Coronary Heart Disease, Peripheral Arterial Disease, and Stroke in Familial Hypercholesterolaemia

Insights From the SAFEHEART Registry (Spanish Familial Hypercholesterolaemia Cohort Study)

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Objective—Heterozygous familial hypercholesterolemia (FH) is the most common premature atherosclerotic cardiovascular disease (ASCVD)-related monogenic disorder, and it is associated with ischemic heart disease. There is limited information whether FH increases the risk of peripheral arterial and cerebrovascular disease. Our aim was to analyze ASCVD prevalence and characteristics in different arterial territories in a large FH population, to compare them with an unaffected control population and to determine which factors are associated to ASCVD.

Approach and Results—SAFEHEART (Spanish Familial Hypercholesterolaemia Cohort Study) is an ongoing registry of molecularly defined patients with heterozygous FH in Spain. ASCVD in the different arterial territories was analyzed, as well as individual characteristics, genetic variables, and lipid-lowering therapies. The study recruited 4132 subjects (3745 ≥18 years); 2,752 of those enrolled were molecularly diagnosed FH cases. Median age was 44.0 years (45.9% men) and 40 years (46.6% men) in FH patients and unaffected relatives (P<0.001). ASCVD was present in 358 (13.0%) and 47 (4.7%) FH patients and unaffected relatives, respectively (P<0.001). History of premature ASCVD was more prevalent in FH patients (9.4% and 2.4% in FH patients and unaffected relatives, respectively; P<0.001). Coronary artery-related manifestations and peripheral artery disease were more prevalent in FH patients than in controls, but no significant differences were found for cerebrovascular events. Age, body mass index, type 2 diabetes mellitus, high blood pressure, previous use of tobacco, and lipoprotein(a) >50 mg/dL were independently associated with ASCVD.

Conclusions—The prevalence of ASCVD is higher, and the involvement of the arterial territories is different in FH patients when compared with their unaffected relatives. Age, male sex, increased body mass index, hypertension, type 2 diabetes mellitus, smoking habit, and lipoprotein(a) >50 mg/dL were independently associated with ASCVD.

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I
Heterozygous familial hypercholesterolemia (FH) is a genetic disorder characterized by autosomal inheritance in genes related to LDL receptor (LDLR) and mutations in other genes, which results in lifelong elevation of LDL-cholesterol (LDL-C). The major clinical manifestation of FH results from the prolonged exposure of the vasculature to high levels of LDL-C, which leads to the development of atherosclerotic lesions. It is the most common premature atherosclerotic cardiovascular disease (ASCVD)-related monogenic disorder, being life expectancy shortened by 20 to 30 years when compared with unaffected subjects. Although the type of mutation is probably the most influential variable in the clinical expression of ASCVD in FH, there are other genetic, environmental, and metabolic risk factors that might play a significant role in modulating the atherosclerotic burden in these individuals but the interaction among these risk factors and LDL-C levels in both FH subjects and their unaffected relatives is not well understood. Published estimates of the magnitude of ASCVD risk are highly variable. Furthermore, many reports on this topic were conducted before the introduction of statins as a standard treatment.

FH is associated with premature ischemic heart disease, but there is limited information whether FH increases the risk for peripheral arterial and cerebrovascular disease. This scarce information makes difficult to establish and develop health policies to decrease the disease burden that this genetic disorder provides. The SAFEHEART (Spanish Familial Hypercholesterolaemia Cohort Study) was designed to improve the knowledge about the prognostic factors and mechanisms that influence the development of ASCVD and mortality in a FH population.

The aim of this work was to analyze ASCVD prevalence, manifestations, and characteristics in different arterial territores in a large FH population, to compare them with an unaffected control population and to determine which factors are associated to ASCVD in FH patients.

Materials and Methods
Materials and Methods are available in the online-only Data Supplement.

