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Incidence of cardiovascular disease in familial combined hyperlipidemia: A 15-year follow-up study

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HIGHLIGHTS

- Three quarters of familial combined hyperlipidemia (FCHL) patients receive lipid lowering treatment.
- The 15-year incidence of cardiovascular disease is still very high in FCHL.
- The SCORE risk factors do not fully account for the increased risk in FCHL.

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ABSTRACT

Background and aims: Familial combined hyperlipidemia (FCHL) is a complex dyslipidemia associated with premature cardiovascular disease (CVD). The present study was conducted to 1) determine the incidence of CVD in FCHL in this era of protocolled, primary prevention; and 2) examine whether cardiovascular risk estimation based on the Systemic Coronary Risk Estimation (SCORE) chart, as proposed in the 2016 ESC/EAS guidelines for the management of dyslipidemia, is justified in FCHL.
Methods: FCHL patients, their normolipidemic (NL) relatives and spouses originally included in our baseline cohort in 1998–2005 (n = 596) were invited for a follow-up visit to determine the incidence of CVD, defined as (non-)fatal coronary artery disease, ischemic stroke and peripheral artery disease requiring invasive treatment.
Results: Follow-up data (median: 15 years) was acquired for 85% of the original cohort. The cumulative incidence of CVD was significantly higher in FCHL patients than in spouses (23.6% *versus* 4.7%; hazard ratio (HR): 5.4, 95%CI: 2.0–14.6; HR after adjustment for risk factors included in SCORE: 4.7, 95%CI: 1.6–13.8), but not in NL relatives compared to spouses (5.8% *versus* 4.7%). The SCORE chart tended to overestimate CVD risk in the spouses (observed [O]/expected [E] ratio:0.2, $p = 0.01$), but not in FCHL patients (O/E:1.3, $p = 0.50$).
Conclusions: Risk of primary CVD is still substantially increased in FCHL patients, despite preventive measures. The overestimation of CVD risk by the SCORE chart – a nowadays frequently observed phenomenon thanks to improved primary prevention – was not seen in FCHL. These results suggest that more aggressive treatment is justified to avoid excessive CVD in FCHL.

1. Introduction

In 1973, Goldstein and colleagues described three types of familial dyslipidemias among young survivors of a myocardial infarction, i.e. familial hypercholesterolemia (FH), familial hypertriglyceridemia (FHTG) and familial combined hyperlipidemia (FCHL) [1]. In the following years, mutations in the LDL receptor and later on mutations in its ligand (apolipoprotein B) and its principal clearance protein

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(proprotein convertase subtilisin kexin type 9; PCSK9) were identified as genetic defects that cause FH. These discoveries have initiated cost-effective cascade screening strategies that have contributed to the prevention of cardiovascular complications in FH pedigrees [2].

The genetic dissection of FCHL has turned out to be more complicated. Although initially assumed to be an autosomal dominant disease, it is a nowadays accepted that FCHL is a complex disease, i.e. the lipid phenotype is the consequence of an interaction between multiple susceptibility genes and unhealthy lifestyle factors [3]. Compared to FH, plasma LDL cholesterol levels are only moderately elevated in FCHL. The higher cardiovascular risk can probably be accounted for by the sum of plasma lipid abnormalities – increased apolipoprotein B and triglycerides levels, reduced HDL cholesterol and presence of small dense LDL particles – and co-segregation with metabolic syndrome related factors such as visceral obesity, insulin resistance, nonalcoholic fatty liver disease and hypertension [3]. To date, only few longitudinal studies have addressed the incidence of cardiovascular disease (CVD) in FCHL pedigrees. Austin et al. conducted a 20-year longitudinal study in the FCHL pedigrees that were originally identified by Goldstein and colleagues and demonstrated that first-degree relatives of FCHL index patients have an increased cardiovascular mortality risk, independent of traditional risk factors [4]. Because that study was initiated in 1970, far before the introduction of statin therapy [4], it is not known how current prevention strategies have affected these incidence rates.

Primary prevention strategies for FCHL have changed in 2016 when the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) issued new guidelines for the management of dyslipidemia [5]. In contrast to the 2011 guideline [6], FCHL is no longer regarded as a 'high cardiovascular risk' group that deserves aggressive lipid lowering treatment, independent of the SCORE (Systemic Coronary Risk Estimation) risk charts [5]. This implies that risk classification of FCHL patients should now be based on the SCORE chart [5]. It is, however, not known whether this change in guideline is justified.

The aims of the present study are therefore two-fold: first, to assess the incidence of CVD in FCHL pedigrees in an era of improved primary prevention strategies; and second, to investigate the impact of the change in the 2016 ESC/EAS guideline for the management of dyslipidemia on CVD risk estimation in FCHL pedigrees.

