European Journal of Human Genetics (2002) 10, 367–374

www.nature.com/ejhg

ARTICLE

# Segregation analysis of HDL cholesterol in the NHLBI Family Heart Study and in Utah pedigrees

Florian Kronenberg*[,1,2], Hilary Coon[3], R Curtis Ellison[4], Ingrid Borecki[5], Donna K Arnett[6], Michael A Province[5], John H Eckfeldt[7], Paul N Hopkins[1] and Steven C Hunt[1]

[1]*Department of Cardiovascular Genetics, University of Utah, Salt Lake City, Utah, USA;* [2]*University of Innsbruck, Institute of Medical Biology and Human Genetics, Innsbruck, Austria;* [3]*Department of Psychiatry, University of Utah, Salt Lake City, Utah, USA;* [4]*Section of Preventive Medicine and Epidemiology, Boston University, School of Medicine, Boston, Massachusetts, USA;* [5]*Division of Biostatistics, University of Washington, St. Louis, Missouri, USA;* [6]*Division of Epidemiology, University of Minnesota, Minneapolis, Minnesota, USA;* [7]*Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, USA*

**We investigated the genetic determination of high-density-lipoprotein cholesterol (HDL-C) levels in the NHLBI Family Heart Study by segregation analysis. Included was a total of 3755 subjects from 560 randomly selected nuclear families and 522 families selected due to a high family risk of coronary heart disease (CHD). In the whole dataset, there was no evidence for an allele at a major gene locus responsible for HDL-C levels lower than the population mean or even for significant bimodality for low levels of HDL-C. However, we observed evidence for a recessive allele that was associated with higher HDL-C levels than average. This evidence for a recessive major gene was independent of triglyceride concentrations and was most strongly observed in families recruited for CHD. The environmental model was rejected ($P$=0.0027) while the codominant and recessive models were not rejected ($P$=0.085 and $P$=0.133, respectively). The dominant model was also rejected ($P<0.0001$). In the recessive segregation model, the means of those inferred to be homozygous for the high HDL-C allele and those without the high HDL-C allele were separated by about 25 mg/dl HDL-C (73.9 $\pm$ 1.99 *vs* 48.2 $\pm$ 0.36 mg/dl). Because these results were unexpected, segregation was tested in a separate sample of 2013 individuals in 85 large pedigrees ascertained for early heart disease deaths, early stroke deaths, and early hypertension in Utah. Similar evidence for an allele at a major gene locus for high HDL-C was found. In summary, we did not find evidence for an allele at a major gene locus associated with low HDL-C levels segregating in pedigrees recruited for the NHLBI Family Heart Study, or in pedigrees ascertained in Utah for early CHD or related phenotypes. Instead we found some evidence for the segregation of an allele associated with high HDL-C.**
*European Journal of Human Genetics* (2002) **10,** 367–374. doi:10.1038/sj.ejhg.5200818

**Keywords:** HDL cholesterol; segregation analysis; atherosclerosis; genetics; Family Heart Study

## Introduction

The concentrations of high-density-lipoprotein cholesterol (HDL-C) have been demonstrated to be negatively associated with the risk for coronary heart disease (CHD).[1–5] Besides environmental and behavior factors, a genetic influence on HDL-C levels has been suggested. Several studies described familial aggregation of HDL-C.[6–13] The correlation between the midparent and the child levels of HDL-C was found to be about 0.40 in an investigation in adolescent children living in the same household as their parents.[9] A study in 139 families consisting of the parents and one child reported a correlation of

*Correspondence: Florian Kronenberg, MD; Institute of Medical Biology and Human Genetics, University of Innsbruck, Schöpfstr. 41, A-6020 Innsbruck, Austria; Tel: (+43) 512 507-3474; Fax: (+43) 512 507-2861; E-mail: Florian.Kronenberg@uibk.ac.at
Received 23 January 2002; revised 5 April 2002; accepted 9 April 2002

0.36 for HDL-C between the average parental and the child value.[10] Similar correlations were reported in the Heritage Family Study.[12] Weak parent–child correlations, however, were described in the Cuenca Study.[13] Results from the NHLBI Family Heart Study (FHS) observed in randomly selected families a correlation of 0.32 and 0.29 for parent–child and sibling–sibling HDL-C, respectively, but less than 10% of this correlation was explained by lifestyle habits such as alcohol consumption, physical exercise and smoking.[11] This is in accordance with findings in dizygotic twins showing a correlation of 0.39 for HDL-C, which was only slightly reduced after adjustment for lifestyle and other environmental variables.[14] Furthermore, the genetic heritability was calculated to be $0.485 \pm 0.029$ in data from the Lipid Research Clinics Program Family Study.[15] While heritability for plasma HDL-C levels appears to be high, efforts to fit genetic models with complex segregation analysis have led to conflicting results. Some found no evidence for a major gene,[16–19] others found evidence for major genes with various modes of transmission[20–26] (for details see Table 1). We therefore analysed the segregation of HDL-C in the NHLBI FHS as well as in a separate set of Utah pedigrees.

