce a stop codon leading to premature truncation of a protein (nonsense), insertions or deletions (indels) of DNA that scramble the protein translation beyond the variant site (frameshift), or point mutations at sites of pre-mRNA splicing that alter the splicing process (splice-site). Predicted damaging variants refer to those *LDLR* predicted to be deleterious by *each* of five *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.



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)





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)		

Appendix:

Diagnostic Yield of Sequencing Familial Hypercholesterolemia Genes in Severe Hypercholesterolemia

Amit V. Khera, MD*,[a,b] Hong-Hee Won, PhD*,[c] Gina M. Peloso, PhD*,[b,d] Kim S. Lawson, MS,[e] Traci M. Bartz, MS,[f] Xuan Deng, MSc,[d] Elisabeth M. van Leeuwen,[g] Pradeep Natarajan, MD, MMSc,[a,b] Connor A. Emdin, HBSc,[b] Alexander G. Bick, BS,[b] Alanna C. Morrison, PhD,[e] Jennifer A. Brody,[h] BA, Namrata Gupta, PhD,[b] Akihiro Nomura, MD,[b, i] Thorsten Kessler, MD,[j] Stefano Duga, PhD,[k] Joshua C. Bis, PhD, [h] Cornelia M. van Duijn, PhD,[g] L. Adrienne Cupples, PhD,[d] Bruce Psaty, MD, PhD,[h,l] Daniel J. Rader, MD,[m] John Danesh, FMedSci,[n] Heribert Schunkert,[j] MD, Ruth McPherson, MD,[o] Martin Farrall, FRCPath,[p] Hugh Watkins, MD,[p] PhD, Eric Lander, PhD,[b] James G. Wilson, MD,[q] Adolfo Correa, MD, PhD,[r] Eric Boerwinkle, PhD,[e] Piera Angelica Merlini, MD,[s] Diego Ardissino, MD,[t] Danish Saleheen, MB,BS,PhD,[u] Stacey Gabriel, PhD,[b] Sekar Kathiresan, MD[a,b]

Supplementary Methods.

Coronary artery disease case-control cohort

The coronary disease case-control exome sequencing was performed as previously described (1). Sequence data of all participants were aligned to a human reference genome (hg19) using the Burrows–Wheeler Aligner algorithm. Aligned non-duplicate reads were locally realigned and base qualities were recalibrated using the Genome Analysis ToolKit (GATK) software version 3.4 (2-4). Variants were jointly called using the GATK HaploTypeCaller and filtered using the Variant Quality Score Recalibration (VQSR), quality over depth metrics, and strand bias. The sensitivity of the selected VQSR threshold was 99.6% for single nucleotide polymorphisms and 95% for insertion/deletion variants as empirically assessed using hapmap controls with known genotypes included in the genotyping call set. Previous studies using similar approaches have estimated a false-positive genotyping error rate of 0.001% (5). We also excluded outlier samples with respect to relatedness with any other samples and number of variants, increased heterozygous to non-reference homozygous genotypes ratio, high missing genotypes, discordance between reported and genotypic gender, or a high discordant rate with

genotype array data when available. Population genetic substructure was assessed by calculation of principal components of ancestry using Eigenstrat 4.2 as previously described (6,7).

Population-based cohort studies

The prevalence of a familial hypercholesterolemia mutation in individuals with a LDL cholesterol > 190 mg/dl was additionally determined in 11,908 participants from five prospective cohort studie: Atherosclerosis Risk in Communities Study (ARIC), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Rotterdam Baseline Study (RS), and Erasmus Rucphen Family Study (ERF) (eTable 2). Whole exome sequencing for 9,866 individuals from ARIC, CHS, and FHS was performed using Illumina HiSeq instruments (San Diego, CA) after exome capture with VCRome 2.1 (NimbleGen Inc., Madison, WI) using chemistry recommended by the manufacturer at Baylor College of Medicine. Sequence alignment and variant calling were carried out via the Mercury pipeline in the DNAnexus platform. Whole exome sequencing for 2,042 individuals from RS and ERF was performed at Erasmus Medical Center, Rotterdam, The Netherlands using Illumina HiSeq instruments (San Diego, CA). Fasting LDL cholesterol in mg/dL was used from the earliest available exam in each contributing study. For participants known to be on lipid-lowering therapy, we estimated the untreated LDL cholesterol value. This approach has been demonstrated to perform well in accounting for treatment effects in studies of quantitative traits. Statins are the most widely used treatment to lower plasma lipids and a statin at average dose reduces total cholesterol by 20% and LDL cholesterol by 30%. Statins became routinely used after the publication of the seminal 4S randomized control trial in 1994. If the sample for LDL cholesterol was collected after 1994, we accounted for lipid-lowering medication in the following manner: the treated total cholesterol value was divided by 0.8. No adjustment was done on data collected before 1994 unless specific information on statin use was