Results
Four thousands one hundred and thirty-two subjects were enrolled. Of the total population, 3,745 were ≥18 years. Of them, 2,752 were FH cases (Figure 1). Median age was 44.0 (34.0–57.0) and 40 (29.0–53.0) years in FH patients and unaffected relatives, respectively (P<0.001). At enrollment, 1264 FH patients (45.9%) and 463 unaffected relatives (46.6%) were men (P=0.7).

Clinical history of ASCVD was present in 358 (13.0%) and 47 (4.7%) FH patients and unaffected relatives, respectively (P<0.001). Median age of onset of clinical cardiovascular events was 48.0 years (38.0–57.0) in FH patients and 55.0 years (48.0–61.0) in unaffected relatives (P=0.001). Among FH subjects, median age of onset of clinical cardiovascular events was 45.5 years (37.8–53.3) in male patients and 52.0 (41.0–64.8) in female patients (P<0.001). In FH patients, mean age of onset of clinical cardiovascular events was 46.45±10.78 years in male patients and 52.04±14.30 in female patients (P<0.001), and in unaffected relatives, mean age of onset of clinical cardiovascular events was 52.5±11.05 years in male patients and 60.36±6.97 in female patients (P=0.03). Main characteristics for FH patients and unaffected relatives are shown in Table 1. Median age at inclusion was significantly higher in FH patients than in unaffected relatives (44.0 years in FH patients and 40.0 years in FH patients and unaffected relatives, respectively; P<0.001). History of premature ASCVD was more prevalent in FH patients (9.4% and 2.4% in FH patients and unaffected relatives, respectively; P<0.001). Nevertheless, there were no statistically significant differences in the history of premature familial ASCVD (22.4% and 20.7% in FH patients and unaffected relatives, respectively; P=0.28). Active smoking was more prevalent in unaffected relatives than in FH patients (26.4% and 33.9% in FH patients and unaffected relatives, respectively; P<0.001). Total cholesterol, LDL-C, non–high-density lipoprotein cholesterol, APOB and lipoprotein(a) (Lp(a)) were significantly higher in FH patients than in unaffected relatives (44.0 versus 40.0 years in FH patients and unaffected relatives, respectively; P<0.001).

The presence of previous ASCVD

Table 2 shows the main characteristics and their differences between FH patients with and without ASCVD. A statistically significant higher prevalence of male sex, history of
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premature familial ASCVD, type 2 diabetes mellitus (T2DM), hypertension, corneal arcus, and xanthomas was found among FH patients with ASCVD. On the contrary, a statistically significant lower prevalence of active tobacco smoking was seen in those FH subjects with ASCVD. Age, body mass index, triglycerides, and Lp(a) levels were higher in FH patients with ASCVD. Two thousand two hundred and fourteen FH patients (80.45%) and 236 unaffected relatives (22.76%) were on lipid-lowering therapy (LLT) at enrolment. Total cholesterol, LDL-C, high-density lipoprotein cholesterol, non–high-density lipoprotein cholesterol cholesterol, and Apo B were significantly higher in FH patients without ASCVD. The use of different high-intensity LLT regimens is shown in Table 2. A significantly higher prevalence of use of ezetimibe, maximum combined therapy, and maximum LLT was found among FH patients with ASCVD when compared with FH patients without ASCVD.

### Functional Mutations and LLT

We identified 209 different functional mutations in LDLR and APOB genes. Distribution and types of mutations are depicted in Tables 1 and 2. No significant differences in the type of mutations were found between FH patients with and without previous ASCVD (Table 2).

