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Genetic covariance between γ–glutamyl transpeptidase and fatty liver risk factors and *B2-adrenergic receptor* genetic variations in twins

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Abstract

Background—Plasma levels of γ-glutamyl transpeptidase (GGT) are associated with risk factors for nonalcoholic fatty liver disease (NAFLD) such as dyslipidemia, insulin resistance (IR), and hypertension. Limited data exist on whether there is genetic covariance between plasma levels of GGT and NAFLD risk factors. Variants of β2-adrenergic receptor gene (*ADRB2*) have been associated with dyslipidemia, IR, and hypertension, but its affect on GGT secretion is not known. We estimated the heritability of GGT using a twin-study design and examined the genetic co-variance between GGT levels, IR, hypertension, levels of low-density lipoproteins and triglycerides, and *ADRB2* variants.

Methods—We studied phenotypes of 362 twins; the heritability of increased GGT activity and genetic covariance with NAFLD risk factors were estimated by variance-component methodology. *ADRB2* genotype associations with plasma GGT activity were examined using generalized estimating equations (GEE) to account for intra-twinship correlations.

Results—Increased GGT activity was heritable in 49%±8% of the twin cohort and had significant covariance with IR; insulin, triglyceride, and uric acid levels; and diastolic blood pressure. In multivariate GEE models, the most common haplotypes of *ADRB2* were significantly associated with plasma GGT activity; 5 single nucleotide polymorphisms (SNPs) within *ADRB2* were associated with increased GGT activity (28.6 IU/L for 1 or 2 copies of the *ADRB2* haplotype

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vs. 23.0 IU/L for 0 copies; P=0.02). Five SNPs in *ADRB2* were associated with levels of GGT; *ADRB2* haplotypes were pleiotropic for GGT and triglyceride levels.

Conclusions—In a twin study, GGT shared genetic co-determination with traits of metabolic syndrome. The *ADRB2* gene had pleiotropic effects on plasma levels of GGT and triglycerides, indicating linked pathways (e.g. adrenergic) between genetic susceptibility to NAFLD and metabolic syndrome.

Keywords

GGT; NAFLD; ADRB2; metabolic syndrome; LDL; TG

INTRODUCTION

Gamma-glutamyl transpeptidase (GGT) is a hepatic and biliary enzyme synthesized by hepatocytes as well as epithelial cells of intra-hepatic bile ducts (1–4). Elevated plasma GGT enzymatic activity is a marker of fatty liver disease resulting from either obesity or alcohol in the general population (5,6). Recent studies suggest that plasma GGT is a significant predictor of the metabolic syndrome, independent of alcohol intake (7,8), as well as its hepatic manifestation, non-alcoholic fatty liver disease (NAFLD) (9,10). Several studies have shown that plasma GGT is cross-sectionally and longitudinally associated with metabolic syndrome risk factors such as diabetes, insulin resistance, dyslipidemia, elevated uric acid, hypertension and NAFLD (7,8,11–13). It is unclear whether the association between plasma GGT, as a marker of NAFLD, and metabolic syndrome traits is genetic and/or environmental in origin. Therefore, we propose to examine whether plasma GGT shares genetic or environmental covariance with metabolic syndrome risk factors in a well-characterized, US twin cohort (14,15).

Wessel et al. showed that C-reactive protein (an acute phase protein made in hepatocytes), which correlates with plasma GGT, is associated with multiple features of the metabolic syndrome and is linked to genes that regulate adrenergic activity and metabolism (15). Several studies have shown that genetic variation at *ADRB2* is associated with insulin resistance, regulation of glucose metabolism in liver, hypertriglyceridemia, and hypertension (16–20). Such metabolic traits are significant correlates of GGT and NAFLD (21). Therefore, we also propose to examine whether common variants at *ADRB2* in twins is associated with plasma GGT. Our study is based upon the hypotheses that: 1) Plasma GGT shares genetic determination (co-variance) with metabolic risk factors such as insulin resistance (measured by homeostasis model of insulin resistance, HOMA-IR), hypertension, obesity, and elevated plasma triglycerides. 2) Genetic variants within the *ADRB2* gene predict plasma GGT. To test these hypotheses, we conducted a cross-sectional twin-study design in a previously well-defined and characterized twin cohort. STROBE (22) guidelines for reporting cross-sectional studies were followed.

METHODS

Participants and study design

The University of California at San Diego twin cohort recruitment has been previously described (14,15,23,24). In brief, the cohort was recruited by access to a twin-birth registry and newspaper advertisements. This study included 380 Caucasian twins with 128 mono-zygotic twins (24 male pairs and 104 female pairs) and 62 di-zygotic twins (14 male pairs, 36 female pairs and 12 male-female pairs). None of the twins had diabetes, and the prevalence of obesity (BMI ≥30 kg/m^2) was 13.9%. Zygosity was confirmed by use of either >100 microsatellites (chromosomes 1 and 2) for self-identified DZ twins, or single

nucleotide polymorphism (SNP) data (11–177 SNPs) as well as the *TH* $(TCAT)_n$ microsatellite (24) for self-reported MZ and DZ pairs. To qualify for analysis, twins of white (European) ancestry identified not only themselves but also both parents and all four grandparents as being of that biogeographic ancestry group. Twins were between 18–81 years of age. None of the twins had a history of renal failure, and plasma creatinine concentrations were ≤1.5 mg/dl. None of the twins provided a history of known liver disease. Definitions of subject characteristics have been previously published in prior manuscripts from our laboratory (15). Study participants were twin volunteers from urban southern California (principally the San Diego area). Written informed consent was obtained from each participant, and the research protocol was approved by the institutional review board of UCSD.

