



The association of alcohol intake with gamma-glutamyl transferase (GGT) levels: Evidence for correlated genetic effects

Jenny H.D.A. van Beek^{a,b,*}, Marleen H.M. de Moor^{a,b}, Lot M. Geels^{a,b}, Michel R.T. Sinke^a, Eco J.C. de Geus^{a,b,c}, Gitta H. Lubke^{a,d}, Cornelis Klufft^e, Jacoline Neuteboom^e, Jacqueline M. Vink^{a,c}, Gonneke Willemsen^{a,b}, Dorret I. Boomsma^{a,b,c}

^a Department of Biological Psychology, VU University Amsterdam, Van der Boeorchorststraat 1, 1081 BT Amsterdam, The Netherlands

^b EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands

^c Neuroscience Campus Amsterdam, Amsterdam, The Netherlands

^d University of Notre Dame, Department of Psychology, 118 Haggard Hall, Notre Dame, IN 46556, USA

^e Good Biomarker Sciences, Zernikedreef 8, 2333 CL Leiden, The Netherlands

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ABSTRACT

Background: Blood levels of gamma-glutamyl transferase (GGT) are used as a marker for (heavy) alcohol use. The role of GGT in the anti-oxidant defense mechanism that is part of normal metabolism supposes a causal effect of alcohol intake on GGT. However, there is variability in the response of GGT to alcohol use, which may result from genetic differences between individuals. This study aimed to determine whether the epidemiological association between alcohol intake and GGT at the population level is necessarily a causal one or may also reflect effects of genetic pleiotropy (genes influencing multiple traits).

Methods: Data on alcohol intake (grams alcohol/day) and GGT, originating from twins, their siblings and parents ($N=6465$) were analyzed with structural equation models. Bivariate genetic models tested whether genetic and environmental factors influencing alcohol intake and GGT correlated significantly. Significant genetic and environmental correlations are consistent with a causal model. If only the genetic correlation is significant, this is evidence for genetic pleiotropy.

Results: Phenotypic correlations between alcohol intake and GGT were significant in men ($r=.17$) and women ($r=.09$). The genetic factors underlying alcohol intake correlated significantly with those for GGT, whereas the environmental factors were weakly correlated (explaining 4–7% vs. 1–2% of the variance in GGT respectively).

Conclusions: In this healthy population sample, the epidemiological association of alcohol intake with GGT is at least partly explained by genetic pleiotropy. Future longitudinal twin studies should determine whether a causal mechanism underlying this association might be confined to heavy drinking populations.

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1. Introduction

Blood levels of the liver enzyme gamma-glutamyl transferase (GGT) are used as a biomarker for heavy drinking (Peterson, 2004). GGT is implicated in alcohol use by keeping intracellular glutathione, the body's most abundant anti-oxidant, at adequate levels to protect cells from oxidative stress resulting during metabolism (e.g. that of alcohol) (Whitfield, 2001). Experimental studies support a causal relation between heavy alcohol use and increased GGT

levels, but also in experimental settings response of GGT to alcohol varies depending on individual characteristics, such as sex, age, and previous drinking habits (Whitfield, 2001). This inter-individual variability in GGT levels in response to alcohol may reflect the effect of genetic differences between individuals. The association of alcohol use and GGT levels at the population level (Conigrave et al., 2003) may then not necessarily reflect a causal effect of alcohol use on GGT, but additionally effects of genes on alcohol use that are shared with those on GGT (genetic pleiotropy; genes influencing multiple traits).

