SAFEHEART risk-equation and cholesterol-year-score are powerful predictors of cardiovascular events in French patients with familial hypercholesterolemia

Antonio Gallo[a,1], Sybil Charriere[b,1], Alexandre Vimont[c], M. John Chapman[d], Denis Angoulvant[e], Franck Boccara[f], Bertrand Cariou[g], Valérie Carreau[a], Alain Carrié[h], Eric Bruckert[a], Sophie Béliard[i,j,*], for the French REgistry of Familial hypERCHOLesterolemia (REFERCHOL) investigators (Denis Angoulvant, Sophie Beliard, Franck Boccara, Eric Bruckert, Bertrand Cariou, Valérie Carreau, Alain Carrie, Sybil Charriere, Yves Cottin, Mathilde DI. Filippo, Sonia Dulong, Vincent Durlach, Michel Farnier, Emile Ferrari, Dorota Ferrieres, Jean Ferrieres, Antonio Gallo, Philippe Giral, Sophie Gonbert, Regis Hankard, Jocelyn Inamo, Olga Kalmykova, Michel Krempf, Philippe Moulin, François Paillard, Noel Peretti, Agnes Perrin, Jean Pierre Rabes, Ariane Sultan, Patrick Tounian, René Valero, Bruno Verges, Cecile Yelnik, Olivier Ziegler)

[a] Department of Endocrinology and Prevention of Cardiovascular Disease, Institute of Cardio Metabolism and Nutrition (ICAN), La Pitié-Salpêtrière Hospital, AP-HP, Paris, France
[b] Hospices Civils de Lyon, Endocrinology Department, Bron, University of Lyon 1, France
[c] Public Health Expertise, Paris, France
[d] Sorbonne University, INSERM and Endocrinology-Metabolism Division, Pitie-Salpetriere University Hospital, Paris, France
[e] Tours University & CHRU de Tours, Cardiology Department & EA4245 T2i, Loire Valley Cardiovascular Collaboration, F37000, Tours, France
[f] AP-HP, Hôpitaux de L'Est Parisien, Hôpital Saint-Antoine, Service de Cardiologie Faculty of Medicine, Sorbonne Université, National Institute of Health and Medical Research, INSERM, UMR_S 938, UPMC, Paris, France
[g] L'institut Du Thorax, Endocrinology Department, CIC 1413 INSERM, CHU de Nantes, Nantes, France
[h] Sorbonne University, Inserm, Institute of Cardiometabolism and Nutrition (ICAN), UMR_S1166, APHP, Department of Biochemistry, Obesity and Dyslipidemia Genetics Unit, Hôpital de La Pitié, Paris, France
[i] Aix Marseille University, INSERM, INRA, C2VN, Marseille, France
[j] Department of Nutrition, Metabolic Diseases, Endocrinology, La Conception Hospital, Marseille, France

HIGHLIGHTS

- SAFEHEART-risk equation can now be used in other European population of HeFH patients to predict CV events.
- The cholesterol-year-score is a robust predictor of CV events in HeFH patients.
- SAFEHEART-RE and the cholesterol-year-score are valid in primary prevention heFH patients.

ARTICLE INFO

ABSTRACT

Background and aims: Patients with heterozygous familial hypercholesterolemia (HeFH) present elevated cardiovascular (CV) risk. Current CV risk stratification algorithms developed for the general population are not adapted for heFH patients. It is therefore of singular importance to develop and validate CV prediction tools, which are dedicated to the HeFH population.
Methods: Our first objective was to validate the Spanish SAFEHEART-risk equation (RE) in the French HeFH cohort (REFERCHOL), and the second to compare SAFEHEART-RE with the low-density-lipoprotein-cholesterol

* Corresponding author. APHM, Department of Nutrition, Metabolic diseases, Endocrinology, La Conception Hospital, Aix Marseille University, INSERM, INRA, C2VN, Marseille, France 147 boulevard Baille, 13005, Marseille, France.
  E-mail address: sophie.beliard@ap-hm.fr (S. Béliard).
  [1] equally contributed to the work.

(LDL-C)-year-score for the prediction of CV events in the HeFH French population.

Results: We included HeFH (n = 1473) patients with a genetic or clinical diagnosis (DLCN score ≥ 8). Among them, 512 patients with a 5-year follow-up were included to validate the 5 year-CV-RE. A total of 152 events (10.3%) occurred in the entire population of 1473 patients during a mean follow-up of 3.9 years. Over the five-year follow-up, non-fatal CV events occurred in 103 patients (20.2%). Almost all the parameters used in the SAFEHEART-RE were confirmed as strong predictors of CV events in the REFERCHOL cohort. The C-statistic revealed a satisfactory performance of both the SAFEHEART-RE and LDL-C-year-scores in predicting CV events for all the patients (primary and secondary prevention) (C-index 0.77 and 0.70, respectively) as well as for those in primary prevention at inclusion (C-index 0.78 and 0.77, respectively).

Conclusions: This analysis represents the first external validation of the SAFEHEART-RE and demonstrated that both SAFEHEART-RE and the LDL-C-year-score are good predictors of CV events in primary prevention HeFH patients.

1. Introduction

Heterozygous familial hypercholesterolemia (HeFH) is one of the most prevalent autosomal dominant genetic diseases, and is characterized by mutations in genes encoding proteins involved in low-density-lipoprotein-cholesterol (LDL-C) catabolism. As a consequence, HeFH patients feature elevated circulating levels of LDL-C from birth. This condition remains underdiagnosed and undertreated [1–3] and is characterized by premature cardiovascular (CV) events driven by life-long exposure of the arterial wall to hypercholesterolemia [4].

Traditional risk factors such as smoking and hypertension may further increase CV risk in HeFH patients. Indeed, cumulative traditional CV risk factors are currently used to classify HeFH patients into categories of lesser or greater severity [5,6]. Cardiovascular risk is however expressed heterogeneously among HeFH patients [7]. It is therefore essential to identify the more severe patients early in life, and to initiate treatment to the guideline-recommended goal of LDL-C commensurate with their level of risk [8,9]. Moreover, it is of particular importance to precisely evaluate CV risk in primary prevention HeFH patients, to guide the choice of the type and the frequency of CV explorations, the initiation of aspirin or PCSK9 inhibitors.

Classical CV risk equations (RE), such as the European SCORE equation or the US Framingham RE were formulated with data from the general population and are not therefore recommended for application to FH patients, as they underestimate their CV risk [1]. Moreover, the SCORE RE is only valid for persons older than 40 years.

Recently, Pérez de Isla et al. developed the first specific CV-RE dedicated to genetically-confirmed HeFH patients, in primary or in secondary prevention: the SAFEHEART-RE [10,11]. This algorithm is constituted of traditional risk factors: age, gender, smoking, hypertension, body mass index, a history of CV events, and levels of LDL-C and lipoprotein (a) (Lp(a)). To date, the SAFEHEART RE has not been validated in another HeFH cohort and has not been tested in patients free of CV events at inclusion. An external validation is critical for the extension of this CV-RE to other HeFH populations.

