Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Large-Scale Screening for Monogenic and Clinically Defined Familial Hypercholesterolemia in Iceland

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OBJECTIVE: Familial hypercholesterolemia (FH) is traditionally defined as a monogenic disease characterized by severely elevated LDL-C (low-density lipoprotein cholesterol) levels. In practice, FH is commonly a clinical diagnosis without confirmation of a causative mutation. In this study, we sought to characterize and compare monogenic and clinically defined FH in a large sample of Icelanders.

APPROACH AND RESULTS: We whole-genome sequenced 49 962 Icelanders and imputed the identified variants into an overall sample of 166 281 chip-genotyped Icelanders. We identified 20 FH mutations in *LDLR*, *APOB*, and *PCSK9* with combined prevalence of 1 in 836. Monogenic FH was associated with severely elevated LDL-C levels and increased risk of premature coronary disease, aortic valve stenosis, and high burden of coronary atherosclerosis. We used a modified version of the Dutch Lipid Clinic Network criteria to screen for the clinical FH phenotype among living adult participants (N=79 058). Clinical FH was found in 2.2% of participants, of whom only 5.2% had monogenic FH. Mutation-negative clinical FH has a strong polygenic basis. Both individuals with monogenic FH and individuals with mutation-negative clinical FH were markedly undertreated with cholesterol-lowering medications and only a minority attained an LDL-C target of <2.6 mmol/L (<100 mg/dL; 11.0% and 24.9%, respectively) or <1.8 mmol/L (<70 mg/dL; 0.0% and 5.2%, respectively), as recommended for primary prevention by European Society of Cardiology/European Atherosclerosis Society cholesterol guidelines.

CONCLUSIONS: Clinically defined FH is a relatively common phenotype that is explained by monogenic FH in only a minority of cases. Both monogenic and clinical FH confer high cardiovascular risk but are markedly undertreated.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: genetic screening ■ genetics ■ hypercholesterolemia ■ lipids ■ mutation

Familial hypercholesterolemia (FH) is a genetic disorder characterized by markedly elevated levels of LDL-C (low-density lipoprotein cholesterol), leading to premature cardiovascular disease and death.[1] Despite advances in genetic diagnostics and the availability of effective cholesterol-lowering treatment, FH remains underdiagnosed and undertreated in most countries.[2]

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FH is classically defined as an autosomal dominant, monogenic disease caused by highly penetrant mutations in the genes encoding the LDL receptor (*LDLR*), apolipoprotein B (*APOB*), or proprotein convertase

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Nonstandard Abbreviations and Acronyms

CAD	coronary artery disease
DLCN	Dutch Lipid Clinic Network
FH	familial hypercholesterolemia
HDL-C	high-density lipoprotein cholesterol
ICD	*International Classification of Diseases*
LDL-C	low-density lipoprotein cholesterol
PCSK9	proprotein convertase subtilisin/kexin type 9
WGS	whole-genome sequencing

Highlights

- Monogenic familial hypercholesterolemia (FH) was found in 199 of 166 281 genotyped Icelanders, a prevalence of 1 in 836.
- Monogenic FH associated with high lifetime cumulative exposure to LDL-C (low-density lipoprotein cholesterol), increased risk of coronary disease and aortic valve stenosis, but not ischemic stroke.
- Clinically defined FH (using the Dutch Lipid Clinic Network criteria) was observed in 2.2% of adults with available cholesterol measurements. Only small minority (5.2%) had monogenic FH.
- Both monogenic FH and clinically defined FH were severely undertreated with cholesterol-lowering medications.

subtilisin/kexin type 9 (*PCSK9*).[3] The prevalence of monogenic FH has been traditionally estimated to be 1 in 500 but recent genetic studies in European and North American populations indicate that the prevalence may be >1 in 250.[4–9] Such estimates, however, depend on the criteria used for defining FH mutations and may differ between populations.

In practice, FH is most commonly diagnosed on the basis of clinical presentation and genetic testing is rarely performed to confirm the diagnosis.[10] Among individuals who undergo genetic testing for FH in tertiary lipid clinics, only 40%–50% are found to have a monogenic cause.[11–13] A substantial fraction of those with a clinical diagnosis of FH but no demonstrable FH mutation may have a polygenic basis for hypercholesterolemia, but environmental and lifestyle factors also play a role.[11–13] Thus, in general, the term FH encompasses 2 partially overlapping entities: classical monogenic FH and the more complex FH clinical phenotype. The use of genetic testing to identify individuals with monogenic FH has important implications for clinical decisions involving family screening, genetic counseling, risk stratification, and therapeutic choices.[10]

In this study, we investigated the prevalence and characteristics of monogenic FH and clinically defined FH in Iceland. First, we examined the prevalence and impact of monogenic FH in over 160 000 genotyped Icelanders. We then determined the prevalence of clinical FH and estimated the contribution of monogenic FH and polygenic burden toward clinical FH, using a subsample of over 79 000 participants. Finally, we assessed the contemporary use and effectiveness of cholesterol-lowering treatment in individuals with monogenic FH and clinical FH.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

This study is based on a genotyped sample of 166 281 Icelandic participants. This sample comprises voluntary participants of various genetic research projects at deCODE genetics, Reykjavík, Iceland, and this study population has been described in detail previously.[14] All analyses presented in this study were conducted in the entire sample or relevant subsamples. All participants donated samples for genotyping and provided informed consents. The study was approved by the National Bioethics Committee of Iceland (VSNb2015080003-03.01 and VSNb2015010033-03.12 with amendments). Personal identities of the participants were encrypted with a third-party system, provided by the Data Protection Authority of Iceland. Genotype information was not disclosed to the study participants.

Laboratory Measurements

Measurements of total cholesterol, HDL-C (high-density lipoprotein cholesterol) and triglycerides, taken between 1990 and 2019, were obtained from Landspítali - The National University Hospital (LUH) in Reykjavík, the largest and only tertiary referral hospital in Iceland; the Laboratory in Mjódd, Reykjavík; Akureyri Hospital, a Regional Hospital in North Iceland, and from the deCODE genetics laboratory. Measurements were taken either in a fasting or nonfasting state. Levels of LDL-C were calculated using the Friedewald[15] equation for triglyceride levels <4.00 mmol/L. Lipoprotein(a) was measured at the laboratory at deCODE genetics using a Tina-quant Lipoprotein(a) Gen.2 (Roche Diagnostics) immunoturbidimetric assay.

Atherosclerotic Diseases

Cases were defined as described below. Unless otherwise noted, diagnostic codes and information obtained from clinical registries were not validated.

