Title: Lipid Phenotype and Heritage Pattern in Families with Genetic Hypercholesterolemia Not Related to *LDLR, APOB, PCSK9* or *APOE*

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Running title: Lipid Heritage in non-FH Genetic Hypercholesterolemia

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Total number of figures and tables: 9 (2 of them Supplemental Tables)

Abstract

Background. A substantial proportion of individuals clinically diagnosed as familial hypercholesterolemia (FH) do not carry pathogenic mutations in candidate genes. Whether in them the high cholesterol trait is transmitted monogenically has not been studied.
Objectives. We assessed the inheritance pattern, penetrance and expression of high LDL-cholesterol in families with genetic hypercholesterolemia (GH) without known causative mutations (non-FH-GH).
Methods. The study included probands with a clinical diagnosis of FH and their families attending two lipid clinics in Spain. Inclusion criteria for probands were: LDL-cholesterol >95th percentile, triglycerides <90th percentile, at least one first-degree family member with LDL-cholesterol >90th percentile, >5 points in the Dutch Lipid Clinic Network criteria score, and absence of mutations in *LDLR*, *APOB*, *PCSK9* or *APOE.* Eleven FH families with a *LDLR* mutation were also examined for comparison.
Results. We analyzed 49 non-FH-GH probands and 277 first and second-degree relatives. LDL-cholesterol was >90th percentile in 37.8% of blood relatives, at concentrations similar to those of probands. LDL-cholesterol had a normal distribution in non-FH-GH families, in contrast with a bimodal distribution in FH families. When a dominant model was tested, family-based association tests gave much lower heritability values for total cholesterol and LDL-cholesterol in non-FH-GH (0.39 and 0.32, respectively) than in FH (0.78 and 0.61, respectively).
Conclusion. Non-FH-GH families have a milder lipid phenotype than genetically defined FH. The heritage pattern of LDL-cholesterol in non-FH-GH does not fit with a monogenic disorder. Our findings support the concept that most non-FH-GH are polygenic hypercholesterolemias.

Keywords: Familial Hypercholesterolemia, hypercholesterolemia, family study, segregation, heritability

Introduction

Familial hypercholesterolemia (FH) is a genetic disorder clinically characterized by very high plasma concentrations of total cholesterol (TC) due to increased LDL cholesterol (LDLc) and high prevalence of cardiovascular disease (CVD), mainly as coronary heart disease. Classical studies described about 50% of first degree relatives affected and an estimated prevalence of nearly 1:500 in the general population.[1] Recent data reveal a wide variability in FH prevalence, from 1:70 estimated among populations with a founder gene effect in South Africa to 1:200 described in the Danish population of the Copenhagen General Population study using clinical diagnosis based on the Dutch Lipid Clinic Network (DLCN) score.[2,3] However, in the latter study, only 20% of those with a clinical diagnosis of FH had a functional mutation in *LDLR* or *APOB* although only the most frequent mutations in LDLR and APOB were screened for in their population.[3] These data could be explained by either the existence of other genes involved in the pathogenesis of FH[4] or the lack of specificity of clinical diagnostic criteria for FH.[5] In fact, some of these criteria, such as CVD or elevated LDLc, are frequently present in the general population.[6] In contrast, the presence of tendon xanthomas increases the specificity for genetically defined monogenic FH.[5]

Defects in three different genes are well-defined causes of FH: *LDLR,* the gene coding for the LDL receptor; *APOB,* coding for apolipoprotein (apo) B; and *PCSK9,* which codes for the enzyme proprotein convertase subtilisin/kexin type 9.[2,4] Recently, defects in two new putative genes causing FH have been identified: the p.Leu167del mutation in *APOE,* [7] and several functional mutations in the signal transducing adaptor family member STAP1.[8] However, in spite of extensive genetic searching, including exome analyses,[9] up to 40% of clinically diagnosed FH cases do not harbor major disease-causing mutations.[3,5,10] This group of patients, henceforth named “Non-FH Genetic Hypercholesterolemias” (non-FH-GH) show milder phenotypes than FH subjects with a positive genetic defect, both regarding risk for CVD and LDLc concentrations,[3,5] and it has been suggested that their raised LDLc might have a polygenic cause.[11] However, the clinical characteristics and lipid phenotype of offspring and the penetrance and heritability of HC in non-FH-GH subjects, which would support a monogenic disorder, have not been described. Hence, there is increasing evidence that a single gene defect may not underlie this clinical phenotype. To test this hypothesis, we selected families where, in addition to the proband, at least one related family member had to have a lipid phenotype that also fulfilled the criteria for FH. We further selected pedigrees where no mutations in candidate genes were identified. We then tested assembled pedigrees for patterns of inheritance and estimated genetic contribution. ~~to clinical to non-FH-GH.~~

Material and Methods

Selection of probands.