One way to test the nature of the population association between alcohol use and GGT and compare the hypothesis of full causality versus full genetic pleiotropy is by conducting a bivariate genetic analysis using data from twins and their family members. Twin(-family) studies can dissect phenotypic trait variation as well

* Corresponding author at: VU University Amsterdam, Department of Biological Psychology, Van der Boeorchorststraat 1, 1081 BT Amsterdam, The Netherlands. Tel.: +31 20 598 8787; fax: +31 20 598 8832.
E-mail address: J.H.D.A.van.Beek@vu.nl (J.H.D.A. van Beek).

as covariation between traits into effects that can be ascribed to genetic and environmental effects (Van Dongen et al., 2012). If alcohol use is causally influencing GGT levels, then genetic and environmental factors that influence alcohol use also influence GGT, with the size of the effects depending on the strength of the causal relation. If a genetic correlation between alcohol intake and GGT is present, but not an environmental correlation, or vice versa, this argues against a causal model (De Moor et al., 2008). If there is a genetic correlation in the absence of an environmental correlation, the phenotypic correlation results from genetic pleiotropy, where the same gene, or set of genes, influences multiple traits.

Two twin(-family) studies have investigated whether genetic and environmental factors influencing alcohol use are correlated with those for GGT (Whitfield and Martin, 1985; $N = 411$; Sung et al., 2011, $N = 1678$). In both studies, alcohol use significantly predicted GGT levels among males ($r = .19-.39$), but not consistently among females (Whitfield and Martin, 1985; $r = .05$, n.s.; Sung et al., 2011; $r = .09$, $p < .05$), underlining that GGT is a less sensitive marker of alcohol use in women. Regarding the results for men, genetic and environmental factors underlying GGT were correlated with those for problematic alcohol use in Koreans (Sung et al., 2011), in line with a causal effect of alcohol use on GGT. Among Australians however, genetic factors underlying alcohol intake and GGT, but not environmental factors, were correlated, thus pointing at effects of shared genes (Whitfield and Martin, 1985). The discrepancy in findings may be explained by differences in sample size, ethnicity, and/or phenotype. If the effect of problematic alcohol use on GGT is not a mere reflection of (extreme) alcohol intake, then a different mechanism may be at play with a different etiology.

The aim of this study is to examine the mechanism that underlies the epidemiological association of alcohol intake with GGT in a predominantly healthy Dutch population sample. By modeling data from 6465 twins and their family members, it is tested whether the association of alcohol use with GGT necessarily results from a causal mechanism or is additionally influenced by shared genes. Sex differences in the mechanism underlying the association are examined.

2. Methods

2.1. Participants

Data on alcohol intake and GGT levels originated from adult twins and their family members registered with the Netherlands Twin Register (NTR; Boomsma et al., 2002; Willemssen et al., 2013). Information on GGT levels determined in plasma was present for 8754 participants (aged ≥ 18) in the NTR biobank study conducted between 2004 and 2008 (Willemssen et al., 2010). The biobank study protocol was approved by the Medical Ethical Committee of the VU University Medical Center, Amsterdam (IRB number IRB-2991 under Federal-wide Assurance-3703; IRB/institute codes, NTR 03-180). Participants consented to the linkage of information obtained during the biobank project with the longitudinal surveys they completed. Data on alcohol intake came from the 2002, 2004 and 2009 surveys of the longitudinal survey study on health, personality and lifestyle. Data of 2289 individuals were excluded. Reasons include missing data on alcohol use ($N = 1687$), being an abstainer ($N = 121$), or having known liver disease ($N = 11$) (see Supplementary Material for a complete overview of excluded individuals). Analyses were performed on data from 6465 individuals for whom data on alcohol intake and levels of GGT were present (3193 twins, 1304 siblings, and 1968 parents from 2815 families). Individuals were categorized into five zygosity by sex groups (see Table 1), based on the zygosity and sex status of the twin pair. Zygosity of same-sex twins was determined by DNA comparison. Overall, 64.9% was female (year of birth: 1915–1988, full range; 1942–1977, 80% range).

2.2. Measures

GGT levels were determined in blood collected between 7.00 and 10.00 a.m. after an overnight fast at the participant's home. Participants were asked to refrain from smoking one hour before the home visit, and to abstain from physical exertion and medication on the day of the home visit, if possible. Blood was collected in heparin plasma tubes that were turned gently 8–10 times immediately after collection to prevent clotting. During transportation, heparin plasma tubes were stored in melting ice. When the samples arrived at the laboratory, plasma was collected and

six samples of 500 μL were snap-frozen and stored at -30°C . Levels of GGT were determined with Vitros assays (Vitros 250, Ortho-Clinical Diagnostics; Johnson and Johnson, Rochester, USA) in units per liter (U/L; Willemssen et al., 2010). Acceptance criteria were: inter-assay CV $< 5.0\%$, intra-assay CV $< 3.5\%$.

