Diagnosis of Familial Combined Hyperlipidemia Based on Lipid Phenotype Expression in 32 Families

Results of a 5-Year Follow-Up Study

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Abstract—Familial combined hyperlipidemia (FCH) is characterized by a variable expression of hypercholesterolemia and/or hypertriglyceridemia. We evaluated the variability in lipid phenotype expression over a 5-year period and studied factors affecting the lipid phenotype expression. A total of 32 families (299 subjects) were studied in 1994 and in 1999. Subjects were classified as having FCH when total cholesterol and/or triglyceride levels exceeded the 90th percentile adjusted for age and sex. In 1994, 93 (31%) of the 299 subjects were affected, whereas 206 (69%) of the subjects were unaffected relatives. In 1999, a diagnosis of FCH was consistent in 69 (74%) of the 93 subjects. So, 26% of the FCH subjects in 1994 showed a sporadic normolipidemic pattern (ie, total cholesterol and/or triglycerides <90th percentile) in 1999. Among the 206 unaffected relatives in 1994, 178 (86%) remained unaffected in 1999, and 28 (14%) developed an FCH lipid phenotype. Multiple regression analysis showed that sex (odds ratio 2.03, 95% CI 1.09 to 3.87; \( P=0.03 \)) and body mass index (odds ratio 1.14, 95% CI 1.05 to 1.24; \( P<0.01 \)) significantly contributed to the variability in lipid phenotype expression. Thus, a diagnosis of FCH, based on plasma total cholesterol and/or triglyceride levels, is consistent in only 74% of the subjects over a 5-year period. Two other major characteristics of our FCH group, compared with the unaffected relatives, included elevated apolipoprotein B (apoB) levels and the presence of small dense low density lipoprotein (LDL), as reflected by a low value of the parameter \( K \) (apoB 1461 ± 305 versus 997 ± 249 mg/L, respectively \( P<0.001 \); \( K \) value −0.22 ± 0.19 versus −0.02 ± 0.19, respectively \( P<0.001 \)). We now report that the apoB concentration and the \( K \) value show less variability in time and are more consistently associated with FCH, inasmuch as affected FCH subjects, compared with the unaffected relatives, persistently show a higher apoB level and a lower value of parameter \( K \), reflecting small dense LDL, even when they present a sporadic normolipidemic pattern. In conclusion, our results emphasize the need for reevaluation of the diagnostic criteria for FCH. We demonstrate that apoB and small dense LDL are attractive new candidates for defining FCH. Further studies are indicated to evaluate the role of apoB and small dense LDL as diagnostic criteria for FCH. (Arterioscler Thromb Vasc Biol. 2002;22:274-282.)

Key Words: familial combined hyperlipidemia ■ follow-up study ■ diagnosis ■ apolipoprotein B ■ small dense LDL

Familial combined hyperlipidemia (FCH) was first described as a new autosomal inherited lipid disorder in 1973 by Goldstein et al.\(^1\) FCH is the most common form of heritable lipid disorders, with an estimated prevalence of 1.0% to 2.0% in the general population and 10% to 20% in survivors of myocardial infarction.\(^2\) Hyperlipidemia is characterized by elevations of plasma total cholesterol (TC) and/or triglyceride (TG) concentration; therefore, it is also known as “multiple-type hyperlipidemia.” Such a lipid profile is frequently associated with an unfavorable decrease in HDL cholesterol (HDL-C) concentration, an elevated apoB concentration, and a preponderance of atherogenic small dense LDL particles.\(^3–7\) In general, FCH is thought to be caused by hepatic VLDL overproduction\(^8,9\) with or without impaired clearance of TG-rich lipoproteins\(^10,11\) of exogenous and endogenous origin. Over the past few years, several other metabolic pathways in the pathogenesis of FCH have been proposed, as recently reviewed.\(^12\) Other syndromes appear to exhibit overlapping etiologies with FCH, such as hyperapolipoproteinemia, the atherogenic lipoprotein phenotype, familial dyslipidemic hypertension, and syndrome X.\(^13–15\)

FCH was originally assumed to be an autosomal dominant trait. However, subsequent reanalyses of the original data of Goldstein et al\(^1\) have provided evidence consistent with a multigenic mode of inheritance. Since then, a number of segregation analyses with multiple sets of families have
indicated complex inheritance. These studies have suggested major gene effects for serum TG levels\textsuperscript{16} and apoB levels.\textsuperscript{17,18} Bivariate analysis has suggested common genetic mechanisms influencing LDL particle size and apoB levels.\textsuperscript{19} Despite extensive research in metabolic and genetic fields, there is still no specific metabolic or genetic marker for FCH. Therefore, family studies are still necessary to establish a diagnosis of FCH in a single patient.