Blood pressure and heart rate phenotyping

Brachial cuff BP (mmHg) and heart rate (beats/min) were obtained in seated subjects with a DynaPulse oscillometric device (PulseMetric, San Diego, California, USA), as described previously (25), to obtain blood pressure (in mmHg) and heart rate (beats/min) in the seated position. The recordings were validated by triplicate determinations of BP and heart rate, until each value was within 10% of the mean value.

Genomics

Details regarding genotyping methodology used in our laboratory have been previously published (15,23,24). SNP selection across the ADRB2 locus proceeded as we have previously described for studies of hypertension (16). The same SNPs were also described by Drysdale et al (26) and McGraw et al (27). Genomic DNA was isolated from blood leukocytes. SNP polymorphisms were scored in a two-stage assay (28). In stage one, PCR primers flanking the polymorphism were used to amplify the target region from 5 ng of genomic DNA. In stage two, an oligonucleotide primer flanking the variant was annealed to the amplified template, and extended across the variant base. The mass of the extension product (wild-type versus variant) was scored by MALDI-TOF mass spectrometry (low mass allele versus high mass allele).

Biochemical phenotyping

Plasma GGT activity—GGT enzymatic activity in plasma was measured by an enzymatic rate method (29) in the UCSD Medical Center clinical chemistry laboratory. The lower limit of detection of the assay was 5 IU/L and the repeated sample correlation coefficient (r) was 0.9985. The precision of the assay was robust with CV <3.5%. Elevated GGT was defined as >40 IU/L in men or >30 IU/L in women. The prevalence of elevated GGT was 19.3% in men and 15.8% in women.

Catecholamines, cytokines, and lipids—Samples of plasma for measurement of catecholamines, cytokines and lipids were quickly frozen at −70 C. Details of methods for the assays have been previously published (15).

Insulin resistance—Fasting plasma glucose and plasma insulin levels were obtained. Insulin resistance was measured by HOMA-IR, the homeostasis assessment model of insulin resistance, where HOMA-IR (mmol/L × μU/ml) = fasting glucose (mmol/L) × fasting insulin (μU/ml)/22.5).

Statistical analyses

Baseline descriptive statistics were obtained in all the twins. Twins were then classified into two groups by dividing about the median GGT value into lower versus upper quantiles,

which were compared using generalized estimating equation (GEE: PROC GENMOD) using SAS version 9.1, SAS Institute , Cary, NC; to account for within twin-pair correlations.

Heritability (h^2) was estimated based upon following equation: $h^2 = V_G/V_P$, where V_G indicates additive genetic variance and V_P is total phenotypic variance, by variance-component methodology implemented in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package (available at http://www.sfbr.org.solar/). This method maximizes the likelihood assuming a multivariate normal distribution of phenotypes in twin pairs (monozygotic versus dizygotic) with a mean dependent on a particular set of explanatory variables. Heritability ($h^2 = V_A/V_P$) is the proportion of trait variance (V_P) that is explained by the genetic variation V_G (relative to environmental variation V_E) in a population. Heritability and environmentability are relative concepts regarding genetic or environmental trait determination in a population, and do not give any information regarding which genes influence the trait. The null hypothesis (H_0) of no heritability is tested by comparing the full model, which assumes genetic variation (V_G), and a reduced model, which assumes no genetic variation, using a likelihood ratio test. Heritability estimates were adjusted for age and sex, because of the effects of these covariates on several traits. The primary study outcome for gene effects on plasma GGT was based upon haplotype analysis in age/sex-adjusted GEE models in Caucasian twins, and p-values ≤0.05 were considered significant. We examined the association between *ADRB2* haplotype-1 (AGGCCGGGC, the most common haplotype) and plasma GGT concentrations. Haplotypes were inferred from diploid genotypic data using the HAP algorithm (30) (http://research.calit2.net/hap); haplotypes at a locus were numbered in order of frequency (1: most frequent). Patterns of linkage disequilibrium (LD) between/among SNPs across the *ADRB2* locus were visualized with Haploview (31).

SOLAR was also used to examine the contribution of allelic variation at the locus to the heritability (i.e., locus-specific V_G) heritability, by comparing models including or excluding the genotype as a covariate. During allele, haplotype and diplotype (haplotype pair) associations, multivariable analyses were carried out to control for confounding by age and sex.

Pleiotropy (shared genetic determination; genetic covariance for two correlated, heritable traits) was estimated as RhoG (ρ_G) and the environmental covariance (shared environmental determination) as parameter RhoE (ρ_E) using SOLAR.

RESULTS

Heritability (h^2) of GGT in twins

Heritability was estimated from twin pairs based upon correlations among monozygotic and dizygotic twins as shown in Figure 1. Heritability was significant for plasma GGT, at 49±9% of trait variance ($p < 0.001$). Figure 1 displays the heritability estimates of other associated metabolic syndrome traits in this twin cohort; such heritability estimates were similar to other reports.

The average (± SEM) plasma GGT in the twin cohort was 23.7 ± 1.2 IU/L. The prevalence of elevated plasma GGT (≥40 IU/L in men or ≥30 U/L in women) was 16.6% in this cohort.

