Am J Hum Genet 32:1–7, 1980

On the Heritability of Serum High Density Lipoprotein in Twins

PERTTI SISTONEN[1] AND CHRISTIAN EHNHOLM

SUMMARY

To estimate the relative contributions of hereditary vs. environmental factors in the variation of high density lipoprotein, we measured the concentrations of its main apoprotein components, apoprotein A-I (apo A-I) and apoprotein A-II (apo A-II), in serum samples from 65 monozygotic (MZ) and 70 dizygotic (DZ) like-sexed twin pairs.

Evidence for a genetic component of variance was found for apo A-II, giving heritability (h^2) estimates of .35 and .30 for males and females, respectively. No genetic contribution to the variance of apo A-I could be demonstrated. Additionally, males had lower concentrations of apo A-I, but higher of apo A-II, than females.

INTRODUCTION

Many epidemiological studies have firmly established the association between elevated plasma cholesterol level and increased risk of premature coronary heart disease. Normally, about 17% of the total cholesterol in fasting plasma is transported in high density lipoprotein (HDL), 70% in low density lipoprotein (LDL), and 13% in very low density lipoprotein (VLDL).

It has been proposed that HDL serves a carrier function, clearing cholesterol from arterial tissue [1]. Both decreased HDL and HDL cholesterol levels have been shown to be correlated to the incidence of coronary heart disease [2–4]. Furthermore, recent studies indicate that high plasma HDL concentrations have a protective effect against atherosclerosis [5, 6].

The protein moiety of human HDL is composed of two major and several minor

Received January 10, 1979; revised July 23, 1979.

This study was supported by grants from the Nordisk Insulinfond and the Sigrid Jusélius Foundation.

[1] Finnish Red Cross Blood Transfusion Service, Helsinki, Finland and the Central Public Health Laboratory, Helsinki.

polypeptides. The major peptides, apo A-I and apo A-II, serve cooperatively to maintain the structural integrity of HDL [7].

Since HDL has been implicated in atherosclerosis as a predisposing as well as a protective factor, it was of interest to study the influence of heredity on serum HDL levels. To obtain an estimate of the relative importance of genetic vs. environmental factors in determining the variation of human quantitative traits, comparisons between MZ and DZ twins have been used. To study the h^2 of HDL, we determined the concentrations of the two main apoproteins of serum HDL in a twin sample.

MATERIALS AND METHODS

We studied a nonselected sample of like-sexed twins in the Finnish Twin Registry born before 1958, and drawn from this population were those twins who lived at the time of this study in Helsinki or its suburban towns (Espoo, Vantaa, Kauniainen). Two-thirds of the specimens were taken in January–March (spring), 1976, and the remaining one-third in September (autumn), 1976.

For zygosity diagnosis the following genetic markers were determined: blood groups A_1A_2BO, MNSs, Rh (CcDEe), and Fy^a; serum groups Hp, Gc, and Gm [1, 2, 5]; and the isozymes AK, AcP, PGM, and GPT. In combination with the information from questions aimed at the ascertainment of zygosity, zygosity diagnosis in each case can be regarded as correctly established [8].

Sera from 65 MZ and 70 DZ twin pairs were tested. Subjects' mean age was 36 years (range = 20–69 years).

Measurement of Apo A-I and A-II Concentrations

The radial immunodiffusion (RID) procedure for the quantitation of apo A-1 and A-11 was similar to that described by Cheung and Albers [9]. A given volume of serum, usually 50 μl, was diluted with an equal volume of tetramethylurea (TMU). The TMU-serum mixture was, in turn, diluted 1:15 by adding 650 μl of 0.01 M Tris, 6 M urea buffer, pH 8.0, and incubated overnight at room temperature. Four μl samples were pipetted in duplicate on agarose plates containing antiserum (4% anti-A-I or 8% anti-A-II) and 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl, 1 mM disodium EDTA, and 1% bovine serum albumin. After 48 hrs of diffusion, the immunoprecipitates were measured with a calibrating viewer (Kallestad Laboratories, Chaska, Minn.). Standard preparations of apo A-I and A-II in five dilutions were included on all plates.

