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Do the MN and Jk systems influence environmental variability in serum lipid levels?

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Significant heterogeneity in the distribution of within pair variances of serum total cholesterol, HDL cholesterol, non HDL cholesterol and triglyceride levels has been found in one or both of two samples of MZ twins. We have found some support for the observation of Magnus et al. (1981) that M^- pairs have greater environmental variability in cholesterol levels than M^+ pairs and weaker evidence that Jk^{a+} pairs are more variable than Jk^{a-} pairs. However, these effects appear to be more striking on triglyceride levels. The low power of the variance ratio test is advanced as a possible reason for the inconsistencies in these findings.

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An apparent influence of the MN blood group system on variance in serum cholesterol levels within monozygotic (MZ) pairs of twins has been reported by Magnus et al. (1981). They found that the intrapair variance for cholesterol in 22 MZ pairs who were M^- (ie. blood group N) was significantly greater than in 75 pairs who were M^+ (M & MN). They also reported a similar but less striking difference concerning the Kidd blood group system where Jk^{a-} pairs had a smaller intrapair variance than Jk^{a+} pairs.

If substantiated, these findings would provide a striking example of the kind of specific genotype-environment interaction whose existence is often speculated upon but rarely demonstrated. They may also shed light on the nature of genetic differences in susceptibility to coronary heart disease.

Here we attempt to replicate the findings of Magnus et al. (1981) in two further (but much younger) samples of MZ twins for whom MN and Jk^a typing, and cholesterol, high density lipoprotein (HDL) cholesterol and triglyceride levels are available. Levels in many of the twins were measured on two occasions and we have also assessed the effect of marker type on intra-individual variation. One sample of MZ twins took part in a cotwin control study of the effect of vitamin C on, *inter alia*, serum lipid levels and the effect of marker type in response to this treatment has been examined. Although we find some support for the hypothesis of marker effects on environmental variability, our results are equivocal and we estimate the sample sizes required to reliably detect effects of the magnitude postulated.

Subjects and Methods

Blood typing and serum lipid measurements were available for two samples of twins.

(i) *Alcohol Study Twins*

Serum lipid measurements were available for 85 MZ pairs (out of a total of 207 pairs of all types) who took part in a study on the genetics of psychomotor susceptibility to alcohol (Martin et al. 1981, Whitfield & Martin 1983). From the MZ and DZ pairs who took part in the alcohol study, 88 individuals were tested on a second occasion between one and seventeen months (mean 4.5) after the first visit. Lipid measurements were available on both occasions for 85 of these individuals.

Twins who took part in the alcohol study were all of European extraction living in the Sydney area and were aged between 18 and 34 years. Blood samples in the alcohol study were all taken at 10 a.m. and before alcohol ingestion. Twins had been instructed to have only a light, non-fatty breakfast before 8 a.m. prior to taking part in the study.

(ii) *Vitamin C Study Twins*

A double-blind MZ cotwin control study was conducted to test the effect of a 1 g daily dose of vitamin C on incidence and severity of the common cold. A blood sample was taken and serum lipid levels measured immediately before commencement and then again towards the end of the 100 day trial. Details of the study and results of the treatment on respiratory symptoms have been reported elsewhere (Carr et al. 1981a). Small effects of the vitamin C treatment in lowering serum cholesterol levels were found but none of these was quite significant at the 5% level (Carr et al. 1981b).

Complete serum lipid measurements on both occasions were available for 106 MZ pairs (aged 14–64 years) who took part in this study. The time of day and dietary state of individuals when blood samples were taken was not closely controlled in this study, and the additional variation that this introduces could make the results from this sample less trustworthy.

Although there were 39 MZ pairs who took part in both the alcohol and vitamin C studies, the two studies and the measurements taken (except blood grouping) were quite independent; this overlap has been ignored in the ensuing analysis.

Zygosity Determination

All twins from both studies were blood typed with the following antisera; anti-A, A_1, B, C, c, D, E, e, M, N, S, s, Fy^a and K and were typed for the serum enzyme, alpha-1-antitrypsin (Pi). Jk^a typing was available for 63 of the alcohol study MZ twin pairs and 100 of the vitamin C study pairs. Twins were diagnosed as DZ on the basis of a difference in sex, at least one marker locus or, in a few cases, large differences in height, colouring or other morphological features. In remaining cases of doubtful zygosity several more genetic markers were typed. It is possible, however, that there are a few pairs diagnosed as MZ who, on still further typing, would prove to be DZ.

Analytical Methods

Plasma total cholesterol and triglycerides were measured on a Technicon SMAC by enzymatic methods (Lie et al. 1976, Bucolo & David 1973). HDL cholesterol was measured on plasma samples to which EDTA had been added, by enzymatic assay after polyethylene glycol precipitation (Allen et al. 1979).

