Genetic Covariation between Serum γ-Glutamyltransferase Activity and Cardiovascular Risk Factors

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Background: Several studies have shown that variation in serum γ-glutamyltransferase (GGT) in the population is associated with risk of death or development of cardiovascular disease, type 2 diabetes, stroke, or hypertension. This association is only partly explained by associations between GGT and recognized risk factors. Our aim was to estimate the relative importance of genetic and environmental sources of variation in GGT as well as genetic and environmental sources of covariation between GGT and other liver enzymes and markers of cardiovascular risk in adult twin pairs.

Methods: We recruited 1134 men and 2241 women through the Australian Twin Registry. Data were collected through mailed questionnaires, telephone interviews, and by analysis of blood samples. Sources of variation in GGT, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) and of covariation between GGT and cardiovascular risk factors were assessed by maximum-likelihood model-fitting.

Results: Serum GGT, ALT, and AST were affected by additive genetic and nonshared environmental factors, with heritabilities estimated at 0.52, 0.48, and 0.32, respectively. One-half of the genetic variance in GGT was shared with ALT, AST, or both. There were highly significant correlations between GGT and body mass index; serum lipids, lipoproteins, glucose, and insulin; and blood pressure. These correlations were more attributable to genes that affect both GGT and known cardiovascular risk factors than to environmental factors.

Conclusions: Variation in serum enzymes that reflect liver function showed significant genetic effects, and there was evidence that both genetic and environmental factors that affect these enzymes can also affect cardiovascular risk.

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In addition to its diagnostic uses, serum γ-glutamyltransferase (GGT) activity has substantial epidemiologic significance (1). Prospective studies have shown a significant relationship between increased GGT and subsequent mortality and morbidity (2–7) and between GGT and development of specific conditions, including myocardial infarction (4), stroke (8), non-insulin-dependent diabetes (9), and hypertension (10). Major effects of body mass index (BMI) on serum GGT have been found, and associations between GGT and multiple cardiovascular risk factors, including serum lipids, blood pressure, smoking, and impaired glucose tolerance or insulin resistance, have been reported [summarized in Ref. (1)]. The known associations with other risk factors account for some, but not all, of the predictive value of GGT, so that it must in part be considered an independent risk factor or a marker of some type of risk that has not yet been characterized.

GGT shows significant correlations within the general population with both serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Some of the epidemiologic studies that showed high GGT to be a risk

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*Nonstandard abbreviations: GGT, γ-glutamyltransferase; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HDL-C and LDL-C, HDL- and LDL-cholesterol, respectively; and apo, apolipoprotein.
factor or to be associated with risk factors also measured and evaluated AST or ALT. In a German study of construction workers (6), AST and ALT, as well as GGT, were significant predictors of disability and death. In a British study of predictors of type 2 diabetes (9), AST was significant, but less predictive than GGT. Both AST and ALT, as well as GGT, are positively associated with BMI (11–14), which may be a reflection of the increased prevalence of fatty liver in more obese individuals.

For all these reasons, the sources of variation in GGT and their overlap with sources of variation in aminotransferases as well as the sources of covariation with cardiovascular risk factors are important. In this study, we assessed the genetic and environmental factors affecting variation in serum GGT activity and the degree to which similar factors affect AST or ALT. We also examined the genetic and environmental causes of covariation between the concentrations of these enzymes in serum and multiple biochemical or physiologic cardiovascular risk factors.

**Participants and Methods**

**Participants**
The characteristics of the participants in this study were described in a previous report (15). They completed a questionnaire in 1989, a telephone interview in 1993–1994, and provided a blood sample in 1993–1996. All participants were twins, born between 1903 and 1964, but in some cases only one member of a twin pair provided blood. Zygosity was determined from responses to questions about physical similarity and the inability of others to tell them apart, supplemented by blood group information. Participants gave informed consent to the questionnaire, interview, and blood collection, and the studies were approved by the appropriate Ethics Committees.