One of the characteristic features of FCH is its variability in lipid and lipoprotein pattern among family members and even in the individual patient.\textsuperscript{4} To our knowledge, there are no data published about the variability in lipid phenotype expression over a period of several years. In this 5-year follow-up study, we evaluate the variability in lipid phenotype expression, apoB level, and small dense LDL in a large cohort of 32 well-defined FCH families, and we study the factors affecting the lipid phenotype expression.

Methods

Study Population

The study population consisted of kindreds and probands of families with known FCH who were recruited in 1994 and followed up in 1999. The recruitment of FCH kindreds took place in 1994 via known affected probands who were attending the outpatient clinic as described previously.\textsuperscript{20} The probands exhibited a combined hyperlipidemia (with TC and TG concentrations exceeding the 90th percentile, according to the PROCAM study\textsuperscript{21}, which was confirmed by repeated measurement; these percentiles are adjusted for age and sex. Unaffected relatives were defined by TC and TG levels classified as having FCH when plasma TC and/or TG levels exceeded the 90th percentile, corrected for age and sex. None of the probands was homozygous for the apo e2 allele, and none of the relatives had diabetes mellitus, hypothyroidism, and hepatic or renal impairment). None of the hyperlipidemia was diagnosed in the proband (ie, diabetes mellitus, hypothyroidism, and hepatic or renal impairment). Families were excluded when a secondary cause of hyperlipidemia was present. Families were included when a multiple-type hyperlipidemia, the presence of at least 1 first-degree relative with hypertriglyceridemia (HTG), hypercholesterolemia (HC), or combined hyperlipidemia was obligatory. Furthermore, at least the proband or 1 of the first-degree relatives should have premature cardiovascular disease (CVD) before the age of 60 years. In addition, the 95th percentile for plasma TC and TG was present in women. BMI was determined for all subjects. After withdrawal of lipid-lowering medication for 4 weeks and after an overnight fast, fasting blood samples were drawn by venipuncture. The subjects were provided in 1994 and 1999 were included. In the present study, all individuals were white. This resulted in a total of 32 families, providing 299 subjects (excluding spouses). At both points of measurement (1994 and 1999), all subjects filled out a questionnaire providing data on sex, age, smoking, alcohol consumption, current medications, and family history of cardiovascular disease. The subjects were classified as having FCH when plasma TC and/or TG levels exceeded the 90th percentile, according to the PROCAM study\textsuperscript{21}.

Plasma Lipid, Lipoprotein, and Apolipoprotein Analysis

Plasma TC and TG concentrations were determined by enzymatic commercially available reagents (catalog No. 237574 [Boehringer-Mannheim] and No. 6639 [Sera Pak, Miles], respectively). VLDL cholesterol (VLDL-C) was isolated from whole plasma by ultracentrifugation at density (d)\textsuperscript{23} 1.006 g/mL for 16 hours at 36 000 rpm with a fixed angle rotor (TFT 45.6 rotor, Kontron) in a Beckman L7-55 ultracentrifuge. HDL-C was determined by the polyethylene glycol 6000 method.\textsuperscript{24} LDL cholesterol (LDL-C) was calculated by subtraction of VLDL-C and HDL-C from plasma TC. Total plasma apoB concentrations were determined by immunonephelometry as recently described in detail elsewhere.\textsuperscript{25-27} To achieve accurate results in relation to the Center for Disease Control Standardization Program, obtained values were recalculated on the basis of an exchange of sera (Dr Marcovina, Northwest Lipid Research Laboratory, Seattle, Wash).

LDL Subfraction Profile Analysis

LDL subfractions were separated by single-spin density gradient ultracentrifugation. Each individual LDL subfraction profile was defined by a continuous variable, K, as described in detail previously.\textsuperscript{28} Briefly, after ultracentrifugation, the LDL subfractions were visible as distinct bands in the middle of the tube. Up to 5 LDL subfractions could be distinguished: LDL1 (d = 1.030 to 1.033 g/mL), LDL2 (d = 1.033 to 1.040 g/mL), LDL3 (d = 1.040 to 1.045 g/mL), LDL4 (d = 1.045 to 1.054 g/mL), and LDL5 (d = 1.049 to 1.054 g/mL). The subfractions were measured using a Beers law method by means of a Pasteur pipette. The volumes were calculated by weighing after correction for the densities. Subsequently, cholesterol was determined in each fraction, and the concentrations were corrected for dilution and incomplete recoveries. The relative cholesterol concentrations in the LDL subfractions were used to calculate parameter K as a continuous variable that best described each individual LDL subfraction profile. A negative K value (K < 0) reflected a more dense LDL subfraction profile, and a positive K value (K > 0) reflected a more buoyant profile.

