

GENETICS OF PLASMA CHOLESTEROL AND TRIGLYCERIDES: A STUDY OF ADULT MALE TWINS*

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Fasting plasma triglycerides and cholesterol, as well as cholesterol in very low density, low density and high density lipoproteins, were measured in 250 MZ and 264 DZ adult male twin pairs. A new method was used to choose an estimate of genetic variance. This includes an estimate of genetic variance for use when the total variances of MZ and DZ twins are unequal. DZ twins had greater total variance for cholesterol. When genetic variance was estimated by subtracting the within-MZ-sets mean square from the within-DZ-sets mean square, all of the lipids had significant estimates of genetic variance; however, when genetic variance was estimated by a method designed to correct bias due to unequal total variances of MZ and DZ twins, only triglycerides had significant genetic variance. The heritability of plasma triglycerides was calculated to be 0.68.

INTRODUCTION

The importance of plasma cholesterol as a risk factor for coronary heart disease has led to a number of studies to partition the population variation of plasma cholesterol into genetic and environmental components. While family studies have generally shown significant covariance of plasma cholesterol among relatives, the strongest evidence for genetic influence upon plasma cholesterol levels has come from twin studies. Table 1 summarizes the findings of eight previously reported twin studies of plasma cholesterol. When male and female twins were combined and using only like-sexed twins, seven of these studies revealed mean squares within DZ twin pairs significantly greater ($p < 0.05$) than within MZ twin pairs. In the study of Blomstrand and Lundman (1966) the within DZ mean square was larger, but not significantly so.

There have been fewer twin studies of plasma triglycerides, probably because they are more difficult to quantitate than cholesterol and for meaningful values samples must be taken after a fast. Jensen et al. (1965) quantitated triglycerides in 67 sets of adult twins and Blomstrand and Lundman (1966) in 193 sets and found that the within DZ mean square was significantly larger than the within MZ mean square. In contrast Rifkind et al. (1968) found no significant difference between the within MZ and DZ mean squares in 108 sets of adult twins.

Several types of primary familial hyperlipidemias have been described (Frederickson and Levy 1972) whose characteristic expression is an increase in the plasma concentrations of cholesterol or triglyc-

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erides. These disorders have been defined by plasma lipoprotein patterns and are thought to be due to mutations of genes that regulate diverse aspects of lipid, protein or carbohydrate metabolism. These familial hyperlipidemias give further evidence for genetic control of plasma lipid levels.

METHODS

The design of the National Heart and Lung Institute Twin Study, a multi-center investigation of adult, white male veteran twins (ages 42-56) has previously been described in detail (Feinleib et al. 1974).

Each of the twins came to the examination centers in the morning after an overnight fast of at least 14 hours. An effort was made to have both twins come in on the same day. Blood lipids were done at special study laboratories in Framingham, Massachusetts; Indianapolis, Indiana; and San Francisco, California. The laboratories exchanged specimens to maintain standardization. Plasma triglycerides as well as plasma total, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol were measured. VLDL cholesterol was obtained by subtracting the amount of cholesterol in the infranate of a sample spun in the preparative ultracentrifuge at plasma density from total plasma cholesterol. LDL cholesterol was obtained by subtracting HDL (the cholesterol remaining after heparin precipitation) from this same infranate (Fredrickson and Levy 1972). There was no significant difference between the means of MZ and DZ twins for any of the lipids studied. Table 2 compares the results obtained from three geographic areas. Only HDL cholesterol had significant variation among the laboratory means.