Calculation and Transformation of Variables

Non-HDL cholesterol was calculated as Total cholesterol − HDL cholesterol and the ratio of HDL cholesterol to this fraction (HDL/NonHDL) was also calculated. The

Table 1

Intercorrelations of lipid variables (log_{10} transformed) and age for alcohol study MZ pairs

			FEMALES				
		1	2	3	4	5	6
MALES	1 Age	–	0.12	0.06	−0.13	0.05	−0.12
	2 Cholesterol	0.36**	–	0.29**	0.23*	0.95***	−0.50***
	3 Triglyceride	0.37**	0.50***	–	−0.44***	0.45***	−0.61***
	4 HDL Cholesterol	0.14	0.10	−0.19	–	−0.07	0.72***
	5 NonHDL Cholesterol	0.35**	0.97***	0.56***	−0.12	–	−0.74***
	6 HDL/NonHDL	−0.16	0.63***	0.52***	0.71***	−0.79***	–

* $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$; two tailed tests.

distributions of all lipid fractions were significantly positively skewed. This skewness biases tests for differences in means and variances so all raw lipid variables were transformed to a log_{10} scale. All the results reported below are based on this transformation.

Results

Correlations of Variables

Intercorrelations of the log_{10} transformed lipid variables and age are shown in Table 1 for the alcohol study MZ twins. Correlations for the vitamin C sample were similar. There are no significant correlations between age and lipid levels in females but there are significant correlations in the male sample. There is a large (0.5) correlation between total cholesterol and triglyceride levels in males but this is less striking in females. The correlation of total and nonHDL cholesterol is almost complete in both sexes but their correlations with HDL cholesterol are very small. These intercorrelations must be taken into account when assessing the results below of analysis of variance by marker type.

Means and Total Variances

Means and standard variations for age and log_{10} lipid variables by sex and marker type are shown in Table 2 for the MN system and for Jk^a type in Table 3. M^- pairs in the alcohol study are just significantly older. M^- males and Jk^{a-} males in the vitamin C study are significantly more variable in age as are Jk^{a+} females from this study. Despite the correlations in males between some lipid levels and age, these differences in age distribution are not large enough to produce differences in variances between marker types of the magnitudes which we have the statistical power to detect. Note, however, that our twins are much younger than the 33–40 and 57–61 year old samples employed by Magnus et al. (1981).

There are several differences between marker types in means and variances of lipid levels, but none of these is consistent between sexes or between studies. If there are differences between marker types in intrapair variances then we expect this to be reflected in their total variances. But unless such an effect is large, we would expect it to be undetectable against the background of a large amount of between pairs variation, whether of genetic or family environmental origin.

Heterogeneity in the Distribution of MZ Intrapair Differences

If marker type has a substantial effect on MZ within pair variances then we might

Table 2

Means and standard deviations for age and $\log_{10}$ transformed lipid variables by sex and M type. Significance of differences between marker types is indicated

		FEMALES				MALES				FEMALES + MALES			
		M^+		M^-		M^+		M^-		M^+		M^-	
Variable	Study	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	A	23.2	4.4	26.1	5.7	24.6	5.2	25.5	5.8	23.8*	4.8	25.7*	5.7
	V	24.9	11.5	26.7	12.1	22.7	8.2***	27.5	15.7***	24.0	10.3*	27.1	13.6*
Cholesterol	A	0.724*	0.099**	0.765*	0.044**	0.775**	0.085	0.718**	0.086	0.747	0.096	0.735	0.077
	V1	0.730*	0.098**	0.761*	0.055**	0.712	0.078	0.737	0.082	0.723*	0.090*	0.751*	0.068*
	V2	0.715	0.089*	0.742	0.056*	0.694	0.076	0.727	0.082	0.707*	0.084	0.736*	0.067
Triglyceride	A	−0.149	0.153	−0.085	0.166	0.022	0.275	−0.048	0.260	−0.072	0.232	−0.061	0.230
	V1	−0.001	0.219	0.010	0.197	0.167	0.227	0.085	0.291	0.067	0.237	0.042	0.240
	V2	−0.009*	0.218	0.095*	0.223	0.192	0.237	0.084	0.243	0.072	0.245	0.090	0.228
HDL	A	0.102	0.109	0.082	0.099	0.028	0.094	0.033	0.105	0.070	0.109	0.050	0.104
Cholesterol	V1	0.113	0.090	0.105	0.097	0.018	0.110	0.049	0.113	0.075	0.108	0.080	0.106
	V2	0.104	0.103	0.108	0.119	0.021	0.112	0.023	0.120	0.071	0.114	0.072	0.126
NonHDL	A	0.583***	0.114**	0.661***	0.047**	0.681*	0.107	0.612*	0.109	0.626	0.121	0.629	0.095
Cholesterol	V1	0.610	0.122*	0.648	0.079*	0.610	0.097	0.630	0.107	0.610	0.113	0.640	0.092
	V2	0.586	0.117	0.619	0.088	0.584	0.096	0.625	0.101	0.585	0.109	0.622	0.093
HDL/NonHDL	A	−0.483*	0.163	−0.579*	0.111	−0.652	0.150	−0.579	0.162	−0.557	0.178	−0.579	0.144
	V1	−0.497	0.145	−0.543	0.153	−0.592	0.154	−0.581	0.177	−0.535	0.156	−0.560	0.163
	V2	−0.481	0.161	−0.511	0.187	−0.564	0.158	−0.603	0.173	−0.514	0.164	−0.549	0.185

A = alcohol study MZ twins; V = vitamin C study twins – V1 first visit, V2 second visit.