The cholesterol-year-score is a simple tool which evaluates the duration and the intensity of vascular exposure to elevated cholesterol levels [12], and in a similar manner to that in which pack-year is used to measure lifelong exposure to tobacco. An increased prevalence of early atherosclerotic burden has been observed in young HeFH subjects who present a high vascular cholesterol burden [13]. The cholesterol-year-score was first used in homozygous FH subjects [12] but its validation in HeFH patients has not been reported to date. To our knowledge, the predictive power of the cholesterol-year-score in such subjects with respect to CV events has not yet been evaluated.

Our first objective was the validation of the Spanish SAFEHEART-RE in our French cohort of HeFH patients (REFERCHOL cohort), and the second, a comparison of the predictive power of SAFEHEART-RE with that of the LDL-C-year-score for CV events, in the whole cohort and in primary prevention HeFH patients.

2. Patients and methods

2.1. Study design and population

The national French Register of FH (REFERCHOL) was established in 2015 by the *Nouvelle Société Francophone d'Athérosclérose* (NSFA) as previously described [14]. For the present study, the population was prospectively enrolled between November 2015 and December 2018 and retrospective data were recorded from 2005 to 2015. A total of 2311 adults (≥ 18 years old) with either a genetic or a clinical diagnostic (DLCN score ≥ 8) of HeFH were enrolled. Among them, 1473 patients with at least two visits in the participating lipid clinics were retained for the analysis (Fig. 1). For the validation of the 5 year CV-RE, we included 512 patients with either 5 years of follow-up, or a CV event within the 5 years following the enrollment visit.

Registry REFERCHOL was declared to the ANSM (the French National Agency for Medicines safety) and received an ID-RCB declarant number: 2014-A01549-38.

2.2. Collection of patient data

Demographic and clinical characteristics at the enrollment visit (baseline) were used in this study. For most patients, DNA was isolated and the genetic diagnosis of FH was evaluated. Maximum statin dose was defined as previously described (atorvastatin 40 or 80 mg/day, rosuvastatin 20 or 40 mg/day, or simvastatin 80 mg/day) [15]. Maximum combined lipid-lowering therapy was defined as maximum statin dose plus ezetimibe 10 mg/day. Assessment of biological parameters, including a plasma lipid profile, were performed in the clinical
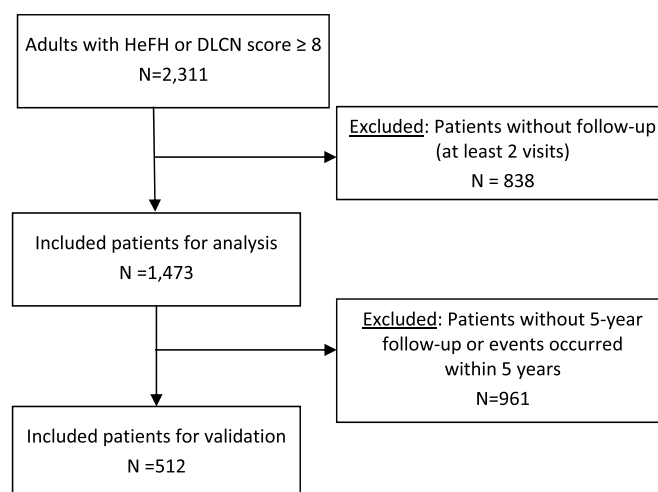
Fig. 1. Patient flowchart for the REFERCHOL cohort for the analysis study and the validation study. HeFH = heterozygous familial hypercholesterolemia. DLCN = Dutch Lipid Clinic Network.

laboratory attached to each lipid clinic, using assay methods subject to quality control. The majority of Lp(a) measurements were made using an immunonephelometric method (Siemens-France). However, some Lp (a) measurements were made with alternative methods in laboratories of other University Medical Centres, the latter corresponding to the location of heFH patients. The LDL-C-year-score was calculated as previously described by multiplying the highest LDL-C value by the patient's age at clinical diagnosis or at the initiation of statin therapy, to which was added the LDL-C value at the inclusion visit in the registry multiplied by the time spent until the inclusion visit, as follows: LDL-C-year-score = *LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at diagnosis/initiation of statin therapy]*.

2.3. Definition of outcomes

Cardiovascular events included coronary heart disease (acute coronary syndromes, myocardial infarction and unstable angina, percutaneous coronary intervention or coronary artery bypass graft), stroke or transient ischemic attack, peripheral artery disease (defined as carotid endarterectomy, carotid angioplasty, peripheral artery angioplasty or bypass), resuscitated sudden death and cardiovascular death [16]. A family history of early CV events was defined as the occurrence of the first event before 55 years of age in men and before 65 years of age in women in first-degree relatives. The duration of follow-up was defined as the time from the enrollment visit to the last available visit at the Lipid Clinic.

2.4. Statistical analysis

These analyses were conducted in accordance with the TRIPOD (Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis) requirements [17]. Univariate effects were analyzed with Cox models by hazard ratios and their 95% confidence intervals. For multivariate analysis, several Cox models were adjusted. REFERCHOL equation A was estimated by forcing variables derived from SAFEHEART-RE in order to compare effect size regardless of statistical significance. REFERCHOL equation B included only independent significant factors with a p value < 0.05 from the analysis population. We modified some parameters as compared to the SAFEHEART cohort. First, maximal LDL-cholesterol (ie LDL-C before treatment) was not extrapolated from LDL-C under lipid lowering therapy but obtain from patient's medical record. Secondly, the segmentation for ranks according to LDL-C levels was modified in view of differences in sample sizes: there were only 91 patients with LDL <100 mg/dL and 427 between 100 and 160 mg/dL. New cut-points for LDL-C were updated from <100 mg/dl in SAFEHEART-RE to ≤160 mg/dL as the reference level, to 160–200 mg/dL and to >200 mg/dL. LDL-C-year-score and the variables included in its calculation (age at initiation of statin treatment, maximum LDL-C before therapy) were also tested in the univariate analysis and LDL-C-year-score was included in the REFERCHOL Equation B.

As in SAFEHEART-RE, the Kaplan-Meier estimator from the analysis population to obtain the 5-year risk was used according to the method described by D'Agostino [18]. To compare ROC curves and C-indexes, the Kendall's Tau-b was computed to measures the ranking association between risk equations, and evaluated the concordance between pairs of estimated risks from two RE for every given risk threshold, ranging from −1 (negative association) to +1 (positive association). For additional validation of the Safeheart-RE and the REFERCHOL equation A, the degree of over-optimism (standard deviation from the standard error of risk estimations) on the REFERCHOL analysis population was estimated with bootstrap resampling of 100 randomized samples as recommended by TRIPOD [17].

Table 1
Characteristics of the French REFERCHOL HeFH population at the enrolment visit.

