









ORIGINAL RESEARCH

# Lipoprotein(a) Genotype Influences the Clinical Diagnosis of Familial Hypercholesterolemia

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**BACKGROUND:** Evidence suggests that *LPA* risk genotypes are a possible contributor to the clinical diagnosis of familial hypercholesterolemia (FH). This study aimed at determining the prevalence of *LPA* risk variants in adult individuals with FH enrolled in the Italian LIPIGEN (Lipid Transport Disorders Italian Genetic Network) study, with (FH/M+) or without (FH/M-) a causative genetic variant.

**METHODS AND RESULTS:** An Ip(a) [lipoprotein(a)] genetic score was calculated by summing the number risk-increasing alleles inherited at rs3798220 and rs10455872 variants. Overall, in the 4.6% of 1695 patients with clinically diagnosed FH, the phenotype was not explained by a monogenic or polygenic cause but by genotype associated with high Ip(a) levels. Among 765 subjects with FH/M- and 930 subjects with FH/M+, 133 (17.4%) and 95 (10.2%) were characterized by 1 copy of either rs10455872 or rs3798220 or 2 copies of either rs10455872 or rs3798220 (Ip(a) score  $\geq 1$ ). Subjects with FH/M- also had lower mean levels of pretreatment low-density lipoprotein cholesterol than individuals with FH/M+ (*t* test for difference in means between FH/M- and FH/M+ groups  $<0.0001$ ); however, subjects with FH/M- and Ip(a) score  $\geq 1$  had higher mean (SD) pretreatment low-density lipoprotein cholesterol levels (223.47 [50.40] mg/dL) compared with subjects with FH/M- and Ip(a) score = 0 (219.38 [54.54] mg/dL for), although not statistically significant. The adjustment of low-density lipoprotein cholesterol levels based on Ip(a) concentration reduced from 68% to 42% the proportion of subjects with low-density lipoprotein cholesterol level  $\geq 190$  mg/dL (or from 68% to 50%, considering a more conservative formula).

**CONCLUSIONS:** Our study supports the importance of measuring Ip(a) to perform the diagnosis of FH appropriately and to exclude that the observed phenotype is driven by elevated levels of Ip(a) before performing the genetic test for FH.

**Key Words:** cardiovascular risk ■ clinical diagnosis ■ familial hypercholesterolemia ■ lipoprotein(a)

Familial hypercholesterolemia (FH) is a genetic inherited disorder mainly caused by variants in genes encoding for the LDL receptor (LDLR), apoB (apolipoprotein B), or PCSK9 (proprotein convertase subtilisin/kexin type 9). FH is characterized by lifelong exposure to elevated low-density lipoprotein cholesterol (LDL-C) levels and subsequent premature cardiovascular disease development.<sup>1</sup> Depending upon the

diagnostic criteria used, a causative variant in candidate genes can be detected in 40% to 80% of clinically defined patients with FH. In all other cases, the cause for the clinical phenotype of hypercholesterolemia remains undefined.<sup>2</sup> In some cases, these patients likely carry a cluster of common polymorphisms affecting several loci associated with raised LDL-C levels, and a polygenic cause can be suspected.<sup>3</sup> In all other cases,

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\*A complete list of the LIPIGEN Study Group members can be found in the Appendix at the end of the article.

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## CLINICAL PERSPECTIVE

### What Is New?

- In the 4.6% of patients with clinically diagnosed familial hypercholesterolemia, the phenotype was not explained by a monogenic or polygenic cause but by the genotype associated with high Lp(a) [lipoprotein(a)] levels.
- The adjustment of low-density lipoprotein cholesterol levels based on Lp(a) concentration reduced from 68% to 42% the proportion of subjects with low-density lipoprotein cholesterol levels  $\geq 190$  mg/dL (or from 68% to 50% considering a more conservative formula).

### What Are the Clinical Implications?

- In patients with a clinical suspicion of familial hypercholesterolemia, Lp(a) should be measured to perform an appropriate diagnosis and to exclude the possibility that the observed phenotype is driven by elevated levels of Lp(a) before performing the genetic test.
- The differentiation of familial hypercholesterolemia, elevated Lp(a), or the combination of both is crucial for an accurate risk stratification.
- In the future, patients with both elevated Lp(a) and familial hypercholesterolemia condition may be shown to benefit from interventions to reduce both low-density lipoprotein cholesterol and Lp(a) plasma levels.

