Journal of the American Heart Association

ORIGINAL RESEARCH

Clinical Implications of Monogenic Versus Polygenic Hypercholesterolemia: Long-Term Response to Treatment, Coronary Atherosclerosis Burden, and Cardiovascular Events

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BACKGROUND: Familial hypercholesterolemia (FH) may arise from deleterious monogenic variants in FH-causing genes as well as from a polygenic cause. We evaluated the relationships between monogenic FH and polygenic hypercholesterolemia in influencing the long-term response to therapy and the risk of atherosclerosis.

METHODS AND RESULTS: A cohort of 370 patients with clinically diagnosed FH were screened for monogenic mutations and a low-density lipoprotein-rising genetic risk score >0.69 to identify polygenic cause. Medical records were reviewed to estimate the response to lipid-lowering therapies and the occurrence of major atherosclerotic cardiovascular events during a median follow-up of 31.0 months. A subgroup of patients (n=119) also underwent coronary computed tomographic angiography for the evaluation of coronary artery calcium score and severity of coronary stenosis as compared with 135 controls. Two hundred nine (56.5%) patients with hypercholesterolemia were classified as monogenic (FH/M+), 89 (24.1%) as polygenic, and 72 (19.5%) genetically undefined (FH/M–). The response to lipid-lowering therapy was poorest in monogenic, whereas it was comparable in patients with polygenic hypercholesterolemia and genetically undetermined. Mean coronary artery calcium score and the prevalence of coronary artery calcium >100 units were significantly higher in FH/M+ as compared with both FH/M– and controls. Finally, after adjustments for confounders, we observed a 5-fold higher risk of incident major atherosclerotic cardiovascular events in FH/M+ (hazard ratio, 4.8; 95% CI, 1.06–21.36; P_{adj}=0.041).

CONCLUSIONS: Monogenic cause of FH is associated with lower response to conventional cholesterol-lowering therapies as well as with increased burden of coronary atherosclerosis and risk of atherosclerotic-related events. Genetic testing for hypercholesterolemia is helpful in providing important prognostic information.

Key Words: atherosclerosis ■ cardiovascular disease ■ genetics ■ hypercholesterolemia ■ therapy

Familial hypercholesterolemia (FH) has been recognized as the most common dominant genetic disorder of lipid metabolism affecting ≈1:250 to 300 individuals in the general population. Most frequently, it is caused by deleterious variants in genes coding for the low-density lipoprotein receptor (*LDLR*), the

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Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.018932

For Sources of Funding and Disclosures, see page 12.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- Monogenic familial hypercholesterolemia is associated with lower responsiveness to conventional low-density lipoprotein cholesterol lowering therapies and increased preclinical coronary atherosclerosis when compared with patients with hypercholesterolemia of polygenic or unknown genetic cause.

What Are the Clinical Implications?

- Genetic testing for familial hypercholesterolemia is helpful in providing important prognostic information and improving atherosclerosis risk stratification, thus potentially helping physicians in tailoring therapies to patients' needs.

Nonstandard Abbreviations and Acronyms

DLCNC	Dutch Lipid Clinic Network criteria
FH	familial hypercholesterolemia
FH/M–	negative for monogenic mutations patients with hypercholesterolemia
FH/M+	monogenic patients with hypercholesterolemia
LIPIGEN	Lipid Transport Disorders Italian Genetic Network
LLT	lipid-lowering therapies
MACE	major adverse cardiovascular events
PCSK9i	proprotein convertase subtilisin/kexin type 9 inibitor
PHC	polygenic hypercholesterolemia
PRS	polygenic risk score

apolipoprotein B (*APOB*), and the proprotein convertase subtilisin/kexin type 9 (*PCSK9*).[1,2] These variants significantly impair LDLR-mediated removal of low-density lipoprotein (LDL) particles from circulation, thus leading to a lifelong exposure to elevated plasma levels of LDL cholesterol (LDL-C) and, thereby, increased risk of atherosclerotic cardiovascular disease (ASCVD).[1] However, only a portion of patients with clinically diagnosed FH can be recognized as carriers of single variants in major FH-causing genes (monogenic FH).[3] Indeed, it has been recently recognized that the FH phenotype may be polygenic because of the concomitant presence in the same individual of several, common LDL-C raising single nucleotide polymorphisms (SNPs).[4] As the actual levels of LDL-C has been indicated as the main guide for FH management, the clinical utility of differentiating the monogenic from the polygenic form of hypercholesterolemia remains controversial.[1,4] It has been suggested that the presence of monogenic FH is associated with a more severe ASCVD prognosis,[1,3,5] but the consequence of carrying monogenic or polygenic FH-causing variants on the development of atherosclerotic coronary damage has never been carefully evaluated. Moreover, whether these 2 types of hypercholesterolemia respond differently to LDL-C lowering medications has been poorly investigated.[3]

Therefore, we aimed to investigate the effect of genotype, namely monogenic versus polygenic hypercholesterolemia (PHC), in influencing the response to treatment, the risk of subclinical coronary atherosclerosis, and the occurrence of major adverse cardiovascular events (MACE) during follow-up.

METHODS

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

Study Design

The study population consisted of 370 unrelated patients with clinically defined FH followed at the Lipid Clinic of the Department of Internal Medicine and of Translational and Precision Medicine, Sapienza University of Rome, which were consecutively enrolled into the LIPIGEN-FH (Lipid Transport Disorders Italian Genetic Network-FH) Registry in the period between April 2013 and December 2018.

The LIPIGEN-FH is an observational, multicenter, retrospective and prospective study aimed at identifying and registering patients with FH in Italy. Details about this study have been reported elsewhere.[6,7] In brief, patients were invited to enroll into the registry if they had a clinical diagnosis of "possible," "probable," or "definite" FH, according to the Dutch Lipid Clinic Network criteria (DLCNC).[6,8] Patients were excluded if they were unwilling or unable to sign the informed consent form or had a secondary cause of hypercholesterolemia. Patients with homozygous FH mutations were excluded from present analysis. The LIPIGEN-FH protocol was approved by the Ethics Committee of Sapienza University of Rome (Approval Code #2469) and patients provided written consent.

After enrollment, patients with FH underwent clinical examination and blood drawing for genetic analysis. In addition, patients with FH >30 years of age and without clinical signs or past medical history of ASCVD were also invited to receive coronary tomography angiography (CTA) to evaluate the coronary atherosclerosis burden. Only patients who have returned at least once after enrollment into the LIPIGEN registry (n=322; 87%) were included in the analysis of response to lipid-lowering therapies

(LLT),[9] coronary atherosclerosis burden, and occurrence of MACE.[10]

Clinical Assessment of Patients With FH

Medical records of enrolled patients were reviewed by 3 authors (D.C., L.D., and M.M.). Clinical information including major coronary risk factors, all available lipid measurements, pharmacological treatments, and history of MACE were retrieved. Last follow-up data were defined as those at the time of the last clinic visit as of April 30, 2019.[9] For 99 subjects for whom pretreatment lipid levels were not available in the medical records, the untreated LDL-C was estimated according to the revert algorithm.[11] Diagnosis of type 2 diabetes mellitus was performed in accordance with World Health Organization criteria[12] and that of hypertension following the criteria recommended by the National Cholesterol Educational Program Adult Treatment Panel III Expert Panel of the US National Cholesterol Panel.[12]

Lipids measurements at the time of first visit at the Lipid Clinic (baseline), before initiation of LLT (untreated), at enrollment in the LIPIGEN study (at entry), and at last visit were considered. According to the intensity of LLT, patients were divided into 1 of the following mutually exclusive groups[8,13]: (1) untreated; (2) low-moderate intensity that was defined as the use of low-moderate intensity therapy, namely atorvastatin 10 or 20 mg or rosuvastatin 5 or 10 mg without ezetimibe, simvastatin 20 mg with or without ezetimibe, simvastatin 40 mg and pravastatin 40 or 80 mg, lovastatin 40 or 80 mg, fluvastatin 40 mg with or without ezetimibe, monacolin K plus ezetimibe or ezetimibe only; (3) high-intensity, defined as the use of rosuvastatin (20 or 40 mg), atorvastatin (40 or 80 mg) without ezetimibe or atorvastatin 10 or 20 mg or rosuvastatin 5 or 10 mg with ezetimibe, simvastatin 40 plus ezetimibe, lovastatin 40 plus ezetimibe and fluvastatin 80 mg plus ezetimibe; and (4) maximal intensity, including rosuvastatin (20 or 40 mg) or atorvastatin (40 or 80 mg) plus ezetimibe. As during follow-up, inhibitors of PCSK9 (proprotein convertase subtilisin/kexin type 9; PCSK9i) were made available for reimbursement in Italy based on DLCNC score >8,[14,15] and 37 patients with FH were prescribed with either evolocumab or alirocumab. Then, these were categorized in patients taking low-moderate intensity LLT plus PCSK9i and maximal intensity LLT plus PCKS9i. Unfortunately, no data on adherence to LLT were available in medical records so this information is lacking.