### Atherosclerotic Cardiovascular Disease

Main ASCVD manifestations are shown in Table 3. The estimated risk of ASCVD among patients with FH was estimated as follows: odds ratio (OR) 3.01 (2.20–4.12) for FH patients compared with patients who do not have FH. Every coronary artery–related manifestation was statistically significant higher in FH patients than in controls. The same significance was found for peripheral artery disease. Nevertheless, no significant differences were found for those events related to the cerebrovascular system. Figure 2 shows the relative distribution of cardiovascular territories clinically involved in FH patients and their unaffected relatives.
Multivariable analysis showed that age (OR, 1.07; 95% confidence interval [CI], 1.06–1.09), body mass index (OR, 1.05; 95% CI, 1.02–1.09), T2DM (OR, 1.58; 95% CI, 0.95–2.61), high blood pressure (OR, 1.83; 95% CI, 1.27–2.63), previous use of tobacco (OR, 2.50; 95% CI, 1.82–3.44), and Lp(a) >50 mg/dL (OR, 2.14; 95% CI, 1.57–2.92) were independently associated with the presence of ASCVD in FH patients. Furthermore, female sex (OR, 0.27; 95% CI, 0.20–0.38) was an independent protective factor. Interestingly, familial history of premature ASCVD and the type of mutation (null or defective) were not independently associated to the existence of ASCVD.

### Table 2. FH Patient’s Characteristics Depending on the Presence Atherosclerotic Cardiovascular Disease

<table>
<thead>
<tr>
<th></th>
<th>FH Cases and ASCVD (+), Median (Q1–Q3)/n (%)</th>
<th>FH Cases and ASCVD (−), Median (Q1–Q3)/n (%)</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>358</td>
<td>2394</td>
<td>...</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>242 (67.6)</td>
<td>1021 (42.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age, y</td>
<td>59.0 (49.0–70.0)</td>
<td>42.0 (32.0–54.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of premature ASCVD</td>
<td>260 (72.6)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Premature familiar ASCVD</td>
<td>105 (29.3)</td>
<td>512 (21.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Corneal arcus</td>
<td>195 (54.6)</td>
<td>721 (30.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Xanthomas</td>
<td>98 (27.5)</td>
<td>278 (11.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T2DM</td>
<td>47 (13.2)</td>
<td>72 (3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>135 (37.8)</td>
<td>262 (10.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Active tobacco smoker</td>
<td>47 (13.2)</td>
<td>678 (28.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>174 (48.6)</td>
<td>494 (20.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.3 (25.6–31.3)</td>
<td>25.5 (22.6–28.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C receptor-negative mutation</td>
<td>142 (39.7)</td>
<td>851 (35.5)</td>
<td>0.29</td>
</tr>
<tr>
<td>LDL-C receptor defective mutation</td>
<td>132 (36.9)</td>
<td>1008 (42.1)</td>
<td></td>
</tr>
<tr>
<td>LDL-C receptor UKF mutation</td>
<td>71 (19.8)</td>
<td>455 (19.0)</td>
<td></td>
</tr>
<tr>
<td>APOB mutation</td>
<td>13 (3.6)</td>
<td>77 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>213.5 (183.0–251.0)</td>
<td>240.2 (208.0–285.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>145.7 (120.6–180.0)</td>
<td>170.0 (141.0–212.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>45.0 (37.0–53.0)</td>
<td>50.0 (42.0–58.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>98.3 (73.0–128.9)</td>
<td>82.9 (62.0–114.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-HDL cholesterol, mg/dL</td>
<td>189.9 (158.6–202.0)</td>
<td>188.4 (158.6–234.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoAI, mg/dL</td>
<td>129.2 (114.3–149.0)</td>
<td>136.0 (118.8–154.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>195.0 (137.0–238.0)</td>
<td>110 (91.3–135.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>39.4 (14.9–82.5)</td>
<td>20.6 (8.2–51.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patients on maximum statin dose</td>
<td>248 (69.3)</td>
<td>797 (33.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patients on ezetimibe</td>
<td>240 (67.0)</td>
<td>758 (31.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patients on maximum combined therapy</td>
<td>183 (51.1)</td>
<td>410 (17.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Patients on maximum LLT</td>
<td>287 (80.2)</td>
<td>1042 (43.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

See details in text for treatment classification. ASCVD indicates atherosclerotic cardiovascular disease; BMI, body mass index; FH, familial hypercholesterolemia patient; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LLT, lipid-lowering therapy; Lp(a), lipoprotein(a); T2DM, type 2 diabetes mellitus; TG, triglycerides; and UKF mutation, mutation without functionality assays.