**Procedures**

Blood was collected from 1134 men and 2241 women. Immediately before blood collection, participants filled in a brief questionnaire that included a table asking how many drinks containing alcohol (10 g) they had consumed on each of the preceding 7 days, divided into beer, wine, spirits, fortified wine, or “other”. The numbers of drinks were summed to obtain a total for the past week. Participants also reported the time of their last meal, and the triglyceride, glucose, and insulin results were adjusted for the elapsed time between the last meal and blood collection. Samples were not taken in the fasting state, but participants reported the time of their last meal, and the triglyceride, glucose, and insulin results were adjusted for the elapsed time between the last meal and blood collection.

Initial analysis of the results revealed highly significant (P < 0.001) correlations between GGT results and numerous biochemical, physiologic, and alcohol-related characteristics. Because the participants were twins and therefore not genetically independent, the effective number of individuals for any characteristic with substantial heritability would be less than the actual number of participants, and therefore, the significance (but not the magnitude) of correlations may be overestimated. More detailed examination of the sources of variation in GGT and of the reasons for covariation with the variables that showed significant correlations in the exploratory analysis was performed using the Mx program, Ver. 1.30 (16), which is designed for analysis of twin and family data and overcomes this problem.

This analysis, like all studies based on twin pairs reared together, depends on the assumption that environments are equally similar for monozygotic and dizygotic co-twins. For biochemical and physiologic characteristics and for individuals ≥30 years of age and living independently, the equal-environments assumption is generally accepted.

After allowing for the effects of demographic variables such as age and sex, the residual correlations between co-twins, by zygosity, were estimated. The data were fitted to models of sources of variation in GGT, ALT, and AST. These models may contain additive and dominance genetic variation and shared and nonshared environmental variation; models that show a significantly worse fit with the data are rejected, and the most parsimonious model that does not yield a worse fit than the full model is chosen. For example, the model containing only addi-
tive genetic and nonshared environmental sources of variation (AE model) will be accepted if the model containing only shared environmental and nonshared environmental sources of variation (CE model) gives a significantly worse fit to the data and if addition of either shared environmental or dominance genetic sources of variation fails to produce significant improvement in the goodness of fit.

Because of the significant correlations between GGT and many other variables, the sources of covariation between them were modeled in a series of multivariate analyses. This led to estimates of the common and unique paths from genetic and environmental sources of variation for the variables included. This was an extension of the univariate model fitting, and in addition to estimating the proportions of variance attributable to genetics and environment, it provided estimates of the extent to which the genetic or environmental effects were specific to one variable (e.g., GGT) and the extent to which they affected more than one variable (e.g., GGT and ALT, or GGT and ALT and AST). Because simultaneous analysis of a large number of variables is computationally intensive, several separate analyses were conducted. The covariation between GGT, ALT, and AST was first analyzed, followed by the covariation between GGT, BMI, and the lipid and lipoprotein concentrations ([triglycerides, HDL- and LDL-C, apolipoprotein (apo) A-II, B, and E]; between GGT, BMI, glucose, and insulin; between GGT, BMI, and ferritin; between GGT, BMI, and urate; and between GGT, BMI, and systolic and diastolic blood pressure.

Results

Heritability of Serum GGT, ALT, and AST

Adjustments were made for demographic and sample-related variables that affected GGT, ALT, and AST activity. Mean values were higher in men than in women, and age had significant effects in both sexes. Mean values for these enzymes and for the other variables measured are shown in Data Supplement Table DS1. (All data supplementary tables are available with the online version of the article at http://www.clinchem.org/content/vol48/issue8/.) The adjusted within-pair correlations by zygosity for GGT, ALT, and AST are shown in Data Supplement Table DS2, with the higher correlations in monozygotic than in dizygotic pairs suggesting significant genetic effects.

The results of testing of models of sources of variation, including additive genetic effects (A), dominance genetic effects (D), common environmental effects shared by members of a twin pair regardless of zygosity (C), and nonshared environmental effects (E), are shown in Data Supplement Table DS3. The models containing only C and E were rejected because the goodness-of-fit between the model and the data was significantly worse, for each of the enzymes, than for a model containing A and E only. Models containing A, C, and E or A, D, and E as sources of variation showed no significant improvements over the AE models, and under the ACE models, the C component was estimated as zero. Therefore, we conclude that there is no evidence that nonadditive genetic effects or shared environmental effects contribute to interindividual variation in GGT, ALT, or AST. The age- and sex-adjusted heritabilities for log-transformed enzyme activities were 0.52 for GGT, 0.48 for ALT, and 0.32 for AST.

