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Determinants of Variation in Plasma Alkaline Phosphatase Activity: A Twin Study

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SUMMARY

Plasma alkaline phosphatase activity has been measured in 204 pairs of twins aged 18–34. Estimated genetic variance was the same in men and women, after exclusion of the youngest men, but environmental variance was greater in men so that the heritability was .90 ± .02 in the women and .71 ± .05 in the 19–34-year-old men. About 15% of the genetic variance was associated with the ABO blood group polymorphism, which is known to affect intestinal alkaline phosphatase. Differences in plasma alkaline phosphatase activity between normal people may be mainly due to genetically determined proportions of isoenzymes of differing stability.

INTRODUCTION

The alkaline phosphatase (E.C.3.1.3.1) activity of plasma has been extensively studied because it is frequently used as a medical diagnostic test; for a constituent that is presumably not under any feedback control mechanism, it displays a remarkable constancy within each individual [1, 2]. Only two studies appear to have considered the contributions of inheritance and environment to the activity of this enzyme. The first involved men, aged between 42 and 56 [3], and it was concluded that no estimate of heritability could be derived, but that environmental factors shared within twin pairs were the most important source of variation. The other [4] was on young people, aged 8–20, and found a high intraclass correlation in both monozygotic (MZ) and dizygotic (DZ) pairs. One other factor known to influence plasma alkaline phosphatase activity is the presence or absence of an intestinal isoenzyme; this is associated with ABO blood group and secretor status

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[5], possibly because of absorption of enzyme by red blood cells that are A or B positive [6].

We measured plasma alkaline phosphatase activity in 204 pairs of male and female twins, aged from 18 to 34, and have derived estimates of the effects of genes, including the ABO blood group polymorphism, shared and nonshared environment, and age on variation in this activity in men and women. Use of DZ opposite-sex pairs has allowed a powerful test of the similarity or difference in factors operating in men and women.

SUBJECTS AND METHODS

Subjects

Pairs of MZ and DZ twins, aged between 18 and 34 (mean 23.1), were recruited from the Australian NH&MRC Twin Registry for a study of alcohol metabolism and susceptibility to intoxication [7]. Both members of a twin pair attended on the same day. Eighty-nine individuals (50 men and 39 women) attended on more than one occasion, and the results from these 89 are used to assess the repeatability of the measurements within an individual.

All twins were blood typed with the following antisera: anti-A, A_1, B, C, c, D, E, e, M, N, S, s, Fy^a, K, and Jk^a, and they were also typed for serum alpha-1- antitrypsin (Pi). 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, coloring, 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.

Of the 205 twin pairs for whom measurements were available, there were 42 MZ female, 42 MZ male, 44 DZ female, 38 DZ male, and 39 DZ pairs of opposite sex (DZOS). There were no substantial differences in age distribution between the five zygosity groups.

Experimental Methods

The twins arrived at about 9 A.M., having had a light, nonfatty breakfast at about 8 A.M., and blood was collected soon after arrival and before any alcohol was administered. Fifteen ml of venous blood, taken into a tube containing heparin, was centrifuged within 2 hrs of collection, and the plasma used, *inter alia*, for alkaline phosphatase assay. Plasma alkaline phosphatase activity was measured by the rate of hydrolysis of *p*-nitrophenyl-phosphate in amino methyl propanol buffer [8] on a Technicon SMAC, after storage at 4°C for between 24 and 48 hrs.

Fitting Models of Variation

The statistics to which models of variation are fitted are the between- and within-pair mean squares (WMS) from an analysis of variance of each separate group of n twin pairs.

Source	Degrees of freedom	Expected mean squares
Between pairs.....	$n - 1$	$S_w^2 + 2S_b^2$
Within pairs	n	S_w^2

A large difference in the means of males and females will inflate the WMS of DZOS pairs by an amount $n/2(\bar{M} - \bar{F})^2$, where there are n pairs, $\bar{M}$ is the male mean, and $\bar{F}$ is the female mean. The DZOS WMS is thus corrected for this amount, and the corresponding degree of freedom is removed.

Models of variation to explain these mean squares can now be fitted using the method of iterative weighted least squares, described extensively elsewhere [9, 10].

A simple model for variation in MZ and DZ mean squares is shown in table 1. E_1 is environmental variance within families, and as such it is specific to the individual and will include error variance. E_2, on the other hand, includes sources of environmental variance shared by members of a family but differing between families. It will, thus, include the lasting effects of cultural and class differences and parental rearing practices. Here it may include dietary or exercise habits that both members of a twin pair share in common, but which differ between pairs. V_A is that part of the genetic variation due to the additive effects of genes in the absence of assortative mating. The appropriateness of different models is tested by the chi-square criterion. A model is elaborated only if a simpler one fails or a significant improvement is made by adding a further parameter.