available. LDL cholesterol was calculated using the Friedewald equation (LDL cholesterol = total cholesterol – high-density lipoprotein cholesterol – (triglycerides/5)) for those with triglycerides <400 mg/dl. If triglycerides were ≥400 mg/dl, calculated LDL cholesterol was set to missing.

Longitudinal Analysis of LDL Cholesterol Exposure

In order to determine whether the cumulative exposure to LDL cholesterol differed according to familial hypercholesterolemia mutation status, individuals with a familial hypercholesterolemia mutation and LDL cholesterol ≥ 130 mg/dl were identified in ARIC and FHS Offspring Study cohorts. ARIC is a prospective, community-based sample of 15,792 adults ages 45–64 years recruited from four US communities between 1987 and 1989 (8). Participants attended a baseline examination (visit 1) and follow-up examinations in 1990–1992 (visit 2), 1993–1995 (visit 3), and 1995–1998 (visit 4). Lipid levels from visits 1-4 were available for analysis. All ARIC phenotypic and sequence data was retrieved from NCBI dbGaP (accession: phs000090.v3.p1 and phs000572.v6.p4). The FHS Offspring Cohort consisted of 5,124 children of the original cohort and their spouses and has been examined every three to eight years. Lipid levels from exam 1 (1971-1975) to exam 7 (1998-2001) were available for analysis. All FHS phenotypic and sequence data used in the longitudinal analysis were retrieved from NCBI dbGaP (Accession: phs000007.v26.p10 and phs000572.v6.p4).

Exome sequencing data from 1091 FHS Offspring individuals and 5727 ARIC participants were downloaded from NCBI dbGaP. The sequences were generated from three independent sequencing efforts, the NHLBI Exome Sequencing Project, the Alzheimer's Disease Sequencing Project and the CHARGE consortium, as previously described (9,10). Sequences were mapped to the human genome assembly hg19 human reference with BWA and single-

nucleotide variants and small indel variants were jointly called in each cohort with GATK version 3.4 using the haplotype caller tool and subsequently filtered using GATK best practices.

Additionally, in the FHS Offspring Study, NCBI dbGaP data were downloaded for a set of 1,623 unrelated FHS Offspring Cohort individuals resequenced for 200 cardiovascular disease genes including APOB, LDLR and PCSK9, as previously described (11). Sequence reads were first aligned to human genome assembly hg19 with BWA, recalibrated with GATK and used for variant calling by the UnifiedGenotyper module. Samples from analysis with below 95% concordance with prior SNP array data were removed.

We excluded outlier samples with respect to relatedness with other samples, number of variants, increased heterozygous/non-reference homozygous ratio, high missing genotypes, discordance between reported and genotype-derived gender, or a high discordant rate with genotype array data when available. Individuals were included in the longitudinal analysis if missing a LDL cholesterol value at no more than one study visit. In those missing a single visit value, the last measured value was carried forward.

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Online Table 1. Coronary Artery Disease Definitions Across Cohorts

Cohort	Controls	Cases	CAD Definition	Control Definition	Reference
ATVB	1050	1248	MI in men or women ≤ 45y	No history of thromboembolic disease	12
EOMI	1213	189	MI (men ≤ 50y or women ≤ 60y)	Hospital-based, no report of MI by history	13
JHS	599	13	Combination of prevalent CHD (self-reported or electrocardiographic evidence of MI) and incident CHD (MI or coronary revascularization)	Free of CHD during follow-up	14
Munich-MI	272	341	MI in men ≤40y or women ≤55y	Controls without CAD, men ≥ 65y and women ≥ 75y	15
OHS	889	386	MI or CABG or angiographic disease (>50% stenosis) in men ≤ 50 y or women ≤ 60 y)	Asymptomatic	16
PROCARDIS	870	560	MI (men ≤ 50 y or women ≤ 60 y)	No history of CAD	17
PROMIS	3684	2803	MI, age 30-80y	Age and gender frequency-matched. No history of MI/CVD	18