2. Materials and methods

2.1. Study participants

Participants who were originally included in the Maastricht FCHL cohort between 1998 and 2005 were re-invited between 2014 and 2016 for participation in the present study. The initial FCHL diagnosis was based on the presence of at least two types of hyperlipidemias, i.e. hypercholesterolemia (total cholesterol ≥ 6.5 mmol/L), hypertriglyceridemia (≥2.3 mmol/L) and/or combined hyperlipidemia within one family, as well as premature coronary artery disease (≤60 years). Secondary causes of hyperlipidemia in the (adult) proband, i.e. body mass index (BMI) > 30 kg/m^2, type 2 diabetes mellitus (T2DM), hypothyroidism, kidney or liver disease, familial dysbetalipoproteinemia or familial hypercholesterolemia, were excluded [7,8]. Once the FCHL diagnosis was established, all additional adult family members were eligible for participation.

Diagnostic criteria for FCHL changed in 2002 by adopting apolipoprotein B levels as a diagnostic criterion, which have been shown to be less variable in time and, hence, detect affected patients more reliably [9,10]. Therefore, family members who fulfilled the plasma lipid criteria apolipoprotein B > 1.2 g/L and triglycerides > 1.5 mmol/L) at baseline were reclassified as FCHL patients while those who did not were defined as normolipidemic (NL) relatives. Their spouses served as the reference group.

All individuals with a follow-up assessment – except for those with a history of CVD at baseline – were included for the primary objective of this study, i.e. to assess the incidence of primary cardiovascular events in FCHL patients and their relatives. For the secondary objective, i.e. to assess the agreement between the observed risk and (SCORE-based) expected risk, we additionally excluded patients with T2DM and individuals with markedly elevated single risk factors (total cholesterol > 8.0 mmol/L or blood pressure ≥ 180/110 mmHg) at baseline, since they are automatically classified as 'high to very high risk' according to the 2016 ESC/EAS guidelines [5]. Furthermore, because the SCORE chart applies to an age range between 40 and 70 years [11], we only included individuals aged ≥ 35 and ≤75 years for these analyses. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Medical Ethics Committee of Maastricht University Medical Centre. All individuals gave written informed consent.

2.2. Measurements

Baseline measurements have been described in detail elsewhere [10]. In short, participants completed a detailed questionnaire on smoking habits, alcohol consumption, medical history and use of medication. They underwent several anthropometric measurements (height, weight, waist, and hip circumference). Blood pressure was measured at both arms in sitting position after 10 min of rest. Blood was drawn after an overnight fast, 3 days abstinence from alcohol and 2 weeks withdrawal from any lipid lowering medication. Total cholesterol, HDL cholesterol, triglycerides, apolipoprotein B and glucose measurements were done as described previously [10]. Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald formula [12].

At follow up, participants were asked to visit the research ward to undergo the same set of measurements. Lipid lowering medication was continued, in contrast to the baseline visit. Plasma lipids were now measured at the university hospital laboratory on a Cobas 8000 involving an enzymatic reaction combined with a spectrophotometric measurement. Individuals who were unwilling or unable to visit our ward were asked to complete a questionnaire regarding medical history and medication use.

2.3. Assessment of incident cardiovascular events

The main outcome measure was fatal and non-fatal cardiovascular disease, which was defined as acute coronary syndrome, percutaneous coronary intervention, coronary artery bypass grafting, ischemic stroke and/or peripheral artery disease requiring percutaneous intervention or bypass grafting. These self-reported CVD events were confirmed by review of the medical records. Cause of death was based on information provided by family members.

2.4. Statistical analysis

Data are presented as mean ± SD, or as median with interquartile range in case of non-normal distribution. Age- and sex-adjusted differences in baseline characteristics between FCHL patients and NL relatives versus spouses were analyzed with linear and logistic regression for continuous and dichotomous outcomes, respectively. Skewed variables were log transformed prior to analyses. Cox's proportional hazard regression models were used to calculate the hazard ratios (HR) for incident CVD in FCHL patients and their NL relatives, entered as dummy variables. Since the hyperlipidemic state can change over time and/or develop later in life in FCHL [13,14], all analyses were also conducted for FCHL family members, i.e. FCHL patients and NL relatives combined, entered as a dummy variable.

Follow-up time was defined as time from baseline visit until the first CVD event or time from baseline until follow-up for censored cases. Hazard ratios were subsequently adjusted for risk factors that are

included in the current SCORE chart, i.e. age (years), sex (male/female), current smoking (yes/no), systolic blood pressure (mmHg), and total cholesterol/HDL cholesterol ratio [5], all assessed at baseline.

