Genetically confirmed familial hypercholesterolemia in patients with acute coronary syndrome.

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

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ABSTRACT

Background: Genetic screening programs in unselected individuals with increased LDL-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 acute coronary syndrome (ACS) patients and compares 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 ACS patients, aged ≤65 years and LDL-cholesterol (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% CI, 4.3–16.4%; n=9), while 29% (95%CI, 18.5–42.1%; n=18) of patients without FH variants showed a score highly suggestive of polygenic hypercholesterolemia. The prevalence of probable/definite FH according to DLC criteria was 27.2% (95%CI, 19.1–37%; n=28), whereas SB criteria identified 27.2% (95%CI, 19.1–37%; n=28) patients with possible/definite FH. DLC and SB algorithms failed to diagnose 4 (44%) and 3 (33%) of the genetically confirmed FH patients, respectively. Cascade genetic testing in first-degree relatives identified 6 additional FH individuals.

Conclusions: The prevalence of genetically confirmed FH in ACS patients aged ≤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 ACS patients with high LDL-C to allow prompt identification of FH patients and relatives at risk.

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Condensed abstract

Genetic screening programs in unselected individuals with increased LDL-cholesterol (LDL-C) have shown modest results in identifying individuals with familial hypercholesterolemia (FH). This study assessed the prevalence of genetically confirmed FH in acute coronary syndrome (ACS) patients and compares the diagnostic performance of FH clinical criteria with FH genetic testing. The prevalence of genetically confirmed FH in ACS patients aged ≤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 ACS patients with high LDL-C to allow prompt identification of FH patients and relatives at risk.

Keywords: Cholesterol, Familial Hypercholesterolemia, Genetics, Dutch Lipid Clinic, Simone Broome criteria.

Abbreviations

FH: Familial hypercholesterolemia
ACS: acute coronary syndrome
LDL-C: LDL-cholesterol
DLC: Dutch Lipid Clinic
SB: Simon Broome Criteria
CHD: Coronary Heart Disease
DNA: Deoxyribonucleic acid
STEMI: ST elevation myocardial infarction
VUS: Variants of unknown significance
SNP: Single nucleotide polymorphism

INTRODUCTION

Familial hypercholesterolemia (FH) is an autosomal dominant inherited genetic disorder with a prevalence historically estimated to be in the order of 1 in 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, and 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 and an aggressive cholesterol-lowering treatment regimen could prevent the occurrence of cardiovascular events by reducing the long-term exposure of these patients and their 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 high-risk individuals from the overall population is low (between 0.3% and 1.7%) (10,11). This low prevalence found in hypercholesterolemic individuals from the overall population suggests that other high-risk groups of patients with higher FH genetic testing uptake should be identified. As such, patients with an acute coronary syndrome (ACS) could represent an optimal group to develop FH screening programs (12).

While the prevalence of genetically-confirmed FH in patients with ACS has not been studied in detail, recent European data based on clinical algorithms showed a prevalence between 1.6% and 8.3% in this group of patients (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 with multiple lipid-lowering therapies.

Prompt identification of FH among ACS patients could be extremely useful to allow early intensification of lipid-lowering treatment and could also lead to early identification of relatives with FH who have not yet experienced cardiovascular events and who would benefit from early initiation of intense lipid-lowering therapies (15).

We sought to determine the prevalence of genetically confirmed FH in patients with ACS and to evaluate the diagnostic performance of FH clinical criteria in comparison with FH genetic findings.

METHODS

Inclusion Criteria and Study Population

Clinical records of all patients aged 65 years or less, hospitalized at Hospital Universitario Puerta de Hierro (Madrid, Spain) with an ACS from January 1 2012 to March 31 2016 were reviewed. 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 (16-18). The effect of other lipid-lowering drugs was not considered.

Levels of LDL-C were calculated according to Friedewald's formula (19). 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 were also excluded.

Whole blood or saliva samples for DNA analysis were collected from patients who accepted to participate in the study and, simultaneously, data about their personal and family history were collected. The patients' 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 National Institute for Health and Care Excellence guidelines, considers a diagnosis of possible FH as that with both total cholesterol >290 mg/dL or LDL-C >190 mg/dL, and 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 USA, the International FH Foundation, and the European Atherosclerosis Society, considers LDL-C levels, physical signs and a personal or family history of premature CHD (Tables S1 and S2 online-only Data Supplement). A possible FH is defined by a score of 3 to 5, and a probable/definite FH by a score of ≥6. Both 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 done with a SureSelect custom resequencing solution commercialized by Ferrer inCode (Lipid inCode®). The design was based on the human reference genome (hg19) and 120 bp-length RNA biotinylated baits were defined to extensively cover all the regions of interest.

