Enzyme
Editors: C. Bachmann, Bern; J.P. Colombo, Bern; H. Eppenberger, Zürich; O. Greengard, New York, N.Y.; O. Sperling, Petah Tikva; U. Wiesmann, Bern
Original Papers

Reprint
Publisher: S. Karger AG, Basel
Printed in Switzerland

Enzyme 33: 61–69 (1985)

0013–9432/85/0332–0061$2.75/0

Individual Differences in Plasma ALT, AST and GGT: Contributions of Genetic and Environmental Factors, Including Alcohol Consumption

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Key Words. Alanine aminotransferase · Alcohol · Aspartate aminotransferase · γ-Glutamyl transferase · Genetics · Twins

Abstract. The causes of individuality of the plasma enzymes alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1) and γ-glutamyl transferase (GGT; EC 2.3.2.2) were investigated in a study of 206 pairs of twins. Between-person variance was greater in men than women, while within-person variation was similar in both sexes. Plasma ALT and AST levels were affected by genetic factors, while GGT was affected by some environmental factor shared by cotwins. In the men, alcohol intake had a significant but small effect on all three enzyme levels, and since alcohol consumption was highly heritable, this appeared as a genetic influence on enzyme activities. The major factors involved in the observed correlations between these enzymes were a non-shared environmental factor other than alcohol affecting ALT, AST and GGT, and a genetic factor affecting only ALT and AST.

Introduction

It is well established that enzyme activities in plasma differ between healthy people, and that there is less variation within a person over time than there is between people [1, 2]. Previous studies which have demonstrated constancy within individuals have not addressed the sources of differences between individuals, which may arise from a number of genetic or environmental causes. Knowledge of the sources of variation may allow closer definition of the appropriate reference range for each person, with consequent improvement in clinical usefulness.

In general, these sources may include genetic variation due to polymorphism at a major locus, as is found for example with red cell alanine aminotransferase [3] and acid phosphatase [4]. This is to be distinguished from additive genetic variation derived from the effects of many genes of small effect, as is found for many constituents of plasma such as cholesterol [5], uric acid [6], and alkaline phosphatase [7]. Another cause of familial aggregation may be environmental sources of

variation shared by members of a family but differing between families, with effects persisting into adult life (such as dietary habits). With appropriate experimental design, shared family environment can be distinguished from environmental sources of variation peculiar to each person. Repeatability studies of the same individuals on different occasions allow partitioning of individual environmental variance into a part which is persistent over time and a part due to short-term fluctuations and errors of measurement. One of the best designs for distinguishing environmental and genetic sources of variation is the study of monozygotic (MZ) and dizygotic (DZ) twins. Furthermore, measured factors known or suspected of influencing the variable being considered can be incorporated into the study, so that correlations between variables and the measured factor can be assessed as primarily genetic or environmental in origin.

The determinants of plasma enzyme activities in the normal population and of correlations between the activities of different enzymes are not well established. Sex differences in mean values exist, and it is also possible that the relative importance of the various factors differs between men and women. Some (notably alkaline phosphatase) are age-dependent, and again the effect of various genetic or environmental factors may vary with age. Alcohol consumption is certainly one factor which influences aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), and the correlation between them [8, 9] and which is likely to affect alanine aminotransferase (ALT) also. However, a correlation between AST and GGT levels exists even in those who profess to be nondrinkers [9]. The activities of both ALT and AST may also be affected by the availability of their coenzyme pyridoxal phosphate [10], while GGT can be shown to increase after the ingestion of microsomal enzyme-inducing drugs [11], and possibly other environmental chemicals which increase drug metabolism [12] could increase plasma GGT also.

We have studied ALT, AST and GGT levels in 206 pairs of normal young twins, some of them on more than one occasion, and this paper addresses the questions of the sources of the individuality of, and of the correlations between, these enzymes.

Subjects and Methods

Subjects

206 pairs of MZ and DZ twins, between 18 and 34 years old (mean 23.1 years) were recruited from the Australian NH & MRC Twin Registry for a study of alcohol metabolism and susceptibity to intoxication [13]. There were 42 MZ male pairs, 43 MZ female, 38 DZ male, 44 DZ female and 39 DZ pairs of opposite sex. Zygosity was determined as previously described [5], primarily by blood typing. Both members of a twin pair attended on the same day. 89 twins (50 men and 39 women) attended on more than one occasion, with an interval between visits of 1–17 months (mean 4.5 months), and the results of these 89 are used to assess the repeatability of the measurements within an individual. Blood was taken for these and other measurements before the subjects ingested any alcohol.