Calibration of the SCORE chart, i.e. the agreement between the expected (E) 10-years risk of fatal and non-fatal CVD (SCORE) and the observed (O) 10-years risk, was evaluated in FCHL patients, NL relatives and spouses. The individual, expected risk was calculated with the Dutch adjusted version of the SCORE risk chart (SCORE-NL), which is based on two Dutch cohorts that were collected between 1987 and 1997. The SCORE-NL estimates both fatal and non-fatal CVD risk, in contrast to other country-specific SCORE versions [11]. The overall number of expected events was subsequently calculated as the sum of all individual SCORE risks at baseline, e.g. 50% risk in subject A translates to 0.5 events, 15% risk in subject B translates to 0.15 events, etcetera. A χ^2 was obtained by summing $(O\text{-}E)^2/E$ in those with and without a CVD event [15]. Agreement between the expected and observed risk was also tested with the Hosmer-Lemeshow statistic [15,16].

In a sensitivity analysis, we repeated the O/E analyses with a statin-adjusted SCORE-risk, as described in detail elsewhere [17]. The reason for this was that statin therapy was first introduced during the collection of the cohorts on which the SCORE-chart was based (1987–1997) and subsequently became widespread during the collection and follow-up of our FCHL pedigrees (1998–2005) [18]. We reasoned that this difference in statin prescription trends could result in a reduced observed (O) risk relative to the expected (E) risk.

In short, the expected statin-mediated cardiovascular risk reduction was calculated as:

$$(1–0.78^{n}) \times \text{SCORE risk}$$

where n is the expected, statin-mediated LDL-cholesterol reduction, which depends on the statin type and dose at baseline, and LDL-cholesterol concentration at baseline. Since each 1 mmol/L reduction in LDL-cholesterol has been associated with a decrease in CVD risk to 0.78 of that in the control group (with a hazard ratio of 1), the 1 minus $0.78^{\text{LDL}-\text{C reduction}}$ represents the expected percent point reduction in CVD risk [17].

Finally, receiver operating characteristics (ROC) curves were constructed to study the discriminatory ability of the SCORE chart to predict CVD among FCHL family members.

All analyses were performed using SPSS 21.0 (SPSS statistics, IBM Corp, Armonk, NY, USA).

3. Results

3.1. Study population

Of the 596 individuals in our baseline cohort, follow-up data was acquired for 506 (85%) individuals derived from 48 families (Supplementary Fig. 1). Baseline characteristics were not different between those who were lost to follow-up (n = 90) and those who participated in the follow-up study (n = 506; Supplementary Table 1).

After exclusion of patients with CVD at baseline, the final cohort that was included for analysis of the primary objective consisted of 463 individuals (Supplementary Fig. 1). Cardiovascular risk factors were only moderately elevated in FCHL patients (n = 86) when compared to their spouses (n = 121) (Table 1). Use of lipid lowering medication and off-treatment total cholesterol levels were at baseline significantly higher in NL relatives (n = 256) than in spouses.

The median follow-up duration was 15 years (interquartile range: 14–16 years). During follow-up, BMI and waist circumference increased in both spouses and NL relatives, but not in FCHL patients (Supplementary Table 2). Use of lipid lowering and anti-hypertensive medication increased in all three groups. Three quarters of the FCHL patients were treated with a statin at the end of follow-up. On-treatment LDL-cholesterol levels did not differ between spouses, NL relatives and FCHL patients (Supplementary Table 2).

Table 1
Baseline characteristics of familial combined hyperlipidemia (FCHL) patients, normolipidemic (NL) relatives and spouses.

	Spouses	NL relatives	FCHL patients
Male/female	56/65	107/149	41/45
Age, years	48 ± 14	40 ± 13[a]	49 ± 13
Smoking, n (%)	30 (26)	74 (29)	32 (37)
Alcohol, units/week	3.3 (0.1–10)	4.0 (0.5–10)	3.0 (0–10)
BMI, kg/m^2	25.3 ± 3.6	25.1 ± 4.0	28.0 ± 4.2[b]
Waist circumference, cm	89.9 ± 11.3	88.8 ± 11.8	98.3 ± 12.2[b]
Total cholesterol, mmol/l	5.3 ± 1.0	5.2 ± 1.1[b]	6.9 ± 1.1[b]
HDL cholesterol, mmol/l	1.1 ± 0.2	1.0 ± 0.2	0.9 ± 0.2[b]
LDL Cholesterol, mmol/l	3.7 ± 1.0	3.6 ± 1.1	4.8 ± 1.1[b]
Triglycerides, mmol/l	1.2 (0.8–1.5)	1.1 (0.9–1.4)	2.4 (1.8–2.8)[b]
Apolipoprotein B, g/l	1.0 ± 0.2	1.0 ± 0.2	1.5 ± 0.2[b]
Lipid lowering medication, n (%)	3 (2)	20 (8)[b]	34 (40)[b]
Systolic BP, mmHg	132 ± 19	130 ± 19	142 ± 25[b]
Diastolic BP, mmHg	83 ± 11	83 ± 11	90 ± 12[b]
Anti-hypertensive medication, n (%)	10 (9)	17 (7)	19 (23)[b]