**Discussion**

This study shows the high prevalence of ASCVD in FH subjects (13.0%), which is 3-fold higher than in unaffected relatives, although in this control population, the cardiovascular factors burden, except for LDL-C, was significantly higher. To our knowledge, this is the largest work describing the prevalence and location and the variables associated to ASCVD in a molecularly well-defined FH cohort, the SAFEHEART registry, which is a nationwide study based on data obtained from real-life practice. The differences between FH patients and unaffected relatives and FH patients with and without ASCVD are extensively described. Furthermore, this is the only work...
in which unaffected relatives are used as the control population. Our results also show how age, male sex, the presence of other cardiovascular risk factors (mainly increased body mass index, hypertension, T2DM, and smoking habit), and Lp(a) >50 mg/dL are independently associated to ASCVD in FH patients. LDL-C association with ASCVD was not assessed because the baseline lipid profile (without treatment) was not available in a huge number of patients and, in concrete, among those patients with previous ASCVD. Nevertheless, LDL-C level is the principal prognostic marker in FH patients and always should be considered to establish risk assessment.

Recent data from the CASCADE FH registry in the United States have shown a high prevalence of ASCVD. However, the main difference with SAFEHEART is the inclusion criteria of FH patients. LDL-C association with ASCVD was not assessed because the baseline lipid profile (without treatment) was not available in a huge number of patients and, in concrete, among those patients with previous ASCVD. Nevertheless, LDL-C level is the principal prognostic marker in FH patients and always should be considered to establish risk assessment.

The estimated risk of ASCVD among patients with FH has been previously described by Benn et al in a population-level survey undertaken in Denmark. This study reported an OR of 13.2 (10.0–17.4) for FH patients not receiving LLT and 10.3 (7.8–13.8) for FH patients receiving LLT, compared with patients who do not have FH and are not receiving LLT. Our group previously conducted a cross-sectional study on 811 FH patients to estimate the risk factors associated with the development of premature ASCVD. The authors compared

| Table 3. Atherosclerotic Cardiovascular Disease Manifestations in FH Patients and Unaffected Relatives |
|-----------------------------------------------|----------------|----------------|----------------|
|                                               | FH Cases, n (% Total FH Cases) | Unaffected Relatives, n (% Total Unaffected Relatives) | \( P \) Values |
| n                                             | 2752            | 993            | \( <0.001 \)  |
| Angina                                         | 205 (7.5)       | 22 (2.2)       | \( <0.001 \)  |
| AMI                                           | 178 (6.5)       | 21 (2.1)       | \( <0.001 \)  |
| Coronary angioplasty/stent                     | 139 (5.0)       | 20 (2.0)       | \( <0.001 \)  |
| CABG                                          | 110 (4.0)       | 4 (0.4)        | \( <0.001 \)  |
| Any atherosclerotic coronary artery disease    | 325 (11.8)      | 36 (3.6)       | \( <0.001 \)  |
| Stroke                                         | 21 (0.8)        | 9 (0.9)        | 0.40           |
| TIA                                           | 23 (0.8)        | 4 (0.4)        | 0.12           |
| Carotid stent                                  | 8 (0.3)         | 3 (0.3)        | 0.60           |
| Carotid surgery                                | 7 (0.3)         | 0              | 0.12           |
| Any atherosclerotic cerebrovascular disease    | 49 (1.8)        | 15 (1.5)       | 0.34           |
| Peripheral artery revascularization            | 14 (0.5)        | 0              | \( <0.01 \)   |
| Any atherosclerotic peripheral artery disease  | 39 (1.4)        | 2 (0.2)        | \( <0.001 \)  |

AMI indicates acute myocardial infarction; CABG, coronary artery bypass graft; FH, familial hypercholesterolemia; and TIA, transient ischemic accident.

