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CLINICAL RESEARCH
Dyslipidaemias

Lipoprotein(a) levels in children with suspected familial hypercholesterolaemia: a cross-sectional study

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Abstract

Aims Familial hypercholesterolaemia (FH) predisposes children to the early initiation of atherosclerosis and is preferably diagnosed by DNA analysis. Yet, in many children with a clinical presentation of FH, no mutation is found. Adult data show that high levels of lipoprotein(a) [Lp(a)] may underlie a clinical presentation of FH, as the cholesterol content of Lp(a) is included in conventional LDL cholesterol measurements. As this is limited to adult data, Lp(a) levels in children with and without (clinical) FH were evaluated.

Methods and results Children were eligible if they visited the paediatric lipid clinic (1989–2020) and if Lp(a) measurement and DNA analysis were performed. In total, 2721 children (mean age: 10.3 years) were included and divided into four groups: 1931 children with definite FH (mutation detected), 290 unaffected siblings/normolipidaemic controls (mutation excluded), 108 children with probable FH (clinical presentation, mutation not detected), and 392 children with probable non-FH (no clinical presentation, mutation not excluded). In children with probable FH, 32% were found to have high Lp(a) [geometric mean (95% confidence interval) of 15.9 (12.3–20.6) mg/dL] compared with 10 and 10% [geometric means (95% confidence interval) of 11.5 (10.9–12.1) mg/dL and 9.8 (8.4–11.3) mg/dL] in children with definite FH ($P = 0.017$) and unaffected siblings ($P = 0.002$), respectively.

Conclusion Lp(a) was significantly higher and more frequently elevated in children with probable FH compared with children with definite FH and unaffected siblings, suggesting that high Lp(a) may underlie the clinical presentation of FH when no FH-causing mutation is found. Performing both DNA analysis and measuring Lp(a) in all children suspected of FH is recommended to assess possible LDL cholesterol overestimation related to increased Lp(a).

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Structured Graphical Abstract

Key Question

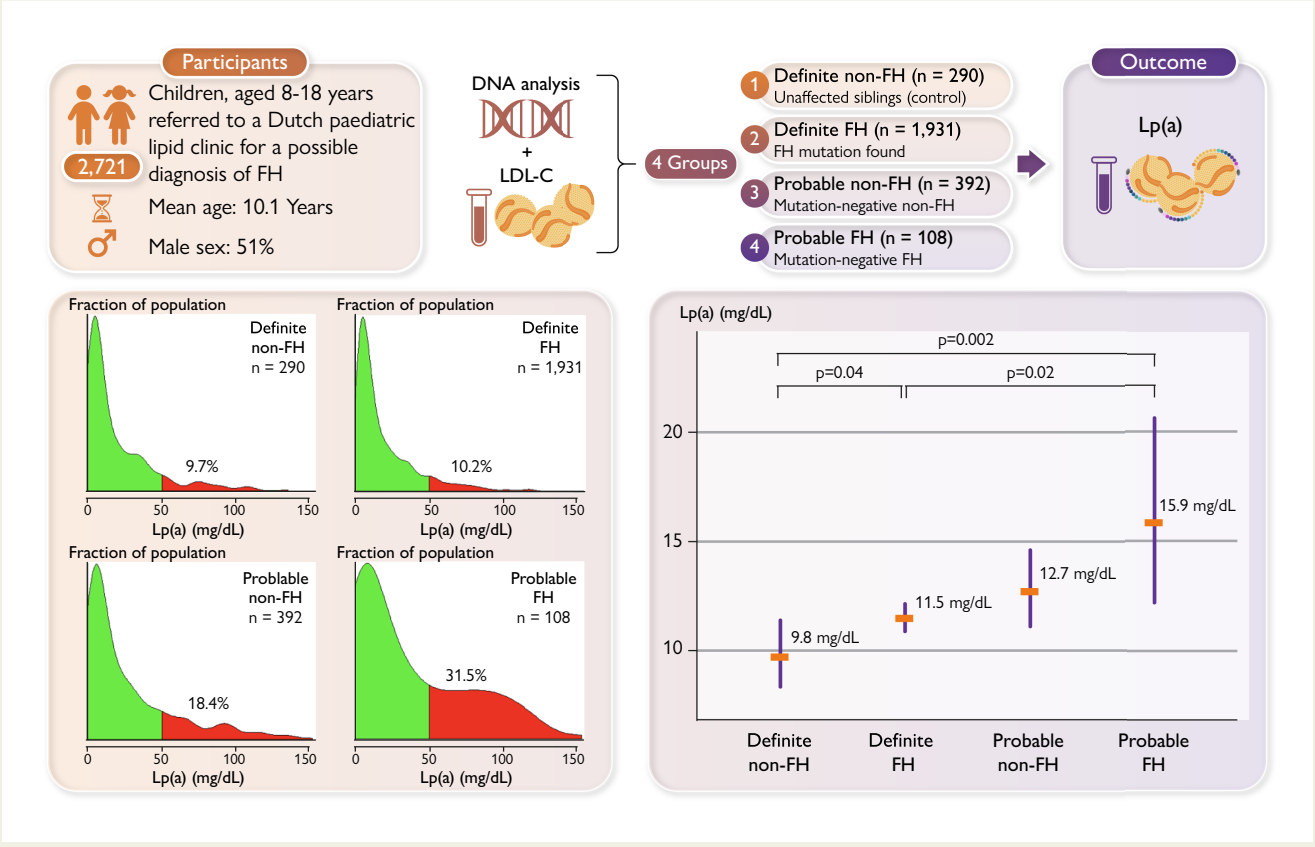
Familial hypercholesterolaemia (FH) is preferably diagnosed with DNA analysis. However, in many children suspected of FH, no mutation can be found. Adult data shows that high lipoprotein(a) [Lp(a)] may underlie this clinical presentation. Whether or not the same applies for the paediatric population, is unknown.