Data are expressed as mean ± SD or as medians with interquartile range between parentheses.
[a] $p < 0.05$ *versus* spouses and FCHL patients, independent-samples t-test.
[b] $p < 0.05$ *versus* spouses, age and sex adjusted.

3.2. Incidence of cardiovascular disease

The primary endpoint, composed of fatal and non-fatal CVD, was reached in 17 of 86 FCHL patients (cumulative incidence: 23.6%), which was substantially higher than observed in spouses (5 of 121, cumulative incidence: 4.7%; HR: 5.4, 95%CI: 2.0–14.6; Fig. 1). This difference was mainly explained by a higher incidence of non-fatal coronary artery events in FCHL patients (Supplementary Table 3). Despite the fact that NL relatives were on average eight years younger than spouses, the incidence of CVD did not differ between both groups (5.8 versus 4.7%; HR: 1.3, 95%CI: 0.5–3.6). Subsequent adjustment for age and sex revealed a higher risk in NL relatives than in spouses, although it was not statistically significant (HR: 2.5; 95%CI: 0.9–7.3, $p = 0.09$).

Next, all individual SCORE components, i.e. age, sex, smoking

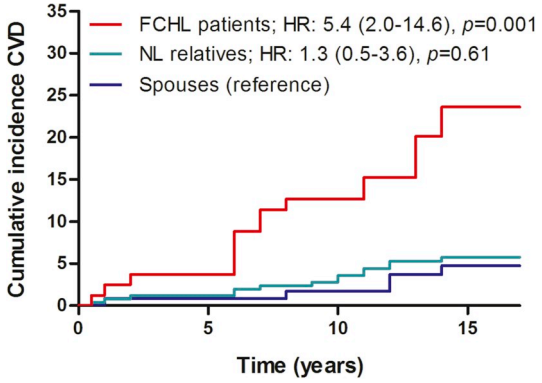

Fig. 1. Cumulative incidence of cardiovascular disease (CVD) in patients with familial combined hyperlipidemia (FCHL), normolipidemic (NL) relatives and their spouses.
The endpoint of CVD was composed of fatal and non-fatal atherosclerotic events, i.e. acute coronary syndrome, percutaneous coronary intervention, coronary artery bypass grafting, ischemic stroke and peripheral artery disease requiring percutaneous intervention or bypass grafting.
Data are presented as hazard ratios (HR) with 95% confidence intervals, calculated with Cox's proportional hazard regression models.

Table 2
Cox proportion hazard regression models of first CVD event (fatal and non-fatal).

Model	Adjusted for	NL relatives *vs.* spouses			FCHL patients *vs.* spouses			FCHL family members *vs.* spouses[a]		
		HR	95% CI	*p*-value	HR	95% CI	*p*-value	HR	95% CI	*p*-value
1	Crude	1.3	0.5–3.6	0.61	5.4	2.0–14.6	0.001	2.2	0.9–5.7	0.10
2	Age and sex	2.5	0.9–7.3	0.09	6.4	2.3–17.5	< 0.001	4.0	1.5–10.6	0.006
3	SCORE risk factors[b]	2.1	0.7–6.3	0.17	4.7	1.6–13.8	0.004	3.0	1.1–8.2	0.03

[a] FCHL family members = FCHL patients and NL relatives combined.
[b] SCORE risk factors are: age (years), sex (male/female), current smoking (yes/no), systolic blood pressure (mmHg) and total cholesterol/HDL cholesterol ratio, all determined at baseline.

status, systolic blood pressure and total cholesterol/HDL cholesterol ratio, were included into the Cox's proportional hazard regression model. In this adjusted model, CVD risk remained higher in FCHL patients than in spouses (HR: 4.7; 95%CI: 1.6–13.8; Table 2). In addition to FCHL-patient status (yes/no), age, male sex and total cholesterol/HDL cholesterol ratio were also independent predictors of incident CVD in this cohort. Similar results were obtained when the analyses were repeated with FCHL family members, i.e. FCHL patients and NL relatives combined (Table 2). Additional adjustment for alcohol consumption at baseline, an important secondary cause of hypertriglyceridemia, did not materially change the outcomes for FCHL patients and NL relatives (data not shown).

