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Severe heterozygous familial hypercholesterolemia and risk for cardiovascular disease: A study of a cohort of 14,000 mutation carriers

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ABSTRACT

Background: Some recently emerged lipid-lowering therapies are currently restricted to patients with homozygous familial hypercholesterolemia (HoFH), and studies are underway to also assess these therapies in patients with 'severe heterozygous FH (HeFH)'. However, no uniform definition of 'severe HeFH' exists, although untreated low-density lipoprotein cholesterol (LDL-C) levels above 8 mmol/L (309 mg/dl) have been historically used to define this phenotype. Our aim was to define severe HeFH, to establish its prevalence and CVD risk, and to study the relative contribution of classical risk factors to CVD risk in HeFH patients.

Methods and results: We analysed a cohort of 14,283 patients with molecularly defined HeFH, identified by the national FH screening programme in the Netherlands. Age and gender specific percentiles of untreated LDL-C were determined. The percentile corresponding to an LDL-C level of 8 mmol/L (309 mg/dL) in men aged 36–40 years (90th percentile) was selected as the cut-off value for severe HeFH. By applying this percentile-criterion to the whole cohort, 11% of the HeFH patients could be considered as having severe HeFH. Combined with an estimated HeFH prevalence of 1:300 in the Netherlands, this would translate into a prevalence of approximately 1:3,000 for severe HeFH. CVD risk was significantly increased in severe HeFH patients compared to non-severe HeFH patients (adjusted hazard ratio: 1.25 [95% CI: 1.05–1.51], $p = 0.015$). In line, male gender, increased age, increased BMI, smoking, hypertension, diabetes, high LDL-C and low high-density lipoprotein cholesterol were independent CVD risk factors in HeFH per se.

Conclusions: We changed the commonly used static LDL-C level of 8 mmol/L for the identification of severe HeFH into an age and gender corrected percentile. This definition would theoretically result in a prevalence of 1:3,000 for severe HeFH. Patients with severe HeFH are at increased CVD risk compared to non-severe HeFH patients, which underscores the need for more aggressive LDL-C lowering these patients.

1. Introduction

Familial hypercholesterolemia (FH) is a prevalent autosomal dominant disorder of lipoprotein metabolism, caused by mutations in the genes encoding for the low-density lipoprotein (LDL) receptor, apolipoprotein B (ApoB) or proprotein convertase subtilisin/kexin-type 9 (PCSK9). Patients with FH are characterized by elevated serum LDL-cholesterol (LDL-C) levels and an increased risk for premature cardiovascular disease (CVD) [1]. LDL-C levels vary widely amongst FH patients, and patients with LDL-C levels at the upper extreme tail of the distribution are considered to suffer from 'severe FH'.

In literature, heterozygous FH (HeFH) patients with a plasma LDL-C level above 8 mmol/L (309 mg/dL), prior to lipid-lowering therapy (LLT), have been considered to suffer from severe HeFH. This level is, however, not adjusted for age and gender, and this might be particularly important given the fact that LDL-C levels are not uniform across characteristics such as age.

The exact prevalence and CVD risk of severe HeFH are unknown. These issues are of particular interest given the potential market authorisation of novel therapeutic LDL-C lowering agents for

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patients with severe HeFH. Lastly, the relative CVD risk compared to non-severe HeFH patients has not been quantified so far.

Although it is pivotal to emphasize that CVD risk in HeFH is largely driven by LDL-C levels, CVD risk also varies widely amongst patients with HeFH. These interindividual differences are probably caused by the aggregate of variation in LDL-C levels and the total burden of other risk factors, such as smoking, hypertension and diabetes [2,3]. It is, however, not fully elucidated what the relative contribution of these latter factors is in HeFH patients.

Therefore the aims of the current study were to: 1) define severe HeFH and establish its prevalence; 2) describe the CVD risk in patients with severe HeFH compared to non-severe HeFH patients; 3) study the contribution of classical risk factors to CVD risk in HeFH patients. We studied an unprecedented number of patients with molecularly defined HeFH to answer these important clinical questions. Here we present our results.

2. Methods

2.1. Study population and collection of data

All data in the current study were collected for the FH screening program in the Netherlands. The aim of this nationwide and government subsidized cascade screening program is to identify all FH patients. The screening cascade starts with the identification of a carrier of an FH mutation ("index patient"), and subsequent analysis in first degree relatives of this patient. A blood sample is drawn to perform DNA analysis and since 2004, blood is withdrawn in a fasting state to also measure lipids and lipoproteins. This is a cross-sectional analysis and as such, all data used for this study were collected at the moment the individual was visited by the genetic field worker. As a consequence, only persons that were alive could participate in the screening program. Subjects were eligible for the current study if a molecular defect underlying FH was identified. Patients were excluded in case a lipid profile was not available or if no data were present on the use of LLT. Homozygous patients were excluded as well. All patients provided written informed consent. This study was approved by the Medical Ethical Committee of the Academic Medical Centre.