## Subjects and methods

### Population

This analysis is based on the subjects recruited for the FHS, a population-based, multicenter study aimed to identify genetic and nongenetic determinants of cardiovascular disease. Probands were identified from three parent cohort studies, the Forsyth, (NC) and Minneapolis, (MN) cohorts of the ARIC study, the Framingham Heart Study Offspring Cohort, and the Utah Family Tree Study. Extended information about design and objectives of FHS is given elsewhere.[27]

In Phase I of FHS, 2000 randomly selected population-based participants and 2000 with family histories of CHD (about 500 from each group and each of the field centres) were identified among 14 592 middle-aged participants in epidemiological studies. Medical histories from these individuals, their parents and siblings, were used to calculate family risk scores which compared the number of reported and validated CHD events with the number expected based on the size, sex and age of family members. In phase II of the study, a total of 661 families with the highest risk scores and early onset of CHD, and 592 randomly sampled families (about 150 from each group and each of the field centres) had a detailed clinical and laboratory investigation including interviews by experienced interviewers. Clinical data obtained included anthropometry, blood pressure, blood samples for laboratory tests, electrocardiogram, pulmonary function, ultrasound of carotid arteries, and questionnaires supplying information about socio-economic status, lifestyle, medical history, medication use, food intake, nicotine and alcohol consumption, physical exercise, family structure, and psychosocial characteristics.

The analyses were repeated using data from 2013 individuals in 85 Utah extended pedigrees. The majority of these subjects (most within large pedigrees) were ascertained through sibships with two or more coronary heart disease deaths before the age of 55 (18 pedigrees), or stroke deaths before the age of 75 (9 pedigrees). These families were selected through a statewide file of 140 000 death certificates computer-linked to genealogical records for 1.2 million people in Utah. The rest of the sample (most within small pedigrees) was ascertained through probands selected

**Table 1** Studies investigating the segregation of HDL-C

| *Reference* | *Study subjects* | *Ascertainment by* | *Finding* |
|---|---|---|---|
| [16] | 31 kindreds with 351 individuals[a] | Probands with high HDL | No major gene for high HDL |
| [17] | 1 pedigree with 654 members | Multiple cases of myocardial infarction | No major gene |
| [20] | 23 families, 132 members | Probands having primary hypoalphalipoproteinemia | Recessive major gene for low HDL |
| [18] | 78 nuclear families with 292 members | 50 families by proband with maturity-onset diabetes mellitus and 28 control families | No major gene |
| [21] | 14 nuclear families, 64 members | Probands having primary hypoalphalipoproteinemia | Major gene for low HDL, mode of transmission unknown |
| [22] | 1 high-risk pedigree with 196 members | Child with positive family history of CHD | Major gene with neither dominant nor recessive expression |
| [23] | 3074 nuclear families with 1–2 children | Screening of all 17-year-olds | Recessive major gene for low HDL |
| [24] | 3074 nuclear families with 1–2 children | Screening of all 17-year-olds | Inconclusive for high HDL |
| [37] | 1806 families with 6821 members | 1146 families selected at random, 483 by hypercholesterolemic and 177 by proband with HDL | Major factor for low HDL transmitted from parent to offspring but unclear whether Mendelian |
| [19] | 69 families with 390 members | Probands undergoing diagnostic cardiac catheterization | No single gene for HDL levels |
| [25] | 28 families with 277 members | Random | Both Mendelian and non-Mendelian models fitted the data for high HDL |
| [26] | 25 families with 526 members | Random | Major codominant gene for low HDL |

[a]Analysis includes the data of the studies[39,40]

randomly from participants of the Utah center for the Hypertension Detection and Follow-up Program. Detailed characteristics of the sample are described elsewhere.[28] The subjects in this sample are 98% Caucasian; analyses were not performed on the small number of non-Caucasian subjects. There was no overlap between these families and the sample of families from Utah recruited for the FHS study.

**Measurement of HDL-C and other phenotypes**

After fasting for 12 h, blood samples were collected from FHS subjects in tubes with no additives. After low-speed centrifugation, serum samples were stored at $-70^{\circ}$C until sufficient numbers of samples accumulated for shipment to the FHS Central Biochemistry Laboratory at the University of Minnesota for processing. HDL-C was measured in the supernatant after dextran sulphate-magnesium precipitation using enzymatic methods.[29,30] Samples from Utah subjects were processed at the University of Utah Cardiovascular Genetics Program using similar methods.[14] Anthropometric measurements were collected with subjects wearing scrub suits. Weight was measured using a balance scale, and height was measured using a vertical ruler mounted to a wall. Subjects were asked to bring medications to their clinic visit, and medication use was assessed through a review of these medications and through an interview. All other variables were collected through interviews performed by trained interviewers.

**Statistical analysis**

We adjusted HDL-C levels within gender for age, amount of alcohol consumption, current smoking status, body mass index, waist/hip ratio, amount of TV watching as a measure of physical inactivity, estrogen substitution for postmenopausal reasons, menopausal status, and recruitment centre by the SAS GLM procedure.[31] Adjusted HDL-C values for segregation analysis were available from 560 randomly recruited Caucasian families (1896 subjects) and from 522 Caucasian families (1859 subjects) with high family risk score for CHD.

In the Utah sample, the same covariates were used with the exception of waist/hip ratio, TV watching, and recruitment centre. Waist circumference, physical activity by questionnaire, and watts needed to maintain 80% of maximum heart rate from a 4-minute bicycle test were used in the Utah HDL-C level adjustment. These adjustments were performed to ensure that a major gene effect was not disguised nor explained by effects of covariates. Other variables were not included in the final analysis after it was shown that they did not contribute to the regression model. Corrections for covariates were done separately within the FHS and Utah samples. Adjusted HDL-C levels were calculated by adding the overall mean HDL-C concentration to the calculated residuals of each individual.