Statistical Analysis

Differences in baseline characteristics for anthropometric measurements, lifestyle variables (including lipid-lowering medication use [yes/no], smoking [yes/no], and consumption of >2 alcoholic beverages per day [yes/no]), CVD (yes/no), lipid and lipoprotein parameters, apoB levels, and the value of parameter K between subjects with FCH and their unaffected relatives were tested for statistical significance by using the 2-tailed Fisher exact test for dichotomous variables and the t test for continuous variables.

Before the final analyses, we were interested in determining whether changes in time in each group were different between men and women. We included sex in the linear mixed model for each dependent variable separately. We were particularly interested in the third-order interaction term among sex, time, and group. We found that this item was never significant; therefore, the sex variable was removed from the final model.

A linear mixed model with repeated measurements was used to test differences in lipid and lipoprotein concentrations, apoB level, K value, and BMI for statistical significance between the points of measurement (1994 and 1999) and between the groups (I, II, III, and IV) for each dependent variable separately. The interaction between time and group and among time, group, and sex was also included in the model. The dependent variables were TC, TG, LDL-C, HDL-C, and apoB levels, parameter K, and BMI. The independent variables were time (1994 and 1999) and group (I, II, III, and IV) and their interactions. Group I was defined by subjects who were affected (FCH) in 1994 and showed a normolipidemic pattern in 1999, defined by TC and TG levels <90th percentile, corrected for age and sex. Group II was defined by subjects who were affected in 1994 and in 1999. Group III consisted of normolipidemic FCH relatives in 1994 who developed an FCH lipid phenotype in 1999, and group IV consisted of those subjects who were not affected, ie, TC and TG <90th percentile in 1994 and in 1999. The mean levels by time and group were estimated by using the appropriate least square means. Adjusted probability values according to Tukey-Kramer were presented, and a value of P < 0.05 was considered to be significant.
A total of 32 families (299 subjects) were studied in 1994 and in 1999. In 1994, 93 (31%) of the 299 subjects were affected (FCH group), whereas 206 (69%) of the subjects were unaffected, with TC and TG levels <90th percentile. Table 1 shows the anthropometric measurements, lifestyle variables (including smoking habits and use of alcohol and lipid-lowering medication), lipid and lipoprotein variables, apoB levels, and parameter K values for FCH and non-FCH (unaffected) relatives in 1994. The ratio of women to men was not statistically significantly different between the affected and unaffected FCH family members. The mean age of the group of affected subjects, compared with the group of unaffected FCH family members, was significantly higher, and they also had a higher BMI. This group also had more subjects with CVD, and the consumption of >2 alcoholic beverages per day was more frequent. In contrast, smoking habits and the use of oral contraceptives, including postmenopausal estrogen replacement therapy, was not significantly different between the affected and unaffected subjects. In 1994, 106 (68%) of the women were premenopausal, and 23 (22%) had FCH, whereas 83 (78%) were unaffected. In 1994, among the 50 (32%) postmenopausal women, 27 (54%) had FCH, and 23 (46%) were unaffected. Fifteen (14%) of the premenopausal women in 1994 were postmenopausal in 1999; this finding was associated with a switch from an unaffected to an affected FCH lipid phenotype expression in 3 women. Twelve women did not show a change in lipid phenotype expression (7 women were unaffected, and 5 women had FCH in 1994 and also in 1999). Thus, 20% of the women who were premenopausal in 1994 and postmenopausal in 1999 showed a switch in lipid phenotype expression, which was not significantly different from the percentage of lipid phenotype switchers (18%) observed among the postmenopausal women in 1994 and 1999. Therefore, the change to postmenopausal status is not the only reason for the switch in lipid phenotype expression.

All affected FCH subjects with CVD used lipid-lowering medication, whereas 8 subjects used lipid-lowering medication for primary prevention of CVD. In the group of the unaffected subjects, 10 subjects used lipid-lowering medication; 4 of these subjects used it for secondary prevention, whereas the other 6 subjects without a history of CVD and without increased TC and/or TG levels used lipid-lowering medication. In total, 5 unaffected subjects with CVD did not use lipid-lowering medication.

In 1994, 3 (1%) of the relatives had diabetes mellitus. Of these 3 relatives, 2 had diabetes mellitus type II and were classified as having FCH, and 1 relative had diabetes mellitus type I and was unaffected. In 1999, 6 subjects had developed diabetes mellitus type II (de novo), which was not associated with a change in lipid phenotype expression; 2 subjects remained unaffected in 1994 and also in 1999, whereas 4 subjects had FCH in 1994 and also in 1999. Note that TC and TG values of these 6 subjects were well within the range of the unaffected and affected FCH group, respectively.