ANALYSIS OF GENETIC VARIANCE

Table 3 shows the analysis of variance model used (Christian et al. 1974). In this model, genetic-environmental covariance within and between the two members of DZ twin pairs are assumed to be equal ($\sigma_{ge} = \sigma_{g'e}$). The environmental covariances of MZ and DZ twins are also assumed to be equal and symbolized by C ($C_{MZ} = C_{DZ} = C$). If it is further assumed that $\sigma^2_{eMZ} = \sigma^2_{eDZ}$, then a complex fraction of genetic variance may be estimated by subtracting the W_{MZ} mean square from the W_{DZ} mean square. The significance of this estimate (\widehat{G}_{WT}) may be tested by the F ratio W_{DZ}/W_{MZ} . Table 4 shows \widehat{G}_{WT} and the probabilities for the five lipids studied. All of the lipids quantitated had highly significant estimates. However, one of the assumptions required was $\sigma^2_{eMZ} = \sigma^2_{eDZ}$. This assumption is testable by comparing the sums of the within and among mean squares of MZ and DZ twins by a two-tailed F' test. The results of this comparison are shown in Table 5 with the approximate degrees of freedom calculated after Cochran (1951) and the probability found by multiplying the probability in the usual F table by 2, as this is a two-tailed test. For all plasma cholesterol fractions this probability was less than 0.05 indicating that the assumption $\sigma^2_{eMZ} = \sigma^2_{eDZ}$ is not valid. This inequality of the sum of the mean squares was consistent for the four cholesterol fractions measured with the DZ sum of the mean squares greater than the MZ sum of the mean squares. In contrast, the sum of the MZ mean squares for triglycerides was somewhat larger than the sum of the DZ mean squares.

When the F' test of total variance rejects the equality of σ^2_{MZ} and σ^2_{DZ} the use of \widehat{G}_{WT} to estimate genetic variance is questionable because this estimate is biased by the difference between σ^2_{eDZ} and σ^2_{eMZ} . In addition to the within twin-pair estimate of genetic variance a second and independent estimate of genetic variance may be obtained by subtracting the among DZ mean square from the among MZ mean square. This estimate has been called the among twin-pair estimate of genetic variance (\widehat{G}_{AT}). When the environmental variance component for DZ twins is greater than the environmental variance component for MZ twins, \widehat{G}_{WT} will be biased upward and the \widehat{G}_{AT} will be biased downward by the same amount. However, the arithmetic mean of these two estimates would be an unbiased estimate of genetic variance even when $\sigma^2_{eMZ} \neq \sigma^2_{eDZ}$. This third estimate has been called the component estimate (\widehat{G}_{CT}) because it is identical to Falconer's (1960) between twin-pairs component estimate of genetic variance.

Table 1. Previous twin studies of plasma cholesterol

Authors	Year	No. of sets		Within pair mean squares		F ratio
		DZ	MZ	W_{DZ}	W_{MZ}	W_{DZ}/W_{MZ}
Osborne et al.	1959	28	43	670	338	2.0*
Gedda and Poggi	1960	50	50	78	6	11.6**
Meyer	1962	17	24	745	297	2.5*
Jensen et al.	1965	17	31	2,661	1,110	2.4**
Blomstrand and Lundman	1966	102	91	2,046	1,592	1.3
Pikkarainen et al.	1966	54	65	1,374	793	1.7*
Rifkind et al.	1968	41	67	632	306	2.1**
Kang et al.	1971	37	45	1,641	433	3.8**

* $p < 0.05$, ** $p < 0.01$.

Table 2. Mean lipid values for study laboratories in the NHLI twin study (mg/100 ml)

Variable	Framingham (55 MZ, 50 DZ)	California (127 MZ, 141 DZ)	Indiana (68 MZ, 73 DZ)	Total (250 MZ, 264 DZ)
Total cholesterol	219.8	222.1	217.7	220.4
HDL cholesterol**	41.3	46.6	46.2	45.4
LDL cholesterol	143.0	143.9	143.1	143.5
VLDL cholesterol	34.4	31.7	29.4	31.6
Triglycerides	141.7	143.4	144.5	143.3

** Variation among laboratories significant at the 0.01 level.

Table 3. Analysis of variance model for twin studies, assuming $\sigma_{ge} = \sigma^*_{ge}$ and $C_{MZ} = C_{DZ} = C$