MACE events were defined as angina pectoris, acute myocardial infarction, coronary, carotid or peripheral revascularization (as well as hemodynamic stenosis without revascularization), ischemic stroke, and cardiovascular death. MACE events were identified by self-reported medical history or previous hospital admission and adjudicated by using available medical records. Events occurred after baseline were used to calculate incident MACE and were censored on the date of event or, if individuals remained event free, up to April 30, 2019.[9] In case of multiple MACEs occurred in the same period (eg, angina followed by coronary revascularization), we considered only the ischemic event for the final adjudication.

Genetic Analysis

Genomic DNA was extracted from circulating leukocytes and sequenced according to the LIPIGEN-FH protocol.[7] In brief, the promoter and coding DNA sequences as well as exon-intron boundaries regions (±25 base pairs) of *LDLR*, *APOB,* apolipoprotein E (*APOE*), *PCSK9*, low density lipoprotein receptor adaptor protein 1 (*LDLRAP1),* Signal Transducing Adaptor Family Member 1 (*STAP1*) and Lipase A, Lysosomal acid (*LIPA*) genes were analyzed by Next Generation Sequencing using MiSeq (Illumina) equipment. Sanger sequencing was also performed to confirm identified genetic variants. DNA samples negative for FH-causing mutations were further screened by Multiplex Ligation-dependent Probe Amplification analysis according to manufacturer's protocol (MRC-Holland, Amsterdam, The Netherlands) to detected copy number variations.

Variants were annotated according to the American College of Medical Genetics classification. In doing this, we used VarSome, a data aggregator and variant data discovery tool,[16] which implemented 21 American College of Medical Genetics criteria for automated interpretation of the clinical significance of sequence variants. A manual adjustment step for all variants was also performed according to Chora et al.[17] Variants predicting nonsense mutations and copy number variations were classified as pathogenic.

According to these criteria, patients carrying pathogenic or likely pathogenic variants were included in the FH monogenic group (FH/M+); those who resulted negative for monogenic mutations were classified as FH/M–.

LDL-C Raising Genetic Risk Score Calculation

A weighted LDL-C-raising polygenic risk score (PRS) was calculated based on 6 SNPs (rs4299376, rs1367117, rs6511720, rs629301, rs7412, rs429358), as reported.[4] These SNPs were genotyped in all patients clinically diagnosed with FH as well as in 1046 subjects with normolipemia, used as reference population. Healthy individuals with normolipemia were selected from 2069 blood donors[12] based upon LDL-C and plasma total triglycerides levels below the 75th

age- and gender-specific percentiles[18] to avoid potential bias of including individuals carrying FH-causing variants. Genotyping was performed using 7900HT Fast Real-Time PCR System, according to manufacturing protocol (Applied Biosystems TaqMan). In agreement with previous reports,[1,4] LDL-C raising PRS correlates with LDL-C values in individuals with normolipemia (Figure S1) and it explains 2.5% of LDL-C variation in the study population with clinically diagnosed FH after excluding those with monogenic FH (β=0.17, *P*=0.023).

To generate a cutoff value of LDL-C raising PRS reasonably appropriate to classify patients with PHC, we used receiver operating characteristic analysis to identify the best value discriminating FH/M– from healthy individuals with normolipemia. The result of area under the receiver operating characteristic curve indicated 0.646 (95% CI, 0.602–0.691; *P*<0.001) as the best discriminating value and, therefore, we decided to select the upper value of >0.69. This value corresponded to the 70th percentile of PRS distribution in the population with normocholesterolemia.

Therefore, patients with FH/M– with LDL-C raising PRS >0.69 were classified as those with the higher probability to have a polygenic cause of their hypercholesterolemia and defined as PHC. Conversely, patients with FH/M– with LDL-C raising PRS ≤0.69 were classified as those with unknown genetic causes of hypercholesterolemia (FH/M– undetermined).

Coronary Computed Tomography Angiography Evaluation

During follow-up, 124 patients agreed to undergo coronary CTA. In 5 patients, the exam was not performed (in 4 subjects for heart rate >70 bpm, despite treatment with oral β blockers and in 1 patient for technical problems) so that the final cohort of imaging study consisted of 119 patients with clinically defined FH.

Two populations were included as controls for the coronary imaging study. In these groups coronary CTA were performed by using the same tomographic scanner and images were analyzed by the same 2 readers blinded for genotype and clinical characteristics of the patients.

The first cohort consisted in 58 subjects classified to be at high risk among subjects participating in a working community-based screening program for atherosclerosis risk factors and voluntary underwent a coronary CTA examination. The medical assessment of these individuals has been detailed elsewhere.[19] In this group, the exclusion of FH was carried out only on a clinical basis.

The second group comprised 77 subjects who participated in a population-based survey[20] for detecting the coronary atherosclerosis burden in free-living individuals. In these subjects, a complete medical workup, including 12-lead ECG and fasting blood samples for laboratory determinations and DNA analysis, were obtained. All subjects included in these control groups provided specific written consent and the Ethics Committee of Sapienza University of Rome reviewed and approved corresponding study protocols (Approval Code #4086).

Coronary CTA was performed using a dual-source 64-detector row CT scanner (SOMATOM Definition, Siemens AG, Forcheim, Germany). All exams were ECG-gated, either retrospectively or prospectively, the decision being taken singularly for each patient depending on the heart rate. Unenhanced scans were performed for determination of coronary artery calcium (CAC). Ninety mL of a high concentration iodine contrast agent (4.5 mL/s rate, 400 mgI/100 mL, Iomeron 400, Bracco, Milan, Italy) were then administered through an automatic dual-head power injector and an 18 gauge cannula in the right antecubital vein, followed by 40 mL of saline to flush the contrast. Bolus tracking technique was used to optimize timing of image acquisition. Scanning parameters for the CTA included heart rate-dependent pitch (0.2–0.45), 0.33 seconds gantry rotation time, 100 to 120 kV tube voltage, and 350 to 800 mA tube current.

All images were transferred to a dedicated workstation (Vitrea2 Vital Images, Minnetonka, MN) for semiautomatic calculation of the Agatston score[21] and for image analysis. Several techniques were applied for estimation of plaque severity, including standard axial images, multiplanar reformat, maximum intensity projection, and cross-sectional reconstructions. Images were analyzed independently by 2 readers blinded to the FH status and LDL-C level of the patients, with a joined session to solve any disagreement.

Atherosclerotic disease was quantified at CTA for each patient using the Coronary Artery Disease-Reporting and Data System (CAD-RADS),[22] which stratifies patients according to their most severe stenosis.

Statistical Analysis

Descriptive statistics, such as mean (±SD) and median (interquartile range) or number (percentage), were calculated for all variables, as appropriate. Continuous variables were compared by Student *t* test, or ANOVA, Mann-Whitney, and Kruskal-Wallis tests if normally or not-normally distributed, respectively; categorical variables were compared by χ^2 test or Fisher exact test. The change of LLTs from baseline to the last follow-up were assessed by using χ^2-test. Paired *t* test and Mann-Whitney tests were respectively used to compare LDL-C values in the whole group of patients with FH and between subgroups.

Because of the high frequency of zero values, CAC scores were compared between groups after transformation using the formula log (CAC score +1) as reported.[23] They were transformed back to the original scale and presented as means (+SD). As sensitivity analysis, the distribution of FH genotypes according to CAC categories 0, 1 to 100, and >100 Agatston units was also evaluated. Linear regression was used to identify variables associated with an increase in the log (CAC+1). Odds ratio through logistic regression analysis was estimated to assess groups with the higher risk of having moderate-severe coronary stenosis.