### Nonstandard abbreviations and acronyms

<b>FH</b>	familial hypercholesterolemia
<b>FH/M-</b>	negative at FH genetic test
<b>FH/M+</b>	positive at FH genetic test
<b>LDLR</b>	LDL receptor
<b>LIPIGEN</b>	Lipid Transport Disorders Italian Genetic Network
<b>Lp(a)</b>	lipoprotein(a)
<b>LPA</b>	lipoprotein(a) coding gene

the presence of unknown genetic or environmental factors could be assumed, which results in a FH phenotype consistent with that observed in monogenic FH. This clinical and genetic heterogeneity can make the FH diagnosis very complex.

It has been already described that patients clinically diagnosed with FH have elevated levels of Lp(a) [lipoprotein(a)],<sup>4,5</sup> which is a causal genetic risk factor for atherosclerotic cardiovascular disease, but the reasons for this remain unclear. It has been suggested, however, that high Lp(a) cholesterol could be responsible for clinical FH.<sup>4,6</sup>

Lp(a) levels are 75% to 95% heritable and predominantly determined by single-nucleotide variants at the *LPA* gene and copy number variants specifically in the kringle IV type 2 domain.<sup>7</sup> To date, 2 specific genetic variants in the *LPA* gene have been shown to significantly influence Lp(a) plasma values: rs3798220 (Ile4399→Met) and rs10455872 (intronic A/G polymorphism).<sup>8</sup> A single copy of the rs10455872-G allele, which is common in European populations, is known to associate with extremely elevated Lp(a) that can result in a phenotype similar to FH.<sup>9</sup> This evidence points out the possibility that *LPA* genotypes are a possible cause of clinical FH, especially in those individuals presenting a low LDL-C polygenic predisposition.

The objective of this study was to determine the prevalence of *LPA* risk variants in a large cohort of Italian subjects clinically diagnosed with FH, by comparing this distribution in subjects with or without causative variants.

## METHODS

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

This analysis was performed in patients enrolled in the LIPIGEN (Lipid Transport Disorders Italian Genetic Network) study, an observational, multicenter, retrospective, and prospective ongoing study started in 2012 and aimed at identifying and registering patients with FH in Italy.<sup>10</sup> Detailed information about the procedures of LIPIGEN study has been previously published.<sup>10</sup> The LIPIGEN project and its amendments were approved by the Ethics Committee of the Coordinating Unit (Ethical Committee IRCCS MultiMedica, Via Milanese 300—20099, Sesto San Giovanni, Milan, September 13, 2011 and June 23, 2015) and by ethics committees of each participating institution. Eligible patients who were able to understand the study procedures and who voluntarily agreed to participate provided written informed consent.

In brief, patients with hypercholesterolemia attending lipid clinics were enrolled in the registry if they had a clinical diagnosis of FH. The clinical diagnosis may be guided by clinical scores, such as the Dutch Lipid Clinic Network Score,<sup>11</sup> and ultimately based on the decision of the lipid specialist, supported by an evocative lipid profile or by a familial history of premature cardiovascular disease. After the visit, patients fulfilling the aforementioned criteria are referred for genetic testing of the appropriate candidate genes.