Therefore, an attempt is made first to fit E_1 alone. Failure of this most simple model will indicate that there is significant between-families variation. A model incorporating E_1 and E_2 will test whether the between-families variation is entirely environmental in origin, while the E_1V_A model will test whether it is entirely genetic. If both two-parameter models fail, then a model incorporating all three sources of variation must be considered.

There is no necessary reason why the components of variation will be the same in both males and females, so models are first fitted to the sexes separately and then to the eight statistics together. At this stage, a heterogeneity chi square for k df can be calculated by adding the two male and female chi squares for $4 - k$ df and subtracting from the chi square ($8 - k$ df) for the corresponding model fitted to all eight statistics. The heterogeneity chi square for k df will indicate whether the same parameters are appropriate for both sexes. If it is not significant, then the DZOS data may be added and the same model fitted to all 10 statistics. If there is significant heterogeneity revealed when the same model is fitted to both sexes, then different parameters may need to be fitted for males and females. Different E_1 parameters are found to be required in the analysis of alkaline phosphatase variation below, and the expectations for the male and female effects, E_{1_M} and E_{1_F}, are shown in table 1.

RESULTS

Scale

The frequency distribution of alkaline phosphatase shows some skewness: in males, the skewness statistic was 1.42; and in females, 0.86. Departure from the

TABLE 1

MODEL FOR TWIN MEAN SQUARES

Mean squares	E_1	E_{1_F}	E_{1_M}	E_2	V_A
MZ females:	1	1	0	2	2
Between.....	1	1	0	0	0
Within.....					
MZ males:	1	0	1	2	2
Between.....	1	0	1	0	0
Within.....					
DZ females	1	1	0	2	½
Between.....	1	1	0	0	½
Within.....					
DZ males:	1	0	1	2	½
Between.....	1	0	1	0	½
Within.....					
DZ opposite-sex:	1	½	½	2	½
Between.....	1	½	½	0	½
Within.....					

normal distribution violates one of the assumptions of the models, but it was found that the results of model-fitting were qualitatively similar for raw and log-transformed data, and the chi-square test showed a slighlty worse fit for the transformed data. Therefore, the results below were calculated from the raw data. No subjects were excluded from the initial analysis, although some extreme values were encountered; trial exclusion of one or two extreme outliers was found not to affect the outcome.

Repeatability

Within-individual (S_{wi}^2) and between-individual (S_{bi}^2) components of variance were calculated for men and for women from the results for 89 twins attending on more than one occasion. The intraclass correlation coefficient, or repeatability $[S_{bi}^2/(S_{bi}^2 + S_{wi}^2)]$, was .93 for men and .36 for women, indicating that there was "individuality" for alkaline phosphatase in men. The low repeatability for women was due both to a smaller between-individual and a greater within-individual variation than were found in the men, and was not due to a large between-occasion difference in a small proportion of the women but rather to moderate differences in all.

Analytical Error

The variance due to analytical error, derived from an external quality control program (Wellcome Clinical Chemistry Quality Control Programme) averaged 72 $(IU/l)^2$ over the period of this study. This may be considered an upper limit, since many of these control samples were at an elevated, pathological level rather than in the normal range. Within-batch analytical variance was considerably lower, at 19 $(IU/l)^2$.

Means and Variances

Males had significantly greater means (85.1 vs. 62.3, $P < .001$) and total variances (1005 vs. 424, $P < .001$) than females. However, no significant differences were found between MZ and DZ groups of a given sex. We may thus proceed to estimate the relative importance of genetical and environmental factors in plasma alkaline phosphatase activity.

Fitting Models of Variation

Between- and within-pairs mean squares and their degrees of freedom for each of the five twin groups are shown in table 2. The results of model fitting are shown in table 3. The two environmental models (E_1 and E_1E_2) were strongly rejected in both men and women. In women, the favored model is E_1V_A, which fitted the data well ($\chi^2_2 = 0.88$) and produced a high heritability estimate of .90 $\pm$ .02. In men, the E_1V_A model also fitted the data adequately but the slightly better fit of the $E_1E_2V_A$ model suggests that both family environmental and genetical factors may be important. Parameter estimates were very much larger for males so, not surprisingly, attempts to combine the results for men and women resulted in rejection of all models.

