Genetically Confirmed Familial Hypercholesterolemia in Patients with Acute Coronary Syndrome

Running Title: Genetically Confirmed FH in ACS

Almudena Amor-Salamanca, MD,[a] Sergio Castillo, PHD,[b] Emiliano Gonzalez-Vioque, PHD,[c] Fernando Dominguez, MD,[a,d] Lucía Quintana, PHD,[b] Carla Lluís-Ganella, PHD,[b] Juan Manuel Escudier, MD,[a] Javier Ortega, MD,[a] Enrique Lara-Pezzi, PHD,[d,e] Luis Alonso-Pulpon, MD, PHD,[a,d] Pablo Garcia-Pavia, MD, PHD[a,d,f]

From the [a]Inherited Cardiac Diseases Unit. Department of Cardiology. Hospital Universitario Puerta de Hierro, Madrid, Spain; [b]Gendiag.exe, Inc/Ferrer inCode, Inc, Barcelona, Spain; [c]Department of Biochemistry, Hospital Universitario Puerta de Hierro, Madrid, Spain; [d]CIBER in Cardiovascular Diseases (CIBERCV), Madrid, Spain; [e]Myocardial Biology Program, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; and [f]Faculty of Health Sciences, University Francisco de Vitoria (UFV), Pozuelo de Alarcon, Madrid, Spain.

Sources of Funding: This work was supported in part by the Instituto de Salud Carlos III (ISCIII) [grants RD012/0042/0066 and CB16/11/00432], by the Spanish Ministry of Economy and Competitiveness [grant SAF2015-71863-REDT] and by Alexion through an Investigator Initiated Research Grant. Grants from ISCIII and the Spanish Ministry of Economy and Competitiveness are supported by the Plan Estatal de I+D+I 2013-2016 – European Regional Development Fund (FEDER) "A way of making Europe". Funders played no role in the design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication.

Disclosures: SC, CL-G, and LQ are employees of Gendiag.exe/Ferrer in Code. Other authors have nothing to disclose.

Acknowledgements: We gratefully acknowledge Kenneth McCreath, PhD for English editing and Ana Royuela, PhD for statistical assistance.

Address for Correspondence:
Pablo Garcia-Pavia, MD, PhD
Department of Cardiology
Hospital Universitario Puerta de Hierro
Manuel de Falla, 2. Majadahonda
Madrid, 28222, Spain
Facultad de Ciencias de la Salud, Universidad Francisco de Vitoria (UFV),
Ctra. M-515 Pozuelo-Majadahonda Km 1,800
28223, Pozuelo de Alarcón, Madrid, Spain
Telephone: +34-911917297
Fax: +34-911917718
E-mail: pablogpavia@yahoo.es

ABSTRACT

BACKGROUND Genetic screening programs in unselected individuals with increased 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 with FH genetic testing.

METHODS Genetic study of 7 genes (*LDLR, APOB, PCSK9, APOE, STAP1, LDLRAP1, 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 ≥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), while 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-definite FH according to DLC criteria was 27.2% (95% CI: 19.1% to 37%; n = 28), whereas SB criteria identified 27.2% patients (95% CI: 19.1% to 37%; n = 28) with possible-definite FH. DLC and SB algorithms failed to diagnose 4 (44%) and 3 (33%) of 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 ≥160 mg/dl is high (around 9%). FH clinical algorithms do not accurately classify FH patients. Genetic testing should be advocated in young patients with ACS and high LDL-C to allow prompt identification of FH patients and relatives at risk.

CONDENSED ABSTRACT

Genetic screening programs in unselected individuals with increased low-density lipoprotein cholesterol (LDL-C) have shown modest results in identifying individuals with familial hypercholesterolemia (FH). 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 with FH genetic testing. The prevalence of genetically confirmed FH in patients with ACS age ≤65 years and with LDL-C ≥160 mg/dl is high (8.7%). FH clinical algorithms do not accurately classify FH patients. Genetic testing should be advocated in young patients with ACS with high LDL-C to allow prompt identification of FH in patients and in relatives at risk.

KEY WORDS cholesterol, genetics, Dutch Lipid Clinic, genetics, low-density lipoprotein cholesterol, Simon Broome criteria

ABBREVIATIONS AND ACRONYMS

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 Criteria
VUS = variants of unknown significance

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 LDL-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). 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 FH identification. Among the most widely used FH clinical criteria are those of the Simon Broome Register Group in the United Kingdom (8) and the Dutch Lipid Clinic Network (9).

Advances in genetic testing have made FH genetic testing affordable, but recent studies have shown that FH diagnosis by 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) could represent an optimal group for whom FH screening programs could be developed.

While the prevalence of genetically confirmed FH in patients with ACS has not been studied in detail, recent European data showed a prevalence between 1.6% and 8.3% in this group of patients when employing 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 could 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).