GGT associations with other traits

Table 1 provides a description of this twin cohort by dividing the cohort into two groups (quantiles) around the GGT median. As expected, plasma GGT increased with age and was higher in men than women. Participants with higher GGT showed significant ($p<0.05$) trait

differences with markers of inflammation, adrenergic activity, and the metabolic syndrome: body mass index, epinephrine, IL-6, P-selectin, triglycerides, HDL, glucose, insulin and HOMA-IR, homocysteine, uric acid, butyrylcholinesterase (BChE), and blood pressure. Sex-specific data is also provided in the appendix supplementary table.

Shared heritability in twins: Genetic covariance between GGT and metabolic risk factors

Metabolic syndrome traits that correlated with plasma GGT were tested for shared genetic determination (pleiotropy) with the GGT trait (Table 2). The following traits showed significant ($p<0.05$) genetic covariance with plasma GGT: diastolic blood pressure (ρ_G 27%, $p<0.05$), uric acid (ρ_G 46%, $p<0.005$), insulin (ρ_G 50%, $p<0.005$), HOMA-IR (ρ_G 39.6%, $p<0.02$), and triglycerides (ρ_G 53%, $p<0.005$). The only trait to share significant environmental covariance with GGT was BChE (ρ_E-24%, $p<0.025$).

β_2-adrenergic receptor gene effect on GGT in twins

We examined the effect of SNPs across the *ADRB2* locus on plasma GGT. Graphical representation of linkage disequilibrium (LD) across the *ADRB2* locus (Figure 2) suggests that the SNPs we evaluated lie within a single block of LD. Hardy-Weinberg Equilibrium was obtained for each SNP across *ADRB2* except promoter T-47C, at p=0.02 (Table 3).

Five SNPs across *ADRB2* were significantly associated with plasma GGT, independent of age and sex in GEE models, as shown in Table 3. Each of the five GGT-associated SNPs occurred in functional domains: four in the promoter region (G-1023A, C-468G, T-367C, and T-47C), and one non-synonymous coding variant (Glu27Gln). Since the SNPs across *ADRB2* have strong pair-wise correlations (Figure 2), it is plausible that one or more functional variants in this region (e.g., T-47C (32)) may play a dominant role in plasma GGT regulation; however, previous functional studies of the ADRB2 promoter (32) indicate that the effects of individual SNPs on gene expression are interactive (i.e., are best explained by haplotypes rather than individual SNPs).

In this twin cohort, there was an association between β_2-adrenergic receptor (*ADRB2*) haplotype-1 (the most frequent haplotype: AGGCCGGGC) and plasma GGT in age/sex-adjusted GEE models ($p<0.024$), in a dominant model (Figure 3A).

Genetic co-determination by *ADRB2* of metabolic traits

ADRB2 haplotype-1 (the most frequent haplotype: AGGCCGGGC) predicted not only GGT but also plasma triglycerides (p=0.0071). Figure 3B illustrates joint effect of *ADRB2* haplotype-1 on both plasma GGT ($p<0.024$) and triglycerides (p=0.0071), suggesting pleiotropic effects of the gene on multiple metabolic traits.

We conducted sensitivity analyses after excluding the HWE-violating SNP (-47 T/C). We examined the association between ADRB2 haplotype-1 (excluding -47 T/C SNP) and plasma GGT as well as pleiotropic effects on plasma GGT and triglyceride. The results remained consistent and statistically significant (please see supplemental figure).

DISCUSSION

Main findings

Using a twin study design, we confirmed that plasma GGT is a heritable trait (at h^2 = 49±8%, $p<0.00001$; Figure 1) and demonstrate genetic covariance (or shared heritable determination) between GGT, a marker of fatty liver disease, and metabolic syndrome traits such as insulin resistance, increased triglycerides, uric acid, and blood pressure (Table 2). This is the first study to report an association between plasma GGT and beta$_2$-adrenergic

receptor genetic variation (Figure 3A). The findings of phenotypic pleiotropy (Table 2) as well as the joint effect of *ADRB2* variants on both GGT and lipids (Figure 3B) thus contribute to a genetic basis for the clinical observation that NAFLD patients display elevations of both GGT and triglycerides. The results provide a potential mechanistic link between adrenergic genetic variation and shared genetic susceptibility between NAFLD and the metabolic syndrome.

Our study suggests that GGT shares significant genetic covariance with uric acid, insulin, HOMA-IR, triglycerides, and BP (Table 2). These traits are common to the metabolic syndrome and frequently associated with NAFLD, and document the contribution of common genetic variation at one particular adrenergic locus: *ADRB2*. Our study thus suggests that the adrenergic system may be a key mediator linking metabolic risk factors with NAFLD.

Elevated GGT is a marker of future risk of diabetes, the metabolic syndrome and all-cause cardiovascular (CVD) and liver disease mortality (7). *ADRB2* genotypes predict not only BP (16) but also survival after acute coronary syndrome (33) underscoring the prognostic significance of such variants. Therefore, the associations we described between adrenergic activity (Tables 1&2), *ADRB2* genotype and plasma GGT (Figures 2&3) may ultimately have clinical implications, opening novel pathways to probe the emerging link between NAFLD and CVD.