Table 3

Means and standard deviations by sex and Jk^a type

		FEMALES				MALES				FEMALES + MALES			
		Jk^{a+}		Jk^{a-}		Jk^{a+}		Jk^{a-}		Jk^{a+}		Jk^{a-}	
Variable	Study	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	A	23.1	5.7*	22.7	3.4*	24.4	5.5	24.7	6.3	23.8	5.6	23.6	4.9
	V	25.7	12.3	24.3	10.7	22.2	7.4***	26.6	16.0***	24.3	10.7	25.1	12.8
Cholesterol	A	0.710	0.095	0.750	0.076	0.757	0.089	0.740	0.060	0.735	0.095	0.745	0.068
	V1	0.743	0.096	0.714	0.087	0.707	0.075	0.732	0.083	0.728	0.090	0.721	0.085
	V2	0.726	0.086	0.699	0.085	0.690*	0.073	0.730*	0.085	0.711	0.082	0.710	0.086
Triglyceride	A	−0.145	0.183**	−0.121	0.089**	−0.024	0.293*	0.056	0.151*	−0.080	0.255**	−0.040	0.149**
	V1	0.018	0.207	−0.036	0.236	0.134	0.232	0.209	0.238	0.066	0.224	0.055	0.263
	V2	0.034	0.243**	−0.030	0.156**	0.174	0.248	0.205	0.201	0.092	0.253	0.057	0.207
HDL	A	0.097	0.115	0.135	0.095	0.017**	0.092	0.077**	0.057	0.053*	0.110	0.108*	0.084
Cholesterol	V1	0.101	0.092	0.136	0.083	0.014	0.113	0.009	0.082	0.066	0.109	0.087	0.102
	V2	0.090*	0.108	0.134*	0.100	0.007	0.112	0.038	0.112	0.056*	0.116	0.099*	0.114
NonHDL	A	0.578	0.132	0.627	0.081	0.665	0.112	0.632	0.068	0.625	0.129**	0.629	0.074**
Cholesterol	V1	0.634**	0.113	0.570**	0.125	0.604	0.094	0.635	0.109	0.622	0.107	0.595	0.122
	V2	0.605*	0.109	0.551*	0.125	0.584	0.090	0.622	0.119	0.596	0.102*	0.578	0.126*
HDL/NonHDL	A	−0.481	0.191**	−0.491	0.086**	−0.648**	0.159**	−0.556**	0.067**	−0.571*	0.192***	−0.521*	0.083***
	V1	−0.533**	0.137	−0.434**	0.157	−0.589	0.151	−0.626	0.169	−0.556	0.145*	−0.508	0.186*
	V2	−0.515**	0.160	−0.417**	0.173	−0.577	0.151	−0.584	0.198	−0.541*	0.159*	−0.479*	0.198*

expect that the distribution of absolute pair differences should be heterogeneous, being a mixture of two or more populations of such differences. Fisher (1925) provided a test for such heterogeneity and this was applied by Jensen et al. (1965) to several lipid measurements made on a sample of 31 pairs of MZ twins. Although their published calculations contain errors, they demonstrated significant departures from homogeneity of within pair differences for total and free cholesterol and for glyceride glycerol, suggesting that there were at least two distributions of differences for each of these measures. Fisher (1925) describes his test thus:

"If d is the difference of any one pair, found by subtracting the less measurement from the greater, and $\bar{d}$ stand for the mean difference, $\overline{d^2}$ for the mean of the squared difference, then for a large sample of normally distributed values we should have

$$\overline{d^2} = \frac{\Pi}{2}\bar{d}^2$$

whereas, for a mixture of two such populations, with different mean differences, $\overline{d^2} - \frac{\Pi}{2}\bar{d}^2$ should be positive. To utilize this fact it is necessary to know the standard error of $\overline{d^2} - \frac{\Pi}{2}\bar{d}^2$ and this is found to be

$$\frac{\overline{d^2}}{\sqrt{n}}\sqrt{(2\Pi-6)} = \frac{\overline{d^2}}{\sqrt{n}} \times \cdot 5321"$$

(Note that Fisher omits in error the $\sqrt{}$ over $(2\Pi-6)$ in his original paper (Bennett 1972)).

We have applied this test to the distributions of MZ absolute pair differences for each variable. The t values and their one-tailed significance for $t>0$ are shown in Table 4. Many of the variables show heterogeneity in the distribution although only nonHDL cholesterol does so consistently in both the alcohol and vitamin C studies.