2.2. Lipid profile and mutation analysis

The lipid profile was measured with the LDX-analyser (Cholestech Corporation, Hayward, USA) [4]. LDL-C levels were subsequently calculated with the Friedewald formula, unless triglycerides were above 4.5 mmol/L [5]. We calculated untreated LDL-C levels in patients using LLT at the time of screening, on the basis of dose and type of medication. The correction factors for each therapy, and the scientific foundation for these, are provided in Supplement A. The maximal LDL-C lowering efficacy of LLT was considered to be 50%.

DNA of the tested individuals was isolated from 10 ml of freshly collected blood containing EDTA as anticoagulant. The method of mutation analysis has been described previously [6,7].

2.3. Definition of severe HeFH

We redefined severe HeFH while applying age and gender specific percentiles with the data of the current study (see Supplement B). Males between 36 and 40 years were considered to be most frequently represented in our and previous cohort studies on severe HeFH and we therefore established the percentile corresponding to the LDL-C level of 8 mmol/L (309 mg/dL) in these specific patients. We transposed this percentile to the remainder of the total population, thereby defining an age and gender specific LDL-C level for the definition of "severe HeFH".

2.4. Cardiovascular disease

CVD was defined as: myocardial infarction; coronary artery bypass surgery; percutaneous transluminal coronary angioplasty; angina pectoris; and/or ischaemic stroke.

2.5. Statistical analyses

Differences in baseline characteristics between non-severe HeFH and severe HeFH patients were evaluated using logistic (dichotomous variables) and linear (continuous variables) regression models.

We constructed cumulative survival curves for patients with severe HeFH as well as for patients not suffering from severe HeFH by the Kaplan–Meier method. Differences between the curves were tested with the log-rank test. Cox proportional hazards models were used to estimate hazard ratios (HRs) while adjusting for gender, birth-year and LLT (time-dependent). To account for family ties, the Cox model was fitted with a random intercept per family. Follow-up started at birth and ended for each individual at the date of the first CVD event or censoring (date when subject was visited for enrolment in the screening program), whichever came first. Since several potential confounders could only be measured at screening, we also adjusted for these factors by means of a logistic regression analysis.

The association between CVD and demographic and clinical characteristics was first explored using univariate logistic regression analysis. The following variables were entered into the analyses: gender, age, body mass index (BMI), smoking, hypertension, diabetes mellitus and lipid profile. Stepwise multiple logistic regression analysis was used to assess the independent effect of these variables on the presence of CVD.

All logistic and linear regression analyses were performed using the generalized estimating equation method to account for correlations within families. The exchangeable correlation structure was used for these models.

Continuous variables with a skewed distribution were log transformed before the analyses. A p-value less than 0.05 was considered to be statistically significant. Analyses were carried out using SPSS 20 for Windows (IBM Software, NY, USA) and R Statistics 3.0.1.

Table 1
Demographic and clinical characteristics of 14,283 heterozygous FH patients.

Male gender – no. (%)	6,848 (48.0)
Age (years) – mean (SD)	38.33 (21.16)
Body mass index (kg/m2) – mean (SD)	23.67 (5.10)
Current smoking – no. (%)	3,731 (26.7)
Hypertension – no. (%)	1,619 (11.3)
Diabetes mellitus – no. (%)	401 (2.8)
Lipid-lowering therapy – no. (%)	5,381 (37.7)
Total cholesterol (mmol/L) – mean (SD)	5.97 (6.47)
HDL-cholesterol (mmol/L) – mean (SD)	1.21 (1.88)
LDL-cholesterol (mmol/L) – mean (SD)	4.13 (1.38)
Untreated LDL-cholesterol (mmol/L) – mean (SD)	5.17 (1.98)
- Non-severe HeFH patients – mean (SD)	4.72 (1.40)
- Severe HeFH patients – mean (SD)	8.84 (2.15)
Triglycerides (mmol/L) – median [IQR]	1.1 [0.75–1.64]
Cardiovascular disease in medical history – no. (%)[a]	1,310 (9.2)

HDL, high-density lipoprotein; LDL, low-density lipoprotein; no., number; SD, standard deviation.

[a] Defined as myocardial infarction, coronary bypass surgery, percutaneous transluminal coronary angioplasty, angina pectoris and/or ischaemic stroke in the medical history.