Figure 2. Relative distribution of cardiovascular territories clinically involved in familial hypercholesterolemia (FH) patients and their unaffected relatives. \( P \) value for the difference between FH patients and unaffected relatives: coronary artery disease \( P<0.001 \); cerebrovascular disease \( P=0.34 \); and peripheral artery disease \( P<0.001 \).
the results from this registry with the overall Spanish population. Their data show a calculated 8.4-fold increased cardiovascular risk. Nevertheless, in that study, only index cases were included and only premature ASCVD was considered.

Prevalence of risk factors different to elevated LDL-C levels as well as their management and impact likely differ between FH patients and non-FH patients. The OR found for the presence of ASCVD in the present study was smaller than those previously described. Probably, the reason for that is the high prevalence of other cardiovascular risk factors different to elevated LDL-C plasma levels present in the unaffected relatives population enrolled. As can be seen, the prevalence of active smoking is significantly higher among unaffected relatives than among FH patients with ASCVD. This finding presumably reflects the perceived importance about their disease that FH patients feel and the impact that their physicians' advice causes in their lifestyle. An interesting point is the lack of significant differences found in the prevalence of familial history of premature ASCVD. It is well understood if we keep in mind that all the enrolled subjects, with and without the mutation, belong to the same families.

Recently, it has been published the risk of coronary artery disease on the presence or absence of a mutation related with FH in coronary artery disease case–control studies showing an OR of 22 in subjects with a FH mutation and LDL-C of >190 mg/dL compared with those with LDL-C of <130 mg/dL and no mutation. After adjusting for LDL-C levels, the OR was 4.2, slightly higher than that in our study. Besides differences in the design and analyzed population between both studies, the prevalence of other cardiovascular risk factors like T2DM, hypertension, current smokers, and meanage is higher in the study by Khera et al. This fact could explain the difference in coronary artery disease risk.

FH is known to be associated with elevated LDL-C levels and increased risk of premature coronary heart disease. Because increased LDL-C levels lead to atherosclerosis, FH has also been proposed as a risk factor for peripheral vascular and ischemic cerebrovascular diseases. Currently, the association between clinical FH and risk of stroke is unclear. Some studies, focused on estimating the stroke risk, have provided limited data, because of the lack of a control group, the small sample size, and the lack of a molecular diagnosis.

A prospective study of FH by the United Kingdom–based Simon Broome Register Group did not find an increased risk of stroke mortality for subjects with FH. By contrast, the prevalence of peripheral arterial disease is increased from 5- to 10-fold in FH subjects compared with non-FH controls. Our data show a clearly increased prevalence of coronary heart disease among those patients with FH and an increased prevalence of peripheral artery disease when compared with their unaffected relatives. Among those patients with ASCVD, 13.7% FH patients and 31.9% unaffected relatives had cerebrovascular involvement. Nevertheless, in our study, no significant differences were found for atherosclerotic cerebrovascular disease prevalence between FH patients and their relatives. Once more, the different prevalence of cardiovascular risk factors different to elevated LDL-C plasma levels might have played a role for not achieving differences between both groups. A better understanding of the association between FH and the incidence of atherosclerotic cerebrovascular disease–related events could have a public health impact by improving the diagnosis and management of individuals with FH.

Interestingly, our results might show a decreased prevalence of ASCVD when compared with pretreatment studies, as the effective LDL-C lowering achieved by statins and ezetimibe is associated with a significant reduction in the prevalence of ASCVD.

Strengths and Limitations

We acknowledge some strengths and limitations of our study. To our knowledge, this is the largest study of a molecularly characterized heterozygous FH population that describe the development of atherosclerotic lesions in the heart, brain, and peripheral arteries. A potential limitation is the fact that only clinical events have been considered; the detection of subclinical atherosclerotic disease might have room to improve the management of these patients. This is a large follow-up study of FH patients in which no intervention different to the provided by the patient’s physician was done. Thus, a reliable baseline lipid profile in this registry is missing because some patients were already on treatment when they were enrolled. This work compares genetically defined FH patients with their genetically confirmed non-FH relatives, who are not completely free from other lipid disorders such as combined familial hypercholesterolemia or polygenic hypercholesterolemia.