By definition, the FCH group had significantly higher TC and TG concentrations. The FCH group also exhibited significantly higher concentrations of VLDL-C, VLDL-TGs, and LDL-C and lower concentrations of HDL-C. Furthermore, significantly higher levels of apoB and lower values of parameter K, reflecting small dense LDL, were found in the FCH group compared with the group of unaffected relatives (Table 1).

### FCH Diagnosis in 1994 Versus 1999

In 1994, 93 (31%) of the 299 subjects were affected (FCH). These affected subjects presented with HC (n=26 [28%]),
TABLE 2. Number of Subjects in 32 FCH Families (299 Subjects) Stratified by Lipid Phenotype Expression in 1994 and in 1999

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<th>1994</th>
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<td>HC</td>
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<td>8</td>
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HC indicates FCH based on TC >90th percentile; HTG, FCH based on TGs >90th percentile; and NL, FCH relatives with TC and TG levels ≤90th percentile. Values are absolute number of subjects.

HTG (n=31 [33%]), or HC+HTG (n=36 [39%]; Table 2). In 1999, a diagnosis of FCH was consistent with the diagnosis in 1994 in only 69 (74%) of the 93 subjects. These subjects were affected in 1994 and also in 1999 according to HC (n=9), HTG (n=20), or combined HC+HTG (n=10) levels, and 30 subjects remained affected by FCH but changed in lipid phenotype, as shown in Table 2. However, most important, a total of 24 subjects (26%) with FCH in 1994 according to HC (n=8), HTG (n=6), or HC+HTG (n=10) levels showed a normolipidemic pattern in 1999. Of the 206 unaffected relatives in 1994, 178 (86%) of them remained unaffected in 1999; however, 28 (14%) of the normolipidemic subjects (ie, TC and TG levels in the <90th percentile) in 1994 were affected (FCH) in 1999 according to HC (n=1), HTG (n=26), and HC+HTG (n=1) levels. The Figure shows the pedigree of 1 FCH family at both points of measurement (1994 and 1999), indicating the individual change in lipid phenotype expression.

In total, 83 (28%) of the 299 subjects showed a switch in lipid phenotype for any reason (Table 2). The numbers of switches in lipid phenotype that were due to change in only TC concentration (n=27) were not significantly different from the numbers of switches in lipid phenotype that were due to change in only TG concentration (n=37, P>0.05 by McNemar’s test). The cholesterol level of 45 participants in the present study fell above the cutoff point (90th percentile) in 1994 and below this point in 1999, or vice versa. So, they underwent a switch in phenotype according to the cholesterol level. The mean change in the cholesterol level of these subjects was 1.10±0.66 mmol/L. Fifty-six participants switched in phenotype according to the TG level (mean change 1.49±1.15 mmol/L). These results show that the switch in lipid phenotype is based on significant TC and/or TG changes that cannot be simply explained by the intrindividual and analytical variation. Therefore, we studied 4 subgroups (groups I, II, III, and IV) defined by the switch in lipid phenotype as described in Statistical Analysis.

**Anthropometric Measurements and Lipid and Lipoprotein Concentrations in 1994 Versus 1999**

Table 3 shows the anthropometric measurements and mean values of lipid and lipoprotein concentration by the 4 subgroups, with each defined by lipid phenotype expression in 1994 versus 1999. Note that a normolipidemic lipid profile is defined by TC and TG levels <90th percentile, corrected for age and sex. The mean age of the unaffected subjects in 1994 and 1999 (group IV) was statistically significantly lower than the mean age of the other groups. The results of the linear mixed model showed that there was a statistical significant difference in each of the lipids and lipoprotein values and of the BMI between 1994 and 1999 and between the groups and that the time differences were different between the groups (ie, interaction). This result indicates that the variability in lipid and lipoprotein concentration observed, especially among FCH subjects (groups I, II, and III), is not simply the result of either random biological or analytical variation, or a time effect.

The BMI of the subjects who were affected in 1994 and had a normolipidemic pattern in 1999 (group I) did not change, whereas the BMI of the subjects of the other groups increased significantly from 1994 to 1999 (groups II, III, and IV).

