Genetics and therapy of familial hypercholesterolemia

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CHAPTER 2

Prevalence and significance of cardiovascular risk factors in a large cohort of patients with familial hypercholesterolemia

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ABSTRACT

BACKGROUND

Patients with familial hypercholesterolemia (FH) vary widely in terms of onset of cardiovascular disease (CVD). The association between cardiovascular risk factors and prevalent CVD was examined in a cross-sectional study in order to elucidate their contribution to atherogenesis.

METHODS AND RESULTS

Patients were recruited from 37 Dutch Lipid Clinics. The diagnosis of FH was based on a uniform diagnostic protocol, confirmed by DNA analysis in 62% of the cases. All patients were investigated free from any lipid-lowering drug for at least 6 weeks. A total of 526 patients were assessed and more than 37% had a history of CVD with a mean age of onset of 46.8 years. Mean LDL-cholesterol (LDL-C) levels were severely elevated (8.38 $\pm$ 2.13 mmol/L). In univariate analysis, age, presence of hypertension or diabetes, body mass index, triglycerides (TG) and low HDL-C were all significantly associated with CVD. Also in multivariate analysis, all these risk factors, except TG and diabetes, were significantly linked to CVD.

CONCLUSIONS

A high CVD risk in this large well-documented characterised sample of FH patients is not only conferred by elevated LDL-C but also by low HDL-C.

INTRODUCTION

Familial hypercholesterolemia (FH) is an autosomal dominant disorder of lipoprotein metabolism and affects approximately 1 in 400 people in the Netherlands.[1] Mutations in the LDL-receptor gene, located on the short arm of chromosome 19, cause a reduction in the clearance of LDL-cholesterol (LDL-C) in these patients which consequently leads to a rise in LDL-C levels and predisposes to the development of atherosclerosis.[2] Therefore, FH patients are at increased risk of developing premature cardiovascular disease (CVD). Typically, approximately 45% of male and 20% of female patients have documented coronary artery disease (CAD) by the age of 50.[3]

Over 150 different mutations in the LDL-receptor have been identified in the Netherlands to date and novel defects are continuously detected.[4] FH is characterised by large differences in the expression of the clinical phenotype. Even FH patients with identical mutations and LDL-C levels can have significant differences in clinical outcome, such as widely different ages of onset of CVD.[5] Both genetic and environmental factors contribute to this phenomenon, as is illustrated by the fact that, FH patients living in China have significantly lower LDL-C levels compared to family members who have emigrated to Canada. Furthermore, neither xanthomas nor premature CAD were seen in rural Chinese patients but did occur in Canadian relatives.[6] However, solid data addressing the contribution of environmental and genetic factors to the variable phenotypic expression of FH are scarce. In order to assess these issues, we firstly collected a large cohort of FH patients with an otherwise homogeneous ethnic background. Subsequently, clinical and environmental variables were recorded in order to assess the association between cardiovascular risk factors and prevalent CVD in a cross-sectional design in order to elucidate their contribution to atherogenesis. Here we present the cross-sectional prevalence data and the significance of different risk factors for CVD in this large well-documented FH cohort.

METHODS

Study design, subjects and organisation

FH patients were recruited from 37 Lipid Clinics in the Netherlands. When patients were on previous lipid-lowering therapy, they had a 6-week washout period. All patients were subsequently included in a study to receive simvastatin 80 mg treatment. The

following inclusion criteria had to be met: all patients had to have either a molecular diagnosis for FH or were diagnosed with definite FH and had to have 6 or more points, according to an algorithm (to allow standardisation of the diagnosis of FH based on clinical findings, personal and familial clinical history and biochemical parameters).[7] Patients were excluded if they: were less than 18 years of age, pregnant or nursing women, had hyperlipidemia Type I, III, IV or V or homozygous FH; had secondary hypercholesterolemia due to any cause; had inadequately controlled diabetes. The Ethics Institutional Review Boards Committees of all the 37 centres approved the protocol and written informed consent was obtained from all participants.
Medical history, physical examination and additional risk factors for cardiovascular disease as well as laboratory analysis of lipid and lipoprotein levels were obtained in all patients.
Cardiovascular disease was considered to be present if subjects met one of the following criteria: Subjects who had 1. A myocardial infarction, proven by electrocardiogram (ECG) abnormalities and enzyme changes; 2. An ischemic stroke; 3. A diagnosis of clinically documented angina pectoris; 4. A history of intermittent claudication documented by ultrasound; 5. Coronary bypass surgery or percutaneous coronary intervention; 6. A clinically significant stenosis on the coronary angiogram; 7. An unequivocally positive exercise ECG.