Alcohol intake was measured by the question 'How many glasses a week do you drink on average?'. In the 2002 and 2004 surveys, response categories were: 'less than 1 glasses', '1–5 glasses a week', '6–10 glasses a week', '11–20 glasses a week', '21–40 glasses a week', and 'more than 40 glasses a week'. In the 2009 survey, individuals were asked to report the number of glasses of beer, wine and spirits they drank for each day of the week. These numbers were summed and categorized as in the 2002 and 2004 surveys. In the analyses described below, alcohol intake was analyzed as the average amount of (grams of) alcohol consumed per day. This was obtained from the question given above by taking the median number of drinks per week for each category (0, 3, 8, 15, 30.5 or 46), multiplied by 14 grams of alcohol per glass, divided by seven (days in the week). The last category ('more than 40 glasses a week') was given the value of 46 based on the median number of drinks among individuals who reported to consume 41 or more drinks per week in the 2009 survey (in which number of drinks was reported as a continuous measure). If alcohol data were available from two or more surveys, the survey was selected for which the time interval to the biobank visit was smallest. The time interval between alcohol use assessment and blood collection ($M = 15.1$ months, $SD = 13.3$) was not considered to influence the results to a large extent since the stability of alcohol intake over time was high ($r = .80$ for over a two year period; $r = .67$ for over a six year period). Alcohol intake and GGT were highly skewed and log-transformed to approximate normality (Van Beek et al., 2013a,b). Age effects were regressed out prior to the analyses.

2.3. Statistical analyses

Bivariate genetic analyses of alcohol intake with GGT levels were conducted in Mx (v1.54; Neale et al., 1994, 2006). The analyses consisted of two steps. First, a saturated model (model 1) was fitted that estimated the familial cross-trait correlations as well as the familial within-trait correlations for alcohol intake and GGT. Means and variances were modeled as in Van Beek et al. (2013a,b). For alcohol intake, one variance was estimated (equal over sex) and two means (for males, females). For GGT, four means were estimated (for parents and offspring, separately over sex) and three variances (male offspring, female offspring, parents; see also Supplementary Material). The significance of the cross-trait correlations between alcohol intake and GGT was evaluated in an overall model (model 2) and separately by sex (models 2a and 2b). Sex differences in the magnitude of the cross-trait correlations were examined (model 2c). Model comparison was based on a likelihood ratio test (Bentler and Bonett, 1980) with a significance level of .01. This conservative significance level was chosen to take multiple testing into account.

In a second step, by structural equation modeling it was estimated what part of the correlation of alcohol intake with GGT could be ascribed to correlations between the genetic factors (genetic correlations) and what part to the correlation between the environmental factors (environmental correlation) underlying alcohol intake and GGT. The correlations between genetic and environmental factors influencing alcohol intake and GGT are calculated from the genetic and environmental variances and covariances for these traits. This was done in bivariate genetic factor models (Neale et al., 1994).

The heritability and genetic correlation of alcohol intake (ALC) and GGT can be estimated because family members share their genetic and environmental background to different degrees. MZ twin pairs share (nearly) all of their genetic material, whereas DZ twin and sibling pairs share half of their segregating genes on average. Parents and their offspring share exactly 50% of their segregating genes. Non-additive genetic influences that reflect effects of interacting risk alleles due to dominance and/or epistasis (Keller et al., 2010) can be estimated because these are correlated 1 in MZ pairs, whereas DZ twins and sibling pairs share on average a quarter (.25) of the non-additive genetic factors. Parent-offspring pairs share none of the non-additive genetic factors. Environmental factors that are not shared between family members are estimated as the remainder of the (co)variance that is not explained by genetic effects. A bivariate model was specified that included additive genetic (A), non-additive genetic (D) and environmental factors (E) (model 3; see Figure S1 in the Supplementary Material), informed by the fact that common environmental factors (C) shared by family members did not influence alcohol intake levels (Van Beek et al., 2013b). The Supplementary Material offers further details on the bivariate variance-covariance decomposition of alcohol intake and GGT.

