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Value of Measuring Lipoprotein(a) During Cascade Testing for Familial Hypercholesterolemia

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ABSTRACT

BACKGROUND Familial hypercholesterolemia (FH) and elevated lipoprotein(a) [Lp(a)] are inherited disorders associated with premature atherosclerotic cardiovascular disease (ASCVD). Cascade testing is recommended for FH, but there are no similar recommendations for elevated Lp(a).

OBJECTIVES This study investigated whether testing for Lp(a) was effective in detecting and risk stratifying individuals participating in an FH cascade screening program.

METHODS Family members (N = 2,927) from 755 index cases enrolled in SAFEHEART (Spanish Familial Hypercholesterolemia Cohort Study) were tested for genetic FH and elevated Lp(a) via an established screening program. Elevated Lp(a) was defined as levels ≥50 mg/dl. The authors compared the prevalence and yield of new cases of high Lp(a) in relatives of FH probands both with and without high Lp(a), and prospectively investigated the association between elevated Lp(a) and ASCVD events among family members.

RESULTS Systematic screening from index cases with both FH and elevated Lp(a) identified 1 new case of elevated Lp(a) for every 2.4 screened. Opportunistic screening from index cases with FH, but without elevated Lp(a), identified 1 individual for 5.8 screened. Over 5 years' follow-up, FH (hazard ratio [HR]: 2.47; $p = 0.036$) and elevated Lp(a) (HR: 3.17; $p = 0.024$) alone were associated with a significantly increased risk of experiencing an ASCVD event or death compared with individuals with neither disorder; the greatest risk was observed in relatives with both FH and elevated Lp(a) (HR: 4.40; $p < 0.001$), independent of conventional risk factors.

CONCLUSIONS Testing for elevated Lp(a) during cascade screening for FH is effective in identifying relatives with high Lp(a) and heightened risk of ASCVD, particularly when the proband has both FH and elevated Lp(a). (J Am Coll Cardiol 2019;73:1029–39) © 2019 by the American College of Cardiology Foundation.

Familial hypercholesterolemia (FH) is an autosomal codominant disorder associated with substantially elevated low-density lipoprotein (LDL)-cholesterol and the early onset of atherosclerotic cardiovascular disease (ASCVD) (1). Although a diagnosis of FH can be made using established clinical criteria, including the Dutch Lipid Clinic Network and Simon Broome criteria, genetic testing is recommended and offers the most precise diagnosis (2). This is because a pathogenic FH-causing mutation reflects a lifetime burden of high LDL-cholesterol, and hence, individuals with genetically defined FH have an increased likelihood of developing ASCVD when compared with individuals

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ABBREVIATIONS AND ACRONYMS

apo(a) = apolipoprotein(a)

ASCVD = atherosclerotic cardiovascular disease

BMI = body mass index

FH = familial hypercholesterolemia

HR = hazard ratio

LDL = low-density lipoprotein

Lp(a) = lipoprotein(a)

with the same LDL-cholesterol level, but without a pathogenic mutation (3). In patients with FH, early detection and intervention is crucial for mitigating the time-dependent risk of exposure to high LDL-cholesterol.

Lipoprotein(a) [Lp(a)] is composed of an LDL-like moiety that is covalently bound to apolipoprotein(a) [apo(a)], a large and hydrophilic glycoprotein. Lp(a) is highly polymorphic, and accordingly, levels vary widely (~1,000-fold) between individuals (4). The findings from numerous large epidemiological (5) and Mendelian randomization studies (6–8) have conclusively established Lp(a) as an important risk factor for ASCVD and have demonstrated a curvilinear association with ASCVD risk at levels exceeding 30 mg/dl. Approximately 25% of the general population have Lp(a) levels in the atherogenic range (4).

SEE PAGE 1040

Although FH and elevated Lp(a) are both inherited disorders associated with an increased risk of ASCVD, they have distinct genetic bases. Most individuals with molecularly defined monogenic FH are found to have a pathogenic mutation in the gene encoding the LDL receptor (*LDLR*), with missense mutations in *APOB* and gain-of-function mutations in *PCSK9* accounting for most other cases (9). Levels of Lp(a) are estimated to be between 70% and 90% heritable with most of the variability in plasma levels currently explained by genetic variation in *LPA*, the gene encoding apo(a) (10,11).

The weight of evidence is that Lp(a) is not elevated specifically in FH (12–14). However, elevated Lp(a) has been shown to be an important predictor of ASCVD in FH (13–16). The recently developed SAFEHEART (Spanish Familial Hypercholesterolemia Cohort Study) Risk Equation, which includes Lp(a) levels, has also been shown to predict ASCVD events in patients with FH with significantly greater accuracy than other conventional cardiovascular disease risk equations (17).

Nevertheless there is widespread lack of awareness of the conjoint role of FH and Lp(a) in the acceleration of ASCVD, and most cases in the community remain undiagnosed (18). Cascade screening, that is, the screening of close family members of an index case, is a cost-effective approach for identifying new cases of FH and for preventing ASCVD (19,20). Elevated Lp(a) is also a heritable risk factor that is transmitted independently of FH and may be suitable for cascade testing. However, the effectiveness of Lp(a) cascade screening has not been assessed.

Our hypothesis was that testing for Lp(a) is effective in detecting and risk stratifying individuals participating in an FH cascade screening program. To address this hypothesis, we compared the prevalence and yield of new cases of high Lp(a) in relatives of probands with FH and high Lp(a), with relatives of probands with FH and normal Lp(a). We also prospectively investigated the association between elevated Lp(a) and ASCVD events among all family members tested for high Lp(a).