Biochemical analysis

Blood samples were taken in the morning after an overnight fast. Total plasma cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG) were routinely determined in the different laboratories and standardised by a virtual central laboratory. LDL-C was calculated using the Friedewald formula.[8] Apolipoprotein A1 (apoA1) and apolipoprotein B (apoB) were determined by an immunological rate-nephelometric procedure using a polyclonal goat anti-human antibody (Array protein system, Beckman Coulter, Netherlands).[9] Lipoprotein (a) (Lp(a)) samples were measured by an immunological rate-nephelometric procedure using a polyclonal rabbit antibody directed against Lp(a) (Array protein system, Beckman Coulter, Netherlands).[10] In order to determine plasma total homocysteine (tHcy), samples were pre-treated with tri-n-butylphosphine to release homocysteine from plasma proteins and tHcy was measured by a sensitive and selective HPLC method using pre-column derivatisation with 7-fluoro-2-oxa-1,3-diazole-4-sulfonate.[11]

Statistical analysis

Mean values in lipids, lipoproteins and homocysteine between subgroups were compared using the independent sample t-test. Other parameters (TG, Lp(a), tHcy), were compared by the non-parametric Mann-Whitney U test, because they had a skewed distribution.

The relation between cardiovascular disease and baseline variables was explored using logistic regression analysis. All statistical analyses were performed using the SPSS package (version 10.1, Chicago, Illinois). A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Demographics and baseline characteristics

A total of 546 FH patients were considered for inclusion. Of these, 526 patients finally met the inclusion criteria. Baseline characteristics are given in table 1. Ages ranged from 18 to 80 years. Slightly more males than females were included. More than 37% of all patients had a history of cardiovascular disease with a mean age of onset of 46.8 years (51.4 years in women and 43.6 years in men). Xanthomas were present in 43.5% of all patients.

We also evaluated demographics and baseline characteristics of patients with and without cardiovascular disease. Mean age in patients with CVD was higher than in FH patients without CVD (55.5 years vs. 42.7 years; $p<0.0001$). Also, slightly more males were present in the cardiovascular group, but this was not statistically significant. In addition, mean body mass index (BMI) was significantly higher in patients with a past cardiovascular event compared to the non CVD group (26.7 kg/m^2 vs. 25.4 kg/m^2; $p<0.0001$). Strikingly, fewer smokers were seen in the CVD group than in the group without CVD (18.4% vs. 31.7%; $p=0.001$).

Lipid, lipoprotein and homocysteine levels

From the total of 526 patients, we obtained lipid levels after the washout period in 516 patients. Mean baseline lipid, lipoprotein and homocysteine levels of the whole study group are shown in table 1. Mean TC (10.50 ± 2.17 mmol/L) and LDL-C (8.38 ± 2.13 mmol/L) levels were, as can be expected in FH patients, severely elevated.

Table 1. Demographic and physical baseline characteristics.

Demographics	Age (year)	47.5	(± 13.2)
	Male gender (%)	55.6	
Cardiovascular History	Cardiovascular Disease (%)	37.2	
	Mean age of onset (year)	46.8	(± 9.8)
	Coronary Artery Disease (%)	91.3	
	Peripheral Artery Disease (%)	20.9	
	Both (%)	12.2	
Concomitant Medication	Aspirin (%)	29.5	
	Coumarin derivatives (%)	7.6	
	Beta-blockers (%)	19.8	
	Calcium antagonist (%)	11.2	
	Diuretics (%)	7.4	
	ACE inhibitors & AII antagonists (%)	10.8	
	Nitrates (%)	9.3	
	Oral contraceptives (%)	7.4	
	Hormone replacement therapy (%)	1.9	
Risk factors	Smoking Current (%)	26.8	
	Non smoking (%)	73.2	
	Family history of premature CAD	65.8	
	Diabetes (%)	2.1	
	Hypertension (%)	15.4	
Physical examination	Weight (kg)	77.8	(±13.5)
	Height (m)	1.73	(± 0.1)
	BMI (kg/m^2)	25.9	(± 3.5)
	Xanthomas (%)	43.5	
	Achilles tendon xanthomas	32.3	
	Other xanthomas	22.7	
	Both	45.0	
	Arcus cornealis (%)	30.6	
Laboratory	Total cholesterol (mmol/L)	10.50	(± 2.17)
	LDL cholesterol (mmol/L)	8.38	(± 2.13)
	HDL cholesterol (mmol/L)	1.22	(± 0.35)
	Triglycerides (mmol/L)	1.80	(1.2-2.4)
	Apolipoprotein A1 (g/L)	1.21	(± 0.21)
	Apolipoprotein B (g/L)	1.98	(± 0.45)
	Lp(a) (mg/L)	137	(47-380)
	Homocysteine (µmol/L)	12.00	(10.20-14.28)