Methods

Heparinised blood was centrifuged within 2 h of venipuncture and plasma was stored at 4 °C for up to 2 days before analysis. ALT, AST and GGT were assayed using two- or three-point methods on a SMAC multichannel analyser (Technicon Equipment Corporation, Tarrytown, N.Y.). Habitual alcohol consumption was assessed by the twins answering a questionnaire as previously described [14].

Transformations

This study is concerned with the causes of variation in plasma enzyme activities in normal subjects, but it was found that with the original untransformed

data a small number of outlying results, perhaps resulting from some illness, could have a disproportionate effect on the conclusions. This raised the question of whether these high values are part of the normal range and should be included, or due to a disease state in which case they might reasonably be excluded. We tried several approaches to overcome this problem. One was to truncate the frequency distribution at some point and analyse only the results from subjects below that limit; another was to perform log transformation, and in some cases both were tried.

In fact, for ALT, AST, GGT and weekly alcohol consumption, it was found that logarithmic transformation of the raw data improved the distributional properties sufficiently to allow the analysis to be carried out on the full data set, without exclusion of any values.

Correction for age was also considered but because the age range of our sample was narrow, little regression on age of any of the variables was found and so no such correction has been made.

Repeatability

The resemblance of the results for 1 person on 2 occasions was assessed by analysis of variance of the log-transformed results. Repeatability, or test-retest reliability, was assessed by calculation of the intraclass correlation coefficient (the between-person variance as a proportion of the total variance). This proportion can be compared with the variance ascribed to genetic and shared environmental effects, which together should not exceed it. If these two effects together are less than the intraclass correlation, then the difference can be attributed to individual habits constant over the timespan of the study.

Genetic and Environmental Models of Variation

If MZ twin pairs are more similar than DZ pairs, this may be taken as evidence that genetic factors are influencing that characteristic. Similarity has often been assessed by correlation between the members of the pairs, and the role of genetic and environmental factors derived by comparison of these correlations in MZ and DZ pairs. Heritabilities are often calculated using these methods without any test of whether genetic factors contribute significantly to variation at all, whether genetic factors are equally important in men and women, or whether environmental factors are all specific to the individual as opposed to being shared by siblings and other members of the family (as may be the case for dietary factors). To avoid these inadequacies, we have employed a hypothesis-testing approach [15] in which different models, to account for variation within and between MZ and DZ twin pairs, are fitted by the method of interative weighted least-squares and are tested by the χ^2 criterion of goodness-of-fit. This approach can be extended to the testing of models of covariation between variables [16].

Results

Frequency Distributions

As expected, the enzymes showed positively skewed frequency distributions. Statistics summarising the raw data, and the means and variances of the log-transformed results, for all subjects are shown in table I, separately for men and women.

Repeatability

The repeatability or individuality of the plasma enzyme activities is shown in table II. These are calculated from the log-transformed results and, because of the mean differences between men and women, are shown for the two sexes separately. The calculated within-person and between-person components of variance are also given separately for the men and the women.

Causes of Variation in Enzyme Levels

Table III gives the within- and between-pair mean squares, and their degrees of freedom, for the log-transformed data by sex and zygosity. These are used to test hypotheses about sources of variation, the simplest being that all variation is due to individual environmental effects (E1). This hypothesis is rejected for all three enzymes in both sexes, so combinations of either individual environmental and shared environmental effects (E1 and E2), or individual environmental and additive genetic effects (E1 and VA) are tested. The

Table I. Summary statistics for all subjects by sex

	ALT		AST		GGT	
	male (n = 199)	female (n = 214)	male (n = 195)	female (n = 210)	male (n = 199)	female (n = 215)
Raw data						
Mean ± SD, μmol/min/l	25.9 ± 30.5	17.6 ± 12.4	25.6 ± 19.5	20.4 ± 7.8	16.3 ± 17.1	9.5 ± 9.4
Skewness	5.8	3.8	5.9	0.7	2.2	1.8
Kurtosis	39.2	23.3	43.4	0.8	5.6	4.7
Log-transformed						
Mean	1.30	1.17	1.35	1.28	1.05	0.87
SD	0.30	0.26	0.20	0.18	0.42	0.37

Mean values for all variables differ significantly ($p < 0.001$) between males and females, both before and after log-transformation. The variances differ significantly for all variables before transformation, and for GGT after transformation.