From January 2010 to December 2014, consecutive non-FH-GH subjects attending the lipid clinics of HUMS in Zaragoza and Hospital Clínic in Barcelona were invited to participate in this family study. All participants, probands and family members, signed an informed consent to the protocol approved by the ethical institutional review boards of the two institutions. This work has been carried out in accordance with the Declaration of Helsinki for experiments involving humans.

Inclusion criteria for the probands included: age older than 18 y, TC and LDLc >95th percentile and triglycerides (TG) <90th percentile according to the age-sex distribution of the Spanish population,[12] at least one first degree family member with LDLc >90th percentile, a score >5 points in the DLCN criteria, at least 3 first degree family members alive and available for testing, and absence of FH pathogenic mutations in *LDLR*, *APOB*, *PCSK9* (searched with the Lipochip® platform, Progenika-Biopharma Grifols, Derio, Spain)[13] and *APOE.*[14] The specificity and sensitivity of Lipochip® for the detection of 118 different mutations in the LDLR is 99.7% and 99.9% respectively. Furthermore, samples with a negative result underwent large rearrangement analysis by quantitative fluorescence-based multiplex PCR; and if this analysis was also negative, DNA sequencing was carried out to identify new disease-causing variations.[13] Patients with secondary causes of HC such as hypothyroidism (TSH >6 mU/L), renal disease with glomerular filtration rate <30 ml/min, liver disease (ALT >3 times upper normal limit), nephrotic syndrome, pregnancy, obesity (body mass index, BMI, >30 kg/m2), autoimmune disease, type-2 diabetes, or use of drugs known to raise LDLc were excluded as probands for the study. The presence of an *APOE* ε2/ε2 genotype was also a criterion for exclusion.

Selection of family members.

We attempted recruiting the largest possible number of relatives of each proband, including parents, siblings, spouses, children, nephews and nieces. However, adopted family members were excluded in further analysis except spouses for the inheritance model analysis.

Clinical assessment.

Probands and family members were assessed for CVD risk factors (smoking, hypertension, overweight), alcohol consumption, current medical treatment and personal history of CVD. The physical examination included anthropometric measurements, blood pressure and a search for superficial lipid deposits (arcus cornealis, xanthelasmas, and tendon xanthomas). BMI was calculated as weight (kilograms) divided by height (meters) squared.

Lipid profile.

A fasting (8-10 h) blood sample was obtained in all participants after 6 weeks without lipid-lowering drugs. In subjects with prior CVD or calculated very high CVD risk (European guidelines) baseline lipid values were obtained from medical records. TC and TG were determined by conventional enzymatic assays. High-density lipoprotein cholesterol (HDLc) was measured by an immunoprecipitation assay. Lipoprotein (a) (Lp(a)), apo A-I, apo B and C-reactive protein (CRP) were determined by nephelometry using a IMMAGE-Immunochemistry System (Beckmann Coulter). LDLc was calculated with the Friedewald formula.

Definition of hyperlipidemia.

HC was defined by LDLc values above 90th age-sex specific percentiles.[12] Subjects with TG above 90th percentile were classified as high TG. Mixed hyperlipidemia was defined when both HC and high TG were present. Secondary causes of hyperlipidemia were also exclusion criteria for family members.

Probands and families with genetically confirmed familial hypercholesterolemia.

For the purpose of comparing the inheritance pattern and pedigree characteristics, 11 FH families of a proband with a functional *LDLR* mutation were selected at random from a group of 158 genetically confirmed FH patients concomitantly evaluated at the lipid clinics. These probands and their first-degree relatives were assessed following the same study protocol used in non-FH-GH families.

Statistical analyses.

All statistical analyses were performed using the SPSS software v.20 (SPSS Inc. Chicago, IL), except when noted. Data are presented as mean ± standard deviation (SD) for continuous variables, as median and interquartile range for variables with a skewed distribution, and as a frequency or percentages for categorical variables. Differences in mean values of variables with a normal distribution were assessed using t-tests or ANOVA, and the Mann-Whitney U-test or H- Kruskall-Wallis test were used for variables with a skewed distribution. Categorical variables were compared using the chi-square test.

Genetic models and heritability of hypercholesterolemia.

