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ORIGINAL RESEARCH

Twelve Variants Polygenic Score for Low-Density Lipoprotein Cholesterol Distribution in a Large Cohort of Patients With Clinically Diagnosed Familial Hypercholesterolemia With or Without Causative Mutations

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BACKGROUND: A significant proportion of individuals clinically diagnosed with familial hypercholesterolemia (FH), but without any disease-causing mutation, are likely to have polygenic hypercholesterolemia. We evaluated the distribution of a polygenic risk score, consisting of 12 low-density lipoprotein cholesterol (LDL-C)-raising variants (polygenic LDL-C risk score), in subjects with a clinical diagnosis of FH.

METHODS AND RESULTS: Within the Lipid Transport Disorders Italian Genetic Network (LIPIGEN) study, 875 patients who were FH-mutation positive (women, 54.75%; mean age, 42.47±15.00 years) and 644 patients who were FH-mutation negative (women, 54.21%; mean age, 49.73±13.54 years) were evaluated. Patients who were FH-mutation negative had lower mean levels of pretreatment LDL-C than patients who were FH-mutation positive (217.14±55.49 versus 270.52±68.59 mg/dL, $P<0.0001$). The mean value (±SD) of the polygenic LDL-C risk score was 1.00 (±0.18) in patients who were FH-mutation negative and 0.94 (±0.20) in patients who were FH-mutation positive ($P<0.0001$). In the receiver operating characteristic analysis, the area under the curve for recognizing subjects characterized by polygenic hypercholesterolemia was 0.59 (95% CI, 0.56–0.62), with sensitivity and specificity being 78% and 36%, respectively, at 0.905 as a cutoff value. Higher mean polygenic LDL-C risk score levels were observed among patients who were FH-mutation negative having pretreatment LDL-C levels in the range of 150 to 350 mg/dL (150–249 mg/dL: 1.01 versus 0.91, $P<0.0001$; 250–349 mg/dL: 1.02 versus 0.95, $P=0.0001$). A positive correlation between polygenic LDL-C risk score and pretreatment LDL-C levels was observed among patients with FH independently of the presence of causative mutations.

CONCLUSIONS: This analysis confirms the role of polymorphisms in modulating LDL-C levels, even in patients with genetically confirmed FH. More data are needed to support the use of the polygenic score in routine clinical practice.

Key Words: familial hypercholesterolemia ■ molecular diagnosis ■ polygenic risk score

Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder characterized by life-long exposure to elevated low-density lipoprotein cholesterol (LDL-C) levels, with an estimated prevalence as high as 1 in 200 people.[1,2] Patients with FH are at significantly higher risk of premature coronary disease

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CLINICAL PERSPECTIVE

What Is New?

- This analysis investigated the impact of a low-density lipoprotein polygenic score on low-density lipoprotein cholesterol levels in a large cohort of subjects with familial hypercholesterolemia with or without a causative mutation.
- Our findings indicate that the polygenic or monogenic causes of hypercholesterolemia are not mutually exclusive, but rather interacting entities.
- Our results led us to hypothesize that variants in multiple low-density lipoprotein cholesterol–raising genes can play a role in determining low-density lipoprotein cholesterol levels even in patients with monogenic familial hypercholesterolemia.

What Are the Clinical Implications?

- These data can integrate the evidence on polygenic scores associated with hypercholesterolemia, deepening the knowledge about the genetic basis of this pathology.
- Our results undermine the clinical benefit of the evaluation of the low-density lipoprotein polygenic score to guide diagnosis.
- More data are needed to investigate the association between the polygenic score and the risk of atherosclerotic cardiovascular disease to support the use of the score in clinical practice to accurately assess the risk.

Nonstandard Abbreviations and Acronyms

FH	familial hypercholesterolemia
FH/M+	mutation-positive FH
FH/M–	mutation-negative FH
LDLc-score	polygenic low-density lipoprotein cholesterol risk score

compared with the general population; however, a timely diagnosis and initiation of efficacious LDL-C–lowering therapies can significantly delay the onset of cardiovascular events and even normalize life expectancy.[3]

The most common FH-causing variants involve mutations in the *LDLR* gene, followed by mutations in the genes encoding the APOB (apolipoprotein B-100) and PCSK9 (proprotein convertase subtilisin/kexin type 9),[4] or biallelic mutations in the gene encoding for LDLRAP1 (low-density lipoprotein receptor adaptor protein 1).[5] Several attempts to identify new genes that could account for the FH phenotype[6] failed to detect single variants exhibiting effects equaling those in the previously mentioned genes, though mutations in *APOE* and *LIPA* genes have been reported to play a role.[7,8] Depending upon the diagnostic criteria used, a causative mutation in candidate genes can be detected in 40% to 80% of clinically defined patients with FH.[9–11] In all the other cases, the cause for the clinical phenotype of hypercholesterolemia remains undefined.