Coronary Artery Disease

Coronary artery disease (CAD) was defined as previously described,[16] primarily on the basis of *International Classification of Diseases* (*ICD*) codes indicative of CAD (including myocardial infarction). Cases with CAD were identified based on discharge diagnoses from LUH (*ICD-9* codes 410.*, 411.*, 412.*, and 414.* or *ICD-10* codes I20.0, I21.*, I22.*, I23.*, I24.*, and I25.*), documentation of obstructive CAD in nationwide coronary angiography registries at LUH[17] and relevant surgical procedure codes

from LUH. CAD case status was also assigned based on the same *ICD* codes for CAD listed as the cause or contributing cause of death, in the Icelandic death registry. Early-onset CAD was defined as CAD occurring before age 50 years for men and 60 years for females.

Coronary Revascularization

All procedures were performed at LUH, the only center for interventional cardiology and cardiothoracic surgery in Iceland. Individuals who underwent percutaneous coronary intervention (years 1985–2017) were identified using nationwide coronary angiography registries[17] and relevant procedure codes, and those who underwent coronary artery bypass surgery (years 1987–2017) were identified through relevant surgical procedure codes.

Peripheral Artery Disease

Cases were identified based on discharge diagnoses (*ICD-10*: I70.2, I70.9, and I73.9) and relevant surgical procedure codes at LUH between years 1998 and 2016. A subset of cases (ascertained during years 1998–2006) was clinically validated by a vascular surgeon, as previously described.[18]

Ischemic Stroke

Cases were identified from either a registry of individuals with a validated diagnosis of ischemic stroke or transient ischemic attack at LUH during the years 1993 to 2013, as described previously,[19] or relevant discharge diagnoses at LUH between years 2014 and 2016 (*ICD-10* codes: I63 and G45).

Aortic Valve Stenosis

Cases were identified based on relevant discharge diagnoses (*ICD-10* codes I35.0 or I35.2) or the relevant NOMESCO classification of surgical procedure codes (FMA, FMD, and subcodes) at LUH, between years 1983 and 2016, as previously described.[20]

Extent of Coronary Atherosclerosis

Coronary Angiography

Individuals were identified in the Swedish Coronary Angiography and Angioplasty Registry, which holds data on all consecutive individuals undergoing coronary angiography and percutaneous coronary intervention in Iceland from January 1, 2007.[17,21] Here, we used data through December 31, 2017. Obstructive CAD was defined as having ≥50% diameter stenosis in one or more epicardial coronary artery, including the left main stem. Multivessel disease was defined as having ≥50% diameter stenosis in at least 2 epicardial coronary arteries or left main disease.

Coronary Artery Calcium

Individuals underwent coronary artery calcium (CAC) scanning for any indication at Röntgen Domus, the largest privately operated medical imaging clinic in Iceland. Imaging was performed between January 4, 2009, and October 31, 2017.[17] CAC was assessed using cardiac-gated multidetector computed tomography scanners (Aquilion, Toshiba Medical Systems) with a slice thickness of 0.5 to 3 mm. Scans were read by radiologists and CAC was quantified using a CAC score (Agatston score[22]).

Genotyping and Whole-Genome Sequencing

The methods used for whole-genome sequencing (WGS), calling of single-nucleotide polymorphisms and small insertions/deletions (up to a length of 60 bp), long-range phasing and imputation were as described previously.[14,23,24] Briefly, a total of 166 281 Icelanders were genotyped using various Illumina single-nucleotide polymorphism chips and their genotypes phased using long-range phasing. A subsample of 49 962 underwent WGS (median depth, 39×), and the identified DNA sequence variants were imputed into the overall sample. Individuals were chosen for WGS based on various conditions, including extremes of cholesterol levels.[25] Consequently, the WGS subsample is enriched for individuals with high LDL-C as well as various cardiovascular phenotypes (Table I in the Data Supplement). We searched for copy-number variants (eg, deletions) in *LDLR* using several methods based on WGS data (PopDel,[26] DELLY,[27] Graphtyper,[28] and Manta[29]), single-nucleotide polymorphism genotypes (PennCNV[30]) and long-read sequences of 3622 Icelanders.[31]

Genotype imputation was performed as previously described,[14,23] as outlined in the Data Supplement. We used Sanger sequencing to validate the genotypes of all predicted carriers based on imputation, in addition to confirming the genotypes of carriers who had undergone WGS. Furthermore, we used the comprehensive Icelandic genealogical database[32] to direct extensive Sanger sequencing among relatives of carriers, to validate their imputed genotypes and search for additional carriers. The directly assessed genotypes were then used as a training set for reimputation of the variants. The majority of FH mutations (13/20) had imputation information of at least 0.89, reflecting accurate imputation (Table II in the Data Supplement). We were unable to impute 5 mutations (4 singletons and 1 with 2 carriers) as the genotypes could not be placed onto haplotypes with high confidence and thus were imputed to a 0% frequency.

Definition of FH Mutations

Mutations were considered to potentially cause FH if they met one of the following criteria:

1. Predicted loss-of-function mutations in *LDLR*. All predicted loss-of-function mutations in *LDLR* were considered to be FH mutations, that is, nonsense mutations (premature stop-codon), essential splice variants (donor or acceptor), insertion/deletion (indels) that cause frameshift or larger copy-number variants (eg, deletions) involving exons.
2. Reported FH mutations in ClinVar. We retrieved data from ClinVar for variants in *LDLR*, *APOB,* and *PCSK9* (http: https://www.ncbi.nlm.nih.gov/clinvar/, accessed November 11, 2019). Variants were considered if they were annotated as either Pathogenic or Likely pathogenic. Variants with Conflicting interpretations of pathogenicity were considered if at least half of submissions annotated the variant as Pathogenic or Likely pathogenic.
3. *LDLR* missense mutations at the same position as pathogenic mutations. We considered rare *LDLR* missense variants that cause an amino acid change at the same position as a mutation designated as Pathogenic or Likely pathogenic in ClinVar.

Mutations meeting the above criteria were manually curated and excluded if the allele frequency in our data was inconsistent with FH (eg, >0.1%) or if the phenotypes of the carriers were grossly inconsistent with FH (eg, low or normal levels of LDL-C if not on lipid-lowering medications). The selection process is outlined in Figure I in the Data Supplement.

Search for Additional FH Mutations

We searched for other, potential FH mutations by assessing rare sequence variants (allele frequency below 0.1%) in *LDLR*, *APOB*, *PCSK9*, *APOE*, *LDLRAP1*, *ABCG5*, and *ABCG8*. Of the identified variants, none associated with a large increase in LDL-C levels (ie, at least 1 mmol/L at $P<0.05$, under additive and recessive models) among 104 828 genotyped Icelanders. In brief, we did not identify additional mutations in these genes that are likely to cause FH in Iceland.