To detect inheritance patterns, TC, LDLc, HDLc and TG data were analyzed with FBAT (Family-Based Association Tests) using the PBAT module implemented by Golden Helix SNP & Variation Suite v7.7.8 software (Golden Helix, Bozeman, MT), thereby seeking to find a plausible model of transmission.[15] This analysis is based on a generalization of the original method of transmission disequilibrium test (TDT), which uses trios, the simplest design to test associations in family studies.[16] The trios include an affected offspring and both parents. Homozygous parents are discarded and only transmissions from heterozygous parent to offspring are considered (informative trio). The expected distribution is constructed using Mendel's segregation laws taking into account any confounding variable (covariates),

hence the usual problems caused by incorrect specification of the model as well as the mixture or population stratification are avoided.

The choice of a genetic model is based on the observed segregation analysis in families. The dominant model tested the association of carrying at least one pathogenic allele versus carrying none. The recessive model tested the association of carrying the pathogenic allele in homozygous form versus carrying one o none. TC, TG, HDLc, and LDLc serum values were considered as dependent variables to test the inheritance model. Sex, age, BMI and the *APOE* genotype were considered as covariates. The three *APOE* alleles (ε2, ε3 and ε4) were introduced as combinations of two non-synonymous SNVs, rs429358 (T->C) and rs7412 (C->T), which codify amino acids at positions 112 and 158, respectively. Except for the analysis of TG, the Lp(a) concentration (as natural logarithm: LN Lp(a)) was also a covariate in every case; the HDLc concentration was a covariate in LDLc analyses; and the LDLc concentration was included as covariate in HDLc analyses. For comparing results obtained by FTAB when analyzing a well-established genetic disease, data from the 11 FH probands and 67 first-degree relatives were included.

Heritability (h^2) is defined as the proportion of phenotypic variance explained by the analyzed marker.[17] A negative sign h^2 indicates an inverse correlation between phenotype and number of disease alleles transmitted in accordance with the considered genetic model. H^2 was estimated by FBAT, while the General Linear Model (GLM, implemented in SPSS software v.20) was used for comparing TC, TG, HDLc and LDLc concentrations (dependent variables) among groups. The models included the above covariates and *a posteriori* simple contrasts analyses were performed.[18] Eta-squared (η^2) measures the effect size from ANOVA. Partial eta squared (partial η^2) was estimated for dependent and significant covariates, while removing the effects of any significant variables.[19]

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Sample size.

According to Laird and Lange,[20] a sufficiently large sample for FBAT analysis could consist of at least 10 informative trios. To improve statistical power and to assess a large enough number of non-FH-GH subjects, representative of the heritability and phenotype of these families, a total of 50 families were projected for the final analysis.

Results

Probands and relatives.

During the study period, a total of 1648 unrelated patients with the clinical diagnosis of primary GH were assessed at the two lipid clinics, and 243 probands fulfilled inclusion and exclusion criteria. Those who met inclusion criteria were consecutively invited to participate until the projected number of 50 families was reached. After the initial characterization of probands, one family was excluded because of a complex assignation of parenthood. The

clinical characteristics of the 49 probands finally selected were similar to the rest of non-FH-GH evaluated in our lipid clinics (data not showed). The 49 families studied included a total of 277 first and second-degree relatives, 249 blood relatives (89.9%), and 28 spouses (10.1%). The median number of relatives included per family was 7 (range 3-12). A total of 94 subjects (37.8% of blood relatives) had LDLc above the 90th percentile. In this group of blood relatives, 23 (9.3%) subjects belonging to 17 families (34.7% of the families) showed mixed hyperlipidemia.

Characteristics of non-FH-GH probands.

The characteristics of the 49 probands included in the study are described in Table 1. There were more women (63%) than men and the median age was 53 y (range, 18-86 y). TC and LDLc concentration were similar in men and women. There were no between-sex differences in other lipid parameters except for a lower HDLc in men. BMI (nearly significantly) and waist circumference (significantly) were higher in men. Few patients had a history of prior CVD, and only one proband showed tendon xanthomas.

Comparison between probands and first degree relatives.

TC and LDLc concentrations were similar between probands and HC relatives, while HC relatives had lower apo B levels (Table 2). When we compared participants with and without HC, non-affected relatives were younger and expectedly had lower TC and LDLc concentrations. Non-affected relatives showed lower values of HDLc and TG than their HC relatives. Lp(a) and CRP values were similar in probands, HC relatives and non-affected relatives (Table 2).

LDL cholesterol distribution.

The distribution of LDLc values for all blood family members from non-FH-GH and FH is showed in Figure 1 (panel A and panel B, respectively). The clinical characteristics of FH subjects is presented in Supplemental Table 1). Compared to non-FH-GH individuals, FH families had higher values that showed a clear bimodal distribution. According to Z of the Kolmogorov-Smirnov test, the distribution of LDLc in non-FH-GH subjects was normal ($p=0.338$), while that of FH subjects was skewed ($p=0.039$). In subjects from non-FH-GH families, the prevalence of HC increased with age from 20% in those younger than 40 y to up to 60% in those older than 60 y (Figure 2). The median proportion of subjects with HC per family was 50% (range, 12.5 to 100). The percentage of HC subjects per family was independent of the number of studied family members.