A possible explanation is that, in clinically diagnosed patients with FH without mutations in the classical genes, elevated LDL-C levels might have a polygenic cause. Such patients likely carry a cluster of common polymorphisms affecting several loci associated with raised LDL-C levels. To address this question, polygenic risk scores have been developed to predict LDL-C levels and atherosclerotic cardiovascular disease risk in these individuals.[12,13] A meta-analysis of genome-wide association studies by the Global Lipid Genetic Consortium identified several loci associated with raised LDL-C levels.[14,15] Talmud et al demonstrated that individuals carrying multiple LDL-C–raising single nucleotide polymorphisms may present with LDL-C levels similar to those observed in patients carrying FH-causative mutations.[16] In addition, in clinically diagnosed patients with FH without known monogenic mutations as compared with healthy controls, an elevated polygenic LDL-C score, calculated by incorporating 12 LDL-C–raising alleles, was reported, suggesting a potential polygenic cause for the hypercholesterolemic phenotype.[13] Even in patients with monogenic FH, a polygenic contribution may subsist, likely contributing to the variable phenotypic expression observed in patients carrying the same FH-causative mutation.[17]

Despite the growing attention to this aspect, several questions still remain to be addressed. It is not well defined to what extent polygenic hypercholesterolemia contributes to the prevalence of clinically suspected FH. Moreover, there is no consensus about the cutoff value of each polygenic score best discriminating FH from polygenic hypercholesterolemia. Finally, the impact of elevated polygenic score in predicting LDL-C levels in patients with or without monogenic variants has been only marginally investigated. These uncertainties still make it unclear whether and to what extent a polygenic involvement should be investigated in the clinical practice. As a consequence, the clinical potential of polygenic risk scores is still debated.[18]

In this study, we aimed at describing the distribution of the polygenic LDL-C risk score proposed by Talmud et al[13] (LDLc-score) in a large cohort of Italian subjects clinically diagnosed with FH, by comparing this distribution in subjects with or without causative mutations and evaluating its correlation with LDL-C levels.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files.

This analysis was performed in patients enrolled in the Lipid Transport Disorders Italian Genetic Network (LIPIGEN) study, an observational, multicenter, retrospective, and prospective ongoing study started in 2012 and aimed at identifying and registering patients with FH in Italy.[19,20]

Detailed information about the procedures of LIPIGEN study has been previously published.[19,21] In brief, patients with hypercholesterolemia attending >50 lipid clinics throughout Italy were enrolled in the registry if they had a clinical diagnosis of FH. The clinical diagnosis may be based either on the application of the clinical score or on the decision of the lipid specialist, supported by anomalies in the lipid profile or by the presence of a familial history of premature cardiovascular disease. After the visit by a specialized physician, patients with clinically suspicious primary hypercholesterolemia are referred for genetic testing of the appropriate candidate genes. Collected data include demographic and clinical data (age, sex, personal and family history of hypercholesterolemia or premature cardiovascular or cerebrovascular events, data from physical examination), pharmacological therapies, and biochemical data.

In the present analysis, we included adults with a clinical diagnosis of FH and with genetic test performed in a centralized laboratory searching for possible mutations in candidate genes (*LDLR*, *APOB*, *PCSK9*, *APOE*, *LDLRAP1*) and evaluating the 12 common LDL-C–raising single nucleotide polymorphisms included in the LDLc-score[13] (rs2479409 [*PCSK9* gene], rs629301 [*CELSR2* gene], rs1367117 [*APOB* gene], rs4299376 [*ABCG8* gene], rs1564348 [*SLC22A1* gene], rs1800562 [*HFE* gene], rs3757354 [*MYLIP* gene], rs11220462 [*ST3GAL4* gene], rs8017377 [*NYNRIN* gene], rs6511720 [*LDLR* gene], rs429358 [*APOE* gene], rs7412 [*APOE* gene]).