Phenotypic Correlations Between Serum GGT, ALT, and AST Activities and Other Characteristics

Highly significant correlations were found between GGT values and many other physiologic or biochemical characteristics. These included other liver enzymes (AST and ALT), alcohol intake and smoking status (but only in men), BMI and biochemical aspects of the metabolic syndrome (triglycerides; HDL- and LDL-C; apo A-II, B, and E; urate; glucose; and insulin), blood pressure (systolic and diastolic), and iron status (ferritin). Similarly, there were significant associations between ALT and AST and many of the variables that showed correlations with GGT. The phenotypic correlations, taking the individual as the unit and making no allowance for gene-sharing within twin pairs, are shown in Data Supplement Table DS4.

The correlations with cardiovascular risk factors tended to be stronger for GGT than for ALT (or even more so) AST. This trend was apparent for BMI, lipids, apolipoproteins [except apo A-I and apo(a), which showed little association with the enzyme values], glucose and insulin, urate, blood pressures, and ferritin. It therefore seems that there are two components to the associations between GGT and the other measurements: one a general hepatic effect shared with ALT and AST, and the other more specific to GGT.

It is also noteworthy that although apo A-II was significantly and positively associated with GGT, ALT, and AST in both men and women, apo A-I was not, and HDL-C tended to show negative correlations. This dissociation between the components of HDL was not seen for the lipids and apoproteins associated with LDL or VLDL.

Common and Unique Genetic and Environmental Influences on GGT, AST, and ALT

Because of the large number of variables associated with GGT as well as with ALT and AST, the relationships between these enzymes and the genetic or environmental causes of these relationships were explored in a series of analyses. The relationships between genetic and environmental sources of variation acting on GGT, AST, and ALT are illustrated in Fig. 1. This path diagram shows additive genetic (A1, A2, and A3) and nonshared environmental (E1, E2, and E3) effects on these variables. The paths between the sources of variation and the observed values are shown in Fig. 1 and consist of A1 and E1, which influence all three variables; A2 and E2, which affect ALT and GGT, but not AST; and A3 and E3, which affect GGT only. Values next to these paths show the proportion of
variance: e.g., the path from A1 to ALT labeled “15” shows that 15% of the variance in ALT is attributable to genes that also affect AST. For GGT, 29% of the observed variance is attributable to genes that affect GGT but not ALT or AST, whereas 20% (12% + 8%) is attributable to genes that also affect ALT or AST or both. Twenty-seven percent of the variance in GGT is attributable to environmental effects not shared with AST and ALT, whereas 25% is attributable to environmental factors that also affect ALT and/or AST. Therefore, GGT is subject to both general “liver enzyme” factors and GGT-specific factors. It is slightly more closely related to ALT than to AST. Fig. 1 considers only the relationships between GGT and the aminotransferases; the genetic and environmental connections with cardiovascular risk factors are considered below. Loci affecting cardiovascular risk factors are expected to affect both the genetic effects unique to GGT and those shared with the other enzymes.

GENETIC AND ENVIRONMENTAL CORRELATIONS BETWEEN GGT AND VARIABLES RELATED TO CARDIOVASCULAR RISK

As stated above, there were multiple highly significant correlations between GGT and known or suspected cardiovascular risk factors. Of these, the strongest relationships were between GGT and variables associated with VLDL (triglycerides, apo B, and apo E), obesity, blood pressure (diastolic and systolic), and insulin resistance (insulin and glucose). There were also significant correlations with HDL-C and apo A-II (but not apo A-I), with urate, and with ferritin. The degrees to which these phenotypic correlations were attributable to genes affecting multiple variables from this list or to nongenetic (environmental) factors with similar multifaceted effects can be seen from the genetic and environmental correlations with GGT, which are shown in Table 1.

Because the phenotypic correlations were the outcome of both genetic and environmental effects, the correlations in Table 1 are broadly similar to those in Table DS4. There was a tendency for the genetic correlations to be greater than the environmental ones, in part because environmental factors included measurement errors and short-term biological variation and these would not usually be correlated across variables. The genetic correlations with GGT were notably stronger than the environmental correlations for triglycerides, apo B and E, and BMI, and to a lesser extent for blood pressure and HDL-C.