We sought 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 age 65 years or younger hospitalized at Hospital Universitario Puerta de Hierro (Madrid, Spain) for ACS from January 1, 2012, to March 31, 2016. All patients with real 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 triglycerides 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 also were 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 physical examination was performed. The patient selection process is represented in the flow chart 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 Simon Broome (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 >290 mg/dl or LDL-C >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 Dutch Lipid Clinic (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 by a DLC criteria score of 3 to 5 and probable-definite FH by a score of ≥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). As genetic information is usually not available for most clinicians and as we wanted to compare the diagnostic performance of genetic testing with the clinical criteria, genetic information was not considered when calculating FH clinical criteria by both algorithms.

DNA SEQUENCING. Genomic DNA was extracted from saliva or peripheral blood samples. Targeted enrichment was performed with a custom resequencing solution (Lipid inCode, Ferrer in Code, 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 Lipid Genetics Consortium (**Online Table 3**), based on 12 LDL-C-raising genetic variants, which determines the likelihood that a patient has polygenic hypercholesterolemia (21). 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 cut-off for the Whitehall II control cohort (21), 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 regions (MaxEntScan, NNSplice, FSPLICE, and GeneSplicer), among others. Moreover, information on more than 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 with an 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 carried out using standardized effect size measures, estimating odds ratios (OR) for categorical variables or Cohen's d for numerical values, as well as their corresponding 95% confidence intervals (CI). The level of statistical significance was set at $p < 0.05$. Statistical analyses were performed using the IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, New York) and Stata / IC v.14.2. (StataCorp LLC, College Station, Texas).

RESULTS

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

After clinical evaluation with 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 (2 patients; 1.9%) or possible (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. Additionally, 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) (**Online Table 4**).

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*; see **Online Table 4** for details) 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 finally 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 age ≤65 years with LDL-C ≥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 by genetic testing against FH clinical criteria, 4 patients (44%) with genetically confirmed FH were not diagnosed by DLC criteria and 3 (33%) failed to be confirmed using SB criteria (**Table 4**). Conversely, 82.1% (95% CI: 62.4% to 93.2%; n = 23) of patients diagnosed by the DLC algorithm and 78.6% (95% CI: 58.5% to 90.9%; n = 22) diagnosed by SB criteria did not show any 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 show genetic variants in FH-causing genes exhibited 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 on 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 our 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**).

DISCUSSION

This study described, for the first time, a complete genetic analysis of genes associated with FH in patients with ACS age ≤65 years and with LDL-C levels ≥160 mg/dl. Our study showed that the prevalence of genetically confirmed FH in these patients is approximately 9%. This 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 demonstrated 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 as 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 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 FH patients, as they can be identified before an event occurs, it requires prior identification of the FH probands, which is not an easy task.

Recent screening studies where participant selection was based solely on a single elevated LDL-C level were disappointing and reported FH mutations in fewer than 2% of severely hypercholesterolemic subjects (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 raised the need to find other clinical scenarios where genetic screening would yield a higher uptake (10). Two approaches, national screening of infants with very high total cholesterol or primary care screening programs during routine immunization visits, have

turned out to be very good strategies, as demonstrated by 2 recent studies from Slovenia and the United Kingdom (25,26). Unfortunately, implementing national screening programs in children is complex and this methodology 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 like 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 using a complete genetic approach, and the only reported study described a very low detection rate (27).

Wald et al. reported a prevalence of FH of 1.3% in young patients (≤50 years) with myocardial infarction at a London hospital (27). Unlike our study, the genetic analysis performed by these authors 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 >271 mg/dl (27). By contrast, we used next-generation sequencing (NGS) 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 and LDL-C ≥160 mg/dl), could explain the differences found between the studies and should be considered when designing genetic screening programs.

The prevalence of clinical familial hypercholesterolemia in ACS patients has been recently studied in Europe 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-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 for men and <60 years for

women) (14). In more than 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-definite FH was 8.3% overall but 15.4% in the 2,212 patients who were <60 years (13). Our study reported FH prevalence of 27.2% (95% CI: 19.1% to 37%) 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. Additionally, data about clinical signs of lipid accumulation in tissue, as well as information on family history of elevated LDL-C, were not available to the SPUM-ACS authors 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 gives more points in the clinical scores) and also obtain data from their 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 our study.

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 using FH clinical scores, while more than three-quarters of patients with ACS diagnosed with FH by clinical scores did not harbor any FH mutation. Our findings aligned with recent publications (2,28) that 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, where 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, 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) (29).

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, 23% 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 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, too. Recent data showed that FH patients identified by 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 FH patients do not reach LDL-C target levels for secondary prevention (12,14,31). These results emphasized 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 suffered 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 of 202 mg/dl was identified in our study (see family 9 in the **Online Appendix**). Given the importance of early diagnosis of FH before an event occurs, we believe that genetic studies constitute a fundamental tool to improve prognosis of FH patients.