Biological rationale and proposed mechanism of adrenergic involvement

The genetic polymorphisms within *ADRB2* that predict plasma GGT and triglyceride concentrations (Table 4) confer functional variation upon the gene: the promoter variants (especially T-47C) alter reporter gene expression in transfected *ADRB2* promoter/luciferase plasmids (32), while non-synonymous variant Gln27Glu enhances *ADRB2* down-regulation in the face of repeated agonist exposure (34). Based upon our findings, we propose a model for the role of the adrenergic pathway in GGT regulation and its association with NAFLD. First of all, *in vitro* and *in vivo* studies demonstrate that hepatocytes and hepatic stellate cells (HSC), as well as intra-hepatic biliary epithelial cells, possess adrenergic receptors and respond to adrenergic stimulation (35–37). Catecholamines such as epinephrine stimulate hepatocytes to synthesize and release C-reactive protein (38), which correlates with elevations of plasma GGT and other features of the metabolic syndrome and NAFLD (Table 1) (15). Additionally, norepinephrine is required for activation of HSC and may be a key mediator of fibrogenesis (37). Both inflammatory and fibrogenic responses may lead to release of GGT by epithelial cells of intrahepatic bile duct and hepatocytes (21,36,39,40). In addition, ADRB2 receptors are responsible for maintaining glucose homeostasis by hepatocytes, as well as release of free fatty acids (41), which play a role in the pathogenesis of NAFLD (42). Taken these findings together, it is plausible to suggest that increased adrenergic activity may predispose to both increased GGT and fibrogenic processes leading to NAFLD. The present study supports the viewpoint that the adrenergic system may be one common denominator between metabolic risk factors and NAFLD, and this nexus may be exploited, in future, to improve our understanding of the development of NAFLD in humans.

Strengths and limitations of the study

The strengths of this study are several: 1. Well-characterized cohort with comprehensive phenotyping of multiple metabolic risk factors (23). 2. Twin study design, which allowed us to examine both the heritability of the GGT trait, as well as genetic and environmental covariances associated with complex inheritance of GGT (and perhaps NAFLD). 3. Documentation of joint (pleiotropic) effects of *ADRB2* genetic variation, both haplotypes

and single SNPs, on both GGT and lipid traits. 4. The trait-associated *ADRB2* SNPs are known to be functional, suggesting mechanistic links among the pathogenic metabolic traits.

Limitations of this study include that only one ethnicity (European ancestry) was examined. Therefore, these data may not be generalizable to individuals of other ethnicities. However, homogeneity of biogeographic ancestry in our study population may alleviate concerns regarding the internal validity of the results. The average BMI in our cohort was 25 kg/m^2 that is lower than the reported average BMI of the US population (NHANES 1999–2002). Our twin population was exclusively white, predominantly female (72% of the population) and mainly derived from residents of Southern California, who may not be representative of entire US population, given demographic differences. Alcohol use, detailed socioeconomic and marital status data were not available. Effect modifications due to gender, alcohol use and age could not be adequately analyzed due to the lack of power. Additionally, only ADRB2 gene effects were examined. However, expanding the study to include a larger number of genes, utilizing a hypothesis-free, genomic approach, creates statistical problems in achieving the necessary modified alpha-threshold to avoid false-positive conclusions.

Results in context with other published studies

Our results suggest that GGT is heritable, and the heritability estimate (at h^2=49±8%; Figure 1) is similar to previously published studies (43–46). The heritability estimate reported in our study is consistent with previous published twin study by Whitfield et al (GGT heritability is 49% in our study and 52% in Whitfield et al. study) (43). The mean age in Whitfield's study was 23 versus 40 years in our study. Therefore, results remain consistent across at least these age groups of 23 through 40 among Caucasian twins. The average BMI was 25 kg/m^2 in the Whitfield study, which is similar to our cohort. Therefore, we believe that our findings are consistent with prior published data.

Furthermore, Bathum et al have shown that the effect of heredity on plasma GGT is independent of BMI and alcohol among 580 Danish twins (47). Valle and colleagues have shown that butyryl-cholinesterase (BChE) has shared genetic association with the metabolic syndrome (41,48), but here we find that GGT and BChE share *environmental* covariance but genetic covariance did not reach statistical significance. However, we only tested covariance models considering additive genetic variance for GGT and BChE, and cannot exclude other potentially shared effects such as dominance genetic variance or gene-by-environment interactions between these two traits. Additionally, a Japanese study of NAFLD showed that the same *ADRB2* non-synonymous SNP that we associated with GGT (Gln27Glu) may be associated with both hypertriglyceridemia and NAFLD susceptibility (17).

A recent genome-wide association conducted in the Dallas Heart Study found that *PNPLA3* (patatin-like phospholipase-3) genotype is associated with fatty liver in that cohort (49). PNPLA3 is a member of a newly recognized family of adipocyte triglyceride lipases (50–52). The previously described adipocyte triglyceride lipase, known as HSL (hormone sensitive lipase) or LIPE (lipase, hormone-sensitive), is positively regulated by catecholamines acting through ADRB2; the expression of LIPE seems to be decreased in obesity despite a rise in catecholamines (increased adrenergic activity), perhaps contributing to a state of catecholamine- and insulin-resistance (51–53). Both the newer family of lipases (including PNPLA3) and LIPE catalyze triglyceride hydrolysis in adipocytes (51,52). Therefore, our study provides an additional mechanistic link between these parallel lipolytic processes and metabolic syndrome traits of increased adrenergic activity, triglycerides and GGT. Since NAFLD is a complex disease with substantial hereditary determination, there are likely several genes that may contribute to genetic susceptibility for the trait (54–56).