To estimate the incident rate of MACE, the first new event that occurred after baseline during follow-up was considered. We estimated the survival from incident MACE event during follow-up by using Kaplan-Meier curves.[10] Cox proportional hazards model was applied to investigate the predictors of incident MACE.[10]

All multiple comparisons adjustments were performed by adding bootstrap correction, with the aim to adjust raw *P* value and obtain more robust estimates of SEs and CIs of the parameters included in the model.[24]

All statistical analyses were performed using SPSS/WIN software version 18.0 (SPSS Inc., Chicago, IL). A 2-sided $P<0.05$ was considered as statistically significant.

Table 1. Characteristics of Clinically Diagnosed FH Patients at Enrollment Into the LIPIGEN Registry

Demographic	
n	370
Age, y	47.1±14.4
Male, n (%)	178 (48.1)
BMI, kg/m^2	24.9±3.8
DLCN S, n (%)	
Possible (3–5 points)	182 (49.2)
Probable (6–8 points)	89 (24.1)
Definite (>8 points)	99 (26.8)
Plasma lipids, mg/dL	
Total cholesterol	249.8±73.1
LDL cholesterol	167.4±67.9
HDL cholesterol	59.2±15.7
Total triglycerides	120.2±77.2
Medical history, n (%)	
History of ASCVD	38 (10.3)
T2DM	13 (3.5)
Family history of ASCVD	122 (33.9)
Smokers	62 (16.7)
HTN	57 (15.4)
Lipid-lowering medications	227 (61.3)

Data are reported as mean (± standard deviation) or number (percentage) as appropriate. ASCVD was defined as the occurrence of any of angina, acute myocardial infarction and coronary, carotid or peripheral revascularization (as well as hemodynamic lesion) as well as of ischemic stroke (see Material and Methods). ASCVD indicates atherosclerotic cardiovascular disease; BMI, body mass index; DLCNC, Dutch Lipid Clinic Network Criteria; FH, familial hypercholesterolemia; HDL, high-density lipoprotein; HTN, hypertension; LDL, low-density lipoprotein; and T2DM, type 2 diabetes.

RESULTS

Characteristics of Patients With Clinically Defined FH

The characteristics of patients with FH at the time of enrollment in the LIPIGEN registry are shown in Table 1. The mean age was 47.1 years (interquartile range 36.5–59.0) and 48.1% were men. About one third (35.6%) of patients had other cardiovascular risk factors such as smoking (16.7%), hypertension (15.4%), or type 2 diabetes mellitus (3.5%). Thirty-eight (10.3%) reported previous premature ASCVD events and family history of ASCVD events was present in 33.9% of patients. According to DLCNC, about 50% of patients had definite or probable FH and the mean LDL-C was 167.4±67.9 mg/dL; 61.3% were taking LLTs.

Diagnostic Yield of Genetic Testing

Within the clinically defined FH cohort, 209 (56.5%) patients were classified as FH/M+ and 161 (43.5%) patients were classified as FH/M–.

The list of causative variants identified in patients with FH/M+ is reported in Table S1. Overall, 205 (98.1%) patients with FH/M+ were carriers of a single mutation, whereas 2 (1.4%) were carriers of double heterozygous mutations (*in cis*) in the *LDLR* gene. Three (1.4%) and 1 (0.4%) patients were found to be carriers of heterozygous mutations in *APOB* and *PCKS9* genes, respectively. No FH-causative variants were found in *LDLRAP1* or *LIPA* genes. We identified 97 unique mutations, 97.9% of which were within the *LDLR* gene. Most of them were already reported, and 15 were novel (Table S2).

Then, we assessed whether the LDL-C raising PRS could explain hypercholesterolemia in patients with FH/M–. To this aim, we compared the LDL-C raising PRS determined in the FH/M– group with that obtained in the group of subjects with normocholesterolemia. In this latter group, mean age was 41.3±12.2 years, the proportion of women 36.6%, and mean LDL-C level 115.3±37.8 mg/dL. The distribution of LDL-C raising PRS among patients with FH/M– was right skewed (toward higher scores) as compared with that among subjects with normocholesterolemia (Figure S2A), thus generating a mean LDL-C raising PRS higher than that in the reference population (0.69±0.20 versus 0.61±0.20; $P<0.001$). Interestingly, the distribution and

the mean value (0.61±0.24) of LDL-C raising PRS in FH/M+ did not differ from that in subjects with normocholesterolemia (Figure S2B). By applying the LDL-C raising PRS >0.69 as cutoff value for polygenic FH, we found that 89 (55.3%) of patients without monogenic cause of FH could be classified as PHC. Conversely, 72 (45.7%) patients with FH/M– showing LDL-C raising PRS ≤0.69 remained genetically undefined and thus classified as FH/M– undetermined.

Figure 1 show the distribution of FH genotypes according to DLCNC score. Among patients classified as "definite FH" 82.8% had a monogenic cause whereas only 10.1% had a polygenic cause of hypercholesterolemia. Conversely, among patients classified as "possible FH" 37.4% were monogenic and 34.1% were polygenic. This latter group contained the largest proportion (28.6%) of patients with genetically undefined FH.

Clinical characteristic of patients with FH according to genotypes are shown in Table S3. Those with monogenic FH were younger, showed higher untreated LDL-C, lower high-density lipoprotein cholesterol and higher prevalence of tendinous xanthomas as compared with polygenic or patients with FH/M– undetermined. By definition, PHC showed the highest LDL-C raising PRS, but monogenic FH also showed higher LDL-C raising risk score PRS than patients with FH/M– undetermined. No significant differences among groups were observed in the classical risk factors. However, when PHC was compared with FH/M– undetermined group, the latter showed significantly lower untreated LDL-C.

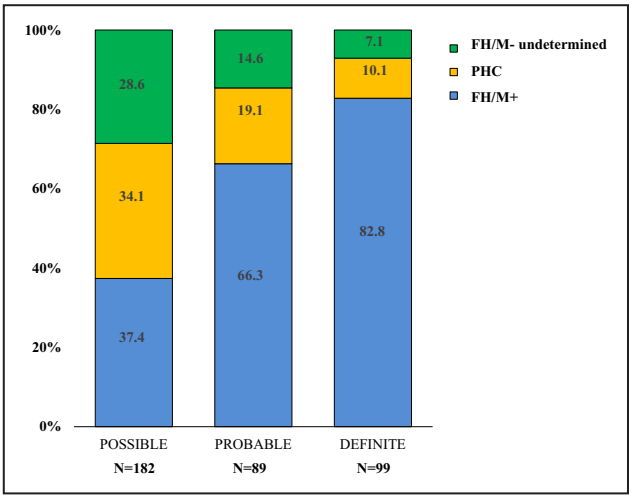

Figure 1. Distribution of genotypes according to Dutch Lipid Clinic Network Criteria scores.
Percentage is reported for each class of DLCNC score. Possible FH are patients with a DLCNC score between 3 and 5 points whereas Probable and Definite FH are those with a DLCNC scores between 6 and 8 points and higher than 8 points, respectively. Patients have been defined as FH/M+ if carrying a monogenic mutation, PHC if having a weighted polygenic risk score >0.69, and FH/M– undetermined if having elevated LDL-C and LDL-C raising genetic risk score ≤0.69 (see Methods). DLCNC indicates Dutch Lipid Clinic Network Criteria; FH, familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; N, number; and PHC, polygenic hypercholesterolemia.

Response to Lipid-Lowering Treatment According to Genotypes

As represented in Table 2, FH/M+ showed higher untreated and baseline LDL-C as compared with FH/M– subgroups. At baseline, patients with monogenic FH were receiving a more intense LDL-C lowering therapy than those with polygenic and undetermined hypercholesterolemia. During follow-up, patients received an intensification of LLT and this translated into an improvement of LDL-C control in all groups (Table 2). As patients with FH/M+ were exposed to more aggressive treatment (Table 2), the response to therapy was evaluated according to LLT intensity. As reported in Figure 2, LDL-C concentration at last visit was consistently higher in FH/M+ as compared with FH/M– across all categories of treatments. Accordingly, FH/M+ genotype was found to be a significant predictor of the last visit LDL-C level, even after adjustment for baseline LDL-C and LLT intensity (β=0.10; 95% CI, 0.004–0.100; P_{adj}=0.034). Of note, patients classified as FH/M– undetermined showed a response to therapy that appeared almost superimposable to that observed in PHC.