Variable	Value	REFERCHOL patients (N = 1473)
Gender (n)	Male	712 (48.34%)
Age (years)	Mean (Sd)	49.61 (16.65)
Age (class)	<30	244 (16.56%)
	30–60	794 (53.90%)
	>60	435 (29.53%)
Mutation (n)	Yes	1032 (70.06%)
	LDL-R	917 (88.86%)
	APOB	80 (7.75%)
	PCSK9	35 (3.39%)
History of ASCVD (n)	Yes	326 (22.13%)
Premature history of familial ASCVD (n)	Yes	379 (32.39%)
Type II diabetes (n)	Yes	61 (4.39%)
HTA (n)	Yes	377 (27.26%)
Current smoker (n)	Yes	369 (27.05%)
BMI (kg/m^2)	Mean (SD)	24.69 (4.76)
TC (mg/dl)	Mean (SD)	277.12 (91.21)
	Median	261.0
Maximum TC (mg/dl)	Mean (SD)	388.78 (102.20)
	Median	376.0
LDL-C (mg/dl)	Mean (SD)	199.88 (83.33)
	Median	186.0
Maximum LDL-C (mg/dl)	Mean (SD)	299.75 (95.12)
	Median	288.5
HDL-C (mg/dl)	Mean (SD)	54.14 (16.43)
	Median	52.0
TG (mg/dl)	Mean (SD)	111.72 (69.24)
	Median	94.0
ApoA1 (mg/dl)	Mean (SD)	144.04 (32.39)
	Median	142.0
ApoB (mg/dl)	Mean (SD)	140.90 (47.02)
	Median	130.0
Lp(a) (mg/dl)	Mean (SD)	44.55 (55.28)
	Median	25.0
Lp(a) > 50 mg/dl(n)	–	265 (31.36%)
Treatment(n)	None	491 (33.33%)
	Statins only	464 (31.50%)
	Bitherapy	517 (35.10%)
Maximum statin dose (n)		364 (24.71%)
Ezetimibe (n)		454 (30.82%)
Maximum combined therapy (n)		226 (15.34%)
LDL-apheresis		40 (2.7%)
Time on statins (years)	Mean (SD)	10.84 (10.73)
	Median	8.0
Age at initiation of statins (years)	Mean (SD)	32.03 (14.28)
	Median	30.9

(continued on next page)

Table 1 (*continued*)

Variable	Value	REFERCHOL patients (N = 1473)
LDL-C-year-score (mg/dl)	Mean (SD)	11.075 (5.863)
	Median	10.424
LDL-C-year-score mg/dl (class)	<6.000	275 (21.62%)
	6.000–16.000	756 (59.20%)
	>16.000	244 (19.18%)

ASCVD = Atherosclerotic cardiovascular disease; SD = standard deviation.
A premature family history of 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 in first-degree relatives. Maximum statin dose was defined as atorvastatin 40 or 80 mg/day, rosuvastatin 20 or 40 mg/day, and simvastatin 80 mg/day [15]. Maximum combined lipid-lowering therapy was defined as maximum statin dose plus ezetimibe 10 mg/day. LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at diagnosis/initiation of statin].

3. Results

3.1. Characteristics of the HeFH REFERCHOL population at the enrollment visit

The mean characteristics of the analysis population are presented in Table 1. The mean age of the patients was 49.6 ± 16.7 years. Genetic causal mutations were found in 70.1% (n = 1032) of the REFERCHOL cohort, with 88.9% in the *LDLR* gene, 7.8% in the *APOB* gene and 3.4% in the *PCSK9* gene. Among patients with a clinical diagnosis (n = 441), more than half (n = 236, 53.5%) did not have a genetic test and a small number of them lacked detectable causal mutations (n = 33, 7.5%). For other patients (n = 172, 39%), genetic tests are in process. At the enrollment visit, a third of patients were not receiving lipid lowering therapy (n = 491, 33.3%). Almost a quarter of patients were receiving maximally tolerated statin therapy and 40% were treated with maximum combined therapy (i.e. maximum tolerated statin therapy plus ezetimibe). The mean age at statin initiation was 32 ± 14.3 years. The characteristics of the SAFEHEART population used for the development of the SAFEHEART risk equation [10] are also presented in Supplemental Data 1.

3.2. Predictive factors for incident CV events in the REFERCHOL cohort

A total of 152 events occurred in the entire population of 1473 patients included for the analysis (10.3%) during a mean follow-up of 3.9 years (SD: 3.4 years; Med: 2.6 years). A large proportion of events occurred in patients with a history of CV events (n = 79/326, 24.2%); a small proportion of events occurred in patients free of CV events at the enrollment visit (n = 73/1147, 6.3%). Kaplan-Meier curves showing freedom from recurrent cardiovascular events in all patients, and patients with history of CV events or patients without history of CV events, are shown in Fig. 2. Table 2 presents univariate analysis of predictors of incident CV events. With the exception of a family history of premature CV event, ApoAI and Lp(a) levels, the predictors observed in the SAFEHEART cohort were statistically significant in the REFERCHOL cohort and the effect size of HRs varied globally over a similar range. Among the additional variables in REFERCHOL, LDL-C-year score was the strongest predictor of CV events. Table 3 shows multivariate analysis for REFERCHOL equations A and B. Data on the HRs for patients from the SAFEHEART study are reported in the first column, and are compared to those of the REFERCHOL cohort. REFERCHOL equations A and B were tested in the entire REFERCHOL population of 1473 patients. The REFERCHOL equation-A was applied by forcing the inclusion of all the significant variables of the SAFEHEART study in the model. Significant variables in the multivariate analysis in the

equation-A model were age, the history of a CV event, active smoking and LDL-C levels. The REFERCHOL equation-B was calculated with the inclusion of only significant variables from the univariate analysis in the REFERCHOL cohort: gender, history of CV events, LDL-C levels and LDL-C-year-score. As maximal LDL-C levels and the age of statin initiation were used for the calculation of the cholesterol-year-score, they were removed from the multivariate analysis. Gender, history of CV events, LDL-C levels and LDL-C-year-score were independent CV risk predictors in the REFERCHOL cohort. LDL-C-year score was a strong predictor for CV events, as HeFH patients with a LDL-C-year-score between 6000 and 16000 mg/dL had a 5.7 fold increased in CV risk (95%CI, *p* = 0.09) and those with a cholesterol-year-score above 16000 mg/dL had a CV risk multiplied by 17.4 fold (95%CI, *p* = 0.006).

3.3. Validation of the SAFEHEART risk prediction model and comparison to REFERCHOL-RE and cholesterol-year-score for predicting CV events in the REFERCHOL cohort

The analysis of the validation population (512 patients with five-year follow-up or a CV event within the five-year following enrollment visit) showed non-fatal CV events occurring in 103 patients (20.1%; 34 nonfatal myocardial infarctions, 28 coronary artery revascularization procedures or bypass, 22 unstable anginas, 18 nonfatal strokes or transient ischemic attacks). Among the 512 patients, 393 were in primary prevention at inclusion and 46 of them had a CV event within the five 5 years of follow-up (11.7%). Among the 119 patients in secondary prevention at inclusion, 57 of them had a recurrent CV event (47.9%) event. Only the first event during follow-up was considered for analysis.