Inheritance model analysis.

When comparing the TC and LDLc distributions in our sample to a hypothetical model of autosomal dominant distribution, non-significant differences were obtained (chi-square=0.014; $p=0.907$). Table 3 shows the results of association analyses based on non-FH-GH families for lipid concentrations as dependent variables respect to a hypothetical

locus with an autosomal dominant inheritance pattern. Both TC and LDLc concentrations showed association with an autosomal dominant allele, with h^2 values of 0.39 for TC and 0.32 for LDLc. TG and HDLc were not associated with the dominant allele. A model for TG segregation was searched with the same procedure. In this case, only subjects within families with at least one member displaying elevated TG were introduced in the model. The TG distribution in our sample agreed with an autosomal recessive inheritance respect to a hypothetical recessive locus (chi-square=0.065; p=0.799). TG heritability fitted a recessive allele (h^2=0.18). We observed a small albeit significant association of this recessive allele, with heritabilities of 0.059 and 0.035 for TC and LDLc, respectively. Although HDLc showed a significant negative association with the recessive allele, the heritability value was negligible (h^2=- 0.01). The same analysis in the 11 FH families showed a significant association for an autosomal dominant model, with heritabilities of 0.78 and 0.61 for TC and LDLc, respectively. Neither HDLc nor TG showed any association with this locus in the FH families (Table 4).

According to the previous inheritance model analysis; subjects were assigned to a genetic group based on their lipid phenotype. These groups refer to the presence of hypothetical causal alleles according to the inheritance model for TC and TG. Non-affected subjects were predicted to show no allele related to HC or high TG. The prediction for the HC phenotype was that affected subjects would carry, at least, one dominant HC causal allele; subjects with isolated high TG were predicted to carry two recessive high TG alleles, and those with mixed hyperlipidemia should carry dominant HC and recessive TG alleles. The combined effect of the genetic group and other cofactors, including age, sex, BMI, and Lp(a) on the lipid phenotype was analyzed by ANOVA (Table 5). The genetic group assignment explained 33.5% ($p<0.05$) of the TC variation (Partial η^2). A significant positive effect of age was also detected ($F=30.904$; $p<0.001$; partial $\eta^2= 0.124$). Similar results were obtained for LDLc, explaining 29.5% of the observed variability. Age explained 8.7% of the variability of LDLc concentrations ($F=22.109$, $p<0.001$), while Lp(a) explained 3.6% ($F=8.799$, $p=0.003$). Significant differences among groups for TG concentrations were detected, explaining 15.4% of TG variability. No confounding variables showed significant effects. No significant differences among groups were detected for HDLc concentrations, for which the power value was low ($p=0.106$; power=0.524).

Discussion

To the best of our knowledge, this is the first study analyzing lipid segregation in a large group of families with GH without mutations in known FH-causing genes. Our results show that elevated LDLc in these families has a genetic component that clearly differs from classical FH. Non-FH-GH is genetically more complex, not fully explained by a monogenic

defect, displaying a milder hypercholesterolemic phenotype and lower penetrance and disease expression than FH. Actually, LDLc in these families has an almost normal distribution that clearly deviates from the bimodal distribution of LDLc expected in a monogenic disease, as shown for FH families with well-defined *LDLR* mutations. Our findings support the concept that many non-FH-GH individuals have polygenic HC.

The most widely used clinical criteria to diagnose FH, the DLCN and Simon Broome scores, have high sensitivity but low specificity for detecting FH.[5] In fact, in many subjects with a clinical diagnosis of FH, particularly those labeled as probable FH who do not carry a mutation in candidate genes, exome analyses do not detect major defects in other genes.[9] Clearly there is an important overlap between the phenotypes of monogenic FH and non-FH-GH, many of whom show a polygenic component. This overlap probably explains the observation in several population studies of a more frequent than expected prevalence of clinical FH, as high as 1:200 in a large Danish cohort;[3] however, only 20% of cases were carriers of FH-causing mutations in this series. These data have been confirmed recently in a larger sample from the same population.[21] A similar unexpected high prevalence of clinically diagnosed FH has been observed in the NHANES survey in the United States.[22]

The first step in the diagnosis of a dominant monogenic disease such as FH is to demonstrate a Mendelian transmission of the HC phenotype. Current diagnostic algorithms focus mostly on the LDLc concentration and the history of CVD rather than the heritage pattern.[2] All HC probands in our study had a clinical diagnosis of "probable or definitive FH" by DLCN criteria above 5, but the inclusion criteria used in our study permitted the recruitment of subjects without a clear monogenic inheritance pattern, as done in most studies of presumed FH populations.[3,5,6,9,21,22] In our study of non-FH-GH there was a substantial number of affected relatives in all cases, but the heritability differed from that characteristic of FH. The mean penetrance in our study was 48.3% (range 12.5-100%) and there were no differences depending on the number of relatives included in each family study.