For TC, a significant decrease was observed in the subjects who were affected in 1994 but had a normolipidemic pattern in 1999 (group I). The decrease in TC concentration of the subjects who remained affected (group II) or unaffected (group IV) and the increase in TC concentration of the subjects who had a normolipidemic pattern in 1994 but were affected in 1999 (group III) did not reach statistical significance. The TG concentration decreased significantly in the subjects who were affected in 1994 but had a normolipidemic pattern in 1999 (group I), whereas the subjects who remained affected (group II) and who had a normolipidemic pattern in 1994 and an FCH phenotype in 1999 (group III) showed a significant increase, most likely reflecting progression of the disease. The unaffected subjects (group IV) showed no significant change in TG concentration. The LDL-C concentration showed a decrease in all 4 groups, reaching statistical significance in the subjects who were affected in 1994 and
TABLE 3. Anthropometric Measurement, Lipid and (Apo)lipoprotein Concentrations, and Value of Parameter K, Reflecting LDL Heterogeneity, in 299 Subjects From 32 FCH Families, Stratified by Switch in Lipid Phenotype Expression in 1994 Versus 1999

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<td>FCH (n=24)</td>
<td>FCH (n=69)</td>
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<td>Male/female,*</td>
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<td>Age (1994),†</td>
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<td>1994</td>
<td>26.7 (25.2–28.1)</td>
<td>27.1 (26.3–27.9)</td>
<td>25.4 (24.1–26.7)</td>
<td>23.0 (22.5–23.5)</td>
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<td>1999</td>
<td>26.5 (25.0–27.9)</td>
<td>28.6 (27.7–29.4)</td>
<td>28.0 (26.7–29.3)</td>
<td>24.2 (23.7–24.7)</td>
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<td>TC,‡ mmol/L</td>
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<td>1994</td>
<td>7.04 (6.61–7.48)</td>
<td>7.05 (6.79–7.30)</td>
<td>5.75 (5.35–6.16)</td>
<td>5.23 (5.07–5.39)</td>
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<td>TGs,‡ mmol/L</td>
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<td>1994</td>
<td>2.85 (2.19–3.50)</td>
<td>3.40 (3.02–3.79)</td>
<td>1.55 (0.93–2.16)</td>
<td>1.10 (0.85–1.34)</td>
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<td>1999</td>
<td>1.86 (1.20–2.52)</td>
<td>4.27 (3.88–4.66)</td>
<td>2.89 (2.28–3.51)</td>
<td>1.20 (0.96–1.45)</td>
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<td>LDL-C,‡ mmol/L</td>
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<td>1999</td>
<td>4.21 (3.78–4.65)</td>
<td>4.13 (3.87–4.38)</td>
<td>3.73 (3.34–4.06)</td>
<td>3.44 (3.29–3.60)</td>
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<td>HDL-C,‡ mmol/L</td>
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<td>1994</td>
<td>0.97 (0.85–1.10)</td>
<td>1.05 (0.98–1.12)</td>
<td>1.03 (0.92–1.15)</td>
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<td>1999</td>
<td>1.0 (0.88–1.12)</td>
<td>0.92 (0.85–0.99)</td>
<td>0.93 (0.81–1.04)</td>
<td>1.23 (1.18–1.27)</td>
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<td>ApoB,§ mg/L</td>
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<td>1994</td>
<td>1434 (1331–1536)</td>
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<td>1174 (1078–1269)</td>
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<td>1999</td>
<td>1271 (1169–1374)</td>
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<td>1006 (969–1044)</td>
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<td>K value§</td>
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<td>1994</td>
<td>−0.25 (−0.34−0.17)</td>
<td>−0.21 (−0.26−0.15)</td>
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<td>0.0 (−0.04−0.03)</td>
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<td>1999</td>
<td>−0.13 (−0.21−0.04)</td>
<td>−0.31 (−0.36−0.26)</td>
<td>−0.26 (−0.34−0.19)</td>
<td>0.05 (0.02−0.08)</td>
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Values are estimated mean (95% CI) and absolute numbers for sex distribution.

*χ² test (P<0.05).
†ANOVA (P=0.05), indicating that only the mean age is significantly lower in group IV compared with all other groups.
‡Statistically significant effect for each of the following: (1) time, (2) group, and (3) time-group interaction with use of a linear mixed model (P<0.05).
§In 1994 and 1999, the mean value in groups I, II, and III is significantly different compared with that in group IV (P<0.05 by adjusted Tukey-Kramer test).
\[P<0.05\] by adjusted Tukey-Kramer for differences between 1994 and 1999.

had a normolipidemic pattern in 1999 (group I) and in the group of subjects who remained affected in 1994 and also in 1999 (group II). The HDL-C concentration in the subjects who had FCH in 1994 but had a normolipidemic pattern in 1999 (group I) did not change. In all the other groups (II, III, and IV), the HDL-C concentration decreased, reaching statistical significance in the subjects who consistently had FCH and in those who were consistently unaffected (groups II and IV, respectively).