Table 4

t values, and their significance for $t>0$. Tests for heterogeneity of MZ within pair differences according to Fisher (1925). t significantly greater than zero implies mixture of more than one distribution of differences

		FEMALES		MALES		FEMALES+MALES	
Variable	Study	N	t	N	t	N	t
Cholesterol	A	43	0.076	42	1.263	85	0.988
	V1	63	−0.298	43	3.441***	106	1.969*
	V2	63	1.680*	43	2.378*	106	2.878**
Triglyceride	A	43	−0.187	42	0.478	85	0.885
	V1	43	1.293	43	1.021	106	1.939*
	V2	63	1.334	43	2.249*	106	3.028**
HDL	A	42	−0.413	39	0.363	81	0.088
Cholesterol	V1	60	2.013*	40	2.343*	100	3.045**
	V2	63	2.542**	43	0.662	106	2.398**
NonHDL	A	41	1.770*	39	2.088*	80	2.755**
Cholesterol	V1	63	0.821	43	3.211**	106	2.681**
	V2	63	3.327**	43	2.349*	106	4.071***
HDL/nonHDL	A	41	0.314	39	0.886	80	1.086
	V1	60	3.523***	40	0.632	100	3.291***
	V2	63	3.403***	43	−0.118	106	2.576**

A=alcohol study MZ twins; V1,V2=vitamin C study twins first and second visits.

Table 5

Within pairs meansquares ($\times 10^5$) by M type and sex. Variance ratio (F) of WMS(M^-)/WMS(M^+) and its one-tailed probability (P) are given

		FEMALES						MALES						FEMALES+MALES					
		M^+		M^-				M^+		M^-				M^+		M^-			
Variable	Study	df	WMS	df	WMS	F	P	df	WMS	df	WMS	F	P	df	WMS	df	WMS	F	P
Cholesterol	A	35	161	8	78	0.49	0.856	29	93	13	311	3.33	0.003	64	130	21	222	1.71	0.053
	V1	52	145	11	63	0.43	0.933	35	111	8	73	0.66	0.724	87	131	19	67	0.51	0.951
	V2	52	116	11	129	1.11	0.371	35	127	8	197	1.55	0.176	87	120	19	158	1.32	0.194
	AR	36	106	2	60	0.57	0.573	35	147	12	101	0.69	0.748	71	126	14	95	0.76	0.710
	VR	104	106	22	156	1.47	0.101	70	134	16	162	1.21	0.284	174	117	38	159	1.36	0.097
Triglyceride	A	35	1203	8	2341	1.95	0.084	29	3115	13	2872	0.92	0.543	64	2069	21	2669	1.29	0.216
	V1	52	1203	11	1774	1.47	0.170	35	1835	8	3763	2.05	0.069	87	1457	19	2612	1.79	0.036
	V2	52	1235	11	1216	0.98	0.472	35	2581	8	1202	0.47	0.872	87	1777	19	1210	0.68	0.828
	AR	36	872	2	2229	2.56	0.092	35	3386	12	1859	0.55	0.867	71	2112	14	1912	0.91	0.557
	VR	104	1694	22	3465	2.05	0.009	70	2571	16	3726	1.45	0.145	174	2047	38	3575	1.75	0.009
HDL	A	34	386	8	552	1.43	0.220	26	270	13	276	1.02	0.461	60	336	21	381	1.14	0.340
Cholesterol	V1	50	260	10	512	1.97	0.057	32	188	8	679	3.61	0.004	82	232	18	586	2.53	0.002
	V2	52	284	11	293	1.03	0.433	35	249	8	455	1.83	0.105	87	270	19	361	1.34	0.182
	AR	32	422	2	366	0.87	0.430	33	296	12	304	1.03	0.447	65	358	14	313	0.87	0.590
	VR	100	158	20	217	1.37	0.154	65	212	16	383	1.81	0.049	165	179	36	291	1.63	0.022
NonHDL	A	33	243	8	70	0.29	0.965	26	121	13	481	3.96	0.001	59	189	21	324	1.71	0.055
Cholesterol	V1	50	242	10	89	0.37	0.955	32	158	8	190	1.20	0.329	82	209	18	133	0.64	0.861
	V2	52	179	11	263	1.47	0.172	35	200	8	340	1.70	0.133	87	188	19	295	1.57	0.083
	AR	32	181	2	65	0.36	0.702	33	272	12	133	0.49	0.906	65	227	14	124	0.54	0.897
	VR	100	161	20	205	1.27	0.215	65	189	16	244	1.29	0.230	165	172	36	222	1.29	0.145
HDL/NonHDL	A	33	711	8	615	0.86	0.555	26	316	13	561	1.77	0.104	59	537	21	581	1.08	0.391
	V1	50	513	10	674	1.31	0.249	32	273	8	1140	4.18	0.002	82	419	18	881	2.10	0.013
	V2	52	462	11	769	1.66	0.108	35	521	8	814	1.56	0.172	87	485	19	788	1.62	0.068
	AR	32	689	2	393	0.57	0.571	33	791	12	409	0.52	0.889	65	741	14	406	0.55	0.894
	VR	100	260	20	311	1.20	0.274	65	465	16	619	1.33	0.206	165	341	36	448	1.31	0.129

A = Alcohol Study MZ twins; V1,V2 = Vitamin C Study MZ twins, Visits 1 & 2; AR,VR = Repeat individuals for Alcohol Study, Vitamin C Study respectively.

It is now appropriate to see the extent to which marker type contributes to this heterogeneity of within pair differences.