Source of variation	DF	Mean squares	Expected value of mean square
MZ twins:			
Among pairs	$n_{MZ} - 1$	A_{MZ}	$2\sigma^2_a + 2\sigma^2_d + 2\sigma^2_i + \sigma^2_{e,MZ} + 4\sigma_{ge} + C$
Within pairs	n_{MZ}	W_{MZ}	$\sigma^2_{e,MZ} - C$
DZ twins:			
Among pairs	$n_{DZ} - 1$	A_{DZ}	$3/2\sigma^2_a + 5/4\sigma^2_d + (1 + f)\sigma^2_i + \sigma^2_{e,DZ} + 4\sigma_{ge} + C$
Within pairs	n_{DZ}	W_{DZ}	$1/2\sigma^2_a + 3/4\sigma^2_d + (1 - f)\sigma^2_i + \sigma^2_{e,DZ} - C$

NOTE. n_{MZ} = number of MZ twin pairs; n_{DZ} = number of DZ twin pairs; σ^2_e = variance component due to additive genetic effects; σ^2_d = variance component due to dominant genetic effects; σ^2_i = variance component due to epistatic genetic effects; $\sigma^2_{e,MZ}$ = variance component due to environmental influence on MZ twins; $\sigma^2_{e,DZ}$ = variance component due to environmental influences on DZ twins; σ_{ge} = covariance between genetic and environmental effects in the same individual; σ^*_{ge} = covariance between genetic effects on one member of a twin pair and environmental effects on the other member of that twin pair; C_{MZ} = covariance among environmental effects between pairs of MZ twins; C_{DZ} = covariance among environmental effects between pairs of DZ twins; and f = one minus the fraction of epistatic variance manifest within DZ twin sets.

Table 4. \hat{G}_{WT} (mg/ml)² calculated for plasma lipids from the NHLI twin study (264 DZ and 250 MZ sets)

Variable	Mean squares		\hat{G}_{WT}	F ratio	p
	W_{DZ}	W_{MZ}			
Total cholesterol	977	434	543	2.2	< 10 ⁻⁶
HDL cholesterol	118	51	67	2.3	< 10 ⁻⁶
LDL cholesterol	892	374	518	2.4	< 10 ⁻⁶
VLDL cholesterol	598	209	389	2.9	< 10 ⁻⁶
Triglycerides	5747	3995	1752	1.4	0.002

Table 5. F' test for equality of total variance calculated for MZ and DZ twins in the NHLI twin study

Variable	DZ pairs		MZ pairs		F' ratio	p
	Total variance	Approx. DF	Total variance	Approx. DF		
Total cholesterol	1717	441	1234	349	1.4	0.001
HDL cholesterol	216	424	163	334	1.3	0.008
LDL cholesterol	1451	443	1138	336	1.3	0.02
VLDL cholesterol	857	466	446	382	1.9	< 10 ⁻⁶
Triglycerides	7990	477	9129	375	1.1	0.17

Table 6. \hat{G}_{CT} (mg/ml)² calculated for plasma lipids from the NHLI twin study (264 DZ and 250 MZ sets)

Variable	$A_{MZ} + W_{DZ}$	Approx. DF	$A_{DZ} + W_{MZ}$	Approx. DF	\hat{G}_{CT}	F' ratio	p
Total cholesterol	3010	446	2890	350	60	1.04	0.34
HDL cholesterol	392	425	365	337	14	1.07	0.25
LDL cholesterol	2793	435	2382	345	205	1.17	0.06
VLDL cholesterol	1280	496	1324	345	0	0.97	0.63
Triglycerides	20,010	421	14,227	429	2891	1.41	0.0002

\hat{G}_{CT} was calculated for the five lipid variables (Table 6) and the significance of this estimate tested by the F' test (Christian et al. 1974), $(A_{MZ} + W_{DZ})/(A_{DZ} + W_{DZ})$, and the approximate degrees of freedom calculated after Cochran (1951). Using \hat{G}_{CT} none of the cholesterol fractions measured had significant genetic variance although LDL cholesterol approached significance (p 0.06). VLDL cholesterol in fact had a negative estimate, shown as zero. In contrast triglycerides had a highly significant \hat{G}_{CT} as well as \hat{G}_{WT} .