Segregation analysis of adjusted HDL-C from the FHS sample was performed in the whole group of families, in the random group, and in the CHD group. For the Utah sample, the entire sample was analysed together. Maximum likelihood segregation analysis[32,33] was done using the Pedigree Analysis Package (PAP) software.[34] This analysis was performed before adjustment of HDL-C for log transformed triglyceride concentrations and again after adjustment. Adjustment for the above variables explained 22% of the HDL-C values in FHS females (11% in Utah females) and 16% in FHS males (12% in Utah males) when triglycerides were not considered. Inclusion of triglycerides in the model increased the sex-specific variance explained to 31% (19% for Utah) and 32% (18% for Utah), respectively. A standard segregation model was tested, in which we assumed the variation in HDL-C was caused by the sum of the independent effects of alleles L and H at a major locus, polygenic inheritance, and random environmental factors specific to each individual. We assumed that the major locus alleles were in Hardy–Weinberg equilibrium. Three HDL-C phenotypic means were defined, one for each genotype ($\mu_{LL}$, $\mu_{LH}$, and $\mu_{HH}$).

Model fitting was performed searching both for an allele at a major gene locus associated with lower than average HDL-C levels as well as for an allele associated with higher than average HDL-C levels. In models investigating low HDL-C, $\mu_{HH}$ represents a mean close to the average HDL-C levels in the pedigree members and $\mu_{LL}$ represents the low HDL-C mean. When searching for evidence of an allele that raises HDL-C, $\mu_{HH}$ represents the mean of higher than average HDL-C levels. Several starting values for the three means were used. As a general rule we decreased the starting values of $\mu_{LL}$ and $\mu_{LH}$ in steps of 2 to 5 mg/dl when fitting a low HDL-C model and increased $\mu_{LH}$ and $\mu_{HH}$ when fitting a high HDL-C model. The gene frequency q is the frequency of the allele associated with the mean furthest from the average HDL-C level in the low and high HDL-C models. Homoscedasticity was assumed by defining one common parameter ($\sigma$) for the standard deviation of each genotypic category. Note that the variation within a genotypic category is divided into a polygenic component and random environmental effects specific to each individual. These effects were assumed to be normally distributed. Three transmission probabilities ($\tau_1$, $\tau_2$, and $\tau_3$) were also defined. The transmission probabilities reflect the chance that a parent will transmit a specific allele to an offspring (1, 1/2, and 0 for Mendelian transmission).

The GEMINI function[35] was used to produce maximum-likelihood estimates of the parameters. Sub-models were compared to a more general model. The difference in $-2\times\ln$(likelihood) of each model is approximated by a $\chi^2$ distribution, with degrees of freedom equal to the difference in the number of parameters estimated in the two models. Tests were performed as follows: first, the most general model with the three $\tau_i$ estimated was generated.

Both a Mendelian model and an environmental model were compared to this general model to test the transmission pattern. If the Mendelian model was accepted and the environmental model was rejected, dominant and recessive models were compared with the codominant model to determine the most likely mode of inheritance.

Ascertainment correction in the FHS group recruited for early onset CHD was performed for the youngest family member with coronary artery disease or for the proband if a family member with CHD was not available for examination. This correction was performed independently whether the CHD families were analysed as a separate group or whether they were analysed together with the random families. Within PAP, the likelihood of the pedigrees given the model parameters is divided by the likelihood of the ascertainment subset to produce the final corrected likelihood. Correction for ascertainment bias will minimize the risk of making false claims of a major gene effect.[36] Analysis in the FHS group was also done without ascertainment correction which showed virtually the same results. Ascertainment corrections of the Utah pedigree data could not be done since ascertainment was on unexamined deceased founders of the extended pedigrees.

## Results

Table 2 presents the variables used in adjusting HDL-C for the FHS data. Model fitting was performed separately for males and females. In the model which included triglycerides, HDL-C increased in both genders with increasing alcohol consumption, physical activity, and decreased with age, body mass index, waist/hip ratio, abdominal girth, triglycerides, smoking, and in males with increasing TV watching time. In females postmenopausal status was associated with lower and estrogen use with higher HDL-C values. The distribution of HDL-C after adjustment had a skewness of 0.73 and a kurtosis of 1.07. Adjustment in the Utah data yielded similar results (data not shown).

**Table 2** Adjustment of variables influencing HDL-C concentrations in the whole FHS study population. The sex-specific variance explained 31 and 32% of HDL-C values in females and males, respectively

| | *Entire sample* | *Females (n=1976)* | | *Males (n=1779)* | |
|---|---|---|---|---|---|
| *Variable* | *Mean*$\pm$SD | *F* | *P* | *F* | *P* |
| Age (years) | 49.6$\pm$13.5 | 10.9 | 0.001 | 7.9 | 0.0049 |
| Body mass index (kg/m$^2$) | 27.5$\pm$5.4 | 64.1 | $<$0.0001 | 23.1 | $<$0.0001 |
| Waist/hip ratio | 0.91$\pm$0.09 | 15.3 | $<$0.0001 | 4.4 | 0.035 |
| Research center[a] | | 9.1 | $<$0.0001 | 19.2 | $<$0.0001 |
| Triglycerides (mg/dl) | 143$\pm$82 | 248.2 | $<$0.0001 | 423.8 | $<$0.0001 |
| Current smokers (percentage) | 15% | 16.1 | $<$0.0001 | 8.8 | 0.003 |
| Alcoholic drinks within 24 hours | 0.26$\pm$0.78 | 50.2 | $<$0.0001 | 73.4 | $<$0.0001 |
| Daily hours of TV watching | 2.08$\pm$1.49 | 0.3 | 0.56 | 6.4 | 0.011 |
| Estrogen use (percentage of women) | 29% | 154.8 | $<$0.0001 | – | – |
| Menopausal status (percentage of women) | pre[b]: 44% peri[b]: 3% post[b]: 53% | 3.0 | 0.052 | – | – |