Considering the 512 patients cohort (primary and secondary prevention patients at the inclusion), the C-index of SAFEHEART-RE for predicting five-year CV events in the REFERCHOL cohort was 0.77, and the C-index of REFERCHOL-RE A and the cholesterol-year-score were respectively 0.78 and 0.70. Fig. 3A shows the ROC curves for the 3 models. The ability of SAFEHART-RE, REFERCHOL-RE and the LDL-C-year-score to predict five-year CV events was quite similar between the 3 models, although a little less effective for the LDL-C-year-score (Supplemental Data 2).

Considering the 393 patients cohort (primary prevention patients), the sensitivity analysis showed that the all three risk prediction models were good predictors of CV events in this patients free of CV event at the inclusion with the following C-indexes: 0.78 for SAFEHEART-RE, 0.79 for the REFERCHOL-RE A and 0.77 for the cholesterol-year-score. (Fig. 3B and Supplemental Data 2). The population of the 119 patients in secondary prevention at the enrollment was too small to be analyzed separately with unstable and uninterpretable results (Supplemental Data 2).

To compare ROC curves and C-indexes, the Kendall's Tau-b was computed to measure the ranking association between risk equations. The results were as follows: Safeheart RE *vs* Referchol RE: 0.92, Safeheart RE *vs* LDL-year score: 0.78, Referchol RE *vs* LDL-year score: 0.83 (Kendall's Tau-b ranging from −1 (negative association) to +1 (positive association)). Additional validation assessed with bootstrap resampling (100 randomized sample of the original dataset) showed a degree of over-optimism of 0.01 and 0.007 for SAFEHART-RE and REFERCHOL-RE, respectively.

4. Discussion

The estimation of CV risk in HeFH has led to the development of dedicated CV-RE derived from the earliest longitudinal, prospective studies of national HeFH cohorts. SAFEHEART-RE was the first European HeFH cohort to develop a prospective model of prediction of CV events in this pathology [10]. However, an external validation has not been performed to date, mainly as a result of the quality of data collection (namely, the lack of a molecular diagnosis of FH), of a relatively shorter follow-up for the comparison of CV outcomes, and of
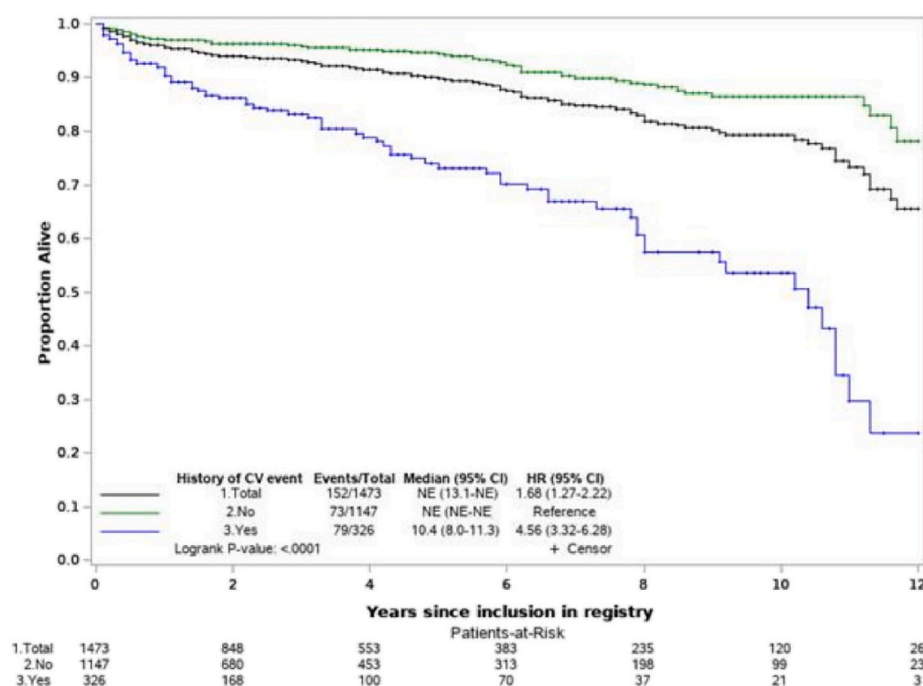
Fig. 2. Kaplan-Meier curves of freedom from cardiovascular event in all HeFH patients (black line), with history of CV event (blue line) or without history of CV event (green line).
CV = cardiovascular.

incompatibility between the databases. Indeed, HeFH is characterized by marked phenotypic variability, which often occurs independently of a molecular diagnosis, and which makes lifelong-cholesterol exposure (the so-called cholesterol burden) potentially a major surrogate marker for future CV disease. Moreover, SAFEHEART-RE was developed in a mixed population of primary and secondary prevention patients.

In the present study, we validated two different tools for the prediction of CV events in HeFH patients, SAFEHEART-RE and the LDL-C-year-score. Both SAFEHEART-RE and LDL-C-year-score performed in a highly satisfactory manner as concerns the prediction of CV events in the whole REFERCHOL cohort and specifically in patients in primary prevention. To our knowledge, this is the first external validation of the Spanish CV risk equation SAFEHEART dedicated to familial hypercholesterolemia, and the first validation of this CV risk equation in the specific population of HeFH patients free of CV events at the inclusion. .

4.1. Differences between the Spanish and the French heFH cohorts

Our REFERCHOL cohort exhibited some differences as compared to that from Spain. First, patients included in the REFERCHOL cohort had either a genetic diagnosis with a heterozygous causal gene mutation or a clinical diagnosis based on the DLCN score, whereas all patients belonging to the SAFEHEART cohort had a genetic diagnosis of HeFH. In order to be included in the study, a DLCN ≥8 was needed, resulting in a more severe CV risk profile in the REFERCHOL cohort, the consequence of the absence of a molecular diagnosis (which itself accounts for a DLCN score = 8). Indeed, 20.1% of the REFERCHOL cohort was in secondary prevention as compared to 10% in the Spanish cohort and a higher prevalence of personal and/or family history of CV events, as well as higher LDL-C levels and/or xanthoma, were observed in the REFERCHOL cohort as compared to that of SAFEHEART. Xanthoma are a well-known CV risk factor in FH as they are correlated with cholesterol burden [19,20]. Moreover, patients in the REFERCHOL cohort were older (49.6 years *vs* 45.5 years) and cumulated more CV risk factors (hypertension prevalence 27.3% *vs* 14%; LDL-C levels at the enrollment visit: 200 mg/dL *vs* 177 mg/dL and Lp(a) levels 44.5 mg/dL *vs* 38 mg/dL) than those in the SAFEHEART cohort. Together, these

differences between the two cohorts may explain the finding that French patients were more clinically severe, but they equally reinforce the fact that the SAFEHEART-RE is valid in different populations of HeFH patients. We observed that one third of HeFH patients were not treated at the enrolment visit, which corresponds to the first visit to a specialist Lipid Clinic. This observation implies that a selection bias may be operative as more severe patients are probably referred to Lipid clinics, while less severe patients are probably followed by their general practitioner. Lipid lowering therapies are typically initiated and/or intensified in specialist lipid clinics. Furthermore, some patients exhibit statin intolerance and are therefore addressed to a specialist lipidologist.