ATVB: Atherosclerosis, Thrombosis and Vascular Biology Italian Study; EOMI: NHLBI Exome Sequencing Project Early-Onset Myocardial Infarction; JHS: Jackson Heart Study; OHS: Ottawa Heart Study; PROCARDIS: Precocious coronary artery disease; PROMIS: Pakistan Risk of Myocardial Infarction Study

MI: myocardial infarction; CAD: coronary artery disease; CABG: coronary artery bypass; y: years of age

Online Table 2. Cohort Descriptions for the Prospective Cohort Studies

Cohort	Descriptions	N	N (%) with LDL Cholesterol > 190 mg/dl
Atherosclerosis Risk in Communities Study (ARIC)	The ARIC study has been described in detail previously.[8] Men and women aged 45-64 years at baseline were recruited from four communities: Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland. A total of 15,792 individuals, predominantly White and African American, participated in the baseline examination in 1987-1989, with three triennial follow-up examinations. 2671 African-American and 5604 European-American individuals with LDL cholesterol were exome sequenced at Baylor University.	2486 (AA)	229 (9%)
		5473 (EA)	428 (8%)

Cardiovascular Health Study (CHS)	The CHS has been described in detail previously.[19] The CHS is a population-based cohort study of risk factors for coronary heart disease and stroke in adults ≥65 years conducted across four field centers. The original cohort of 5201 persons was recruited in 1989-1990 (84% Caucasian) from random samples of the Medicare eligibility lists. DNA was extracted from blood available blood samples drawn on participants at their baseline examination. 732 European-American individuals with LDL cholesterol were exome sequenced at Baylor University. CHS was approved by institutional review commitees at each field center and the coordinating center. Participants gave informed consent including consent to use of genetic information for the study of cardiovascular disease.	732	38 (5%)
Framingham Heart Study (FHS)	The FHS is a three generational prospective cohort that has been described in detail previously.[20] Individuals were initially recruited in 1948 in Framingham, USA to evaluate cardiovascular disease risk factors. The second generation cohort (5124 offspring of the original cohort) was recruited between 1971 and 1975. The third generation cohort (4095 grandchildren of the original cohort) was collected between 2002 and 2005. Fasting lipid levels were measured at exam 1 of the Offspring (1971-1975) and third generation (2002-2005) cohorts, using standard LRC protocols.	1175	47 (4%)
Rotterdam Baseline Study (RS)	The Rotterdam Study is an ongoing prospective population-based cohort study, focused on chronic disabling conditions of the elderly. The study comprises an outbred ethnically homogenous population of Dutch Caucasian origin. The rationale of the study has been described in detail elsewhere.[21] In summary, 7983 men and women aged 55 years or older, living in Ommoord, a suburb of Rotterdam, the Netherlands, were invited to participate. 794 European individuals with LDL cholesterol were exome	794	99 (12%)

	sequenced.		
Erasmus Rucphen Family (ERF) Study	The ERF study has been described in detail previously.[22] A total of approximately 3000 participants descend from 22 couples who lived in the Rucphen region in The Netherlands in the 19th century. 1248 European individuals with LDL cholesterol were exome sequenced.	1248	115 (9%)

SD: standard deviation. SI conversion factor: To convert cholesterol to mmol/L, multiply values by 0.0259. To convert triglyceride levels to mmol/l, multiple values by 0.01129.

Online Table 3. Baseline Characteristics According to CAD Case-Control Status within the Myocardial Infarction Genetics Consortium Studies

	CAD Control (N = 8,577)	**CAD Case (N = 5,540)**
Age	58 (14)	45 (8)
Male Gender	5,871 (68%)	4,550 (82%)
Race		
White	3,908 (46%)	2,672 (48%)
Black	985 (11%)	65 (1%)
South Asian	3,684 (43%)	2,803 (51%)
Hypertension	2,940 (44%)	1,928 (52%)
Diabetes Mellitus	1,715 (23%)	1,655 (32%)
Current Smoking	1,961 (23%)	2,821 (52%)
Total Cholesterol, mg/dl	198 (47)	221 (56)
LDL-Cholesterol, mg/dl	121 (41)	140 (52)
HDL-Cholesterol, mg/dl	44 (15)	39 (13)
Triglycerides, mg/dl	138 (95 – 202)	165 (116 – 242)
Lipid-lowering Medication	342 (4%)	1,502 (27%)

Values represent n (% of individuals with nonmissing data), mean (SD), or median (IQR). CAD – coronary artery disease. SI conversion factor: To convert cholesterol to mmol/L, multiply values by 0.0259. To convert triglyceride levels to mmol/l, multiple values by 0.01129.