STUDY LIMITATIONS. Most of the patients were Caucasian males, 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 on statins or ezetimibe prior to admission. This approach might inaccurately estimate LDL-C given the heterogeneity in drug selection, dosing, and individual response and variability across baseline LDL-C levels or mutation status. Furthermore, NGS testing does not detect inversions and translocations. While these genetic abnormalities probably are not major causes of FH, we cannot address its effect in our cohort. Although cost of FH NGS 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 work 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 age ≤65 years and with an LDL-C ≥160 mg/dl is high (~9%). FH clinical algorithms do not accurately identify FH patients in this setting, with a substantial number of patients with genetically confirmed FH unidentified by clinical criteria, while there are also numerous individuals diagnosed with FH by 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 to allow prompt identification of FH patients and relatives at risk.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: A significant number of patients younger than 65 years with an ACS suffer from familial hypercholesterolemia. FH clinical algorithms do not accurately identify FH patients in this setting, as a substantial number of patients with genetically confirmed FH are unidentified by clinical criteria; also, a high number of individuals diagnosed by FH clinical criteria have polygenic hypercholesterolemia.

COMPETENCY IN PATIENT CARE: FH genetic testing should be advocated in young patients with ACS and high LDL-C to allow prompt identification of FH patients and relatives at risk.

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Figure legends

CENTRAL ILLUSTRATION Results of HF Genetic Screening Programs

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

FIGURE 1 Patient Selection

This flow chart shows the successive steps taken during the study. *Estimated untreated LDL-C for those patients on statins or ezetimibe. ACS = acute coronary syndrome; LDL-C low-density lipoprotein cholesterol.

FIGURE 2 Genetic Testing Results

This diagram explained 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.

TABLE 1 Baseline Characteristics

Number of patients	103
Mean age at admission (years)	54±6.7
Males, n (%)	90 (87.4%)
Caucasian, n (%)	91 (88.3%)
Hypertension, n (%)	42 (40.8%)
Diabetes, n (%)	18 (17.5%)
Smoking, n (%)	58 (56.3%)
Glomerular filtration rate (mL/min/1.73m^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
Triglycerides (mg/dL)	154.2±61.7
On statins at admission, n (%)	39 (37.9%)
Other lipid-lowering agent, n (%)	8 (7.8%)
Unstable angina, n (%)	9 (8.7%)
Non-STEMI, n (%)	47 (45.6%)
STEMI, n (%)	47 (45.6%)
Previous CHD, n (%)	16 (15.5%)
Stroke, n (%)	3 (2.9%)
Peripheral vascular disease, n (%)	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.

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 sex, n (%)	8 (87.2)	82 (88.9)	1.17 (0.13–56.21)
Mean age at admission (years)	55±5.9	54±6.8	0.15 (-0.54-0.83)
Caucasian race, n (%)	8 (88.9)	83 (88.3)	1.06 (0.12–51.26)
Statin at admission, n (%)	4 (44.4)	35 (37.2)	1.35 (0.25–6.71)
Hypertension, n (%)	3 (33.3)	39 (41.5)	0.71 (0.11–3.56)
Diabetes, n (%)	1 (11.1)	17 (18.1)	0.566 (0.012–4.749)
Smoking, n (%)	6 (66.7)	52 (55.9)	1.62 (0.32–10.53)
Previous ischemic heart disease, n (%)	1 (11.1)	15 (16)	0.66 (0.01–5.59)
Stroke, n (%)	1 (11.1)	2 (2.1)	5.75 (0.09–118.39)
Peripheral vascular disease, n (%)	0	6 (6.4)	---
Total cholesterol (mg/dL)	256.6±52.2	239.8±33.7	0.47 (-0.22–1.16)
LDL-cholesterol (mg/dL)	222.3±52.5	186.4±31.1	1.08 (0.38–1.78)
HDL-cholesterol (mg/dL)	40.22±7.2	41.97±10.5	-0.17 (-0.85–0.51)
Triglycerides (mg/dL)	121.9±32.7	157.3±62.9	-0.58 (-1.27–0.11)
Family history of ischemic heart disease (Dutch Lipid Clinic Criteria)	4 (44.4)	17 (18.1)	3.62 (0.64–8.58)
Family history of ischemic heart disease (Simon Broome criteria)	5 (55.6)	31 (33)	2.54 (0.50–13.62)

Values are n (%) or mean ± SD.

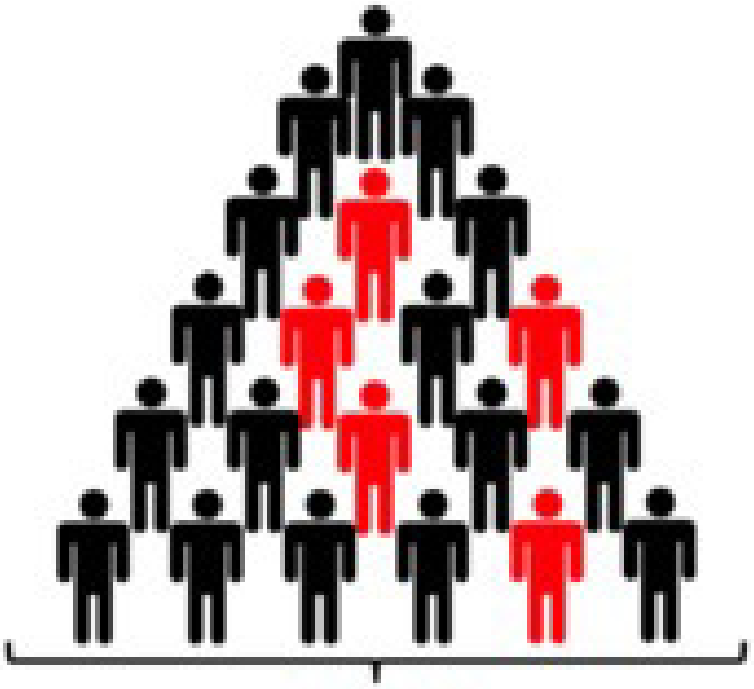
CI = confidence interval; other abbreviations as in Tables 1 and 2.