In our study, the heritability of non-FH-GH corresponding to a single locus was 32.2% for LDLc, far from the 78% figure observed with the same method for FH families. Recently, Talmud et al.[11] analyzed the heritability component of common single nucleotide variations (SNVs) in genes linked to cholesterol metabolism that influence LDLc concentrations, such as *CELSR2* (cadherin EGF LAG 7-pass G-type receptor 2), *APOB, ABCG5/8, LDLR* and *APOE* in subjects with clinically defined FH with and without causal mutations and population controls. Twelve common LDLc raising SNVs were analyzed and a weighted LDLc-raising gene score was constructed. This score was significantly higher in mutation-negative FH subjects than in controls, but the proportion of the LDLc variation explained was low (<20%).[11] Replication analyses in other populations, including children, have shown similar

results.[23,24] We have previously reported that intestinal cholesterol hyperabsorption plays a role in the cause of non-FH-GH,[25] but the co-segregation of LDLc with phytosterols as surrogate markers of cholesterol absorption did not support a monogenic defect.[26]

Whereas high TG was an exclusion criterion for the probands in our study, it was a common feature among family members. However, although high TG was more prevalent in family members with high LDLc, TG segregated within the families independently of the major loci associated with high LDLc. A total of 34.7% of the families included at least one blood member with mixed hyperlipidemia, hence meeting accepted criteria for familial combined hyperlipidemia (FCHL). The subjects with mixed hyperlipidemia had higher BMI than the remaining family members and affected subjects with isolated HC (Supplemental Table 2). Clearly, there is also overlapping between non-FH-GH and FCHL. FCHL is the result of the interaction between a polygenic background and environmental factors, mainly overweight.[27] Given that polygenes affecting TG in FCHL are mostly different from those associated with high LDLc,[28] it is reasonable to surmise, as supported by our results, that FCHL subjects combined a genetic background associated with non-FH-GH, common SNVs associated with high TG and LDLc, and environmental factors, some of them aggregating to explain familial clustering of the phenotype. In summary, our results identify FCHL as a clinical phenotype within non-FH-GH with some subjects influenced by TG polygenes and a more extreme environmental factor, mainly high BMI, as previously reported.[29]

Conclusion.

Non-FH-GH is a heterogeneous entity different from FH. In spite of a positive family history, the heritage pattern does not fit with a monogenic disease, rather with a complex genetic disease with major loci and polygenic influences. Non-FH-GH families have a milder lipid phenotype, tendon xanthomas are seldom present, and LDLc shows a continuous normal distribution. The influence of age favors the phenotypic expression of HC, and the family study often detects members with mixed hyperlipidemia, suggesting that non-FH-GH and FCHL are polygenic lipid disorders sharing many genetic influences.

Acknowledgements

This study was supported by grants from the Spanish Ministry of Economy and Competitiveness PI12/01087, PI12/01703 and PI12/01321 integrated in the Plan Nacional de I+D+I and cofinanced by ISCIII-Subdirección General de Evaluación and Fondo Europeo de Desarrollo Regional (FEDER); Red de Investigación Cardiovascular (RIC) RD12/0042/0055 and CIBEROBN. RIC and CIBEROBN are initiatives of ISCIII, Spain.

Disclosures

Authors report no conflicts of interest related to this work. All authors have approved the final article.

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Table 1. Characteristics of Non-FH-GH probands by sex

	Males N=18	Females N=31	P value*
Age, years	52.7 (±13.7)	53.5 (±8.8)	0.806
Ever smoker, N (%)	11 (61.1)	15 (48.4)	0.390¶
Prior cardiovascular disease, N (%)	3 (16.7)	1 (3.2)	0.109¶
Hypertension, N (%)	3 (16.7)	8 (25.8)	0.464¶
Body mass index, kg/m2	26.3 (±2.62)	24.4 (±3.86)	0.080
Waist circumference, cm	95.3 (±7.30)	82.6 (±10.2)	<0.001
Tendon xanthomas, N (%)	1 (5.6)	0 (0)	--
Total cholesterol, mg/dL	299 (±55)	309 (±41)	0.492
Triglycerides, mg/dL	133 (±58)	103 (±44)	0.051
HDL cholesterol, mg/dL	54 (±15)	67 (±17)	0.009
LDL cholesterol, mg/dL	219 (±52)	221 (±39)	0.873
Lipoprotein(a), mg/dL	31.1 (13.1-71.4)	41.4 (20.7-86.6)	0.267†
Apolipoprotein A1, mg/dL	156 (±34)	176 (±32)	0.057
Apolipoprotein B, mg/dL	156 (±36)	154 (±33)	0.808
C-reactive protein, mg/L	0.54 (±0.89)	1.24 (±1.32)	0.065
Fasting glucose, mg/dL	98 (±16)	93 (±11)	0.410