Utilizing the twin-study design, we demonstrate significant shared genetic and environmental covariance between metabolic risk factors and GGT (Table 2). In addition, we found that five functional (32) SNPs at *ADRB2* are associated with plasma GGT levels in humans. *In vitro* and *in vivo* studies have shown that plasma epinephrine is involved in progression of NAFLD (35,36), but human data to confirm these findings has been lacking. It is well-recognized that adrenergic activity plays a critical role in portal hypertension, and use of β-blockade in the setting of advanced portal hypertension (especially after variceal bleeding with elevated hepatic wedge pressure gradient) improves survival (57,58), and *ADRB2* gene polymorphisms influence hemodynamic response to β-blockade (59) . Our study suggests that increased epinephrine levels are associated with plasma GGT elevation (Table 1). It is thus plausible that increased adrenergic activity may be involved in the pathogenesis of NAFLD, as well as the progression of NAFLD to nonalcoholic steatohepatitis (NASH) and ulimately cirrhosis. Further studies are needed to examine these hypotheses.

Conclusions and perspectives

We conclude that GGT is heritable and shares significant genetic co-determination with multiple features of the metabolic syndrome as well as elevated adrenergic activity in Caucasians. *ADRB2* genetic variation is predictive of plasma GGT concentration in humans, and the *ADRB2* locus may thus participate in the complex heritability of GGT, and perhaps NAFLD in this population. Future longitudinal studies are needed to examine whether *ADRB2* genetic variants increase susceptibility of NAFLD and/or progression from steatosis to steatohepatitis in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

NAFLD	non-alcoholic fatty liver disease
SNP	single nucleotide polymorphism
NASH	non-alcoholic steatohepatitis
GGT	gamma-glutamyl transpeptidase
ADRB2	β_2-adrenergic receptor
IR	insulin resistance
HOMA	homeostasis model of insulin resistance

h^2	heritability
TG	triglycerides
LDL	low density lipoprotein
CRP	C-reactive protein
LD	linkage disequilibrium
ρ_G	Rho-G (genetic co-variance between traits)
ρ_E	Rho-E (environmental co-variance between traits)
HDL	high density lipoprotein
LDL	low density lipoprotein
CVD	cardiovascular disease
GEE	generalized estimating equation

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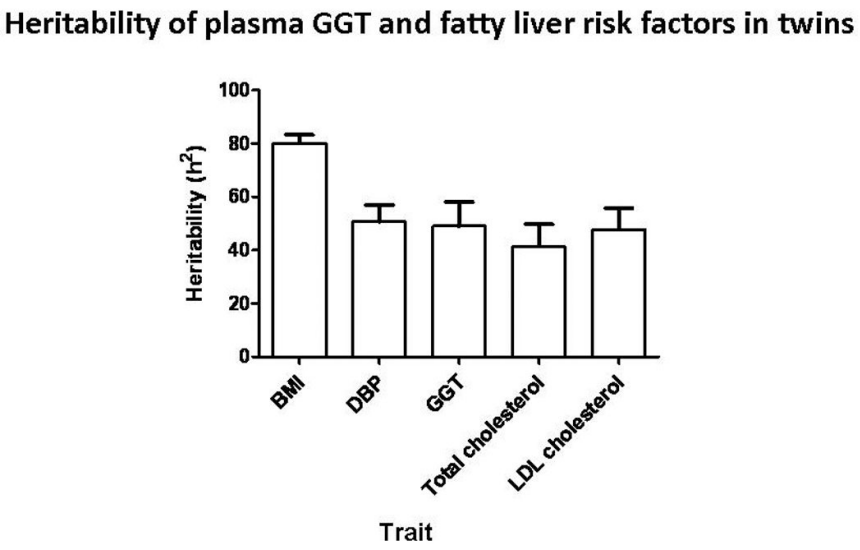

Figure 1. Heritability (h^2) estimates of GGT and fatty liver risk factors shown in the present twin cohort

These h^2 estimates (± SEM) are similar to previously published studies, suggesting generalizability of findings from our cohort. GGT has substantial heritability, at 49±8%. Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; GGT, gamma-glutamyl transpeptidase; LDL, low-density lipoprotein..