The additive genetic correlation $r_{a,ALC,GGT}$, non-additive genetic correlation $r_{d,ALC,GGT}$, and individual-specific environmental correlations $r_{e,ALC,GGT}$ that were tested for significance in overall models and separately over sex (models 4–6), can be expressed as follows:

$$r_{a,ALC,GGT} = \frac{\text{cov}(A_{ALC}, A_{GGT})}{\sqrt{\text{var}(A_{ALC}) \times \text{var}(A_{GGT})}}$$

Table 1
Number of participants per zygosity by sex group.

Zygosity by sex group ^a	Number of participants				
	Twins ^b	Brothers	Sisters	Fathers	Mothers
MZM	487 (67.4%)	42	56	94	112
DZM	252 (56.3%)	29	37	65	83
MZF	1230 (75.0%)	81	124	189	225
DZF	602 (70.8%)	58	76	118	146
DOS	622 (56.3%)	61	89	148	176
Families without twins ^c	0	102	549	267	345
Total	3193	373	931	881	1087

^a MZM, monozygotic males; DZM, dizygotic males; MZF, monozygotic females; DZF, dizygotic females; DOS, dizygotic opposite-sex.

^b Percentage reflects what proportion of twins is part of a complete twin pair.

^c Families in which twins did not participate.

$$r_{d,ALC,GGT} = \frac{cov(D_{ALC}, GGT)}{\sqrt{var(D_{ALC}) \times var(D_{GGT})}}$$

$$r_{e,ALC,GGT} = \frac{cov(E_{ALC}, GGT)}{\sqrt{var(E_{ALC}) \times var(E_{GGT})}}$$

3. Results

3.1. Descriptives

Table 2 shows levels of alcohol intake, GGT, BMI and age separately for males and females. Alcohol intake and GGT were higher in men than women. Inspection of mean GGT levels over drinking categories did not show evidence of a J-shaped function. GGT levels linearly increased with drinking categories. The effect of BMI on alcohol intake was negligible (see Table 2) and therefore not taken into account in the genetic analyses.

3.2. Genetic analysis of alcohol intake with GGT

Alcohol intake predicted GGT levels. The phenotypic correlation of alcohol intake with GGT was larger in men ($r = .17$) than women ($r = .09$). The phenotypic correlations were not dependent on the time difference between alcohol assessment and blood collection (data available upon request). Familial cross-trait correlations were significant for MZ pairs ($r_{MZM} = .14$; $r_{MZF} = .07$), but not for first-degree relatives (Table 3). Model fit statistics of the tested models and conclusions that follow from it are reported in Table 4. The bivariate ADE model that specified additive (A), non-additive (D) and individual-specific environmental factors (E) fitted well, when compared to the saturated model. For both males and females, genetic factors underlying GGT and alcohol were correlated. For men 7.2% of the variance in GGT could be explained by (additive and non-additive) genetic effects that were shared with those for alcohol intake. For women, this was 4.4%.

There was no significant correlation between the environmental factors ($p > .05$ for sex-specific analyses, $p = .04$ for overall analyses). For men, 2.3% of the variance in GGT is explained by environmental factors that are shared with those for alcohol intake. For women, this is 1.4%. This falsifies the hypothesis of full causality, and suggests that additional effects of genetic pleiotropy underlie the epidemiological association between alcohol use and GGT. In exploratory analyses, we investigated whether there are qualitative age differences in the mechanism underlying this association. Rerunning the analyses for the individuals who were born within a 25 year span (1948 and 1973; 50% of the sample) showed that this was not the case: results were similar.