METHODS

THE SAFEHEART STUDY. The study design of the SAFEHEART cohort has been previously published (21). Briefly, the SAFEHEART study is an ongoing, long-term, nationwide prospective cohort study of genetically defined heterozygous FH conducted in 25 outpatient lipid clinics across Spain. Patients were recruited according to the following inclusion criteria: probands with a genetic diagnosis of FH, and relatives of probands over 15 years of age with a genetic diagnosis of FH; relatives over 15 years of age, but without FH, are enrolled as an FH-negative control group. Entire pedigrees were invited to participate in the screening program. All subjects provided informed written consent. Test results were risk communicated by each subject's physician who had been educated about FH and the importance of Lp(a). Ethics approval was granted by local committees.

STUDY DESIGN: CASCADE SCREENING FOR FH AND LP(A) TESTING. Cascade screening for FH was initiated from genetically defined FH probands who had consented to family members being contacted for testing. Family members were contacted by telephone from the Familial Hypercholesterolemia Foundation, and if consent was provided for cascade screening, these individuals attended an appointment at a participating hospital clinic. The prevalence and yield of elevated Lp(a), defined as a level ≥50 mg/dl, was investigated in families in which the proband was defined as having a pathogenic gene variant causative of FH irrespective of the presence of high Lp(a). Because all relatives underwent both FH and Lp(a) testing, this enabled the comparison of 2 separate screening approaches for the detection of elevated Lp(a) in FH families, namely systematic and opportunistic testing. Consistent with the process of cascade screening, systematic testing for high Lp(a) was employed in the relatives of probands with both genetically defined FH and elevated Lp(a). The detection rate from systematic screening was compared with opportunistic screening in which Lp(a) testing was carried out in the relatives of

probands with genetically defined FH but without elevated Lp(a). Both screening methods yielded 4 possible outcomes: FH alone, elevated Lp(a) alone, FH plus elevated Lp(a), and neither disorder.

Demographic, biochemical, and clinical data, including drug treatments and medical history, were collected at baseline and at annual follow-up visits. Cardiovascular risk factors were defined on the basis of European Society of Cardiology guidelines (22). ASCVD events and death from cardiovascular causes were recorded prospectively for up to 5 years (median follow-up 4.4 years). The overall cohort reported in this study included 755 probands with genetically determined FH, both with and without elevated Lp(a), and 2,927 relatives. On average, 4 relatives were screened per index case (range 1 to 29).

BIOCHEMICAL ANALYSES. Plasma lipid profiles and Lp(a) concentrations were determined from fasting venous blood samples as previously described (13). DNA was extracted from whole blood using established protocols, and FH was diagnosed using a DNA-array-based platform (23). Briefly, in patients with a clinical diagnosis of FH, genetic analysis was carried out using the LIPOchip platform (Progenika Biopharma, Derio, Spain), a microarray containing the most frequent *LDLR*, *APOB*, and *PCSK9* FH mutations found in Spain (23). From June 2008, copy-number variations in the *LDLR* gene were also included; before this, *LDLR* copy-number variation analysis was carried out using adapted quantitative multiplex PCR methodology or multiplex ligation-dependent probe amplification (24). If these genetic analyses did not identify an FH-causing mutation, sequencing within the *LDLR* (promoter, translated exon sequences, exon-intron boundaries) and *APOB* (exon 26) genes was carried out (24).

LDL-cholesterol was calculated using the Friedewald formula. Lp(a) was quantified using an isoform-independent assay (Quantia Lp(a) 7K00-01; Tulip Diagnostics, Bambolim, India) run on an Architect autoanalyzer C16000 (Abbott Diagnostics, Lake Forest, Illinois), and was calibrated using the International Federation of Clinical Chemistry (IFCC) reference apo(a) standard (IFCC/SRM 2B); interassay variation was <7% (13).

DEFINITION OF ASCVD. ASCVD was defined as before (13,17) as the presence of any of the following: 1) myocardial infarction, demonstrated by at least 2 of the following: classic symptoms, specific electrocardiographic changes, and increased levels of cardiac biomarkers; 2) angina pectoris, diagnosed as classic symptoms in combination with at least 1 unequivocal result of 1 of the following: exercise test, nuclear scintigram, dobutamine stress ultrasound scan, or >70% stenosis on a coronary angiogram; 3) percutaneous coronary intervention or other invasive coronary procedures as indicated by the treating physician; 4) coronary artery bypass grafting; 5) ischemic stroke demonstrated by computed tomography or magnetic resonance imaging scan or documented transient ischemic attack; 6) peripheral artery disease: intermittent claudication, defined as classic symptoms and at least 1 positive result of an ankle/arm index <0.9, stenosis>50% on angiography or ultrasonography, or abdominal aortic aneurysm; or 7) peripheral arterial revascularization, that is, peripheral artery bypass grafting or percutaneous transluminal angioplasty. ASCVD death was defined as death as a result of a cardiovascular cause.