All values are given as mean levels ± standard deviation, except where given as percentages. Triglycerides, Lp(a) and homocysteine are given as median with the interquartile range between brackets. ACE indicates angiotensin converting enzyme; CAD, coronary artery disease; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a),lipoprotein (a).

In comparison, TC and LDL-C levels were 5.49 and 3.56 mmol/L, respectively in 3403 Dutch controls.[12] HDL-C levels (1.22 ± 0.35 mmol/L) were in the normal range as compared to the controls (1.20 mmol/L) and TG levels (median of 1.80 mmol/L and mean of 2.06 mmol/L) were elevated compared to the mean TG in the controls (1.66 mmol/L). Mean apoA1 levels were 1.21 ± 0.21 g/L and mean apoB 1.98 ± 0.45 g/L. Median Lp(a) levels were elevated (137 mg/L, interquartile range 47-380 mg/L). In 201 apparently healthy unrelated blood donors median Lp(a) levels were 53 mg/L (10-166 mg/L) determined by the N-Latex Lp(a) kit (Behringwerke, Marburg, Germany). (J.Prins, unpublished data) This test has previously been compared with the test we used and no substantial differences were found.[13] Mean tHcy levels (for men 13.47 ± 4.84 and for women 11.89 ± 3.98 μmol/L) are lower than in a random sample (n=3025) of the general Dutch population aged 20-65 y (mean for men 14.6 and for women 13.1 μmol/L).[14]

Table 2 shows lipid, lipoprotein and homocysteine levels in patients with and without CVD. In CVD patients mean TC and LDL-C levels were slightly, but not significantly, higher compared to the non-CVD patients. HDL-C levels were significantly lower in the CVD group (1.18 vs. 1.25 mmol/L; p=0.02), median TG levels were significantly higher (2.00 vs. 1.60 mmol/L; p<0.0001) and median tHcy levels (12.60 vs. 11.80 μmol/L; p=0.02) were significantly higher. ApoB showed a trend towards higher levels in the CVD group compared to the non CVD group (p=0.05).

Table 2. Lipid, lipoprotein and homocystein levels in FH patients with and without cardiovascular disease.

Variable	FH with CVD (n = 190)	FH without CVD (n = 326)	p-value
Total cholesterol (mmol/L)	10.73 ± 2.33	10.37 ± 2.07	0.07
LDL cholesterol (mmol/L)	8.53 ± 2.30	8.29 ± 2.03	0.22
HDL cholesterol (mmol/L)	1.18 ± 0.31	1.25 ± 0.36	0.02
Triglycerides (mmol/L)	2.00 (1.40-2.80)	1.60 (1.10-2.30)	<0.0001
Apolipoprotein A1 (g/L)	1.20 ± 0.19	1.22 ± 0.22	0.21
Apolipoprotein B (g/L)	2.03 ± 0.48	1.95 ± 0.42	0.05
Lp(a) (mg/L)	134 (55-460)	140 (42-348)	0.14
Homocystein (μmol/L)	12.60 (10.60-15.20)	11.80 (10.20-13.80)	0.02

All values are given as mean levels ± standard deviation only triglycerides, Lp(a) and homocysteine are given as median with the interquartile range between brackets. FH indicates familial hypercholesterolemia; CVD, cardiovascular disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a), lipoprotein (a)

The entire cohort of FH patients was also assessed for differences between males and females (table 3). Females had significantly higher LDL-C levels (8.66 ± 2.31 vs. 8.15 ± 1.96 mmol/L; p=0.008), higher HDL-C levels (1.36 ± 0.37 vs. 1.11 ± 0.28 mmol/L; p<0.0001) and lower median TG levels (1.70 vs. 1.80 mmol/L; p=0.004). Median tHcy levels were significantly lower in women compared to men (11.45 vs. 12.80 μmol/L; p<0.0001).

Table 3. Lipid, lipoprotein and homocystein levels in male and female FH heterozygotes.