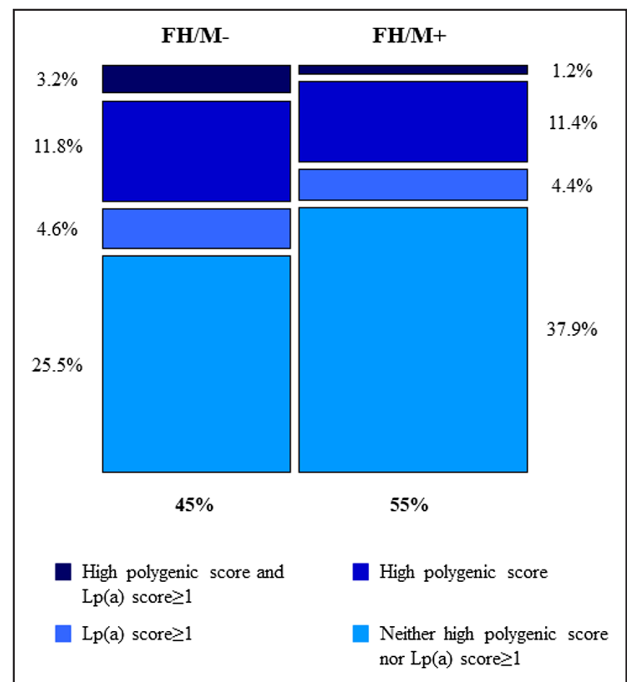
### Statistical Analysis

In the present analysis, we included adult (aged  $\geq 18$  years) patients with FH, with genetic testing

performed in a centralized laboratory searching for possible causative variants in candidate genes and evaluating the 2 common lp(a)-raising single nucleotide polymorphisms (rs3798220 and rs10455872). We defined patients as genetic-positive FH (those with at least 1 causative variant on *LDLR* [FH/M+]), and genetic-negative FH (ie, without causative variants on *LDLR* [FH/M-]). Subjects presenting only variants of uncertain significance were excluded from the analysis (393 individuals, 18.8% of the sample), as well as individuals with homozygous FH. For each subject, a polygenic score (LDL-C-score) including 12 common LDL-C-raising single nucleotide polymorphisms (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]), selected by Talmud et al<sup>12</sup> from the Global Lipid Genetic Consortium for being significantly associated with LDL-C with a *P* value cutoff of  $<5 \times 10^{-8}$ , was also evaluated. Briefly, for each individual, we calculated the specific gene scores using the weighted sum of the risk allele (ie, the LDL-C-raising allele). The weights used were the corresponding per-risk-allele beta coefficients reported by the Global Lipid Genetic Consortium, as proposed by Talmud et al<sup>12</sup> Because the beta-coefficients reported by the Global Lipid Genetic Consortium are for the effect of each minor allele, where the effect is LDL lowering, the other allele is considered the risk allele and the absolute effect size is used as the weight. An LDL-C score value  $\geq 1.08$  (the cutoff that identifies the 80th percentile of the distribution of the score in their general population<sup>12</sup>) was considered as indicative of a high probability of having polygenic hypercholesterolemia. An lp(a) genetic score was calculated for each participant by summing the number risk-increasing alleles inherited at rs3798220 and rs10455872 variants. Four different groups of subjects were then created based on genetic test response and *LPA* genotype (lp(a)/FH groups).

Baseline LDL-C levels were defined as those before initiation of cholesterol-lowering medication. Among the subgroup of patients with measured lp(a) levels available, lp(a)-adjusted LDL-C was calculated as recommended by Dahlen<sup>13,14</sup> as follows (in mg/dL): total cholesterol-(triglycerides/5)-high-density lipoprotein cholesterol-[0.3×lp(a)], and also as recommended by Yeang et al<sup>14</sup> as follows (in mg/dL): total cholesterol-(triglycerides/5)-high-density lipoprotein cholesterol-[0.173×lp(a)], which is a more conservative correction.

Continuous variables are presented as mean±SD, or as median and interquartile range, whereas categorical variables are presented as cases and



**Figure.** Distribution of patients with clinically diagnosed FH by genotype.

The figure describes the distribution of patients with clinically diagnosed FH according to the results of the genetic test (FH/M+: 54.87% of the sample; FH/M-: 45.13%), and based on the presence of a causative variant, polygenic score, or lp(a) genotype. FH/M- indicates negative familial hypercholesterolemia genetic test; FH/M+, positive familial hypercholesterolemia genetic test; and lp(a), lipoprotein A.

percentage rate. Chi-square tests were used for contingency analyses. Normally distributed data were analyzed with unpaired Student’s *t* tests or 1-way ANOVA, whereas nonnormal data were analyzed with Wilcoxon-Mann-Whitney test as appropriate. Analyses were performed in SAS software version 9.4 (SAS Institute, Cary, NC).

Statistical significance was claimed when 2-sided *P* values were  $\leq 0.05$ .