[a]The sample was obtained from four US research centres: Forsyth NC=20%, Minneapolis MN=29%, Framingham MA=21%, Salt Lake UT=30%. [b]pre=premenopausal, peri=perimenopausal, post=menopausal.

### Modelling of an allele for low HDL-C in FHS families

***HDL-C without adjustment for triglyceride concentrations*** Independently of how we set the initial estimates in the search for a locus for low HDL-C (e.g. $\mu_{HH}$=50 mg/dl, $\mu_{LH}$=48 mg/dl, $\mu_{LL}$=38 mg/dl), $\mu_{LL}$ changed to a high value above 70 mg/dl. In fact, $\mu_{LL}$, $\mu_{LH}$, and $\mu_{HH}$ changed to exactly the same values as we found them when using starting estimates favouring an allele that raises HDL-C above average (see below). There was no bimodality initially evident for a mean representing low values. Therefore we decided to omit those individuals with the upper 10% of HDL-C to see if either some outliers or possibly another allele related to high HDL-C caused this phenomenon. Although there was now significant bimodality for a low HDL-C mean in the data, we did not find any evidence for an allele responsible for low HDL-C levels ($P<0.0001$, $P<0.0001$, and $P<0.005$ rejecting the Mendelian *vs* the general model for all families, random families and the high-risk CHD families, respectively) (Table 3).

When testing for low HDL-C levels before adjustment for triglyceride concentrations, the best model in all three FHS groups (whole, random and CHD group) was the environmental model ($P$=0.74, $P$=0.19 and $P$=0.53, respectively) (Table 3). The environmental model parameter estimates for the whole group were as follows: q=0.29, $\mu_{HH}$=43.7, $\mu_{LH}$=55.0, $\mu_{LL}$=33.6, $\sigma$=6.10 and $h^2$=0.91.

***HDL-C adjusted for triglyceride concentrations*** When we reran the analysis after additional adjustment of HDL-C for triglycerides, both the environmental and major gene models were rejected and we accepted only the general model (Table 3). The general model parameter estimates for the whole group were as follows: q=0.35, $\mu_{HH}$=53.3, $\mu_{LH}$=43.7, $\mu_{LL}$=34.8, $\sigma$=5.74 and $h^2$=0.21.

***Modelling of an allele for high HDL-C in FHS families*** Evidence for segregation when modeling a mean for high HDL-C in the FHS families was further examined using all individuals without exclusion of subjects above or below a certain percentile. In the whole group as well as in the random group, the Mendelian and the environmental models were rejected independently of whether we adjusted for triglycerides or not, although the codominant model fitted better than the environmental model (Table 3). The

general model also fitted the best for the CHD group when HDL-C was not adjusted for triglycerides. After adjustment, however, it was no longer possible to reject the codominant and the recessive model in the CHD group (Table 4). In both models, the means of the homozygote allele carriers and those without the allele were separated by about 25 mg/dl HDL-C (74.3 *vs*. 47.6 mg/dl for the codominant model and 73.9 *vs*. 48.2 mg/dl for the recessive model). The gene frequencies (q) for high HDL-C were 0.23 and 0.24 for the codominant and the recessive models, respectively. Interestingly, the mean values of the different genotypes as well as the gene frequencies (Table 3) were similar when compared to the random group of families, considering that the random group should generally have higher mean HDL-C values. The parameter estimates for the codominant model of the random group were q=0.19, $\mu_{LL}$=48.1, $\mu_{LH}$=53.1, $\mu_{HH}$=82.1, $\sigma$=9.71, $h^2$=0.53 and for the recessive model q=0.19, $\mu_{LL}$=49.7, $\mu_{LH}$ and $\mu_{HH}$=81.2, $\sigma$=9.99 and $h^2$=0.56. Only the transmission probabilities and especially $\tau_3$ were markedly different between the CHD, random and whole group of families: $\tau_3$=0.25, 0.007 and fixed to 0, respectively.