The genetic correlation of alcohol intake and GGT was mainly found for non-additive genetic factors. For females, the correlation

between non-additive genetic factors underlying alcohol intake and GGT (r_d) was .47 (95% confidence interval, CI, .09, .59) with the correlation between additive genetic factors affecting alcohol intake and GGT (r_a) estimated at $-.09$ (95% CI $-.29, .13$). For males, all non-additive genetic effects on GGT were modeled to be shared with alcohol intake ($r_d = 1$), with r_a estimated at $-.23$ (95% CI $-.48, .03$).

4. Discussion

This study examined whether the epidemiological association of alcohol intake with GGT in a predominantly healthy Dutch population sample necessarily results from a causal effect of alcohol intake on GGT or reflects additional effects of genetic pleiotropy. The aim of the study was not to show an increase in GGT directly after drinking, but to test whether at the population level individuals who drink more have higher GGT levels and to explain that association. For men, 7.2% of the variance in GGT could be explained by genetic effects that were shared with those for alcohol intake. For women, this was 4.4%. In comparison, environmental factors underlying alcohol intake explained only 2% of the variance in GGT in males and 1% in females. Thus, in this population sample, results did not support the hypothesis of full causality. Additional effects of genetic pleiotropy likely contribute to the population association between alcohol use and GGT, in line with Whitfield and Martin (1985). Results were similar over different ages. This study is the first to show that correlated genetic effects not only contributed to the explanation of the population association of alcohol intake and GGT in males, but also in females. The within-person correlation among females ($r = .09$) was comparable to that in Sung et al. (2011), but by including data of family members other than twins (mothers, sisters), the power to detect this association was increased (Posthuma and Boomsma, 2000).

The difference in results between our study and the study among Koreans (Sung et al., 2011) may suggest that the mechanism underlying the population association of alcohol use with GGT differs over ethnicity. Alternatively, the mechanism underlying the population association is dependent on the level of (heavy) drinking. Although NTR participants are representative to the Dutch population for regular alcohol use (Distel et al., 2007), male NTR participants drank less and not as heavy as the Korean participants (127 grams/week, 16% AUDIT score ≥ 8 versus 181 grams/week, 39% AUDIT score ≥ 8 respectively; Sung et al., 2011). It is possible that some sensitization occurs in the liver during heavy drinking that is absent or different from that in normal drinkers. This sensitization may be triggered by factors that differ between normal drinkers and heavy drinkers, such as certain diet specifics (e.g. low carbohydrate, high fat content), the development of fatty liver, iron overload or certain immune reactions (Whitfield, 2001). Future longitudinal twin studies should test whether a causal effect underlying the

Table 2
Distribution of GGT, BMI and age over drinking categories (in glasses per week), for males and females.

	Drinking level category	N (%)	Mean GGT ^a (SD)	Mean BMI (SD)	Mean age (SD)
Males	<1 glass p/wk	340 (15.0%)	35.7 (29.0)	26.2 (4.6)	47.5 (15.4)
	1–5 glasses p/wk	701 (30.9%)	34.0 (20.7)	25.5 (3.6)	46.3 (15.7)
	6–10 glasses p/wk	506 (22.3%)	38.4 (44.8)	25.4 (3.4)	46.8 (15.6)
	11–20 glasses p/wk	511 (22.6%)	45.7 (41.0)	25.6 (3.2)	48.1 (15.3)
	21–40 glasses p/wk	180 (7.9%)	55.0 (58.7)	25.8 (3.3)	48.2 (15.1)
	>40 glasses p/wk	28 (1.2%)	66.4 (78.8)	24.6 (3.3)	45.9 (15.1)
Females	<1 glass p/wk	1580 (37.6%)	25.8 (31.5)	25.4 (4.9)	42.8 (13.5)
	1–5 glasses p/wk	1463 (34.8%)	24.7 (17.0)	24.7 (4.3)	43.1 (13.5)
	6–10 glasses p/wk	630 (15.0%)	25.4 (16.3)	24.5 (3.8)	45.9 (13.5)
	11–20 glasses p/wk	445 (10.6%)	32.6 (38.5)	24.6 (4.0)	48.9 (13.2)
	21–40 glasses p/wk	77 (1.8%)	64.2 (109.0)	25.3 (3.4)	51.3 (10.4)
	>40 glasses p/wk	4 (.1%)	76.3 (119.8)	25.9 (3.3)	39.6 (12.4)

^a Untransformed values for alcohol in grams/day and GGT.

