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Genetically Confirmed Familial Hypercholesterolemia in Patients With Acute Coronary Syndrome

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ABSTRACT

BACKGROUND Genetic screening programs in unselected individuals with increased levels of low-density lipoprotein cholesterol (LDL-C) have shown modest results in identifying individuals with familial hypercholesterolemia (FH).

OBJECTIVES This study assessed the prevalence of genetically confirmed FH in patients with acute coronary syndrome (ACS) and compared the diagnostic performance of FH clinical criteria versus FH genetic testing.

METHODS Genetic study of 7 genes (*LDLR*, *APOB*, *PCSK9*, *APOE*, *STAP1*, *LDLRAP1*, and *LIPA*) associated with FH and 12 common alleles associated with polygenic hypercholesterolemia was performed in 103 patients with ACS, age ≤65 years, and LDL-C levels ≥160 mg/dl. Dutch Lipid Clinic (DLC) and Simon Broome (SB) FH clinical criteria were also applied.

RESULTS The prevalence of genetically confirmed FH was 8.7% (95% confidence interval [CI]: 4.3% to 16.4%; n = 9); 29% (95% CI: 18.5% to 42.1%; n = 18) of patients without FH variants had a score highly suggestive of polygenic hypercholesterolemia. The prevalence of probable to definite FH according to DLC criteria was 27.2% (95% CI: 19.1% to 37.0%; n = 28), whereas SB criteria identified 27.2% of patients (95% CI: 19.1% to 37.0%; n = 28) with possible to definite FH. DLC and SB algorithms failed to diagnose 4 (44%) and 3 (33%) patients with genetically confirmed FH, respectively. Cascade genetic testing in first-degree relatives identified 6 additional individuals with FH.

CONCLUSIONS The prevalence of genetically confirmed FH in patients with ACS age ≤65 years and with LDL-C levels ≥160 mg/dl is high (approximately 9%). FH clinical algorithms do not accurately classify patients with FH. Genetic testing should be advocated in young patients with ACS and high LDL-C levels to allow prompt identification of patients with FH and relatives at risk. (J Am Coll Cardiol 2017;70:1732–40) © 2017 by the American College of Cardiology Foundation.

Familial hypercholesterolemia (FH) is an autosomal dominant inherited genetic disorder with a prevalence historically estimated to be on the order of 1:500, but recent data suggest that it could be between 1:200 and 1:250 (1-3). Patients with FH have elevated levels of total cholesterol and low-density lipoprotein (LDL) particles, as well as increased low-density lipoprotein cholesterol (LDL-C) arterial deposits, leading to coronary heart disease (CHD) (4,5).

Patients with FH have cardiovascular complications at an early age and a reduced life expectancy (6,7).

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Early diagnosis followed by an aggressive cholesterol-lowering treatment regimen could prevent occurrence of cardiovascular events by reducing the long-term exposure of these patients and their affected relatives to high levels of LDL-C.

Diagnosis of FH was traditionally based on clinical algorithms, and several groups have developed clinical diagnostic criteria for identification of FH. Among the most widely used FH clinical criteria are those of the Simon Broome (SB) Register Group in the United Kingdom (8) and the Dutch Lipid Clinic (DLC) Network (9).

Advances in genetic testing have made FH genetic testing affordable, but recent studies have shown that FH diagnosis with the use of genetic testing in severely hypercholesterolemic individuals from the overall population is low (between 0.3% and 1.7%) (10,11). This low prevalence suggests a need to identify additional high-risk groups of patients for FH genetic testing. As such, patients with an acute coronary syndrome (ACS) may represent an optimal group for whom FH screening programs could be developed.

SEE PAGE 1741

Although the prevalence of genetically confirmed FH in patients with ACS has not been studied in detail, recent European data found a prevalence between 1.6% and 8.3% in this group of patients when using clinical algorithms (12–14). Patients with ACS and FH are at particularly elevated risk for recurrent cardiovascular complications (12), and current management of these patients focuses on aggressive lipid-lowering strategies. Prompt identification of FH among patients with ACS could be extremely useful to allow early intensification of lipid-lowering treatment and might lead to early identification of relatives with FH who have not yet experienced cardiovascular events but who would benefit from early initiation of intensive lipid-lowering therapies (9,15,16).

The goal of the present study was to determine the prevalence of genetically confirmed FH in patients with ACS and to evaluate the diagnostic performance of FH clinical criteria compared with FH genetic findings.