Table II. Within-person (s_W^2) and between-person (s_B^2) components of variance, and intraclass correlation (R_i), for men and women separately

	Men (n = 50)			Women (n = 39)		
	s_W^2	s_B^2	R_i	s_W^2	s_B^2	R_i
log ALT	0.044	0.054	0.55*	0.064	0.021	0.25 NS
log AST	0.020	0.026	0.57*	0.032	0.007	0.18 NS
log GGT	0.092	0.079	0.46*	0.106	0.016	0.13 NS

* $p < 0.001$; NS $p > 0.05$ (not significant).

results for GGT are most compatible with a combination of shared and individual environmental effects, although the model allowing only E1 and VA cannot be rejected. However, for both ALT and AST, the E1E2 model is rejected and a combination of additive genetic and individual environmental effects is the chosen model. For GGT, the same model and the same estimates of the variances are applicable for both sexes.

For both ALT and AST separate E1 and VA parameter estimates for men and women are required. For these variables, there is an anomaly in that the results for the members of DZ pairs of opposite sex are too similar. This leads to an excessively high estimate of the parameter VAMF which represents the covariance of additive genetic effects acting in the male and female members of DZ opposite-sex pairs, for which we have no explanation.

Table III. Within- and between-pair mean squares (MS) and their degrees of freedom (df), by sex and zygosity

	log ALT		log AST		log GGT	
	df	MS	df	MS	df	MS
MZ male						
Between	41	0.1447	39	0.0504	41	0.4122
Within	42	0.0345	40	0.0169	42	0.0807
MZ female						
Between	42	0.0753	40	0.0479	42	0.3253
Within	43	0.0249	41	0.0093	43	0.0725
DZ male						
Between	37	0.0867	37	0.0734	37	0.3497
Within	38	0.0815	38	0.0324	38	0.0770
DZ female						
Between	43	0.1103	43	0.0515	43	0.3196
Within	44	0.0431	44	0.0250	44	0.0806
DZ opposite-sex						
Between	38	0.1328	38	0.0349	38	0.2760
Within	38	0.0298	38	0.0088	38	0.0958

The results of the model-fitting procedure, showing the goodness-of-fit of the rejected and chosen models, and the estimates of the environmental and genetic contributions to variance in the chosen model are shown in table IV.

Causes of Covariation between Enzymes

Correlations between the three liver enzymes (log transformed) are shown in table V. It can be seen that these are considerably greater in males than in females; indeed, the correlation between AST and GGT is significantly negative in women. One possible cause of this striking sex difference might be the greater consumption of alcohol in males causing liver damage and a correlated increase in the serum levels of all three enzymes.

All twins reported on their usual weekly alcohol consumption. The number of standard drinks per week has an extremely skewed distribution, so we use instead the derived variable log ALC, = log (drinks per week + 1). Correlations of the liver enzymes and this measure of alcohol intake are also shown in table V.

As expected, correlations of the enzymes with log ALC are positive and significant in males but negligible in females, presumably reflecting the smaller range of alcohol consumption among women. Alcohol consumption alone, however, cannot explain the considerable correlation found between ALT and AST in both women and men, nor can we tell whether the correlations observed arise primarily from environmental or from genetic causes. To answer these questions, we must use the genetical analysis of covariance structure [16].

This is a maximum likelihood technique which simultaneously tests hypotheses about both the sources and structure of covariation. The details of the procedure and the various models fitted to the data are beyond the scope of this paper; suffice it to say that the results of the most parsimonious acceptable model, fitted to the male data only, are shown in table VI, which gives the percentages of variance due to different sources of variation and covariation in each of the three enzymes and alcohol consumption. Covariation between all four variables must be due to either individual environmental or genetic causes, since it was found, as in the univariate analysis, that shared environmental factors contributed only to variation specific to GGT.