The experimental procedure was performed according to the manufacturer's instructions (Agilent Technologies Inc., Santa Clara, CA, USA) with some modifications as a result of our internal validations. Very briefly, 50 ng of high quality dsDNA from every sample was enzymatically fragmented and, after hybridization to the Lipid inCode® solution and capture, libraries were amplified by PCR and indexed. Final libraries were quantified and their quality was assessed on a 2100 Bioanalyzer using High Sensitivity DNA chips (Agilent

Technologies). All libraries were then pooled and sequenced (up to 40 per run). Sequencing paired-end process was developed on a MiSeq System (Illumina) using 2×75 bp reads length. The Lipid InCode® platform performs 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 Lipid InCode® platform also interrogates a weighted LDL-C-raising gene score identified by the Global Lipid Genetics Consortium (Table S3 online-only Data Supplement), based on 12 LDL-C-raising genetic variants, which determines the likelihood that a patient has polygenic hypercholesterolemia (20). The calculation of the risk score was computed as described in Talmud et al. (20), and was determined in patients without variants in FH-related genes. A gene score ≥1.08, which is the 9th decile cut-off for the Whitehall II control cohort (20), has been proposed as very suggestive of polygenic hypercholesterolemia (21).

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

Variant Data and Pathogenicity Interpretation

Variant data analysis is described in the online-only Data Supplement. Variants with a minor allele frequency (MAF) <1% in the general population were considered as non-common variants. The potential pathogenicity of rare variants was evaluated in silico by considering the recommendations published by the American College of Medical Genetics and Genomics (ACMG) (22), in which different criteria are evaluated: type and variant frequency, functional data if available, scientific support, 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 of Ferrer InCode was also considered to complete the evaluation of genetic variants. Variants with a clinical relevance: pathogenic (Class I), likely pathogenic (Class II), and variants with an unknown significance (VUS; Class III) were reported.

Familial Evaluation and Variant Reclassification

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 relatives of patients with VUS variants that, according to ACMG recommendations, could be reclassified as pathogenic/likely pathogenic if a positive cosegregation is found. 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 and standard deviation (SD). Discrete data are presented as percentages. Analysis on differences in characteristics between groups was carried out using standardized effect size methods, estimating odds ratios (OR) for categorical variables or Cohen´s d for numerical, as well as their corresponding 95% confidence intervals. 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, NY, USA) and Stata / IC v.14.2. (StataCorp2015. Stata Statistical Software: Release 14. College Station, Tx: StataCorp LP).

RESULTS

The study cohort comprised 103 patients (mean age 54±6.7 years, range 37–65; 87.4% males) admitted for an ACS. Forty-seven were admitted for a STEMI, 47 because of a non-STEMI and 9 for unstable angina. Mean LDL-C at admission was 189.5±34.7 mg/dL, but only 39 (37.9%) patients were on statins. Sixteen patients (15.5%) had previous history of CHD, 3 (2.9%) had had a 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. Twenty-eight patients (27.2%) were therefore classified as having probable or definite FH by DLC criteria. Furthermore, 28 patients (27.1%) had definite (2 patients; 1.9%) or possible (26; 25.2%) FH by SB criteria (Table 2).

Genetic testing revealed 9 heterozygous pathogenic/likely pathogenic FH mutations in 9 individuals (8.7%). Seven mutations were found in the *LDLR* gene, 1 in *PCSK9* and 1 in *STAP1* (Table S4 online-only Data Supplement). Five VUS were also found in patients with pathogenic/likely pathogenic FH mutations. Thirty-two patients carried 35 VUS and 62 (60.2%) individuals 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 LA deficiency; Table S4 online-only Data Supplement).

Familial genetic evaluation was offered to first-degree relatives of the 9 patients with pathogenic/likely pathogenic mutations and to the relatives of the 6 patients with VUS (3 in *LDLR*, 2 in *APOB* and 1 in *PCSK9*; see Table S4 for details) that, based on the ACMG recommendations, could have been reclassified (22).

Familial screening was not possible or was rejected in 5 families (2 with pathogenic/likely pathogenic variants and 3 with VUS). Clinical and genetic study of 21 first-degree relatives from 10 families (7 with pathogenic/likely pathogenic mutations and 3 with VUS) was finally performed (Table S5 online-only Data supplement). Familial evaluation did not allow reclassification of any VUS as pathogenic/likely pathogenic according to the ACMG criteria (22). Therefore the final prevalence of genetically confirmed FH among ACS patients aged ≤65 with LDL-C ≥160 mg/dL was 8.7% (95% confidence interval [CI], 4.3–16.4%; n=9) (Figure 2).