*Values are mean (±SD), median (25th–75th), or proportions (%). T-test was used for between-group comparisons of quantitative continuous variables, †U-Mann Whitney for non-parametric variables, and ¶Chi square for qualitative variables.

Table 2. Comparison of probands and affected and non-affected blood relatives according to the lipid phenotype

	Probands N=49	Relatives with high LDLc N=94	Non-affected relatives N=155	P value*
Age, years	53.2 (±10.7)	51.0 (±17.3)	41.3 (±17.6)[b,c]	<0.001
Ever smoker, N (%)	26 (53.1)	50 (54.3)	76 (50)	0.792
Men, N (%)	18 (36.7)	45 (47.9)	82 (52.9)	0.140
Prior cardiovascular disease, N (%)	4 (8.2)	1 (1.1)	6 (4.1)	0.445
Hypertension, N (%)	11 (22.4)	25 (27.2)	22 (14.3)	0.042
Diabetes, N (%)	0 (0)	3 (3.2)	6 (3.9)	0.208
Body mass index, kg/m2	25.1 (±3.54)	25.2 (±4.89)	24.9 (±4.67)	0.815
Waist circumference, cm	87.3 (±11.0)	89.6 (±13.0)	89.6 (±12.8)	0.513
Total cholesterol, mg/dL	305 (±46)	295 (±44)	205 (±37)[b,c]	<0.001
Triglycerides, mg/dL	106 (73-144)	112 (86-161)	88 (66-123)[c]	0.001
HDL cholesterol, mg/dL	62 (±17)	61 (±16)	56 (±14)[c]	0.011
LDL cholesterol, mg/dL	220 (±44)	206 (±46)	128 (±30)[b,c]	<0.001
Lipoprotein(a), mg/dL	33.1 (18.6-77.6)	27.6 (10.6-61.6)	23.9 (9.8-57.3)	0.097
Apolipoprotein A1, mg/dL	169 (±34)	170 (±30)	159 (±27)	0.09
Apolipoprotein B, mg/dL	155 (±34)	142 (±32)[a]	96 (±23)[b,c]	<0.001
C-reactive protein, mg/L	1 (0.55-2.85)	1.3 (0.6-2.85)	1 (0.5-2.5)	0.391
Fasting glucose, mg/dL	87 (81-94)	84 (80-93)	85 (80-93)	0.636
APOE ε2/ε4, N (%)	0 (0)	3 (3.3)	3 (2)	0.101†
APOE ε3/ε2, N (%)	0 (0)	5 (5.6)	12 (8.1)	

APOE ε3/ε3, N (%)	37 (75.5)	58 (64.4)	105 (70.9)
APOE ε3/ε4, N (%)	12 (24.5)	24 (26.7)	26 (17.6)
APOE ε4/ε4, N (%)	0 (0)	0 (0)	2 (1.4)

*Values are mean (±SD), median (25th–75th), or proportions (%). P value for ANOVA comparison among the three groups. † Fisher´s exact test

a: p<0.05 for the comparison between probands and non-affected relatives

b: p<0.05 for the comparison between probands and non-affected relatives

c: p<0.05 for the comparison between relatives with high LDLc and non-affected relatives

Table 3. Heritage pattern analysis and heritability by FBAT in non-FH-GH families

Dependent Variable	Dominant model (number of informative trios=55)			Recessive model (number of informative trios=23)			Covariates
	P value (FBAT)	Power (FBAT)	Heritability	P value (FBAT)	Power (FBAT)	Heritability	
Total cholesterol	3.92E-14	0.999	0.389*	0.001	0.934	0.059*	Sex, age, BMI, *APOE*
Triglycerides	0.114	0.250	0.028	3.79E-06	0.999	0.178*	Sex, age, BMI, *APOE*
HDL cholesterol	0.600	0.855	0.118	0.047	0.124	-0.012*	Sex, age, LDLc, BMI, *APOE*
LDL cholesterol	2.80E-14	0.999	0.322*	0.005	0.674	0.035*	Sex, age, HDLc, BMI, *APOE*

FBAT indicates Family- Based association tests; BMI, body mass index

*Statistically significant model. Variables with significant univariate association with the lipid profile were included as covariates.