## RESULTS

We reported data on 1695 subjects with clinically diagnosed FH. Considering the presence of a causative variant, the polygenic score, and the genetic score for lp(a) as the cause of the FH phenotype (Figure), a total of 54.87% of the sample had a causative variant (FH/M+), 11.80% had only a high polygenic score, and 4.60% had only an lp(a) score  $\geq 1$  (representing 10.2% among individuals with FH/M-), whereas in the 3.24% of the total sample both these conditions were present. The remaining 25.49% were subjects with FH/M- for whom neither polygenic cause nor high lp(a) genetic score were found.

Among 765 subjects with FH/M- and 930 subjects with FH/M+, 632 and 835 individuals presented 0 copies of the variant (Ip(a) score=0), respectively, and 133 (17.4%) and 95 (10.2%) were characterized by 1 copy of either rs10455872 or rs3798220 or 2 copies of rs10455872 or rs3798220 (Ip(a) score ≥1). Demographic and clinical data of Ip(a)/FH groups are shown in the Table 1. We found that subjects with FH/M- had higher levels of Ip(a) than patients in the FH/M+ group (median values 41 mg/dL [9–103] versus 19 mg/dL [8–41], Wilcoxon-Mann-Whitney test <0.0001), with increasing Ip(a) concentrations among subjects with the same FH genetic background based on increasing number of Ip(a) alleles (P<0.0001 for patients with FH/M- and P=0.001 for patients with FH/M+).

FH/M- subjects had also lower mean levels of pretreatment LDL-C than FH/M+ individuals (t-test for difference in means between FH/M- and FH/M+ groups <0.0001); however, FH/M- subjects with Lp(a) score≥1 had higher mean (SD) pretreatment LDL-C levels (223.47 [50.40] mg/dL) compared to FH/M- subjects with Lp(a) score=0 (219.38 [54.54] mg/dL), although not statistically significant. Moreover, patients with FH/M- characterized by 1 copy of either rs10455872 or rs3798220, or 2 copies of either rs10455872 or rs3798220, presented higher prevalence of clinical history of premature coronary heart disease (10.69% versus 7.53%, P=0.23) compared with negative FH individuals presented 0 copies of the variants; a similar trend was observed for cerebral/peripheral vascular disease (6.11% versus 4.39%, P=0.40). Even considering only FH/M+ subjects, having the Lp(a) score≥1 resulted in a higher

prevalence of clinical history of premature coronary (12.63% versus 8.00%, P=0.12) or cerebral/peripheral vascular disease (4.21% versus 3.03%, P=0.53) compared with positive FH individuals presented 0 copies of the variants.

Among individuals with baseline Ip(a) measurement available (138 patients with FH/M- and 179 with FH/M+), applying the adjustment based on Ip(a) levels, LDL-C levels appeared drastically lower in subjects with Ip(a) score ≥1 (-14% in FH/M- and -9% in FH/M+, on average), whereas they were only slightly different in subjects with Ip(a) score=0 (-6% in FH/M- and -3% in FH/M+ on average; Table 2). Using the correction recommended by Yeang et al, LDL-C levels percentage reduction became -8%, -5%, -4%, and -2%, respectively. Considering an LDL-C ≥190 mg/dL as a threshold for the clinical suspicion of FH,<sup>1</sup> this implied that the condition would be recognized in 68% of individuals with FH/M- who had at least 1 copy of either rs10455872 or rs3798220 variants presented; however, this percentage decreased to 42% (or 50% considering a more conservative formula) when the assessment was made considering Ip(a)-adjusted LDL-C values.

## DISCUSSION

Despite considerable recent progress in the knowledge of the genetic basis of hypercholesterolemia, a causative variant is not found in many individuals with a phenotype and a clinical history consistent with that of FH. It has been suggested that, in some of these subjects, the FH phenotype can be explained by the presence of several genetic variants, which are