There is considerable covariation between the three enzyme levels due to individual

Table IV. Results of model-fitting to mean squares data showing rejection of inappropriate models and estimates of the variance attributable to specified sources in the chosen model

log ALT	
Rejected models	E_1 only, $\chi^2_9 = 59.8$ $p < 0.001$; E_1+E_2, $\chi^2_8 = 28.2$ $p < 0.001$; E_1+V_A, $\chi^2_8 = 17.5$ $p < 0.01$
Accepted model	E_{1m} 0.038, E_{1f} 0.025, V_{Am} 0.051, V_{Af} 0.040, V_{Amf} 0.096. $\chi^2_5 = 6.50$ $p = 0.260$.
log AST	
Rejected models	E_1 only, $\chi^2_9 = 63.1$ $p < 0.001$; E_1+E_2, $\chi^2_8 = 29.2$ $p < 0.001$; E_1+V_A, $\chi^2_8 = 22.4$ $p < 0.01$
Accepted model	E_{1m} 0.017,E_{1f} 0.10, V_{Am} 0.023, V_{Af} 0.023, V_{Amf} 0.054, $\chi^2_5 = 10.02$ $p = 0.075$.
log GGT	
Rejected models	E_1 only, $\chi^2_9 = 49.6$ $p < 0.001$; E_1+V_A, $\chi^2_8 = 13.3$ $p = 0.101$
Accepted model	E_1 0.081, E_2 0.128. $\chi^2_8 = 2.67$ $p = 0.954$.

E_1, E_{1m}, E_{1f} are estimates of variance due to non-shared environmental factors; a common estimate for both sexes or separate estimates for males and females. E_2 is the variance attributable to environmental factors shared by siblings regardless of zygosity. V_A, V_{Am}, V_{Af}, V_{Amf} are estimates of variance attributable to additive effects of many genes. See text for discussion of V_{Amf}.

Table V. Correlations of enzyme values and normal weekly alcohol consumption, on $\log_{10}$ transformed data

Men (n = 195–199)	Women (n = 208–212)			
	log ALT	log AST	log GGT	log ALC
log ALT	–	0.33*	0.14***	0.05
log AST	0.53*	–	−0.13***	−0.02
log GGT	0.31*	0.18**	–	0.02
log ALC	0.21**	0.14***	0.19**	–

* $p < 0.001$, ** $p < 0.01$, *** $p < 0.05$.

Table VI. Percentage contributions of genetic and environmental sources to variation and covariation between liver enzyme concentrations and alcohol consumption (see text for explanation)

	Individual environment		Shared environment	Genetic		
	factor	specific		factor I	factor II	specific
log ALT	24	12	–	1	63	–
log AST	39	0	–	4	13	44
log GGT	16	15	45	8	–	15
log ALC	1	26	–	73	–	–

environmental factors, but these are not connected with alcohol use, as is shown by the negligible variance in log ALC (1%) accounted for by the environmental factor affecting all three enzymes. Two genetic factors are allowed in the model; factor I clearly reflects genetic influences on alcohol consumption and the extent to which these contribute to genetic variance in the enzyme levels. (Note that the heritability of log ALC is high in this sample of males: 73%.) Thus, 8% of variance in log GGT and 4% in log AST is due to genetic influences affecting alcohol consumption. However, another genetic factor must be invoked to account for the high correlation between log ALT and log AST. The ascription of factor and specific variance when only two variables are being considered, as here, is arbitrary. However, we can say that 13% of the total variation in log AST is due to the same genes which affect variation in log ALT. A model which ascribes the correlation between ALT and AST to environmental sources does not fit as well.

We also notice that a proportion of the total variance in log GGT (23%) is attributed to genetic factors in this multivariate analysis. This may appear to conflict with the univariate analysis in which a model specifying no genetic variance was accepted. However, it should be emphasised that the power of the univariate analysis to detect a small amount of genetic variance against a background of shared environment is very low [17].