Drug Prescription Data

Prescriptions of cholesterol-lowering medications (ATC code C10) were obtained from a nationwide registry maintained by the Directorate of Health that contains all issued drug prescriptions in Iceland between January 1, 2003, and December 31, 2018. Statin potency was assigned as described in the 2013 American College of Cardiology/American Heart Association cholesterol guidelines[33] (Table III in the Data Supplement).

Definition of Clinical FH

We used a modified version of the Dutch Lipid Clinic Network (DLCN) criteria that exclude physical examination findings and genetic information. In brief, each individual is assigned a score based on family history of hypercholesterolemia or premature cardiovascular disease (maximum 2 points), personal history of premature cardiovascular disease (maximum 2 points), and the maximum documented LDL-C (maxLDL-C) levels for the individual (maximum 8 points; see Table IV in the Data Supplement for details). Family history variables were created using the Icelandic genealogical database[32] (to identify first-degree relatives) coupled with relevant clinical data. Clinical FH was defined as probable FH (score 6–8) or definite FH (score >8). These criteria were applied to genotyped participants that were alive and between the ages of 20 and 80 years, with at least one available LDL-C measurement. Participants with no available LDL-C measurement were excluded.

Polygenic Contribution in Mutation-Negative Clinical FH

We estimated the polygenic contribution in mutation-negative clinical FH using a genetic score for LDL-C. We used a weighted genetic score based on the effects of 345 lipid-associated variants on LDL-C levels in an exome-wide association study of >300 000 individuals,[34] as previously described.[17] In a sample of 98 497 genotyped Icelanders with available information, the genetic score explained 12.3% of the variance (R^2) in maxLDL-C and associated with an increase by 1.04 mmol/L per 1-unit increase in the genetic score ($P<10^{-300}$). A 1-unit increase in the genetic score approximates an increase by one SD in LDL-C levels, based on the aggregate effects of the individual variants.

Statistical Analyses

A generalized form of linear or logistic regression that accounts for the relatedness between individuals and potential population stratification was used to test for associations with quantitative traits and diseases. For association analyses, levels of LDL-C were adjusted for statin use: for individuals who were prescribed statins within one year before measurement, total cholesterol was divided by 0.8 (Liu et al[34]), and the modified value was used for calculation of LDL-C. Measurements taken before January 1, 2003, and after December 31, 2018 (24% of all measurements) were not adjusted for statin use due to unavailable prescription data. Unadjusted values were used in analyses involving cumulative LDL-C exposure and LDL-C target attainment. For lipid traits, residuals were obtained after adjustment for age, age^2, year of birth, sex, measurement site, and county of birth. The adjusted residuals were used as outcome variables in association analyses for lipid traits. For associations with maxLDL-C, raw, non-normalized adjusted residuals were used to better retain information from outliers (ie, individuals with very high maxLDL-C). For lipid traits other than maxLDL-C, the mean values of adjusted residuals (for each individual) were transformed to a normal distribution with a mean of 0 and a SD of 1. Unless otherwise specified, controls in logistic regression analyses comprise noncases for a given phenotype in the overall genotyped population. Data were analyzed using R software (The R Foundation for Statistical Computing), and $P<0.05$ was considered to be statistically significant.

RESULTS

Prevalence of Monogenic FH

We identified 20 FH mutations in 49 962 Icelanders whose genomes had been sequenced. Most of the mutations are located in *LDLR* (3 loss-of-function mutations, 12 missense mutations, and 1 promoter variant), 3 in *PCSK9* (missense mutations) and 1 in *APOB* (missense mutation; Table 1 and Table II in the Data Supplement). These variants were imputed into an additional 116 319 chip-genotyped individuals to identify additional carriers. The genotypes of all identified carriers were confirmed with Sanger sequencing. A diagram showing the structure of the overall genotyped sample and subsamples are shown in Figure II in the Data Supplement.

In the overall sample (N=166 281), we identified 199 heterozygous FH mutation carriers. This corresponds to a monogenic FH prevalence of 1 in 836 (0.12%). Of the 199 identified FH mutation carriers, 98 (49%) had undergone WGS. The prevalence of monogenic FH was ≈2-fold higher among those who underwent WGS (1 in 515 [0.19%]), compared with those who did not (1 in 1149 [0.087%]; Table I in the Data Supplement). This is likely due to the intentional enrichment for individuals with severe hypercholesterolemia (eg, 1.9-fold enrichment for LDL-C >99th percentile) and various cardiovascular phenotypes in the WGS subsample (Table I in the Data Supplement).

Table 1. FH Mutations Found in the Overall Genotyped Sample of 166 281 Icelanders

Gene	Position (hg38)	Alleles*	Mutation	Type	Carriers (N)	Allele frequency† (%)	Previously identified in Iceland
PCSK9	chr1:55043921	C/T	Arg96Cys	Missense	3	9.0×10^{-4}	
	chr1:55044020	G/A	Asp129Asn	Missense	1	3.0×10^{-4}	
	chr1:55052398	G/A	Arg215His	Missense	1	3.0×10^{-4}	
APOB	chr2:21006288	G/A	Arg3527Gln	Missense	10	3.0×10^{-3}	
LDLR	chr19:11089397	C/T	c.-152C>T	Promoter	21	6.3×10^{-3}	
	chr19:11102772	A/T	Asp100Val	Missense	1	3.0×10^{-4}	
	chr19:11105315	G/A	Gly137Ser	Missense	2	6.0×10^{-4}	
	chr19:11105599	C/A	Cys231Ter	Stop gained (LoF)	5	1.5×10^{-3}	Yes[36]
	chr19:11105602	T/C	c.694+2T>C	Splice donor (LoF)	80	0.024	Yes[35]
	chr19:11106640	G/A	Arg257Gln	Missense	2	6.0×10^{-4}	
	chr19:11107493	G/A	Asp307Asn	Missense	20	6.0×10^{-3}	Yes[36]
	chr19:11111577	A/G	Tyr375Cys	Missense	3	9.0×10^{-4}	Yes[36]
	chr19:11113337	C/T	Arg416Trp	Missense	1	3.0×10^{-4}	
	chr19:11113398	T/C	Val436Ala	Missense	5	1.5×10^{-3}	
	chr19:11116125	G/A	Ala540Thr	Missense	20	6.0×10^{-3}	Yes[36]
	chr19:11116198	A/G	Asn564Ser	Missense	1	3.0×10^{-4}	
	chr19:11116880	A/C	Tyr576Ser	Missense	11	3.3×10^{-3}	Yes[36]
	chr19:11120502	A/T	Asp707Val	Missense	5	1.5×10^{-3}	Yes[36]
	chr19:11112202-11114606	Deletion/no deletion	Ex9-10DEL	Deletion (LoF)	5	1.5×10^{-3}	Yes[37]
	chr19:11129598	C/G	Asn825Lys	Missense	2	6.0×10^{-4}	

FH indicates familial hypercholesterolemia; and LoF, loss-of-function.