### Segregation in the Utah pedigrees

As with the FHS data, the Utah data produced estimates indicating a higher than average mean for HDL-C regardless of starting values. Models using HDL-C not adjusted for triglyceride resulted in rejection of both the environmental and codominant models ($P<0.0001$; $P$=0.046, respectively). For HDL-C adjusted for triglycerides,

**Table 3** Gene frequencies (q) and means ($\mu_{LL}$, $\mu_{LH}$, and $\mu_{HH}$) for the different genetic models calculated for the whole, the random and the CHD group of FHS families. Results are given in the order q/$\mu_{LL}$/$\mu_{LH}$/$\mu_{HH}$

| | *General* | *Codominant* | *Environmental* |
|---|---|---|---|
| *Low HDL-C model, not adjusted for triglycerides** | | | |
| Whole group | 0.33/33.6/55.0/43.7 | 0.33/37.8/53.5/44.0† | 0.29/33.6/55.0/43.7 |
| Random group | 0.41/34.5/55.6/44.2 | 0.54/40.1/54.3/44.6† | 0.31/34.4/55.6/44.2 |
| CHD group | 0.26/32.8/54.6/43.1 | 0.25/35.1/42.0/51.6† | 0.27/32.9/54.6/43.1 |
| *Low HDL-C model, adjusted for triglycerides** | | | |
| Whole group | 0.35/34.8/43.7/53.3 | 0.55/40.8/50.3/51.8† | 0.15/31.9/48.1/48.1† |
| Random group | 0.38/36.2/55.0/54.7 | 0.62/42.3/52.2/52.9† | 0.29/36.3/44.9/53.2† |
| CHD group | 0.73/40.4/50.9/51.4 | 0.60/40.5/50.4/51.4† | 0.64/41.1/51.1/51.1† |
| *High HDL-C model, not adjusted for triglycerides* | | | |
| Whole group | 0.20/45.7/58.7/85.2 | 0.22/47.6/51.4/80.3† | 0.10/47.1/62.4/90.6† |
| Random group | 0.35/49.9/48.4/80.9 | 0.21/48.6/50.7/81.2† | 0.20/49.5/49.5/79.8† |
| CHD group | 0.18/44.9/58.1/82.4 | 0.20/46.0/53.1/79.8† | 0.13/45.8/59.5/83.8† |
| *High HDL-C model, adjusted for triglycerides* | | | |
| Whole group | 0.29/48.6/49.9/78.8 | 0.22/47.9/51.2/79.2† | 0.20/49.1/49.1/77.8† |
| Random group | 0.30/49.1/51.0/81.5 | 0.19/48.1/53.1/82.1† | 0.19/49.8/49.8/79.6† |
| CHD group‡ | 0.32/48.5/47.6/73.5 | 0.23/47.6/49.2/74.3 | 0.22/48.1/48.1/72.7† |

The gene frequency q is the frequency of the allele associated with low and high HDL-C levels in the low and high HDL-C models, respectively. *After exclusion of subjects with HDL above the 90th percentile. †Model was rejected compared to the general model. ‡See Table 4 for the extended model and exact parameter estimates in the CHD group.

**Table 4** Genetic model parameters and estimates for high HDL-C levels (after adjustment for triglycerides) in the CHD group of FHS families

| | *General* | *Codominant* | *Recessive* | *Dominant* | *Environmental* |
|---|---|---|---|---|---|
| q | 0.32±0.06 | 0.23±0.03 | 0.24±0.03 | 0.03±0.01 | 0.22±0.02 |
| $\tau_1$ | 0.97±0.05 | (1.0) | (1.0) | (1.0) | =q |
| $\tau_2$ | 0.68±0.11 | (0.5) | (0.5) | (0.5) | =q |
| $\tau_3$ | 0.25±0.19 | (0) | (0) | (0) | =q |
| $\mu_{LL}$ | 48.5±1.19 | 47.6±1.03 | 48.2±0.36 | 48.3±0.37 | 48.1±2.92 |
| $\mu_{LH}$ | 47.6±1.94 | 49.2±1.67 | =$\mu_{LL}$ | 74.2±2.25 | 48.1±0.50 |
| $\mu_{HH}$ | 73.5±1.89 | 74.3±2.17 | 73.9±1.99 | =$\mu_{LH}$ | 72.7±2.02 |
| $\sigma$ | 9.16±0.22 | 9.18±0.24 | 9.21±0.22 | 9.41±0.22 | 9.21±0.22 |
| $h^2$ | 0.48±0.07 | 0.44±0.06 | 0.45±0.06 | 0.42±0.06 | 0.49±0.06 |
| −2 ln(L) | 11863.90 | 11870.52 | 11870.95 | 11895.07 | 11878.02 |
| Parameters | 9 | 6 | 5 | 5 | 6 |
| P value *vs* general model | – | 0.085 | 0.133 | <0.0001 | 0.0027 |

q: gene frequency for high HDL-C, $\tau_1-\tau_3$: transmission parameters, $\mu_{LL}$, $\mu_{LH}$, and $\mu_{HH}$: means for each of the three genotypes, $h^2$: polygenic heritability, −2 ln(L): $-2\times$log-likelihood, parameters: parameters estimated.

support for a major gene was found (Table 5). The environmental model was rejected ($P<0.0001$), but the major gene model was not rejected ($P$=0.22 and $P$=0.054 for the codominant and the recessive models, respectively). In comparison with the general model, the dominant model could be rejected ($P<0.0001$). Parameter estimates for the best-fitting codominant model are remarkably similar to those found in the FHS data: q=0.26, $\mu_{LL}$=45.8, $\mu_{LH}$=49.0, $\mu_{HH}$=67.5, $\sigma$=8.09, $h^2$=0.54, with a separation of about 22 mg/dl HDL-C between the two homozygote means.