3.3. Evaluation of calibration and discrimination of the SCORE-chart in FCHL

Since the use of SCORE chart is not indicated for individuals with markedly elevated single risk factors (i.e. total cholesterol > 8.0 mmol/L or blood pressure ≥180/110 mmHg) or T2DM [5], the second objective was analyzed after exclusion of those individuals who fulfilled one of these criteria at baseline (n = 39, see Supplementary Fig. 1). Of interest, repeat analyses of the Cox proportional hazard regression models, as shown in Table 2, yielded very similar outcomes when these high-risk individuals were excluded (Supplementary Table 4). After additional exclusion of all individuals aged < 35 years and > 75 years (for which the SCORE chart has not been developed, see Materials and methods section), the final cohort for these analyses consisted of 287 individuals, i.e. 53 FCHL patients, 143 NL relatives and 91 spouses (Supplementary Fig. 1).

The 10-year expected (E) number of fatal and non-fatal CVD events, based on the SCORE-chart, was 9.1 in spouses, 7.2 NL relatives and 5.5 in FCHL patients (Table 3). Comparison with the 10-year observed (O) risk demonstrated that the SCORE-chart overestimated risk in spouses (O/E = 0.2, p = 0.01). In contrast, the observed risk was in line with the calculated expected risk in both NL relatives and FCHL patients, or when both groups were combined (O/E = 0.7, $p = 0.40$; O/E = 1.3, $p = 0.50$, and O/E = 0.9, $p = 0.84$, respectively; Table 3). Similar results were obtained with the Hosmer-Lemeshow statistics, in which statistical significance indicates poor agreement ($p = 0.049$ for spouses, $p = 0.54$ for NL relatives and p = 0.53 for FCHL patients).

Table 3
Agreement between observed risk and SCORE-based, expected risk of fatal and non-fatal CVD at 10-year follow-up.

State	n	Number of CV events		O/E	χ^2	*p*
		Observed (O)	Predicted (E)			
Crude						
Spouses	91	2	9.1	0.2	6.5	0.01
NL relatives	143	5	7.2	0.7	0.7	0.40
FCHL patients	53	7	5.5	1.3	0.5	0.50
FCHL family members[a]	196	12	12.7	0.9	0.04	0.84
Adjusted for statin use at baseline[b]						
Spouses	91	2	9.0	0.2	6.0	0.01
NL relatives	143	5	7.0	0.7	0.6	0.44
FCHL patients	53	7	4.6	1.5	1.4	0.24
FCHL family members[a]	196	12	11.6	1.0	0.02	0.90

[a] NL relatives and FCHL patients combined.
[b] See Materials and methods, Supplementary Table 5 and Reference [17] for detailed information.

To study the impact of statin use at baseline on the expected risk, we subsequently calculated the statin-adjusted, expected number of events. Statin use at baseline reduced the expected, absolute number of events by 0.05 in spouses, 0.2 in NL relatives and 0.9 in FCHL patients, respectively. The higher risk reduction in FCHL patients can be attributed to the higher number of statin users and LDL cholesterol levels at baseline (Supplementary Table 5). As a consequence, the O/E ratios did not materially change in spouses and NL relatives, whereas a small increase in this ratio was observed in FCHL patients (O/E = 1.5, $p = 0.24$, Table 3).

Lastly, ROC curves were constructed to evaluate the discriminatory ability of the SCORE chart to predict CVD in FCHL. For reasons of statistical power, ROC curves were only created for FCHL family members, i.e. FCHL patients and NL relatives combined. The diagnostic accuracy, as indicated by the area under the curve, was 0.81 (95%CI: 0.71–0.91; Fig. 2). The number of CVD cases in spouses was too low to construct a reliable ROC curve.

4. Discussion

This 15-year longitudinal study showed that almost 1 in 4 FCHL patients develop new-onset CVD during this time period. Cox's

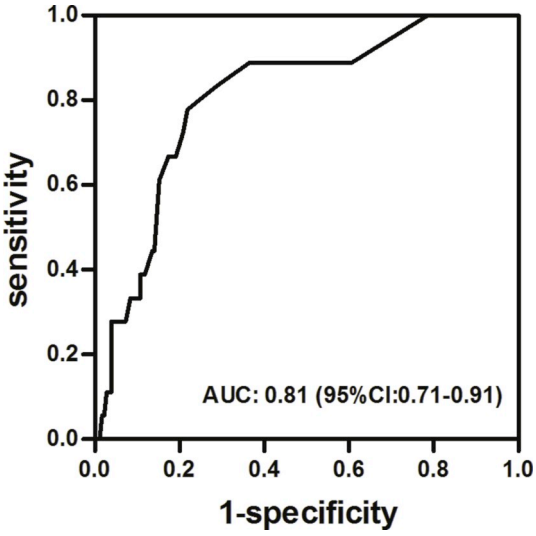

Fig. 2. Receiver operating characteristic (ROC) curve for the prediction of non- and fatal cardiovascular events with SCORE in FCHL family members (i.e. FCHL patients and normolipidemic relatives combined).