*Reference allele/alternative allele.

†Allele frequency in the combined, overall sample of 166 281 genotyped individuals. Mutations were identified in the subsample of 49 962 individuals who underwent both whole-genome sequencing and chip genotyping. The genotypes of the additional 116 319 individuals who were only chip genotyped were imputed. Sanger sequencing followed by reimputation was used to confirm genotypes and improve imputation accuracy (Methods).

The most common single FH mutation was a known founder mutation in Iceland,[35,38] a splice donor mutation in *LDLR* (c.694+2T>C) carried by 80 individuals and thus explaining 40.2% of monogenic FH in the overall sample. Five mutations are likely of a recent foreign origin and appear to have been introduced to the Icelandic gene pool during the last century (Data Supplement). Of the 20 mutations, 12 have not been described previously in Iceland (Table 1).

Lipid Levels in Monogenic FH

The maximum documented LDL-C level (maxLDL-C) in individuals with monogenic FH (N=175) was 7.15 mmol/L on average, compared with 3.94 mmol/L in noncarriers (N=104 653; Figure 1A). Levels of maxLDL-C were adjusted for statin use to approximate untreated levels (Methods). Monogenic FH associated with higher maxLDL-C by 3.37 mmol/L (95% CI, 3.16–3.58, $P<10^{-300}$; Table 2). Influence on maxLDL-C by mutation class are shown in Figure 1B and Table V in the Data Supplement. In addition, monogenic FH associated nominally with higher levels of lipoprotein(a) (β=0.35 SD [95% CI, 0.027–0.68]; P=0.034) but lower levels of triglycerides (β=−0.27 SD [95% CI, −0.41 to −0.16]; $P=2.4\times10^{-4}$) and HDL-C (β=−0.22 SD [95% CI, −0.07 to −0.37]; P=0.0032; Table 2), consistent with previous observations.[39,40]

Cumulative Lifetime Exposure to LDL-C

Figure 2 shows the relationship between monogenic FH and estimated cumulative exposure to LDL-C in adults aged 20 to 80 years in our data, expressed in units of mmol/L years. Cumulative exposure to LDL-C is the cumulative sum of mean LDL-C in mmol/L × years across age groups, based on LDL-C measurements taken over a period of nearly 3 decades (years 1990–2019; Figure III in the Data Supplement). As shown in Figure 2, individuals with monogenic FH have high cumulative LDL-C exposure throughout adult life. For example, the estimated cumulative LDL-C exposure of a 40-year-old individual with monogenic FH is similar to that of a 70-year-old noncarrier.

Atherosclerotic Diseases and Aortic Valve Stenosis

Monogenic FH associated with 3.4-fold greater risk of CAD (OR, 3.43 [95% CI, 2.25–5.22]; $P=9.8\times10^{-9}$)

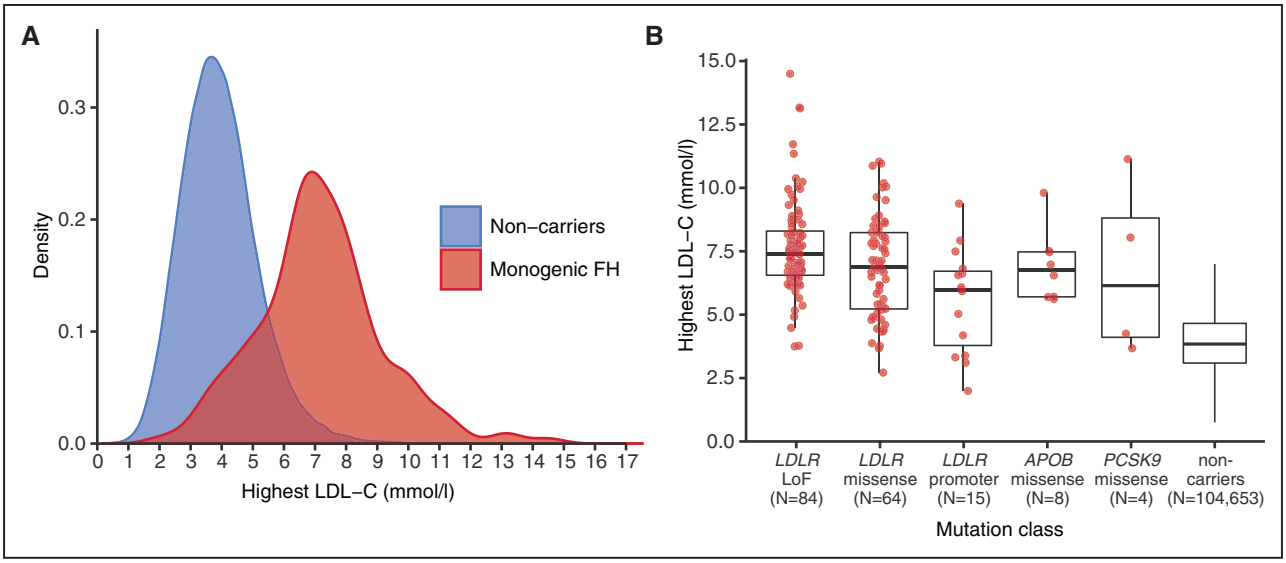

Figure 1. Monogenic familial hypercholesterolemia (FH) and LDL-C (low-density lipoprotein cholesterol) levels. **A** shows the distribution of the maximum documented LDL-C levels (maxLDL-C) in the subsample of 104 828 participants who had available LDL-C measurements. Individuals with monogenic FH are indicated with red (N=175) and FH mutation noncarriers with blue (N=104 653). **B** shows the distribution of maxLDL-C levels by FH mutation class. To convert LDL-C levels from mmol/L to mg/dL, multiply by 38.6.

and 5.1-fold higher risk of early-onset CAD (before age 50 years for men and 60 years for women; OR, 5.14 [95% CI, 2.84–9.28]; P=5.9×10^{-8}; Table 3). Associations stratified by mutation class are shown in Table VI in the Data Supplement. Individuals with monogenic FH were diagnosed with CAD (N=46) at a mean age of 57.7 years (SD 11.4 years), that is 8.4 years earlier than noncarriers (N=19 628, mean 66.1 years [SD 12.9 years]; P=3.7×10^{-7}). In addition, individuals with monogenic FH were more likely to have undergone coronary revascularization with percutaneous coronary intervention or coronary artery bypass surgery (Table 3). We did not observe associations with other atherosclerotic diseases such as peripheral artery disease (OR, 1.05 [95% CI, 0.33–3.38]; P=0.93) or ischemic stroke (OR, 0.88 [95% CI, 0.36–2.15]; P=0.78).