TABLE 1 Clinical Characteristics of the FH Probands and Relatives Screened

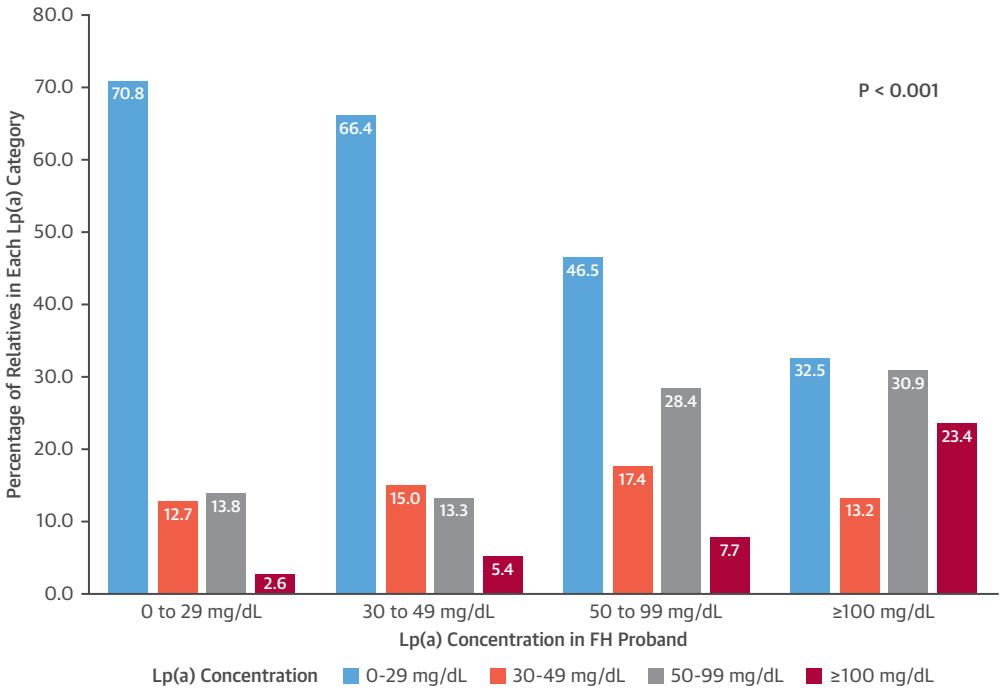
	Probands (n = 755)	Relatives (n = 2,927)	p Value
Age at enrollment, yrs	48.7 ± 13.6	43.6 ± 16.1	**<0.001**
Male	353 (46.8)	1,343 (45.9)	0.668
LDL-C, mg/dl	175.0 ± 61.5	165.7 ± 59.8	**<0.001**
Lp(a)-adjusted LDL-C, mg/dl	163.0 ± 62.4	155.2 ± 59.7	**0.002**
Lp(a), mg/dl*	22.3 (20.5–24.3)	18.8 (18.0–19.6)	**<0.001**
Statin/ezetimibe therapy	709 (94.0)	1,713 (58.6)	**<0.001**
LDL-C apheresis	5 (0.7)	4 (0.1)	**0.009**
Years treated with statins and/or ezetimibe	15.2 ± 7.0	8.6 ± 8.7	**<0.001**
BMI, kg/m^2	26.8 ± 4.7	26.2 ± 4.9	0.001
Diabetes mellitus	32 (4.2)	129 (4.4)	0.840
Arterial hypertension	121 (16.0)	409 (14.0)	0.152
Smoking			
Current	152 (20.2)	893 (30.6)	**<0.001**
Former	245 (32.5)	596 (20.4)	
Never	357 (47.3)	1,434 (49.1)	
ASCVD present at study enrollment	139 (18.4)	259 (8.8)	**<0.001**

Values are mean ± SD, n (%), or geometric mean and (95% confidence intervals). **Bold** p values are significant.
*Ln transformed.

ASCVD = atherosclerotic cardiovascular disease; BMI = body mass index; FH = familial hypercholesterolemia; LDL-C = low-density lipoprotein cholesterol; Lp(a) = lipoprotein(a).

STATISTICAL ANALYSES. The prevalence of elevated Lp(a) in families in which the proband was defined as having both a pathogenic gene variant causative of FH and elevated Lp(a) (systematic screening) was compared with those in whom the index case has FH, but not elevated Lp(a) (opportunistic screening) using chi-square analyses. Yield was described as the number of relatives screened to detect 1 new case of either genetically determined FH or elevated Lp(a). The association between a diagnosis of FH and elevated Lp(a) was estimated as the concordance and discordance rate with Cohen's Kappa statistic. Group differences in demographic, biochemical and clinical

FIGURE 1 Proportion of Relatives With Elevated Plasma Concentrations of Lp(a) in Relation to Plasma Lp(a) Concentrations in Probands With FH



This figure shows the proportion of screened relatives with Lp(a) concentrations between 0 to 29 mg/dl, 30 to 49 mg/dl, 50 to 99 mg/dl, and ≥100 mg/dl when relatives are grouped according to the Lp(a) concentration of their proband. There was a positive association between proband Lp(a) concentration and that of screened relatives; the proportion of relatives with Lp(a) levels exceeding 50 mg/dl significantly increased with the Lp(a) concentration of the proband. FH = familial hypercholesterolemia; Lp(a) = lipoprotein(a).

characteristics were performed using chi-square analysis, Student's *t*-tests and analysis of variance, as appropriate. Lp(a) exhibited a skewed distribution and was log transformed before analysis, and geometric means (95% confidence intervals) were reported. Associations with the composite outcome of ASCVD events and death were investigated using Kaplan-Meier and Cox proportional hazards modeling. Hazards analysis included a per family cluster adjustment to the variance ('vce cluster' command in Stata) to account for potential correlation between observations in members of the same family. Statistical significance was defined at the 5% level. All analyses were carried out in SPSS Statistics software version 21 (IBM, Armonk, New York) and Stata software version 14.1 (StataCorp, College Station, Texas).