Variable	Males (n = 287)	Females (n = 229)	p-value
Total cholesterol (mmol/L)	10.24 ± 1.98	10.84 ± 2.36	0.002
LDL cholesterol (mmol/L)	8.15 ± 1.96	8.66 ± 2.31	0.008
HDL cholesterol (mmol/L)	1.11 ± 0.28	1.36 ± 0.37	<0.0001
Triglycerides (mmol/L)	1.80 (1.20-2.80)	1.70 (1.10-2.20)	0.004
Apolipoprotein A1 (g/L)	1.16 ± 0.20	1.29 ± 0.20	<0.0001
Apolipoprotein B (g/L)	1.96 ± 0.43	1.99 ± 0.47	0.51
Lp(a) (mg/L)	142 (45-355)	126 (54-407)	0.38
Homocystein (μmol/L)	12.80 (10.80-14.90)	11.45 (9.40-13.10)	<0.0001

All values are given as mean levels ± standard deviation only triglycerides, Lp(a) and homocysteine are given as median with the interquartile range between brackets. FH indicates familial hypercholesterolemia; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Lp(a), lipoprotein (a)

Relation between CVD and risk factors

All risk factors and baseline characteristics were firstly evaluated in a univariate logistic regression model with cardiovascular disease as the outcome variable. Odds ratios and 95% confidence interval (CI) for clinically and statistically significant variables are presented in table 4. Presence of diabetes (OR 17.6; 95% CI 2.25-137.8; p=0.006) or hypertension (OR 3.76; 95% CI 2.29-6.17; p<0.0001) was very significantly related to CVD. Age also had a very strong influence on the presence of CVD (OR1.10; 95% CI 1.08-1.12; p<0.0001). BMI (OR 1.11; 95% CI 1.06-1.17; p<0.0001), TG levels (OR 1.19; 95% CI 1.04-1.37; p=0.01) and low HDL-C levels (OR 0.53; 95% CI 0.31-0.91; p=0.02) were all significantly associated with risk for developing CVD. To evaluate the possible independent association of risk factors for CVD, multivariate backward stepwise logistic regression analyses were carried out. Male gender (OR 1.70; 95% CI 1.05-2.76; p=0.03), age (OR 1.10; 95% CI 1.08-1.13; p<0.0001), presence of hypertension (OR 1.82; 95% CI 1.03-3.22; p=0.04), HDL-C (OR 0.39; 95% CI

0.19-0.80; p=0.01) and BMI (OR 1.07; 95% CI 1.003-1.14; p=0.04) appeared as significant risk factors of CVD. Diabetes and TG levels were not significantly related in this multivariate analysis.

Table 4. Odds ratio (OR) and 95% confidence interval (95% CI) for the presence of a risk factor for cardiovascular disease.

	Univariate			Multivariate		
	OR	95 % CI	p-value	OR	95 % CI	p-value
Male gender	1.18	0.83-1.69	0.36	1.70	1.05-2.76	0.03
Age (years)	1.10	1.08-1.12	<0.0001	1.10	1.08-1.13	<0.0001
Hypertension	3.76	2.29-6.17	<0.0001	1.82	1.03-3.22	0.04
Diabetes	17.61	2.25-137.8	0.006	-	-	-
Body Mass Index (kg/m^2)	1.11	1.06-1.17	<0.0001	1.07	1.003-1.14	0.04
Triglycerides (mmol/L)	1.19	1.04-1.37	0.01	-	-	-
HDL-C (mmol/L)	0.53	0.31-0.91	0.02	0.39	0.19-0.80	0.01

DISCUSSION

Baseline characteristics

It was of pivotal importance that the diagnosis of FH was unequivocal and that the study cohort consisted of sufficient numbers of patients with evident CVD. Therefore, the strictest possible inclusion criteria were applied, which resulted in a high percentage (62%) of patients who also had a molecular diagnosis of FH. Moreover, these criteria resulted in very high mean TC and LDL-C levels (10.50 ± 2.17 and 8.38 ± 2.13 mmol/L, respectively), above reported in other studies.[15,16] In FH patients with a mean age of 50 years, the prevalence of CVD is 45% for males and 20% for females.[3] In our study cohort the expected prevalence would be approximately 35% at a mean age of 50 years. However, the mean age is somewhat lower (47.5 years) and the actual prevalence is somewhat higher (37.2%), although direct comparisons between our cohort and other studies are difficult to make since diagnostic criteria, CAD rates in the background population and treatment intensity will differ. Xanthomas were found in 43.5% of our FH patients, slightly higher than expected, according to a prevalence (in per cent) roughly equal to age minus 10 (RR Williams, unpublished observations, 1996) but less than reported in Canadian FH patients.[15]