These results show that, on average, the subjects who switch from affected to a normolipidemic pattern (group I) improve on all parameters (although less for HDL-C). Most likely, the healthier lifestyle in 1999, as indicated by the stable BMI, contributed to the improved lipid and lipoprotein profile. However, the lipid phenotype remained more atherogenic: TC, TG, and LDL-C were significantly higher and HDL-C was significantly lower than in the unaffected relatives, suggesting that these subjects are genetically predisposed to FCH. Also, on average, the subjects with a normolipidemic pattern in 1994 but with FCH in 1999 (group III) deteriorated. Although the deterioration was not more than that in the unaffected subjects (group IV) for all parameters, the assumption that these subjects already had a predisposition to FCH is more likely because these subjects already had higher baseline levels in 1994.

Because the change in lipid and lipoprotein parameters, as described above, is not simply the result of a random effect (including biological and analytical variation), further statistical analysis was performed to identify whether sex, age, use of lipid-lowering medication, and BMI contribute to the change in lipid phenotype expression (ie, from FCH to a normolipidemic pattern or from a normolipidemic pattern to FCH). The final model, in which we used selection procedures with multivariate logistic regression analyses, showed that only sex (adjusted odds ratio 2.03, 95% CI 1.09 to 3.87; \(P=0.03\)) and BMI (adjusted odds ratio 1.14, 95% CI 1.05 to...
that apoB and parameter group of unaffected relatives (group IV). This result indicates not significantly different from that observed among the parameter between time points (1994 to 1999) in the values of apoB and parameter.

The general observation that men with a high BMI are most likely to vary in lipid phenotype.

**ApoB and LDL Heterogeneity in 1994 Versus 1999**

ApoB levels and values of parameter K, reflecting LDL heterogeneity, are shown in Table 3, stratified by 4 groups, defined by switch in lipid phenotype expression in 1994 versus 1999.

In contrast to the lipid and lipoprotein levels, the difference between time points (1994 to 1999) in the values of apoB and parameter K among FCH subjects (groups I, II, and III) was not significantly different from that observed among the group of unaffected relatives (group IV). This result indicates that apoB and parameter K are less variable in time than are TC and TG levels. Although variability in apoB levels and the value of parameter K is present, it is most intriguing to observe that, on average, subjects with an affected FCH phenotype in 1994 and/or 1999 (groups I, II, and III) have significantly higher apoB concentrations and lower values of parameter K, reflecting small dense LDL, than do the unaffected relatives (group IV), even when they have a sporadic normolipidemic phenotype (group I in 1999 and group III in 1994, Table 3).

**Presence of CVD, Use of Lipid-Lowering Medication, and Smoking/Drinking Habits in 1994 Versus 1999**

Table 4 shows the use of lipid-lowering medication, smoking and drinking habits, and the presence of CVD by group (I, II, III, and IV) in 1994 and 1999. The percentage of those consuming >2 alcoholic beverages per day and smokers was not statistically significantly different between points of measurement (1994 and 1999). The percentage of those consuming >2 alcoholic beverages per day was significantly higher in the group of subjects who remained affected (group II) compared with the subjects who remained normolipidemic between 1994 and 1999 (group IV). The percentage of smokers in the group of subjects who were affected in 1994 but had a normolipidemic pattern in 1999 (group I) was significantly higher than the percentage of smokers in the group of subjects who remained affected (group II) and in the group of subjects who remained normolipidemic (group IV).

The percentage of subjects using lipid-lowering medication significantly increased (odds ratio 3.28, 95% CI 1.96 to 5.5; P<0.001) in 1999 compared with 1994 for all groups, by multivariate logistic regression.

### Table 4. Smoking and Drinking Habits, the Use of Lipid-Lowering Medication, and the Presence of CVD in 299 Subjects From 32 FCH Families Stratified by Switch in Lipid Phenotype Expression in 1994 Versus 1999

<table>
<thead>
<tr>
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<th>1994→1999</th>
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<tbody>
<tr>
<td></td>
<td>FCH→NL (I n=24)</td>
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<tr>
<td></td>
<td>Alcohol (&gt;2 U/d), n (%)</td>
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<td></td>
<td>3 (13)</td>
</tr>
<tr>
<td></td>
<td>1994</td>
</tr>
<tr>
<td></td>
<td>Smoking (&gt;1 cigarette/d), n (%)</td>
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<tr>
<td></td>
<td>1994</td>
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<td>1999</td>
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<tr>
<td></td>
<td>LL med, n (%)</td>
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<tr>
<td></td>
<td>1994</td>
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<td>1999</td>
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<td></td>
<td>CVD, n (%)</td>
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<td></td>
<td>1994</td>
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<td>1999</td>
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</table>

LL med indicates lipid-lowering medication. Values are absolute numbers (percentages).

*The use of alcohol is significantly higher in group II compared with group IV, by multivariate logistic regression.

†Smoking is significantly higher in group I compared with groups II and IV, by multivariate logistic regression.