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			
Unlikely FH	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 (probable or definite)	**5 (55.5%)**	**23 (24.5%)**	**3.86 (0.75–20.86)**
Score Simon Broome			
Unlikely	3 (33.3%)	72 (76.6%)	
Possible FH	6 (66.7%)	20 (21.3%)	
Definite FH	0	2 (2.1%)	
Score Simon Broome (possible or definite)	**6 (66.7%)**	**22 (23.4%)**	**6.54 (1.25–42.79)**

Values are n (%).

Abbreviations as in Table 2.



Adults
LDL-C ≥ 190 mg/dL

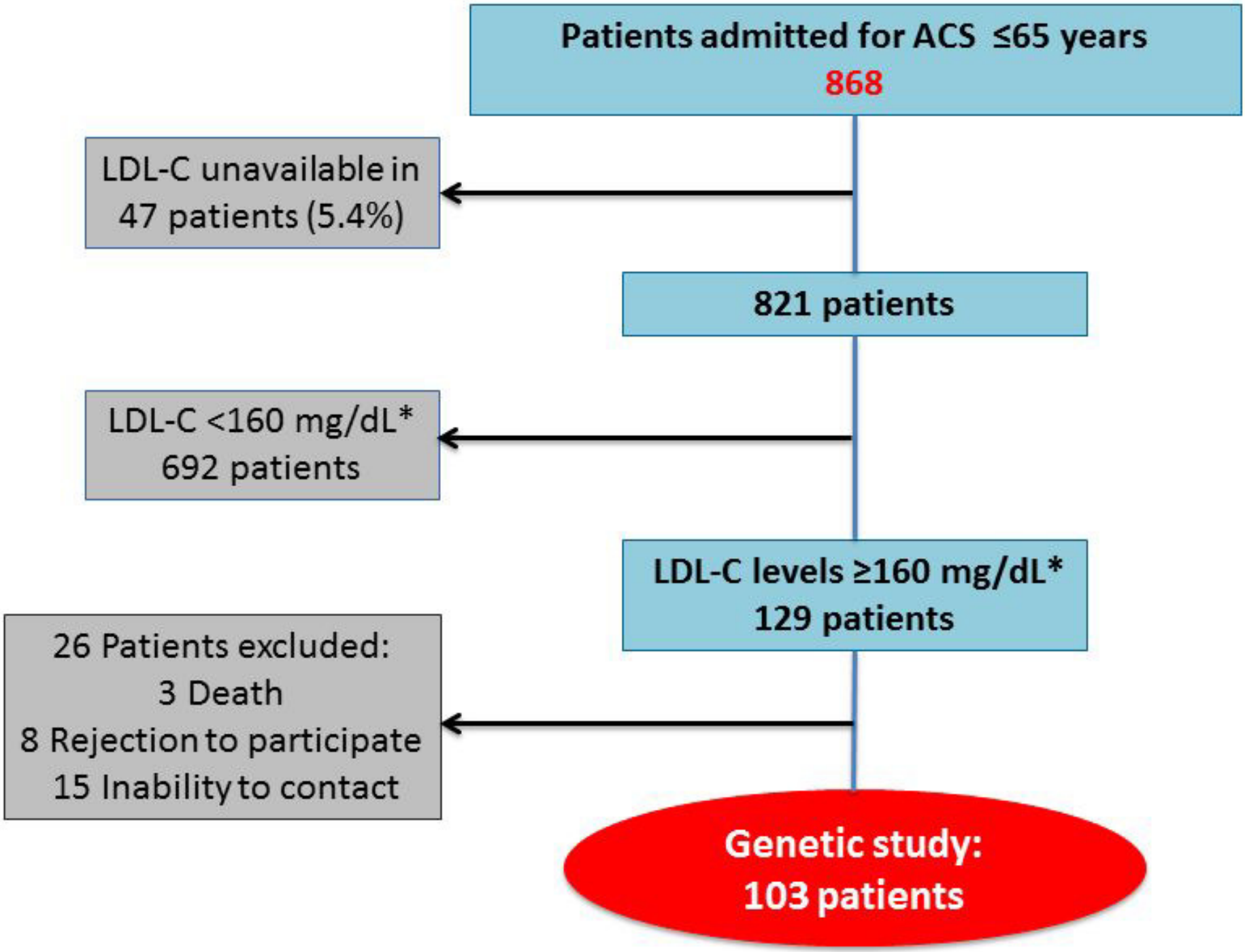
1.7% FH patients (11)