Many of the cardiovascular risk factors that showed phenotypic, genetic, and environmental correlations with GGT were also correlated with each other. The strongest GGT correlation was with triglyceride concentration. This led us to consider whether there might be a single underlying cardiovascular factor associated with GGT, largely based on triglycerides and VLDL. This was examined by ordering the multivariate analysis so that genetic factors affecting both triglycerides and GGT were estimated and that, for other lipid or lipoprotein variables, only the components not affecting triglycerides and GGT would appear. The outcome is shown in Table 2. Although each of the variables listed had reasonably strong genetic correlations with GGT (Table 1), the genes affecting both triglycerides and GGT (including genes that also

<table>
<thead>
<tr>
<th>Variable</th>
<th>Genetic correlation</th>
<th>Environmental correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (log)</td>
<td>0.35</td>
<td>0.34</td>
</tr>
<tr>
<td>AST (log)</td>
<td>0.42</td>
<td>0.49</td>
</tr>
<tr>
<td>BMI</td>
<td>0.34</td>
<td>0.23</td>
</tr>
<tr>
<td>Triglycerides (log)</td>
<td>0.45</td>
<td>0.29</td>
</tr>
<tr>
<td>apo B</td>
<td>0.37</td>
<td>0.17</td>
</tr>
<tr>
<td>apo AI</td>
<td>−0.04</td>
<td>−0.03</td>
</tr>
<tr>
<td>apo AIi</td>
<td>0.22</td>
<td>0.19</td>
</tr>
<tr>
<td>apo E</td>
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<td>0.19</td>
</tr>
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<tr>
<td>HDL-C</td>
<td>−0.25</td>
<td>−0.15</td>
</tr>
<tr>
<td>apo(a) (log)</td>
<td>−0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>Glucose³</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>Insulin (log)³</td>
<td>0.25</td>
<td>0.22</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.22</td>
<td>0.28</td>
</tr>
<tr>
<td>Diastolic BP³</td>
<td>0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.25</td>
<td>0.15</td>
</tr>
<tr>
<td>Iron</td>
<td>0.05</td>
<td>−0.12</td>
</tr>
<tr>
<td>Transferrin</td>
<td>0.10</td>
<td>0.07</td>
</tr>
<tr>
<td>Ferritin (log)</td>
<td>0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* Glucose and insulin results have been adjusted for time since last meal, collection-to-processing time, and storage time.

³ BP, blood pressure.
affect apo B, BMI, and so forth) accounted for 9.6% of the variance in GGT, the genes affecting both apolipoprotein B and GGT (including genes that also affect BMI, apo E, and so forth, but not triglycerides) accounted for 1.7% of the variance in GGT, and the other variables listed had only minor effects on GGT except through pathways shared with triglycerides. Genetic effects on GGT that were not shared by any of the other variables listed accounted for 36.6% of the GGT variance. Similarly, the nonunique environmental effects on GGT mainly affected triglycerides, and paths between other variables and GGT that excluded effects on triglycerides were negligible.

Discussion

There are only limited previous data on the heritability of serum GGT and the aminotransferases. In our previous work (17), all participants were between 18 and 35 years of age with a mean age of 23 years. AST and ALT were affected by genetic and nonshared environmental factors, some of which affected both enzymes, whereas GGT was affected by both shared and nonshared environmental factors but not genetic ones. A recent study on Danish twins (18) reported data from individuals 73–102 years of age and found a mixture of genetic and nonshared environmental influences on GGT and ALT; AST was not measured. Our current results are consistent with the latter report and confirm the presence of genetic effects on these serum enzymes in adults after the age of 30.

This result implies that either the release of enzymes from liver cells or the rate of clearance of the enzymes from the circulation is subject to genetic effects. Because a considerable proportion of the genetic effect is shared by the three enzymes, which probably have different rates of clearance, we favor the former explanation. The genetic component unique to GGT may reflect variation in GGT activity at the hepatocyte surface exposed to the circulation.

