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⁺⁺ pairs are more variable than Jk⁺⁻ 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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Key words: Cholesterol; coronary heart disease; genotype-environment interaction; Jk blood group; MN blood group; MZ twins; triglyceride.

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⁺⁻ pairs had a smaller intrapair variance than Jk⁺⁺ 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⁺ 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 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, A1, B, C, c, D, E, e, M, N, S, s, Fya and K and were typed for the serum enzyme, alpha-1-antitrypsin (Pi). Jka 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

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
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<tr>
<td>FEMALES</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 Age</td>
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<td>0.06</td>
<td>–</td>
<td>0.05</td>
<td>–</td>
</tr>
<tr>
<td>2 Cholesterol</td>
<td>0.36**</td>
<td>–</td>
<td>0.29**</td>
<td>0.23*</td>
<td>0.95***</td>
<td>–</td>
</tr>
<tr>
<td>3 Triglyceride</td>
<td>0.37**</td>
<td>0.50***</td>
<td>–</td>
<td>–</td>
<td>0.44***</td>
<td>0.45***</td>
</tr>
<tr>
<td>MALES</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 HDL Cholesterol</td>
<td>0.14</td>
<td>0.10</td>
<td>–</td>
<td>0.19</td>
<td>–</td>
<td>–</td>
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<tr>
<td>5 NonHDL Cholesterol</td>
<td>0.35**</td>
<td>0.97***</td>
<td>0.56***</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6 HDL/NonHDL</td>
<td>–</td>
<td>0.16</td>
<td>0.63***</td>
<td>0.52***</td>
<td>0.71***</td>
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* 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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study</th>
<th>FEMALES M+</th>
<th>FEMALES M-</th>
<th>MALES M+</th>
<th>MALES M-</th>
<th>FEMALES+MALES M+</th>
<th>FEMALES+MALES M-</th>
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<tr>
<td></td>
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<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age</td>
<td>A</td>
<td>23.2</td>
<td>4.4</td>
<td>26.1</td>
<td>5.7</td>
<td>24.6</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>24.9</td>
<td>11.5</td>
<td>26.7</td>
<td>12.1</td>
<td>22.7</td>
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<td>A</td>
<td>0.724*</td>
<td>0.099**</td>
<td>0.765*</td>
<td>0.044**</td>
<td>0.775**</td>
<td>0.085</td>
</tr>
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<td></td>
<td>V1</td>
<td>0.750*</td>
<td>0.098**</td>
<td>0.761*</td>
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</tr>
<tr>
<td></td>
<td>V2</td>
<td>0.715</td>
<td>0.089*</td>
<td>0.742</td>
<td>0.056*</td>
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<tr>
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<td>-0.149</td>
<td>0.153</td>
<td>-0.085</td>
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<td>0.022</td>
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<tr>
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<tr>
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<td>0.105</td>
<td>0.097</td>
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<td>0.114**</td>
<td>0.661***</td>
<td>0.047**</td>
<td>0.681*</td>
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<tr>
<td></td>
<td>V1</td>
<td>0.610</td>
<td>0.122*</td>
<td>0.648</td>
<td>0.079*</td>
<td>0.610</td>
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<td>-0.579*</td>
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<td></td>
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<td>-0.592</td>
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<td>0.161</td>
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<td>-0.564</td>
<td>0.158</td>
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</table>

*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* type

<table>
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<tr>
<th>Variable</th>
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<th>Mean</th>
<th>SD</th>
<th>Mean</th>
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<td>Jk*+</td>
<td>Jk*+</td>
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<td>Jk*+</td>
<td>Jk*+</td>
<td>Jk*+</td>
<td>Jk*+</td>
</tr>
<tr>
<td>Age</td>
<td>A</td>
<td>23.1</td>
<td>5.7*</td>
<td>22.7</td>
<td>3.4*</td>
<td>24.4</td>
<td>5.5</td>
<td>24.7</td>
<td>6.3</td>
<td>23.8</td>
<td>5.6</td>
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<td></td>
<td>V</td>
<td>25.7</td>
<td>12.3</td>
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<td>10.7</td>
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<td>7.4***</td>
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<td>Cholesterol</td>
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<td>0.710</td>
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<td></td>
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<td>0.743</td>
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<td>0.707</td>
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<td>A</td>
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<td>0.183***</td>
<td>-0.121</td>
<td>0.089***</td>
<td>-0.242*</td>
<td>0.293*</td>
<td>0.056</td>
<td>0.151*</td>
<td>-0.080</td>
<td>0.255**</td>
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<tr>
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<td>0.207</td>
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<td>0.134</td>
<td>0.232</td>
<td>0.209</td>
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<td>0.224</td>
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<td>A</td>
<td>0.097</td>
<td>0.115</td>
<td>0.135</td>
<td>0.095</td>
<td>0.017**</td>
<td>0.092</td>
<td>0.077**</td>
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<td>0.014</td>
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<td>0.0082</td>
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<td>0.627</td>
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<td>0.665</td>
<td>0.112</td>
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<td>0.625</td>
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<td>0.570**</td>
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<td>0.604</td>
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<td>HDL/NonHDL</td>
<td>A</td>
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<td>0.191***</td>
<td>-0.491</td>
<td>0.066***</td>
<td>-0.648**</td>
<td>0.169***</td>
<td>-0.556**</td>
<td>0.067**</td>
<td>-0.571*</td>
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</tr>
<tr>
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<td>V1</td>
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<td>0.137</td>
<td>-0.434**</td>
<td>0.157</td>
<td>-0.589</td>
<td>0.151</td>
<td>-0.626</td>
<td>0.169</td>
<td>-0.556</td>
<td>0.145*</td>
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<td>-0.515**</td>
<td>0.160</td>
<td>-0.417**</td>
<td>0.173</td>
<td>-0.577</td>
<td>0.151</td>
<td>-0.584</td>
<td>0.196</td>
<td>-0.541*</td>
<td>0.159*</td>
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</table>
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