proportional hazard regression analyses demonstrated that the increased CVD risk in FCHL patients compared to spouses was not fully explained by the risk factors that underlie the SCORE chart. This finding automatically implicates that the calibration of the Dutch SCORE chart, i.e. the ability of a model to align the number of predicted and observed events, is suboptimal for either FCHL (i.e. underestimation) and/or spouses (i.e. overestimation). Formal analyses showed that the latter is the case.

Our results are in line with a previous 20-year longitudinal study by Austin et al. who reported that first-degree relatives of FCHL index patients have an increased risk to die from CVD independent of the traditional cardiovascular risk factors [4]. The residual risk in that and our study may be explained by the fact that premature CVD is a diagnostic criterion of FCHL [1,9]. This could have led to enrichment of CVD susceptibility genes that contribute to CVD through other pathways than plasma lipids in FCHL pedigrees. The Malmö Diet and Cancer Study showed that a genetic risk score composed of 50 established coronary artery disease (CAD) gene variants predicted new-onset CAD, independent of traditional risk factors [19]. These findings emphasize the importance of assessing the family history for CVD in CVD risk estimation. The aforementioned longitudinal study by Austin et al. was conducted *before* the widespread introduction of preventive measures, including statin therapy. The present study therefore shows that, despite these preventive strategies, FCHL patients are still at high risk to develop CVD.

In the current study, the discriminatory power of the SCORE chart, i.e. how well the model discriminates between individuals who develop a cardiovascular event and those who do not, was good. The area under the ROC curve (0.81; 95%CI: 0.71–0.91) was very much in line with other low- and high-risk populations [20]. Furthermore, calibration was good as well (O/E = 1.3). At first sight, these model properties suggest that the change in the 2106 ESC/EAS guideline for the management of dyslipidemia to no longer automatically regard FCHL as a 'high risk' group and to use the SCORE chart for CVD risk estimation instead [5], is justified. There is, however, one important caveat that deserves further discussion.

As already outlined in the methods section, the time window in which the SCORE chart was developed is substantially different from the present longitudinal study. Previous studies have shown that CVD risk can be systematically overestimated when risk scores derived from older cohorts are applied to modern cohorts [21,22]. Potential reasons for this overestimation are a change in significance of risk factors over time and the presence of more effective, preventive measures [22]. As such changes have most likely also occurred in our cohort, it probably explains why the SCORE chart tended to overestimate the cardiovascular risk in the spouses. In this light it is remarkable that the agreement between the observed and expected events was 'only' good in FCHL. This suggests that before the introduction of effective, preventive measures, SCORE *underestimated* CVD risk in FCHL. Indeed, correction for one preventive measure, i.e. statin therapy at baseline, increased the O/E ratio to some extent in FCHL patients, indicating a trend towards underestimation of CVD risk. It should be noted that this correction is still a conservative adjustment as it did not take into account any statin therapy initiated during follow-up (75% of the FCHL patients received lipid lowering treatment at follow-up), nor did we correct for other preventive measures. Based on this argumentation, our data actually suggest that – in contrast to what has been proposed in the 2016 ESC/EAS guideline for the management of dyslipidemia – the SCORE chart may be unsuitable for FCHL, similar to FH. Replication of the current findings in another FCHL cohort is therefore highly warranted.

The consequences of regarding FCHL as a 'high-risk' disease regardless of SCORE – similar to FH – would be that LDL cholesterol treatment targets should be below 2.6 mmol/L [5]. When these targets are applied to the current FCHL cohort, in which LDL cholesterol levels at follow-up were on average 3.1 mmol/L (Supplementary Table 2), it can be calculated (with the formula presented in the methods section [17]) that this would lead to 1.4% point reduction in the 10-years CVD risk, corresponding to the prevention of one CVD case in the cohort of FCHL patients, as presented in Table 3. It is expected that even further LDL cholesterol reduction below 1.8 mmol/L would prevent two to three CVD cases. Such a reduction can nowadays be achieved in the majority of hyperlipidemic patients with combined statin and PCSK9-antagonist therapy [23].