## Discussion

There is clear evidence of a high heritability of HDL-C concentrations.[6,9–13,15] The mode of inheritance, however, is controversial, as can be seen in Table 1. Friedlander *et al* studied 3074 nuclear families with 1–2 children and found evidence for a recessive major gene for low HDL-C.[23] The analysis remained inconclusive for high HDL-C in the same study population.[24] Bucher *et al*[37] proposed a factor for low HDL-C levels transmitted from parents to offspring in a large sample of 1806 families with nearly 7000 members. It was unclear whether this transmission followed a Mendelian pattern. Byard *et al* described a recessive major gene for low HDL-C concentrations.[20] A study in 526 Mexican American individuals in 25 randomly ascertained families reported evidence for a major locus for low HDL-C with a codominant model.[38] Others reported either evidence for a major gene with unknown mode of transmission[21,22,25] or no evidence for a major gene locus.[16,18,19] The reasons for these divergent results might be found in differences in study design, differences in ethnic compositions of study populations, recruitment conditions and data adjustment.

Although we investigated a large data set of families we did not find any evidence for a major gene allele that lowers HDL-C concentrations below average, independently of whether we adjusted for triglyceride concentrations or not. Bimodality for low HDL-C levels was not observed unless the highest 10% of HDL-C values were excluded. We accepted only the environmental model for low HDL-C before adjustment for triglycerides. The rejection of the environmental model after adjustment for triglycerides is in accordance with the inverse relation between triglyceride and HDL-C concentrations and the pronounced environmental influence on triglyceride levels.

It is surprising at first glance that we found evidence for a major allele leading to higher than average HDL-C values only in the group of families recruited for clustering of CHD, the group one would least expect to find it. We observed a recessive or codominant transmission of the inferred gene after adjusting for the effects of triglycerides on HDL-C. The mean separation and standard deviation of the different HDL-C genotype distributions as well as the gene frequencies were similar in the random and the whole group of families when compared to the CHD group. The major differences were found in the transmission probabilities which suggests that the models between the groups of families are very similar from the physiological point of view. A more pronounced confounding and/or heterogeneity in the random group of families resulting in different transmission probabilities could be an explanation for these findings. In other words, the random and CHD models for high HDL-C are very similar resulting in mild evidence for a high HDL-C allele in the entire dataset. There appears, however, to be some other factor that may be confounding the segregation in both groups but is just a little stronger in the random group (or by chance appears to be stronger).

Although we used a large number of families, these somewhat small family structures might have limited our analysis providing poor estimates of the transmission probabilities. The ascertainment correction for the FHS CHD families may not have been correct, given the complicated selection of these families. However, the correction made

**Table 5** Genetic model parameters for analyses of HDL-C levels (after adjustment for triglycerides) in the Utah extended pedigrees

| | *General* | *Codominant* | *Recessive* | *Dominant* | *Environmental* |
|---|---|---|---|---|---|
| q | 0.26±0.03 | 0.26±0.03 | 0.27±0.03 | 0.05±0.01 | 0.21±0.03 |
| $\tau_1$ | 0.73±0.14 | (1.0) | (1.0) | (1.0) | =q |
| $\tau_2$ | 0.52±0.05 | (0.5) | (0.5) | (0.5) | =q |
| $\tau_3$ | 0.0‡ | (0) | (0) | (0) | =q |
| $\mu_{LL}$ | 45.6±0.74 | 45.8±0.67 | 47.1±0.37 | 47.0±0.40 | 47.5±0.96 |
| $\mu_{LH}$ | 49.3±1.23 | 49.0±1.05 | =$\mu_{LL}$ | 64.5±1.63 | 47.5±1.68 |
| $\mu_{HH}$ | 67.5±1.59 | 67.5±1.67 | 66.2±1.49 | =$\mu_{LH}$ | 67.0±1.74 |
| $\sigma$ | 8.01±0.25 | 8.08±0.23 | 8.24±0.20 | 8.36±0.21 | 8.53±0.20 |
| $h^2$ | 0.56±0.05 | 0.54±0.05 | 0.59±0.05 | 0.52±0.05 | 0.63±0.04 |
| −2 ln(L) | 14424.24 | 14428.61 | 14433.54 | 14456.67 | 14461.93 |
| parameters | 9 | 6 | 5 | 5 | 6 |
| P value *vs* general model | – | 0.22 | 0.054 | <0.0001 | <0.0001 |

q: gene frequency for high HDL-C, $\tau_1-\tau_3$: transmission parameters, $\mu_{LL}$, $\mu_{LH}$ and $\mu_{LH}$: means for each of the three genotypes, $h^2$: polygenic heritability, −2 ln(L): −2 × log-likelihood, parameters: parameters estimated. ‡Maximized at its boundary.

virtually no difference in the results when compared to an analysis without ascertainment correction (data not shown). To test for reproducibility, we obtained similar results from a set of large extended Utah pedigrees ascertained for early death due to stroke or CHD, or due to early hypertension. These similar results in an independent sample with greater segregation information support the evidence for a major allele for high HDL-C in subjects ascertained for CHD risk.

## Conclusion

It appears that there is little or no evidence for a major gene allele for lower than average HDL-C levels in these data sets and that an environmental model fits best for low HDL-C. Surprisingly, the model that showed the most evidence for an allele leading to high HDL-C occurred in the CHD group, supported by similar results obtained in a Utah sample ascertained for risk of CHD and related phenotypes.