We evaluated the association between monogenic FH and measures of the extent of coronary atherosclerosis, as assessed by conventional coronary angiography (34 individuals with monogenic FH and 11 212 noncarriers) or noninvasive CAC scanning (18 individuals with monogenic FH, 5844 noncarriers). Characteristics of the samples are shown in Table VII in the Data Supplement. We observed an association with higher risk of having obstructive angiographic CAD (OR, 2.44 [95% CI, 1.11–5.36]; P=0.026) and left main disease (OR, 4.81 [95% CI, 2.02–11.44]; P=0.00038), adjusting for age and sex (Table VIII in the Data Supplement). Monogenic FH associated with the presence of coronary calcium (CAC score >0; OR, 5.68 [95% CI, 1.67–19.30]; P=0.0053) and CAC score >400 (OR, 11.48 [95% CI, 3.64–36.18]; P=3.1×10^{-5}), adjusting for age and sex (Table VIII in the Data Supplement). Thus, monogenic FH associated with greater burden of coronary atherosclerosis as assessed by either coronary angiography or CAC scanning.

An association between monogenic FH and increased risk of aortic valve stenosis was recently reported in Norway.[41] We tested for association with aortic valve stenosis and found that individuals with monogenic FH had 3.4-fold higher risk of aortic valve stenosis than noncarriers (OR, 3.41 [95% CI, 1.16–10.05]; P=0.026; Table 3).

Table 2. Association of Monogenic FH With Blood Lipid Levels

		Monogenic FH		Noncarriers		Adjusted difference	
Lipid trait	N total*	N	Mean (SD)	N	Mean (SD)	β (95% CI)	*P* value
LDL-C (maximum), mmol/L†	104 828	175	7.15 (2.02)	104 653	3.94 (1.23)	+3.37 mmol/L (3.16 to 3.58)	<10^{-300}
Triglycerides, mmol/L	109 550	178	1.24 (0.64)	109 372	1.44 (0.80)	−0.27 SD (−0.41 to −0.16)	2.4×10^{-4}
HDL-C, mmol/L	110 076	177	1.35 (0.38)	109 899	1.45 (0.42)	−0.22 SD (−0.07 to −0.37)	0.0032
Lipoprotein(a), nmol/L	24 257	36	61.1 (102)	24 221	41.5 (63.2)	+0.35 SD (0.027 to 0.68)	0.034

Values for LDL-C, HDL-C, and triglycerides are given in mmol/L. To convert to mg/dL, multiply by 38.6 for LDL-C and HDL-C, and by 88.6 for triglycerides. FH indicates familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

*No. of genotyped participants with at least one available measurement of the relevant lipid trait.

†Adjusted for statin use (Methods).

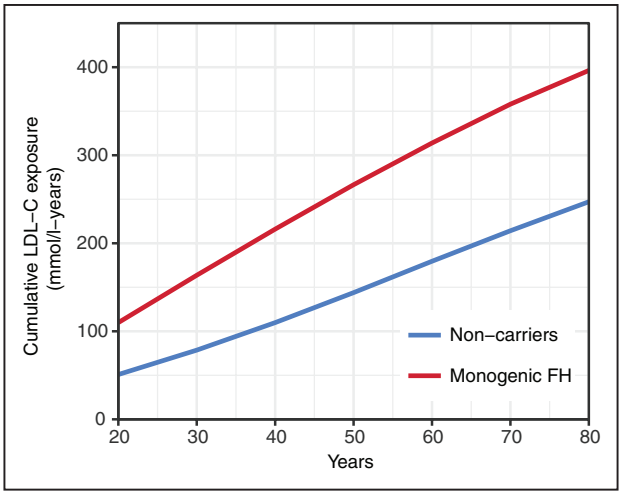

Figure 2. Cumulative lifetime exposure to LDL-C (low-density lipoprotein cholesterol).
Shown is the estimated average lifetime cumulative exposure to LDL-C, in units of mmol/L years. Individuals with monogenic familial hypercholesterolemia (FH; N=175) are shown in red, and non-carriers (N=104 653) in blue. Here, LDL-C measurements were not adjusted for statin use and thus reflect actual exposure to LDL-C. To convert mmol/L years to mg/dL years, multiply by 38.6.

Lifespan

Among individuals who lived to be at least 50 years old and were born after 1880 (N=48 628), individuals with monogenic FH had shorter lifespan by an average of 3.6 years (95% CI, 1.0–6.2; *P*=0.0066).

Prevalence by Maximum LDL-C Level and Diagnosis of Early-Onset CAD

We assessed the prevalence of monogenic FH by different strata of maxLDL-C levels and by diagnosis of early-onset CAD (Table IX in the Data Supplement). The prevalence was 1 in 599 (0.17%) among genotyped individuals with at least one LDL-C measurement available (N=104 828). Among those with maxLDL-C≥4.9 mmol/L (N=20,507), the prevalence was 1 in 134 (0.75%) and among those with both maxLDL-C≥4.9 mmol/L and early-onset CAD (N=1247), the prevalence was almost 2-fold higher (1 in 69 or 1.44%). The highest prevalence (11.4%) was observed in individuals with maxLDL-C over 8.5 mmol/L (N=325).

Clinically Defined FH and the Contribution of Monogenic FH

We screened for the clinical FH phenotype using a modified version of the DLCN criteria that exclude genotype data and physical examination findings. We screened a subsample of the overall genotyped sample, consisting of 79 058 living participants between the ages of 20 and 80 years that had at least one LDL-C measurement (summarized in Figure IV in the Data Supplement). Their mean age was 57.7 years and 45.0% were male (Table X in the Data Supplement). The prevalence of monogenic FH in this sample was 0.18% (Table XI in the Data Supplement).

A total of 1736 (2.2%) individuals fulfilled the criteria for clinical FH (probable or definite FH). The prevalence of clinical FH increased with age and was highest in those between the ages of 70 and 80 years (3.8%; Table XII in the Data Supplement). Overall, only 5.2% (N=90) of individuals with clinical FH were found to have monogenic FH (20.3% [N=29] of individuals with definite FH and 3.8% [N=61] with probable FH, Table XI in the Data Supplement).