Intrapair Variances of M^+ and M^- Groups
Within pairs meansquares (WMS) for M^+ and M^- MZ pairs from the alcohol study (A) and the vitamin C study first (V1) and second (V2) visits are shown in Table 5. Intra-individual meansquares for the individuals for whom two measurements were taken in the alcohol study (AR) and for all the individuals in the vitamin C study (VR), are also shown in Table 5. The meansquares are compared, separately for females, males and the sexes combined, by one-tailed variance ratio (F) tests, and the probability of the observed ratio of the M^- meansquare to its M^+ counterpart is given.

A glance at the results reveals a high degree of inconsistency, between sexes, between studies and between the within MZ pair and within individual comparisons. Nevertheless, disregarding their lack of independence, of twenty-five variance ratio comparisons on the combined female and male data, ten are significant at the 10% level, five at the 5% level and two at the 1% level in the expected direction. Only one comparison is significant at the 5% level in the opposite direction to that expected.

In the alcohol study twins, all five comparisons yield F ratios greater than one, those for total and nonHDL cholesterol being almost significant at the 5% level. These two ratios are highly significant in the male twins but less than one, significantly so for nonHDL cholesterol in the female twins. In the vitamin C study twins there is great inconsistency between the ratios for first and second visits except for HDL cholesterol and the HDL/nonHDL ratio where a 5% significant result is found on the first and almost so on the second visit.

In the two repeat studies, the M^-/M^+ variance ratio is less than one for all five variables in the alcohol study, but greater than one for all five variables in the vitamin C study, significantly so for three variables at the 10% level.

Intrapair Variances for Jk^{a+} and Jk^{a-} Groups
These are shown in Table 6 and the variance ratio $WMS(Jk^{a+})/WMS(Jk^{a-})$ and its one-tailed significance are also given. If there is any effect of the Jk locus on environmental variability it is even less consistent and less striking than the effect of the MN locus. Of twenty-five comparisons on the combined sex data, three are significant at the 1% level in the expected direction and one is significant at the 5% level in the direction opposite to that reported by Magnus et al. (1981). The effect, if there is one, is perhaps strongest for triglyceride, although even there the results are inconsistent.

Differential Effect of Vitamin C on Cholesterol Levels Between Marker Types
One further contrast can be extracted from the vitamin C data. Analysis of the total data set showed almost significant effects of vitamin C in lowering serum levels of total cholesterol ($P=0.054$), HDL cholesterol (0.085) and non-HDL cholesterol (0.068) (Carr et al. 1981b). We postulate that vitamin C will have a larger effect on serum lipid levels in M^- than in M^+ individuals, in Jk^{a+} than Jk^{a-} individuals and in M^-Jk^{a+} than M^+Jk^{a-} individuals. Consequently we calculated the difference of the occasion differences between the vitamin C treatment group (C) and their cotwins who were allocated (blindly) to the placebo treatment (P) i.e. $D=(C_1-C_2)-(P_1-P_2)$. If the vitamin C treatment has lowered cholesterol levels more than the placebo treatment this quantity should be negative. We compared these D values in each of the pairs of marker types stated above but no significant differences were found.

Table 6

Within pairs meansquares ($\times 10^5$) by Jk^a type and sex. Variance ratio (F) of WMS(Jk^{a+})/WMS(Jk^{a-}) and its one-tailed probability (P) are given

		FEMALES						MALES						FEMALES+MALES					
		Jk^{a+}		Jk^{a-}				Jk^{a+}		Jk^{a-}				Jk^{a+}		Jk^{a-}			
Variable	Study	df	WMS	df	WMS	F	P	df	WMS	df	WMS	F	P	df	WMS	df	WMS	F	P
Cholesterol	A	23	127	7	281	0.45	0.923	27	183	6	91	2.01	0.195	50	157	13	193	0.81	0.712
	V1	43	135	17	115	1.17	0.371	30	108	10	74	1.46	0.270	73	124	27	100	1.24	0.271
	V2	43	98	17	188	0.52	0.957	30	169	10	84	2.01	0.122	73	127	27	150	0.85	0.717
	AR	15	157					27	109	5	145	0.75	0.719	42	126	5	145	0.87	0.651
	VR	86	127	34	97	1.31	0.191	60	135	20	102	1.32	0.247	146	130	54	99	1.31	0.126
Triglyceride	A	23	1309	7	963	1.36	0.356	27	2700	6	2253	1.20	0.446	50	2060	13	1558	1.32	0.300
	V1	43	1252	17	1468	0.85	0.675	30	2111	10	2991	0.71	0.780	73	1605	27	2032	0.79	0.788
	V2	43	1343	17	1009	1.33	0.266	30	3023	10	713	4.24	0.010	73	2033	27	900	2.26	0.010
	AR	15	856					27	3301	5	3495	0.94	0.595	42	2428	5	3495	0.69	0.770
	VR	86	2373	34	1267	1.87	0.021	60	3275	20	1629	2.01	0.043	146	2743	54	1401	1.96	0.003
HDL	A	23	416	7	509	0.82	0.670	27	326	6	146	2.23	0.160	50	367	13	342	1.07	0.472
Cholesterol	V1	41	310	16	284	1.09	0.442	27	321	10	249	1.29	0.349	68	314	26	270	1.16	0.342
	V2	43	322	17	235	1.37	0.244	30	299	10	281	1.06	0.488	73	313	27	252	1.24	0.269
	AR	15	606					27	208	5	179	1.16	0.480	42	350	5	179	1.96	0.234
	VR	82	168	32	146	1.15	0.335	55	252	20	240	1.05	0.471	137	202	52	182	1.11	0.340
NonHDL	A	23	203	7	356	0.57	0.854	27	271	6	118	2.30	0.151	50	240	13	246	0.98	0.557
Cholesterol	V1	41	211	16	245	0.86	0.663	27	177	10	99	1.79	0.168	68	198	26	189	1.05	0.463
	V2	43	117	17	411	0.28	0.999	30	272	10	133	2.05	0.116	73	181	27	308	0.59	0.962
	AR	15	282					27	206	5	150	1.37	0.392	42	233	5	150	1.55	0.332
	VR	82	196	32	119	1.65	0.058	55	184	20	186	0.99	0.535	137	191	52	144	1.33	0.123
HDL/NonHDL	A	23	806	7	637	1.27	0.397	27	496	6	178	2.79	0.102	50	638	13	426	1.50	0.216
	V1	41	517	16	689	0.75	0.776	27	497	10	356	1.40	0.298	68	509	26	561	0.91	0.636
	V2	43	406	17	851	0.48	0.975	30	617	10	499	1.24	0.378	73	493	27	721	0.68	0.898
	AR	15	1057					27	582	5	70	8.31	0.013	42	752	5	70	10.74	0.007
	VR	82	302	32	166	1.82	0.030	55	500	20	531	0.94	0.587	137	382	52	306	1.25	0.182