Conclusions

This study shows the high prevalence of ASCVD and the differential involvement of the arterial territories in FH patients when compared with their unaffected relatives. Age, male sex, increased body mass index, hypertension, T2DM, smoking habit, and Lp(a) of >50 mg/dL were independently associated with the presence of ASCVD, not forgetting LDL-C level as the principal driver for its development.

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References

Highlights
• Prevalence of ASCVD in FH patients is high and the involvement of the arterial territories is different to that of their unaffected relatives.
• Age, male sex, increased body mass index, hypertension, T2DM, smoking habit, and Lp(a) of >50 mg/dL were independently associated with the presence of ASCVD.
• LDL-C level remains the principal driver for ASCVD development.
Coronary Heart Disease, Peripheral Arterial Disease, and Stroke in Familial Hypercholesterolaemia: Insights From the SAFEHEART Registry (Spanish Familial Hypercholesterolaemia Cohort Study)

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MATERIALS AND METHODS

Study design and population

SAFEHEART is an open, multicentre, long-term prospective cohort study in a molecularly defined FH population (1). It was carried out in 28 outpatient lipid clinics in Spain. FH subjects and their unaffected relatives were enrolled since 2004 and the enrolment is still ongoing. Inclusion criteria were: 1) index cases with genetic diagnosis of FH, 2) relatives over 15 years old with genetic diagnosis of FH and 3) relatives over 15 years old with a negative genetic diagnosis of FH (control group). Relatives with high cholesterol values due to other reasons in the control group were not excluded. Data analysed for this work were obtained between January 2004 and November 2013 and only subjects ≥ 18 years old were analyzed. This study was approved by the local ethics committees and all eligible subjects gave written informed consent.

Cardiovascular disease definitions

Cardiovascular Disease was defined as the presence of any of the following: 1) Myocardial infarction: proved by at least two of the following: classic symptoms (> 15 minutes), specific electrocardiographic changes and increased levels of cardiac biomarkers (> 2× upper limit of normal); 2) angina pectoris: diagnosed as classic symptoms in combination with at least one unequivocal result of one of the following: exercise test, nuclear scintigram, dobutamine stress ultrasound scan or > 70% stenosis on a coronary angiogram; 3) percutaneous coronary intervention or other invasive coronary procedures; 4) coronary artery bypass grafting; 5) ischemic stroke demonstrated by computed tomography or magnetic resonance imaging or documented transitory ischemic attack; 5) Peripheral arterial disease: intermittent claudication, which was defined as classic symptoms and at least one positive result of an ankle/arm index <0.9 or stenosis >50% on angiography or ultrasonography or abdominal aortic aneurism; 6) peripheral arterial revascularization: peripheral artery bypass grafting or percutaneous transluminal angioplasty. Premature ASCVD was defined as the occurrence of the first event before 55 years of age in men and before 65 years of age in women. The same cut-off points were used for the definition of premature familial ASCVD.

Measures and blood samples

Age, medical history focused on ASCVD, classic cardiovascular risk factors (hypertension, type 2 diabetes mellitus [T2DM], smoking habit), physical examination and treatment at entry for hypercholesterolemia and other risk factors were registered. History of ASCVD, premature ASCVD and familial premature ASCVD were obtained from medical records provided by the patients. Venous blood samples were taken after 12 hours of fasting. Serum, plasma and DNA samples were aliquoted and preserved at -80°C in a biobank located at the Cardiovascular Research Center in Barcelona. DNA was isolated from whole blood using standard methods and the genetic diagnosis of FH is made using a DNA-microarray (2). Serum total cholesterol, triglycerides and HDL-cholesterol levels were measured in a centralized laboratory using enzymatic methods. Serum LDL-C concentration was calculated using the Friedewald formula.