It is also worth mentioning that, despite maximizing the conventional LLT, the vast majority of patients with FH did not reach the recommended LDL-C target.[8] We observed that only the addition of PCSK9i to maximal LLT was able to bring LDL-C within the suggested target and this appeared to be independent from genotype[8] (Figure 2).

Impact of Genotypes on Coronary Atherosclerosis Burden

Among the 119 patients with clinically ascertained FH investigated with CTA, 79 were classified FH/M+ and 40 FH/M–. Their characteristics in comparison with those of pooled CTA study reference populations (controls, n=135) are shown in Table 3. As a whole, patients with FH were younger and thinner and reported higher prevalence of smoking; conversely, controls showed higher prevalence of hypertension and diabetes mellitus. As expected, on-treatment LDL-C was significantly higher in patients with monogenic FH as compared with controls, whereas it was comparable between patients with FH/M– and controls.

Data for CAC score are reported in Table 3. Median CAC score was significantly higher in patients with monogenic FH as compared with both patients with FH/M– and controls, whereas it did not differ between

Table 2. LDL-C Burden and LLT Changes in Clinically Defined FH Patients According to Genotypes During Follow-Up

	FH/M+ Monogenic (N=182)		FH/M– Whole (N=140)		PHC (N=75)		FH/M– Undetermined (N=65)		*P* Value FH/M+ vs FH/M–		*P* Value FH/M+ vs PHC		*P* Value FH/M+ vs FH/M– Undetermined		*P* Value PHC vs FH/M– Undetermined	
Duration of follow-up, mo	33.5 (14.0–72.0)		29.0 (12.0–60.0)		34.0 (12.0–60.0)		24.0 (11.0–61.0)		ns		ns		ns		ns	
LDL-C, mg/dL																
Untreated	258.5 (216.5–323.2)		207 (181.2–232.0)		213.8 (187.6–236.0)		195.0 (173.8–224.0)		<0.001		<0.001		<0.001		0.014	
Baseline	198.5 (162.7–239.1)		172.0 (139.4–207.8)		174.3 (143.2–209.6)		171.2 (139.4–204.0)		<0.001		0.001		0.002		ns	
Best on-treatment	103.9 (76.3–137.9)		103.1 (84.9–124.9)		107.2 (89.0–124.9)		99.0 (79.1–128.6)		ns		ns		ns		ns	
On-treatment	132.4 (107.4–167.2)		123.8 (104.0–149.5)		125.4 (112.6–149.0)		121.5 (93.9–151.0)		ns		ns		ns		ns	
Last visit	117.8 (92.5–159.6)		115.6 (95.2–149.4		116.2 (99.4–148.1)		109.2 (82.4–149.4)		ns		ns		ns		ns	
Last visit % reduction	41.4 (14.3–58.3)		25.8 (3.0–45.7)		23.5 (2.3–45.5)		27.6 (7.4–48.7)		0.001		0.001		0.04		ns	
LLT Intensity, n (%)																
	FH/M+ Monogenic (N=182)		**FH/M– Whole (N=140)**		**PHC (N=75)**		**FH/M– Undetermined (N=65)**		***P* Value FH/M+ vs FH/M–**		***P* Value FH/M+ vs PHC**		***P* Value FH/M+ vs FH/M– Undetermined**		***P* Value PHC vs FH/M– Undetermined**	
	Baseline	**Last Visit**	**Baseline**	**Last Visit**	**Baseline**	**Last Visit**	**Baseline**	**Last Visit**	**Baseline**	**Last Visit**	**Baseline**	**Last Visit**	**Baseline**	**Last Visit**	**Baseline**	**Last Visit**
Untreated	65 (35.7)	20 (11.0)	64 (46.0)	27 (19.6)	34 (45.3)	11 (14.9)	31 (47.7)	15 (23.1)	0.002	<0.001	0.010	<0.001	0.025	<0.001	ns	ns
Low-moderate	61 (33.5)	42 (23.2)	59 (42.4)	65 (47.1)	33 (44.0)	38 (51.4)	26 (40.0)	28 (43.1)								
High	38 (20.9)	40 (22.1)	12 (8.6)	32 (23.2)	5 (6.7)	17 (23.0)	7 (10.8)	16 (24.6)								
Maximal	17 (9.3)	46 (25.4)	4 (2.9)	10 (7.2)	3 (4.0)	6 (8.1)	1 (1.5)	4 (6.2)								
Low-moderate+PCKS9i	1 (0.5)	4 (2.2)	…	3 (2.2)	…	1 (1.4)	…	2 (3.1)								
Maximal+PCKS9i	…	29 (16.0)	…	1 (0.7)	…	1 (1.4)	…	…								

Data are reported as mean (± standard deviation), median (interquartile ranges) or number (percentage) as appropriate. Patients have been defined as FH/M+ monogenic if carrying a monogenic mutation and FH/M– if they were PHC or FH/M– undetermined (see Materials and Methods). For definition of LLT intensity see Materials and Methods. FH indicates familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; LLT, lipid lowering therapies; ns, not significant; PCSK9i, proprotein convertase subtilisin/kexin type 9 inhibitors; and PHC, polygenic hypercholesterolemia.

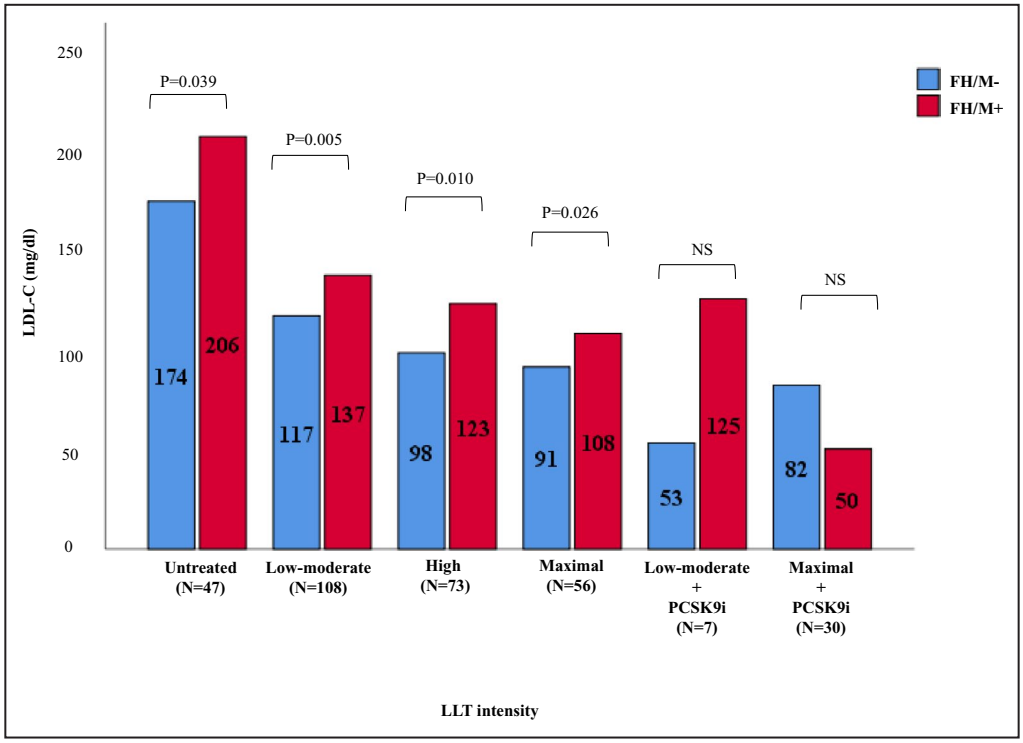

Figure 2. LDL-C levels at last visit according to intensity of lipid-lowering therapy.
We have reported the median LDL-C levels obtained at last visit according with genotypes and intensity of lipid-lowering therapies. Patients have been classified as FH/M+ if carrying a monogenic mutations and FH/M– if they were PHC or FH/M– undetermined (see Methods). For definition of LLT intensity see Methods. FH indicates familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; LLT, lipid-lowering therapies; PCKS9i, proprotein convertase subtilisin/kexin type 9 inhibitors; and PHC, polygenic hypercholesterolemia.

these latter groups. Figure 3A shows the percentage of patients with monogenic and FH/M– in each CAC categories. A CAC score above 100 occurred more frequently among patients with monogenic FH than in the other groups (χ^2=12.46, P=0.002$_{\text{for trend}}$). It is worth to mention that CAC score was also available for 15 patients with FH/M– undetermined having a polygenic risk score below our cutoff for PHC designation. Interestingly, an exploratory analysis suggested that these patients had CAC distribution that was similar to that in PHC (data not shown). In a multivariate linear regression analysis (stepwise method) including age, LLT use, current smoking, LDL-C at the time of imaging, and gender as covariates, Log (CAC+1) was significantly associated with monogenic FH (β=0.28; 95% IC, 0.32–1.02; P_{adj}<0.001), age (β=0.62; 95% IC, 0.04–0.07; P<0.001) and gender (β=0.25; 95% IC, 0.24–0.93; P_{adj}=0.001). These associations persisted even after adjustment for multiple testing (all bootstrap P_{adj}<0.001).