Table 3
Cross-trait correlations for alcohol intake with GGT, for male-male, female-female and opposite-sex pairs.

	Cross correlations alcohol intake with GGT (95% confidence intervals)		
	Male–male	Female–female	Opposite-sex
Within-person	0.169 (0.127, 0.210)	0.093 (0.062, 0.123)	
MZ	0.140 (0.064, 0.214)	0.072 (0.025, 0.118)	
DZ/sib	0.007 (–0.071, 0.084)	–0.035 (–0.084, 0.014)	–0.041 (–0.096, 0.015)
Parent–offspring	–0.015 (–0.084, 0.054)	0.005 (–0.040, 0.050)	–0.040 (–0.092, 0.011) ^a –0.041 (–0.103, 0.020) ^b

^a Cross-trait correlation for father–daughter.

^b Cross-trait correlation for mother–son.

epidemiological association of alcohol use and GGT is confined to heavy drinking populations.

The rather low within-person correlations of alcohol intake with GGT (.09–.17) might in part be due to the time difference between the blood draw and the assessment of alcohol intake (15 months on average). Other studies detected higher correlations for alcohol intake with GGT for males ($r = .20$ – $.40$), although not for females ($r = .00$ – $.30$; Conigrave et al., 2003; Whitfield and Martin, 1985; Sung et al., 2011). The influence of this time interval on the results is considered to be minor however. First, the surveys inquired about alcohol consumption in the past year and were therefore assumed to capture regular alcohol use, the type of drinking pattern that is most clearly associated with GGT (Conigrave et al., 2003). Stability of alcohol intake over time was large ($r = .80$ for over a two year period, corresponding to our situation of a ~15 month time difference) and remains high for over a long period ($r = .67$ for over six years). Second, the correlation between alcohol intake and GGT was not dependent on the time difference (data available upon request). The lower correlations are more likely explained by variation in whether or not participants drank alcohol during the days preceding the home visit. Although participants abstained from food and drinks from 10 p.m. the night before the blood collection, for individuals who had been drinking heavily earlier that evening, GGT levels would have been temporarily increased, leading to noise in the overall prediction (which is not equal to bias, but indicates increased variance). Indeed, when restricting the analyses to individuals who reported to drink at least several times per week (who are more likely to have been drinking in the days preceding blood collection) to reduce variance (in whether or not individuals had been drinking the night before the home visit), results were similar (data available upon request).

The current study compared the hypothesis of full causality versus full genetic pleiotropy. The power to detect correlated genetic effects (additive and non-additive) was very good (.95 for $p = .01$), but was lower for correlated environmental effects (.51 for $\alpha = .01$; .74 for $p = .05$). Since the environmental correlation was close to significant ($p = .04$), a causal effect of alcohol intake on GGT

may play a role. The reality may be complex, with a combination of genetic pleiotropy and causality explaining the association at the population level. Additionally, the mechanism of association may vary over drinking level with genetic pleiotropy explaining the association in a low drinking population, while causality explains the association in a heavy drinking population. This may suggest gene by alcohol interaction, which presents an interesting venue for future research.