DISCUSSION

The finding of highly significant genetic variance for plasma triglycerides indicates detectable genetic effects on the level of fasting plasma triglycerides. In this age group, using the Frederickson and Levy (1972) criteria, it should be fruitful to identify single genes causing hypertriglyceridemia from which one could study specific environmental modifications which influence the genetic tendency to abnormally high plasma triglyceride levels.

By making the further assumptions that σ_d^2 and $\sigma_i^2 = 0$, \widehat{G}_{CT} may be transformed into an estimate of total population genetic variance by multiplying it by two. In addition, an estimate of heritability may be obtained by dividing this estimate of total population genetic variance by an estimate of total population variance $[(W_{MZ} + A_{MZ} + W_{DZ} + A_{DZ})/4]$. This estimate of heritability was 0.68 (5,824/8,522).

These results don't rule out relatively rare mutations which affect plasma cholesterol levels; for example, familial hypercholesterolemia which is estimated to affect one in 200 individuals (Fredrickson and Levy 1972) may not be detected in a study of this type. The among component estimate of genetic variance (\widehat{G}_{CT}) for LDL cholesterol approached significance ($p 0.06$) and may well represent true genetic variance. However, this study indicates that the majority of variation of plasma cholesterol cannot be explained by genetic influences.

REFERENCES

- Blomstrand R., Lundman T. 1966. Serum lipids, smoking and heredity. *Acta Med. Scand.* [Suppl.], 455: 51-60.
- Christian J.C., Kang K.W., Norton J.A. Jr. 1974. Choice of an estimate of genetic variance from twin data. *Am. J. Hum. Genet.*, 26: 154-161.
- Cochran W.G. 1951. Testing a linear relation among variances. *Biometrics*, 7: 17-32.
- Feinleib M., Christian J.C., Borhani N.O., Rosenman R.J., Wagner J., Kannel W.B., Hrubec Z., Schwartz J.T. 1974. The National Heart and Lung Institute Twin Study of Cardiovascular Disease Risk Factors: organization and methodology. *Proc. 1st Int. Congr. Twin Studies, Rome.* *Acta Genet. Med. Gemellol.* (Roma), 25: 125-128.
- Falconer D.A. 1960. *Quantitative Genetics*. New York: Ronald Press.
- Fredrickson D.S., Levy R.I. 1972. Familial hyperlipoproteinemia. In J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson (eds.): *The Metabolic Basis of Inherited Disease* [pp. 545-614]. New York: McGraw Hill.
- Gedda L., Poggi D. 1960. Sulla regolazione genetica del colesterolo ematico. Uno studio su 50 coppie gemellari MZ e 50 coppie DZ. *Acta Genet. Med. Gemellol.* (Roma), 9: 135-154.
- Jensen J., Blankenhorn D., Chin H.P., Sturgeon P., Nare A.G. 1965. Serum lipids and serum uric acid in human twins. *J. Lipid Res.*, 6: 193-205.
- Kang K.W., Taylor G.E., Greves J.H., Staley H.L., Christian J.C. 1971. Genetic variability of human plasma and erythrocyte lipids. *Lipids*, 6: 595-600.
- Meyer K. 1962. Serum cholesterol and heredity. A twin study. *Acta Med. Scand.*, 172: 401-404.
- Osborne R.H., Adlersberg D., De George F.V., Wang C. 1959. Serum lipids, heredity and environment: a study of adult twins. *Am. J. Med.*, 26: 54-59.
- Pikkarainen J., Jakkunen J., Kulonen E. 1966. Serum cholesterol in Finnish twins. *Am. J. Hum. Genet.*, 18: 115-126.
- Rifkind B.M., Boyle J.A., Gale M., Greig W., Buchanan W.W. 1968. Study of serum lipid levels in twins. *Cardiovasc. Res.*, 2: 148-156.