Clinical, analytical and treatment characteristics of ACS patients with and without FH mutations were compared. (Table 3).

When confronting FH diagnosis by genetic testing against FH clinical criteria, 4 (44%) patients with genetically confirmed FH were not diagnosed by DLC criteria and 3 (33%) by SB criteria (Table 4), while 82.1% (95%CI, 62.4–93.2%; n=23) of patients diagnosed by the DLC algorithm and 78.6% (95%CI, 58.5%–90.9%; n=22) by SB criteria did not show any FH mutation. Furthermore, 29.03% (95%CI, 18.5–42.13%; 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 elevated LDL-levels or were already on statins (Table S5 on-line Supplement).

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 the guidelines. Two patients had levels between 70 and 100, and 6 patients had LDL-C levels >100 mg/dL, despite the fact

that the majority of them were taking high doses of lipid-lowering drugs (Table S6 on-line Data Supplement).

DISCUSSION

This study describes for the first time a complete genetic analysis of genes associated with FH in patients with ACS aged ≤65 years and with LDL-C levels ≥160 mg/dL. Our study shows that the prevalence of genetically confirmed FH in these patients is around 9%. This value 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 demonstrates that FH clinical algorithms do not accurately identify FH subjects among patients with ACS, and that FH genetic testing in this population is useful to facilitate early diagnosis of patients and 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 counselling 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 underline the utility of identifying causal mutations to facilitate cascade screening (23). Although cascade screening is the best mean 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 participants were selected solely on the basis of a single elevated LDL-C level were disappointing, and reported FH mutations in fewer than 2% of severely hypercholesterolemic subjects (11,24). This low yield of FH diagnosis questioned

the utility of genetic screening programs in unselected patients with high LDL-C levels, and raised the need to find other clinical scenarios where genetic screening would yield a higher uptake (24). As such, national screening of infants with very high total cholesterol or primary care screening programs during routine immunization visits has turned out to be very good strategies, as demonstrated by two 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 an 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 with 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 in that study included a panel of 48 known FH mutations and whole exon deletions/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 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-old and LDL-C ≥160 mg/dL) could explain the differences found between both 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 cohort that

included 4,778 ACS patients, 1.6% (95%CI: 1.3–2.0%) of patients fulfilled criteria of probable/definite FH according to DLC criteria (14). The prevalence of clinical FH was 4.8% in 1,451 ACS patients with premature CHD (<55 years old for men and <60 years old for women) (14). In more than 7,000 European patients with CHD from the Euroaspire IV study, the prevalence of probable/definite FH was 8.3%, and was 15.4% in the 2,212 patients who were <60 years old (13). Our study reports FH prevalence of 27.2% (95%CI: 19.1–37%) according to the DLC and the SB criteria. We think that the higher prevalence found in our cohort is partly related with the LDL threshold used, which selects individuals with higher pre-test probability. In addition, data about clinical signs of lipid accumulation in the tissue, as well as information on family history of elevated LDL-cholesterol, were not available to the SPUM-ACS authors and they decided that missing information counted zero in the Dutch Lipid Clinic algorithm (14). In contrast, in our work, 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 two critical factors (LDL-C threshold and clinical/familial information) could explain the higher FH prevalence as determined by clinical criteria found in our study.

Nevertheless, one of the main findings of our study is the demonstration of the inability of FH clinical scores to correctly identify ACS patients with and without FH. As shown here, 30–40% of patients with confirmed FH mutations were not detected using FH clinical scores, while more than three quarters of ACS patients diagnosed with FH by clinical scores did not harbour any FH mutation. Our findings are in line with recent publications (2,28) that have also shown that clinical FH criteria are inaccurate to identify FH individuals when confronted with FH genetic testing. Our results must be taken in the context of ACS setting where available information about FH prevalence is currently restricted to FH clinical criteria (13, 14).

Recently, several opinion leaders in FH alerted that three 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 show 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 favour 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). As a result of the FH genetic screening performed in this study, clinical FH screening is no longer necessary in relatives of a large number of patients who did not present FH mutations irrespective of the clinical criteria findings of the proband.

The present study also provides some data on the impact of identifying genetically confirmed FH among ACS patients. At one-year follow-up, only 1 FH proband presented recommended LDL-C levels <70 mg/dL despite the fact that most were receiving high doses of statin, and in some cases also ezetimibe. Recent data show 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), and 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 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 ACS individuals with higher risk and who should be treated aggressively soon after the ACS event.