We selected patients with mutation-positive FH, with at least 1 causative mutation in 1 of the candidate genes (FH/M+), and patients with mutation-negative FH, without mutations in any of these genes[22] (FH/M–). Subjects presenting only variants of uncertain significance were excluded from the analysis.[22]

The LIPIGEN study has been approved by the Institutional Review Board of each participating center and conducted in accordance with the principles of the Helsinki Declaration, the standards of Good Clinical Practice (ICH GCP), the data protection laws, and other applicable regulations. Patients of any age and sex, with clinical suspicion of familial hypercholesterolaemia, who are able to understand the study procedures and who voluntarily agree to participate by providing written informed consent may be included into the study.

Statistical Analysis

Continuous variables are presented as mean±SD, whereas categorical variables are presented as cases and percentage rate. To compare mean LDLc-score values between FH/M+ and FH/M– groups, the Student *t* test was applied when the distributions did not fail the assumptions of normality.

A receiver operating characteristic analysis was also performed to determine whether the LDLc-score might discriminate individuals with a causative mutation from subjects who were mutation-negative. Sensitivity and specificity are presented as the measures to assess the effectiveness of the polygenic score, which indicates the ability of the LDLc-score to discriminate FH/M+ from subjects who were FH/M–. The Youden index method was applied to define the optimal cut point, the point maximizing the Youden function, which is the difference between true positive rate and false positive rate over all possible cut point values.

Correlations between the LDLc-score and LDL-C levels in subjects who were FH/M+ and FH/M– were assessed using the Spearman rank coefficient, and the Loess procedure was used to fit a smooth curve to the data, which attempts to capture the general pattern. The correlation was also tested among patients sharing the same mutation, selecting the most frequent one.

All analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC). Statistical significance was set at the 0.05 level for every analysis performed.

RESULTS

A total of 875 patients who were FH/M+ and 644 patients who were FH/M– were identified. Demographic and clinical data of FH/M– and FH/M+ groups are shown in the Table.

Although all patients who were FH/M– had a clinical phenotype consistent with a diagnosis of FH, they had lower mean levels of total cholesterol and pretreatment LDL-C, and higher levels of HDL cholesterol, triglycerides, and lipoprotein(a) than patients in the FH/M+ group (Table). Conversely, there were no statistically significant differences in the clinical history of premature coronary heart disease, or premature cerebral or peripheral vascular disease between individuals who were FH/M– and FH/M+ . Instead, the prevalence of tendinous xanthomata and arcus cornealis were significantly higher in the FH/M+ group (17.49% versus 4.19% and 13.71% versus 10.56%, respectively). No significant differences in lipid-lowering therapies were observed.

The distribution of the LDLc-score by genetic diagnosis is reported in Figure 1A. The mean value of the LDLc-score was 1.00 (±0.18) in patients who were FH/M– and 0.94 (±0.20) in patients who were FH/M+ (*P* value for the difference between means<0.0001, Figure 1B). Stratifying by pretreatment LDL-C levels,

Table. Clinical, Demographic, and Biochemical Profile of Adults With and Without an Identified Causative Mutation

	FH/M–, N=644	FH/M+, N=875	
	Mean [SD]/median [IQR]*	Mean [SD]/median [IQR]*	*P* value
Age at baseline, y	49.73 [13.54]	42.47 [14.96]	<0.0001
Total cholesterol, mg/dL	272.69 [72.63]	313.05 [86.26]	<0.0001
Triglycerides, mg/dL*	126 [89–177]	98 [71–137]	<0.0001
HDL-C, mg/dL	59.88 [17.12]	56.1 [15.07]	<0.0001
Lp(a), mg/dL*†	39.95 [8.4–98]	19.05 [8.55–37]	0.003
Glucose, mg/dL‡	94.78 [23.78]	89.29 [18.44]	0.0002
Pretreatment LDL-C, mg/dL	217.14 [55.49]	270.52 [68.59]	<0.0001
	N (%)	N (%)	*P* value
Women	348 (54.21)	478 (54.75)	0.83
First-degree relative with premature CHD	214 (33.23)	352 (40.23)	0.0205
First-degree relative with LDL-C >95th percentile	437 (67.86)	752 (85.94)	<0.0001
First-degree relative with tendinous xanthomata and/or arcus cornealis	19 (2.95)	101 (11.54)	<0.0001
Children <18 years with LDL-C >95th percentile	80 (12.42)	197 (22.51)	<0.0001
Clinical history of premature CHD	50 (7.76)	76 (8.69)	0.52
Clinical history of premature cerebral or peripheral vascular disease	27 (4.19)	35 (4.00)	0.85
Tendinous xanthomata	27 (4.19)	153 (17.49)	<0.0001
Arcus cornealis before age 45 y	68 (10.56)	120 (13.71)	0.07
Pretreatment LDL-C value			
155–190 mg/dL	93 (14.44)	52 (5.94)	<0.0001
191–250 mg/dL	335 (52.02)	308 (35.20)	
251–325 mg/dL	124 (19.25)	322 (36.80)	
>325 mg/dL	20 (3.11)	171 (19.54)	
Lipid-lowering therapy	198 (30.75)	268 (30.63)	0.96