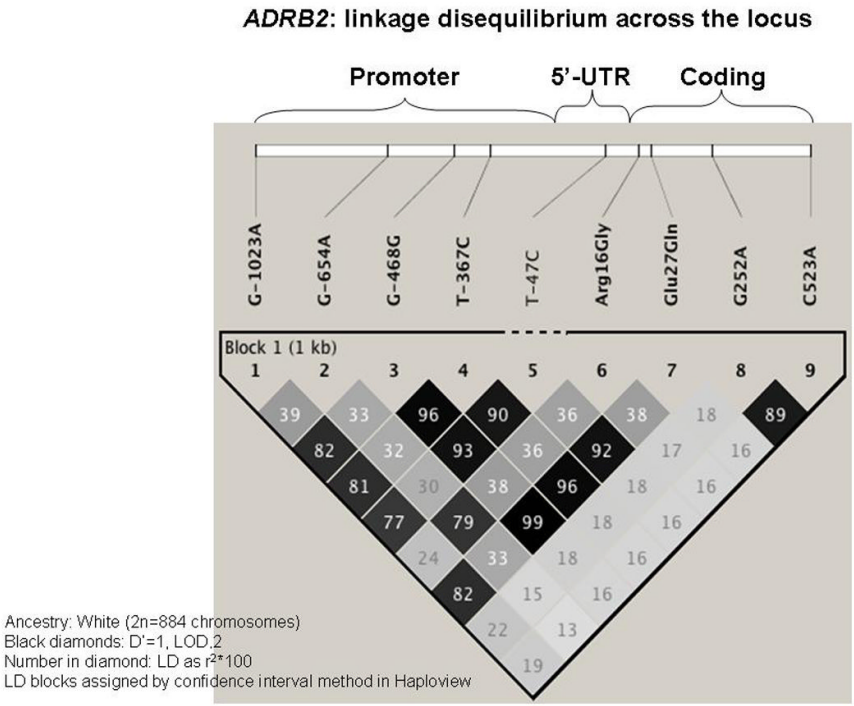

Figure 2. Linkage equilibrium (LD) across the *ADRB2* locus using Haploview

Darker block color denotes stronger pair-wise correlation among SNPs, within one major LD block.