Online Table 4. Variant Characteristics and Frequency in Coronary Artery Disease Controls vs. Cases

CHR:POS_REF/ALT	GENE	Consequence	Amino Acid Change	Loss of Function	Predicted Damaging	ClinVar Pathogenic	N Controls	N Cases
1:55518037_G/A	PCSK9	Missense	Asp204Asn	--	--	Yes	0	1
2:21229068_G/A	APOB	Missense	Arg3558Cys	--	--	Yes	8	8
2:21229160_C/T	APOB	Missense	Arg3527Gln	--	--	Yes	2	4
19:11210912_C/G	LDLR	Missense	Cys27Trp	--	Yes	--	0	1
19:11210970_G/A	LDLR	Missense	Asp47Asn	--	Yes	--	1	1
19:11210928_C/T	LDLR	Premature Stop	Gln33Ter	Yes	--	--	0	1
19:11210974_G/A	LDLR	Missense	Gly48Asp	--	Yes	--	1	0
19:11211016_C/T	LDLR	Missense	Thr62Met	--	Yes	--	4	3
19:11213418_A/G	LDLR	Missense	Asp90Gly	--	Yes	--	0	1
19:11213450_G/A	LDLR	Missense	Glu101Lys	--	Yes	Yes	0	1
19:11213453_C/T	LDLR	Premature Stop	Gln102Ter	Yes	--	--	0	1
19:11213463_G/A	LDLR	Splice Donor		Yes	--	--	0	4
19:11213463_G/T	LDLR	Splice Donor		Yes	--	--	0	1
19:11215908_G/A	LDLR	Missense	Cys109Tyr	--	Yes	--	0	1
19:11215937_G/A	LDLR	Missense	Gly119Arg	--	Yes	--	0	1
19:11215974_A/G	LDLR	Missense	Asp131Gly	--	Yes	--	0	1
19:11215991_G/A	LDLR	Missense	Gly137Ser	--	Yes	--	1	0
19:11215992_G/T	LDLR	Missense	Gly137Val	--	Yes	--	0	1
19:11215995_C/G	LDLR	Premature Stop	Ser138Ter	Yes	--	--	0	2
19:11216000_G/T	LDLR	Premature Stop	Glu140Ter	Yes	--	--	0	2
19:11216011_C/A	LDLR	Premature Stop	Cys143Ter	Yes	--	--	1	0
19:11216090_G/A	LDLR	Missense	Asp170Asn	--	Yes	--	1	0
19:11216102_G/T	LDLR	Premature Stop	Glu174Ter	Yes	--	--	0	1

19:11216112_C/T	LDLR	Missense	Ser177Leu	--	Yes	Yes	0	2
19:11216242_C/CTG	LDLR	Frameshift	Asp221TrpfsTer45	Yes	--	--	1	0
19:11216244_A/G	LDLR	Missense	Asp221Gly	--	Yes	--	0	8
19:11216264_G/T	LDLR	Premature Stop	Glu228Ter	Yes	--	Yes	0	1
19:11217344_T/A	LDLR	Missense	Asp266Glu	--	Yes	--	1	1
19:11218077_G/C	LDLR	Missense	Cys276Ser	--	Yes	--	0	1
19:11218096_C/A	LDLR	Missense	Phe282Leu	--	Yes	--	0	1
19:11218136_T/TA	LDLR	Premature Stop	Cys296Ter	Yes	--	--	0	1
19:11218148_A/G	LDLR	Missense	Arg300Gly	--	Yes	--	0	1
19:11221334_A/G	LDLR	Missense	Asn316Ser	--	Yes	--	1	1
19:11221354_G/A	LDLR	Missense	Gly323Ser	--	Yes	--	1	0
19:11221366_C/T	LDLR	Missense	His327Tyr	--	Yes	--	2	5
19:11221390_G/A	LDLR	Missense	Gly335Ser	--	Yes	--	1	1
19:11221391_G/A	LDLR	Missense	Gly335Asp	--	Yes	--	1	0
19:11221406_G/T	LDLR	Missense	Cys340Phe	--	Yes	--	0	2
19:11221435_C/T	LDLR	Premature Stop	Arg350Ter	Yes	--	--	0	1
19:11221449_T/G	LDLR	Splice Donor		Yes	--	--	0	1
19:11222190_A/G	LDLR	Missense	Asp354Gly	--	Yes	--	0	1
19:11222295_C/T	LDLR	Missense	Thr389Met	--	Yes	--	0	1
19:11222305_C/A	LDLR	Premature Stop	Cys392Ter	Yes	--	--	0	1
19:11223962_G/A	LDLR	Missense	Ala399Thr	--	Yes	--	0	1
19:11223983_C/T	LDLR	Missense	Arg406Trp	--	Yes	--	1	0
19:11224005_C/T	LDLR	Missense	Thr413Met	--	Yes	--	0	1
19:11224013_C/T	LDLR	Missense	Arg416Trp	--	Yes	--	0	1
19:11224019_G/A	LDLR	Missense	Glu418Lys	--	Yes	--	0	1
19:11224024_C/G	LDLR	Premature Stop	Tyr419Ter	Yes	--	--	0	1
19:11224052_G/A	LDLR	Missense	Val429Met	--	Yes	Yes	0	1