CHD indicates coronary heart disease; FH, familial hypercholesterolemia; FH/M+, patients with mutation-positive FH; FH/M–, patients with mutation-negative FH; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; and Lp(a), lipoprotein(a).

*Median [interquartile range].

†N=124 (FH/M–) and N=172 (FH/M+).

‡N=392 (FH/M–) and N=495 (FH/M+).

higher mean LDLc-score levels (Figure 2) were observed among patients who were FH/M– having LDL-C levels between 150 and 350 mg/dL. However, when the receiver operating characteristic analysis was performed (Figure S1A), the area under the curve predicting polygenic hypercholesterolemia was 0.59 (95% CI, 0.56–0.62), with sensitivity and specificity being 77% and 36%, respectively, at 0.905 as a cutoff value (Figure S1B).

When we explored the correlation between LDLc-score and LDL-C levels in subjects who were FH/M+ or FH/M–, a positive trend was observed among subjects who were FH/M– (*R*, 0.13; *P*=0.0006) that was even more marked among subjects who were FH/M+ (*R*, 0.15; *P*<0.0001) (Figure 3). We also confirmed this correlation in a subgroup of patients carrying the most frequent mutation affecting *LDLR* (c.662A>G, p. Asp221Gly [N=72, Figure S2]; *R*, 0.31; *P*=0.009).

DISCUSSION

In recent years, because of the methodological advances in genetic analysis techniques and to genome-wide association studies, several polygenic scores for LDL-C have been proposed that may help explain the significant proportion of patients having a clinical phenotype of FH but with a negative genetic test for mutations of known causative genes.[18]

Summary of Our Results and Comparisons

In the present analysis, the polygenic score proposed by Talmud et al[13] was tested in a population of adults with a clinical diagnosis of FH. Compared with the original publication, our study population was larger, but with comparable LDL-C levels and mean values of LDLc-score (1.00 in FH/M– and 0.94 FH/M+ in

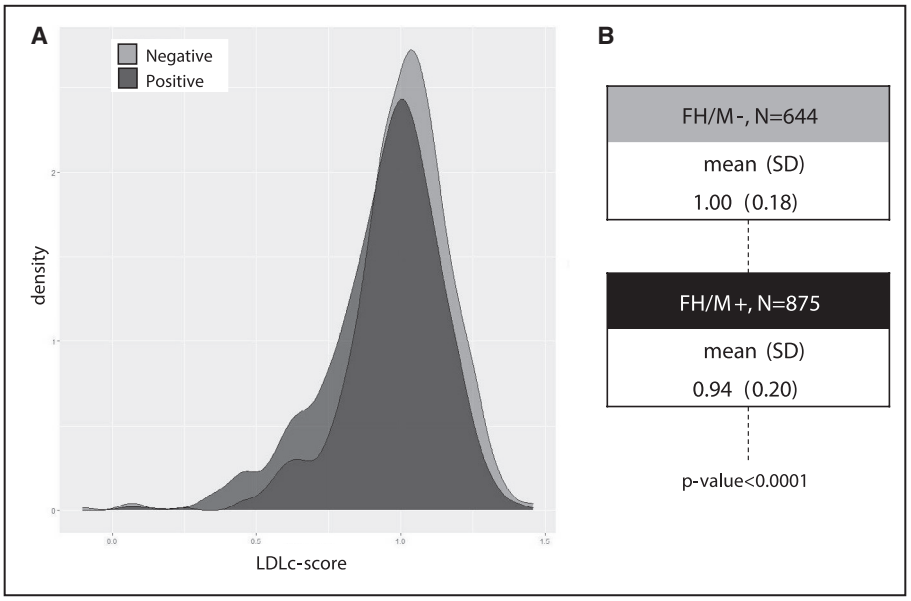

Figure 1. Distribution (A) and mean (SD) values (B) of the LDLc-score in FH/M– and FH/M+ patients with FH.
FH indicates familial hypercholesterolemia; FH/M+, patients with mutation-positive FH; FH/M–, patients with mutation-negative FH; and LDLc-score, polygenic low-density lipoprotein cholesterol risk score.