Figure 3A

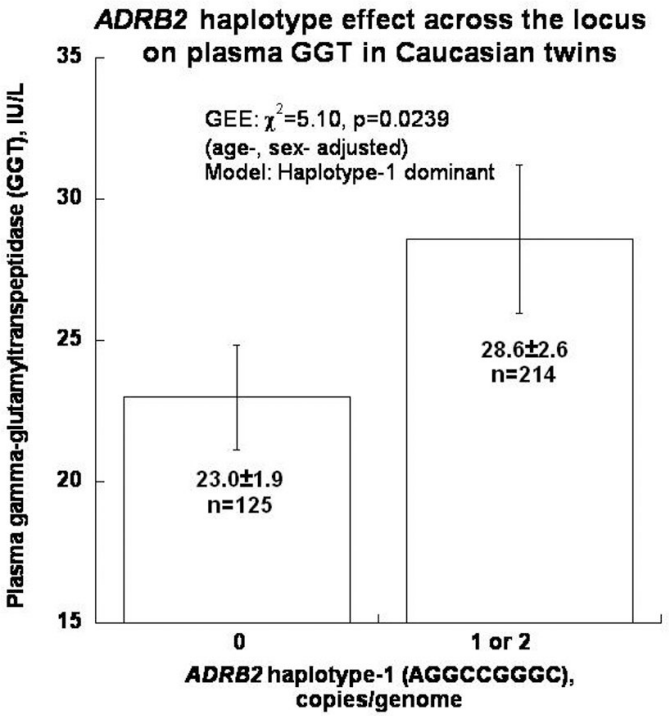

Figure 3B

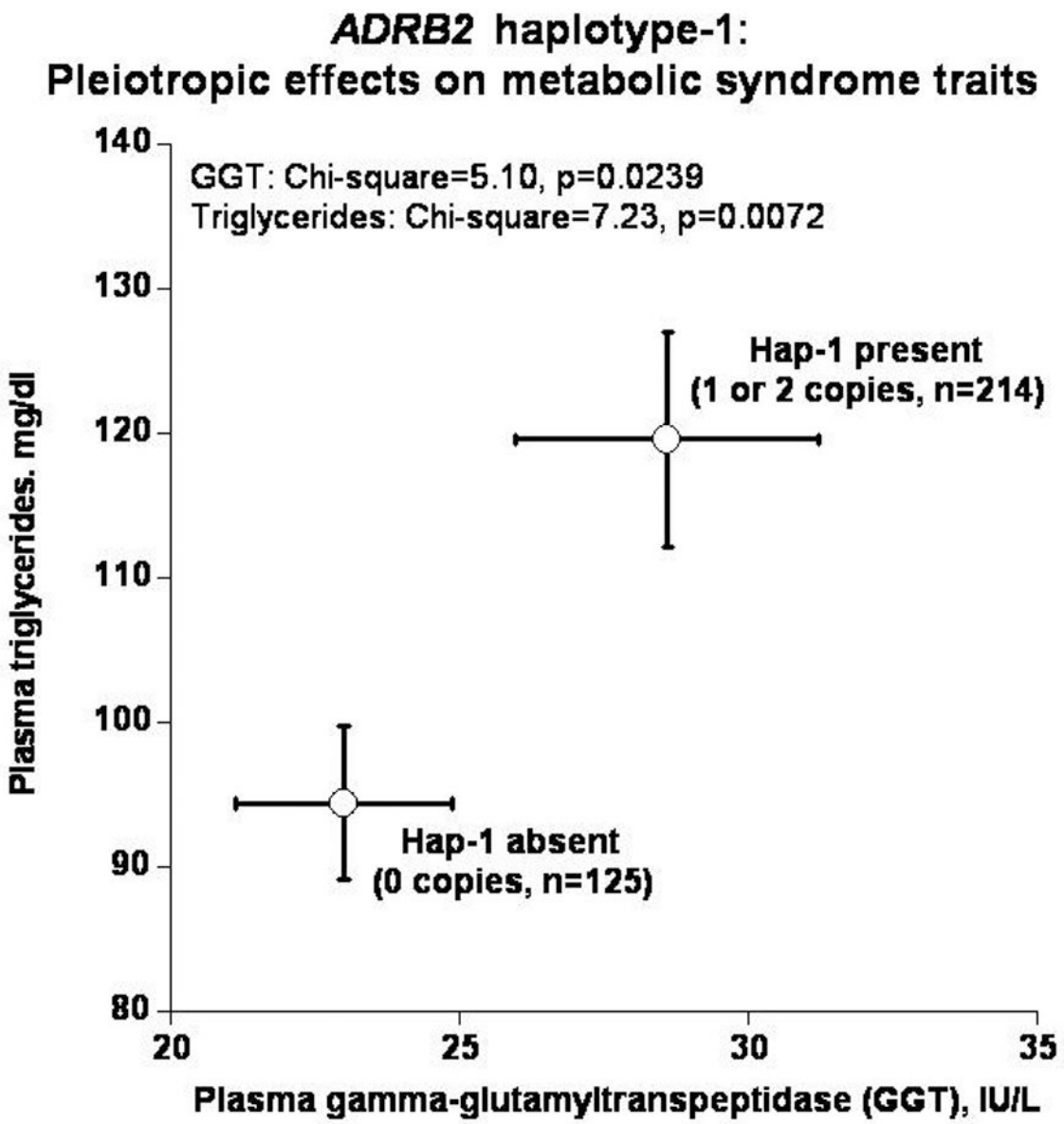

Figure 3. Figure 3A. *ADRB2* haplotype-1 (AGGCCGGGC) effect on GGT in age-/sex-adjusted models

Results demonstrate a significant association ($p < 0.024$), suggesting that *ADRB2* plays a role in regulation of GGT secretion.

Figure 3B. *ADRB2* haplotype-1 (AGGCCGGGC) increases both GGT and triglycerides in plasma. Results illustrate the pleiotropic effect of *ADRB2* genetic variation on fatty liver risk factors.

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Table 1

Gamma-glutamyl transpeptidase (GGT) stratification into upper and lower quantiles: Association with demographic, biochemical and physiological traits in twins.

Trait	GGT lower quantile, ≤16.6 IU/L (n=182)		GGT upper quantile, >16.6 IU/L (n=180)		
	Mean	**SEM**	**Mean**	**SEM**	**p-value**
Demographic					
Age (years)	37.7	1.2	43.7	1.2	**0.001**
Sex (M/F)	30/152		50/130		**0.01**
Ethnicity	All Caucasian		All Caucasian		--
State (CA/non CA)	178/4		176/4		0.99
Obesity (Obese: BMI≥30 kg/m^2) Non-obese/Obese	164/14		147/33		**0.003**
Physical					
BMI (kg/m^2)	24.3	1.3	25.6	0.4	**0.016**
Physiological					
Systolic blood pressure (mmHg)	129	1.1	132	1.3	0.08
Diastolic blood pressure (mmHg)	70	0.7	72.3	0.8	**0.03**
Biochemical (plasma)					
C-reactive protein (ng/ml)	2214	235	2610	318	0.3
Uric acid (mg/dl)	3.9	0.1	4.5	0.1	**<0.0001**
Homocysteine (μmol/L)	6.2	0.2	7.0	0.3	**0.02**
Butyryl-cholinesterase (IU/L)	9.4	0.15	10.0	0.2	**0.03**
IL-6 (pg/ml)	1.6	0.1	2.2	0.2	**0.019**
vWF (%)	53.2	3.8	60.2	4.3	0.2
P-selectin (ng/ml)	101	4.5	125.9	6.4	**0.001**
Metabolic (plasma)					
Glucose (mg/dl)	79	0.6	84.8	1.9	**0.006**
Insulin (U/L)	11.5	0.6	15.7	1.2	**0.001**
HOMA-IR (score)	2.3	0.13	3.5	0.3	**0.0003**
Gamma-glutamyl transpeptidase (IU/L)	10.5	0.3	37.1	2.1	**<0.0001**

Trait	GGT lower quantile, ≤16.6 IU/L (n=182)		GGT upper quantile, >16.6 IU/L (n=180)		
	Mean	**SEM**	**Mean**	**SEM**	**p-value**
Total cholesterol (mg/dl)	174.6	3	180.1	2.8	0.19
Triglycerides (mg/dl)	97.3	3.8	124.7	7.3	**0.001**
HDL-cholesterol (mg/dl)	54.4	1.3	50.6	1.2	**0.037**
LDL-cholesterol (mg/dl)	100.7	2.5	105	2.5	0.222
Adrenergic (plasma)					
Epinephrine (pg/ml)	24.4	1.3	30.0	1.9	**0.013**
Norepinephrine (pg/ml)	312.3	12.2	348.6	14.1	0.052
Autonomic					
Baroreflex coupling at 0.05–0.15 Hz, msec/mmHg	17.0	1.5	13.0	0.8	**0.018**

Descriptive statistics for twin study population are provided above. Values are means ± one SEM (standard error of the mean) or n and (percentage) derived from generalized estimating equations (GEEs; PROC GENMOD) in SAS software version 9.1, to account for intra-pair correlations within twinships. GGT (gamma-glutamyl transpeptidase, dichotomized around the median concentration of 16.6 IU/L) is listed for all individuals. The mean or N (%) gives the mean for continuous variables, or percentage of the trait observed for dichotomous variables (listed in parentheses). A two-tailed p-value ≤ 0.05 is considered statistically significant (in **bold** type). Abbreviations: BMI, body mass index; CRP; C-reactive protein; BChE, butylcholinesterase; IL-6, interleukin-6; vWF, Von-Willebrand factor; HOMA-IR, homeostasis assessment model of insulin resistance (HOMA-IR (mmol/L × μU/ml) = fasting glucose (mmol/L) × fasting insulin (μU/ml)/22.5); HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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Table 2

Shared genetic and environmental determination for metabolic and adrenergic traits with gamma-glutamyl transpeptidase in twins.