RESULTS

SAFEHEART SUBJECT CHARACTERISTICS. The baseline characteristics of all probands (n = 755) and screened relatives (n = 2,927) are shown in **Table 1**. The average age of probands was 48.7 years and relatives, 43.6 years ($p < 0.001$). Approximately 46% of study participants were male. Although LDL-cholesterol was only marginally higher in probands (175.0 mg/dl) compared with relatives (165.7 mg/dl; $p < 0.001$), a higher frequency of probands were receiving statin and/or ezetimibe therapy at the time of study inclusion (94.0% vs. 58.6%; $p < 0.001$) and had been receiving therapy for a significantly longer period than cascade-screened relatives (15.2 years vs. 8.6 years; $p < 0.001$). No individuals were receiving a PCSK9 inhibitor. Lp(a) levels were higher in probands compared with relatives (22.3 mg/dl vs. 18.8 mg/dl; $p < 0.001$). A previous ASCVD event was present at study inclusion in a significantly greater proportion of probands (18.4%) than relatives (8.8%; $p < 0.001$).

There were no major differences in clinical characteristics between relatives of FH probands with elevated Lp(a) and those from FH probands without elevated Lp(a) (Online Table 1). Mean Lp(a) levels

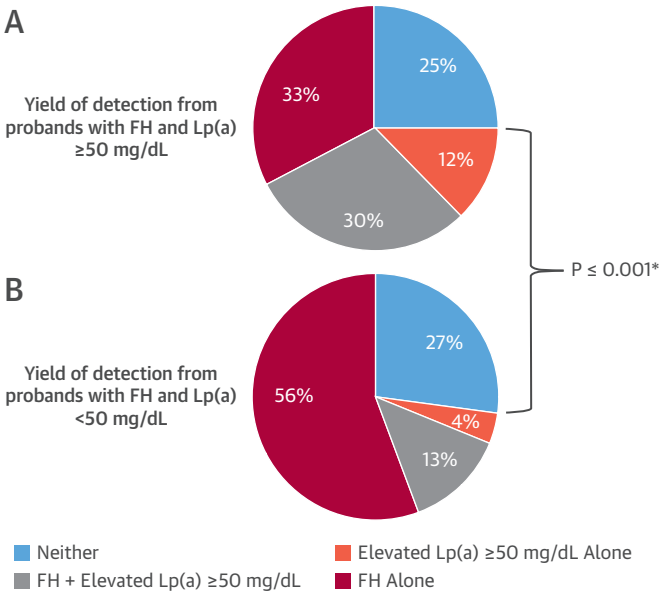
were not significantly different when comparing individuals with an *LDLR* and *APOB* FH mutation (Online Table 2). No *PCSK9* mutations were detected. There was no significant difference in the degrees of separation from the proband for relatives screened with the systematic versus opportunistic screening approach (Online Table 3).

CASCADE SCREENING FOR FH AND DETECTION OF ELEVATED LP(A). Lipoprotein(a) levels in relatives and the relationship with proband concentration. In the total cohort of screened relatives (N = 2,927), 60.7% had Lp(a) <30 mg/dl, 14.2% had levels between 30 and 49 mg/dl, 18.5% between 50 and 99 mg/dl, and 6.6% had an Lp(a) level exceeding 100 mg/dl. As expected, on the basis of the mode of inheritance, the Lp(a) concentration of screened relatives was positively associated with that of the proband ($p < 0.001$) (**Figure 1**) and the degree of separation from the proband (Online Table 4).

Systematic testing: FH plus elevated Lp(a) probands. Consistent with the protocol for cascade testing, this approach focused on probands (n = 222) with both FH and elevated Lp(a). Screening was carried out in 879 relatives. The frequency of the 4 possible diagnostic outcomes in screened relatives were as follows: genetic FH alone (32.8%), elevated Lp(a) alone (12.5%), genetic FH plus elevated Lp(a) (29.6%), or neither disorder (25.1%) (**Figure 2**). These frequencies were confirmed in an independent cascade screening program from the Lipid Disorders Clinic in Perth, Australia (Online Figure 1). Systematic screening identified 1 new case of FH for every 1.6 relatives screened and 1 new case of elevated Lp(a) for every 2.4 relatives screened. The yield of FH plus elevated Lp(a) was 1 in 3.4 (**Central Illustration**). The concordance rate between a diagnosis of FH and elevated Lp(a) among the relatives screened was 54.7%, with a corresponding kappa statistic of 0.128 indicating poor agreement (Online Table 5); the gene variants employed to diagnose FH have been described elsewhere (23,24).

Opportunistic testing: FH index cases without elevated Lp(a). The detection of elevated Lp(a) in relatives of index cases with molecularly defined FH, but without elevated Lp(a), reflects the transmission of elevated Lp(a) from the nonscreened parent as opposed to the proband. In 1,919 relatives from 533 probands with FH, but without elevated Lp(a), we identified genetic FH alone in 55.7% of individuals, elevated Lp(a) alone in 4.0%, genetic FH plus elevated Lp(a) in 13.1%, and neither disorder in 27.2% of relatives. Opportunistic screening identified 1 new case of FH for every 1.5 relatives screened and 1 new case of elevated Lp(a) for every 5.8 relatives screened. For every 7.6 relatives screened, 1 new case of FH plus elevated Lp(a) was identified (**Central Illustration**).