the LIPIGEN cohort, and 1.00 and 0.95, respectively, in Talmud et al). These results validate the use of the LDLc-score in a different population of European ancestry.[23]

Talmud et al also described the correlation between the LDLc-score and LDL-C concentrations in a healthy group of White men and women from the UK Whitehall II study,[13] showing increasing levels of

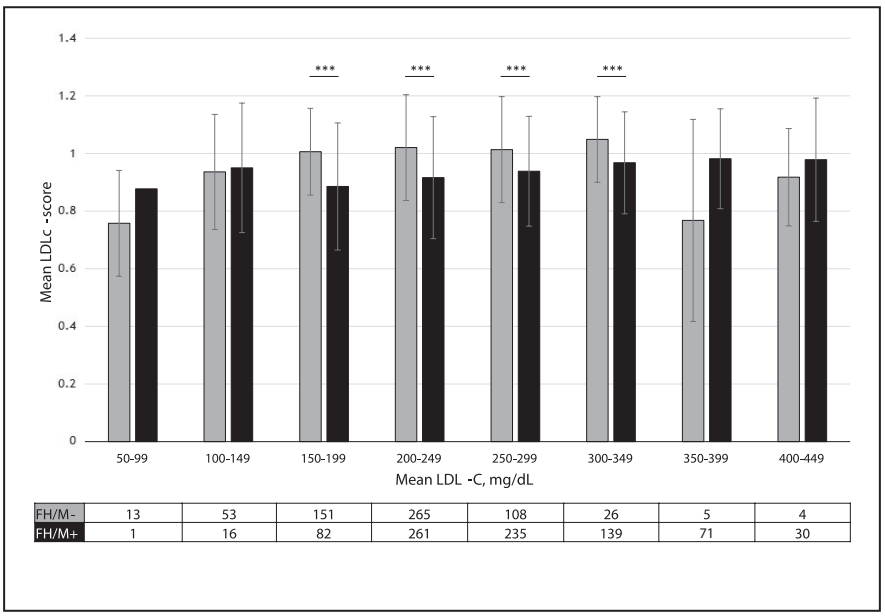

Figure 2. Mean values of LDLc-score by LDL-C classes in patients with FH/M– and FH/M+ FH.
FH indicates familial hypercholesterolemia; FH/M+, patients with mutation-positive FH; FH/M–, patients with mutation-negative FH; LDL-C, low-density lipoprotein cholesterol; and LDLc-score, polygenic LDL-C risk score. *** means *P*value for differences among gentic classes lower than 0.001 (*P* value<0.001).

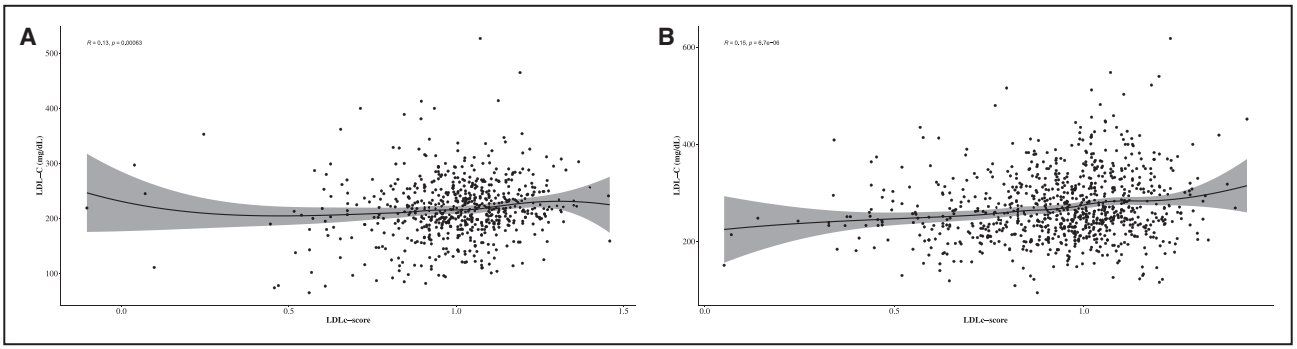

Figure 3. Correlation between LDL-C levels and LDLc-score in mutation-negative (A) and mutation-positive (B) FH patients.
FH indicates familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; and LDLc-score, polygenic LDL-C risk score.