Comparing Monogenic FH and Mutation-Negative Clinical FH

We explored the differences between individuals with a purely genetic diagnosis of FH (ie, monogenic FH) and those with clinical diagnosis of FH where no causative mutation is found. For this analysis, we compared the characteristics of individuals with monogenic FH (irrespective of DLCN classification) and those with mutation-negative clinical FH, defined as the subsample of individuals with clinical FH who did not carry an FH mutation (N=1736–90=1646). As individuals with

Table 3. Association of Monogenic FH With Atherosclerotic Diseases and Aortic Valve Stenosis

Disease	Cases*	Controls†	OR (95% CI)	*P* value
Coronary artery disease	19 674 (46)	129 508	3.43 (2.25–5.22)	9.8×10^{-9}
Coronary artery disease, early onset‡	3473 (19)	145 415	5.14 (2.84–9.28)	5.9×10^{-8}
Percutaneous coronary intervention	4067 (15)	139 646	4.14 (2.14–8.04)	2.6×10^{-5}
Coronary artery bypass surgery	3747 (15)	144 764	5.05 (2.55–10.03)	3.6×10^{-6}
Peripheral artery disease	2601 (3)	144 735	1.05 (0.33–3.38)	0.93
Ischemic stroke	5156 (5)	144 400	0.88 (0.36–2.15)	0.78
Aortic valve stenosis	1662 (5)	144 941	3.41 (1.16–10.05)	0.026

FH, familial hypercholesterolemia; and OR, odds ratio.
*No. of cases that have monogenic FH are given within parentheses.
†Controls are noncases for a given phenotype from the overall genotyped sample.
‡Age at diagnosis <50 y for men and <60 y for women.

mutation-negative clinical FH were alive by definition, we included only living individuals with monogenic FH (N=166) for this comparison.

Individuals with monogenic FH were younger than individuals with mutation-negative clinical FH (mean age, 53.9 versus 66.4 years, $P<0.0001$). Individuals with monogenic FH were more likely to have extreme hypercholesterolemia (maxLDL-C≥8.5 mmol/L; 19.3% versus 10.9%, $P=0.00064$) and family history of either hypercholesterolemia (maxLDL-C above 95th percentile; 71.1% versus 62.3%, $P<0.0001$) or clinical FH (68.1% versus 52.2%, $P<0.0001$), but lower prevalence of early-onset CAD (10.2% versus 33.2%, $P<0.0001$), hypertension (21.7% versus 51.3%, $P<0.0001$), and ever smoking (28.3% versus 47.1%, $P=0.0091$), with P values adjusted for age and sex (Table XIII in the Data Supplement).

We evaluated prescribing patterns of cholesterol-lowering drugs using nationwide drug prescription data for lipid-lowering medications prescribed from 2003 to 2018. During this period, individuals with monogenic FH were less likely than individuals with mutation-negative clinical FH to have received a prescription of any statin (75.9% versus 96.9%, $P<0.0001$) but were more likely to have received a high-potency statin (55.4% versus 46.2%, $P=0.00015$), ezetimibe (28.9% versus 11.1%, $P<0.0001$), and PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors (3.0% versus 0.73%, $P=0.048$), with P values adjusted for age and sex (Table XIII in the Data Supplement). At the time of first prescription of a lipid-lowering medication, individuals with monogenic FH were on average 9.9 years younger than those with mutation-negative clinical FH (mean age, 44.8 versus 54.4 years; adjusted difference, −9.9 years; $P<0.0001$), after accounting for sex.

Prescription Patterns and Effectiveness of Cholesterol-Lowering Treatment

Figure 3 shows the latest unadjusted LDL-C measurement (years 2004–2018) by prescription of statins (highest potency class) and ezetimibe in the preceding year for living individuals with monogenic FH (N=135, mean age 56.0 years) and mutation-negative clinical FH (N=1508, mean age 66.4 years). Individuals that did not have an LDL-C measurement during this time period were not included. During the year preceding the measurement, high-potency statins were prescribed to 40.0% and 21.9% of individuals with monogenic FH and mutation-negative clinical FH, respectively. The fraction of those who received neither statins nor ezetimibe was 28.1% and 17.9%, respectively. Only 11.0% of individuals with monogenic FH and 24.9% with mutation-negative clinical FH attained an LDL-C level <2.6 mmol/L, the target endorsed by the 2016 European Society of

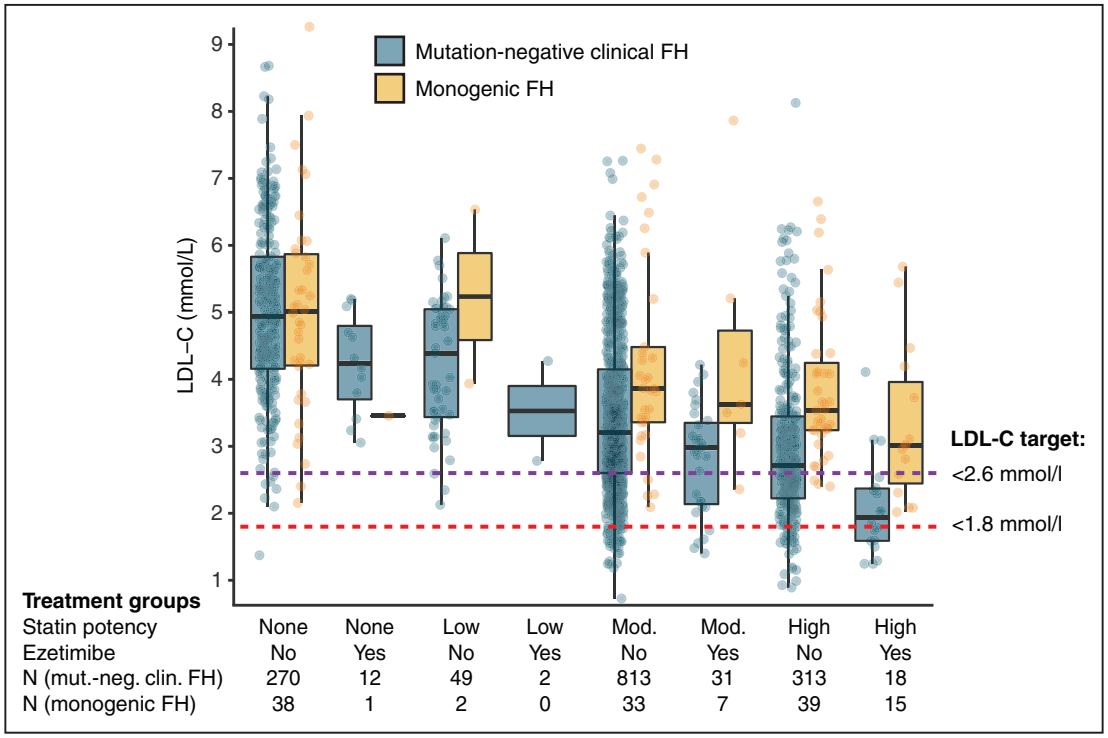

Figure 3. Prescription patterns and effectiveness of cholesterol-lowering therapy in living individuals with monogenic familial hypercholesterolemia (FH; yellow, N=135) and mutation-negative clinical FH (blue, N=1508).
Shown is the latest available LDL-C (low-density lipoprotein cholesterol) measurement (years 2004–2018) as a function of potency of the prescribed cholesterol-lowering therapy (ie, prescriptions of statins and ezetimibe) during the preceding year. Here, LDL-C values were not adjusted for statin use. Horizontal lines indicate the recommended target levels for primary prevention in FH according to the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) guidelines from 2016 (purple, <2.6 mmol/L)[42] and 2019 (red, <1.8 mmol/L).[43]

Cardiology/European Atherosclerosis Society Guidelines for the management of dyslipidaemias[42] for primary prevention in FH (Figure 3). No individual with monogenic FH and only 5.2% with mutation-negative clinical FH attained an LDL-C level <1.8 mmol/L, the target recommended by the 2019 European Society of Cardiology/European Atherosclerosis Society guidelines[43] for primary prevention in FH, in the absence of atherosclerotic disease and other major cardiovascular risk factors. These data demonstrate that both individuals with monogenic FH and individuals with mutation-negative clinical FH are markedly undertreated.