FIGURE 2 The Frequency of FH and Elevated Lp(a) in Relatives Who Were Screened for FH and Elevated Lp(a)

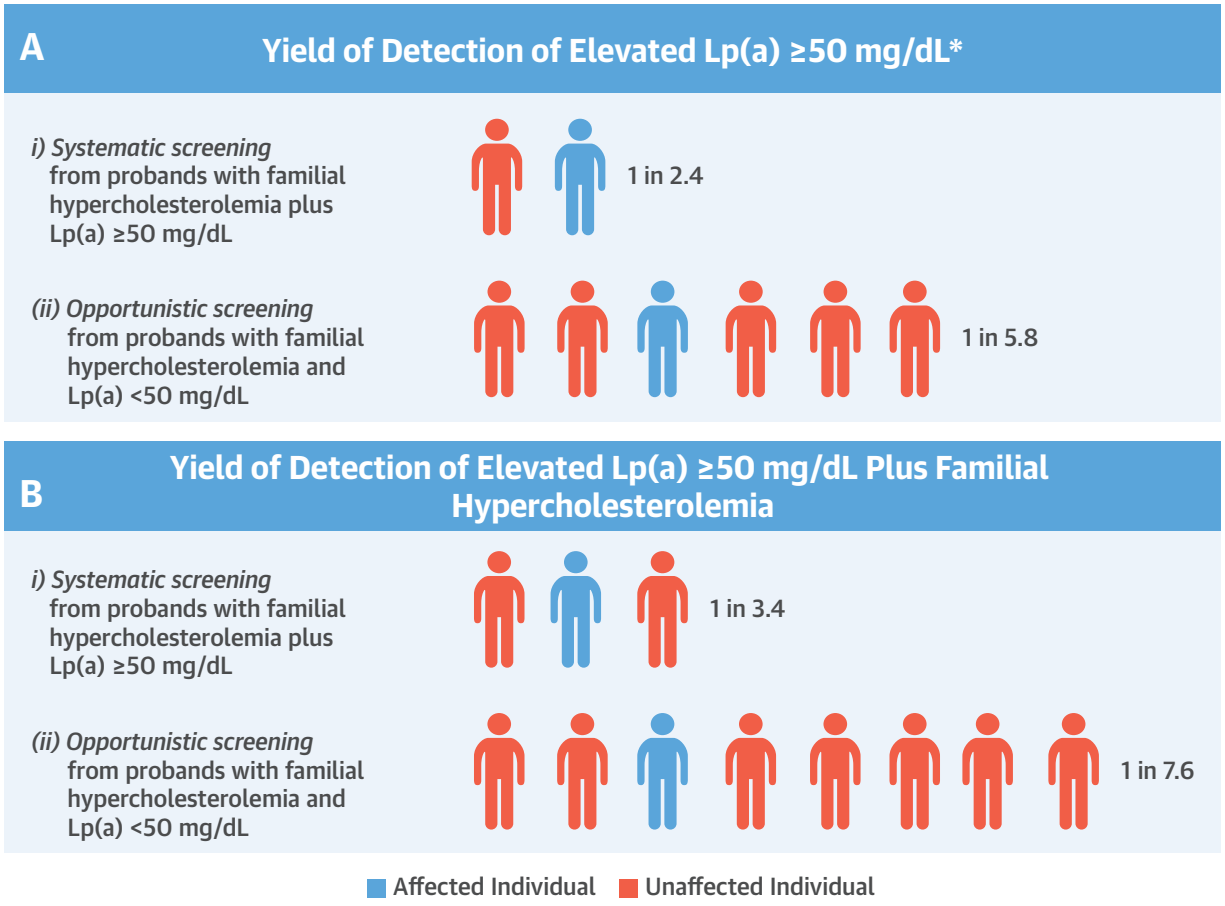


The frequency of FH and elevated Lp(a) is shown in relatives who were screened for FH and elevated Lp(a) from **(A)** probands with Lp(a) ≥50 mg/dl and **(B)** probands with Lp(a) <50 mg/dl. *The p value refers to the comparison of proportions between **(A)** and **(B)**. There were 4 possible outcomes of cascade screening for FH and elevated Lp(a), that is, elevated Lp(a) alone, FH alone, FH plus elevated Lp(a), or neither disorder. The frequency of these disorders were compared between the systematic screening **(A)** and opportunistic screening **(B)** approaches. The frequency of elevated Lp(a) ≥50 mg/dl, either alone or in combination with FH, was significantly lower in relatives tested with opportunistic cascade screening. Abbreviations as in **Figure 1**.

The yield of elevated Lp(a) was significantly lower with opportunistic screening than with systematic screening ($p < 0.001$). The yield of elevated Lp(a) from opportunistic screening was concordant with the prevalence of 1:5 reported in large studies of the general population (4).

CLINICAL CHARACTERISTICS BASED ON FH AND LP(A) DIAGNOSES IN SCREENED RELATIVES. The baseline patient characteristics of screened relatives based on their FH and elevated Lp(a) diagnosis are shown in **Table 2**. A significant difference in age at study enrollment was observed with patients with neither disorder on average 2 to 6 years younger than patients with FH and/or elevated Lp(a) ($p < 0.001$). As expected, LDL-cholesterol ($p < 0.001$) and Lp(a) levels ($p < 0.001$) were higher in those

CENTRAL ILLUSTRATION **Detection of Elevated Lipoprotein(a) in Familial Hypercholesterolemia Families**



Ellis, K.L. et al. J Am Coll Cardiol. 2019;73(9):1029-39.