Key Finding

Nearly 3,000 children suspected of FH were included. Lp(a) levels were more often elevated and significantly higher in children with probable FH (clinical presentation, no mutation) compared to children with definite FH (mutation found) and unaffected siblings.

Take Home Message

High Lp(a) may underlie the clinical presentation of FH in children in whom no mutation is found. To distinguish between FH and high Lp(a), and to identify children with both risk factors, performing DNA analysis as well as measuring Lp(a) is recommended in all children suspected of FH.



Evaluation of Lp(a) levels in nearly 3000 children suspected of FH suggests that high levels of Lp(a) may underlie the clinical presentation of FH in children in whom no FH-causing mutation was found. FH, familial hypercholesterolaemia; LDL-C, LDL cholesterol; Lp(a), lipoprotein(a); mg/dL, milligrammes per deciliter.

Keywords Lipoprotein(a) • Lp(a) • Familial hypercholesterolaemia • FH • Children • DNA analysis

Introduction

Familial hypercholesterolaemia (FH) is an autosomal dominant disorder of lipoprotein metabolism that affects around 1 in 310 individuals.[1] It is caused by mutations in the *LDLR*, *APOB*, or *PCSK9* genes that lead to impaired clearance of LDL cholesterol (LDL-C) from the circulation and, consequently, very high LDL-C levels from birth onwards.[2] If left untreated, early signs of atherosclerosis can already be observed in childhood,[3–5] emphasizing the importance of early diagnosis and treatment initiation.[6]

The preferred method for diagnosing FH is genetic testing.[7–9] In many countries, however, FH is often diagnosed clinically with the use of diagnostic tools such as the Dutch Lipid Clinic Network Criteria (DLCNC). In children, on the other hand, these tools are not validated, and consequently, genetic testing is even more important for diagnosing FH in the paediatric population. However, even when DNA analysis is performed, a considerable group of children remain with a clinical presentation (or phenotypic/clinical diagnosis) of FH in whom no FH-causing mutation can be found, suggesting that other factors may underlie this clinical presentation.[10,11] In fact, several adult studies have shown that a considerable part of the clinical diagnoses of FH could be explained by high lipoprotein(a) [Lp(a)] levels.[12–14]

Elevated Lp(a) is a genetic risk factor for cardiovascular disease (CVD), independent of conventional risk factors such as LDL-C.[15–17] With a population prevalence of approximately 20%, it is one of the most prevalent inherited dyslipidaemias.[18] Despite no pharmacological treatment for lowering Lp(a) levels being available in children, measuring Lp(a) levels is important to allow further optimization of other CVD risk factors, including a more aggressive lowering of LDL-C and lifelong adoption of a healthy lifestyle.[19,20] Due to the LDL-like core of Lp(a), conventional LDL-C assays capture both the cholesterol content of LDL particles and of Lp(a) particles, and thus, if Lp(a) levels are high, measured LDL-C levels can be elevated as well.[21–23] As a result, FH may be clinically diagnosed, while it is actually high Lp(a) levels that underlie this presentation.[24] Yet this is mainly based on adult data, and whether high Lp(a) levels may underlie a clinical presentation of FH in children is unknown. Therefore, we compared Lp(a) levels of children with a clinical presentation of FH in whom no mutation was found to Lp(a) levels of both children with an FH-causing mutation and their unaffected siblings.

Methods

Study population and design

In this cross-sectional study, we included children who were referred to the paediatric lipid clinic of the Amsterdam UMC—Location Academic Medical Center between June 1989 and January 2020 for a tentative diagnosis of FH (a family history of premature CVD, FH in family members, or high LDL-C levels in the child or family members). As part of routine care, a lipid profile, including Lp(a) levels, was obtained, and DNA analysis for FH was performed. We excluded children if Lp(a) was not measured or measured after the age of 18; if Lp(a) was measured and analysed in external laboratories; if no DNA analysis was performed; or if children had homozygous FH. At the first visit, demographic and clinical characteristics were collected, including medical history and family history of premature CVD (defined as any cardiovascular event before the age of 60 years).

We divided the final study population into four groups, as depicted in *Figure 1*. If an FH-causing mutation was found with DNA analysis, children were classified as 'definite FH' (mutation detected). If no FH-causing mutation was found while a parent or sibling was known to have a mutation, FH could be definitively ruled out and these unaffected siblings were included as normolipidaemic control group ('definite non-FH'). If no FH-causing mutation was found but a child did have a clinical presentation of FH (i.e. LDL-C levels above 5 mmol/L or between 4 and 5 mmol/L with a family history of premature CVD), the child was classified as 'probable FH' (clinical presentation, mutation not detected).[8] Finally, if no FH-causing mutation was found and the child had no clinical presentation of FH (i.e. LDL-C below 4 mmol/L or between 4 and 5 mmol/L without a family history of premature CVD), the child was classified as 'probable non-FH' (no clinical presentation, mutation not excluded).