Table 4. Heritage pattern analysis and heritability by FBAT in genetically defined FH families

Dependent variable	Dominant model (number of informative trios=19)			Covariates
	P value (FBAT)	Power (FBAT)	Heritability	
Total cholesterol	1.762E-06	1	0.780*	Sex, age, BMI
Triglycerides	0.214	0.464	0.129	Sex, age, BMI
HDL cholesterol	0.392	0.308	0.068	Sex, age, LDLc, BMI
LDL cholesterol	0.0041	0.999	0.606*	Sex, age, HDLc, BMI

FBAT indicates Family- Based association tests; BMI, body mass index

*Statistically significant model. Variables with significant univariate association with the lipid profile were included as covariates.

Table 5. Lipid profile depending on the genetic group related to the lipid phenotype calculated by General Lineal Model*

Variable	Genetic group				F (mean comparison)	P value	Partial η^2	Power
	Non-affected (n=137)	High LDLc (n=117)	High TG (n=10)	Mixed hyperlipidemia (n=23)				
Total cholesterol, mg/dL	204 (±35.2)[a]	305 (±52.4)[c]	248 (±33.1)[b]	319 (±37.8)[c]	36.6	<0.001	0.335	1.000
Triglycerides, mg/dL	93.9 (±34.7)[a]	108 (±36.2)[a]	395 (±408)[b]	269 (±140)[b]	13.4	<0.001	0.154	1.000
HDLc, mg/dL	56.1 (±13.2)[a]	64.9 (±15.8)[a]	43.1 (±12.3)[a]	49.6 (±11.5)[a]	2.06	0.106	0.026	0.524
LDLc, mg/dL	129 (±29.7)[a,]	218 (±52.8)[b]	153 (±36.4)[a]	219 (±32.9)[b]	32.5	<0.001	0.295	1.000

*Covariates included for every dependent variable: sex, age, BMI, and *APOE* genotype. Lp(a) was also included for TC, HDLc and LDLc; LDLc for HDLc; and HDLc for LDLc .

a,b,c, : Values within a row with different superscripts differ significantly at P <0.05.

Partial η^2 refers to the percentage of the variation in each lipid variable adjusted for covariates explained the genetic group assignment

Figure 1. LDL cholesterol distribution in non-FH-GH (panel A) and FH families (panel B)

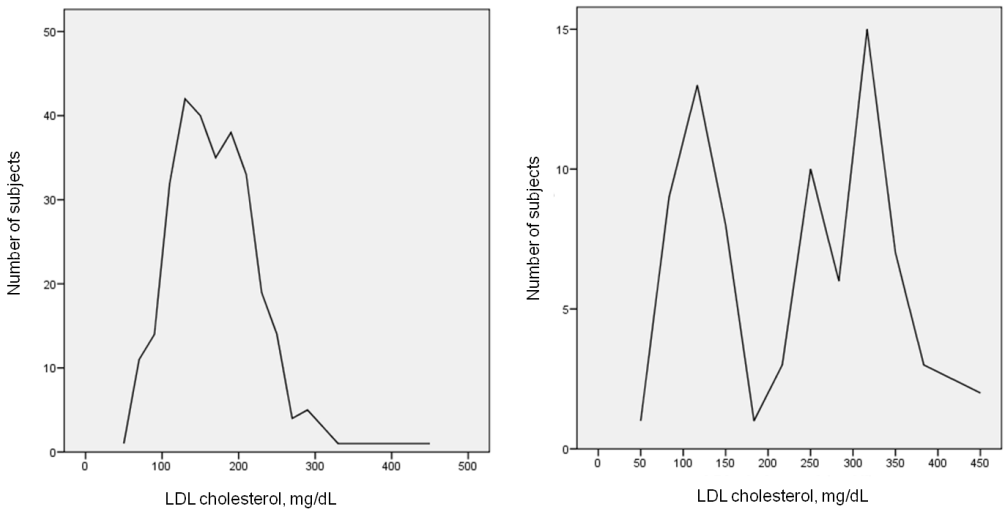
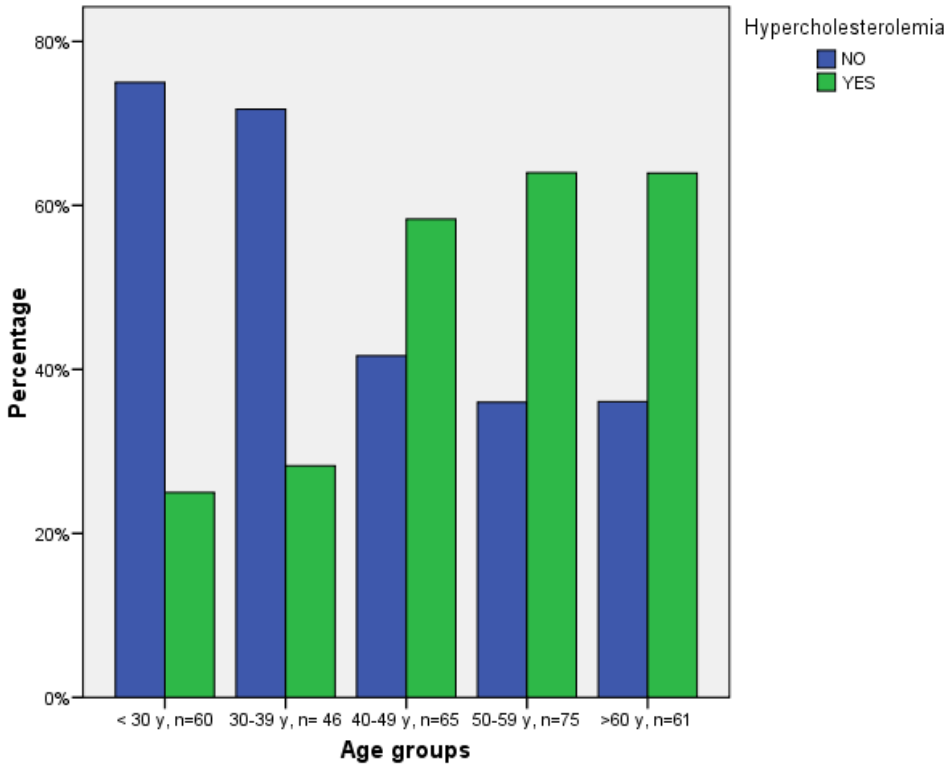