Lipid lowering therapy (LLT) classification.

Maximum statin dose was defined as atorvastatin 40 to 80 mg/day or rosuvastatin 20 to 40 mg/day, which were considered high-intensity statin doses. Maximum combined therapy was defined as maximum statin dose plus ezetimibe 10 mg/day. Maximum lipid-lowering therapy was defined as any LLT expected to
produce at least a 50% reduction in LDL-C baseline levels: simvastatin 20, 40, or 80 mg/day plus ezetimibe 10 mg/day; pravastatin 40 mg/day in combination with ezetimibe 10 mg/day; fluvastatin 80 mg/day plus ezetimibe 10 mg/day; atorvastatin 40 or 80 mg/day with or without ezetimibe 10 mg/day; atorvastatin 10 or 20 mg/day plus ezetimibe 10 mg/day; rosuvastatin 20 or 40 mg/day with or without ezetimibe 10 mg/day; rosuvastatin 10 mg/day plus ezetimibe 10 mg/day; and pitavastatin 4 mg/day in combination with ezetimibe 10 mg/day (3).

Genetic analysis
LDLR mutations were classified according to the effect they have on LDL receptor protein function as null (receptor-negative) and defective mutations (receptor-defective). Variants leading to the complete absence or truncation of the protein (loss of function) demonstrated by in vitro functional analysis or computed simulated analysis were classified as receptor-negative. This includes: 1) point mutations that cause a premature stop codon; 2) missense mutations affecting the fifth cysteine rich repeat in the ligand binding domain of the LDLR gene (class 2A mutation); 3) small deletions or insertions causing a frame shift and a premature stop codon and 4) large rearrangements. Receptor-defective mutations were the rest of inframe point mutations and inframe small deletions and insertions. All mutations without known functionality analysis by means of in vitro studies or computed simulated analysis were classified as “unknown functionality” (UKF) because we cannot assure if the effect on the receptor will be negative or defective. However, we consider them as pathogenics because all subjects carrying one of them have hypercholesterolaemia, and relatives without the mutation, have normal cholesterol levels.

Statistical analysis
Statistical analysis was carried out using SPSS version 18.0. Variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test. Quantitative data was expressed as median and inter-quartile range (IQR) and qualitative data as absolute number and percentage. Comparisons of frequencies between qualitative variables were carried out using the Chi-squared test and Fisher’s exact test when appropriate. Median values of quantitative variables were compared with the Mann-Whitney non-parametric test. A multivariate forward binary logistic regression analysis was conducted to determine the variables independently associated with the presence of ASCVD among FH subjects. We included variables that were statistically significant in univariate analyses, as well as a priori associated variables and confounders: age, gender, T2DM, previous smoking habit, high blood pressure, type of mutation (null or defective) and Lp(a) levels. LDL-C levels were not included as patients with previous ASCVD were more intensively treated. Differences were considered statistically significant when a p value <0.05 was obtained.

References

Heterozygous familial hypercholesterolemia (FH) is the most common premature atherosclerotic cardiovascular disease (ASCVD) related monogenic disorder. Our aim was to analyze ASCVD prevalence and characteristics in different arterial territories in a large FH population, to compare them with an unaffected control population and to determine which factors are associated to ASCVD. SAFEHEART is an ongoing registry of molecularly-defined patients with heterozygous FH. Coronary artery-related manifestations and peripheral artery disease were more prevalent in FH patients than in controls, but no significant differences were found for cerebrovascular events. Age, body mass index, T2DM, high blood pressure, previous use of tobacco, and Lp(a)>50 mg/dl were independently associated with ASCVD. The figure above shows relative distribution of cardiovascular territories clinically involved in FH patients and their unaffected relatives. Thus, the prevalence of ASCVD is higher and the involvement of the arterial territories is different in FH patients when compared with their unaffected relatives.