LDL-C, ranging from 145 mg/dL to 190 mg/dL, from the first to the last LDLc-score decile (0.43–1.23). In our cohort, we confirmed this correlation both in subjects who were FH/M+ and FH/M–, with LDL-C levels ranging from 182 to 230 mg/dL and from 258 to 281 mg/dL, respectively, from the first to the last LDLc-score decile (Figure S3).

The impact of the polygenic score seems to be more relevant among subjects who were FH/M+, as demonstrated by the Pearson correlation coefficient. The additional impact of the polygenic score in subjects with monogenic FH is also supported by the positive correlation between LDLc-score and LDL-C levels in subjects bearing the same causative mutation (Figure S2). These findings indicated that the polygenic or monogenic causes of hypercholesterolemia are not mutually exclusive, but rather interacting entities.[18] Although in both groups the mean LDL-C levels increased as the score increased, mean LDL-C levels in subjects who were FH/M+ were ≈30% higher than in subjects who were FH/M– within the same deciles of the LDLc-score.

In addition, our results led us to hypothesize a possible involvement of additional factors (ie, diet, lifestyle habits) in determining the variability of LDL-C levels. In the absence of a known causative mutation, the increase in LDL-C levels would be expected to be driven by polymorphisms in multiple genes included in the LDLc-score. However, the difference in the polygenic score among FH/M– and FH/M+ individuals is modest both in our analysis and in other studies.[13,24] Moreover, the comparison between LIPIGEN subjects who were H/M– and the healthy subjects from the Whitehall II study showed that the LDL-C level in the former is about 30% higher than in the latter group, despite comparable polygenic score values. These observations suggest that, in subjects with the FH phenotype but lacking a causative mutation (FH/M–), lipid levels may be determined by the interplay between genetic factors, including the polymorphisms evaluated in our study but also other unknown genetic determinants, and environmental factors.

A multifactorial nature, with the concurrent participation of genetic, epigenetic, and environmental factors, has been suggested for cardiovascular disease[25] as well as for many other human disorders such as cancer or diabetes.[26,27] The exposure to environmental factors can modulate a genetic predisposition, and in turn, genetic predisposition can modify the effects of the environment.[28,29] The still incomplete understanding of the genetic basis of cardiovascular disease and the crucial impact that genes, environment, and their unceasing interaction exert on this condition can explain, at least in part, the relative failure of lifestyle interventions to prevent and treat cardiovascular disease and its risk factors. This calls for a better understanding of the complex interplay between genomic, environmental, and epigenetic contributions to cardiovascular disease with the aim of improving diagnosis, treatment, and the approach to each patient.[30]

Clinical Implications

Many studies have addressed the issue of the diagnostic and prognostic usefulness of genetic scores and their clinical potential in the management of patients with hypercholesterolemia.

The rationale is related to the need to identify subjects having a lifetime exposure to high levels of LDL-C, who unveil an increased risk of early cardiovascular events. However, this point calls into question a further aspect that is missing in the current literature, namely, the characterization of the trend of LDL-C levels from youth to adulthood in subjects with polygenic hypercholesterolemia. In these individuals, in whom genetic susceptibility plays a relevant role, additional environmental factors may contribute the increase in LDL-C levels later in life. Thus, another area deserving further research is the possible gene–environment interaction, which implies that the

effect of the polygenic score would be affected by environmental risk factors.[31]

Previous studies have established that polygenic scores for LDL-C independently associate with the risk of CVD. In a study including participants from the UK Biobank cohort, the CV risk increased in a dose-dependent manner with increasing LDL-C polygenic score, with a hazard ratio of 1.35 (95% CI, 1.30–1.40) for the 10th decile compared with the first decile of the polygenic score.[32] Therefore, improving current risk models by means of these scores, which allow quantifying the lifelong cumulative burden of LDL-C, might be crucial in clinical practice, because it can improve both diagnosis and long-term prognosis, especially in young subjects, or highlight the long-lasting exposure to high cholesterol levels, which may be relevant in patients diagnosed later in life. This, in turn, may help in defining which patients are likely to benefit most from pharmacological interventions.[31,33]