A = Alcohol Study MZ twins; V1,V2 = Vitamin C Study MZ twins, Visits 1 & 2; AR,VR = Repeat individuals for Alcohol Study, Vitamin C Study respectively.

Intrapair Variances by M and Jk^a Type

If both M and Jk^a type influence environmental variability, more striking differences in within pairs meansquares should be detected by considering both markers jointly. Within pairs meansquares and their degrees of freedom are given for all four phenotypes in Table 7. Because the cell sizes are smaller with four classes, only the combined female and male WMS's are given. There were no consistent differences in means or total variances for age or any of the lipid variables between the four phenotypes.

For each variable and study, the meansquares have been tested for homogeneity using Bartlett's test (Snedecor & Cochran 1980) and its χ^2 and significance are also shown in Table 7. Of the twenty-five tests, nine are significant at the 10% level, five at the 5% and three at the 1% level.

We would predict that the M^-Jk^{a+} group should have a larger variance than the M^+Jk^{a-} group and a direct test of this, omitting the alcohol repeat group with very small numbers of either phenotype, yields eighteen out of twenty F values greater than one of which six are significant at the 10%, five at the 5% and one at the 0.1% level.

Table 7

Within pairs meansquares ($\times 10^5$) by M and Jk^a type. χ^2 for Bartlett's test of heterogeneity of variance and its significance are given

		FEMALES + MALES									
		$M^+ Jk^{a+}$		$M^+ Jk^{a-}$		$M^- Jk^{a+}$		$M^- Jk^{a-}$			
Variable	Study	df	WMS	df	WMS	df	WMS	df	WMS	df	χ^2
Cholesterol	A	38	94	11	225	12	356	2	19	3	11.93**
	V1	58	139	24	107	15	67	3	44	3	3.83
	V2	58	115	24	152	15	174	3	129	3	1.34
	AR	35	146	4	86	7	26			2	5.75†
	VR	116	120	48	94	30	170	6	140	3	3.34
Triglyceride	A	38	1850	11	806	12	2726	2	5696	3	5.36
	V1	58	1389	24	1746	15	2441	3	4323	3	3.86
	V2	58	2198	24	932	15	1398	3	644	3	6.65†
	AR	35	2531	4	4069	7	1912			2	0.73
	VR	116	2371	48	1442	30	4184	6	1078	3	12.12**
HDL	A	38	346	11	374	12	434	2	165	3	0.69
Cholesterol	V1	53	238	24	222	15	585	2	854	3	7.61†
	V2	58	313	24	194	15	311	3	711	3	3.39
	AR	35	396	4	174	7	121			2	3.63
	VR	107	187	48	157	30	253	4	485	3	4.40
NonHDL	A	38	146	11	290	12	537	2	9	3	13.05**
Cholesterol	V1	53	212	24	203	15	146	2	23	3	3.08
	V2	58	147	24	308	15	311	3	310	3	6.52†
	AR	35	270	4	78	7	48			2	7.07*
	VR	107	179	48	139	30	236	4	207	3	2.63
HDL/NonHDL	A	38	593	11	481	12	783	2	119	3	2.18
	V1	53	377	24	557	15	974	2	610	3	5.83
	V2	58	426	24	662	15	751	3	1190	3	3.96
	AR	35	858	4	75	7	221			2	8.83*
	VR	107	387	48	247	30	361	4	1013	3	6.21

A = Alcohol Study MZ twins; V1, V2 = Vitamin C Study MZ twins, Visits 1 & 2; AR, VR = Repeat individuals for Alcohol Study, Vitamin C Study respectively.