TABLE 2

OBSERVED MEAN SQUARES USED IN MODEL FITTING

	ALL SUBJECTS		EXCLUDING 18-YEAR-OLD MALES	
	df	Mean squares	df	Mean squares
MZ males:				
Between.....	41	1,598	36	1,189
Within.....	42	168	37	173
MZ females:				
Between.....	41	761	...	...
Within.....	42	44	...	...
DZ males:				
Between.....	37	2,175	26	751
Within.....	38	532	27	302
DZ females:				
Between.....	43	776	...	...
Within.....	44	254	...	...
DZ opposite-sex:				
Between.....	38	515	30	403
Within.....	38	430	30	261

This suggestion of an E_2 effect in men but not in women is unexpected, and consideration of the major source of plasma alkaline phosphatase (bone) in normal subjects, and the slightly later growth spurt associated with puberty in males than in females [11], suggests that the possible E_2 factor in men is age-related. Figure 1 shows plasma alkaline phosphatase levels plotted against age for men: there was a significant negative correlation ($r = -.30$, $P < .001$) in men but none in women ($r = .09$, N.S.), and comparison of alkaline phosphatase in 18-year-old men with 19–34-year-old men showed a significant difference in means ($F_{1,197} = 52.1$, $P < .001$) and variances ($F_{40,159} = 3.25$, $P < .001$).

We therefore recalculated the mean squares after exclusion of all 18-year-old males (table 2), and models were fitted again to these revised values. The results (table 3) show a better fit for men on the E_1V_A model ($\chi^2_2 = 1.13$), supporting the association of the E_2 effect with age-dependent bone growth. The male estimate of V_A has also been almost halved with exclusion of the 18-year-olds, indicating that there is considerable age-dependent genetic variance for alkaline phosphatase activity in adolescent males. However, the heterogeneity chi square ($\chi^2_2 = 8.35$, see SUBJECTS AND METHODS) still indicates that it is inappropriate to attempt to obtain common parameter estimates for males and females.

Inspection of the new parameter estimates showed similar values of V_A for men and women but a large difference in the E_1 estimates. We therefore fitted a model to all 10 mean squares, including those for the opposite-sex pairs, which included a common V_A but different E_1 parameters, E_{1M} and E_{1F}, for males and females. This model [10] is shown in table 1, and the estimates derived from it are shown in table 3. A good fit was obtained (χ^2_7 5.76, $P = .56$), a great improvement over an attempt to fit an E_1V_A model to all 10 statistics ($\chi^2_8 = 22.2$, $P = .005$). The heritability estimates are different for men and women because the male $\hat{E}_1$ is almost four times as great as the female estimate.

TABLE 3

SUMMARY OF MODEL FITTING TO DATA FOR ALKALINE PHOSPHATASE

Data set	Model	$\hat{E}_1$	$\hat{E}_2$	$\hat{V}_A$	χ^2	df	P	h^2
Females	E_1	457*	...	...	41.10	3	.000	...
	E_1E_2	152*	309*	...	20.60	2	.000	...
	E_1V_A	44*	...	423*	0.88	2	.645	.90 ± .02
	$E_1E_2V_A$	45*	26[NS]†	401*	0.78	1	.376	.85 ± .22
Males	E_1	1097*	...	...	42.22	3	.000	...
	E_1E_2	342*	765*	...	12.27	2	.002	...
	E_1V_A	165*	...	938*	4.18	2	.124	.85 ± .04
	$E_1E_2V_A$	172*	307[NS]	660*	2.21	1	.137	.58 ± .21
Males (excluding 18-year-olds)	E_1	611*	...	...	29.81	3	.000	...
	E_1E_2	228*	389*	...	3.96	2	.138	...
	E_1V_A	165*	...	432*	1.13	2	.569	.72 ± .07
	$E_1E_2V_A$	169*	137[NS]	302[NS]	0.72	1	.396	.50 ± .30

Data set	Model	$\hat{E}_{1_M}$	$\hat{E}_{1_F}$	$\hat{V}_A$	χ^2	df	P		h^2
10 statistics (including all types of pairs except those including 18-year-old males)	$E_{1_M}E_{1_F}V_A$	161*	45*	393*	5.76	7	.570	Male Female	.71 ± .05 .90 ± .02

* $P < .001$.
† NS = $P > .05$.