	Shared genetic determination (pleiotropy): ρ_G			Shared environmental determination: ρ_E		
Trait	**ρ_G estimate**	**SEM**	**P value**	**ρ_E estimate**	**SEM**	**P value**
Metabolic pathway						
BMI	0.03	0.10	0.8	−0.08	0.09	0.8
Blood pressure*	0.06	0.14	0.7	−0.02	0.09	0.8
Systolic	0.27	0.13	**0.04**	−0.07	0.09	0.4
Diastolic						
Total cholesterol	0.23	0.18	0.2	−0.14	0.10	0.07
LDL-cholesterol	0.40	0.28	0.12	−0.19	0.10	0.5
HDL-cholesterol	−0.21	0.13	0.09	0.11	0.10	0.2
Triglycerides	0.53	0.19	**0.004**	0.21	0.08	0.6
Homocysteine	0.31	0.21	0.2	0.02	0.12	0.9
Uric acid	0.46	0.14	**0.002**	−0.023	0.10	0.8
Glucose	0.12	0.16	0.4	0.06	0.09	0.5
Insulin	0.50	0.16	**0.003**	−0.07	0.11	0.5
HOMA-IR	0.40	0.15	**0.011**	−0.02	0.11	0.9
IL-6	−0.01	0.28	1	0.06	0.09	0.5
P-selectin	0.15	0.12	0.2	0.14	0.10	0.2
Butyryl-cholinesterase	0.21	0.12	0.07	−0.24	0.10	**0.02**
Adrenergic pathway						
Epinephrine	0.02	0.16	0.9	0.04	0.10	0.7
Norepinephrine	−0.03	0.12	0.8	0.01	0.10	0.9
Baroreflex coupling at 0.05–0.15 Hz	−0.05	0.20	0.8	−0.1	0.10	0.4

*Also involved in the adrenergic pathway.

Significant ($p<0.05$) coefficients are designated in **bold** type. ρ_G indicates genetic co-variance that detects the shared genetic determination of two traits. ρ_E indicates environmental co-variance that suggests the shared environmental determination of two traits. Abbreviations and units: see Table 1.

Table 3

ADRB2 allele frequency and Hardy-Weinberg Equilibrium (HWE) for single nucleotide polymorphisms across the *ADRB2* locus.

Domain	RefSNP	N	Major allele freq (p)	Minor allele freq (q)	HWE χ^2	HWE p
Promoter G-1023A	rs2053044	161	0.55	0.45	2.90	0.09
Promoter G-654A	none	162	0.65	0.35	0.20	0.65
Promoter C-468G	rs11168070	165	0.55	0.45	1.51	0.22
Promoter T-367C	rs11959427	166	0.55	0.45	2.48	0.12
Promoter T-47C	rs1042711	162	0.56	0.44	5.63	**0.02**
Gly16Arg, G265A	rs1042713	159	0.66	0.34	1.13	0.29
Glu27Gln, G298C	rs1042714	164	0.55	0.45	1.72	0.19
Leu84Leu, G252A	rs1042717	164	0.80	0.20	1.88	0.17
Arg175Arg, C523A	rs1042718	159	0.82	0.18	0.001	0.97

Footnote: Domains are numbered with respect to the translational start (AUG) codon. All *ADRB2* SNPs except promoter T-47C fulfill the criteria of Hardy-Weinberg equilibrium. One member of each twinship was included in the analysis.

Table 4

Genotype-phenotype association between *ADRB2* (beta-2 adrenergic receptor) polymorphisms and gamma-glutamyl transpeptidase in twins.

SNP domain	Diploid genotype code	Gamma-glutamyl transpeptidase, IU/L		Diploid genotype bases	N	P value
		Mean	SEM			
Promoter G-1023A	1	18.2	1.4	G/G	109	**0.025**
(rs2053044)	2	23.3	1.7	G/A	136	
	3	24.9	3.3	A/A	80	
Promoter C-468G	1	19.4	1.1	C/C	106	**0.015**
(rs11168070)	2, 3	24.7	1.9	C/G or G/G	221	
Promoter T-367C	1	19.6	1.1	T/T	112	**0.021**
(rs11959427)	2, 3	24.7	2.0	T/C or C/C	219	
Promoter T-47C	1	19.5	1.1	T/T	117	**0.012**
(rs1042711)	2, 3	24.8	1.9	T/C or C/C	213	
Glu27Gln, G298C	1	19.5	1.1	C/C	104	**0.016**
(rs1042714)	2, 3	24.8	1.9	C/G or G/G	224	

Footnote:

This table shows that five SNPs in the beta-2-adrenergic receptor gene (*ADRB2*) predict with plasma GGT, a marker of fatty liver. SNP statistics for twin study population are derived from generalized estimating equations (GEEs; PROC GENMOD) in SAS, version 9.1 (SAS Institute, Cary, NC) to account for correlations within twinships. Results are displayed for trait (plasma GGT) mean (IU/L, ± one SEM). All analyses are adjusted for age and sex. GGT was treated as a continuous outcome variable. A two-tailed p-value of ≤0.05 was considered statistically significant.