However, the major topics of interest are not so much the variations in enzyme activities in the serum as the variations in the underlying physiologic or pathophysiologic processes and the ways in which these processes interact with cardiovascular risk. All three enzymes show associations with cardiovascular risk factors, although these associations are strongest for GGT and only GGT has a convincing body of evidence connecting it with outcomes in prospective studies (2–10). The pattern of relationships among the three liver enzymes suggests that GGT in part reflects the same processes as ALT or AST and in part is independently determined.

Although all three enzymes are associated with cardiovascular risk factors, the role of GGT in replenishing intracellular glutathione, and possibly in controlling apoptosis and proliferation in atheromatous plaques (19, 20), may give it added significance. It is clear that increased GGT is associated with an increased probability of death from cardiovascular causes, development of type 2 diabetes, and development of hypertension and stroke (4, 8–10). Most probably, it is associated with fatty liver, insulin resistance, and oxidative stress (21, 22). Because it is possible that GGT plays a role in the proliferation of atheromatous plaques, some of the circulating GGT may come from such plaques. Thus, there are two possible (but not necessarily exclusive) explanations for the association between serum GGT and cardiovascular risk: either GGT comes from the atheromatous plaques, which will be more common and extensive in patients with adverse cardiovascular risk profiles, or GGT is associated with the risk factors even before the plaques are fully developed. Given the age range and essentially healthy status of our participants, the latter seems more likely.

It was notable that the associations between serum GGT and variables related to cardiovascular risk, reported by others and confirmed by us, had a strong genetic component and were less influenced by environmental variation (see Tables 1 and 2). It seems likely that a genetic predisposition to abdominal obesity and insulin resistance is associated with fatty liver, lipid abnormalities (particularly increased VLDL), and increased liver enzymes. The genetic component in the underlying factor of abdominal obesity or metabolic syndrome will lead to genetic correlations between its multiple consequences. Similar reasoning in relation to blood pressure (also associated with insulin resistance) could explain the genetic correlations between GGT and systolic and diastolic blood pressures.

Two other analytes are worthy of comment because of their correlations with both the liver enzymes and the cardiovascular risk factors. Urate was significantly correlated (all \( P < 0.01; \) data not shown) with GGT, ALT, and AST as well as with BMI, blood pressures, triglycerides, apo B, and apo E in both women and men. Ferritin was significantly correlated with the three enzymes and with BMI, triglycerides, and apo A-II in both sexes and additionally with blood pressures, LDL-C, apo B, and apo E in the women. The associations of serum urate with liver enzymes and with cardiovascular risk are not unexpected (21, 23, 24), although they are unexplained. The association between ferritin and liver enzymes may reflect adverse effects of iron on the liver or the release of ferritin from hepatocytes in situations that also cause release of enzymes, but the additional association with cardiovascular risk factors suggests that either iron overload in-

Table 2. Genetic and environmental paths from lipids to GGT.\(^a\)

<table>
<thead>
<tr>
<th>Lipid</th>
<th>apo B</th>
<th>BMI</th>
<th>apo E</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>apo All</th>
<th>Unique</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>9.6</td>
<td>1.7</td>
<td>1.7</td>
<td>0.9</td>
<td>0.2</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>E</td>
<td>3.9</td>
<td>0.2</td>
<td>0.9</td>
<td>0.2</td>
<td>0.5</td>
<td>0.1</td>
<td>1.7</td>
</tr>
</tbody>
</table>

\(^{a}\) Each column shows the percentage of variance in GGT that can be accounted for by the additive genetic (A) and environmental (E) effects that affect the variable (and also those in columns to the right). The last column shows the contributions of genetic and environmental variance unique to GGT after covariance with lipids is taken into account. The genetic effects sum to 52% and the environmental effects to 48%, consistent with the univariate estimate of GGT heritability.
creases the probability of liver dysfunction, insulin resistance, and lipid changes or vice versa. The relationships between iron overload and components of the insulin resistance syndrome have been investigated and discussed recently (25–27), but which is cause and which is effect is still uncertain.

In conclusion, clarification of the genetic associations between liver function (as indicated by the three enzymes studied) and the combined cardiovascular risk factors requires incorporation of a large number of variables and leads to a computationally intensive analysis. These relationships, which raise such issues as the effect of liver function in the broad sense on cardiovascular risk, will be the subject of further work.

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References