19:11224061_C/G	LDLR	Missense	Leu432Val	--	Yes	--	0	3
19:11224066_C/G	LDLR	Missense	Asp433Glu	--	Yes	--	1	0
19:11224126_G/A	LDLR	Splice Donor		Yes	--	--	0	1
19:11224296_G/A	LDLR	Missense	Asp482Asn	--	Yes	--	0	2
19:11224326_G/A	LDLR	Missense	Asp492Asn	--	Yes	--	0	1
19:11224354_C/T	LDLR	Missense	Ala501Val	--	Yes	--	0	1
19:11224419_G/A	LDLR	Missense	Val523Met	--	Yes	Yes	0	1
19:11224422_G/A	LDLR	Missense	Val524Met	--	Yes	--	0	1
19:11224428_C/T	LDLR	Missense	Pro526Ser	--	Yes	--	1	0
19:11224437_G/C	LDLR	Missense	Gly529Arg	--	Yes	--	0	2
19:11226781_G/A	LDLR	Premature Stop	Trp533Ter	Yes	--	--	0	1
19:11226801_G/A	LDLR	Missense	Ala540Thr	--	Yes	--	1	1
19:11226829_G/A	LDLR	Missense	Gly549Asp	--	--	Yes	0	4
19:11227549_C/T	LDLR	Missense	Arg574Cys	--	Yes	--	0	1
19:11227576_C/G	LDLR	Missense	His583Asp	--	Yes	--	0	1
19:11227576_C/T	LDLR	Missense	His583Tyr	--	Yes	--	1	1
19:11227590_C/G	LDLR	Missense	Ser587Arg	--	Yes	--	0	1
19:11227603_G/A	LDLR	Missense	Gly592Arg	--	Yes	--	1	0
19:11227604_G/A	LDLR	Missense	Gly592Glu	--	Yes	Yes	0	2
19:11227613_G/A	LDLR	Missense	Arg595Gln	--	Yes	--	0	1
19:11227645_G/T	LDLR	Missense	Ala606Ser	--	--	Yes	4	2
19:11227676_T/C	LDLR	Splice Donor		Yes	--	--	0	1
19:11230888_C/A	LDLR	Missense	His656Asn	--	Yes	--	1	0
19:11231057_T/C	LDLR	Missense	Cys667Arg	--	Yes	--	0	1
19:11231084_G/A	LDLR	Missense	Gly676Ser	--	Yes	--	1	0
19:11231087_T/C	LDLR	Missense	Cys677Arg	--	Yes	--	0	1
19:11231112_C/T	LDLR	Missense	Pro685Leu	--	Yes	Yes	1	3
19:11231118_T/TC	LDLR	Frameshift	Asn688GlnfsTer29	Yes	--	--	1	0
19:11231154_C/T	LDLR	Missense	Pro699Leu	--	Yes	--	0	1
19:11231159_G/A	LDLR	Missense	Gly701Ser	--	Yes	--	2	2
19:11231198_G/T	LDLR	Premature	Glu714Ter	Yes	--	--	0	1