This study complies with the Declaration of Helsinki. As this study was not subject to the Medical Research Involving Human Subjects Act, approval from an ethics committee in the Netherlands was not required.

DNA analysis

Genomic DNA was prepared from 5 mL of whole blood on an AutopureLS apparatus according to a protocol provided by the manufacturer (Gentra Systems, Minneapolis, MN). Mutation identification in the *LDLR*, *APOB*, and *PCSK9* genes was performed by direct Sanger sequencing; identification of large rearrangements in the *LDLR* gene was done by multiplex ligation-dependent probe technique as described previously in more detail.[25]. Sequence analysis was performed by direct sequencing with the Big Dye Terminator ABI Prism Kit, version 1.1 (Applied Biosystems, Foster City, CA). Products of sequence reactions were run on a genetic analyser 3730 (Applied Biosystems), and sequence data were analysed using the sequencer package (GeneCodes Co, Ann Arbor, MI). Mutations were described according to the nomenclature proposed by den Dunnen and Antonarakis.[26]

Lipids and lipoproteins

Blood samples were obtained after an overnight fast and were freshly analysed. Plasma levels of total cholesterol concentrations and triglycerides concentrations were determined by standardized enzymatic procedures (Boehringer, Mannheim, Germany), and HDL cholesterol concentrations in plasma were measured by an automated method (Roche Diagnostics, Basel, Switzerland). LDL-C levels were calculated using Friedewald's equation.[27]

Serum levels of Lp(a) were collected and freshly analysed in the clinical laboratory of the Amsterdam UMC—Location AMC. Between 1989 and 2020, two assays were used to determine Lp(a): before 01 January 2009, the immunonephelometric assay (Abbott Architect ci8200) was used, and hereafter, the immunoturbidimetric assay (Prospec Siemens) was used. The total mass of Lp(a)-cholesterol [Lp(a)-C] was estimated at 30% of the total measured Lp(a)-mass. Subsequently, LDL-C corrected for Lp(a)-C was estimated by subtracting Lp(a)-C from the total LDL-C.[28,29]

Statistical analysis

Data are presented as means and standard deviations, or 95% confidence intervals (95% CIs), for normally distributed variables, and medians and interquartile ranges for variables with a skewed distribution. The normality of variables was evaluated by visual inspection of distribution plots and Q-Q plots. As Lp(a) is highly skewed, we log-transformed Lp(a) levels before the analyses and geometric means were presented, unless stated differently. Differences in baseline characteristics between groups were tested using ANOVA (continuous variables), the Kruskal–Wallis rank sum test (continuous variables), or χ^2test (categorical variables), as appropriate.

We assessed the association between Lp(a) levels and different groups using linear regression analysis adjusted for age, sex, body mass index, and measurement assay.[30,31] We used generalized estimating equations to account for correlations within families (exchangeable correlation structure). The association between CVD in first-degree relatives and Lp(a) levels was evaluated using logistic regression analysis. A generalized estimating equation was used to account for correlations within families. By means of sensitivity analysis, we also performed a logistic regression analysis with CVD in first-degree relatives, excluding children referred as a result of cascade screening.

All *P*-values are two sided, and *P*-values below 0.05 were considered statistically significant. All analyses were performed using SPSS software v26.0 (IBM SPSS Statistics 26.0) and Rstudio version 1.2.1335.