**Table 1. Clinical, Demographic, and Biochemical Profile of Included Subjects**

	FH/M- and Ip(a) score=0	FH/M- and Ip(a) score≥1	P value	FH/M+ and Ip(a) score=0	FH/M+ and Ip(a) score≥1	P value
N	632	133		835	95	
Age at baseline, y; mean (SD)	49.08 (13.59)	49.47 (13.77)	0.76	41.25 (14.92)	43.83 (15.16)	0.11
Female sex, %	53.81	60.15	0.18	54.32	51.58	0.61
Ip(a), mg/dL; median (IQR) <sup>†</sup>	16.95 (7.00–72.75)	89.00 (62.00–136.00)	<0.0001	19.00 (8.00–34.00)	64.00 (27.00–113.50)	0.001
Pretreatment low-density lipoprotein cholesterol, mg/dL; mean (SD)	219.38 (54.54)	223.47 (50.40)	0.43	265.13 (58.26)	264.43 (60.09)	0.91
Triglycerides, mg/dL; median (IQR)	129.00 (90.00–180.00)	118.50 (89.00–158.00)	0.20	97.00 (69.00–136.00)	100.00 (78.00–138.00)	0.45
Lipid-lowering therapy, %	25.47	37.59	0.005	27.07	34.74	0.11
First-degree relative with premature CHD	36.17	43.65	0.11	42.75	43.33	0.92
Clinical history of premature CHD	7.53	10.69	0.23	8.00	12.63	0.12
Clinical history of premature cerebral or peripheral vascular disease	4.39	6.11	0.40	3.03	4.21	0.53

CHD indicates coronary heart disease; FH, familial hypercholesterolemia; FH/M+, patients with genetic-positive FH; FH/M-, patients with genetic-negative FH; and Ip(a), lipoprotein(a). To convert Ip(a) values to nanomoles per liter, multiply by 2.15.

<sup>†</sup>N=100 (FH/M- & Ip(a) score=0), N=38 (FH/M- & Ip(a) score≥1), N=166 (FH/M+ & Ip(a) score=0), and N=13 (FH/M+ & Ip(a) score≥1).

**Table 2. LDL Cholesterol Profile (Before Initiation of Cholesterol-Lowering Medication and Lp(a)-Adjusted Values) Among Lp(a)/FH Groups**

	FH/M- and Ip(a) score=0	FH/M- and Ip(a) score≥1	P value	FH/M+ and Ip(a) score=0	FH/M+ and Ip(a) score≥1	P value
No.	100	38		166	13	
Pretreatment LDL-C, mg/dL; mean (SD)	217.58 (62.72)	213.80 (51.15)	0.74	275.01 (560.23)	260.49 (48.32)	0.40
Patients with pre-treatment LDL-C≥190mg/dL; %	67.00	68.42	0.87	95.18	92.31	0.65
Lp(a)-adjusted LDL-C*; mean (SD)	203.75 (62.68)	183.52 (50.47)	0.08	266.64 (59.22)	236.42 (54.92)	0.08
Patients with Lp(a)-adjusted LDL-C* ≥190mg/dL; %	55.00	42.11	0.18	93.37	92.31	0.88
Lp(a)-adjusted LDL-C†; mean (SD)	209.60 (62.10)	196.34 (49.92)	0.24	270.18 (59.46)	246.61 (51.18)	0.17
Patients with Lp(a)-adjusted LDL-C† ≥190mg/dL; %	59.00	50.00	0.34	94.58	92.31	0.73

FH indicates familial hypercholesterolemia; FH/M+, patients with genetic-positive FH; FH/M-, patients with genetic-negative FH; LDL-C, low-density lipoprotein cholesterol; and Ip(a), lipoprotein(a).

\*Lp(a) corrected levels of LDL-C calculated based on Dahlen formula.<sup>13</sup>

†Lp(a) corrected levels of LDL-C calculated based on the study by Yeang et al.<sup>14</sup>

individually associated with a slight impact on LDL-C, but if present concomitantly can considerably raise LDL-C values.<sup>12,15–17</sup> Considering the 80th percentile score in the general population as indicative of a high probability of having hypercholesterolemia of polygenic cause, in our cohort this characteristic was present in one third of the subjects with FH/M-. Of note, a high polygenic score was also found in 23% of subjects with FH/M+, which is in agreement with recent literature that has shown that the 2 criteria are independent and are both able to modulate LDL-C levels in an additive manner.<sup>2</sup> Moreover, the distribution illustrated in the Figure shows how the pathogenetic picture in a population clinically diagnosed with FH is particularly complex and heterogeneous, emphasizing the need for a comprehensive clinical and genetic assessment.