		Stop						
19:11234017_C/T	LDLR	Premature Stop	Gln770Ter	Yes	--	--	0	1
19:11238683_G/T	LDLR	Splice Acceptor		Yes	--	--	0	1
19:11238706_AG/A	LDLR	Frameshift	Gly779GlufsTer9	Yes	--	--	0	1
19:11240210_T/TG	LDLR	Frameshift	Val806GlyfsTer11	Yes	--	--	1	2
19:11240239_C/T	LDLR	Missense	Arg814Trp	--	Yes	--	0	1
19:11240278_G/A	LDLR	Missense	Val827Ile	--	Yes	--	4	1

CHR: Chromosome; POS: Chromosomal positions based on the hg19 build of the human reference genome; REF: Reference allele; ALT: Alternate allele.

Online Table 5. Baseline Characteristics According to Familial Hypercholesterolemia Mutation Status within the Myocardial Infarction Genetics Consortium Studies

	Familial Hypercholesterolemia Mutation Negative (N = 13,954)	**Familial Hypercholesterolemia Mutation Positive (N = 164)**
Age	53 (13)	46 (12)
Male Gender	10,291 (74%)	130 (79%)
Race		
White	6,462 (46%)	118 (72%)
Black	1,044 (7%)	6 (4%)
South Asian	6,447 (46%)	40 (24%)
Hypertension	4,832 (47%)	36 (46%)
Diabetes Mellitus	3,351 (27%)	19 (13%)
Current Smoking	4,722 (34%)	60 (37%)
Total Cholesterol, mg/dl	206 (51)	263 (84)
LDL-Cholesterol, mg/dl	130 (46)	190 (84)
HDL-Cholesterol, mg/dl	42 (15)	41 (14)
Triglycerides, mg/dl	150 (102 – 218)	137 (102 – 212)
Lipid-lowering Medication	1,812 (14%)	37 (20%)

Values represent n (% of individuals with nonmissing data), mean (SD), or median (IQR). SI conversion factor: To convert cholesterol to mmol/L, multiply values by 0.0259. To convert triglyceride levels to mmol/l, multiple values by 0.01129.

Online Table 6. Coronary artery disease status by category of observed LDL cholesterol and FH mutation status within Myocardial Infarction Genetics Consortium Studies

LDL Cholesterol Category	FH Mutation Status	N	N CAD-free Controls	N CAD Cases
< 130 mg/dl	Mutation +	7,485	5,175	2,310
< 130 mg/dl	Mutation –	44	22	22
≥ 130 – 160 mg/dl	Mutation +	3,325	1,978	1,347
≥ 130 – 160 mg/dl	Mutation –	28	12	16
≥ 160 – 190 mg/dl	Mutation +	1,879	954	925
≥ 160 – 190 mg/dl	Mutation –	19	6	13
≥ 190 – 220 mg/dl	Mutation +	784	288	496
≥ 190 – 220 mg/dl	Mutation –	22	3	19
≥ 220 mg/dl	Mutation +	51	5	46
≥ 220 mg/dl	Mutation –	480	134	346

Online Table 7. Matching characteristics of average LDL cholesterol exposure analysis. Values, mean (SD) or N (%), refer to characteristics at time of most recent study visit.

Atherosclerosis Risk in Communities	**Familial Hypercholesterolemia Mutation Carriers (n = 18)**	**Familial Hypercholesterolemia Mutation Noncarriers (n = 18)**
Age, years	63 (5)	64 (6)
Male Gender	9 (50%)	9 (50%)
Statin use	9 (50%)	9 (50%)
LDL Cholesterol	195 (11)	196 (11)
Framingham Offspring Study	**Familial Hypercholesterolemia Mutation Carriers (n = 7)**	**Familial Hypercholesterolemia Mutation Noncarriers (n = 7)**
Age, years	66 (8)	67 (9)
Male Gender	2 (29%)	2 (29%)
Statin use	2 (29%)	2 (29%)
LDL Cholesterol	161 (9)	158 (8)

Online Table 8. Sensitivity Analysis Including Only Those Not on Lipid-lowering Therapy (N = 11,739 MIGen Participants): Risk of Coronary Artery Disease in those with Elevated LDL cholesterol (≥190 mg/dl) According to Familial Hypercholesterolemia Mutation Status.