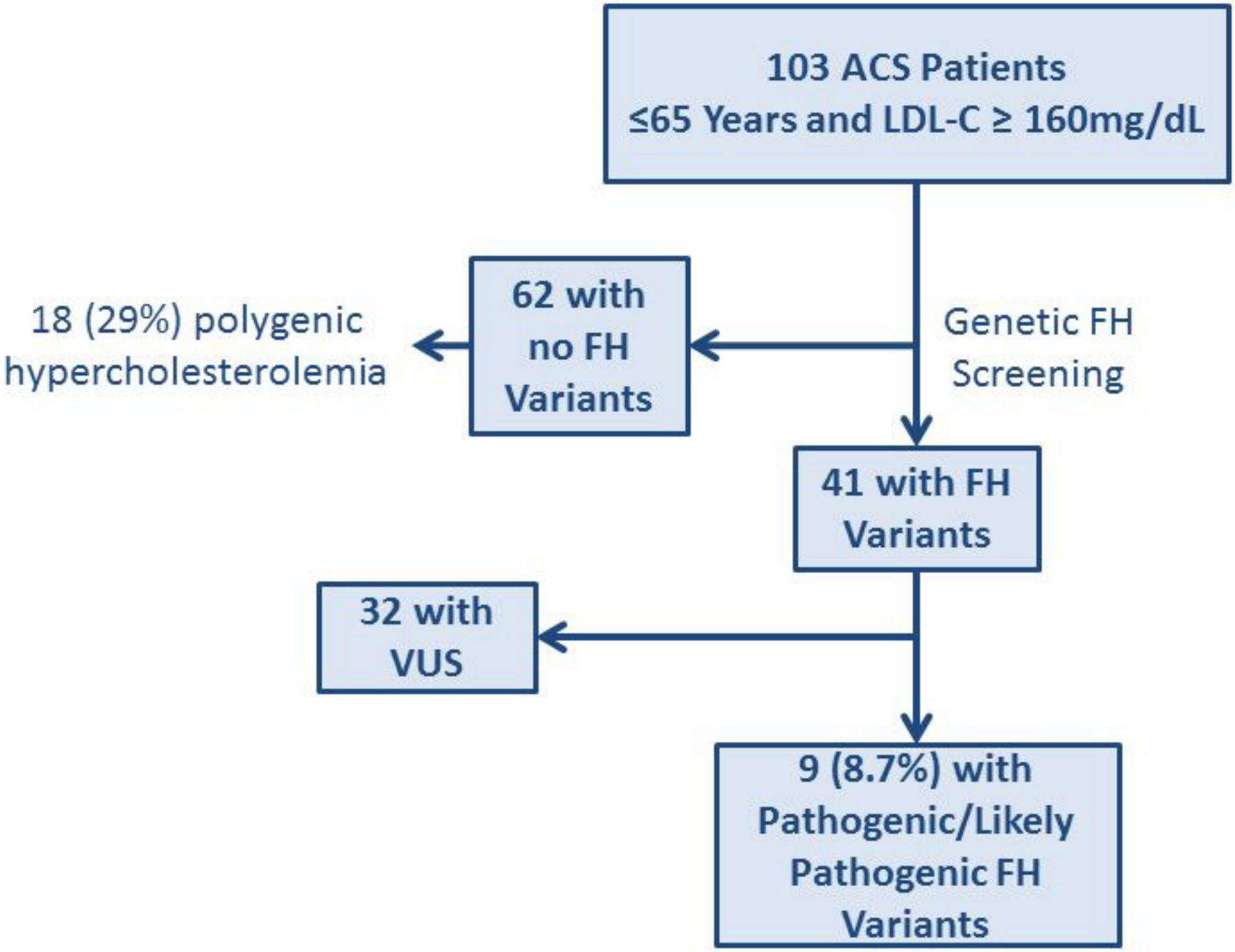
Children 1-2 years old

0.4% FH patients (26)

ACS patients ≤ 65 years
LDL-C ≥ 160 mg/dL

Present work:
8.7% FH patients


Patients admitted for ACS ≤65 years
868
LDL-C unavailable in
47 patients (5.4%)
821 patients
LDL-C <160 mg/dL*
692 patients
LDL-C levels ≥160 mg/dL*
129 patients
26 Patients excluded:
3 Death
8 Rejection to participate
15 Inability to contact
Genetic study:
103 patients


103 ACS Patients
≤65 Years and LDL-C ≥ 160mg/dL
Genetic FH Screening
62 with no FH Variants
18 (29%) polygenic hypercholesterolemia
41 with FH Variants
32 with VUS
9 (8.7%) with Pathogenic/Likely Pathogenic FH Variants

Supplementary material

Genetically confirmed familial hypercholesterolemia in patients with acute coronary syndromes.