† $0.05 < P < 0.10$; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$.

Table 8

Orthogonal comparisons of within pairs meansquares (WMS) for a mean environmental effect (E_1), an environmental effect due to M type (E_1M), to Jk^a type (E_1J) and to the interaction of M and Jk^a types (E_1MJ)

WMS	E_1	E_1M	E_1J	E_1MJ
M^+Jk^{a+}	1	1	1	1
M^+Jk^{a-}	1	1	−1	−1
M^-Jk^{a+}	1	−1	1	−1
M^-Jk^{a-}	1	−1	−1	1

A more satisfactory test of the hypothesis that both M and Jk^a types affect environmental variance is gained by fitting a model specifying these effects to all four meansquares. A complete model including a mean environmental effect (E_1), an environmental effect of M type (E_1M) and of Jk^a type (E_1J), and an interaction effect of the two types (E_1MJ) is shown in Table 8. As specified, these four contrasts should be orthogonal but their estimates are far from independent because the degrees of freedom of the four meansquares to which they are fitted are unequal.

Fitting all four parameters to the four meansquares provides a perfect fit solution with no degrees of freedom left to test the fit of the model. We used the method of iterative weighted least squares (Eaves & Eysenck 1975) to fit four models: E_1; E_1,E_1M; E_1,E_1J and E_1,E_1M,E_1J. The fit of E_1 model provided a residual chi-square very close to the Bartlett homogeneity χ^2 of Table 7. Strictly speaking, one is only justified to fit more elaborate models if this simplest model fails, thus demonstrating that there is heterogeneity of the four WMS's. However, for completeness the fit of the three parameter E_1,E_1M,E_1J model is shown in Table 9 for all twenty sets of meansquares (the alcohol repeat individual

Table 9

Parameter estimates and their significance for a model including a mean environmental effect and additional environmental effects due to M type and Jk^a type. Fit of model (χ^2_1) is shown

Variable	Study	$\hat{E}_1$	$\hat{E_1M}$	$\hat{E_1J}$	χ^2_1
Cholesterol	A	263***	−112*	−56	1.04
	V1	89***	34**	14	0.02
	V2	156***	−24	−16	0.21
	VR	132***	−25	13	0.00
Triglyceride	A	2242***	−921†	460*	1.65
	V1	2215***	−627†	−213	0.31
	V2	1311***	231	555**	0.34
	VR	2492***	−578†	527**	1.48
HDL Cholesterol	A	369***	−20	7	0.44
	V1	422***	−191*	6	0.19
	V2	320***	−64	43	2.48
	VR	225***	−52†	10	1.60
NonHDL Cholesterol	A	372***	−164*	−60	1.12
	V1	152***	53*	27	1.09
	V2	299***	−75†	−76*	0.17
	VR	189***	−29	20	0.00
HDL/NonHDL	A	546***	−19	99	0.88
	V1	737***	−274*	−84	0.22
	V2	721***	−173	−124	0.05
	VR	380***	−61	48†	6.35*

† $0.05<P<0.10$; * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$ (1 tail tests).

WMS's were omitted because there were no M^-Jk^{a-} individuals).

The fit of this model, as judged by the residual χ^2_1, is highly satisfactory in all cases except the HDL/nonHDL ratio for the vitamin C repeat individuals (VR), where a significant E_1MJ interaction term was estimated in the perfect fit solution.

From the results of Magnus et al. (1981) we expect $\hat{E_1}M$ to be negative and $\hat{E_1}J$ to be positive. In fact seventeen out of twenty values of $\hat{E_1}M$ are negative, nine at the 10% and five at the 5% levels. Two of the estimates of $\hat{E_1}M$ are significant in the non-expected direction. Thirteen of the twenty values of $\hat{E_1}J$ are positive, four at the 10%, three at the 5% and two at the 1% level. One value of $\hat{E_1}J$ is significantly negative.

The results for total cholesterol provide only moderate support for the original observation of Magnus et al. (1981). However, the results for triglyceride are more striking with reasonably consistent evidence of an increasing effect on environmental variance of both N and Jk^a alleles.

Discussion

Although our results contain many inconsistencies they do provide some support for the finding of Jensen et al. (1965) that there is heterogeneity in MZ pair variances for some lipid levels. There is also some support for the hypothesis that M^- individuals are more susceptible than M^+ to environmental influences on serum lipid levels but rather less evidence for the greater environmental variability of Jk^{a+} than Jk^{a-} individuals as found by Magnus et al. (1981). While these authors only considered cholesterol levels, our results are more consistent for serum triglyceride. One reason for the less striking results in our samples may arise from the doubtful procedure employed by Magnus et al. (1981) of testing differences between mean absolute differences. Since absolute differences have highly skewed distributions, such tests may give quite misleading results and the correct procedure is to square the differences and compare the resulting meansquares by variance ratio test.