As expected, patients with clinically defined FH showed a higher number of plaques in coronary arteries as compared with controls (Table 3). Nevertheless, the number of lesions was significantly greater in the group with monogenic FH than in the group with FH/M– (7.8±8.9 versus 3.5±5.6; P<0.024) (Table 3). Moreover, 66.7% of patients with FH/M+ were showing more than 2 lesions as compared with 44.4% of patients with FH/M– and 29.2% of controls. Also, the proportion of patients with monogenic FH showing moderate-to-severe stenosis was greater, albeit not statistically significant, as compared with the other groups (24.1% in monogenic, 12.5% in FH/M–, and 9.6% in controls; $P_{\text{for trend}}$=0.075) (Figure 3B). In the logistic regression analysis (stepwise method) with the population with FH/M– as the reference group and age, gender, and smoking included as covariates, the risk of CAD-RADS score above 3 was 4-fold higher in patients with FH/M+ than FH/M– (odds ratio [OR], 4.2; 95% CI, 1.1–15.6; P_{adj}=0.034). After adjustment for multiple testing, patients with FH/M+ still showed 3.6-fold higher risk of having CAD-RADS score above 3 when compared with patients with FH/M– (bootstrap P_{adj}=0.042). However, when we added into the model the LDL-C value at the time of CTA the association between FH genotype and CAD-RADS score was only marginally significant (OR, 3.6; 95% CI, 0.94–13.95; bootstrap P_{adj}=0.06).

When considering controls as the reference population in the same multivariate model, the risk of having

Table 3. Characteristics of Individuals Participating to the Coronary CTA Study

	FH/M+ (N=79)	FH/M– (N=40)	Controls (N=135)	*P* for FH/M+ vs FH/M–	*P* for FH/M+ vs Controls	*P* for FH/M– vs Controls
Demographic						
Age, y	47.5±12.9 (19–73)	50.4±10.7 (29–72)	56.0±10.2 (30–79)	ns	<0.001	0.004
Males n, %	45 (57.0)	25 (62.5)	78 (57.8)	ns	ns	ns
BMI, kg/m^2	25.6±3.6	25.5±3.1	29.6±4.2	ns	<0.001	<0.001
Plasma lipids, mg/dL						
Total cholesterol	225.6±62.9	219.1±54.4	201.6±49.5	ns	ns	ns
HDL cholesterol	53.9±13.2	58.8±14.9	50.5±15.5	ns	0.05	0.002
LDL cholesterol	149.2±56.8	136.4±45.9	123.8±37.0	ns	0.003	ns
Total triglyceride	108.9±58.4	123.1±130.2	183.3±150.9	ns	<0.001	0.006
Medical history, n (%)						
T2DM	3 (3.8)	1 (2.6)	19 (14.1)	ns	0.017	0.047
Smokers	42 (53.2)	17 (43.6)	48 (36.6)	ns	0.042	ns
HTN	17 (21.5)	7 (17.9)	72 (53.3)	ns	<0.001	<0.001
LLT intensity, n (%)						
Untreated	6 (7.6)	11 (27.5)	118 (90.8)*	<0.001	<0.001	<0.001
Low-moderate	18 (22.8)	20 (50.0)	12 (8.9)*			
High	26 (32.9)	7 (17.5)	…			
Maximal	25 (31.6)	2 (5.0)	…			
Low-moderate plus PCKS9i	2 (2.5)	…	…			
Maximal plus PCSK9i	2 (2.1)	…	…			
Coronary atherosclerotic burden						
CAC score†	14.5 (0–161.6)	0 (0–31.8)	0 (0–7.6)	0.050	0.002	ns
No. of coronary lesions	7.8±8.9	3.5±5.6	1.9±4.7	0.024	<0.001	ns

Data are reported as mean (± standard deviation) or number (percentage) as appropriate. Plasma lipids are those measured at the time of CTA study. Patients have been defined as FH/M+ monogenic if carrying a monogenic mutation and FH/M– if they were PHC or FH/M– undetermined (see Materials and Methods). Smokers variables included active or past smoking. For definition of LLT intensity see Materials and Methods. Controls results from the combination of the reference populations (controls) for the coronary CTA study (see Material and Methods). BMI indicates body mass index; FH, familial hypercholesterolemia; HDL, high-density lipoprotein; HTN, hypertension; LDL, low-density lipoprotein; LLT, lipid-lowering therapies; ns, not significant; PCSK9i, proprotein convertase subtilisin/kexin type 9 inhibitors; PHC, polygenic hypercholesterolemia; and T2DM, type 2 diabetes.

*Information about the assumption of lipid-lowering therapies at the time of CTA was missing in 5 subjects from the reference population.

†CAC score was available in 77 subjects from controls. CAC scores are reported as mean (± standard deviation) of original scale values. However, they were compared between groups after log(CAC+1) transformation, as described in Material and Methods.

a CAD-RADS score above 3 was ≈12-fold higher in patients with monogenic FH/M+ (OR, 12.4; 95% CI, 3.07–37.35; P_{adj}<0.001) as compared with controls. This association was maintained even after bootstrap correction (OR, 10.0; 95% CI, 1.8–35.5; bootstrap P_{adj}<0.001). Conversely, no differences in CAD-RADS score were found after comparing patients with FH/M– and controls (OR, 3.4; 95% CI, 0.92–12.47; P_{adj}=0.06, bootstrap P_{adj}=0.19).

Impact of FH Genotypes on MACE

During a median follow-up period of 31.0 months (interquartile range 13.0–68.0 months), 18 MACE were registered among 322 patients with clinically diagnosed FH, representing 11.7 events per 10 000 person-year.[10,25] Sixteen were coronary end points (15 coronary revascularisations and 1 fatal myocardial infarction) and 2 were carotid stenting. Of these, 11 occurred in patients who were in primary prevention and 7 in patients who had a history of ASCVD at enrollment.

Figure 4 shows the Kaplan-Meier survival curves for MACE according to FH/M+ and FH/M– genotypes. The risk of incident MACE was 4-fold higher in patients with monogenic than in FH/M– (hazard ratio [HR], 4.1; 95% CI, 0.98–18.5; *P*=0.06). The results of multivariate analysis are reported in Table S4. The model including age at last visit and gender (Model 1) showed that the risk for MACE associated with the diagnosis of FH/M+ was about 5 times higher (HR, 4.8; 95% CI, 1.06–21.36; P_{adj}=0.041). This risk was even higher (HR, 9.1; 95% CI, 1.18–70.9; P_{adj}=0.034) when MACE at baseline, age at last visit, active smoking, and gender were considered as covariates (Model 2). Finally, these results remained almost unchanged even after adding

