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

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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.

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

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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; Willemsen 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 (Willemsen 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; Willemsen 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 glass', '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:

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