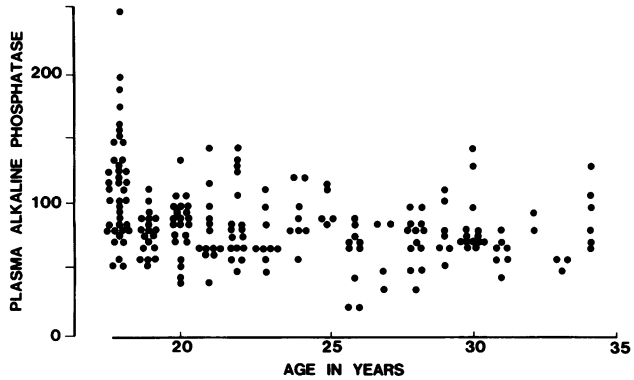

FIG. 1.—Influence of age on alkaline phosphatase in male subjects

Association with the ABO Polymorphism

An analysis of variance of alkaline phosphatase activity between the four ABO blood groups revealed a highly significant between-groups effect in both females ($F_{3,204} = 6.80$, $P = .0002$) and in males over the age of 18 years ($F_{3,151} = 4.83$, $P = .0031$). Means and standard deviations for the four groups are shown in table 4. As found previously [5, 6], blood group O is associated with high levels and blood group A with low levels. The N's for B and AB groups are too small to place any reliance upon. Because twins, particularly MZ ones, cannot be regarded as independent observations, the significance, but not the size of the effect, is overestimated. In females, the between-groups effect is largely due to the B vs. not −B contrast ($P = .015$), while in males, it is largely due to the A vs. not −A contrast ($P = .040$). Nevertheless, the size of the between-groups component of variance (S_{BG}^2) is almost identical in females (56.2) and males (58.8), in line with the common estimate of V_A for both sexes. We can thus estimate that about 15% of the genetic variance in alkaline phosphatase activity is due to the ABO polymorphism, representing 13% of the total variance in females or 11% in males. Typing for the Lewis blood group or secretor system was not available to check the reported association with secretor status [5].

DISCUSSION

Two aspects of these results are particularly striking; first, the common estimate of additive genetic variation for males and females highlights differences between the sexes in the contribution of individual environmental influences to the total variance; second, the heritability estimates obtained for plasma alkaline phosphatase activity are very high, especially in females. Few continuously variable characteristics show a higher heritability than our estimate of $h_F^2 = .90 \pm .02$.

The low repeatability found in women is surprising in this context and different from the results of other workers [1, 2]. This is probably a chance result due to the small number of women (39) attending a second time. The estimate of the environmental contribution to variance in women is close to the estimate for analytical error, leaving very little room for environmental influences on the person (as opposed to apparent E_1 influences acting on the blood sample). The

repeatability should be greater than or equal to the heritability, and the great disparity between these two in our sample might be equally due to a low estimate of E_1 from the twins as to a high estimate of S_{wi}^2 from the repeating women. It must be remembered that these were young women, to a large extent selected for good health, who would be unlikely to be subject to subclinical bone or liver conditions that might increase E_1 in a group of older women.

The nature of the environmental factor that is so much more important in men (at least three times) is unknown, but given the similar V_A components, it is this which leads to the greater overall variance in men. It may be noted that the E_1 estimate for males is almost the same with or without the 18-year-old group.

Previous studies have not differentiated between males and females and have studied either older or younger subjects. These studies suggested shared environment as an important influence on plasma alkaline phosphatase. In subjects aged 8–20 [4], the intraclass correlation coefficients were .97 for MZ pairs and .80 for DZ pairs, taking males and females together. This is quite close to our results when the 18-year-old males are included, and, as Hosenfeld and Drossler point out, the age distribution of their subjects meant that age-dependent pubertal bone growth would be shared within twin pairs regardless of zygosity. The other study [3] considered only adult men, so this source of variation was absent; intraclass correlations of .77 for MZ pairs and .59 for DZ pairs were found. Because twins who saw each other frequently were more similar in their alkaline phosphatase levels than those who did not, the authors concluded that environment was important, but they also stated that MZ pairs were more likely to see each other frequently than were DZ pairs. This argument does not, therefore, rule out a genetic influence in middle-aged men.

The nature of the genetic factors influencing alkaline phosphatase is uncertain; part of the effect (about 15% of the genetic variation or 12% of the whole) is associated with the presumed presence or absence of intestinal isoenzyme, associated with the ABO blood group polymorphism. The remainder could be associated with isoenzyme composition through the rate of loss of activity in the circulation by thermal inactivation at 37°C, which is thought to be the route of loss of enzyme activity, the inactivated enzyme protein being removed subsequently [12].

TABLE 4

MEANS AND STANDARD DEVIATIONS OF ALKALINE PHOSPHATASE ACTIVITY (IU/l) BY ABO BLOOD GROUP

	FEMALES			MALES (> 18 YRS)		
	No.	Mean	SD	No.	Mean	SD
O.....	99	63.5	20.3	63	85.2	22.9
B.....	16	81.0	19.1	26	78.4	21.5
A.....	89	57.4	19.2	62	70.3	23.4
AB	4	68.0	24.0	4	66.8	6.9
	$F_{3,204} = 6.80; P = .0002;$ $S_{BG}^2 = 56.2$			$F_{3,151} = 4.83; P = .0031;$ $S_{BG}^2 = 58.8$		

Extensive studies, aimed at distinguishing between the isoenzymes of alkaline phosphatase, have shown that bone alkaline phosphatase has a comparatively low thermal stability and that liver and intestinal isoenzymes are more stable. There is, however, some person-to-person variation in the half-life of each isoenzyme [13]. Also, the proportions of bone, liver, and intestinal isoenzyme in each person differ, so that although the predominant isoenzyme is bone (on average about 60% of the total [14]), this will also lead to differences in the mean half-life of the total alkaline phosphatase between different people.

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