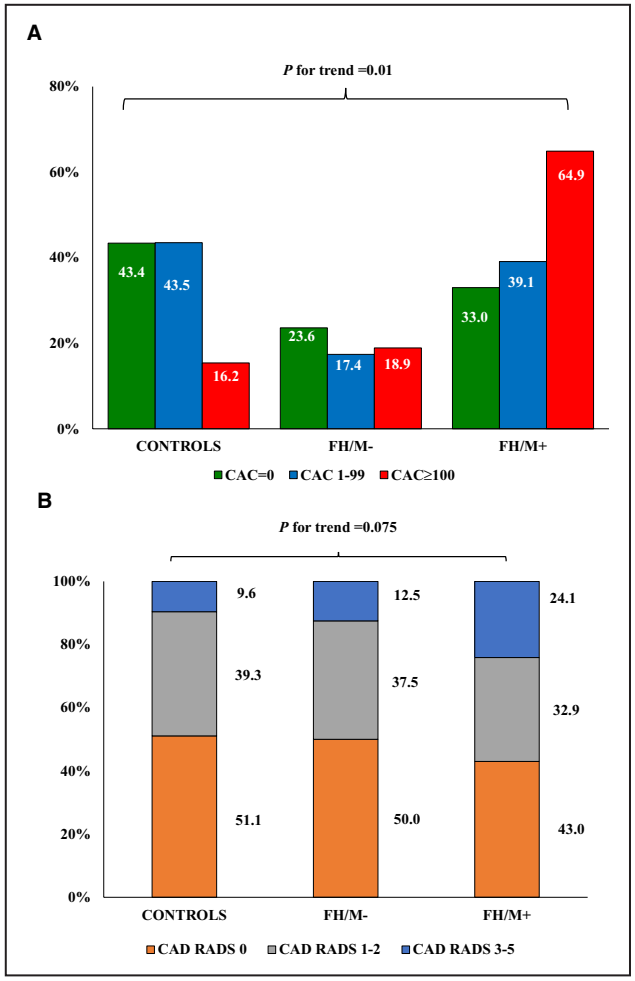

Figure 3. Coronary atherosclerosis burden according to genotypes.
A, Distribution of genotypes according to CAC categories. **B**, Distribution of severity of coronary stenosis expressed by CAD-RADS categories according to FH genotypes. Patients have been classified as FH/M+ if carrying a monogenic mutation (N=79), FH/M– if they were defined as PHC or FH/M– undetermined (N=40), and controls (N=135). CAC values were available in only 77 controls. For definition of CAD-RADS see Methods. CAC indicates coronary artery calcium; CAD-RADS, Coronary Artery Disease-Data and Reporting System; FH, familial hypercholesterolemia; and PHC, polygenic hypercholesterolemia.

into the model untreated LDL-C values (Model 3), the on-treatment LDL-C (Model 4) or after adjustment for multiple comparisons (Model 4; HR, 8.02; 95% CI, 1.01–63.5; bootstrap P_{adj}=0.048).

DISCUSSION

In this study, we have highlighted that monogenic FH was associated with lower responsiveness to conventional LDL-C lowering therapies, increased preclinical coronary atherosclerosis, and risk of atherosclerotic-related events when compared with patients with hypercholesterolemia with polygenic or unknown genetic cause. Our findings are in line with the growing body of evidence indicating that patients with monogenic FH may have a poorer prognosis than those without monogenic FH.[1,3,5,23] However, they add some elements of novelty. First, we found that asymptomatic, patients with treated hypercholesterolemia with a carefully defined monogenic status showed an increased burden of coronary atherosclerosis as compared with those whose hypercholesterolemia had a polygenic or a genetically undetermined cause. It is worth to note that in our cohort the coronary atherosclerosis burden was estimated by assessing at the same time CAC score and the severity of coronary luminal stenosis, measures that both have been demonstrated to be predictors of the risk of occurrence of ischemic clinical events.[1,8,23,26] This is consistent with the observation that in our series the presence of monogenic FH was associated with almost 4 times higher risk of occurrence of new MACE during about 3-years follow-up period. It is remarkable that the excess risk of atherosclerosis associated with monogenic FH was not completely captured by either baseline or on-treatment LDL-C levels observed in patients carrying this genotype. A clear explanation for this is not easily available. The simplest reason could be that the increase in LDL-C observed in monogenic FH may occur earlier in life,[10] thus determining a higher cumulative lifetime LDL-C exposure as compared with what occurs in patients without pathogenic mutations in FH-causing genes. In addition, the residual elevated on-treatment LDL-C levels in patients with monogenic FH during conventional LLT made patients carrying this genotype more susceptible to the long-term atherosclerotic damage.[3] An alternative explanation could lie in the mechanism of LDL-C elevation in monogenic FH, which is definitively due to reduction of LDLR activity unlike what occurs in PHC, where a variety of largely unknown mechanisms may be present.[2,3] Although additional investigations are needed to clarify these possibilities, our findings further confirm the importance of identifying the genotype underlying hypercholesterolemia, for example, monogenic versus polygenic. This allow to better guide the therapeutic choices in patients with molecularly confirmed monogenic hypercholesterolemia in whom a more intensive and earlier intervention is required.[1,9,27]

The second new piece of information provided by our study is represented by the demonstration that, during conventional LLT, patients who carry a pathogenic FH mutation (FH/M+) had a poorer response to the treatment compared with patients without monogenic mutations (PHC and FH/M-undetermined), whereas a comparable response was found in the 2 mutation negative groups. This was present even when high intensity

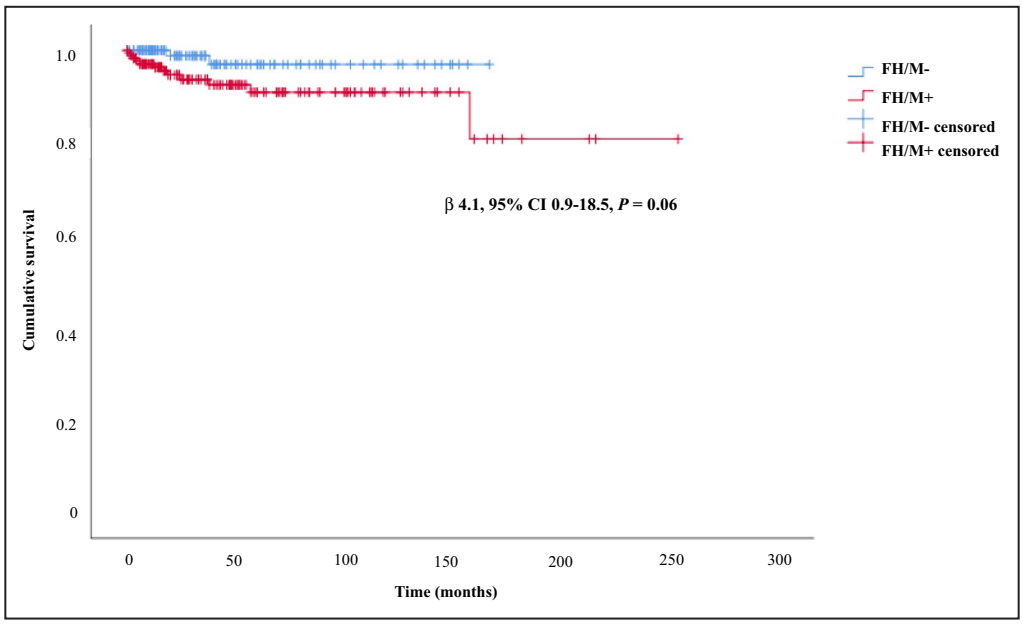

Figure 4. MACE-free survival curves according to genotypes.
Kaplan-Meier survival curves for incident major adverse cardiovascular events (MACE) occurred during follow-up in the cohort of clinical FH patients (322 subjects) according to genotypes. Patients have been defined as FH/M+ monogenic if carrying a monogenic mutation (N=182) and FH/M– if they are PHC or FH/M– undetermined (N=140). MACE have been defined as reported in Methods. FH indicates familial hypercholesterolemia; IC, confidence interval; and PHC, polygenic hypercholesterolemia.

or maximal LLT was prescribed. Previous studies have evaluated the effect of cholesterol-lowering treatments in heterozygous FH[3,9,28,29] but none was carried out among FH carefully classified as having a monogenic versus polygenic basis. Nevertheless, present data confirm that, overall, only a small proportion of monogenic (heterozygous) FH treated with conventional LLT reach the recommended target.[8] In this context, it is remarkable that only the use of PCSK9i allowed reaching the LDL-C targets in our patients with heterozygous FH. In addition, our survey confirms the observation of a comparable response to PCSK9i in both monogenic and polygenic patient groups. Previous studies have investigated this aspect,[3,30,31] but the present investigation has the advantage of being carried out in relatively larger and genetically classified group of patients with FH. Indeed, Lee et al[30] had published comparable biochemical responsiveness to PCSK9i in both monogenic (heterozygous) and polygenic hypercholesterolemia, but in smaller patient groups. Also, Brunham's group reported data about LLT response changes for patients with FH in pre- and post-PCSK9i era but without genetic classification.[3]