METHODS

Clinical records were reviewed for all patients ≤65 years of age hospitalized at Hospital Universitario Puerta de Hierro (Madrid, Spain) for ACS from January 1, 2012, to March 31, 2016. All patients with actual or estimated LDL-C levels ≥160 mg/dl (4.14 mmol/l) on admission were contacted and offered FH genetic testing. In all patients receiving statin therapy or ezetimibe before admission, LDL-C levels were estimated by multiplying their LDL-C level on treatment with correction factors considering the drug and its dose, as previously reported (17–19). The effect of other lipid-lowering drugs was not considered.

Levels of LDL-C were calculated according to the Friedewald formula (20). Patients were excluded from the study if their triglyceride levels were >350 mg/dl (4 mmol/l). Patients without information on cholesterol levels at admission and those with lipid disorders secondary to renal, thyroid, or liver diseases were also excluded.

Whole blood or saliva samples for deoxyribonucleic acid (DNA) analysis were collected from patients who were accepted into the study and, simultaneously, data about their personal and family history were collected, and a physical examination was performed. The patient selection process is represented in the flowchart in **Figure 1**. The study protocol complied with the Declaration of Helsinki and was approved by the ethics committee of Hospital Universitario Puerta de Hierro. All participants gave written informed consent to participate in the study.

FH CLINICAL CRITERIA. The clinical diagnosis of FH was based on 2 widely used FH clinical criteria recommended by international guidelines. The SB criteria (8), recommended by the United Kingdom's National Institute for Health and Care Excellence guidelines, considers a diagnosis of possible FH as a total cholesterol level >290 mg/dl or LDL-C level >190 mg/dl, plus a family history of premature coronary artery disease. A definite FH diagnosis requires the aforementioned cholesterol levels and the presence of tendon xanthomas in the patient or relatives (physical signs of hypercholesterolemia). The DLC criteria (9), endorsed by the European Society of Cardiology, the National Lipid Association in the United States, the International FH Foundation, and the European Atherosclerosis Society, considers LDL-C levels, physical signs, and a personal or family history of premature CHD (Online Tables 1 and 2). Possible FH is defined according to a DLC criteria score of 3 to 5 and probable to definite FH by using a score ≥6. Both sets of criteria include genetic findings among the parameters to consider (which would, per se, at least for DLC clinical criteria, generate a definite diagnosis of FH). Because genetic information is usually not available for most clinicians, and because we wanted to compare the diagnostic performance of genetic testing versus clinical criteria, genetic information was not considered when calculating FH clinical criteria by both algorithms.

ABBREVIATIONS AND ACRONYMS

ACMG = American College of Medical Genetics and Genomics

ACS = acute coronary syndrome

CHD = coronary heart disease

DLC = Dutch Lipid Clinic

DNA = deoxyribonucleic acid

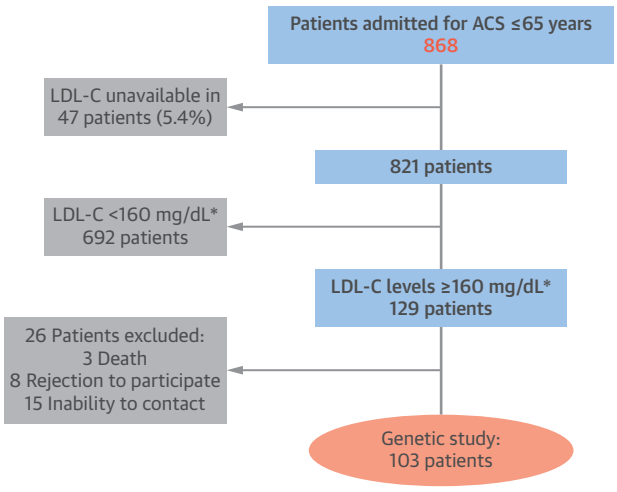
FH = familial hypercholesterolemia

LDL-C = low-density lipoprotein cholesterol

SB = Simon Broome

VUS = variants of unknown significance

FIGURE 1 Patient Selection



Flowchart showing the successive steps taken during the study. *Estimated untreated low-density lipoprotein cholesterol (LDL-C) levels for those patients taking statins or ezetimibe. ACS = acute coronary syndrome.

DNA SEQUENCING. Genomic DNA was extracted from saliva or peripheral blood samples. Targeted enrichment was performed with a custom resequencing solution (Lipid inCode, Ferrer inCode, Barcelona, Spain). The design was based on the human reference genome (hg19), and 120 bp-length ribonucleic acid biotinylated baits were defined to extensively cover all regions of interest.

The experimental procedure was performed according to the manufacturer's instructions with some modifications as a result of our internal validations. Very briefly, 50 ng of high-quality double-stranded DNA from every sample was enzymatically fragmented and, after hybridization to the solution and capture, libraries were amplified by polymerase chain reaction and indexed. Final libraries were quantified and their quality assessed on a bioanalyzer using high-sensitivity DNA chips. All libraries were then pooled and sequenced (up to 40 per run). The sequencing paired-end process was developed on an integrated sequencing system using 2 × 75 bp reads length.