	N (N CAD-free Controls / N CAD Case)	**OR for CAD (95%CI) P-value***	**P-value (FH Mutation + vs. –)[y]**	**LDL Cholesterol-Adjusted OR for CAD (95%CI) P-value***	**P-value (FH Mutation + vs. –) [y]**
LDL Cholesterol ≥ 190 mg/dl					
Familial Hypercholesterolemia Mutation –	731 (342 / 389)	3.1 2.6 – 3.7)	< 0.001	1.4 (1.1 – 1.9) P < 0.001	0.001
Familial Hypercholesterolemia Mutation +	55 (5 / 50)	23.5 (9.6 – 72.5)		7.8 (3.0 – 24.6) P < 0.001	
LDL Cholesterol < 130 mg/dl and Familial Hypercholesterolemia Mutation –	6,698 (4,773 / 1,925)	Reference		Reference	

Odds ratios (OR) for coronary artery disease (CAD) calculated via logistic regression with adjustment for gender, cohort, and principal components of ancestry relative to a reference category of LDL cholesterol <130 mg/dl without a familial hypercholesterolemia (FH) mutation. Odds ratio values with and without additional adjustment for observed LDL cholesterol, expressed as a continuous variable, are provided.

* P-value for difference in OR compared to reference category.

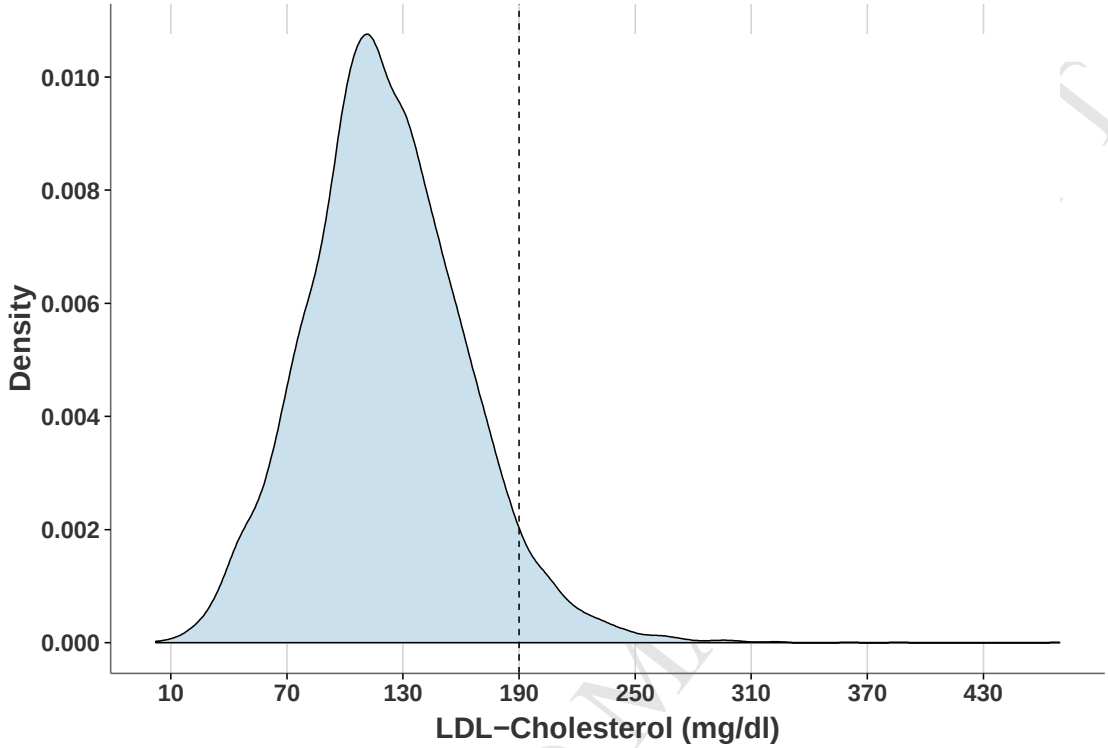
y P-value for difference in OR between FH Mutation + vs. FH Mutation – among participants with LDL cholesterol (≥190 mg/dl)

Online Figure Titles and Legends

Online Figure 1. LDL Cholesterol Values in MIGen Control Participants (n=8,577)

Online Figure 2. Frequency of Familial Hypercholesterolemia Mutations According to LDL Cholesterol Level and Coronary Artery Disease Status within MIGen Consortium Studies.

Online Figure 1.



Online Figure 2.