The correlated genetic effects detected in this study may reflect genetic effects on cardiometabolic traits that are associated with alcohol use and GGT levels. Light to moderate alcohol consumption has been associated with a reduced risk of cardiovascular disease and type 2 diabetes, whereas heavy alcohol use is associated with an increased risk for these diseases (Dawson, 2011). The *CDH13* gene associated with alcohol dependence (Morozova et al., 2012), has been associated with traits such as high blood pressure (Johnson et al., 2011) and metabolic syndrome (Fava et al., 2011) and the *DSCAML1* gene linked to alcohol phenotypes in humans and other species (Morozova et al., 2012), to levels of triglycerides (Pollin et al., 2008). In reverse, genetic risk factors for GGT have been linked to biomarkers of cardiovascular disease such as levels of cholesterol (LDL, HDL), triglycerides, glucose, and insulin resistance (Chambers et al., 2011; Kim et al., 2011; Whitfield et al., 2002). GGT has been coined a marker for fatty liver (Targher, 2009) as well as oxidative stress (Lim et al., 2004) based on its pro-oxidant properties by generating free radicals in the presence of iron (Lee et al., 2004), in addition to its role in the antioxidant defense by maintaining adequate levels of intracellular glutathione (Whitfield, 2001). Alcohol use and conditions of the cardiometabolic syndrome have similar effects on liver functioning through induction of oxidative stress via mitochondrial defects (Mantena et al., 2008). The correlated genetic effects on alcohol intake and GGT may then reflect that the genetic etiology of alcohol-induced and obesity-induced fatty liver disease is partly shared causing an association between alcohol use and GGT at the population level.

A first look at the correlations between alcohol intake and GGT with cardiovascular biomarkers collected in the NTR biobank study

Table 4
Model fit statistics for genetic models of alcohol intake with GGT.

	Model	NP	−2LL	df	vs	$\Delta\chi^2$	Δdf	p	Conclusion ^a
1	Saturated model ^b	40	67,929.817	12,890					
2	All correlations with GGT set to 0	29	68,066.526	12,901	1	136.71	11	<.001	Alcohol intake predicts GGT
2a	Within male person cross-trait corr set to 0	39	67,990.590	12,891	1	60.77	1	<.001	Sign corr alcohol intake and GGT males
2b	Within female person cross-trait corr set to 0	39	67,964.927	12,891	1	35.11	1	<.001	Sign corr alcohol intake and GGT among females
2c	Within male = female person cross-trait corr	39	67,938.380	12,891	1	8.56	1	.003	Correlation alcohol intake and GGT is larger among males than females
3	ADE model for cross-trait variation	30	67,949.553	12,900	1	19.74	10	.032	ADE model fits well
4	Drop genetic correlation D to 0 i.e. AE model for cross-trait variation	28	67,969.541	12,902	3	19.99	2	<.001	Significant non-additive genetic correlation alcohol intake and GGT ^c
4a	Drop genetic correlation D to 0 males only	29	67,962.920	12,901	3	13.37	1	<.001	Sign non-additive genetic correlation alcohol intake and GGT among males ^c
4b	Drop genetic correlation D to 0 females only	29	67,955.657	12,901	3	6.10	1	.013	Non-additive genetic correlation alcohol intake and GGT not sign among females ^c
5	Drop genetic correlation, A + D, to 0 i.e. E model for cross-trait variation	25	67,974.150	12,905	3	24.60	5	<.001	Significant (additive + non-additive) genetic correlation ^d
5a	Drop genetic correlation (A + D) to 0 males only	28	67,964.175	12,902	3	14.62	2	.001	Significant (additive + non-additive) genetic correlation among males ^d
5b	drop genetic correlation (A + D) to 0 females only	28	67,959.173	12,902	3	9.62	2	.008	Significant (additive + non-additive) genetic correlation among females ^d
6	Drop environmental correlation (E) to 0 i.e. AD model for cross-trait variation	28	67,956.008	12,902	3	6.46	2	.040	Environmental correlation not significant ^e
6a	Drop environmental correlation (E) to 0 males only	29	67,953.244	12,901	3	3.69	1	.055	Environmental corr not sign males ^e
6b	Drop environmental correlation (E) to 0 females only	29	67,952.306	12,901	3	2.75	1	.097	Environmental corr not sign females ^e

NP = number of parameters in statistical model, −2LL = −2 log likelihood, fit function, for model with *df* degrees of freedom, vs = baseline model used as comparison model, $\Delta\chi^2$ = model fit statistic: difference in −2LL of two nested models approximately distributed as χ^2 with *df* = Δdf , the difference in NP between the models; *p*-value was regarded significant when <.01, sign = significant (at $\alpha = .01$); corr = correlation.