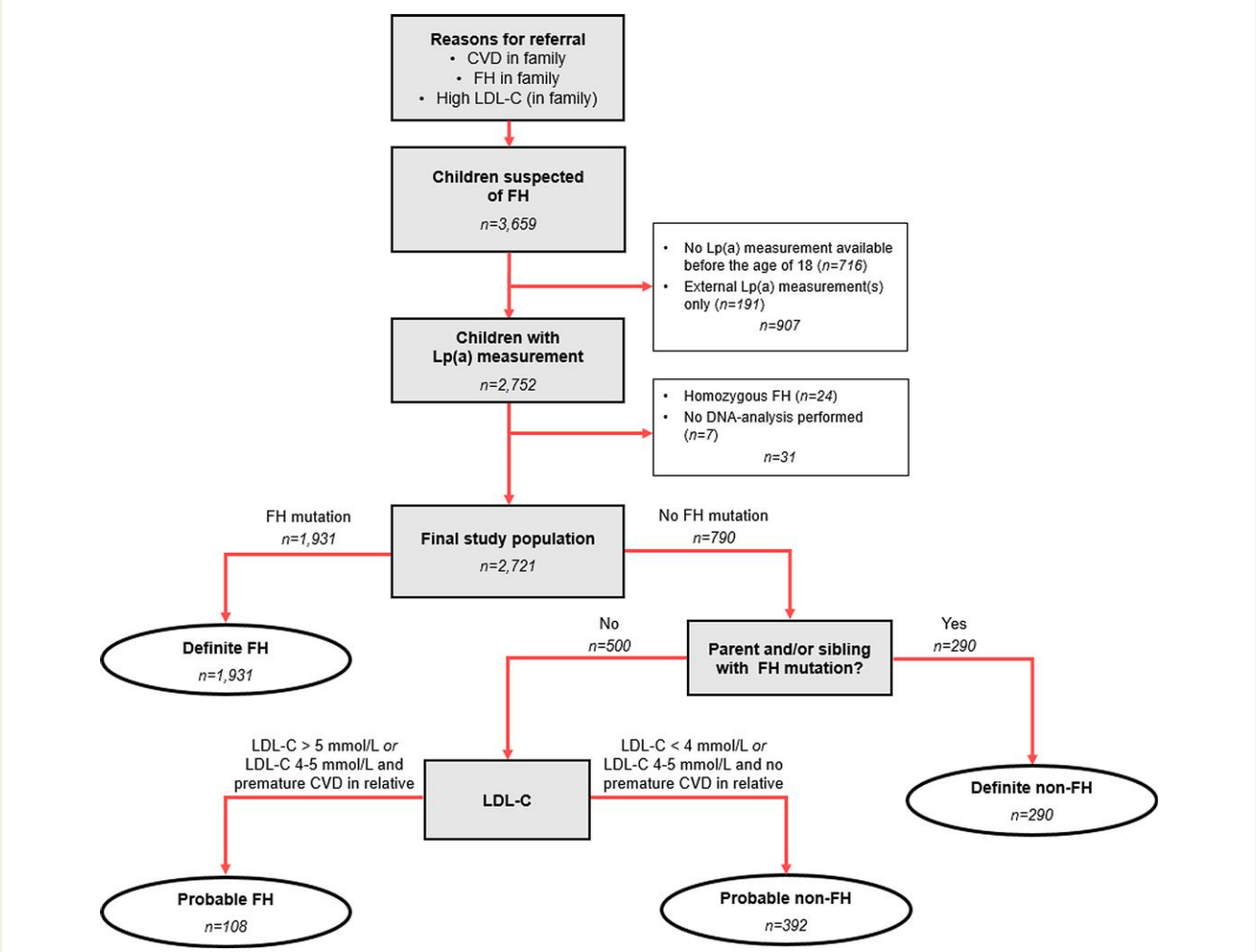


Figure 1 Flowchart illustrating the selection process of eligible children for the current study and the selection of the different groups.

Results

Study population

Between June 1989 and January 2020, a total of 3659 children were referred to the paediatric lipid clinic for review of suspected FH (*Figure 1*). Lp(a) measurements before the age of 18 years were unavailable in 716 children, and in 191 children, Lp(a) was measured in external laboratories. Of the remaining 2752 children, we excluded 24 children with homozygous FH and 7 children because no DNA analysis was performed. The remaining 2721 children comprised our study population. In total, 1378 (51%) children were boys, and mean age (range) at the first visit to the clinic was 10.1 (0.7–17.9) years.

Lp(a) levels in the paediatric referral population

The mean (standard deviations) age at the first Lp(a) measurement in the total population was 10.3 (3.6) years, and 123 (4.5%) children were taking lipid-lowering medication at the first measurement. Of these children, 119 (97%) were taking statins, and in four children (3%), ezetimibe was added to statin therapy. The geometric mean (95% CI) Lp(a) level at the first visit was 11.5 (11.0–12.1) mg/dL ranging from 0.2 mg/dL to 171.0 mg/dL. Supplementray material online, *Figure S1* displays the distribution of Lp(a) levels in the cohort. In total, 665 (24.4%) children had Lp(a) levels of over 30 mg/dL, and in 331 (12.2%) children, Lp(a) levels exceeded 50 mg/dL.

Demographic and clinical characteristics of the different groups

In 1931 (71%) children, a mutation was found (definite FH). In 290 (11%) children, an FH-causing mutation known to be present in a parent and/or sibling was not found in the child, and FH could be definitively excluded (definite non-FH/normolipidaemic controls). Additionally, in 108 (4%) children, no FH-causing mutation was detected, whereas they did have a clinical presentation of FH (probable FH). In 392 (14%) children, no FH-causing mutation and no clinical presentation of FH were found, and they were classified as probable non-FH. The demographic and clinical characteristics of the children in the different groups are summarized in *Table 1*.

The mean (95% CI) LDL-C levels of children with definite non-FH, definite FH, probable non-FH, and probable FH were 2.5 (2.5–2.6) mmol/L, 5.3 (5.3–5.4) mmol/L, 2.9 (2.9–3.0) mmol/L, and 4.4 (4.3–4.5) mmol/L, respectively. Mean LDL-C levels corrected for Lp(a)-C

Table 1 Baseline characteristics of the included children with and without FH at first visit to the clinic