Statistical Methods

The general twin statistics used are those of Kempthorne and Osborne [10] with notations adopted from Christian et al. [11] and Kang et al. [12]. The intraclass correlation coefficient and its sampling variance are given by Kang et al. [12]. The F' test used for the assumption of equal total variances in both twin classes is that proposed by Kempthorne and Osborne [10], Haseman and Elston [13], and Christian et al. [11].

RESULTS

To test whether the HDL apoprotein concentrations were normally distributed and comparable with the distribution found in singleton populations, histograms with only one twin from a pair (the first one in their pair-wise order) was constructed. Figures 1 and 2 show the distributions for apo A-I and A-II, respectively, grouped according to the sex and season of sampling. Table 1 gives the means and variances of the corresponding groups. As none of the distributions deviated significantly from normal

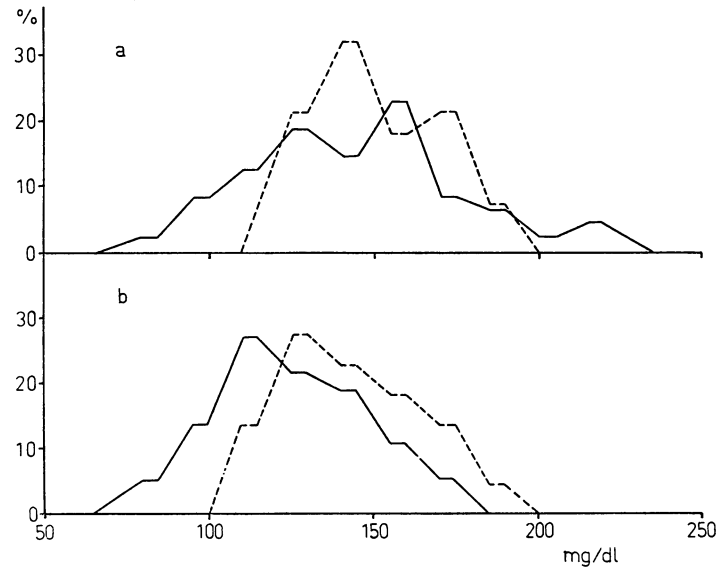

FIG. 1.—Distribution of serum HDL apo A-I concentration of first twins in a pair; *a*, apo A-I concentrations for females; *b*, apo A-I concentrations for males. *Solid line* = samples collected in spring (females, no. = 48; males, no. = 37); *broken line* = samples collected in autumn (females, no. = 28; males, no. = 22).

(chi-square test), no transformations to change the shapes of the distributions were used in the final twin analysis of variance.

Males had a significantly lower mean apo A-I concentration (136.3 mg/dl ± 23.0 mg/dl) than females (151.9 mg/dl ± 27.0 mg/dl) ($P < .001$). When the material was grouped according to season, the spring samples showed lower apo A-I levels than those collected in autumn (fig. 1, table 1). This seasonal difference was significant only for males ($P = .013$). The variances were significantly different for the female groups ($P = .006$).

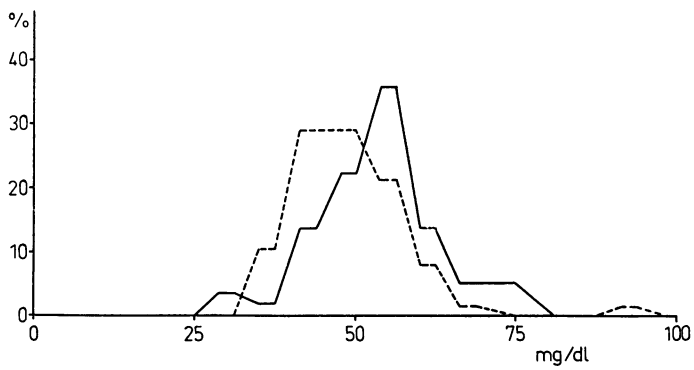

FIG. 2.—Distribution of serum HDL apo A-II concentrations of first twins in a pair. *Solid line*: males, no. = 59; *broken line*: females, no. = 76.