\[ \overline{d^2} = \frac{\Pi}{2} \overline{d^2} \]

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

\[ \frac{\overline{d^2}}{\sqrt{n}} \sqrt{(2\Pi - 6)} = \frac{\overline{d^2} x \cdot 5321}{Vn} \]

(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

<table>
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<tr>
<th>Variable</th>
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<th>N</th>
<th>( t )</th>
<th>N</th>
<th>( t )</th>
<th>N</th>
<th>( t )</th>
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A = alcohol study MZ twins; V1,V2 = vitamin C study twins first and second visits.
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**Table 5**

Within pairs means squares (× 10^6) by M type and sex. Variance ratio (F) of WMS(M^-)/WMS(M^+) and its one-tailed probability (P) are given.

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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 \(Jk^{**}\) and \(Jk^{*}\) Groups**

These are shown in Table 6 and the variance ratio WMS(\(Jk^{**}\))/WMS(\(Jk^{*}\)) 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 \(Jk^{**}\) than \(Jk^{*}\) individuals and in \(M^-Jk^{**}\) than \(M^+Jk^{**}\) 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* type and sex. Variance ratio (F) of WMS(Jk*)/WMS(Jk*) and its one-tailed probability (P) are given.

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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 Type

If both M and Jk 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 $\chi^2$ 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 $M^- Jk^+$ group should have a larger variance than the $M^+ Jk^-$ 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 type. $\chi^2$ for Bartlett’s test of heterogeneity of variance and its significance are given.

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<th>M+ Jk-</th>
<th>M- Jk++</th>
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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.
Orthogonal comparisons of within pairs mean-squares (WMS) for a mean environmental effect (E), an environmental effect due to M type (E,M), to Jk type (E,J) and to the interaction of M and Jk types (E,MJ).

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<th>E,MJ</th>
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<td>-1</td>
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<tr>
<td>M^-Jk^+</td>
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<td>M^-Jk^-</td>
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<td>-1</td>
<td>-1</td>
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A more satisfactory test of the hypothesis that both M and Jk types affect environmental variance is gained by fitting a model specifying these effects to all four mean-squares. A complete model including a mean environmental effect (E), an environmental effect of M type (E,M) and of Jk type (E,J), and an interaction effect of the two types (E,MJ) 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 mean-squares to which they are fitted are unequal.

Fitting all four parameters to the four mean-squares 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,M; E_1,J and E_1,M,E_1,J. The fit of E_1 model provided a residual chi-square very close to the Bartlett homogeneity $\chi^2$ 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 mean-squares (the alcohol repeat individual

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<th>E,J</th>
<th>$\chi^2$</th>
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<td>20</td>
<td>0.00</td>
</tr>
</tbody>
</table>

† 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−Jkα− individuals.

The fit of this model, as judged by the residual $\chi^2$, 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 $E_1M$ to be negative and $E_1J$ to be positive. In fact seventeen out of twenty values of $E_1M$ are negative, nine at the 10% and five at the 5% levels. Two of the estimates of $E_1M$ are significant in the non-expected direction. Thirteen of the twenty values of $E_1J$ are positive, four at the 10%, three at the 5% and two at the 1% level. One value of $E_1J$ 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 Jkα 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 Jkα+ than Jkα− 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 ($\beta$) of the F test with degrees of freedom $0.2n, 0.8n$ to detect a difference between within pairs meansquares of a given magnitude $\lambda$ at a given one-tail $\alpha$ level.

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

**Table 10**

<table>
<thead>
<tr>
<th>$\lambda$</th>
<th>1.5</th>
<th>2.0</th>
<th>2.5</th>
<th>3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>M $n$</td>
<td>790</td>
<td>250</td>
<td>155</td>
<td>120</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.33</td>
<td>0.65</td>
<td>0.83</td>
<td>0.92</td>
</tr>
<tr>
<td>Jkα $n$</td>
<td>660</td>
<td>220</td>
<td>140</td>
<td>100</td>
</tr>
<tr>
<td>$\beta$</td>
<td>0.31</td>
<td>0.65</td>
<td>0.86</td>
<td>0.95</td>
</tr>
</tbody>
</table>
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 type 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 glycoporphin A, while the S and s determinants are associated with glycoporphin 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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References


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