	Definite		Probable		
	Non-FH[a]	FH	Non-FH	FH	*P*-value
No. of patients (%)	290	1931	392	108	
Male—no. (%)	149 (51.4)	990 (51.3)	189 (48.2)	50 (46.3)	0.55
Age (years)—mean (95% CI)	9.4 (9.0–9.8)	10.0 (9.8–10.2)	10.4 (10.0–10.8)	11.5 (10.8–12.2)	<0.001
Body mass index (kg/m^2)—mean (95% CI)	17.2 (16.8–17.5)	17.8 (17.7–18.0)	19.6 (19.1–20.1)	22.1 (21.3–23.0)	<0.001
Systolic blood pressure (mmHg)—mean (95% CI)	106 (104–109)	109 (108–110)	113 (111–115)	116 (113–119)	<0.001
Diastolic blood pressure (mmHg)—mean (95% CI)	63 (61–65)	64 (63–64)	67 (65–68)	68 (66–71)	<0.001
Lipid profiles (mmol/L)					
Total cholesterol—mean (95% CI)	4.3 (4.2–4.4)	7.0 (7.0–7.1)	4.8 (4.7–4.9)	6.3 (6.2–6.4)	<0.001
LDL-C—mean (95% CI)	2.5 (2.5–2.6)	5.3 (5.3–5.4)	2.9 (2.9–3.0)	4.4 (4.3–4.5)	<0.001
LDLcor-C[b]—mean (95% CI)	2.4 (2.3–2.5)	5.2 (5.1–5.2)	2.7 (2.7–2.8)	4.2 (4.0–4.3)	<0.001
HDL-C—mean (95% CI)	1.4 (1.4–1.5)	1.4 (1.3–1.4)	1.5 (1.4–1.5)	1.3 (1.3–1.4)	<0.001
Triglycerides—median [IQR]	0.55 [0.39–0.79]	0.65 [0.45–0.91]	0.70 [0.50–1.05]	1.0 [0.68–1.52]	<0.001
First degree relative premature CVD—no. (%)[c]	91 (31.3)	415 (21.5)	182 (46.5)	43 (40.2)	<0.001
Age at first event relative—mean (95% CI)	38.0 (36.6–39.5)	39.2 (38.5–40.0)	38.0 (37.1–38.9)	37.8 (35.8–39.9)	0.15
Smoking—no. (%)	9 (3.1)	45 (2.3)	9 (2.3)	3 (2.8)	0.87
Lipid-lowering drugs—no. (%)	0 (0)	51 (2.6)	2 (0.5)	2 (1.9)	0.003
Stigmata of FH—no. (%)	0 (0)	45 (2.3)	0 (0)	3 (2.8)	0.001

CVD, cardiovascular disease; CI, confidence interval; FH, familial hypercholesterolaemia; HDL-C, HDL cholesterol; IQR, interquartile range; LDL-C, LDL cholesterol.
[a]Group of unaffected siblings that serve as a normolipidaemic control group.
[b]Total LDL-C corrected for Lp(a)-C with Lp(a)-C being estimated as 30% of Lp(a) mass.
[c]Any CVD before the age of 60 years.

were 2.4 mmol/L, 5.2 mmol/L, 2.7 mmol/L, and 4.2 mmol/L, respectively. The majority of children (91%) with definite FH had a mutation in the *LDLR* gene, whereas mutations in the *APOB* and *PCSK9* genes were found in 172 (9%) and 4 (0%) children, respectively. In addition, premature CVD in a first-degree relative was found in 91 (31%) and 415 (22%) children with definite non-FH and FH, respectively, and in 182 (47%) and 43 (40%) children with probable non-FH and FH, respectively.

Lp(a) levels in the different groups

Figure 2 displays the geometric mean and 95% CI of Lp(a) levels per group adjusted for age, body mass index, sex, and measurement assay. The geometric mean (95% CI) Lp(a) of children with probable FH was 15.9 (12.3–20.6) mg/dL. This was significantly higher than Lp(a) levels of children with both definite FH [11.5 (10.9–12.1) mg/dL; $P = 0.02$] and definite non-FH (normolipidaemic controls) [9.8 (8.4–11.3) mg/dL; $P = 0.002$]. In children with probable non-FH, the geometric mean (95% CI) Lp(a) was 12.7 (11.1–14.6) mg/dL. *Figure 3* displays the distribution of Lp(a) levels in the different groups. High Lp(a) (>50 mg/dL) was most often found in children with probable FH. In these children, 34 (31.5%) had Lp(a) levels of over 50 mg/dL compared with 197 (10.2%) children with definite FH ($P < 0.0001$).

Geometric mean (95% CI) Lp(a) levels of children with an *LDLR* mutation ($n = 1755$) were 11.1 (10.6–11.8) mg/dL and 12.9 (10.8–15.4 mg/dL) in children with a mutation in the *APOB* gene ($P = 0.11$).

Lp(a) levels and CVD in first-degree relatives

The geometric mean (95% CI) Lp(a) in children with one or both parent(s) with premature CVD was 12.7 (11.7–13.9) mg/dL, compared with 11.1 (10.0–12.2) mg/dL in children with no parent(s) with premature CVD ($P = 0.005$).

Significantly more children with probable FH had a first-degree relative with premature CVD compared with children with definite FH (40.2% vs. 21.5%, $P < 0.0001$). This difference remained significant after excluding children ($n = 983$) that were referred as a result of cascade screening (40.2% vs. 28.4%, $P = 0.02$). In children with probable FH and non-FH ($n = 500$), the association between Lp(a) levels in children and CVD in first-degree relatives showed a trend towards significance [OR: 1.15 per 1 unit increase of Lp(a) on the log-scale, 95% CI: 1.00–1.31, $P = 0.05$], whereas this was not the case in children with definite FH and non-FH [OR: 1.06 per 1 unit increase of Lp(a) on the log-scale, 95% CI: 0.96–1.16, $P = 0.21$]. However, the strength of the association did not differ significantly between the two groups (probable vs. definite, *P*-for-interaction = 0.34).