However, to translate this evidence into personalized indications for the patients in the clinical practice, some aspects need to be taken into consideration. First, polygenic hypercholesterolemia, unlike monogenic hypercholesterolemia, is not a dichotomous diagnosis but rather a continuous scale that confers cardiovascular disease risk in a dose-dependent manner. Second, whereas a mutation that negatively impacts LDL receptor activity necessarily leads to an increase in circulating LDL-C levels, the polygenic score is indicative of a greater probability of having high LDL-C concentrations. In this context, where the impact of other determinants, such as diet, lifestyle, or comorbidities may be larger, the approach to the patient must be highly personalized. Third, cascade screening and analysis of segregation pattern are crucial in monogenic FH, where only 1 mutation is responsible for the clinical phenotype,[34–36] but polygenic hypercholesterolemia does not follow an autosomal dominant pattern of inheritance, resulting in a disruption of the cascade screening.

Strengths and Limitations

The major strength of this study is the analysis of an LDL-C polygenic score on a large sample of subjects with a clinical diagnosis of FH. Furthermore, the participation of all specialists in the LIPIGEN network and the centralized laboratory assure a shared approach for the clinical diagnosis and a unique analytical procedure.

We used the score proposed by Talmud et al,[13] including 12 single nucleotide polymorphisms and based on Global Lipid Genetic Consortium data from >100 000 participants.[14] Even if it is likely an unbiased and robust genetic instrument for LDL-C–raising alleles, we cannot exclude the possibility of further refinements.

Finally, another limitation of the study is that we cannot address the contribution of the LDLc-score to the development of atherosclerotic cardiovascular disease in terms of future events, because the collection of follow-up data is still ongoing.

CONCLUSIONS

The results of this analysis, which applied a polygenic risk score to a large sample of subjects with a clinical diagnosis of FH, confirmed the role of polymorphisms in modulating LDL-C levels, and suggested that variants in multiple LDL-C–raising genes can play a role in determining LDL-C even in patients with monogenic FH. These data support the application of polygenic risk scores to refine the diagnosis and the prediction of future cardiovascular risk.

APPENDIX

LIPIGEN Study Group

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Supplemental Material

Figures S1–S3

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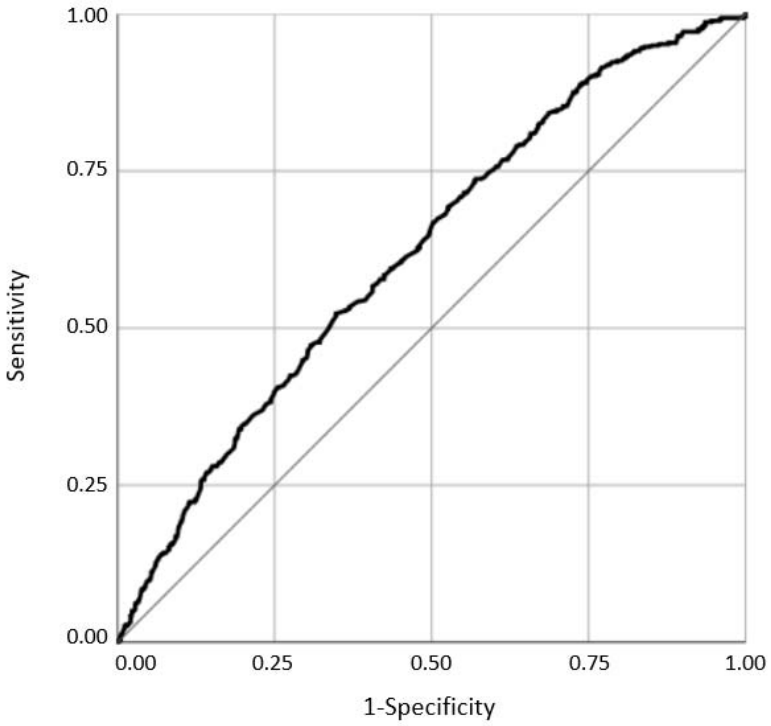
SUPPLEMENTAL MATERIAL

Figure S1. Receiver operating characteristic (ROC) curve for LDLc-score for the diagnosis of a polygenic aetiology (A) and classification of adults with (FH/M+) and without (FH/M-) according to a 0.905 cut off in LDLc-score (B).