The in vitro diagnostic platform used performed the complete analysis of promoters, coding regions, and exon-intron boundaries of 5 genes associated with FH (*LDLR*, *APOB*, *PCSK9*, *APOE*, and *STAP1*) and 2 genes associated with other conditions that have partially overlapping clinical features with FH (autosomal recessive hypercholesterolemia [*LDLRAP1*] and lysosomal acid lipase deficiency [*LIPA*]).

The diagnostic platform used also interrogated a weighted LDL-C-raising gene score identified by the Global Lipids Genetics Consortium (Online Table 3), based on 12 LDL-C-raising genetic variants; this score determines the likelihood that a patient has polygenic hypercholesterolemia. The calculation of the risk score was computed as described in Talmud et al. (21) and determined in patients without variants in FH-related genes. A gene score ≥1.08, which is the ninth decile cutoff for the Whitehall II control cohort, has been proposed as highly suggestive of polygenic hypercholesterolemia (22).

Minimum mean coverage was 696 reads per position, and >100% of the fragments (gene regions as well as single nucleotide polymorphisms genotyped) had coverage >30 reads. Sanger sequencing was used to confirm the genetic variants found.

VARIANT DATA AND PATHOGENICITY CLASSIFICATION. Variant data analysis is described in the Online Appendix. Variants with a minor allele frequency <1% in the general population were considered as noncommon variants. The potential pathogenicity of rare variants was evaluated by considering the recommendations published by the American College of Medical Genetics and Genomics (ACMG) (23), in which different criteria are evaluated: type and variant frequency; functional data if available; scientific support; and computational information for predicted pathogenicity in genomic (PolyPhen2, Provean v.1.1.3, and MutationTaster2) or intronic (MaxEntScan, NNSplice, FSPLICE, and GeneSplicer) regions, among others (23). Moreover, information on >2,200 FH-related genomic variants included in a private database was also considered to complete the evaluation of genetic variants. Variants with a clinical relevance were reported as pathogenic (class I), likely pathogenic (class II), and variants of unknown significance (VUS) (class III).

All first-degree relatives of patients with pathogenic and likely pathogenic variants were offered clinical and genetic evaluation. In addition, clinical and genetic evaluation was proposed to first-degree relatives of patients with VUS who, according to ACMG recommendations, could be reclassified as pathogenic or likely pathogenic if a positive cosegregation is found. These VUS were reclassified as pathogenic or likely pathogenic if they segregated with the clinical phenotype in >2 relatives on familial evaluation. VUS without corroborative family screening data remained as VUS.

STATISTICAL ANALYSIS. Continuous data are reported as mean ± SD. Discrete data are presented as percentages. Analysis of differences in characteristics between groups was conducted by using standardized effect size measures, estimating odds ratios for

categorical variables or Cohen's *d* for numerical values, as well as their corresponding 95% confidence intervals (CIs). The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed by using IBM SPSS Statistics for Windows version 22.0 (IBM SPSS Statistics, IBM Corporation, Armonk, New York) and Stata/IC version 14.2 (StataCorp LLC, College Station, Texas).

RESULTS

The study cohort comprised 103 patients (mean age 54 ± 6.7 years; range 37 to 65 years), 87.4% of whom were male, admitted for an ACS. Forty-seven were admitted for ST-segment elevation myocardial infarction, 47 for non–ST-segment elevation myocardial infarction, and 9 for unstable angina. Mean LDL-C level at admission was 189.5 ± 34.7 mg/dl, but only 39 patients (37.9%) were receiving statin therapy. Sixteen patients (15.5%) had a history of CHD, 3 (2.9%) had a history of stroke, and 6 (5.8%) exhibited peripheral vascular disease. None of the patients had been diagnosed with FH previously by their primary care physicians or treating physicians. Other clinical characteristics are presented in **Table 1**.

After clinical evaluation using the DLC algorithm, 12 patients (11.7%) fulfilled criteria for definite FH, and 16 patients (15.5%) had probable FH. Thus, DLC criteria classified 28 patients (27.2%) with probable or definite FH. Based on SB criteria, 28 patients (27.1%) had definite (n = 2; 1.9%) or possible (n = 26; 25.2%) FH (**Table 2**).