Children with definite FH and elevated Lp(a) levels (>50 mg/dL) appeared to be more likely to have one or both parent(s) with premature CVD compared with children with definite FH alone (26.7% vs. 21.0%), although this difference was not statistically significant ($P = 0.07$). When excluding children who were referred as a result of cascade screening

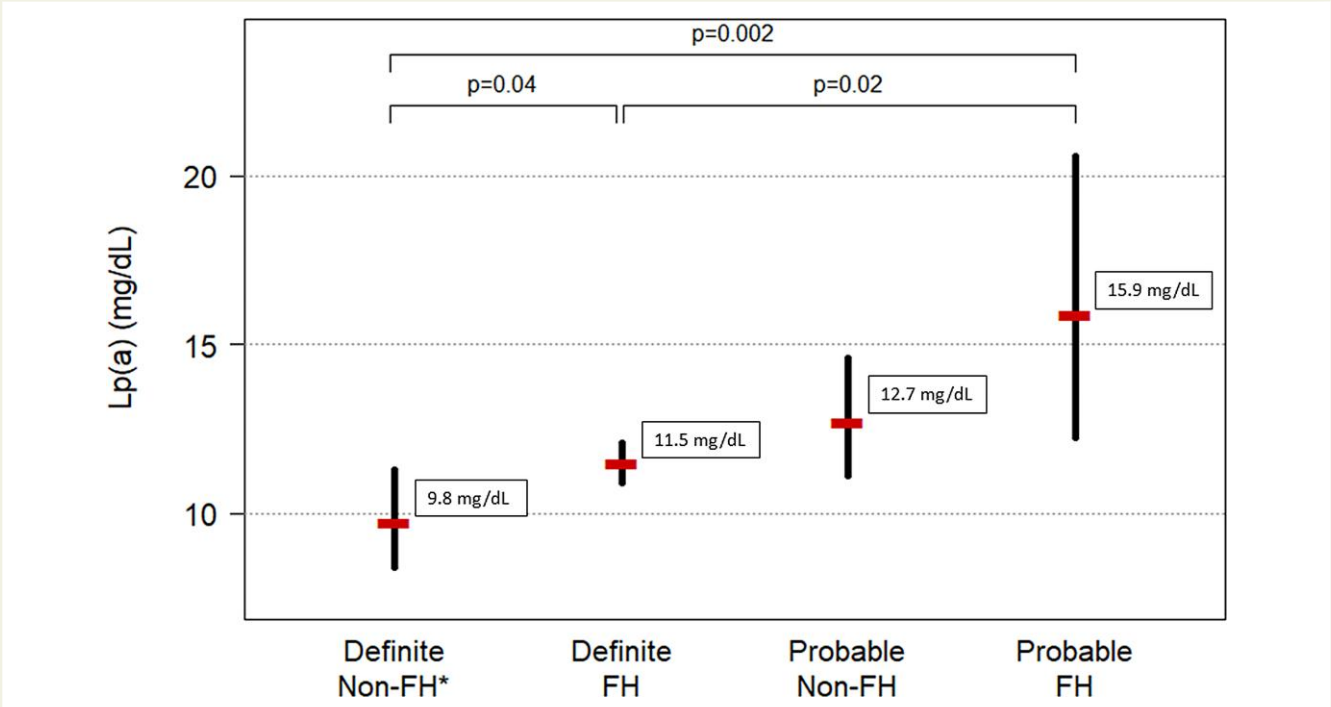


Figure 2 Geometric mean lipoprotein(a) levels and the corresponding 95% confidence intervals per group. The figure shows least square mean lipoprotein(a) levels per group adjusted for age, body mass index, measurement assay, and sex. *Group of unaffected siblings that serve as a normolipidaemic control group.

($n = 983$), this difference did reach statistical significance (37.6% vs. 27.4%, $P = 0.03$).

Discussion

In this study, we evaluated Lp(a) levels in a large referral population of children suspected of FH. We found that Lp(a) levels were significantly higher and more often elevated in children with a clinical presentation of FH in whom no mutation was found (probable FH) when compared with both children with an FH-causing mutation (definite FH) and normolipidaemic controls. Our findings suggest that high Lp(a) levels may underlie the clinical presentation of FH in children in whom no FH mutation is found (*Structured Graphical Abstract*).

To our knowledge, the present study was the first paediatric study of this size to evaluate Lp(a) levels in patients suspected of having FH. Our findings are partly consistent with previously published data, which mainly comes from adults. Different adult studies showed that Lp(a) levels were significantly higher in patients with FH compared with patients without FH or healthy control groups, and that a (clinical) FH diagnosis in at least a part of the FH patients was due to high Lp(a) levels.[13,14,32–35] Yet in most adult studies, FH was diagnosed using diagnostic tools such as the DLCNC, and as a result, these groups include both FH patients with and without FH-causing mutations. In children, however, these diagnostic tools are not validated. Given that key elements of these tools include clinical features of FH (tendon xanthomas, arcus cornealis, a history of premature CVD), and as these features are relatively rare in children, they cannot be used to diagnose FH in the paediatric population. As a result, we distinguished between FH patients with and without a mutation and found that in children with a clinical presentation of FH in whom no mutation was found (probable FH), Lp(a) levels were significantly higher than in children with an FH-causing mutation as well as in normolipidaemic controls. These results are in line with a study from Marco-Benedí et al. that also found significantly higher Lp(a) levels in mutation-negative FH patients ($n = 860$) than in adults with an FH-causing mutation as well as in healthy controls.[14] Moreover, Ellis *et al.*[12] showed in 206 adult patients suspected of FH without an FH-causing mutation that elevated Lp(a) was highly prevalent (44.7%) and that elevated Lp(a) levels predicted coronary artery disease independently of other established risk factors. Results of these adult studies are more or less consistent with our findings and suggest that high Lp(a) levels may underlie the clinical presentation of patients suspected of having FH in whom no FH mutation can be found.