Discussion

Others have previously found that individuals show characteristic plasma enzyme activities [1, 2]. Such an effect was also demonstrable in our subjects, but when the data for men and women were treated separately (as they must be when the two sexes have different means), the between-person component of the variances was less in the women than in the men and only reached statistical significance in the men (table II). *Williams* et al. [2] provide figures for variance components from which the intraclass correlations can be calculated; in the 18- to 35-year age group they also found greater individuality in men than women for AST and GGT (ALT was not measured). Again the difference arises because of higher between-individual variance rather than lower within-person variation. *Van Steirtegheim* et al. [1] also found significant differences between people in their long-term enzyme levels, but did not distinguish between men and women. This sex difference reflects the wider range of values for each of the enzymes in men, as seen from the differences in the standard deviations in table I. Therefore, the definition of individual reference ranges for men would seem reasonable in concept, even if difficult in practice, but the smaller differences between individuals in women would make it less useful in that sex.

It is also clear that members of twin pairs are more similar to each other than would be expected for randomly selected pairs, and this is true for both sexes and all the enzymes studied. Such family resemblance can, however, be due to either common genes or common upbringing, experiences, and habits, and here the three enzymes differ, so we need to consider each enzyme in turn.

The two aminotransferases do show common features; both appear to exhibit significant heritability and share the anomalous high similarity of the opposite-sex DZ pairs. They are also quite strongly correlated, both

in men and women. For each the repeatability (as intraclass correlation) is around 0.5 and the heritability estimated from the log-transformed data is the same or even a little greater; it seems, therefore, that the individuality of plasma ALT and AST is due to genetic influences (table IV). The correlation between them, on the other hand, appears to be partly due to short-term environmental factors which affect all three enzymes and partly due to genetic causes acting only on ALT and AST (table VI).

The univariate results for GGT clearly indicate shared environment as the cause of within-pair similarity. Since the shared environmental effect is rather greater than the repeatability, between-batch analytical variation may account for some of this effect. The possible nature of this shared environmental effect is obscure; we can only speculate that some dietary similarity or an environmental chemical to which some pairs are exposed but not others could be involved. In the multivariate analysis, which was performed only on the data for the men, a small genetic effect (23%) was detected.

In males, increased alcohol consumption appears to be responsible only to a small degree for the variation in, and correlations between, liver enzyme levels. The correlations between alcohol consumption and the enzyme activities are lower than those found in another group in Sydney, but younger people such as the twins in this sample show less effect of drinking on AST and GGT than do people aged over 30 [18]. The covariation due to this cause appears to be primarily genetic in origin. In other words, genes affecting a behavioural trait (alcohol consumption) also contribute to variation in the plasma levels of these liver enzymes. Individual environmental factors are also important in the covariation of these three enzymes, but they do not appear to be related to alcohol. It has previously been found [9] that correlation between plasma GGT and AST levels exists even in those who claim not to drink, and combination of the results from that study and this one suggests a baseline correlation due to environmental factors other than alcohol, with an increasing alcohol-induced association at higher levels of alcohol consumption.

In addition to these causes of covariation of all three enzymes, there appear to be independent genetic factors which jointly influence ALT and AST levels, but not GGT (genetic factor II in table VI). The methods used for ALT and AST determination did not include the addition of excess pyridoxal phosphate as coenzyme and, therefore, differences in circulating pyridoxal phosphate [10] could account for some or all of the observed correlation; this would suggest genetic differences in pyridoxine absorption or metabolism. However, there is no direct evidence from this study that coenzyme availability is involved.

In conclusion, the plasma ALT, AST and GGT concentrations which are characteristic of each person are determined by factors which are shared by members of a family (in this case a pair of twins), but these are primarily genetic for ALT and AST but environmental for GGT. It also appears that there are environmental factors involved which affect all three enzymes, but alcohol consumption plays only a minor part in this covariation when the group considered is young and contains few heavy drinkers. The high correlation of ALT and AST on the other hand may also be affected by genetic factors, possibly related to the availability of pyridoxal phosphate.

Acknowledgements

We are grateful to the twins of the Australian NH & MRC Twin Registry who participated in this study, to *Janet Craig* who organised the collection of the samples and to the staff of the Department of Clinical Biochemistry, Royal Prince Alfred Hospital, for analysing them. The project of which this was a part was funded by the Australian Associated Brewers.

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Received: October 3, 1984
Accepted: November 13, 1984

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