It has been suggested that the clinical diagnosis of FH must be based on DLCNC score.[8] In agreement with others,[32] we found that the likelihood of detecting FH-causing variants is greatest in patients with higher DLCNC scores; in our cohort more than 50% of patients with monogenic FH were in the DLCNC score categories of "definite" and "probable." We are increasingly becoming aware that there are important limitations to the currently used algorithms for the clinical diagnose of FH, namely DLCNC and Simon Broome Register.[32] Indeed, the clinical manifestations (premature corneal arcus, xanthomas, xanthelasma) are seen less frequently in modern practice, family history is sometimes unavailable or unreliable, baseline LDL-C (untreated) level is often difficult to reconstruct because of the use of LLT, and, finally, some subjects with causal mutation in FH-related genes might have an LDL-C <95th percentile. To overcome these difficulties, efforts to develop new and simpler algorithms to define FH are under way.[33] Indeed, a still open question is whether DLCNC has the same ability to predict the response to therapy and the risk of atherosclerosis of genetic testing. Even though this issue is out the scope of present work, preliminary data from our cohort indicate that DLCNC is globally less efficient than genotyping in predicting clinical outcomes in these patients with hypercholesterolemia (data not shown). However, further investigation into this aspect is urgently needed.

Study Limitations

We should acknowledge some strengths and limitations of current study. First, its retrospective, cross-sectional observational design is a key limitation. Moreover,

follow-up data were available only on the 322 index cases who have returned at least once after baseline visit and we were not able to assess patient's adherence to LLT. Despite this, our findings were based on one of the largest cohorts of Italian patients with clinically ascertained FH and were largely consistent with previous investigations. In particular, our on-treatment LDL-C and occurrence of MACE are in accordance with similar data from contemporary large FH registries.[28,29] Second, the assessment of pathogenicity of FH-causative variants was mostly based on in silico prediction, while no direct functional testing was carried out. However, it must be noted that most annotated variants have been already reported to be associated with the FH phenotype and some of the novel types have been segregated within families (data not reported). The LDL-C raising PRS was generated by using a simplified 6-SNP panel as opposed to other investigations where a much larger number of LDL-C raising common SNPs was employed.[3,34] This leaves the possibility that other relevant LDL-C rising variants were not considered in the present analysis. Nevertheless, the use of the 6-SNP panel to predict a polygenic basis in patients with FH not carrying monogenic mutations has been already validated by others.[3,4] Furthermore, one of our objectives was to verify if the use of a simplified genetic approach to the diagnosis of PHC could have relevant implications from a clinical point of view. The LDL-C raising PRS cutoff value for PHC was identified as the best discriminator between patients with FH/M– and individuals with normocholesterolemia in the Italian population. We believe this approach is the most robust and it can better reflect the interplay of population-specific genetic and environmental factors. Indeed, in our reference population with normocholesterolemia LDL-C level increases by an unadjusted value of 31 mg from the first to the last decile of PRS distribution (Figure S1). Third, we obtained coronary imaging in a small subgroup of patients who were not randomized to CTA evaluation, thus possibly leading to a bias. However, it must be noted that we have included in the atherosclerosis burden evaluation only patients who were completely asymptomatic at the time of examination and were not aware of their genetic FH status. Moreover, to strengthen CTA findings we have compared patients with FH with individuals belonging to the general population as well as to a high-risk population without FH. Finally, we have to acknowledge that only a small number of events occurred during follow-up and this might limit the soundness of our estimation of MACE associated to monogenic or polygenic hypercholesterolemia.

CONCLUSIONS

The main finding of this study is that the genetic background has a significant impact in determining the response to conventional LLT as well as the atherosclerosis burden in treated patients with clinically diagnosed FH. These results clearly demonstrate that genetic testing is crucial to improve atherosclerosis risk stratification in FH and might be helpful in planning more intensive therapeutic strategies. In this context, the advent of novel LLT, such as PCSK9i, might be useful in reducing the gap between the genetic forms of hypercholesterolemia.

ARTICLE INFORMATION

Received August 13, 2020; accepted February 19, 2021.

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Acknowledgments

We thank Dr Manuela Casula, Veronica Zampoleri, and Marta Gazzotti for their unparalleled work in the coordination and management of the LIPIGEN Registry.

Sources of Funding

The LIPIGEN-FH Study is an initiative of the SISA Foundation supported by an unconditional research grant from Sanofi.

Disclosures

D'Erasmo has received personal fees for public speaking, consultancy, or grant support from Amryt Pharmaceuticals, Akcea Therapeutics, Pfizer, Amgen, and Sanofi; Arca has received research grant support from Amryth Pharmaceutical, Amgen, IONIS, Akcea Therapeutics, Pfizer, and Sanofi; has served as a consultant for Amgen, Aegerion, Akcea Therapeutics, Regeneron, Sanofi, and Alfasigma and received lecturing fees from Amgen, Amryth Pharmaceutical, Pfizer, Sanofi, and AlfaSigma. The remaining authors have no disclosures to report.

Supplementary Material

Tables S1–S4
Figures S1–S2

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SUPPLEMENTARY MATERIAL

Table S1. List of variants considered for monogenic HeFH classification.

Mutation Type	N. of patients 209	LDL-C mg/dl	Number of Unique Mutations	Novel to This Study
***LDLR* (single mutations)**	**205**	**272.2 ± 83.6**	**91**	**15**
Nonsense	19	318.5 ± 108.0	9	2
Missense (and small in-frame deletion)	116	260.3 ± 76.4	48	4
Frameshift	29	288.6 ± 101.9	14	4
Splicing	18	265.6 ± 63.8	7	1
Copy number variation	21	283.9 ± 70.4	7	2
5'UTR	2	271 ± 33.2	2	-
***LDLR* (2 mutations in cis)**	**2**	**376.5 ± 48.8**	**4**	**2**
***APOB* (single mutations)**	**3**		**1**	**-**
Missense (and small in-frame deletion)	3	193.7 ± 9.7	1	-
***PCSK9* (single mutations)**	**1**		**1**	**-**
Missense (and small in-frame deletion)	1	252.0	1	-

LDL-C values are represented as mean (± standard deviation).

LDL-C, low-density lipoprotein cholesterol; *LDLR*, LDL receptor gene;5' UTR, 5' untranslated region of the gene; *APOB*, apolipoprotein B gene; *PSCK9*, proprotein convertase subtilisin/kexin type 9 gene.

Table S2. Novel variants identified in the *LDLR* gene in the present cohort.

Genetic Identifier	Proteic Identifier (sp)	*ACMG classification*
c.80_81delinsAA	p.Cys27*	Pathogenic
c.109G>A	p.Gly37Arg	Likely Pathogenic
c.169_182del	p.Glu58Valfs*67	Pathogenic
c.191-? 2311+?dup	p.(?)	Pathogenic
c.313+4_313+16del13	p.(?)	Pathogenic
c.363C>A	p.Cys121*	Pathogenic
c.628A>T	p.Ile210Phe	Likely Pathogenic
c.641G>A	p.Trp214*	Pathogenic
c.1299C>G	p.Asp433Glu	Likely Pathogenic
c.1360del	p.Thr454Profs*53	Pathogenic
c.1530_1532delGTT	p.Leu511del	Pathogenic
c.2054dup	p.Gln686Alafs*31	Pathogenic
c.2120A>C	p.Asp707Ala	Likely Pathogenic
c.2141-?_2583+?del	p.(?)	Pathogenic
c.2574_2575insA	p.Val859Serfs*66	Pathogenic

Novel variants identified in the *LDLR* gene and never associated with FH clinical phenotype in HGMD or UCL FH databases are shown.

Missense novel variants were deemed as monogenic FH-causing variants if reported as likely pathogenic or pathogenic according to the ACMG classification and following specific FH assumption as previously reported [16-17]. Variants predicting nonsense mutations or CNVs were classified as pathogenic. The NM_000527.4 was considered for all annotations.