Genetic testing revealed 9 heterozygous pathogenic or likely pathogenic FH mutations in 9 individuals (8.7%). Seven mutations were found in the *LDLR* gene, 1 in *PCSK9*, and 1 in *STAP1* (Online Table 4). Five VUS were also found in patients with pathogenic or likely pathogenic FH mutations. Thirty-two patients carried 35 VUS, and 62 individuals (60.2%) had no genetic variation in FH-related genes. In addition, 7 patients were heterozygous for variants in *LDLRAP1* (autosomal recessive hypercholesterolemia), and 5 patients carried heterozygous variants in the *LIPA* gene (homozygous mutations in this gene cause lysosomal acid lipase deficiency).

Familial genetic evaluation was offered to first-degree relatives of the 9 patients with pathogenic or likely pathogenic mutations and to the relatives of the 6 patients with VUS (3 in *LDLR*, 2 in *APOB*, and 1 in *PCSK9*) (Online Table 4) that, based on the ACMG recommendations, could have been reclassified (23).

Familial screening was not possible or was rejected in 5 families (2 with pathogenic or likely pathogenic variants, and 3 with VUS). Clinical and genetic study of 21 first-degree relatives from 10 families (7 with pathogenic or likely pathogenic mutations, and 3 with VUS) was ultimately performed (Online Table 5). Familial evaluation did not allow reclassification of any VUS as pathogenic or likely pathogenic according to ACMG criteria (23). Therefore, the final prevalence of genetically confirmed FH among ACS patients ≤65 years of age with LDL-C levels ≥160 mg/dl was 8.7% (95% CI: 4.3% to 16.4%; n = 9) (**Figure 2**).

Clinical, analytical, and treatment characteristics of ACS patients with and without FH mutations were compared (**Table 3**). When comparing FH diagnosis according to genetic testing versus FH clinical criteria, 4 patients (44%) with genetically confirmed FH were not diagnosed according to DLC criteria, and 3 (33%) failed to be confirmed by using SB criteria (**Table 4**). Conversely, 82.1% (95% CI: 62.4% to 93.2%; n = 23) of patients diagnosed by using the DLC algorithm and 78.6% (95% CI: 58.5% to 90.9%; n = 22) diagnosed by using the SB criteria did not exhibit any

TABLE 1 Baseline Characteristics (N = 103)

Mean age at admission, yrs	54.0 ± 6.7
Male	90 (87.4)
White race	91 (88.3)
Hypertension	42 (40.8)
Diabetes	18 (17.5)
Smoking	58 (56.3)
Glomerular filtration rate, ml/min/1.73 m^2	93.3 ± 18.2
Total cholesterol, mg/dl	241.3 ± 35.7
LDL-cholesterol, mg/dl	189.5 ± 34.7
HDL-cholesterol, mg/dl	41.8 ± 10.0
Triglycerides, mg/dl	154.2 ± 61.7
On statins at admission	39 (37.9)
Other lipid-lowering agent	8 (7.8)
Unstable angina	9 (8.7)
Non-STEMI	47 (45.6)
STEMI	47 (45.6)
Previous CHD	16 (15.5)
Stroke	3 (2.9)
Peripheral vascular disease	6 (5.8)

Values are mean ± SD or n (%).
CHD = coronary heart disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; STEMI = ST-segment elevation myocardial infarction.

TABLE 2 Prevalence of FH Based on Clinical Scores Versus Genetic Study

Dutch Lipid Clinic Criteria	Simon Broome Criteria	Genetic Study
Unlikely FH: 23 (22.3)	Unlikely FH: 75 (72.8)	Negative: 62 (60.2)
Possible FH: 52 (50.4)	Possible FH: 26 (25.2)	VUS: 32 (31.1)
Probable FH: 16 (15.5)	Definite FH: 2 (1.9)	Pathogenic: 9 (8.7)
Definite FH: 12 (11.7)		

Values are n (%).
FH = familial hypercholesterolemia; VUS = variants of unknown significance.

FIGURE 2 Genetic Testing Results

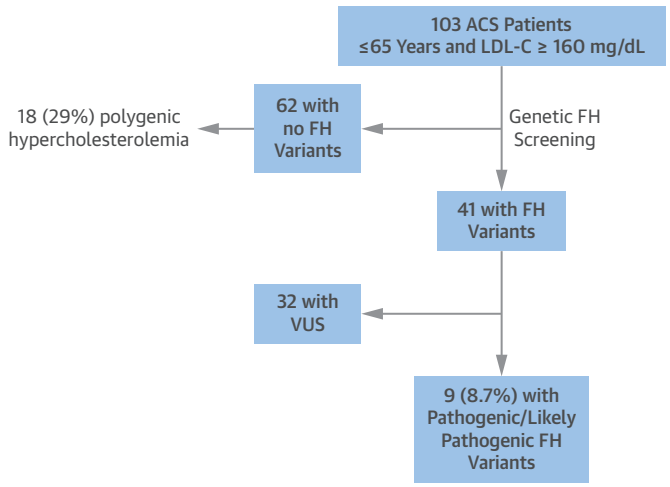


Diagram explaining the results of the genetic study performed in patients with ACS. FH = familial hypercholesterolemia; VUS = variants of unknown significance; other abbreviations as in Figure 1.