Figure 2. Percentage of non-FH-GH blood family members with and without hypercholesterolemia (LDLC >90th percentile) distributed by age groups



Supplemental Table 1. Characteristics of affected and non-affected subjects from genetically confirmed FH families

	Affected N=46	Non-affected N=32	P value*
Age, years	44.9 (±19.6)	49.2 (±19.9)	0.467
Men, N (%)	18 (39.1)	19 (59.4)	0.078
Body mass index, kg/m2	23.7 (±4.05)	25.7 (±5.30)	0.111
Prior cardiovascular disease, N (%)	4 (8.7)	0 (0)	0.087
Tendon xanthomas, N (%)	14 (30.4)	0 (0)	0.001
Total cholesterol, mg/dL	379 (±63.6)	212 (±40.1)	<0.001
Triglycerides, mg/dL	100 (±51.3)	116 (±57.6)	0.206
HDL cholesterol, mg/dL	57.6 (±13.1)	54.4 (±10.5)	0.257
LDL cholesterol, mg/dL	305 (±57.7)	121 (±38.0)	<0.001
Lipoprotein(a), mg/dL	49.5 (24.5-86.2)	31.3 (12.3-72.4)	0.267

*Values are mean (±SD), median (25th–75th), or proportions (%). T-test was used for between-group comparisons of quantitative continuous variables, †U-Mann Whitney for non-parametric variables, and ¶Chi square for qualitative variables.

Supplemental Table 2. Characteristics of affected subjects from non-FH-GH families with isolated hypercholesterolemia and mixed hyperlipidemia.

	Isolated HC N=117	Mixed hyperlipidemia N=23	P value*
Age, years	50.9 (±16.5)	47.6 (±13.9)	0.290
Men, N (%)	50 (42.7)	18 (78.3)	<0.001
Body mass index, kg/m2	24.3 (±3.92)	28.3 (±5.25)	<0.001
Prior cardiovascular disease, N (%)	4 (3.4)	1 (4.3)	0.692
Diabetes, N (%)	2 (1.7)	2 (8.70)	0.091
Total cholesterol, mg/dL	302 (±52.9)	293 (±50.2)	0.404
Triglycerides, mg/dL	106 (±36.8)	302 (±250)	<0.001
HDL cholesterol, mg/dL	64.6 (±16.4)	48.1 (±12.0)	<0.001
Non-HDL cholesterol, mg/dL	237 (±50.2)	246 (±47.5)	0.375
Lipoprotein(a), mg/dL	31.6 (14.1-61.6)	16.8 (3.09-44.5)	0.123
Glucose, mg/dL	87.1 (±11.6)	97.4 (±49.3)	0.239

HC denotes hypercholesterolemia. *Values are mean (±SD), median (25th–75th), or proportions (%). T-test was used for between-group comparisons of quantitative continuous variables, †U-Mann Whitney for non-parametric variables, and ¶Chi square for qualitative variables.