Polygenic Contribution in Mutation-Negative Clinical FH

We estimated the polygenic contribution in mutation-negative clinical FH using an LDL-C genetic score based on 345 lipid-associated variants (Methods). These analyses were performed in a subsample of 72 926 individuals from the overall genotyped sample who (1) were classified using the DLCN criteria, (2) had an available genetic score, and (3) did not have monogenic FH. This sample consists of 1564 individuals with mutation-negative clinical FH and 71 362 controls (ie, unlikely or possible FH according to the DLCN criteria).

An increase in the genetic score corresponding to 1-SD increase in LDL-C (≈1.04 mmol/L increase in maxLDL-C) was associated with about 9-fold higher risk of mutation-negative clinical FH (OR, 9.25, P=3.5×10^{-138}) and 2-fold higher risk of early-onset CAD (OR, 1.94, P=7.6×10^{-31}) but was not associated with risk of ischemic stroke (Table XIV in the Data Supplement). A total of 78.7% of mutation-negative clinical FH cases had values above the 50th percentile in the overall distribution, 58.7% above the 70th percentile, 26.2% above the 90th percentile, and 15.0% above the 95th percentile (Figure 4A). These results show that a large fraction of mutation-negative clinical FH individuals has a high polygenic burden of LDL-C-raising sequence variants.

We compared the risk of mutation-negative clinical FH by percentiles of the genetic score, relative to individuals in the middle quintile (40–59th percentile), adjusting for age and sex. There was a trend toward higher maxLDL-C and higher risk of mutation-negative clinical FH with increasing percentiles of the genetic score (Figure V in the Data Supplement and Figure 4B). Individuals with a genetic score at or above the 99.9th percentile (N=58) had a mean maxLDL-C of 5.0 mmol/L and the prevalence of mutation-negative clinical FH in this percentile was 9.6% (OR, 5.74, P=1.6×10^{-5}). Compared with monogenic FH, individuals with a genetic score at or above the 99.9th percentile had lower estimated cumulative lifetime exposure to LDL-C (Figure VI in the Data Supplement).

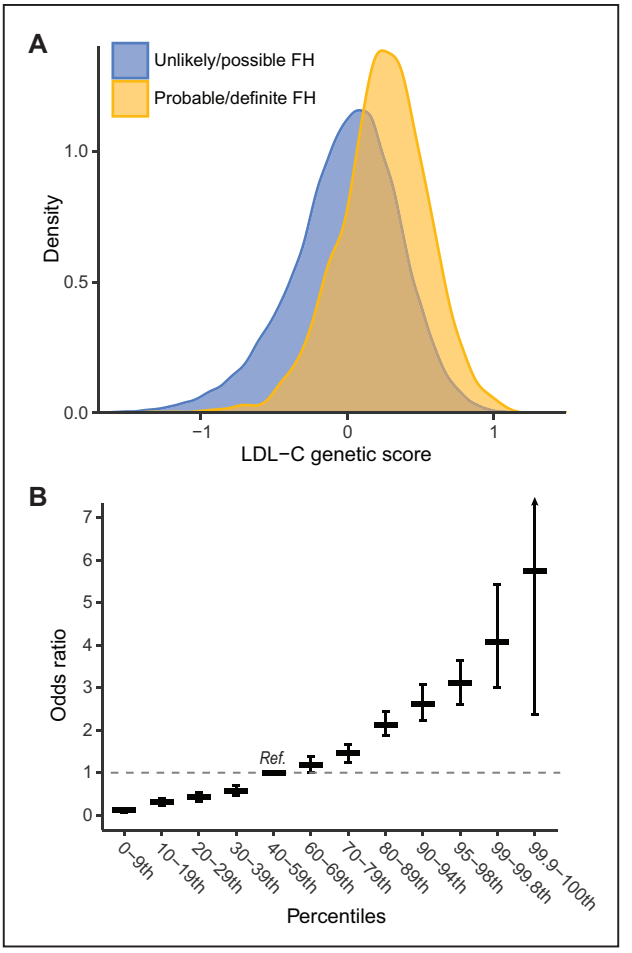

Figure 4. Polygenic contribution to mutation-negative clinical familial hypercholesterolemia (FH).
A shows the distribution of the LDL-C (low-density lipoprotein cholesterol) genetic score by clinical FH status according to a modified version of the Dutch Lipid Clinic Network criteria, excluding individuals with monogenic FH. Yellow indicates clinical FH (probable or definite FH, N=1564) and blue indicates controls (unlikely or possible FH, N=71 362). **B** shows odds ratios for clinical FH by percentiles of the LDL-C genetic score, given relative to the middle quintile (40–59th percentile). 95% CIs are presented.

DISCUSSION

We found that the prevalence of monogenic FH was 1 in 836 in the overall sample of 166 281 genotyped individuals, representing a large fraction of the Icelandic population (364.134 inhabitants on January 1, 2020, Statistics Iceland). We observed a higher prevalence in the nonrandom subsample of 49 962 individuals who had been selected for WGS (1 in 515). This is expected due to the intentional enrichment for individuals with high LDL-C and various cardiovascular phenotypes in this subsample. Thus, the prevalence of monogenic FH in the WGS subsample likely overestimates the true prevalence in the Icelandic population. Because WGS was not performed on all study participants, we may have missed ultra-rare and private FH mutations that are only present in the 116 319 individuals who did not undergo WGS, resulting

in underestimation of the prevalence in the overall genotyped sample. Nevertheless, our data suggest that the prevalence of monogenic FH in Iceland is considerably lower than recent estimates from large genetic studies in Denmark[6] (1 in 217), the United States[5,7,9] (from 1 in 260 to 1 in 211) and the UK Biobank[8] (1 in 176). The comparatively low prevalence of monogenic FH in our study may be related the geographic isolation and genetic homogeneity of the Icelandic population.[44] In addition, because we applied a conservative approach in the selection of mutations assumed to be causative of FH (eg, in-silico predictions were not considered) we may have missed some true FH mutations. Taken together, our findings suggest that the prevalence of monogenic FH in the Icelandic population is likely lower than recent estimates in several European populations.