Running Title: **Genetically confirmed FH in ACS.**

Authors:

Almudena Amor-Salamanca, MD[1]; almuamor2@yahoo.es

Sergio Castillo, PhD[2]; sergio.castillo@gendiag.com

Emiliano Gonzalez-Vioque, MD, PhD[3]; egvioque@gmail.com

Fernando Dominguez, MD[1,4]; fdominguezrodriguez@gmail.com

Lucía Quintana, PhD[2]; lucia.quintana@gendiag.com

Carla Lluís-Ganella, PhD[2]; clluis@ferrerincode.com

Juan Manuel Escudier, MD[1]; jescudiervilla@yahoo.es

Javier Ortega, MD[1]; tegamar@yahoo.es

Enrique Lara-Pezzi, PhD[4,5]; elara@cnic.es

Luis Alonso-Pulpon, MD, PhD[1,4]; luispulpon@secardiologia.es

Pablo Garcia-Pavia, MD, PhD[1,4,6]; pablogpavia@yahoo.es (corresponding author)

Affiliation:

[1] Department of Cardiology. Hospital Universitario Puerta de Hierro, Madrid, Spain.

[2] Gendiag.exe, Inc/Ferrer inCode, Inc, Barcelona, Spain.

[3] Department of Biochemistry, Hospital Universitario Puerta de Hierro, Madrid, Spain.

[4] CIBER in Cardiovascular Diseases (CIBERCV), Madrid, Spain.

[5] Myocardial Biology Program, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain.

[6] Francisco de Vitoria University, Madrid, Spain.

Address for Correspondence:

Pablo Garcia-Pavia, MD, PhD

Department of Cardiology.

Hospital Universitario Puerta de Hierro,

Manuel de Falla, 2. Majadahonda, Madrid, 28222, Spain

Tel: +34-911917297, Fax: +34-911917718

Email: pablogpavia@yahoo.es

Supplementary Material

Table S1. Dutch Lipid Clinic Network Clinical Criteria

Group 1: Family history	Points
First-degree relative with known premature CHD, or	1
First-degree relative with known LDL-C>95th percentile by age and gender for country	1
First-degree relative with tendon xanthoma and/or corneal arcus, or	2
Children<18 years with LDL-C>95th percentile by age and gender for country	2
Group 2: Clinical history	
Subject has premature CHD	2
Subject has premature cerebral or peripheral vascular disease	1
Group 3: Physical examination	
Tendon xanthoma	6
Corneal arcus in a person<45 years	4
Group 4: Biochemical results (LDL-C)	
>325 mg/dL (>8.5 mmol/L)	8
251–325 mg/dL (6.5–8.4 mmol/L)	5
191–250 mg/dL (5.0–6.4 mmol/L)	3
155–190 mg/dL (4.0–4.9 mmol/L)	1
Group 5: Molecular genetic testing	
Causative mutation shown in the LDLR, APOB or PCSK9 genes	8

>8 points Definite FH
6–8 points Probable FH
3–5 points Possible FH
0–2 points Unlikely FH

Table S2. Simon Broome diagnostic criteria for Familial Hypercholesterolemia

Definite Familial Hypercholesterolemia

Required laboratory=high cholesterol levels

- Adult: Total cholesterol levels>290 mg/dL (7.5 mmol/L) or LDL-C>190 mg/dL (4.9 mmol/L)
- Child<16 years: Total cholesterol levels >260 mg/dL (6.7 mmol/L) or LDL-C>155 mg/dL (4.0 mmol/L)

Plus at least one of two:

1. Physical finding: tendon xanthomas, or tendon xanthomas in first or second degree relative
2. DNA-based evidence of an LDLR mutation, familial defective apo B-100 or a PCSK9 mutation

Possible Familial Hypercholesterolemia

Laboratory=high cholesterol levels

- Adult: Total cholesterol levels>290 mg/dL (7.5 mmol/L) or LDL-C>190 mg/dL (4.9 mmol/L)
- Child<16 years: Total cholesterol levels >260 mg/dL (6.7 mmol/L) or LDL-C>155 mg/dL (4.0 mmol/L)

Plus at least one of the two:

1. Family history of at least one of the following:
    - Family history of myocardial infarction at:
        - Age 60 years or younger in first degree relative
        - Age 50 years or younger in second-degree relative

OR

2. Family history of elevated total cholesterol
    - Greater than 290 mg/dL (7.5 mmol/L) in adult first- or second-degree relative
    - Greater than 280 mg/dL (6.7 mmol/L) in child, brother or sister aged younger than 16 years

Variant data analysis

FASTQ files were processed by an internal pipeline developed by Gendiag.exe and implemented with Gendicall software (an informatics tool developed by Gendiag.exe). Data analysis included as a first step trimming of adaptors and low quality bases; then, resultant reads were mapped with BWA-MEM.[1] Duplicates were removed using Picard,[2] and the resultant BAM file was used for variant calls that are performed with a combination of SAMtools v.1.2[3] and private scripts. Also, internal algorithms were applied to detect breakpoints and CNVs. Variants annotation is based on HGVS standards[4] using Ensemble isoforms and considers information contained in public databases,[5] the 1000 Genomes browser Phase 3 data release[6] (http://www.1000genomes.org/), and the Exome Aggregation Consortium (ExAC)[7] v.0.3 data release (http://exac.broadinstitute.org/) and Exome Variant Server.[8]