The yield of detection of **(A)** elevated Lp(a) ≥50 mg/dl and **(B)** elevated Lp(a) ≥50 mg/dl plus FH in relatives tested from designated probands using **(i)** systematic and **(ii)** opportunistic screening. *Refers to the yield of detection of elevated Lp(a) ≥50 mg/dl as an isolated abnormality or in combination with FH. One new case of elevated Lp(a) was detected for every 2.4 individuals screened with systematic cascade screening. A lower yield of detection of 1 new case of elevated Lp(a) for every 5.8 individuals screened was observed for opportunistic cascade screening. Similarly, the yield of detection of FH plus elevated Lp(a) was higher for systematic compared with opportunistic cascade screening (1 in 3.4 vs. 1 in 7.6). Lp(a) = lipoprotein(a).

with FH and elevated Lp(a), respectively. Statin and/or ezetimibe therapy was present at a greater frequency in patients with an FH diagnosis compared with those without FH ($p < 0.001$). Furthermore, individuals identified as having genetic FH had been receiving lipid-lowering therapy for a substantially longer duration ($p < 0.001$). The frequency of the secondary cardiovascular risk factors: diabetes mellitus, hypertension, smoking status, and body mass index (BMI) were compared between groups. Hypertension was more common in patients with elevated Lp(a) ($p = 0.007$), and BMI was higher in patients with both FH and elevated Lp(a) compared with those with neither disorder ($p = 0.016$). There was no significant difference across groups with regard to diabetes mellitus ($p = 0.402$) and smoking status ($p = 0.053$).

ASSOCIATION WITH ASCVD IN SCREENED RELATIVES. During follow-up, 3.2% ($n = 95$) of screened relatives experienced an ASCVD event or died from a cardiovascular cause. Kaplan-Meier survival analysis revealed a significant association between the FH and Lp(a) diagnoses of relatives and ASCVD events (Figure 3) (log-rank $p < 0.001$). Cox proportional hazards analysis identified that the association with ASCVD events/death was independent of the established cardiovascular risk factors: age, sex, hypertension, diabetes mellitus, statin

TABLE 2 Comparison of Clinical Characteristics of the Relatives

	FH and Elevated Lp(a) (n = 531)	FH Alone (n = 1,413)	Elevated Lp(a) Alone (n = 203)	Neither (n = 780)	p Value
Age at enrollment, yrs	47.2 ± 17.0	43.2 ± 15.8	44.1 ± 15.7	41.6 ± 15.6	**<0.001**
Male	236 (44.4)	651 (46.1)	83 (40.9)	373 (47.8)	0.298
LDL-C, mg/dl	186.0 ± 64.6	181.0 ± 60.8	142.0 ± 38.7	130.1 ± 37.2	**<0.001**
Lp(a)-adjusted LDL-C, mg/dl	158.8 ± 64.6	175.9 ± 60.7	117.7 ± 39.5	125.0 ± 37.1	**<0.001**
Lp(a), g/l*	84.0 (81.4–86.6)	11.6 (11.0–12.2)	77.0 (74.0–80.2)	11.2 (10.5–12.1)	**<0.001**
Statin/ezetimibe therapy	432 (81.4)	1061 (75.2)	56 (27.6)	164 (21.1)	**<0.001**
LDL-C apheresis	3 (0.6)	1 (0.1)	0 (0.0)	0 (0.0)	**0.030**
Years treated with statins and/or ezetimibe	12.6 ± 8.2	11.4 ± 8.5	3.5 ± 6.7	2.2 ± 5.1	**<0.001**
BMI, kg/m^2	26.7 ± 5.1	26.1 ± 4.8	26.4 ± 5.2	25.9 ± 4.9	**0.016**
Diabetes mellitus	30 (5.6)	55 (3.9)	10 (4.9)	34 (4.4)	0.402
Arterial hypertension	95 (17.9)	178 (12.6)	36 (17.7)	100 (12.8)	**0.007**
Smoking					
Current	141 (26.6)	421 (29.9)	67 (33.0)	264 (33.9)	0.053
Former	111 (20.9)	296 (21.0)	31 (15.3)	158 (20.3)	
Never	279 (52.5)	693 (49.1)	105 (51.7)	357 (45.8)	
ASCVD present at study enrolment	93 (17.5)	122 (8.6)	7 (3.4)	37 (4.7)	**<0.001**
Follow-up, yrs	3.6 ± 1.7	3.4 ± 1.8	3.4 ± 2.0	3.5 ± 1.9	0.210
ASCVD/death during follow-up	40 (7.5)	40 (2.8)	6 (3.0)	9 (1.2)	**<0.001**

Values are mean ± SD, n (%), or geometric mean (95% confidence intervals). **Bold** p values are significant. Elevated Lp(a) is defined as ≥50 mg/dl. *Ln transformed. Abbreviations as in Table 1.

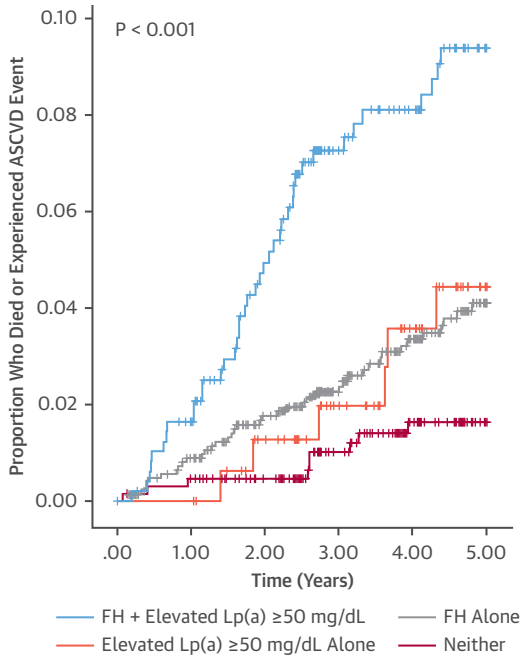
therapy, BMI, smoking, and previous history of ASCVD (Table 3). Adjusting for these univariate predictors of increased risk and family cluster revealed that patients with elevated Lp(a) alone (hazard ratio [HR]: 3.17; p = 0.024) and FH alone (HR: 2.47; p = 0.036) were at heightened risk when compared with individuals with neither disorder. The greatest risk of ASCVD was observed in relatives diagnosed with both genetically determined FH and elevated Lp(a), with these individuals >4 times more likely to have experienced an ASCVD event or died during the follow-up period (HR: 4.40; p < 0.001).