This study has several strengths and weaknesses. Strengths of this study are the long-term follow up of a large number of FCHL families and the high retention rate (85%), which makes the likelihood of selection bias relatively small. Despite the fact that longitudinal studies in FCHL are scarce, the number of participants and events in this study is still relatively low, particularly the numbers of events in the group of spouses. It can therefore not be ruled out that chance has contributed to the overestimation of CVD risk by the SCORE risk chart in spouses. The outcomes of this study should therefore first be replicated before any revision of guideline can be implemented. Second, the diagnosis of FCHL is complex, since there is no single gene or plasma biomarker that is specific for this entity. Furthermore, previous studies have shown that the lipid phenotype within an individual can change over time [10,13], which makes the distinction between the normolipidemic and affected state at a distinct point in time arbitrary. We therefore performed additional analyses in all FCHL family members, i.e. normolipidemic and hyperlipidemic individuals combined, which also revealed an increased incidence of CVD, independent of classical risk factors (Table 2). Another issue that relates to the complicated diagnosis of FCHL is that its lipid phenotype can overlap with the FH lipid phenotype. Civeira and colleagues have previously shown that genetic defects in the LDL receptor are more common in FCHL pedigrees [24]. We therefore cannot exclude that similar defects are present in our FCHL cohort. This is, however, very similar to daily clinical practice: patients who do not fulfil the clinical criteria for FH genetic testing [5] – but carry a mutation in the LDL receptor, apolipoprotein B or PCSK9 gene – can fulfil the FCHL diagnostic criteria and, as such, be clinically diagnosed with FCHL. In the present study, the average total cholesterol and LDL cholesterol levels in FCHL patients were below the criteria for FH genetic testing. Notably, repeat analysis of the primary objective after exclusion of individuals with markedly elevated total cholesterol levels (> 8.0 mmol/L) revealed similar outcomes (Supplementary Table 3). We therefore believe that the potential inclusion of patients with FH related mutations has not largely influenced the outcomes of our study. Third, the observational nature of this study may have introduced several types of bias. We have tried to reduce the risk of information bias by verifying all self-reported CVD events with the general practitioner or medical specialist. Causes of death were, however, based on information provided by family members instead of death certificates, which may have led to misclassification and should therefore be regarded as a limitation. Fourth, in the present study we included the spouses as a reference group, since FCHL family members and their spouses are anticipated to have fairly comparable lifestyle habits. Furthermore, there are also practical advantages to invite FCHL family members simultaneously with their spouses for participation in our studies. Comparison of the spouses with a large population-based cohort derived from the same geographical region with a comparable age and sex-distribution reveals consistent similarities with regard to BMI, plasma lipids and frequency of use of antihypertensive and lipid lowering medication [25]. Finally, the awareness of being identified as a high-risk individual in a high-risk pedigree may have resulted in a change in behavioral habits during the follow-up period, which could lead to confounding that is difficult to correct for. The stable BMI in the follow-up period – as observed in the FCHL patients but not in their NL relatives or spouses (Supplementary Table 2) – could be an example thereof. It is, however, anticipated that such a change in lifestyle habits will further contribute to an underestimation of the O/E ratio in FCHL patients.

In conclusion, this study shows that despite all current preventive

measures FCHL patients are still at an increased risk to develop non- and fatal cardiovascular events, independent of the traditional risk factors that are incorporated in the SCORE risk chart. These results suggest that more aggressive treatment strategies are justified to avoid excessive CVD in FCHL.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.11.013.