Table S3. Genomic variants associated with polygenic elevated LDL cholesterol levels

	Gene	Genetic Variant
Polygenic cause of elevated LDL cholesterol levels	*ABCG5-G8*	rs4299376:G>T
	APOB	rs1367117:G>A
	APOE	rs429358:T>C
	APOE	rs7412:C>T
	CELSR2	rs629301:G>T
	HFE	rs1800562:G>A
	LDLR	rs6511720:G>T
	MYLIP	rs3757354:C>T
	NYNRIN	rs8017377:G>A
	PCSK9	rs2479409:G>A
	SLC22A1	rs1564348:T>C
	ST3GAL4	rs11220462:G>A

Table S4. Genetic variants found in ACS patients

PATHOGENIC/LIKELY PATHOGENIC MUTATIONS
LDLR GENE
LDLR - c.514G>A, p.(Asp172Asn)
LDLR - c.418G>A, p.(Glu140Lys)
LDLR - c.1618G>A, p.(Ala540Thr)
LDLR - c.1444G>A, p.(Asp482Asn)
LDLR - c.401G>T, p.(Cys134Phe)
LDLR - c.862G>A, p.(Glu288Lys)
LDLR - c.313+1G>C, p.(?)
PCSK9 GENE
PCSK9 - c.-331C>A, p.(?)
STAP1 GENE
STAP1 - c.291G>C, p.(Glu97Asp)
LIPA GENE
LIPA - c.894G>A, p.(298=) †

†Found in two individuals

VARIANTS OF UNKNOWN SIGNIFICANCE
LDLR GENE
LDLR - c.274C>G, p.(Gln92Glu)
LDLR - c.596C>T, p.(Ala199Val)
LDLR - c.892A>G, p.(Met298Val)
LDLR - c.1536C>G, p.(Phe512Leu)
LDLR - c.68-14T>C, p.(?)
LDLR - c.*28C>G, p.(?)
LDLR - c.694+25C>T, p.(?)
APOB GENE
APOB - c.11477C>T, p.(Thr3826Met)
APOB - c.4696T>C, p.(Tyr1566His)
APOB - c.5066G>A, p.(Arg1689His)
APOB - c.10607C>T, p.(Ser3536Phe)
APOB - c.9140C>G, p.(Thr3047Arg)
APOB - c.12794T>C, p.(Val4265Ala) †
APOB - c.9105T>C, p.(Asn3035=)
APOB - c.6639_6641del, p.(Asp2213del)
APOB - c.3383G>A, p.(Arg1128His)
APOB - c.3509-11C>T, p.(?)
APOB - c.8045G>T, p.(Ser2682Ile)
APOB - c.13277T>C, p.(Ile4426Thr)
APOB - c.3712C>A, p.(Leu1238Ile)

APOB - c.2068-4T>A, p.(?)
APOB - c.11354C>T, p.(Thr3785Ile))
APOB - c.1088T>C, p.(Val363Ala)
APOB - c.8462C>T, p.(Pro2821Leu)
APOB - c.7615G>A, p.(Val2539Ile)
APOB - c.694-21C>T, p.(?)
APOB - c.66_67insCTGCTG, p.(Leu22_Ala23insLeuLeu)
APOB - c.2295G>A, p.(Leu765=)
PCSK9 GENE
PCSK9 - c.132C>T, p.(Ala44=)
PCSK9 - c.1354+9G>T, p.(?)
PCSK9 - c.835C>A, p.(Pro279Thr)
PCSK9 - c.1978G>A, p.(Asp660Asn)
PCSK9 - c.1354+12G>A, p.(?)
PCSK9 - c.1247T>G, p.(Ile416Ser)
STAP1 GENE
STAP1 - c.35G>A, p.(Arg12His)
STAP1 - c.-60A>G, p.(?) †
STAP1 - c.693C>T, p.(Ser231=)
LDLRAP1 GENE
LDLRAP1 - c.-92G>T, p.(?)
LDLRAP1 - c.602C>G, p.(Pro201Arg)
LDLRAP1 - c.604_605delTCinsCA, p.(Ser202His)

LDLRAP1 - c.396C>T, p.(Ile132=)
LDLRAP1 - c.672C>T, p.(Ser224=)
LDLRAP1 -c.71_72delGCinsTT, p.(Gly24Val)
LDLRAP1 - c.811G>A, p.(Val271Ile)
LIPA GENE
LIPA - c.891C>T, p.(Ser297=)
LIPA - c.-218C>A, p.(?)
LIPA - c.754A>T, p.(Ile252Leu)
APOE GENE
APOE - c.369C>T, (Gly123=)

† Found in two individuals

Table S5. Familial evaluation in patients with FH pathogenic/likely pathogenic mutations or VUS