DISCUSSION

Cascade screening of close relatives of probands is widely recognized as effective for detecting new cases of FH. The present study is the first to our knowledge to investigate the outcome of testing relatives for elevated Lp(a) within an FH cascade screening program. Systematic cascade testing for elevated Lp(a) in families in which the proband was defined as having both a pathogenic gene variant causative of FH and elevated Lp(a) was compared with an opportunistic approach in which the index case had FH, but not elevated Lp(a). We demonstrated that screening for elevated Lp(a) using a systematic approach has a higher yield than an opportunistic approach, with opportunistic screening useful for identifying probands and triggering systematic cascade screening (Central Illustration). The association between elevated Lp(a) and ASCVD, particularly in the presence of FH, suggests value in identifying a subgroup of FH relatives at heightened risk. We suggest that testing for elevated Lp(a) should be incorporated routinely into cascade screening programs for FH, irrespective of whether the proband has elevated Lp(a). Although the focus of the present study was on FH, the findings do not exclude the intrinsic merit of cascade screening for elevated Lp(a) outside of FH. This warrants further investigation, however.

Systematic testing for elevated Lp(a) identified 1 in 2.4 with elevated Lp(a) and 1 in 3.4 with FH plus elevated Lp(a). As expected, opportunistic testing had a lower yield, but the findings were comparable to the frequency of elevated Lp(a) reported in the general population (4). Our analysis of concordance in the detection of FH and of elevated Lp(a) are consistent with the notion of 2 genetically orthogonal conditions. This supports previous population-based genetic studies that have identified that *LPA* alleles, expressed in a codominant manner, explain most of the variation in Lp(a) levels (10,11). Although several other genetic loci, including *APOE,* have been found to exhibit smaller effects on plasma Lp(a) levels, these associations require confirmation (10,11). FH follows an autosomal codominant pattern of

FIGURE 3 Kaplan-Meier Survival Analysis in Screened Relatives According to the Presence of FH Plus Elevated Lp(a), Elevated Lp(a) Alone, FH Alone, and Neither Disorder



No. at Risk						
FH + Elevated Lp(a) ≥50 mg/dL	531	472	424	336	302	242
Elevated Lp(a) ≥50 mg/dL Alone	203	162	151	132	115	91
FH Alone	1,413	1,217	1,077	851	742	751
Neither	780	645	603	508	430	348

The proportion of cascade-screened relatives who died or experienced an ASCVD event over a 5-year follow-up period was compared between the FH and elevated Lp(a) groups. Individuals with FH plus elevated Lp(a) **(blue line)** were at the greatest risk of ASCVD. Subjects with FH alone **(gray line)** and elevated Lp(a) **(orange line)** exhibited a similar risk; however, FH in this context reflects treated FH because most of these patients were receiving lipid-lowering therapy. Individuals with neither FH nor elevated Lp(a) **(red line)** were at the lowest risk of death and/or ASCVD event.
ASCVD = atherosclerotic cardiovascular disease; other abbreviations as in **Figure 1**.

TABLE 3 Cox Proportional Hazards Analysis of FH and Lp(a) Diagnoses in Screened Relatives Indicating an Independent Association With ASCVD Events/Death

	HR	95% CI	p Value
Age at inclusion, yrs	1.05	1.03–1.07	**<0.001**
Sex, female vs. male	0.63	0.37–1.07	0.085
BMI, kg/m^2	1.05	1.01–1.09	**0.006**
Hypertension, yes vs. no	1.22	0.73–2.05	0.443
Diabetes, yes vs. no	1.13	0.59–2.16	0.719
Smoking status			
Previous vs. never	1.20	0.67–2.18	0.539
Current vs. never	2.80	1.58–4.95	**<0.001**
Statin/ezetimibe, yes vs. no	0.83	0.41–1.66	0.600
Previous ASCVD event, yes vs. no	4.05	2.11–7.80	**<0.001**
FH and Lp(a) status			
Elevated Lp(a) alone vs. neither*	3.17	1.16–8.64	**0.024**
FH alone vs. neither	2.47	1.06–5.74	**0.036**
FH + elevated Lp(a) vs. neither*	4.40	1.92–10.07	**<0.001**

Bold p values are significant. *Elevated Lp(a) defined as ≥50 mg/dL.
CI = confidence interval; HR = hazard ratio; other abbreviations as in **Table 1**.

inheritance and is caused by pathogenic mutations in the *LDLR* gene and to a lesser extent in *APOB* and *PCSK9* (2).

Our finding of particularly heightened risk of ASCVD in relatives with both FH and elevated Lp(a) agrees with earlier investigations of FH that have shown that the presence of elevated Lp(a) independently enhances ASCVD risk (13,15) and has utility in 5- and 10-year predictions of CV events (17). Lp(a) levels have been correlated with the extent of obstructive disease and predict coronary revascularization in patients with elevated LDL-cholesterol, but not in those with LDL-cholesterol below treatment targets (25). An interaction between high Lp(a) and elevated LDL-cholesterol levels that increases risk of premature acute coronary syndrome has also been demonstrated (26). In a large study of women, the association between Lp(a) and cardiovascular disease was apparent only among those with elevated total cholesterol (27).