Table S3. Comparison of clinical characteristics of patients with clinically diagnosed HeFH according to genotypes

Variables	FH/M+ Monogenic N=209	FH/M- Whole n=161		P FH/M+ monogenic vs PHC	P FH/M+ monogenic vs FH/M- undetermined	P PHC vs FH/M- undetermined
		PHC N=89	FH/M- undetermined N=72			
Age, yrs	44.8 ± 15.0	50.4 ± 13.4	49.6 ± 14.4	0.005	0.029	ns
Male, n (%)	99 (47.4)	44 (49.4)	35 (49.2)	ns	ns	ns
BMI, Kg/m2	24.9 ± 4.2	24.7 ± 3.0	25.2 ± 3.	ns	ns	ns
Arcus cornealis, n (%)	23 (11.0)	8 (8.9)	13 (18.1)	ns	ns	ns
Tendinous xanthoma, n (%)	16 (7.68)	2 (2.2)	0	0.041	0.016	ns
6-SNPs Score	0.61 ± 0.23	0.83 ± 0.08	0.52 ± 0.16	<0.001	<0.001	<0.001
Plasma Lipids, mg/dL						
Total cholesterol	353.3 ± 79.9	303.0 ± 45.7	292.3 ± 59.2	<0.001	<0.001	0.037
LDL cholesterol	271.5 ± 83.3	217.2 ± 40.6	205.7 ± 56.9	<0.001	<0.001	0.009
HDL cholesterol	59.0 ± 21.6	60.6 ± 17.0	60.6 ± 17.0	ns	ns	ns
Total triglycerides	127.3 ± 73.6	136.0 ± 75.7	125.5 ± 60.7	ns	ns	ns
Medical History, n (%)						
History of ASCVD	77 (33.6)	8 (8.9)	13 (18.1)	ns	ns	ns
T2DM	5 (2.3)	6 (6.7)	3 (4.1)	ns	ns	ns
Family history of ASCVD	166 (34.6)	28 (26.2)	29 (31.2)	ns	ns	ns
Smokers	38 (16.5)	8 (8.9)	4 (5.6)	ns	ns	ns

HTN	29 (13.8)	19 (21.3)	9 (12.5)	ns	ns	ns

Data are reported as Mean (± standard deviation) or number (percentage) as appropriate. Lipid data indicate untreated values.

Patients have been defined as FH/M+ monogenic if carrying a monogenic mutation and FH/M- if they were PHC or FH/M- undetermined (see Materials and Methods).

FH, Familial Hypercholesterolemia; PHC, polygenic hypercholesterolemia, BMI, Body Mass Index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ASCVD, atherosclerotic cardiovascular disease; T2DM, type 2 diabetes; HTN, hypertension.

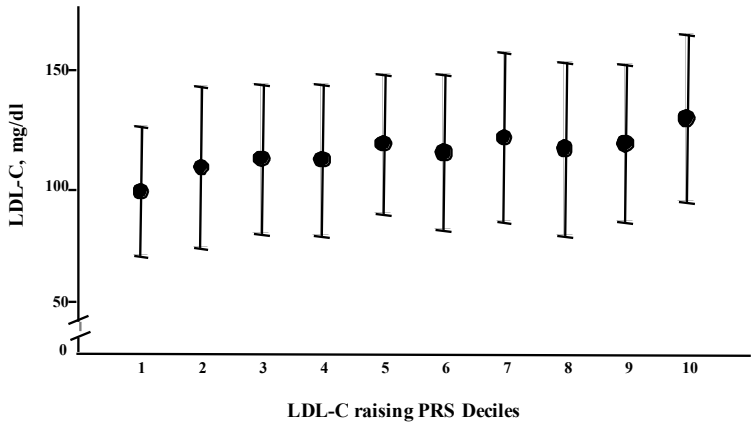
Table S4. Multivariate cox regression analysis (stepwise) for the risk of incident MACE at follow-up

	Model 1	Model 2	Model 3	Model 4
FH/M+	4.772 (1.06-21.36), 0.041	9.151 (1.18-70.99), 0.034	9.151 (1.18-70.99), 0.034	7.975 (1.01-62.41), 0.048
Male sex	ns	ns	ns	ns
Age at last visit	1.007 (1.003-1.011), 0.002	1.008 (1.003-1.013), 0.001	1.008 (1.003-1.013), 0.001	1.007 (1.002-1.012), 0.004
MACE baseline	-	ns	ns	ns
Active smoking	-	ns	ns	ns
Untreated LDL-C	-	-	ns	-
On-treatment LDL-C	-	-	-	ns

Data are expressed as Expβ (95%IC, P). Model 1 includes genotype, gender, age, Model 2 the same covariate of Model 1 plus smoking and MACE at baseline, Model 3 the same covariates of Model 2 plus untreated LDL-C and Model 4 the same covariates of Model 2 plus On-treatment LDL-C. A p value<0.005 is considered as significant.

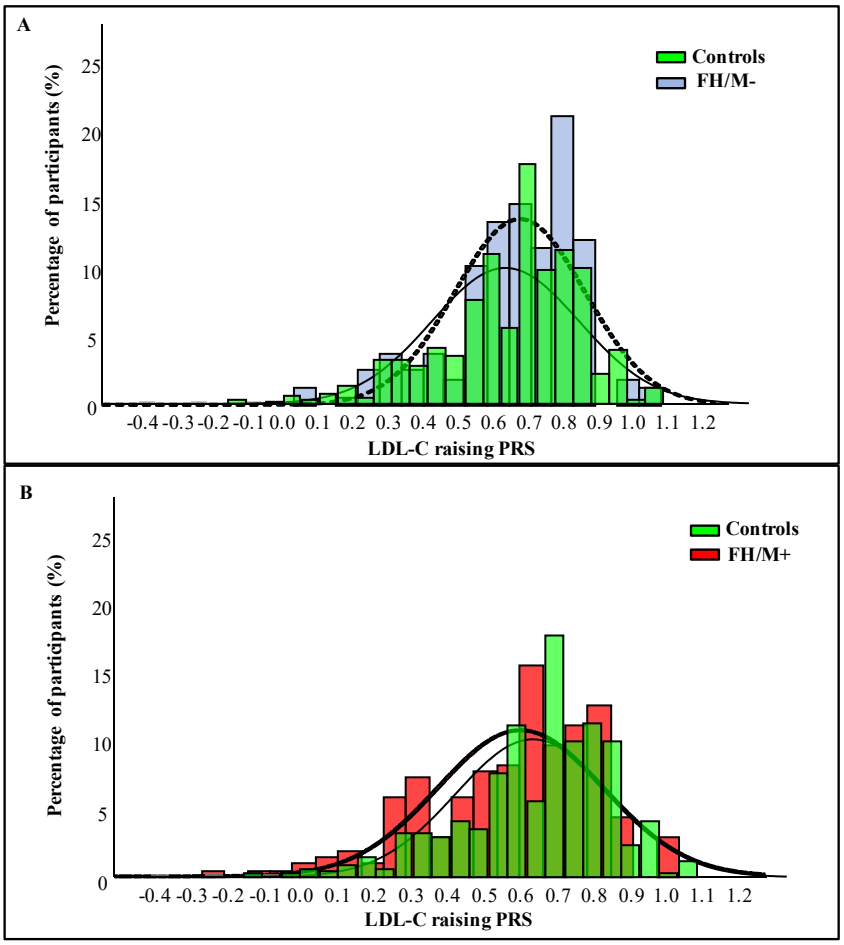
MACE, major cardiovascular events; FH/M+, monogenic hypercholesterolemia; LDL-C, low density cholesterol.

Figure S1. Correlation between LDL-C raising PRS and LDL-C values in normolipemic individuals



In this figure we represented the mean LDL-C values associated with each deciles of LDL-C raising PRS in normolipemic invididuals (Controls).
LDL-C, low density lipoprotein cholesterol; PRS, polygenic risk score.

Figure S2.



Distribution of LDL-C raising Polygenic Risk Score (PRS) in a reference Italian normolipemic population cohort (Controls) in patients carrying a monogenic FH-causing variant (FH/M+) or in mutation-negative (FH/M-) patients.

Panel A: Mutation-negative FH patients (FH/ M-) *vs*. healthy controls (Controls); **Panel B***:* Mutation-positive FH patients (FH/M+) vs. healthy controls (Controls).