Family studies in first-degree relatives of patients with FH pathogenic/likely pathogenic mutation

Patient 1		**LDLR - c.514G>A, p.(Asp172Asn)**
1.1	Daughter	Carrier. Total Cholesterol 218, LDL 148, HDL 56, Tg 70
1.2	Son	Carrier. Total Cholesterol 234, LDL 160, HDL 52, Tg 111
Patient 2		**LDLR - c.418G>A, p.(Glu140Lys)**
2.1	Daughter	Carrier. Total cholesterol 270, LDL 193, HDL 65
Patient 3		**LDLR - c.1618G>A, p.(Ala540Thr)**
3.1	Son	Carrier. Total cholesterol 271, LDL 197, HDL 53
Patient 4		**LDLR - c.1444G>A, p.(Asp482Asn)**
Family study not possible		
Patient 5		**LDLR - c.401G>T, p.(Cys134Phe)**
5.1	Daughter	Non carrier. Total cholesterol 260, LDL 147, HDL 88
Patient 6		**PCSK9 - c.-331C>A, p.(?)**
6.1	Son	Non carrier. Total cholesterol 163, LDL 100, HDL 52, Tg 55
Patient 7		**STAP1 - c.291G>C, p.(Glu97Asp)**
7.1	Daughter	Carrier. Total cholesterol 166, LDL 99, HDL 50
Patient 8		**LDLR - c.862G>A, p.(Glu288Lys)**
Family study not possible		
Patient 9		**LDLR - c.313+1G>C, p.(?)**
9.1	Son	Non carrier. Total cholesterol 133, LDL 78, HDL 40, Tg 73
9.2	Son	Non carrier. Total cholesterol 141, LDL 83, HDL 46, Tg 58
9.3	Daughter	Non carrier. Total cholesterol 172, LDL 100, HDL 56, Tg 81
9.4	Son	Non carrier. Total cholesterol 173, LDL 113, HDL 44, Tg 78
9.5	Daughter	Carrier. Total cholesterol 263, LDL 202, HDL 49

Family studies in first-degree relatives of patients with VUS

Patient 10		**PCSK9 - c.835C>A, p.(Pro279Thr)**
10.1	Daughter	Carrier. Total cholesterol 230, LDL 158, HDL 45, Tg 134
10.2	Son	Non Carrier. Total cholesterol 148, LDL 76, HDL 52, Tg 102
Patient 11		**APOB - c.2068-4T>A, p.(?)**
11.1	Son	Carrier. Total cholesterol 138, LDL 97, HDL 33, Tg 35
11.2	Brother	Non carrier. Total cholesterol 185, LDL 121, HDL 31
11.3	Brother	Non carrier. Total cholesterol 187, LDL 67, HDL 42 Tg 390
11.4	Sister	Non carrier. Total cholesterol 213, LDL 146, HDL 45, Tg 109
Patient 12		**APOB - c.11477C>T, p.(Thr3826Met).**
12.1	Brother	Carrier. On treatment: Total cholesterol 224, LDL 73, HDL 34
12.2	Brother	Non carrier. Total cholesterol 233, LDL 142, HDL 75, Tg 75
12.3	Sister	Carrier. Total cholesterol 257, LDL 171, HDL 70, Tg 83
Patient 13		**LDLR – c.1536C>G, p.(Phe512Leu)**
Family study not possible		
Patient 14		**LDLR - c.68-14T>C, p.(?); PCSK9 - c.1247T>G, p.(Ile416Ser)**
Family study not possible		
Patient 15		**LDLR - c.596C>T, p.(Ala199Val)**
Family study not possible		

Table S6. LDL cholesterol levels one year after acute coronary syndrome in patients with genetically confirmed FH

Patients	Gene	Mutation	LDL cholesterol (mg/dL)	Lipid-lowering treatment
Patient n.1	**LDLR**	**c.514G>A, p.(Asp172Asn)**	112	Rosuvastatin 10 mg
Patient n.2	**LDLR**	**c.418G>A, p.(Glu140Lys)**	117	Atorvastatin 80 mg + Ezetimibe 10 mg
Patient n.3	**LDLR**	**c.1618G>A, p.(Ala540Thr)**	142	Atorvastatin 80 mg
Patient n.4	**LDLR**	**c. 1444G>A, p.(Asp482Asn)**	147	Atorvastatin 40 mg
Patient n.5	**LDLR**	**c.401G>T, p.(Cys134Phe)**	143	Atorvastatin 80 mg + Ezetimibe 10 mg
Patient n.6	**PCSK9**	**c.-331C>A, p.(?)**	83	Rosuvastatin 10 mg
Patient n.7	**STAP1**	**c.291G>C, p.(Glu97Asp)**	45	Rosuvastatin 40 mg + Ezetimibe 10 mg
Patient n.8	**LDLR**	**c.862G>A, p.(Glu288Lys)**	99	Atorvastatin 80 mg
Patient n.9	**LDLR**	**c. 313+1G>C, p.(?)**	149	Pitavastatin 4 mg + Ezetimibe 10 mg

Supplementary Material References

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