Previous investigations have demonstrated that FH is associated with a greater risk of ASCVD than elevated Lp(a). However, in the present study, the risk of ASCVD was similar between those with Lp(a) alone and FH alone. Although counterintuitive, this may be explained by the high rate of statin therapy in those with FH (75%), with the HR for FH reflecting treated as opposed to untreated FH. The HR of 3.2 for elevated Lp(a) alone also appears inflated in the present study when compared with other large epidemiological cohorts, such as the Emerging Risk Factors Collaboration, which have reported a HR of ~1.2 (5–7). The difference in risk between our study and others is likely explained at least in part by our smaller sample size and fewer ASCVD events. It is also worth noting that our study was carried out in clusters of families in whom ASCVD risk may be enriched due to other factors, such as polygenic hypercholesterolemia, and not in an unselected population. This intrinsic difference could also have contributed to the higher HR for elevated Lp(a) in the present study when compared with unselected epidemiological investigations.

There have been several expert guidelines for the detection and management of elevated Lp(a) (4,22,28,29). These guidelines generally propose Lp(a)

screening in individuals at intermediate or high risk of ASCVD, including those with premature CVD, FH, and recurrent events despite receiving optimal lipid-lowering therapy. However, in the absence of clinical outcome data and the lack of therapeutic options for selectively lowering Lp(a), the utility of universal screening for elevated Lp(a) is not currently recognized.

The management of patients with elevated Lp(a) is a therapeutic challenge (18,30). At present, there are no approved therapeutic agents for the selective lowering of Lp(a). Clinical trial data demonstrating a reduction in ASCVD events with interventions specifically targeting elevated Lp(a) are lacking. Nevertheless, currently available therapeutics with Lp(a)-lowering effects include PCSK9 inhibitors, niacin, mipomersen, MTP inhibitors, and lipoprotein apheresis (reviewed in Ellis et al. [18]). An important role for lifestyle intervention in individuals with high Lp(a) has also been recently emphasized (31). In a recent report from the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) trial, after LDL-cholesterol was lowered with evolocumab, Lp(a) remained a predictor of cardiovascular risk, with the lowest risk observed in patients with low LDL-cholesterol plus low Lp(a) (32). However, the greatest reductions in Lp(a) levels have been demonstrated with apo(a) antisense therapy that targets hepatic apo(a) mRNA and reduces Lp(a) concentrations by up to 92.4% with no major adverse effects to date (33). Although additional clinical investigations are required to assess efficacy, safety, and cost-effectiveness in preventing CVD events, RNA-based therapies are likely to be the future treatment of choice to lower very high Lp(a). Findings from genetic studies indicate that future clinical outcome trials designed to test the Lp(a) hypothesis should select individuals with Lp(a) >100 mg/dl and aim to reduce concentrations by 70% (34); others have suggested that a pre-treatment Lp(a) cutoff of 60 mg/dl may be sufficient to see an effect on ASCVD events with antisense therapy (35).

STUDY STRENGTHS. The major strengths of this study are the comparatively large sample size and the availability of prospective follow-up data. The SAFEHEART registry is the largest cohort of molecularly defined FH, allowing us to carry out the most comprehensive examination of the yield of elevated Lp(a) cascade testing in the context of FH. The unique study design also allowed the comparison of 2 methods of screening for Lp(a) within families. The yield of FH and elevated Lp(a) were confirmed in an independent Australian cohort suggesting the generalizability of the results. Furthermore, the diagnosis of FH was made on the basis of the presence of a pathogenic mutation and not clinical criteria, overcoming the potential confounding of the diagnosis by Lp(a) (14).

STUDY LIMITATIONS. Potential weaknesses include the single measurement of Lp(a), the potentially arbitrary cutoff of 50 mg/dl, and the implementation of Lp(a) cascade screening using a plasma threshold and not a molecular diagnosis. Although the atherogenic effects of Lp(a) begin at levels of 30 mg/dl, our definition of elevated Lp(a) was based on current guidelines that recommend an Lp(a) target of below the 80th percentile (< ~50 mg/l) (4,28). Lp(a) was also not measured in parents unaffected by FH. Nevertheless, we have assumed that relatives with high Lp(a) identified through the opportunistic screening approach inherited elevated Lp(a) from the nonscreened parent. The methods for deploying the use of Lp(a) screening and risk communication need to be designed and tested. Challenges include communicating the risk of 2 separate genetic disorders that increase ASCVD, as well as those posed by incidentally identifying elevated Lp(a) and hence the requirement to trigger systematic screening.

CONCLUSIONS

Systematic testing for elevated Lp(a) during cascade screening for FH is highly effective in identifying new cases of high Lp(a). Opportunistic testing had a lower yield but may be a useful approach for detecting probands, with subsequent testing of relatives being more effective employing a systematic approach. The detection of new cases of elevated Lp(a) is important because these individuals are at increased risk of ASCVD, particularly with coexistent FH. Hence, cascade screening programs for FH should incorporate both systematic and opportunistic testing for elevated plasma Lp(a). Our findings also suggest that there may be value in screening for elevated Lp(a) outside of FH; however, this requires further investigation. The practicability, organization, management, and cost-effectiveness of integrated screening strategies for high Lp(a) in families with and without FH remain to be demonstrated. Our study lays the foundations for future research in high-risk families with elevated Lp(a) in an era of novel RNA-based therapies that can selectively and potently lower Lp(a) concentrations.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: Measurement of Lp(a) blood levels during cascade screening for familial hypercholesterolemia can enhance risk assessment.

TRANSLATIONAL OUTLOOK: Further investigation is required to assess the safety and efficacy of selective Lp(a)-lowering therapies in individuals with familial hypercholesterolemia and elevated Lp(a) levels.

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KEY WORDS **atherosclerotic cardiovascular disease, cascade screening, familial hypercholesterolemia, lipoprotein(a)**

APPENDIX **For a supplemental figure and tables, please see the online version of this paper.**