TABLE 1

MEANS AND VARIANCES OF APO A-I AND A-II FOR TWIN GROUPS SHOWN IN FIGURES 1 AND 2

Twin group	No.	Mean (mg/dl)	Variance
	A.) Apo A-I		
Male, spring	37	130.7	530.7
Female, spring	48	149.8	948.4
Male, autumn	22	145.8	398.2
Female, autumn	28	155.4	347.4
	B.) Apo A-II		
Male	59	51.1	93.7
Female	76	46.6	75.2

The distributions of apo A-II concentrations in males and females are shown in figure 2. Male twins had a higher mean apo A-II concentration (51.1 mg/dl ± 9.7 mg/dl) than females (46.6 mg/dl ± 8.7 mg/dl) ($P = .006$). No seasonal differences were observed.

In the calculation of the within-pair mean squares, the influences of sex and the season of sample collection on the HDL apoprotein concentrations were eliminated because only one-sex pairs were used and the blood specimens of a given pair were drawn at the same time. However, as sex and sampling time affect the among-pair mean squares and, thus, the total variances, the concentrations were adjusted for sex (apo A-I) and the groups with equal variances combined (table 1).

The among- and within-pair mean squares, as well as the total variances, of MZ and DZ twins were not significantly different in either of the seasonal groups of apo A-I. Thus, there is no evidence for genetic influence on the variation of apo A-I levels (table 2).

For apo A-II, the within-pair mean squares were significantly different ($P = .003$) in the two classes of female twins (table 3). They also had more divergent total variances ($P = .10$). In males, the within-pair mean squares did not differ significantly, although the difference was near the formal significance limit. These results suggest a genetic component in the variation of apo A-II.

DISCUSSION

Several twin studies have been carried out to clarify the relative contributions of environmental vs. genetic factors to the variation of plasma cholesterol and triglyceride levels. Most of these—some of them summarized by Christian et al. [14]—showed evidence for a proportionately strong genetic variance component. The existence of several primary familial hyper- and hypolipidemias [5] is also in accordance with the concept of genetic regulation of plasma lipid levels.

Our results indicate that the quantitative variation of HDL apo A-II is, in part, genetically controlled. This was especially evident in female twins. The difference in the conspicuousness of the genetic component between male and female twins could be

TABLE 2

ANALYSES OF VARIANCE OF SERUM APO A-I CONCENTRATIONS IN MZ AND DZ TWINS

Twin group	N*	M_A†	M_W‡	F_A§	F_W‖	F'	$\hat{\imath}$#	SD**	h²††
DZ, spring	40	821	606	...	...	...	0.15	0.16	...
DZ and MZ, spring	...	...	...	1.3	1.1	1.1	...	...	*ns*‡‡
MZ, spring	45	1069	549	...	...	...	0.32	0.14	...
DZ, autumn	30	609	210	...	...	...	0.49	0.14	...
DZ and MZ, autumn	...	...	...	1.3	1.3	1.2	...	...	ns
MZ, autumn	20	818	157	...	...	...	0.68	0.12	...