Individuals with monogenic FH are exposed to high plasma LDL from early life and throughout adulthood.[45] Using LDL-C measurements spanning 3 decades for over 100 000 individuals, we demonstrated a high cumulative lifetime exposure to LDL in Icelanders with monogenic FH, consistent with previous studies.[7,46] Monogenic FH was strongly associated with increased risk of premature coronary disease and greater burden of coronary atherosclerosis, as previously described.[5–7,47–49] We did not observe increased risk of ischemic stroke in monogenic FH, in keeping with previous studies,[40,50] indicating that high LDL levels may not influence the development of atherosclerotic lesions to the same extent in all arteries. Our results corroborate recent findings of an increased risk of aortic valve stenosis in monogenic FH,[41] consistent with a causal role of LDL in the development of aortic valve stenosis.[20,51,52]

We observed that 2.2% of 79 000 living adult participants with at least one LDL-C measurement could be classified as having clinical FH, defined as probable or definite FH according to a modified version of the DLCN criteria. Clinical FH was over 10-times more common than monogenic FH in this sample (2.2% versus 0.18%). Of note, individuals with clinical FH were more likely than individuals with monogenic FH to have early-onset CAD (33% versus 10%). Although not entirely clear, this may reflect enrichment for cases of early-onset CAD due to its weight in the DLCN criteria (giving 2 points), older age (mean age, 66 versus 54 years), or both. Previous estimates of the prevalence of clinical FH in large population-based studies, using DLCN criteria, have ranged between 0.35% and 1.2%.[5,6,53,54] The comparatively high prevalence observed in our study may be explained, at least in part, by the use of comprehensive genealogical information providing an accurate family history that is not subject to recall bias. We found that only about 5% of individuals with clinical FH had monogenic FH. This observation is consistent with a study among 46 285 participants in an electronic health records-linked biobank where only about 9% of individuals with clinical FH carried an FH mutation.[5] By contrast, in tertiary lipid clinics, a monogenic cause is commonly found in 40% to 50% of cases.[11–13] This is not surprising, however, as individuals who are referred to lipid clinics represent a highly selected population with high a priori probability of having a causative mutation. Thus, our findings indicate that on the population scale, the clinical FH phenotype is likely caused by monogenic FH in only a small minority of cases.

Our results demonstrate that polygenic susceptibility to elevated plasma LDL-C is an important contributor to development of mutation-negative clinical FH, consistent with previous studies.[11–13] In contrast to one previous study[55] but consistent with a recent report,[56] our study shows that having an extreme value of a LDL-C genetic score is not comparable to having monogenic FH. Compared with monogenic FH, individuals with a genetic score at or above the 99.9th percentile had lower maxLDL-C levels (mean, 5.0 versus 7.15 mmol/L), lower estimated cumulative lifetime exposure to LDL-C and substantially lower prevalence of clinical FH (9.6% versus 64%). These results are also consistent with previous findings showing a greater risk of atherosclerotic cardiovascular disease[8] and higher severity of preclinical atherosclerosis[57] in individuals with monogenic FH, compared with those considered to have polygenic hypercholesterolemia on the basis of a high LDL-C genetic score. Thus, a high LDL-C genetic score is a marker of polygenic predisposition to hypercholesterolemia and the clinical FH phenotype, but it does not have a penetrance comparable to that of monogenic FH.

The present findings have clinical implications. First, our results show that the majority of Icelanders with monogenic and clinically defined FH are markedly undertreated with cholesterol-lowering medications, as is the case in most countries.[2] Here, only a small minority reached a target of LDL-C<2.6 mmol/L (11% and 25%, respectively) as suggested by the 2016 European Society of Cardiology/European Atherosclerosis Society guidelines,[42] and even fewer reached <1.8 mmol/L (0% and 5%) as suggested by the recent 2019 European Society of Cardiology/European Atherosclerosis Society guidelines.[43] Note that these targets are only appropriate for primary prevention in individuals with FH without other major cardiovascular risk factors. Thus, the degree of undertreatment in our data is underestimated by these numbers, as lower targets would apply for those with manifest atherosclerotic disease or otherwise classified at very high risk. The most likely explanation for undertreatment is clinical underdiagnosis due to several factors, including inadequate awareness of FH among clinicians and underuse of genetic testing and family cascade screening. In addition, among individuals with a known diagnosis of FH, lack of appropriate escalation of therapy as well as lack of patient

education and motivation are likely contributing factors. Second, the yield of clinical genetic testing for FH and subsequent family cascade screening in Iceland can be improved by incorporating the panel of FH mutations identified in this study. Third, the obvious underdiagnosis and undertreatment of FH in Iceland calls for public health care initiatives to improve diagnosis and appropriate treatment of FH, including clinician awareness and facilitation of referrals for genetic testing and subsequent family cascade screening.

Limitations

Several limitations to this study deserve mention. We chose a conservative approach in defining FH mutations which limits false-positives but comes at the expense that some very rare mutations that truly cause FH may have been missed. Identification of FH mutations was based on WGS in approximately a third of the overall sample and thus we may have missed FH mutations only present in those that were not sequenced. However, these mutations would likely be extremely rare and thus not have significant impact on the estimated prevalence of monogenic FH. Similarly, we cannot exclude the presence of undetected, potentially pathogenic copy-number variants in *LDLR* in our data. Although widely used in registry studies,[5,6,58] the DLCN criteria were not designed for screening at a population level and may thus not be ideal for this purpose. Analyses were based on LDL-C measurements taken for various clinical indications and thus this sample may be enriched for individuals with high LDL-C levels. Prevalence estimate of clinical FH is subject to an inherent selection bias related to genotyping status and the availability of LDL-C measurements and thus our estimate may be biased upwards. The use of cholesterol-lowering drugs was inferred from drug prescription data and may not accurately reflect the actual use in some cases.

Conclusions

Our findings indicate that the prevalence of monogenic FH in Iceland is lower than many contemporary estimates in European and North American populations. Clinical FH is a relatively common high-risk cardiovascular phenotype that has a strong polygenic basis but is rarely caused by an FH mutation. Both individuals with monogenic FH and individuals with mutation-negative clinical FH are markedly undertreated with cholesterol-lowering agents in Iceland. These results emphasize an urgent need for improved diagnosis and appropriate treatment of monogenic and clinically defined FH.

ARTICLE INFORMATION

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The authors affiliated with deCODE genetics/Amgen, Inc, are employed by the company. The other authors report no conflicts.

Supplemental Materials

Note
Supplementary Methods
Data Supplement Tables I–XV
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