References

[1] J.L. Goldstein, H.G. Schrott, W.R. Hazzard, et al., Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia, J. Clin. Invest. 52 (1973) 1544–1568.
[2] G.K. Hovingh, M.H. Davidson, J.J. Kastelein, et al., Diagnosis and treatment of familial hypercholesterolaemia, Eur. Heart J. 34 (2013) 962–971.
[3] M.C. Brouwers, M.M. van Greevenbroek, C.D. Stehouwer, et al., The genetics of familial combined hyperlipidaemia, Nat. Rev. Endocrinol. 8 (2012) 352–362.
[4] M.A. Austin, B. McKnight, K.L. Edwards, et al., Cardiovascular disease mortality in familial forms of hypertriglyceridemia: a 20-year prospective study, Circulation 101 (2000) 2777–2782.
[5] A.L. Catapano, I. Graham, G. De Backer, et al., ESC/EAS guidelines for the management of dyslipidaemias: the task force for the management of dyslipidaemias of the european society of Cardiology (ESC) and european atherosclerosis society (EAS)developed with the special contribution of the european assocciation for cardiovascular prevention & rehabilitation (EACPR), Eur. Heart J. (2016) 2016.
[6] European Association for Cardiovascular, P, Rehabilitation, Z. Reiner, et al., ESC/EAS guidelines for the management of dyslipidaemias: the task force for the management of dyslipidaemias of the european society of Cardiology (ESC) and the european atherosclerosis society (EAS), Eur. Heart J. 32 (2011) 1769–1818.
[7] M.C. Brouwers, M.M. van Greevenbroek, V.M. Vermeulen, et al., Five-year follow-up of waist circumference, insulin and ALT levels in familial combined hyperlipidaemia, Clin. Sci. (Lond.) 113 (2007) 375–381.
[8] C. Voors-Pette, T.W. de Bruin, Excess coronary heart disease in Familial Combined Hyperlipidemia, in relation to genetic factors and central obesity, Atherosclerosis 157 (2001) 481–489.
[9] A.D. Sniderman, M. Castro Cabezas, J. Ribalta, et al., A proposal to redefine familial combined hyperlipidaemia - third workshop on FCHL held in Barcelona from 3 to 5 May 2001, during the scientific sessions of the European Society for Clinical Investigation, Eur. J. Clin. Invest. 32 (2002) 71–73.
[10] M.J. Veerkamp, J. de Graaf, S.J. Bredie, et al., Diagnosis of familial combined hyperlipidemia based on lipid phenotype expression in 32 families: results of a 5-year follow-up study, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 274–282.
[11] I. van Dis, D. Kromhout, J.M. Geleijnse, et al., Evaluation of cardiovascular risk predicted by different SCORE equations: The Netherlands as an example, Eur. J. Cardiovasc. Prev. Rehabil. 17 (2010) 244–249.
[12] A.J. Tremblay, H. Morrissette, J.M. Gagne, et al., Validation of the Friedewald formula for the determination of low-density lipoprotein cholesterol compared with beta-quantification in a large population, Clin. Biochem. 37 (2004) 785–790.
[13] M.C. Brouwers, J. de Graaf, M.M. van Greevenbroek, et al., Parabolic relationship between plasma triacylglycerols and LDL-cholesterol in familial combined hyperlipidaemia: the multiple-type hyperlipidaemia explained? Clin. Sci. (Lond.) 114 (2008) 393–401.
[14] E. ter Avest, A.D. Sniderman, S.J. Bredie, et al., Effect of aging and obesity on the expression of dyslipidaemia in children from families with familial combined hyperlipidaemia, Clin. Sci. (Lond.) 112 (2007) 131–139.
[15] D. De Bacquer, G. De Backer, Predictive ability of the SCORE Belgium risk chart for cardiovascular mortality, Int. J. Cardiol. 143 (2010) 385–390.
[16] N.R. Cook, Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve, Clin. Chem. 54 (2008) 17–23.
[17] H. Soran, J.D. Schofield, P.N. Durrington, Cholesterol, not just cardiovascular risk, is important in deciding who should receive statin treatment, Eur. Heart J. 36 (2015) 2975–2983.
[18] T. Walley, P. Folino-Gallo, P. Stephens, et al., Trends in prescribing and utilization of statins and other lipid lowering drugs across Europe 1997-2003, Br. J. Pharmacol. 60 (2005) 543–551.
[19] H. Tada, O. Melander, J.Z. Louie, et al., Risk prediction by genetic risk scores for coronary heart disease is independent of self-reported family history, Eur. Heart J. 37 (2016) 561–567.
[20] R.M. Conroy, K. Pyorala, A.P. Fitzgerald, et al., Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project, Eur. Heart J. 24 (2003) 987–1003.
[21] M.T. Cooney, A. Dudina, R. D'Agostino, et al., Cardiovascular risk-estimation systems in primary prevention: do they differ? Do they make a difference? Can we see the future? Circulation 122 (2010) 300–310.
[22] A.P. DeFilippis, R. Young, C.J. Carrubba, et al., An analysis of calibration and discrimination among multiple cardiovascular risk scores in a modern multiethnic cohort, Ann. Intern. Med. 162 (2015) 266–275.
[23] D.J. Blom, T. Hala, M. Bolognese, et al., A 52-week placebo-controlled trial of evolocumab in hyperlipidemia, N. Engl. J. Med. 370 (2014) 1809–1819.
[24] F. Civeira, E. Jarauta, A. Cenarro, et al., Frequency of low-density lipoprotein receptor gene mutations in patients with a clinical diagnosis of familial combined hyperlipidemia in a clinical setting, J. Am. Coll. Cardiol. 52 (2008) 1546–1553.
[25] B.M. Sorensen, A.J. Houben, T.T. Berendschot, et al., Prediabetes and type 2 diabetes are associated with generalized microvascular dysfunction: the Maastricht study, Circulation 134 (2016) 1339–1352.