* N = number of twin pairs.
† M_A = among-pair mean square.
‡ M_W = within-pair mean square.
§ F_A = two-tailed F test for M_A's with $N - 1$ df.
‖ F_W = one-tailed F test for M_W's with DZ mean square the greater and N df.
$\hat{\imath}$ = intraclass correlation coefficient: $M_A - M_W/M_A + M_W$.
** SD = standard deviation of $\hat{\imath}$ [12].
†† h² = heritability: $\hat{\imath}_{MZ} - \hat{\imath}_{DZ}$.
‡‡ ns = not significantly different from zero.

explained reasonably well by the greater number of MZ female than male pairs (40 vs. 25, respectively). However, females also had a greater difference between the total variances of MZ and DZ twins than males as evidenced by the F' test (1.5 vs. 1.1, respectively). Performing the F' test at a higher probability level ($\alpha = .2$), as Christian et al. [11] have proposed, would lead to the rejection of the null hypothesis of equal total variances in female twins ($P = .1$). This in turn could be interpreted as evidence for the violation of the condition of equal environmental components of variance in the two classes of female twins—an assumption inherent in the basic twin model itself. Consequently, the result would be a nonvalid estimate of genetic variance from the within-pair comparison. Nevertheless, we chose not to use the higher than conventional probability level on two grounds. First, it could lead, generally, to many unnecessary type I statistical errors. Second, the independent genetic variance estimate from male twins is suggestive of a genetic variance component, while giving no evidence of an inequality between the total variances. Thus, we contend that the difference in the total variances of MZ and DZ females is caused by sampling and does

TABLE 3

ANALYSES OF VARIANCE OF SERUM APO A-II CONCENTRATIONS IN MZ AND DZ TWINS

Twin group	N	M_A	M_W	F_A	F_W	F'	$\hat{\imath}$	SD	h²
DZ, male	34	116	86	...	...	...	0.15	0.17	...
DZ and MZ, male	...	...	...	1.2	1.8	1.1	...	...	0.35
MZ, male	25	140	47	...	...	...	0.50	0.15	...
DZ, female	36	100	57	...	...	...	0.27	0.16	...
DZ and MZ, female	...	...	...	1.2	2.5*	1.5	...	...	0.30
MZ, female	40	83	23	...	...	...	0.57	0.11	...

NOTE.—See table 2 footnotes for symbol explanation.
* Significant at the 5% level.

not invalidate the genetic variance estimate for apo A-II from the within-pair mean squares.

We were not able to demonstrate genetic variance for apo A-I. The spring-collected twin sample showed almost twice the variance found in the autumn-collected sample; being the larger of the two, this may have contributed to the fact that no genetic variance could be found. The inflated environmental contribution tends to mask any genetic influence, especially if the latter is weak.

Heritability has been used in addition to the genetic variance estimates to evaluate the quantity of genetic contribution to the variances of quantitative traits [15]. The h^2 estimates for HDL apo A-II, calculated from the intraclass correlation coefficients as $\hat{t}_{MZ} - \hat{t}_{DZ}$, give an upper limit of $h^2 = .35 \pm .23$ for males and $h^2 = .30 \pm .19$ for females. This same result would be obtained from Falconer's estimate of $t_{MZ} - t_{DZ} = \frac{1}{2}h^2$ [15] and is the proportion of additive variance to total phenotypic variance, suggesting that approximately 30% of the quantitative variation in HDL apo A-II is genetically determined. Indices of quantity, such as h^2, tell nothing about the underlying genetic mechanisms of the regulation of apoprotein level nor how many genes might be involved—they only give a rough statistical estimate of the amount of the variation that is transmittable to the progeny.

Recently, a seasonal variation was reported for HDL cholesterol [16]. In our material, the apo A-I concentrations were somewhat lower in the spring sample, but this difference was significant only for male twins and may be due to the sample's longer storage. We found higher apo A-I concentrations in females as found previously in both the spring and autumn samples.

The apo A-II concentrations were higher in males than in females. This inverse sex distribution to apo A-I was not observed by Cheung and Albers [9]. There are conflicting reports on whether apo A-I and A-II are catabolized together. Both sex differences and seasonal variations support separate metabolic pathways.

Besides sex and season, several other environmental factors are known to associate with HDL cholesterol and lipoprotein levels [16–19]. The importance of finding and correctly interpreting these factors, as they may be one source for biased genetic variance estimates, is obvious.