4.2. The cholesterol-year-score, a simple tool to predict CV events in HeFH

Cholesterol-year-score is an easy-to-use tool for the estimation of lifelong vascular exposure to elevated levels of lipoprotein cholesterol. The cholesterol-year-score was first used in the nineties as a tool for evaluating the duration and the severity of hypercholesterolemia in homozygous FH [12,21]. Twenty years later, the concept of cholesterol burden is at the forefront of care recommendations in FH, and emphasizes the fact that lipid lowering treatment must be initiated early [1]. An elevated cholesterol burden is associated with an increased atherosclerotic burden. In an earlier study, we showed that up to 40% of young HeFH subjects aged 20–45 years exhibited coronary artery calcification, which was itself frequently associated with a high cholesterol burden [13]. Interestingly, late initiation of lipid-lowering therapy, indirectly resulting in a higher pre-treatment cholesterol burden, was in turn associated with a higher atherosclerotic burden. This relationship has been extensively observed in other FH registries: the risk of CV death related to FH diagnosis was increased despite treatment in subjects in whom the age of initiation of lipid lowering therapy was late. A recent long-term follow-up study of young FH patients and their unaffected siblings has shown that lipid-lowering treatment that is initiated early in life results in a lower subclinical atherosclerotic burden and a significantly lower CV morbidity and mortality as compared to those FH subjects in whom treatment was

Table 2
Univariate analysis with proportional hazard Cox model for predicting CV events.

	Hazard ratio	SAFEHEART patients (N = 2404)				REFERCHOL patients (N = 1473)			
		Hazard ratio	Lower 95% CI	Upper 95% CI	p-value	Hazard ratio	Lower 95% CI	Upper 95% CI	p-value
SAFEHEART variables									
Age	<30	Ref.				Ref			
	30–59	5.88	2.22	15.59	<.001	6.479	2.37	17.714	0.0003
	≥60	12.81	4.83	34.01	<.001	12.479	4.563	34.128	<.0001
Gender (male)		2.76	1.89	4.02	<.001	1.935	1.391	2.692	<.0001
History of CV event		6.64	4.52	9.76	<.001	4.598	3.338	6.334	<.0001
Family history of premature CV event		2.66	1.76	4.05	<.001	1.150	0.805	1.644	0.4433
Diabetes mellitus		3.45	2.03	5.86	<.001	2.177	1.175	4.032	0.0134
High blood pressure		3.38	2.29	4.98	<.001	2.349	1.69	3.266	<.0001
Active smoker		1.77	1.20	2.60	0.004	1.834	1.308	2.571	0.0004
BMI	Normal weight	Ref.				Ref.			
	Overweight	4.69	2.71	8.12	<.001	2.08	1.405	3.079	0.0003
	Obesity	6.12	3.51	10.70	<.001	2.371	1.42	3.958	0.001
TC (unit)		1.002	1.001	1.005	0.05	1.003	1.001	1.004	0.0007
		SAFEHEART patients (N = 2404)				**REFERCHOL patients (N = 1473)**			
LDL-c level	<100 mg/dl	Ref.				Ref.			
	100–159 mg/dl	1.66	0.49	5.57	0.83	0.522	0.253	1.076	0.0781
	≥160 mg/dl	2.06	0.63	6.72	0.23	0.96	0.501	1.84	0.9019
HDL-c (unit)		0.97	0.96	0.98	<.001	0.975	0.964	0.987	<.0001
TG (Unit)		1.004	1.003	1.006	<.001	1.006	1.004	1.007	<.0001
APOA1 (unit)		0.99	0.985	0.997	0.003	0.993	0.983	1.002	0.1358
APOB (unit)		1.007	1.003	1.010	<.001	1.009	1.004	1.014	0.0004
Lipoprotein(a) > 50 mg/dL		2.14	1.51	3.04	<.001	1.400	0.931	2.104	0.1057
Patients on treatment:									
Maximum statin dose		2.08	1.48	2.93	<.001	2.634	1.911	3.629	<.0001
Ezetimibe		3.42	2.38	4.92	<.001	2.272	1.643	3.142	<.0001
Maximum LLT		2.88	2.002	4.15	<.001	2.58	1.802	3.694	<.0001
Years on statins (unit)		1.04	1.01	1.07	0.002	1.021	1.001	1.04	0.0349
REFERCHOL additional variables									
TC-c max	≤350 mg/dl	–				Ref.			
	350–450 mg/dl					1.104	0.747	1.632	0.6186
	>450 mg/dl					2.162	1.480	3.159	<.0001
LDL-c max	≤250 mg/dl	–				Ref.			
	250–350 mg/dl					1.149	0.790	1.671	0.4679
	>350 mg/dl					1.813	1.216	2.703	0.0035
LDL-c	<160 mg/dl					Ref			
	160–200 mg/dl	–				1.491	0.942	2.359	0.0881
	200 mg/dl	–				1.676	1.127	2.494	0.0108
Treatment class	None					Ref			
	Statins	–				1.125	0.729	1.734	0.5947
	Bitherapy	–				2.479	1.646	3.735	<.0001
Age at initiation of statins (unit)	–					1.051	1.037	1.065	<.0001
LDL-C-year-score[a]	≤6.000					Ref			
	6.000–16.000					5.462	2.371	12.580	<.0001
	>6.000					14.095	6.031	32.938	<.0001

A premature family history of 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 in patient's first-degree relatives. Maximum statin dose was defined as atorvastatin 40 or 80 mg/day, rosuvastatin 20 or 40 mg/day, and simvastatin 80 mg/day [15]. Maximum LLT = maximum lipid lowering therapy was defined as maximum statin dose plus ezetimibe 10 mg/day. LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at diagnosis/initiation of statin].

[a] Calculated by multiplying the highest LDL-C value by the patient's age at clinical diagnosis or initiation of statin therapy, to which was added the LDL-C value at the inclusion visit in the registry multiplied by the time spent until the inclusion visit, as follows: LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at diagnosis/initiation of statin].

initiated later [22]. In the present study, the LDL-C-year-score was a strong predictor of CV events in HeFH patients especially in those in primary prevention. The CV risk is multiplied by 5.7 fold for patients with a cholesterol-year-score between 6000 and 16,000 mg/dL and by 17.4 fold for those with a cholesterol year score above 16000 mg/dL. More interestingly, we observed that a LDL-C-year-score above 6000 mg/dL provided a similar CV risk as that in patients who had already presented clinically with a CV event, suggesting that earlier initiation of lipid lowering therapy might contribute to reduction in the incidence of CV events.