## Acknowledgements

*F Kronenberg is supported by the 'Austrian Programme for Advanced Research and Technology' (APART) of the Austrian Academy of Science and by the Austrian Science Fund (P14717-GEN). This study was supported by cooperative agreements from NHLBI: UO1-HL-56563, -56564, -56565, -56566, -56567, -56568, and -56569; two grants from NHLBI: R01HL21088 and R01HL24855; and a grant from NIA: AG18734. This paper is presented on behalf of the investigators of the NHLBI Family Heart Study. Participating institutions and principal staff of the study are as follows: Forsyth County/University of North Carolina/Wake Forest University: Gerardo Heiss, Stephen Rich, Greg Evans; James Pankow; HA Tyroler, Jeannette T Bensen, Catherine Paton, Delilah Posey and Amy Haire; University of Minnesota Field Center: Donna K Arnett, Aaron R Folsom, Larry Atwood, James Peacock, and Greg Feitl; Boston University/Framingham Field Center: R Curtis Ellison, Richard H Myers, Yuqing Zhang, Andrew G Bostom, Luc Djoussé, Jemma B Wilk and Greta Lee Splansky; University of Utah Field Center: Steven C Hunt, Roger R Williams (deceased), Paul N Hopkins, Hilary Coon and Jan Skuppin; Coordinating Center, Washington University, St. Louis: Michael A Province, DC Rao, Ingrid B Borecki, Yuling Hong, Mary Feitosa, Jeanne Cashman, and Avril Adelman; Central Biochemistry Laboratory, University of Minnesota: John H Eckfeldt, Catherine Leiendecker-Foster, Michael Y Tsai, and Greg Rynders; Central Molecular Laboratory, University of Utah: Mark F. Leppert, Jean-Marc Lalouel, Tena Varvil, Lisa Baird; National Heart, Lung, & Blood Institute – Project Office: Phyliss Sholinsky, Millicent Higgins (retired), Jacob Keller (retired), Sarah Knox, and Lorraine Silsbee.*

## References

1 Castelli WP, Doyle JT, Gordon T *et al*: HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. *Circulation* 1977; **55**: 767–772.
2 Pekkanen J, Linn S, Heiss G *et al*: Ten-year mortality from cardiovascular disease in relation to cholesterol level among men with and without preexisting cardiovascular disease. *N Engl J Med* 1990; **322**: 1700–1707.
3 Rubins HB, Robins SJ, Collins D *et al*: Distribution of lipids in 8,500 men with coronary artery disease. Department of Veterans Affairs HDL Intervention Trial Study Group. *Am J Cardiol* 1995; **75**: 1196–1201.
4 Rubins HB, Robins SJ, Collins D: The veterans affairs high-density lipoprotein intervention trial: Baseline characteristics of normocholesterolemic men with coronary artery disease and low levels of high- density lipoprotein cholesterol. *Am J Cardiol* 1996; **78**: 572–575.
5 Jacobs Jr DR, Mebane IL, Bangdiwala SI, Criqui MH, Tyroler HA: High density lipoprotein cholesterol as a predictor of cardiovascular disease mortality in men and women: The follow-up study of the Lipid Research Clinics Prevalence Study. *Am J Epidemiol* 1990; **131**: 32–47.
6 Boulton TJC, Magarey AM, Cockington RA: Serum lipids and apolipoproteins from 1 to 15 years: Changes with age and puberty, and relationships with diet, parental cholesterol and family history of ischaemic heart disease. *Acta Paediatr* 1995; **84**: 1113–1118.
7 Knoblauch H, Busjahn A, Münter S *et al*: Heritability analysis of lipids and three gene loci in twins link the macrophage scavenger receptor to HDL cholesterol concentrations. *Arterioscler Thromb Vasc Biol* 1997; **17**: 2054–2060.
8 Beaty TH, Self SG, Chase GA, Kwiterovich PO: Assessment of variance components models on pedigrees using cholesterol, low-density, and high-density lipoprotein measurements. *Am J Med Genet* 1983; **16**: 117–129.
9 Mjos OD, Thelle DS, Forde OH, Vik-Mo H: Family study of high density lipoprotein cholesterol and the relation to age and sex. The Tromso Heart Study. *Acta Med Scand* 1977; **201**: 323–329.
10 Sosenko JM, Breslow JL, Ellison RC, Miettinen OS: Familial aggregation of total cholesterol, high density lipoprotein cholesterol and total triglyceride levels in plasma. *Am J Epidemiol* 1980; **112**: 656–660.
11 Ellison RC, Myers RH, Zhang Y *et al*: Effects of similarities in lifestyle habits on familial aggregation of high density lipoprotein and low density lipoprotein cholesterol: the NHLBI Family Heart Study. *Am J Epidemiol* 1999; **150**: 910–918.
12 Perusse L, Rice T, Despres JP *et al*: Familial resemblance of plasma lipids, lipoproteins and postheparin lipoprotein and hepatic lipases in the HERITAGE Family Study. *Arterioscler Thromb Vasc Biol* 1997; **17**: 3263–3269.
13 Martinez V, Salcedo AF, Franquelo GR, Jarabo CY, Garcia NP, Dominguez R: Familial aggregation of cardiovascular disease risk factors: the Cuenca Study. *Prev Med* 1999; **28**: 131–137.
14 Hunt SC, Hasstedt SJ, Kuida H, Stults BM, Hopkins PN, Williams RR: Genetic heritability and common environmental components of resting and stressed blood pressures, lipids, and body mass index in Utah pedigrees and twins. *Am J Epidemiol* 1989; **129**: 625–638.
15 Namboodiri KK, Kaplan EB, Heuch I *et al*: The Collaborative Lipid Research Clinics Family Study: biological and cultural determinants of familial resemblance for plasma lipids and lipoproteins. *Genet Epidemiol* 1985; **2**: 227–254.
16 Rao DC, Lalouel JM, Suarez BK, Schonfeld G, Glueck CJ, Siervogel RM: A genetic study of hyper-alpha-lipoproteinemia. *Am J Med Genet* 1983; **15**: 195–203.
17 Hasstedt SJ, Albers JJ, Cheung MC *et al*: The inheritance of high density lipoprotein cholesterol and apolipoproteins A-I and A-II. *Atherosclerosis* 1984; **51**: 21–29.
18 Iselius L, Carlson LA, Morton NE, Efendic S, Lindsten J, Luft R: Genetic and environmental determinants for lipoprotein concentrations in blood. *Acta Med Scand* 1985; **217**: 161–170.
19 Prenger VL, Beaty TH, Kwiterovich PO: Genetic determination of high-density lipoprotein-cholesterol and apolipoprotein A-1 plasma levels in a family study of cardiac catheterization patients. *Am J Hum Genet* 1992; **51**: 1047–1057.
20 Byard PJ, Borecki IB, Glueck CJ, Laskarzewski PM, Third JL, Rao DC: A genetic study of hypoalphalipoproteinemia. *Genet Epidemiol* 1984; **1**: 43–51.