ACKNOWLEDGMENTS

The skillful technical assistance of Miss Seija Puomilahti is gratefully acknowledged. We want to thank Prof. K. Aho, MD, Prof. J. Huttunen, MD, and J. Leikola, MD for valuable advice during the preparation of the manuscript.

REFERENCES

1. MILLER GJ, MILLER NE: Plasma-high-density-lipoprotein and development of ischaemic heart-disease. *Lancet* 1:16–19, 1975
2. MILLER NE, FØRDE OH, THELLE DS, MJØS OD: The Tromsø Heart Study. High density lipoproteins and coronary heart disease: a prospective case-control study. *Lancet* 1:965–967, 1977
3. CASTELLI WP, DOYLE JT, GORDON T, ET AL.: HDL-cholesterol and other lipids in coronary heart disease: the co-operative lipoprotein phenotyping study. *Circulation* 55:767–772, 1977

4. BERG K, BØRRESEN A-L, DAHLÉN G: Serum-high-density-lipoprotein and atherosclerotic heart-disease. *Lancet* 1:499–501, 1976
5. GLUECK CJ, GARTSIDE PS, STEINER PM, ET AL.: Hyperalpha- and hypobeta-lipoproteinemia in octogenarian kindreds. *Atherosclerosis* 27:387–406, 1977
6. LEVY RI, BLUM CB, SCHAEFFER EJ: in *Lipoprotein Metabolism*, edited by GRETEN H, Berlin, Springer, 1976, p 56
7. EISENBERG S, LEVY RI: Lipoprotein metabolism. *Adv Lipid Res* 13:1–86, 1975
8. SARNA S, KAPRIO J, SISTONEN P, KOSKENVUO M: The diagnosis of zygosity by mailed questionnaire. *Hum Hered* 28:241–254, 1978
9. CHEUNG MC, ALBERS JJ: The measurement of apolipoprotein A-I and A-II levels in men and women by immunoassay. *J Clin Invest* 60:43–50, 1977
10. KEMPTHORNE O, OSBORNE RH: The interpretation of twin data. *Am J Hum Genet* 13:320–339, 1961
11. CHRISTIAN JC, KANG KW, NORTON JA: Choice of an estimate of genetic variance from twin data. *Am J Hum Genet* 26:154–161, 1974
12. KANG KW, LINDEMAN JP, CHRISTIAN JC, NANCE WE, NORTON JA: Sampling variances in twin sibling studies of man. *Hum Hered* 24:363–372, 1974
13. HASEMAN JK, ELSTON RC: The estimation of genetic variance from twin data. *Behav Genet* 1:11–19, 1970
14. CHRISTIAN JC, FEINLEIB M, HULLEY SB, ET AL.: Genetics of plasma cholesterol and triglycerides: a study of adult male twins. *Acta Genet Med Gemellol (Roma)* 25:145–149, 1976
15. FALCONER DS: *Introduction to Quantitative Genetics*. Edinburgh and London, Oliver and Boyd, 1960
16. VAN GENT CM, VAN DER VOORT H, HESSEL LW: High-density lipoprotein cholesterol, monthly variation and association with cardiovascular risk factors in 1000 forty-year-old Dutch citizens. *Clin Chim Acta* 88:155–162, 1978
17. ALBERS JJ, WAHL PW, CABANA VG, HAZZARD WR, HOOVER JJ: Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. *Metabolism* 25:633–644, 1976
18. CHRISTIAN JC, KANG KW: Maternal influence on plasma cholesterol variation. *Am J Hum Genet* 20:462–467, 1977
19. COREY LA, KANG KW, CHRISTIAN JC, NORTON JA JR, HARRIS RE, NANCE WE: Effects of chorion type on variation in cord blood cholesterol of monozygotic twins. *Am J Hum Genet* 28:433–441, 1976