In the REFERCHOL cohort, the predictive power of cholesterol-year-score for CV events was as robust as SAFEHEART-RE for patients in primary prevention but quite less robust when considering all patients

(primary and secondary prevention). Nonetheless, these results demonstrated that arterial exposure to cholesterol as evaluated by the LDL-C-year-score is a satisfactory predictor of CV events, especially in patients free of CV events and that it summarized the effect of all other variables. These results reinforce the well-known major role of cholesterol burden in the development of CV disease in FH, as the duration and the severity of the hypercholesterolemia performs as well as the combination of other traditional risk factors in prediction of CV events.

4.3. Other markers or predictors of CV risk in HeFH patients

We did not evaluate the burden of subclinical atherosclerosis in this study: carotid IMT is a valid marker for the assessment of early

Table 3
Multivariate analysis with proportional hazard Cox model for predicting CV events.

		SAFEHEART patients (N = 2404)				REFERCHOL patients (N = 1473)				REFERCHOL patients (N = 1473)			
						Forcing SAFEHEART variables				No forcing (selection of significant variables only)			
		SAFEHEART equation				REFERCHOL Equation – A				REFERCHOL Equation - B			
		Hazard ratio	Lower 95% CI	Upper 95% CI	p-value	Hazard ratio	Lower 95% CI	Upper 95% CI	p-value	Hazard ratio	Lower 95% CI	Upper 95% CI	p-value
Age	< 30					**Ref**							
	30–59	**2.92**	1.14	7.52	0.026	**3.474**	1.235	9.768	0.0182				
	≥ 60	**4.27**	1.60	11.48	0.004	**5.452**	1.883	15.787	0.0018				
Gender (Male)		**2.01**	1.33	3.04	0.001	**1.335**	0.889	2.005	0.1643				
History of CV event		**4.15**	2.55	6.75	<.001	**4.192**	2.783	6.314	<.0001	**5.774**	3.378	9.868	<0.0001
High blood pressure		**1.99**	1.26	3.15	0.003	**1.085**	0.718	1.637	0.6992				
Active smoker		**1.62**	1.08	2.44	0.02	**1.680**	1.129	2.500	0.0106	**2.146**	1.283	3.587	0.0036
BMI	Normal weight	**Ref**				**Ref**							
	Overweight	**2.40**	1.36	4.23	0.002	**1.052**	0.694	1.594	0.8113				
	Obesity	**2.67**	1.47	4.85	0.001	**1.585**	0.914	2.750	0.1011				
LDL-c level[a]	< 100 mg/dl	**Ref**				**Ref**[a]				**Ref**[a]			
	100–159 mg/dl	**2.50**	0.60	10.53	0.21	**2.261**	1.359	3.759	0.0017	**2.925**	1.469	5.826	0.023
	≥ 160 mg/dl	**4.80**	1.15	20.01	0.032	**3.348**	2.094	5.353	<.0001	**3.261**	1.625	6.546	0.0009
Lipoprotein (a) > 50 mg/dl		**1.52**	1.05	2.21	0.028	**1.413**	0.929	2.149	0.1059				
LDL-C-year-score	≤ 6.000									**Ref**			
	6.000–16.000									5.685	0.761	42.491	0.0904
	> 16.000									17.403	2.309	131.172	0.0056

[a] LDL-C level: < 160 mg/dl (Reference); 160–200 mg/dl; ≥ 200 mg/dl for REFERCHOL Equation A and B. LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at diagnosis/initiation of statin].
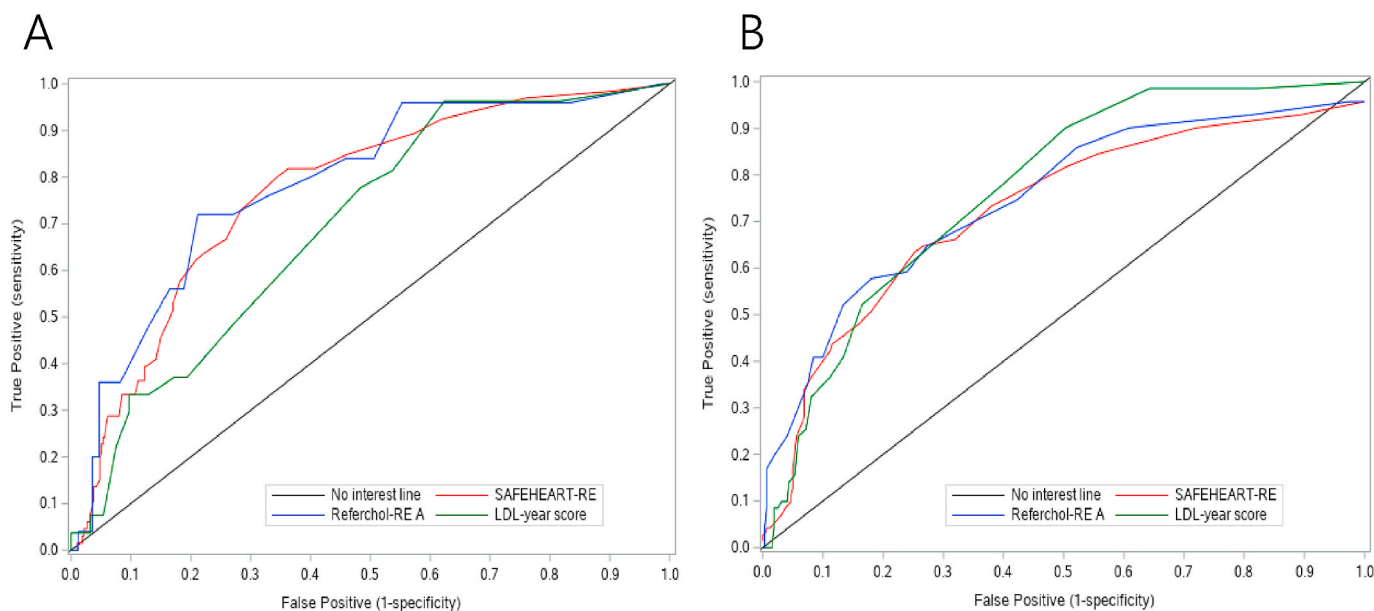
Fig. 3. ROC curves for REFERCHOL-RE A, SAFEHEART-RE, and LDL-C-year-score.
(A) HeFH patients in primary and secondary prevention. (B) HeFH patients in primary prevention. LDL-C-year-score = LDL-C max * [age at diagnosis/initiation of statin] + LDL-C at inclusion * [age at inclusion - age at diagnosis/initiation of statin].

atherosclerosis, namely in FH children [23], although carotid athero-sclerosis (evaluated by non-invasive ultrasound or the presence of plaque) has not proven its efficacy in further stratification of CV risk [24]. Assessment of coronary artery calcium represents a more pro-mising approach. Apart from showing the highest reclassification power in the general population, CAC score was found to be superior in im-proving CV event prediction when added to the Framingham risk score in HeFH subjects [25]. It has also recently been shown that SAFEHE-ART-RE correlates with the CAC Score [26] and with other biological parameters such as circulating microvesicles that may represent sur-rogate biomarkers of coronary calcification in FH patients [27]. Con-firmation of the contribution of CAC in the context of FH-specific CV

risk algorithms is awaited in large populations.