Nonetheless, differences in clinical interpretation exist: some argue that high Lp(a) may be a direct genetic cause of clinical FH,[13,36] whereas we believe that high Lp(a) should be considered a separate entity. In most patients with definite FH, mutations in the *LDLR*, *APOB*, or *PCSK9* genes are involved, whereas variation in the *LPA* gene leads to high Lp(a) levels. In addition, the pathophysiological mechanisms by which FH and high Lp(a) levels lead to high LDL-C levels differ substantially. In most FH patients, a decreased number of functional LDL receptors results in impaired clearance of LDL-C, while the role of the LDL receptor in patients with high Lp(a) is likely modest.[37–40] Moreover, patients with high Lp(a) levels may benefit from different non-therapeutical and therapeutical strategies than patients with FH. In patients with FH, both diet and statin therapy effectively lower LDL-C levels and the risk of CVD, while Lp(a) and its corresponding cholesterol content are not directly affected by diet and statin therapy.[6,41–43] For the treatment of high Lp(a) levels, other therapies

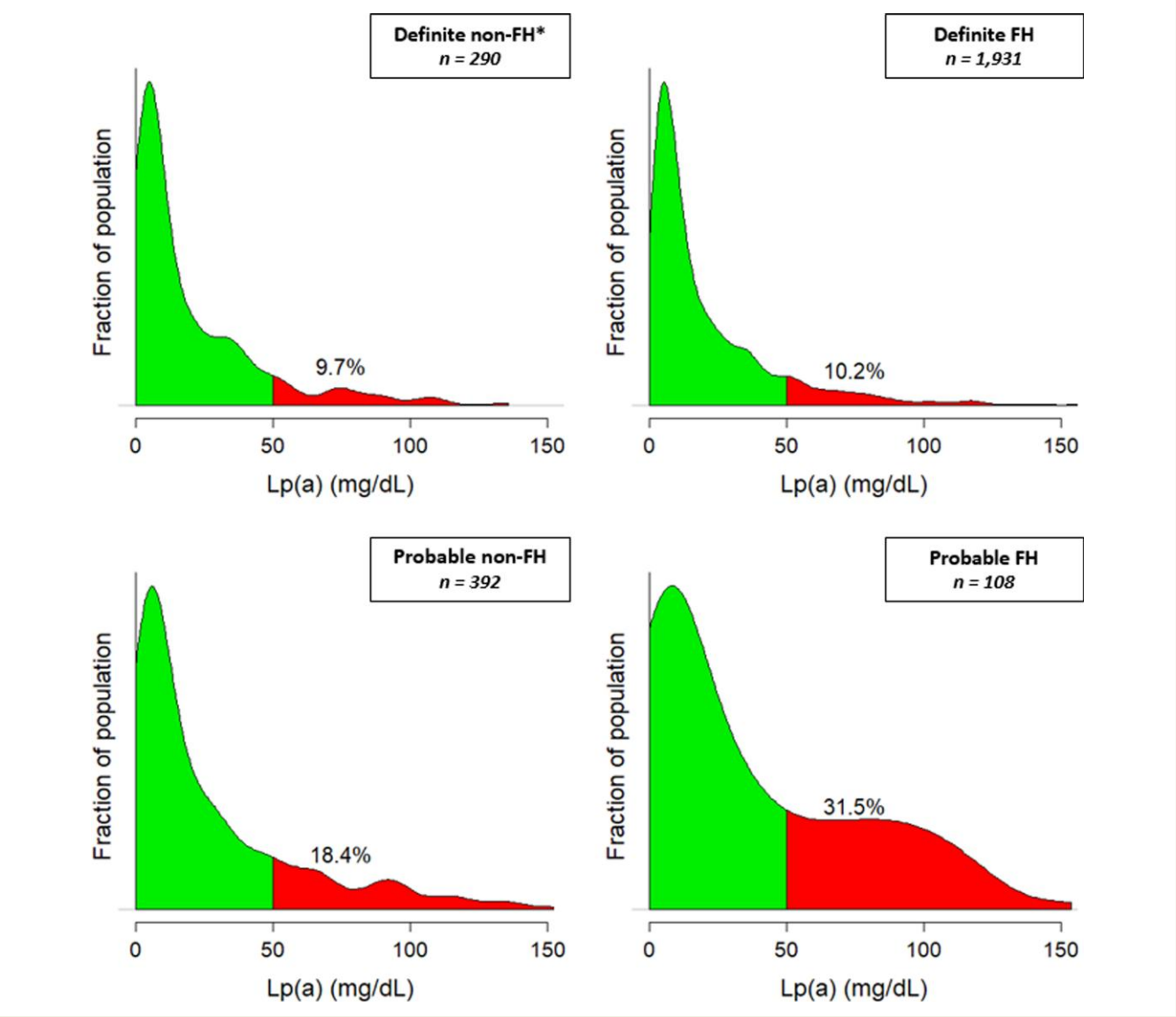


Figure 3 Distribution of lipoprotein(a) levels for the different groups. *Group of unaffected siblings that serve as a normolipidaemic control group.