One considerable obstacle to finding significant evidence for these hypotheses is the low power of the variance ratio test. For example, the 95% confidence limits for a ratio of 1.5 given 15 M^- pairs and 60 M^+ pairs are 0.73 to 3.78. In Northern European populations the frequency of M^- individuals is about 0.2 (Race & Sanger 1968) so in a sample of n pairs of MZ twins, we need to know the power (β) of the F test with degrees of freedom $0.2n, 0.8n$ to detect a difference between within pairs meansquares of a given magnitude λ at a given one-tail α level.

If the true value of the ratio $WMS(M^-)/WMS(M^+)$ is λ, then we require n such that $\Pr(F(\alpha, 0.2n, 0.8n)/\lambda) = \beta$ (Pearson & Hartley 1972). For $\beta = 0.95$ and α(1 tail) $= 0.05$, the total numbers of pairs required for values of λ, (1.5, 2.0, 2.5, 3.0) are given in Table 10. Similar calculation for postulated effects of the Jk^a locus are also shown in Table 10, based upon a frequency of Jk^{a+} of 0.75 (Race & Sanger 1968) so that degrees of freedom for F are $0.75n$, $0.25n$.

Table 10

Number of pairs (n) required to detect a difference in variances of size λ for $\alpha = 0.05$ (1 tail) and $\beta = 0.95$. Power (β) to detect these effects in a sample of 100 pairs for $\alpha = 0.05$ is given. For the M system, $F = WMS\ (M^-)/WMS(M^+)$ and $v_1 = 0.2n$, $v_2 = 0.8n$. For the Jk^a system, $F = WMS(Jk^{a+})/WMS(Jk^{a-})$ and $v_1 = 0.75n$, $v_2 = 0.25n$

	λ	1.5	2.0	2.5	3.0
M	n	790	250	155	120
	β	0.33	0.65	0.83	0.92
Jk^a	n	660	220	140	100
	β	0.31	0.65	0.86	0.95

Also shown is the power of a total sample of 100 pairs, to detect effects of these magnitudes. This is the approximate sample size available in each of the MZ twin samples and in the alcohol repeat sample. It can be seen that if the variance of M^- individuals is 50% greater than that of M^+ individuals, there is only a one third chance of detecting a significant effect at the 5% level of significance and that nearly 800 pairs would be required to detect such an effect with 95% probability. Increasing effects on variance of the Jk^a allele would be similarly difficult to detect. Power is obviously greater for larger effects of marker type on variance, but it can be seen that a three-fold or more effect on variance would be required in order to reliably detect a difference with the total sample sizes available in this study. Of course, splitting the sample into sex or age categories merely substitutes two or more tests of considerably lower power.

The inconsistencies in our data and of ours with the significant effects demonstrated by Magnus et al. (1981) could well be explained by the low power of the variance ratio test. Clearly, much larger samples of twins are required to obtain reliable evidence for or against the hypothesis of the effect of marker types on environmental variability. As Magnus et al. (1981) point out, the effect may be more pronounced in the older twins which they tested and our difficulty in detecting effects as large as they did may be because of our much younger samples. A further strategy might be an intervention study in which twins of different blood groups are placed on high and low lipid diets.

Associations with a marker locus may either be due to pleiotropic effects of the marker locus itself or to effects of other genes in linkage disequilibrium with it. The fact that Magnus et al. (1981) found no effect of the S polymorphism which is closely linked to the MN locus and is in linkage disequilibrium with it, tends to argue for a pleiotropic effect of the MN locus itself.

The MNSs determinants appear to be associated with two human erythrocyte membrane sialoglycoproteins. The M and N determinants are associated with glycophorin A, while the S and s determinants are associated with glycophorin B. There is evidence that MN determinants, or closely related genes, are also expressed in lymphocytes (Blajchman et al. 1982). If these determinants are present on the surface of other cells, one might postulate some interaction with cell surface LDL receptors as a pleiotropic effect. On the other hand, despite the reported lack of association with the S polymorphism, it may be worth checking for associations with other polymorphisms in the MN region of chromosome 4, such as Gc (Falk et al. 1979). We have not looked for an association with the S polymorphism although the typing is available for our samples.

If the M allele decreases environmental variance in plasma lipid levels then we might expect more M^- individuals in the tails of the distribution, even if there is no difference in the means of the two groups. Because hyperlipidaemia is a risk factor for coronary heart disease (Goldstein et al. 1973) we may therefore expect that MN blood group may be as well. Association of MN blood group with another CHD risk factor, hypertension, has been reported by Cruz-Coke et al. (1964) who found a significantly higher diastolic blood pressure in NN than MM individuals and they also observed, but did not comment on, a greater variance in the NN blood group ($P = 0.06$, 2 tail). A number of associations of the MN blood group with hypertension and various forms of coronary heart disease have been reported, although these are inconsistent in that some groups show an elevated risk of blood group N and others of group M (Mourant et al. 1978). It is possible that this

inconsistency is compatible with the theory since in a "safe" environment the environmentally labile group may be better off while in an "unsafe" environment the stable group will be favoured. To the extent that elevated serum levels of cholesterol and its fractions and increased blood pressure are associated with CHD, an association of the MN locus with environmental variability may have clinical importance.

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