21 Borecki IB, Rao DC, Third JL, Laskarzewski PM, Glueck CJ: A major gene for primary hypoalphalipoproteinemia. *Am J Hum Genet* 1986; **38**: 373–381.
22 Amos CI, Wilson AF, Rosenbaum PA *et al*: An approach to the multivariate analysis of high-density- lipoprotein cholesterol in a large kindred: the Bogalusa Heart Study. *Genet Epidemiol* 1986; **3**: 255–267.
23 Friedlander Y, Kark JD, Stein Y: Complex segregation analysis of low levels of plasma high- density lipoprotein cholesterol in a sample of nuclear families in Jerusalem. *Genet Epidemiol* 1986; **3**: 285–297.
24 Friedlander Y, Kark JD: Complex segregation analysis of plasma lipid and lipoprotein variables in a Jerusalem sample of nuclear families. *Hum Hered* 1987; **37**: 7–19.
25 Cupples LA, Myers RH: Segregation analysis for high density lipoprotein in the Berkeley Data. *Genet Epidemiol* 1993; **10**: 629–634.
26 Mahaney MC, Blangero J, Rainwater DL *et al*: A major locus influencing plasma high-density lipoprotein cholesterol levels in the San Antonio family heart study – Segregation and linkage analyses. *Arterioscler Thromb Vasc Biol* 1995; **15**: 1730–1739.
27 Higgins M, Province M, Heiss G *et al*: NHLBI Family Heart Study: objectives and design. *Am J Epidemiol* 1996; **143**: 1219–1228.
28 Williams RR, Hunt SC: Recruitment of members of high-risk Utah pedigrees. *Controlled Clin Trials* 1987; **8**: 105S–114S.
29 Allain CC, Poon LS, Chan CS, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. *Clin Chem* 1974; **20**: 470–475.
30 Warnick GR, Benderson J, Albers JJ: Dextran sulfate-Mg2+ precipitation procedure for quantitation of high- density-lipoprotein cholesterol. *Clin Chem* 1982; **28**: 1379–1388.
31 SAS Institute.: SAS/STAT User's Guide, Version 7, Cary, NC SAS Institute, 1998.
32 Elston RC, Stewart J: A general model for the genettic analysis of pedigree data. *Hum Hered* 1971; **21**: 523–542.
33 Lalouel JM, Rao DC, Morton NE, Elston RC: A unified model for complex segregation analysis. *Am J Hum Genet* 1983; **35**: 816–826.
34 Hasstedt SJ: PAP pedigree analysis package, rev. 4, Salt Lake City: Department of Human Genetics, University of Utah, 1993.
35 Lalouel JM: GEMINI a computer program for optimization of a nonlinear function. Salt Lake City: Department of Medical Biophysics and Computing, University of Utah, 1979.
36 Morton NE, Rao DC: Causal analysis of family resemblance. *Prog Clin Biol Res* 1979; **32**: 431–452.
37 Bucher KD, Kaplan EB, Namboodiri KK, Glueck CJ, Laskarzewski P, Rifkind BM: Segregation analysis of low levels of high-density lipoprotein cholesterol in the collaborative Lipid Research Clinics Program Family Study. *Am J Hum Genet* 1987; **40**: 489–502.
38 Reaven PD: Mechanisms of atherosclerosis: Role of LDL oxidation. *Adv Exp Med Biol* 1994; **366**: 113–128.
39 Glueck CJ, Fallat RW, Millett F, Gartside P, Elston RC, Go RC: Familial hyper-alpha-lipoproteinemia: studies in eighteen kindreds. *Metabolism* 1975; **24**: 1243–1265.
40 Siervogel RM, Morrison JA, Kelly K, Mellies M, Gartside P, Glueck CJ: Familial hyper-alpha-lipoproteinemia in 26 kindreds. *Clin Genet* 1980; **17**: 13–25.