Cardiovascular risk factors

As demonstrated in the Simon Broome Register Group, in which 1185 FH patients were prospectively followed from 1980-1995, relative risk of a fatal coronary event was extremely increased despite treatment (men age 20-39; 48 fold increase and women age 20-39; 125 fold increase).[17] However, within FH patients the age of onset differs because of different other risk factors. The demographic characteristics of our study cohort confirm the risk factor pattern for CVD in FH patients. FH patients with CVD were older, more often male, had a higher BMI and had a higher incidence of hypertension and diabetes. All these risk factors interact to accelerate the onset of CVD in FH. Age and gender have been consistent risk factors in virtually all FH studies.[15,18] Hypertension[19,20], diabetes[19,21] and BMI[16,22] have also been associated with increased coronary artery disease risk in FH patients, but inconsistently. Strikingly, fewer smokers were seen in our FH patients with CVD. This is in contrast to previous reports.[15,19,23] Although we did not collect any data on previous smoking habits, this discrepancy may be explained by a high number of former smokers in the CVD group, who stopped smoking after their first cardiovascular event.

FH patients with CVD had more untoward lipid and lipoprotein levels than FH patients without CVD: higher TC, LDL-C and TG levels and lower HDL-C levels. Previous studies did not always show uniform results concerning differences in lipids between FH patients with and without CVD. Often, total and LDL-C levels were higher in CVD patients[15] but frequently not statistically significant.[16,19,23,24] In contrast, in most studies significant differences were found for low HDL-C[25,26] and high TG levels.[19,27]

Median Lp(a) levels were elevated in our FH cohort but no differences were found between patients with or without CVD. Earlier studies have reported that an increased Lp(a) level would represent the best discriminator between FH subjects with and without CVD[28,29] and that mean and median Lp(a) levels in FH patients are indeed increased compared to controls.[29,30] However, this phenomenon could not always be confirmed.[25,31] Differences in the analytical methods used and the absence of standardisation in Lp(a) measurement may have contributed to the uncertainty regarding Lp(a), CVD and FH.[32] The test used in this study is isoform-dependent and is therefore known to underestimate the Lp(a) concentration of small apolipoprotein (a) (apo(a)) isoforms.[33] Small sized apo(a) is atherogenic and the level of apo(a) is elevated in FH patients with CVD compared to FH patients without CVD.[34]

Mean tHcy levels were normal in this FH cohort but tHcy levels were significantly higher in patients with CVD. Moderate elevations of plasma homocysteine levels are associated with an increased risk for CAD.[35] The few studies addressing homocysteine

levels in FH patients did not reveal elevated levels[18,36-39] and the relation between tHcy levels and the prevalence of CVD in FH remains unclear.

Gender differences

Total, LDL and HDL-C levels were significantly higher and TG significantly lower in women with FH compared to men with FH. Many FH studies have reported significantly higher levels of HDL-C in women compared to men[15,19,24] mostly in combination with elevated LDL-C or in combination with higher TG levels in men. These higher LDL-C levels in women are still poorly understood, since it is known that oestrogen upregulates the LDL-receptor that should lead to lower LDL-C levels.[40] Median Lp(a) level did not reveal a gender difference, in accordance with previous results.[41] This may be explained by the fact that Lp(a) is under strict genetic control and is hardly affected by race, age, sex or diet.[42] Median plasma tHcy was significantly higher in men compared to women, as reported in many previous studies.[43] An explanation for this phenomenon might be the effect of larger muscle mass in men[44] and may reflect higher intake of vitamins in women.

Role of major risk factors in CVD such as male gender, age, hypertension, diabetes, low HDL-C and BMI was confirmed in our study cohort after both univariate and multivariate analysis, in the latter diabetes expected. The characteristics of our study cohort are largely in keeping with those in other, but smaller FH study populations.

In conclusion, our data indicate that we have collected a large and well-documented FH group what will allow us to answer a number of relevant questions. However, it should be acknowledged that a degree of selection bias is inevitable in a sample recruited from Lipid Clinics. But, importantly, our study points to the fact that next to the severely elevated LDL-C levels, other risk factors, notably HDL-C, also make a strong contribution to the presence of CVD in FH patients. Whether lifestyle changes or pharmacological modalities that raise HDL-C are beneficial in FH patients remains to be investigated.

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