‡Statistically significant (P<0.05) increase in 1999 compared with 1994 for all groups, by multivariate logistic regression.
Discussion

This is the first long-term follow-up study of a large cohort of FCH families. The present study shows that a diagnosis of FCH, based on internationally accepted criteria (including plasma TC and/or TG levels above the 90th percentile adjusted for age and sex), is consistent in only 74% of the subjects over a 5-year period. So, most important, 26% of the subjects with FCH in 1994 had a sporadic normolipidemic pattern in 1999, defined by TC and TG levels below the 90th percentile, corrected for age and sex. The literature shows that variability in lipid phenotype expression is a characteristic of FCH.1,2,4 However, this variability is usually determined as a change from HTG to HC, or vice versa. Indeed, in our large cohort of FCH families, 32% of the affected subjects showed such a change in lipid phenotype over a period of 5 years, but the change was still consistent with the diagnosis of FCH. Our present results show that this variability in lipid phenotype may also lead to a sporadic “normolipidemic” pattern, because 26% of the affected subjects in 1994 showed a normolipidemic pattern in 1999, 5 years after the initial diagnosis of FCH (Table 2).

The switch from FCH to a normolipidemic phenotype was due to a significant decrease in plasma TC, LDL-C, and TG levels (Table 3), which could not be explained by a random effect, including biological and analytical variation. Further statistical analysis revealed that BMI contributes to a change in lipid phenotype expression. Indeed, these subjects who switched from FCH to a sporadic normolipidemic pattern did not change in body weight, whereas the subjects who remained affected increased in body weight. Knowledge of the presence of FCH in 1994 might have resulted in the patients’ leading a healthier lifestyle, contributing to a stable body weight and a reduction in lipid and lipoprotein levels. However, this knowledge of having FCH did not affect BMI and lipid and lipoprotein levels in subjects who remained affected in 1994 and also in 1999. From the present study, we cannot deduce which other factors contribute to the confounding effect of knowledge of the presence of FCH.

The subjects who developed an FCH phenotype between 1994 and 1999 showed a significant increase in BMI, which was associated with a significant increase in TG level, whereas TC, LDL-C, and HDL-C levels did not change significantly. As discussed above, BMI influences the FCH lipid phenotype expression. Thus, our data suggest that body weight control in FCH subjects should be an important issue in lifestyle intervention programs. However, the significant increase in BMI among the unaffected subjects in 1994 and 1999 was not associated with expression of the FCH phenotype, suggesting that these subjects are most likely not genetically predisposed to develop FCH. The expression of FCH has been suggested to be age dependent,1 with increased prevalence in adolescence. However, in the present study, the mean age of the subjects who developed an FCH phenotype between 1994 and 1999 was 42.9 years. In additional statistical analysis, age was not an independent variable contributing to FCH expression. Also, Porkka et al20 did not find clear signs of age dependence with TC and/or TG criteria.

With aging, the plasma TC and TG levels usually increase, so it may be surprising that in our cohort after 5 years, the TC concentration did not change and even decreased in some groups (Table 3). The change in TC concentration in our FCH cohort reflects the total Dutch population in which the plasma TC concentration had decreased within 0.5 mmol/L between 1987 and 1997.29

Thus, we demonstrate that the lipid profiles in FCH families are highly variable in time, leading to misclassification in diagnosis. Our results help to explain why, despite extensive research, until now no single diagnostic marker has been identified for FCH. For example, until now, 3 genome screens have been reported in an attempt to locate the genetic region for FCH,30–32 but no unique locus has been found in the different family sets. To be able to delineate the metabolic and genetic factors contributing to the etiology of FCH, it is essential to have consistent diagnostic tools to establish a diagnosis of FCH. Although internationally accepted diagnostic criteria are formulated for FCH, not all research groups use the same criteria to establish the diagnosis as recently reviewed.28,33 For example, in some studies, the 95th percentiles or even absolute values for TC and/or TG levels, not adjusted for age and sex, were used, whereas in other studies, apoB also served as a diagnostic criterion.11 However, a change in threshold or cutoff points for TC and TG levels will not improve the diagnosis of FCH because of the considerable intrapatient variability in lipid phenotype expression in time, as demonstrated by our results; with use of the 90th percentile cut point for TC and/or TG levels adjusted for age and sex, 26% of the FCH subjects in our cohort had a normolipidemic pattern after a 5-year period, leading to a false-negative disease status at that point of time. So, for a diagnosis of FCH, the inclusion of only plasma TC and TG levels is insufficient because of this considerable variability in time.