FH mutation. Furthermore, 29.03% (95% CI: 18.5% to 42.13%; n = 18) of the individuals without FH genetic variants had a genetic score consistent with polygenic hypercholesterolemia. Of note, 3 patients who fulfilled DLC FH clinical criteria and who did not exhibit genetic variants in FH-causing genes had a genetic score suggestive of polygenic hypercholesterolemia. The familial study led to the diagnosis of 6 relatives with FH mutations, of whom 4 presented with elevated LDL-C levels or were already taking statins (Online Table 5).

Finally, the retrospective nature of our study allowed us to analyze 1-year LDL-C levels in patients with ACS and with genetically confirmed FH identified in this study. Only 1 of the 9 patients had LDL-C levels <70 mg/dl, as recommended in guidelines. Two patients had levels between 70 and 100 mg/dl, and 6 patients had LDL-C levels >100 mg/dl, even though most of them were taking high doses of lipid-lowering drugs (Online Table 6).

TABLE 3 Characteristics of Patients With and Without Genetically Confirmed FH

	FH Mutation (n = 9)	No FH Mutation (n = 94)	Standardized Effect Size (95% CI)
Male	8 (87.2)	82 (88.9)	1.17 (0.13 to 56.21)
Mean age at admission, yrs	55.0 ± 5.9	54.0 ± 6.8	0.15 (-0.54 to 0.83)
White race	8 (88.9)	83 (88.3)	1.06 (0.12 to 51.26)
Statin at admission	4 (44.4)	35 (37.2)	1.35 (0.25 to 6.71)
Hypertension	3 (33.3)	39 (41.5)	0.71 (0.11 to 3.56)
Diabetes	1 (11.1)	17 (18.1)	0.57 (0.01 to 4.75)
Smoking	6 (66.7)	52 (55.9)	1.62 (0.32 to 10.53)
Previous ischemic heart disease	1 (11.1)	15 (16.0)	0.66 (0.01 to 5.59)
Stroke	1 (11.1)	2 (2.1)	5.75 (0.09 to 118.39)
Peripheral vascular disease	0 (0.0)	6 (6.4)	-
Total cholesterol, mg/dl	256.6 ± 52.2	239.8 ± 33.7	0.47 (-0.22 to 1.16)
LDL-cholesterol, mg/dl	222.3 ± 52.5	186.4 ± 31.1	1.08 (0.38 to 1.78)
HDL-cholesterol, mg/dl	40.22 ± 7.2	41.97 ± 10.5	-0.17 (-0.85 to 0.51)
Triglycerides, mg/dl	121.9 ± 32.7	157.3 ± 62.9	-0.58 (-1.27 to 0.11)
Family history of ischemic heart disease according to			
Dutch Lipid Clinic criteria	4 (44.4)	17 (18.1)	3.62 (0.64 to 8.58)
Simon Broome criteria	5 (55.6)	31 (33.0)	2.54 (0.50 to 13.62)

Values are n (%) or mean ± SD, unless otherwise indicated.
CI = confidence interval; other abbreviations as in Tables 1 and 2.

DISCUSSION

The present study described, for the first time, a complete genetic analysis of genes associated with FH in patients with ACS who were ≤65 years of age and had LDL-C levels ≥160 mg/dl. Our study showed that the prevalence of genetically confirmed FH in these patients is approximately 9%. This finding is much lower than the estimated FH prevalence as determined by widely accepted clinical FH criteria (27% in our cohort) but at the same time much higher than what has been previously reported in other FH genetic screening studies (**Central Illustration**). Moreover, our study showed that FH clinical algorithms do not accurately identify FH subjects among patients with ACS, but FH genetic testing in this population is useful to facilitate early diagnosis of patients and their relatives at risk.

Early recognition of FH is essential because many patients with FH are unaware of their disease, which is a major cause of early CHD. Identifying FH allows specific counseling for diet and cardiovascular risk factors, and it ensures high-dose statin prescription and appropriate referral of family members for FH screening.

Recent European guidelines for prevention of CHD in FH underlined the utility of identifying causal mutations to facilitate cascade screening (24). Although cascade screening is the best means to identify patients with FH, as they can be identified before an event occurs, it requires earlier identification of the FH probands, which is not an easy task.