Classical cardiovascular risk factors were used to construct the SAFEHEART risk equation: age, gender, smoking, high blood pressure, BMI, history of CV events, LDL-C levels and Lp(a) levels. A recent analysis from the SAFEHEART study has confirmed the independent predictive role of Lp(a) in determining CVD risk in subjects with or without HeFH [28]. High levels of Lp(a) were not confirmed as pre-dictors in the REFERCHOL cohort, although this result might be due to variability in the methods used for its assessment and to the potentially higher genotypic variability, which differentially impacts CV risk [29,30]. Further efforts are needed for the optimal personalized stra-tification of CV risk in HeFH: novel approaches such as Genome Wide

Association Studies have already strengthened the evidence of the role of cholesterol burden on CV risk [31], and have equally integrated the possibility to predict therapeutic response to lipid lowering treatment. Such approaches may be invaluable in the future in further defining the latent CV risk in HeFH [32].

4.4. Differences between the analysis population (n = 1473) and the validation population (n = 512)

HeFH patients with a 5-year follow-up presented a more severe clinical profile than those included for the complete analysis (20.1% of CV events as compared to 10% respectively). This difference may be due to a recruitment bias as more severe patients tend to benefit more frequently from follow-up in specialist Lipid clinics, thereby explaining the longer follow-up.

4.5. Strengths and limitations

This analysis represents the first study to validate a CV risk equation in a population of HeFH patients with either genetic or clinical diagnosis of FH. Furthermore, the cholesterol-year-score, a very simple tool, predicted risk as well as a CV risk equation integrating multiple CV risk factors, especially in primary prevention. Data employed to estimate the LDL-C-year-score were robust, as the score was calculated with the value for the maximal LDL-cholesterol level recorded in the medical dossiers of almost all patients, and was not extrapolated from values under treatment. In addition, the number of CV events in the French population with a five-year-follow-up was similar to that in the SAFE-HEART cohort, ie. 103 and 122 CV events respectively. Only national and international FH registries with lifelong patient follow-up can of course provide robust data on CV risk in this specific population [30]. Finally, we could only validate the SAFEHEART-RE for the 5 years follow-up. However, a longer follow-up would provide greater numbers of both patients and CV events, and would permit a 10-year CV – RE to be constructed for both the in SAFEHEART and REFERCHOL cohorts.

In conclusion, a pragmatic assessment of intermediate and long-term CV risk in HeFH is provided by the SAFEHEART- RE and the cholesterol-year score evaluation, highlighting the importance of early CV risk assessment in guiding more efficient therapeutic strategies. Both SAFEHEART and cholesterol-year score were powerful predictors of CV events in primary prevention. However, in the entire cohort (i.e. including both primary and secondary prevention patients), SAFEHE-ART was superior to the cholesterol-year-score. This first external validation of the SAFEHEART-RE is promising as it validates the wider applicability of SAFEHEART-RE in other HeFH populations.

Financial supports

Author contributions

SB and EB are the principal investigators of the REFERCHOL registry, they contributed to the design and the setting of the registry. AG, SC, SB and EB designed the study and wrote the manuscript. AV made the statistical analyses of the data. JC, DA, FB, BC, VC and AC are co-investigators of the REFERCHOL registry and they made a critical revision of the manuscript. SB and EB are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data.

CRediT authorship contribution statement

Antonio Gallo: Conceptualization, Formal analysis, Investigation, Supervision, Validation, Visualization, Writing - original draft. **Sybil Charriere:** Conceptualization, Investigation, Supervision, Validation, Visualization, Writing - review & editing. **Alexandre Vimont:** Data curation, Formal analysis, Methodology, Software. **M. John Chapman:** Visualization, Writing - review & editing. **Denis Angoulvant:** Investigation. **Franck Boccara:** Investigation, Resources. **Bertrand Cariou:** Funding acquisition, Resources. **Valérie Carreau:** Investigation. **Alain Carrié:** Investigation. **Eric Bruckert:** Funding acquisition, Resources, Supervision, Validation, Visualization, Writing - original draft. **Sophie Béliard:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing.

Declaration of competing interest

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2020.06.011.

References

[1] B.G. Nordestgaard, M.J. Chapman, S.E. Humphries, H.N. Ginsberg, L. Masana, O.S. Descamps, et al., Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society, Eur. Heart J. 34 (45) (2013 Dec) 3478-90a.

[2] M. Benn, G.F. Watts, A. Tybjaerg-Hansen, B.G. Nordestgaard, Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication, J. Clin. Endocrinol. Metab. 97 (11) (2012 Nov) 3956–3964.

[3] P. Benedek, M. Eriksson, K. Duvefelt, A. Freyschuss, M. Frick, P. Lundman, et al., Genetic testing for familial hypercholesterolemia among survivors of acute coronary syndrome, J. Intern. Med. 284 (6) (2018 Dec) 674–684.

[4] L. Mundal, J. Igland, L. Ose, K.B. Holven, M.B. Veierod, T.P. Leren, et al., Cardiovascular disease mortality in patients with genetically verified familial hypercholesterolemia in Norway during 1992-2013, Eur. J. Prev. Cardiol. 24 (2) (2017 Jan) 137–144.

[5] S. Beliard, A. Millier, V. Carreau, A. Carrie, P. Moulin, A. Fredenrich, et al., The very high cardiovascular risk in heterozygous familial hypercholesterolemia: analysis of 734 French patients, J. Clin. Lipidol. 10 (5) (2016 Sep-Oct) 1129–11236 e3.

[6] R.D. Santos, S.S. Gidding, R.A. Hegele, M.A. Cuchel, P.J. Barter, G.F. Watts, et al., Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel, Lancet Diabetes Endocrinol. 4 (10) (2016 Oct) 850–861.

[7] A.D. Sniderman, S. Tsimikas, S. Fazio, The severe hypercholesterolemia phenotype: clinical diagnosis, management, and emerging therapies, J. Am. Coll. Cardiol. 63 (19) (2014 May 20) 1935–1947.

[8] F. Mach, C. Baigent, A.L. Catapano, K.C. Koskinas, M. Casula, L. Badimon, et al., ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk, Eur. Heart J. 41 (1) (2019) 111–188 2019 Aug 31.

[9] R.D. Santos, S.S. Gidding, R.A. Hegele, M.A. Cuchel, P.J. Barter, G.F. Watts, et al., Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel, Lancet Diabetes Endocrinol. 4 (10) (2016 Oct) 850–861.

[10] L. Perez de Isla, R. Alonso, N. Mata, C. Fernandez-Perez, O. Muniz, J.L. Diaz-Diaz,

et al., Predicting cardiovascular events in familial hypercholesterolemia: the SAFEHEART registry (Spanish familial hypercholesterolemia cohort study), Circulation 135 (22) (2017 May 30) 2133–2144.