(A)

Model (AUC): 0.59 [0.56-0.62]

Cut-off: 0.905
Sensitivity: 77%
Specificity: 36%
Youden index: 0.14

	FH/M-	FH/M+	total
≤0.905	148	315	463
	31.97	68.03	
	22.98	36.00	
>0.905	496	560	1056
	46.97	53.03	
	77.02	64.00	
Total	644	875	1519

(B)

Figure S2. Correlation between LDL-C levels and LDLc-score in mutation-positive FH subjects carrying the mutation c.662A>G (p.Asp221Gly).

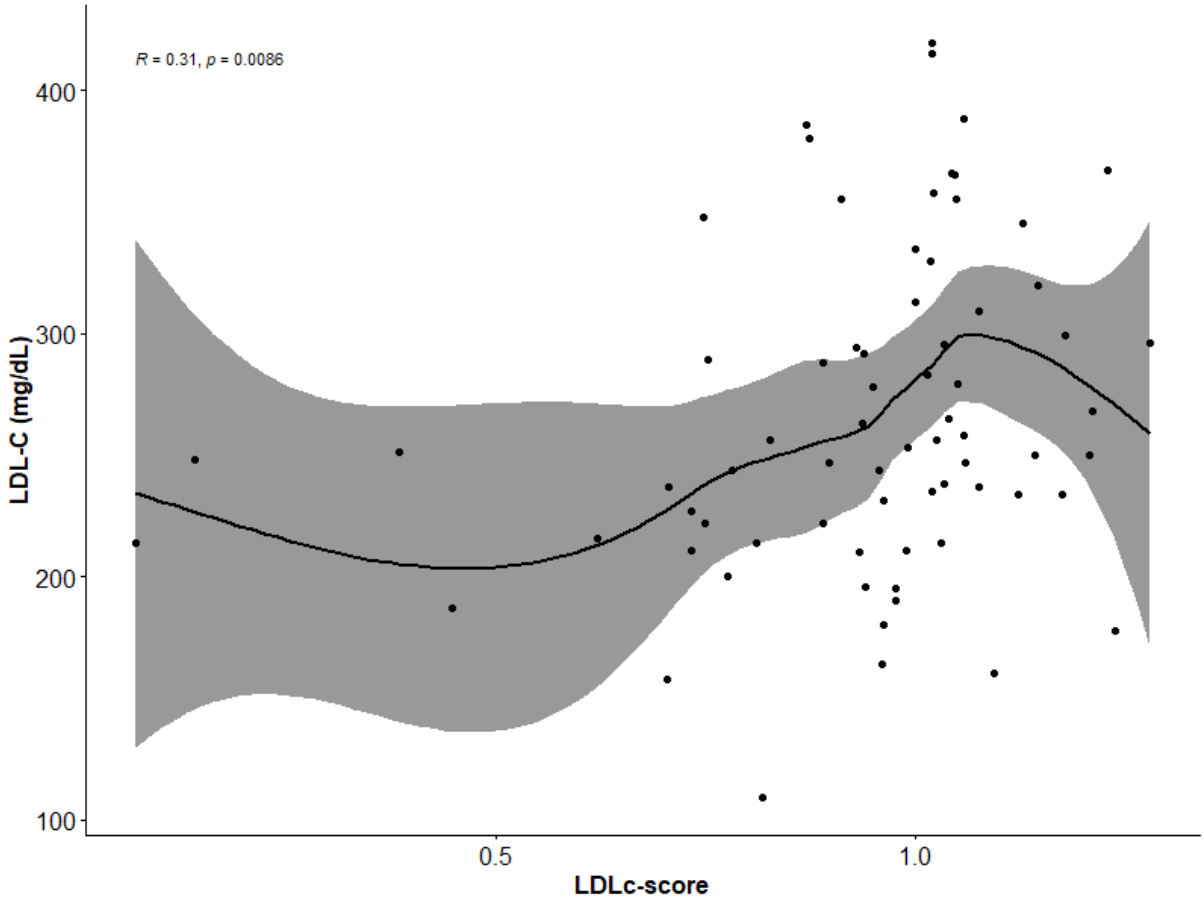


Figure S3. LDL-C levels by LDLc-score deciles (as defined by Talmud et al.[13]) in UK Whitehall II study and in the FH/M- and FH/M+ LIPIGEN cohorts.