Recent screening studies in which participant selection was based solely on a single elevated LDL-C level were disappointing and reported FH mutations in <2% of patients with severe hypercholesterolemia (10,11). This low yield of FH diagnosis called into question the utility of genetic screening programs in unselected patients with high LDL-C levels; plus, it highlighted the need to find other clinical scenarios in which genetic screening would yield a higher uptake

(10). Two approaches (national screening of infants with very high total cholesterol levels or primary care screening programs during routine immunization visits) have turned out to be very good strategies, as shown by 2 recent studies from Slovenia and the United Kingdom (25,26). Unfortunately, implementing national screening programs in children is complex, and this method cannot be applied in many countries. By contrast, identifying FH individuals during hospitalization for ACS could be of great interest in the absence of national FH screening programs. ACS might be the first manifestation of FH, and a hard event such as ACS could have a great impact among relatives, facilitating familial screening. Despite its suspected importance, the prevalence of genetically confirmed FH in ACS has never been investigated by using a complete genetic approach, and the only reported study described a very low detection rate (27).

Wald et al. (27) reported a prevalence of FH of 1.3% in young patients (≤50 years of age) with myocardial infarction at a London hospital. Unlike our study, the genetic analysis performed by these investigators included a panel of 48 known FH mutations and whole exon deletions or duplications of *LDLR* regardless of cholesterol levels, followed by Sanger sequencing of *LDLR* in individuals without mutations and a total cholesterol level >271 mg/dl. By contrast, we used next-generation sequencing to study the promoter, coding, and exon-intron boundary regions of 5 FH-causing genes. These methodological differences, plus a less restrictive patient approach (we included individuals ≤65 years of age and with LDL-C levels ≥160 mg/dl), could explain the differences found between the studies and should be considered when designing genetic screening programs.

The prevalence of clinical FH in ACS patients has recently been studied in Europe by using FH clinical scores (13,14). In the Swiss SPUM-ACS (Special Program University Medicine-Acute Coronary Syndromes) cohort that included 4,778 patients with ACS, 1.6% (95% CI: 1.3% to 2.0%) of patients fulfilled criteria of probable to definite FH according to DLC criteria (14). The prevalence of clinical FH was 4.8% in 1,451 patients with ACS and premature CHD (<55 years of age for men and <60 years of age for women). In >7,000 European patients with CHD from the EUROASPIRE (European Action on Secondary and Primary Prevention through Intervention to Reduce Events) IV study, the prevalence of probable to definite FH was 8.3% overall but 15.4% in the 2,212 patients who were <60 years of age (13). Our study reported an FH prevalence of 27.2% (95% CI: 19.1% to 37.0%) according to the DLC and the SB criteria. We think that the higher prevalence found in our cohort was partly related to the LDL threshold used, which selected individuals with higher pre-test probability. In addition, data about clinical signs of lipid accumulation in tissue, as well as information on family history of elevated LDL-C level, were not available to the SPUM-ACS investigators, and they decided that missing information counted as zero in the DLC algorithm (14). By contrast, in our study, we were able to perform physical examination in all participants (the presence of xanthomas is one of the items that yields more points in the clinical scores) and also obtain data from the participants' personal and family history. These 2 critical factors (LDL-C threshold and clinical or familial information) might explain the higher FH prevalence as determined by clinical criteria found in the present study.