^a If both the genetic correlations (additive + non-additive) and the environmental correlation between alcohol intake and GGT are significant, this is consistent with a causal mechanism between alcohol intake and GGT. If either the (additive or non-additive) genetic correlations or environmental correlation is not significant, a causal effect of alcohol use on GGT levels is less likely.

^b Constraints included two means (males, females) and one variance (equal for males and females) for alcohol intake and four means (male twins/sibs, female twins/sibs, fathers, mothers) and three variances (male twins/sibs, female twins/sibs, parents) for GGT.

^c A significant non-additive genetic correlation means that the non-additive genetic factors underlying alcohol intake and GGT are correlated and that the phenotypic correlation between alcohol intake and GGT can be partly explained by the correlation between the latent non-additive genetic factors.

^d A significant additive genetic correlation means that the additive genetic factors underlying alcohol intake and GGT are correlated and that the phenotypic correlation between alcohol intake and GGT can be partly explained by the correlation between the latent additive genetic factors.

^e A significant environmental correlation means that the environmental factors underlying alcohol intake and GGT are correlated and that the phenotypic correlation between alcohol intake and GGT can be partly explained by the correlation between the environmental factors.

showed that alcohol intake correlated positively with HDL ($r = .2$) and negatively with fibrinogen ($r = -.1$) ($r < .1$ for other parameters). GGT had correlations between .2 and .3 with triglycerides, insulin and CRP, and between .1 and .2 with LDL, total cholesterol, glucose, and fibrinogen ($r < .1$ with HDL) (taking effects of sex and age into account). The differential associations of alcohol intake and GGT with these parameters given that alcohol intake correlates positively with GGT, may be explained by the fact that GGT fractions that correlate highest with cardiometabolic traits (b- and f-GGT) do not entirely overlap with those that correlate highest with alcohol consumption (m-, s- and f-GGT), and because the ratio of the b- to s-GGT (b/s ratio) correlates positively with triglycerides, LDL and fibrinogen, whereas it shows an inverse relation to alcohol use and HDL (Franzini et al., 2013). In future research, the relation

between alcohol use, GGT and cardiometabolic traits could be further explored taking specific GGT fractions into account. In addition, future research should focus on specific drinking patterns. Heavy alcohol use is associated with increased risk for cardiometabolic disease (Dawson, 2011; Costanzo et al., 2011), whereas cardioprotective effects have been observed for light to moderate drinking levels of beer and/or wine (but not spirits; Costanzo et al., 2011) and if the drinking pattern did not include heavy drinking episodes (Rehm et al., 2010).

Despite the correlated genetic effects for alcohol use and GGT, genome-wide association studies for GGT have not yet detected variants that have been implicated in alcohol use (e.g., see Chambers et al., 2011), with the exception of (variants in close linkage with) the *ALDH2*2* allele in East-Asian populations (Kamatani

et al., 2010; Kim et al., 2011; Baik et al., 2011). Based on the current findings it will be interesting to take into account the substantive non-additive genetic variation underlying the association of alcohol intake and GGT by performing gene-finding studies that assume a (2 *df*) genotypic model instead of an (1 *df*) additive model. In addition, prediction models may be fitted that involve complex interactions among the genetic markers, such as random forests (Molinaro et al., 2011).

To conclude, the current study detected that the association between alcohol intake and GGT at the population level is at least partly explained by correlated genes, and that an explanation in terms of full causality of alcohol intake on GGT is unlikely. The observation that the relation between alcohol intake and GGT is largely due to non-additive genetic effects warrants further study and calls for gene-finding efforts that take the possibility of complex gene interactions into account.

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Nothing declared.

Contributors

GW, JV, JvB and LG collected the alcohol data. GW and EdG performed the NTR biobank study (blood collection). CK and JN were involved in the determination of GGT levels. JvB, MdM, GW and DB designed the study. MS and JvB performed the pilot study. JvB analyzed data for the final study and wrote the manuscript. MdM, GW, GL and DB participated in revising the manuscript. All authors have approved the final manuscript.

Conflict of interest

No conflict declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.drugalcdep.2013.09.016>.

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