currently in development, such as gene-silencing therapies, are needed and will likely become the cornerstone of the treatment of high Lp(a) levels, at least in the adult population.[44,45]

Paediatric guidelines recommend measuring Lp(a) as part of CVD risk assessment.[46–48] Not only does measuring Lp(a) lead to significantly improved risk prediction of CVD, it also leads to improved accuracy of FH diagnosis when LDL-C is corrected for Lp(a) cholesterol.[49,50] Our study provides an additional rationale for measuring Lp(a) levels in children and strengthens the idea that DNA analysis as well as measuring Lp(a) levels should preferably be performed in all children suspected of FH. Since no concrete, validated diagnostic tools for diagnosing FH exist in children, performing both is especially important to distinguish children with definite FH from those without an FH mutation but with high Lp(a). More importantly, patients with genetically confirmed FH who also have high Lp(a) levels form another, high-risk group due to a lifelong exposure to two genetic risk factors for CVD[13,32,51,52] and should be identified and monitored as early as possible, preferably during childhood. If high Lp(a) levels are found in children, further optimization of other CVD risk factors is needed, including more aggressive/intensive lipid-lowering treatment and lifelong adoption of a healthy lifestyle.[19,20] Also, family members should be tested for high Lp(a).[52,53]

In our study cohort, 12.2% of the children were found to have elevated Lp(a) levels, with a geometric mean (95% CI) Lp(a) level of 11.5 (11.0–12.1) mg/dL. We were able to include a large group of children compared with most paediatric studies and thereby provide insights into Lp(a) levels in a large paediatric referral population. Other paediatric studies performed in referral populations as well as in healthy children show roughly similar results.[54–58] However, as these studies either did not use median/log-transformed Lp(a) levels in their analysis or used different clinical thresholds for high Lp(a), comparing these results to ours is difficult. In adult studies, it is assumed that 20% of the general population has increased Lp(a) levels of over 50 mg/dL, and that in referral populations, the number of subjects with increased Lp(a) is even higher.[18,32,59] However, in our study cohort, a lower prevalence of elevated Lp(a) was observed compared with adult studies. This might be explained

either by differences in indications for referral or because Lp(a) levels may be lower in children than in adults.[31]

The current study has several limitations that merit discussion. First, our study population comprises a specific group of children, and results may therefore be less generalizable to the general paediatric population. Nonetheless, a number of clinical guidelines state that Lp(a) should be measured in children with an increased risk of CVD, and since our cohort largely comprises children with an increased risk of CVD, the findings of our study are relevant more broadly to the population in whom Lp(a) should be measured. Secondly, two assays were used to measure Lp(a), which may have led to slightly different results over the years. However, we included the measurement assay as a covariate in our analyses, and Lp(a) levels were not significantly different for the two assays. In addition, when adding assay as an interaction term to our model, the association between Lp(a) and the different groups did not change. Therefore, we believe that the impact on our findings was relatively small. Notably, both assays measure the mass-based concentration of Lp(a), which can be influenced by the size of Lp(a) particles as determined by an individual's number of apo(a) kringle repeats. Other assays that measure the molar concentration of Lp(a) are less susceptible to this influence. Thirdly, Lp(a) was missing for some of the children, and therefore they were excluded. However, as demographic and clinical characteristics of children with and without an Lp(a) measurement were similar, we assumed these levels were missing (completely) at random and expected the potential influence of this on our findings to be negligible. Fourthly, because of the observational nature of this study, the association between the clinical groups and Lp(a) may be obscured by confounding. Despite the fact that we adjusted for known (and measured) confounders, it may be possible that some confounders were not measured or taken into account. Finally, approximately 50% of the children with definite FH were referred to the clinic because of an FH-causing mutation was found in family members during cascade screening. As a result of early identification and early treatment initiation in family members, this group may have slightly different characteristics with respect to age at first visit, lifestyle, and in particular, CVD in first degree relatives. To evaluate the consequences of this, we repeated the analyses without this group of children, and results were similar. Therefore, the impact of this potential referral bias seems to be relatively low.

Conclusions

In this study, we provided insights into Lp(a) levels in children with and without probable or definite FH. We demonstrated that Lp(a) levels are significantly higher and more often elevated in children with probable FH than in children with definite FH or normolipidaemic controls. We believe that these children make up a separate patient group that cannot be classified as having FH. We suggest that both DNA analysis and Lp(a) measurement should be performed in all children suspected of FH in order to distinguish between genetically determined FH and high Lp(a) levels, to correct LDL-C for Lp(a) cholesterol, and to identify children that have both definite FH and high Lp(a) levels. Especially in the latter group, which is at even higher risk of CVD, we advise to monitor them carefully, keep LDL-C levels as low as possible, and test family members for high Lp(a) levels.

Supplementary data

Supplementary material is available at *European Heart Journal* online.

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Data availability

The data underlying this article cannot be shared publicly due to privacy reasons. However, on reasonable request, additional analyses can be done after contacting the corresponding author.

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