TABLE 4 Clinical Scores of Patients With or Without FH Mutation

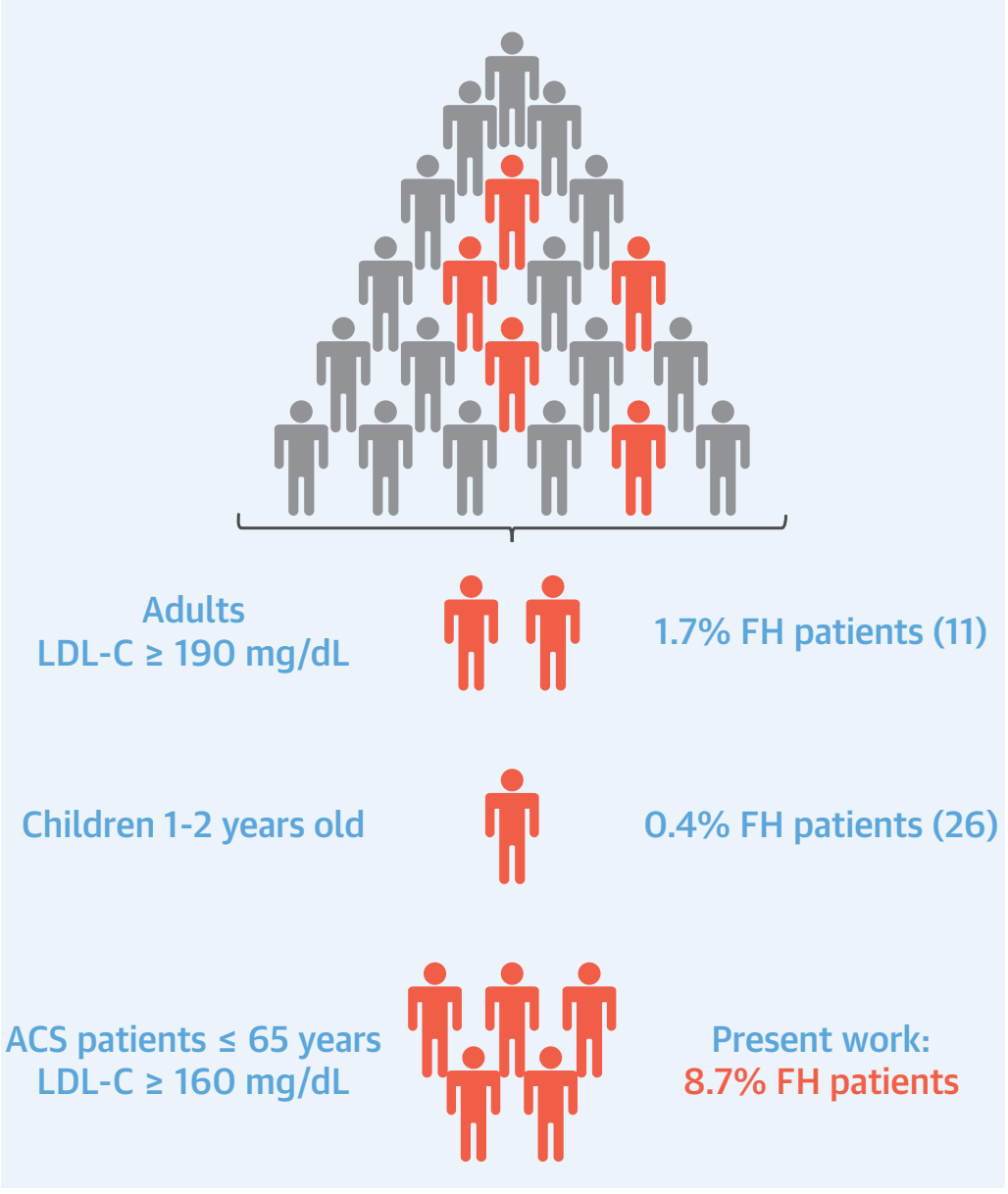
	FH Mutation (n = 9)	No Mutation + VUS (n = 94)	Odds Ratio (95% CI)
Score, Dutch Lipid Clinic criteria			
Unlikely FH	0 (0.0)	23 (24.5)	
Possible FH	4 (44.4)	48 (51.1)	
Probable FH	2 (22.2)	14 (14.9)	
Definite FH	3 (33.3)	9 (9.6)	
Score, Dutch Lipid Clinic criteria (probable or definite)	5 (55.5)	23 (24.5)	3.86 (0.75–20.86)
Score, Simon Broome criteria			
Unlikely	3 (33.3)	72 (76.6)	
Possible FH	6 (66.7)	20 (21.3)	
Definite FH	0 (0.0)	2 (2.1)	
Score, Simon Broome criteria (possible or definite)	6 (66.7)	22 (23.4)	6.54 (1.25–42.79)

Values are n (%), unless otherwise indicated.
Abbreviations as in Table 2.

Nevertheless, one of the main findings of our study was the demonstration that FH clinical scores were unable to correctly identify ACS patients with and without FH. As shown here, 30% to 40% of patients with confirmed FH mutations were not detected by using FH clinical scores, whereas more than three-quarters of patients with ACS diagnosed with FH according to clinical scores did not harbor any FH mutation. Our findings aligned with recent publications (2,28), which have also shown that clinical FH criteria were unable to identify FH individuals compared with genetic testing. Nevertheless, our results must be taken in the context of the ACS setting, in which available information about FH prevalence is currently restricted to FH clinical criteria (13,14).

Recently, several opinion leaders in FH concluded that 3 parts of the FH clinical diagnostic criteria are no longer as useful as they once were (29). With the widespread use of statins over the last 30 years,

CENTRAL ILLUSTRATION **Results of FH Genetic Screening Programs**



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Familial hypercholesterolemia (FH) genetic screening in young patients with acute coronary syndrome (ACS) and high levels of low-density lipoprotein cholesterol (LDL-C) improves detection of FH. Results of FH screening were compared in this cohort of 103 young patients (<65 years of age) with ACS and LDL-C levels >160 mg/dl versus a recent genetic screening study in adults with a single elevated LDL-C level >190 mg/dl (11) and with a primary care genetic screening program in children 1 to 2 years of age during routine immunization visits (26).

average LDL-C levels across the general population are lower, physical examination findings such as xanthomas are found less frequently, and family history information is less useful (i.e., there is the potential for less CHD development in FH families).