[11] P. Mata, R. Alonso, L. Perez de Isla, Atherosclerotic cardiovascular disease risk assessment in familial hypercholesterolemia: does one size fit all? Curr. Opin. Lipidol. 29 (6) (2018 Dec) 445–452.

[12] H.H. Schmidt, S. Hill, E.V. Makariou, I.M. Feuerstein, K.A. Dugi, J.M. Hoeg, Relation of cholesterol-year score to severity of calcific atherosclerosis and tissue deposition in homozygous familial hypercholesterolemia, Am. J. Cardiol. 77 (8) (1996 Mar 15) 575–580.

[13] A. Gallo, P. Giral, A. Carrie, V. Carreau, S. Beliard, R. Bittar, et al., Early coronary calcifications are related to cholesterol burden in heterozygous familial hypercholesterolemia, J. Clin. Lipidol. 11 (3) (2017 May - Jun) 704–711 e2.

[14] S. Beliard, F. Boccara, B. Cariou, A. Carrie, X. Collet, M. Farnier, et al., High burden of recurrent cardiovascular events in heterozygous familial hypercholesterolemia: the French Familial Hypercholesterolemia Registry, Atherosclerosis 277 (2018 Oct) 334–340.

[15] M.R. Law, N.J. Wald, A.R. Rudnicka, Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis, BMJ 326 (7404) (2003 Jun 28) 1423.

[16] K.A. Hicks, K.W. Mahaffey, R. Mehran, S.E. Nissen, S.D. Wiviott, B. Dunn, et al., Cardiovascular and stroke endpoint definitions for clinical trials, J. Am. Coll. Cardiol. 71 (9) (2017) 1021–1034 2018 Mar 6.

[17] G.S. Collins, J.B. Reitsma, D.G. Altman, K.G. Moons, Transparent reporting of a multivariable prediction model for individual Prognosis or diagnosis (TRIPOD), Ann. Intern. Med. 162 (10) (2015 May 19) 735–736.

[18] RB D'Agostino Sr., R.S. Vasan, M.J. Pencina, P.A. Wolf, M. Cobain, J.M. Massaro, et al., General cardiovascular risk profile for use in primary care: the Framingham Heart Study, Circulation 117 (6) (2008 Feb 12) 743–753.

[19] F. Civeira, S. Castillo, R. Alonso, E. Merino-Ibarra, A. Cenarro, M. Artied, et al., Tendon xanthomas in familial hypercholesterolemia are associated with cardiovascular risk independently of the low-density lipoprotein receptor gene mutation, Arterioscler. Thromb. Vasc. Biol. 25 (9) (2005 Sep) 1960–1965.

[20] H. Tada, M.A. Kawashiri, A. Nohara, A. Inazu, H. Mabuchi, M. Yamagishi, Impact of clinical signs and genetic diagnosis of familial hypercholesterolaemia on the prevalence of coronary artery disease in patients with severe hypercholesterolaemia, Eur. Heart J. 38 (20) (2017 May 21) 1573–1579.

[21] J.M. Hoeg, I.M. Feuerstein, E.E. Tucker, Detection and quantitation of calcific atherosclerosis by ultrafast computed tomography in children and young adults with homozygous familial hypercholesterolemia, Arterioscler. Thromb. 14 (7) (1994 Jul) 1066–1074.

[22] I.K. Luirink, A. Wiegman, D.M. Kusters, M.H. Hof, J.W. Groothoff, E. de Groot, et al., 20-Year follow-up of statins in children with familial hypercholesterolemia, N. Engl. J. Med. 381 (16) (2019 Oct 17) 1547–1556.

[23] D.M. Kusters, A. Wiegman, J.J. Kastelein, B.A. Hutten, Carotid intima-media thickness in children with familial hypercholesterolemia, Circ. Res. 114 (2) (2014 Jan 17) 307–310.

[24] H.M. Den Ruijter, S.A. Peters, T.J. Anderson, A.R. Britton, J.M. Dekker, M.J. Eijkemans, et al., Common carotid intima-media thickness measurements in cardiovascular risk prediction: a meta-analysis, J. Am. Med. Assoc. 308 (8) (2012 Aug 22) 796–803.

[25] M.H. Miname, M.S. Bittencourt, A.C. Pereira, C.E. Jannes, J.E. Krieger, K. Nasir, et al., Vascular age derived from coronary artery calcium score on the risk stratification of individuals with heterozygous familial hypercholesterolaemia, Eur. Heart J. Cardiovasc. Imag. 12 (9) (2019 Nov 8) 1797–1804.

[26] L. Perez de Isla, R. Alonso, O. Muniz-Grijalvo, J.L. Diaz-Diaz, D. Zambon, J.P. Miramontes, et al., Coronary computed tomographic angiography findings and their therapeutic implications in asymptomatic patients with familial hypercholesterolemia. Lessons from the SAFEHEART study, J. Clin. Lipidol. 12 (4) (2018 Jul - Aug) 948–957.

[27] G. Chiva-Blanch, T. Padro, R. Alonso, J. Crespo, L. Perez de Isla, P. Mata, et al., Liquid biopsy of extracellular microvesicles maps coronary calcification and atherosclerotic plaque in asymptomatic patients with familial hypercholesterolemia, Arterioscler. Thromb. Vasc. Biol. 39 (5) (2019 May) 945–955.

[28] K.L. Ellis, L. Perez de Isla, R. Alonso, F. Fuentes, G.F. Watts, P. Mata, Value of measuring lipoprotein(a) during cascade testing for familial hypercholesterolemia, J. Am. Coll. Cardiol. 73 (9) (2019 Mar 12) 1029–1039.

[29] N. Perrot, S. Theriault, C. Dina, H.Y. Chen, S.M. Boekholdt, S. Rigade, et al., Genetic variation in LPA, calcific aortic valve stenosis in patients undergoing cardiac surgery, and familial risk of aortic valve microcalcification, JAMA Cardiol. 4 (7) (2019 Jul 1) 620–627.

[30] S. Zewinger, M.E. Kleber, V. Tragante, R.O. McCubrey, A.F. Schmidt, K. Direk, et al., Relations between lipoprotein(a) concentrations, LPA genetic variants, and the risk of mortality in patients with established coronary heart disease: a molecular and genetic association study, Lancet Diabetes Endocrinol. 5 (7) (2017 Jul) 534–543.

[31] B.A. Ference, H.N. Ginsberg, I. Graham, K.K. Ray, C.J. Packard, E. Bruckert, et al., Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur. Heart J. 38 (32) (2017 Aug 21) 2459–2472.

[32] N.A. Marston, F.K. Kamanu, F. Nordio, Y. Gurmu, C. Roselli, P.S. Sever, et al., Predicting benefit from evolocumab therapy in patients with atherosclerotic disease using a genetic risk score: results from the FOURIER trial, Circulation 41 (8) (2019 Nov 11) 616–623.