We also found that in 1 out of 5 subjects with FH/M-, 1 copy of either rs10455872 or rs3798220 or 2 copies of either rs10455872 or rs3798220 were detected, a frequency that was double compared with subjects with FH/M+. Some studies<sup>4,18–20</sup> reported higher Ip(a) levels in subjects with FH/M- compared with those with FH/M+; with a similar approach, our analysis described difference in *LPA* genotype in the population with FH. This genetic feature, associated with higher Ip(a) levels, determined an increase in the reported LDL-C levels, although comparisons were not statistically significant. Our subanalysis showed that patients with Ip(a) score ≥1 had even lower LDL-C levels compared with patients with Ip(a) score=0 when the adjustment by Ip(a) levels was applied. Although this approach could be limited by the inter- and intraindividual variation in the Ip(a) mass,<sup>14</sup> the results strongly support the need to account for the contribution of Ip(a) concentration to the estimated level of LDL-C not to misidentify individuals with clinical diagnosis of FH.

Patients with Ip(a) score ≥1, despite comparable (or lower, when adjusted) levels of LDL-C, presented with

a worse cardiovascular clinical profile, although statistical tests were not significant, possibly due to the low sample size. This aspect may also contribute to suggest a clinical diagnosis of FH, remarking the role of elevated Ip(a) in a phenotype similar to that of subjects with confirmed FH. This evidence warrants further studies on larger samples. Interestingly, a similar trend to higher prevalence of premature coronary, cerebrovascular, or peripheral disease associated with Ip(a) score ≥1 was observed within the FH/M+ cohort, suggesting a role for Ip(a) in increasing cardiovascular risk that is independent not only from the effect of monogenic variants but also from LDL-C levels. Accordingly, Ellis et al evaluated 316 patients admitted to a coronary care unit, identifying elevated Ip(a) and the FH condition in 27.0% and 11.6% of patients, respectively, and both disorders in 4.4% of individuals. They also found that elevated Ip(a) alone conferred a 1.9-fold, FH alone a 3.2-fold, and the combination of both disorders a 5.3-fold increased risk of premature coronary artery disease.<sup>21</sup>

Overall, in our sample of subjects with a clinical diagnosis of FH, after excluding the presence of a causative variant and the polygenic cause, a genotype associated with high Ip(a) levels could explain the observed phenotype in 4.6% of cases. Possibly, these are subjects in whom hypercholesterolemia and cardiovascular risk are essentially driven by high Ip(a) values, and for this reason, in the future, they may potentially benefit from therapies targeting this lipoprotein.

The recently published European Atherosclerosis Society Consensus Statement about the role of Ip(a) in atherosclerotic cardiovascular disease clearly described a continuous relationship between Ip(a) concentration and absolute atherosclerotic cardiovascular disease risk,<sup>22</sup> with an Ip(a) level of 100 mg/dL being associated with a 2-fold risk of atherosclerotic cardiovascular disease in the general population, irrespective of baseline absolute risk. From this point of view, as

also suggested by our results, the assessment of Ip(a) levels is of 2-fold importance in the setting of FH. First, the measurement of Ip(a) allows the FH diagnosis to be refined in cases where it is essentially based on LDL-C levels. Second, regardless of the genetic background on *LDLR*, Ip(a) levels should be used to refine cardiovascular risk assessment, better stratify patients,<sup>23</sup> and identify those requiring more intensive and timely intervention.

## Limitations

Some limitations have to be acknowledged. We have to point out that differences were often not statistically significant. This could be due to the low number of subjects in each group; thus further studies are warranted to confirm our observations. In the LIPIGEN study, the measurement of Ip(a) values was not mandatory and for many centers belonging to the LIPIGEN Network it was not a routine practice. Therefore, the measured values were available for only a subgroup of subjects, which did not allow us to make an in-depth assessment of this parameter. In addition, the methodology was not the same in all centers. However, the genetic score we applied to identify the subjects most likely to have elevated Ip(a) levels was shown to explain about 40% of the variability in Ip(a) levels<sup>8</sup>; the use of the score allowed us to have a larger sample size, as well as to overcome the limitations linked to the heterogeneity of the measurement methods.<sup>24</sup>

## CONCLUSIONS

Our results highlight the importance of measuring Ip(a) to perform the diagnosis of FH appropriately and to exclude the possibility that the observed phenotype is driven by elevated levels of Ip(a) before performing the genetic test that is often requested based on the presence of elevated LDL-C levels.