Our results also showed that FH clinical criteria do not seem to be useful in individuals with premature ACS, and the high FH genetic uptake found in our study would strongly favor the adoption of FH genetic testing strategies over FH clinical criteria in this

clinical setting. Interestingly, in our study, 29% of individuals without FH variants had a high score for polygenic hypercholesterolemia, which is also a relevant finding. Furthermore, 3 patients with a genetic score suggestive of polygenic hypercholesterolemia fulfilled FH clinical criteria and, in the absence of genetic study, their relatives would have had to undergo FH clinical screening according to current guidelines.

The National Institute for Health and Care Excellence cost-effectiveness study found that cascade screening was more efficient when guided by genetic testing for a known FH mutation (30). Because of the FH genetic screening performed in this study, clinical FH screening is no longer necessary in relatives of numerous patients who did not present FH mutations irrespective of the clinical criteria findings of the proband.

The present study also provided some data on the impact of identifying genetically confirmed FH among patients with ACS. At the 1-year follow-up, only 1 FH proband presented with recommended LDL-C levels <70 mg/dl even though most were receiving high doses of statin and, in some cases, ezetimibe as well. Recent data showed that patients with FH identified by using clinical criteria have a >2-fold adjusted risk of coronary event recurrence within the first year after discharge than patients without FH (12); other investigators have shown that a vast majority of patients with FH do not reach LDL-C target levels for secondary prevention (12,14,31). These results emphasize the need for better monitoring and utilization of available medication in patients with FH. Prompt recognition of FH status is extremely important to identify individuals with ACS and higher risk and who should be treated aggressively soon after the ACS event.

Finally, our study showed the benefits of FH genetic screening at the family level, as the maximum usefulness of FH genetic screening is not to identify subjects with FH who have already experienced an event but rather to identify other FH subjects at risk of future events that can be avoided. In our study, FH genetic screening allowed diagnosis of FH in 6 first-degree relatives who otherwise would have remained unidentified by clinical criteria in most cases. As an example of early FH diagnosis prompted by genetic screening in subjects with ACS, a 6-year-old girl with FH and an LDL-C level of 202 mg/dl was identified in our study (see family of 9 in Online Table 5). Given the importance of early diagnosis of FH before an event occurs, we believe that genetic studies constitute a fundamental tool to improve the prognosis of patients with FH.

STUDY LIMITATIONS. Most of the patients were white male subjects, which might limit the external applicability of the results. LDL-C level was measured in the first 48 h after ACS admission, and some evidence suggests that LDL-C levels are decreased during this time. Moreover, untreated LDL-C levels were estimated for those patients who were taking statins or ezetimibe before admission. This approach might inaccurately estimate LDL-C level given the heterogeneity in drug selection, dosing, and individual response and variability across baseline LDL-C levels or mutation status. Furthermore, next-generation sequencing testing does not detect inversions and translocations. Although these genetic abnormalities probably are not major causes of FH, we cannot address their effect in our cohort. Although cost of FH next-generation sequencing genetic testing is now small (~300 to 350 Euros), and cascade FH screening is more efficient when guided by genetic testing, the cost-effective consequences of adopting a large-scale FH genetic screening program in patients with ACS following the criteria used in our study are unknown. Finally, the unicentric and retrospective nature of our research should be taken into consideration, and our results must be replicated, ideally in a large prospective study.

CONCLUSIONS

Prevalence of genetically confirmed FH in ACS patients ≤65 years of age and with an LDL-C level ≥160 mg/dl is high (approximately 9%). FH clinical algorithms do not accurately identify patients with FH in this setting, with a substantial number of patients with genetically confirmed FH unidentified by using clinical criteria, whereas there are also numerous individuals diagnosed with FH by using clinical criteria without FH mutations and with a genetic score consistent with polygenic hypercholesterolemia. Our data support the view that clinical criteria should not be used to identify FH in this setting. Instead, we believe that FH genetic testing should be advocated in young patients with ACS and high LDL-C levels to allow prompt identification of patients with FH and relatives at risk.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Patients with FH commonly develop ACS before 65 years of age. A substantial proportion of patients with genetically confirmed FH are not identified by using clinical criteria, whereas many of those identified by clinical algorithms have polygenic hypercholesterolemia.

TRANSLATIONAL OUTLOOK: Further studies are needed to assess the cost-effectiveness and impact on clinical outcomes of systematic genetic testing for young patients with high LDL-C levels who develop ACS.

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APPENDIX For supplemental tables and material, please see the online version of this article.