Literature has shown that FCH is associated with other traits that may contribute to the increased risk of CVD, including unfavorable increases in plasma apoB and an increased prevalence of atherogenic small dense LDL. Previously, we reported that in FCH, apoB is as effective as lipid levels in classifying subjects at increased risk for CVD.24 In the present study, a significant higher apoB level and a lower value of parameter K, reflecting small dense LDL, was found in the affected FCH group. Most important, we show that the variabilities in the plasma levels of apoB and in the value of parameter K in time are considerably less than those for TC and TG levels and not different from those for unaffected relatives. Most intriguingly, we demonstrate that compared with unaffected subjects, subjects with FCH have significantly higher apoB levels and increased prevalence of small dense LDL (ie, lower value of parameter K), even when they present a normolipidemic phenotype. So, even the affected FCH subjects in 1994 who showed a normolipidemic lipid profile in 1999 had significantly higher levels of apoB and more small dense LDL in 1994 and 1999. Similarly, the subjects who were unaffected in 1994 but who developed the FCH phenotype in 1999 showed a significantly higher apoB concentration and a lower value of K in 1994 and 1999. These data suggest that subjects genetically predisposed to FCH have increased levels of apoB and increased prevalence of small dense LDL, independent of the lipid profile. Together
with less variability in time, apoB and small dense LDL, compared with TC and TGs, could potentially contribute to more consistent diagnostic criteria for FCH, with a better discriminant power to separate affected from unaffected relatives.

FCH is accompanied by an increased risk of CVD. The total percentage of subjects with CVD increased significantly between 1994 and 1999, but the increase was not significantly different between the 4 groups. However, the total percentage of subjects with CVD in the group of subjects who had an FCH phenotype in 1994 and also in 1999 was significantly higher than that in each of the other groups; in the group of subjects who were normolipidemic in 1994 and also in 1999, the percentage of subjects with CVD was significantly lower than that in each of the other groups. It might appear somewhat surprising that the percentage of subjects who develop CVD was similar among FCH subjects despite the switch to a normolipidemic phenotype in 20% of the subjects versus a switch to an FCH phenotype in 23% of the subjects. The explanation to this apparent contradiction is most likely that once a subject shows an FCH lipid phenotype, he or she is genetically predisposed to FCH, associated with an increased risk of CVD. In FCH, several potential risk factors have been suggested in literature to contribute to its increased atherogenicity, including small dense LDL, an increase in apoB, low HDL levels, and insulin resistance. We show that FCH subjects (groups I, II, and III) have an increased apoB level and more small dense LDL (low value of parameter K), independent of the lipid phenotype expression. So, even when a relative with FCH shows a sporadic normolipidemic pattern, increased levels of apoB and lower values of parameter K are found, most likely contributing to an increased risk of CVD and, thus, contributing to the explanation for the similar increase in the percentage of subjects who develop CVD within 5 years.

In 1994, only 23 of the 93 affected subjects used lipid-lowering medication. Thus, <25% of the subjects were treated properly. In 1999, 53% of the affected subjects used lipid-lowering medication, showing that FCH (most likely due to relatively mild hyperlipidemia) is still not treated properly. The recommendations for treating subjects with mild hyperlipidemia in a family with FCH must be stricter than the recommendations for treating subjects with mild hyperlipidemia in a family without FCH, because a positive family history is an independent risk factor for CVD. Besides lipid-lowering medication, lifestyle intervention programs should reinforce a cessation of smoking, because, unfortunately, >20% of all the subjects were still smoking in 1999, despite their known FCH status in 1994.

A major problem in clinical medicine, contributing to the undertreatment of FCH patients, is the difficulty in diagnosing FCH, because with the present diagnostic criteria (TC and TG levels), the diagnosis cannot be made in a single patient without family screening. Therefore, a major effort should be undertaken to redefine the diagnostic criteria of FCH by 1 standardized parameter, which, measured at 1 time point, discriminates between affected and unaffected relatives. We now demonstrate that apoB and small dense LDL are potentially attractive parameters for improving the diagnostic criteria for FCH.

This unique study of a large cohort of FCH families with a 5-year follow-up indicates that the diagnosis FCH, based on plasma TC and/or TG levels above the 90th percentile adjusted for age and sex, is consistent in only 74% of the subjects. Twenty-six percent of the affected subjects showed a normolipidemic pattern after 5 years. Therefore, our results emphasize the need for reevaluation of the diagnostic criteria. Furthermore, we demonstrate that plasma apoB and small dense LDL are potentially valuable diagnostic criteria for FCH. Further studies are indicated to determine the role of apoB and the presence of small dense LDL and, thus, to improve the diagnostic criteria for FCH.

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References


Diagnosis of Familial Combined Hyperlipidemia Based on Lipid Phenotype Expression in 32 Families: Results of a 5-Year Follow-Up Study
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