Clinical and laboratory investigations for differentiating FH, elevated Ip(a), or the combination of both are also important for accurate risk stratification and treatment strategies, as in the future patients with both elevated Ip(a) and FH condition may be shown to benefit from interventions to reduce both LDL-C and Ip(a) plasma levels.

## APPENDIX

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## REFERENCES

- Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, Wiklund O, Hegele RA, Raal FJ, Defeseche JC, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*. 2013;34:3478–3490. doi: 10.1093/eurheartj/ehd273
- Olmastroni E, Gazzotti M, Arca M, Averna M, Pirillo A, Catapano AL, Casula M, Groupdagger LS. 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. *J Am Heart Assoc*. 2022;11:e023668. doi: 10.1161/JAHA.121.023668
- Talmud PJ, Drenos F, Shah S, Shah T, Palmen J, Verzilli C, Gaunt TR, Pallas J, Lovering R, Li K, et al. Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. *Am J Hum Genet*. 2009;85:628–642. doi: 10.1016/j.ajhg.2009.10.014
- Langsted A, Kamstrup PR, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. High lipoprotein(a) as a possible cause of clinical familial hypercholesterolaemia: a prospective cohort study. *Lancet Diabetes Endocrinol*. 2016;4:577–587. doi: 10.1016/S2213-8587(16)30042-0
- Utermann G, Hoppichler F, Dieplinger H, Seed M, Thompson G, Boerwinkle E. Defects in the low density lipoprotein receptor gene affect lipoprotein (a) levels: multiplicative interaction of two gene loci associated with premature atherosclerosis. *Proc Natl Acad Sci USA*. 1989;86:4171–4174. doi: 10.1073/pnas.86.11.4171
- Trinder M, DeCastro ML, Azizi H, Cermakova L, Jackson LM, Frohlich J, Mancini GBJ, Francis GA, Brunham LR. Ascertainment bias in the association between elevated lipoprotein(a) and familial hypercholesterolemia. *J Am Coll Cardiol*. 2020;75:2682–2693. doi: 10.1016/j.jacc.2020.03.065
- Zekavat SM, Ruotsalainen S, Handsaker RE, Alver M, Bloom J, Poterba T, Seed C, Ernst J, Chaffin M, Engreitz J, et al. Deep coverage whole genome sequences and plasma lipoprotein(a) in individuals of European and African ancestries. *Nat Commun*. 2018;9:2606. doi: 10.1038/s41467-018-04668-w
- Clarke R, Peden JF, Hopewell JC, Kyriakou T, Goel A, Heath SC, Parish S, Barlera S, Franzosi MG, Rust S, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N Engl J Med*. 2009;361:2518–2528. doi: 10.1056/NEJMoa0902604
- Trinder M, Uddin MM, Finneran P, Aragam KG, Natarajan P. Clinical utility of lipoprotein(a) and LPA genetic risk score in risk prediction of incident atherosclerotic cardiovascular disease. *JAMA Cardiol*. 2020;6:1–9. doi: 10.1001/jamacardio.2020.5398
- Averna M, Cefalu AB, Casula M, Noto D, Arca M, Bertolini S, Calandra S, Catapano AL, Tarugi P; LIPIGEN Group. Familial hypercholesterolemia: the Italian Atherosclerosis Society Network (LIPIGEN). *Atherosclerosis Suppl*. 2017;29:11–16. doi: 10.1016/j.atherosclerosis.2017.07.001
- Casula M, Olmastroni E, Pirillo A, Catapano AL, Arca M, Averna M, Bertolini S, Calandra S, Tarugi P, Pellegatta F, et al. Evaluation of the performance of Dutch Lipid Clinic Network score in an Italian FH population: the LIPIGEN study. *Atherosclerosis*. 2018;277:413–418. doi: 10.1016/j.atherosclerosis.2018.08.013
- Talmud PJ, Shah S, Whittall R, Futema M, Howard P, Cooper JA, Harrison SC, Li K, Drenos F, Karpe F, et al. Use of low-density lipoprotein cholesterol gene score to distinguish patients with polygenic and monogenic familial hypercholesterolaemia: a case-control study. *Lancet*. 2013;381:1293–1301. doi: 10.1016/S0140-6736(12)62127-8
- Dahlen GJ. Potential significance of Lp (a) lipoprotein clinical methodological aspects. In: Bearn AG, ed. *Genetics of Coronary Heart Disease*. Oslo: Institute of Medical Genetics, University of Oslo; 1992:75–88.
- Yeang C, Witztum JL, Tsimikas S. Novel method for quantification of lipoprotein(a)-cholesterol: implications for improving accuracy of LDL-C measurements. *J Lipid Res*. 2021;62:100053. doi: 10.1016/j.jlr.2021.100053
- Mariano C, Alves AC, Medeiros AM, Chora JR, Antunes M, Futema M, Humphries SE, Bourbon M. The familial hypercholesterolaemia phenotype: monogenic familial hypercholesterolaemia, polygenic hypercholesterolaemia and other causes. *Clin Genet*. 2020;97:457–466. doi: 10.1111/cge.13697
- Narayanaswamy M, Sharma S. Polygenic hypercholesterolemia. *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing; 2022.
- Tada H, Nohara A, Kawashiri MA. Monogenic, polygenic, and oligogenic familial hypercholesterolemia. *Curr Opin Lipidol*. 2019;30:300–306. doi: 10.1097/QCO.0000000000000563
- Alonso R, Argueso R, Alvarez-Banos P, Muniz-Grijalvo O, Diaz-Diaz JL, Mata P. Familial hypercholesterolemia and lipoprotein(a): two partners in crime? *Curr Atheroscler Rep*. 2022;24:427–434. doi: 10.1007/s11883-022-01019-5
- Merino-Ibarra E, Puzo J, Jarauta E, Cenarro A, Recalde D, Garcia-Otin AL, Ros E, Martorell E, Pinto X, Franco M, et al. Hyperlipoproteinaemia(a) is a common cause of autosomal dominant hypercholesterolaemia. *J Inherit Metab Dis*. 2007;30:970–977. doi: 10.1007/s10545-007-0585-z
- Pederiva C, Capra ME, Biasucci G, Banderali G, Fabrizi E, Gazzotti M, Casula M, Catapano AL; LIPIGEN Paediatric Group; Arca M, Averna M, et al. Lipoprotein(a) and family history for