Finally, our study shows 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 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. Given the importance of early diagnosis of FH before an event occurs, we believe that genetic studies constitutes a fundamental tool to improve prognosis of FH patients.

Our study has several limitations. LDL-C level was measured in the first 48 hours after ACS admission, and it has been described that LDL-C levels at this time are decreased. 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. NGS testing does not detect inversions and translocations. Although these genetic abnormalities probably are not major causes of FH, we cannot address its effect in our cohort. Although NGS genetic testing cost is now small (around 300-350 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 ACS patients 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 aged ≤65 years and with a LDL-C ≥160 mg/dL is high (around 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, and also with a large number of individuals diagnosed by clinical criteria suffering from 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 ACS patients with 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 acute coronary syndrome (ACS) suffer from familial hypercholesterolemia (FH). 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, and 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 ACS patients with high LDL-C to allow prompt identification of FH patients and relatives at risk.

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Figure legends

Central illustration: Results of Familial Hypercholesterolemia genetic screening programs in several populations.

Results of FH genetic screening in 103 young ACS patients (<65 years) with high LDL-C >160 mg/dl (present work) were compared with 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 1-2 years-old (25).

FH: Familial Hypercholesterolemia. LDL-C: LDL cholesterol

Figure 1. Selection of patients. Flow chart that shows the successive steps taken during the study. ACS: Acute coronay syndrome. LDL-C: LDL-cholesterol. * Estimated untreated LDL-C for those patients on statins/ezetimibe.

Figure 2. Genetic testing results.

Diagram explaining the results of the genetic study performed in patients with acute coronary syndrome. ACS: Acute coronary syndrome. FH: Familial Hypercholesterolemia. LP: Likely Pathogenic. LDL-C: LDL cholesterol. VUS: Variants of unknown significance.

Table 1. Clinical characteristics of 103 patients with an acute coronary syndrome screened for Familial Hypercholesterolemia

Clinical baseline characteristics	**n=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%)

CHD: coronary heart disease; STEMI: ST 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%)		

FH: Familial hypercholesterolemia

Table 3. Baseline clinical, analytical and treatment characteristics of ACS patients with and without genetically confirmed FH

	FH mutation (n=9)	No FH mutation (n=94)	Standardised effect size (SES) (95% CI)
Male sex, n (%)*	8 (87.2)	82 (88.9)	1.170 (0.134 - 56.213)
Mean age at admission (years)†	55±5.9	54±6.8	0.148 (-0.536 - 0.832)
Caucasian race, n (%)*	8 (88.9)	83 (88.3)	1.060 (0.120 - 51.260)
Statin at admission, n (%)*	4 (44.4)	35 (37.2)	1.348 (0.249 - 6.713)
Hypertension, n (%)*	3 (33.3)	39 (41.5)	0.705 (0.108 - 3.556)
Diabetes, n (%)*	1 (11.1)	17 (18.1)	0.566 (0.012 - 4.749)
Smoking, n (%)*	6 (66.7)	52 (55.9)	1.615 (0.320 - 10.527)
Previous ischemic heart disease, n (%)*	1 (11.1)	15 (16)	0.658 (0.014 - 5.586)
Stroke, n (%)*	1 (11.1)	2 (2.1)	5.750 (0.087 - 118.388)
Peripheral vascular disease, n (%)*	0	6 (6.4)	---
Total cholesterol (mg/dL)†	256.6±52.2	239.8±33.7	0.473 (-0.215 - 1.159)
LDL-cholesterol (mg/dL)†	222.3±52.5	186.4±31.1	1.078 (0.376 - 1.775)
HDL-cholesterol (mg/dL)†	40.22±7.2	41.97±10.5	-0.170 (-0.854 - 0.514)
Triglycerides (mg/dL)†	121.9±32.7	157.3±62.9	-0.580 (-1.267 - 0.110)
Family history of ischemic heart disease (Dutch Lipid Clinic Criteria)*	4 (44.4)	17 (18.1)	3.623 (0.638 - 8.583)

Family history of ischemic heart disease (Simon Broome criteria)*	5 (55.6)	31 (33)	2.540 (0.502 -13.619)

1

2 *OR (CI 95%); †Cohen's d. Effect size: Small <0.2; Medium 0.2-0.5; Large 0.5-0.8; Very
3 large >1.3
4

Table 4. Clinical scores of patients with pathogenic mutation, variants of unknown significance and without FH mutations

	FH MUTATION (n=9)	**NO MUTATION + VUS (n=94)**	***OR (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)**