- 
- cardiovascular disease in paediatric patients: a new frontier in cardiovascular risk stratification. Data from the LIPIGEN paediatric group. *Atherosclerosis*. 2022;349:233–239. doi: [10.1016/j.atherosclerosis.2022.04.021](https://doi.org/10.1016/j.atherosclerosis.2022.04.021)
21. Ellis KL, Pang J, Chieng D, Bell DA, Burnett JR, Schultz CJ, Hillis GS, Watts GF. Elevated lipoprotein(a) and familial hypercholesterolemia in the coronary care unit: between Scylla and Charybdis. *Clin Cardiol*. 2018;41:378–384. doi: [10.1002/clc.22880](https://doi.org/10.1002/clc.22880)
  22. Kronenberg F, Mora S, Stroes ESG, Ference BA, Arsenault BJ, Berglund L, Dweck MR, Koschinsky M, Lambert G, Mach F, et al. Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement. *Eur Heart J*. 2022;43:3925–3946. doi: [10.1093/eurheartj/ehac361](https://doi.org/10.1093/eurheartj/ehac361)
  23. Tromp TR, Ibrahim S, Nurmohamed NS, Peter J, Zuurbier L, Defesche JC, Reeskamp LF, Hovingh GK, Stroes ESG. Use of lipoprotein(a) to improve diagnosis and management in clinical familial hypercholesterolemia. *Atherosclerosis*. 2023;365:27–33. doi: [10.1016/j.atherosclerosis.2022.11.020](https://doi.org/10.1016/j.atherosclerosis.2022.11.020)
  24. Marcovina SM, Albers JJ. Lipoprotein (a) measurements for clinical application. *J Lipid Res*. 2016;57:526–537. doi: [10.1194/jlr.R061648](https://doi.org/10.1194/jlr.R061648)