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The Genetics of Alcohol Dependence: Twin and SNP-Based Heritability, and Genome-Wide Association Study Based on AUDIT Scores

Hamdi Mbarek,[1]* Yuri Milaneschi,[2] Iryna O. Fedko,[1] Jouke-Jan Hottenga,[1] Marleen H.M. de Moor,[1] Rick Jansen,[2] Joel Gelernter,[3,4] Richard Sherva,[5] Gonneke Willemsen,[1] Dorret I. Boomsma,[1] Brenda W. Penninx,[2] and Jacqueline M. Vink[1]

[1]Department of Biological Psychology, VU University Amsterdam, Amsterdam, The Netherlands

[2]Department of Psychiatry and EMGO Institute for Health and Care Research, VU University Medical Center/GGZ inGeest, Amsterdam, The Netherlands

[3]Division of Human Genetics, Department of Psychiatry, Yale University School of Medicine, West Haven, Connecticut

[4]VA CT Healthcare Center, West Haven, Connecticut

[5]Department of Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, Massachusetts

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Alcohol dependence (AD) is among the most common and costly public health problems contributing to morbidity and mortality throughout the world. In this study, we investigate the genetic basis of AD in a Dutch population using data from the Netherlands Twin Register (NTR) and the Netherlands Study of Depression and Anxiety (NESDA). The presence of AD was ascertained via the Alcohol Use Disorders Identification Test (AUDIT) applying cut-offs with good specificity and sensitivity in identifying those at risk for AD. Twin-based heritability of AD-AUDIT was estimated using structural equation modeling of data in 7,694 MZ and DZ twin pairs. Variance in AD-AUDIT explained by all SNPs was estimated with genome-wide complex trait analysis (GCTA). A genome-wide association study (GWAS) was performed in 7,842 subjects. GWAS SNP effect concordance analysis was performed between our GWAS and a recent AD GWAS using DSM-IV diagnosis. The twin-based heritability of AD-AUDIT was estimated at 60% (55–69%). GCTA showed that common SNPs jointly capture 33% (SE = 0.12, $P = 0.002$) of this heritability. In the GWAS, the top hits were positioned within four regions (4q31.1, 2p16.1, 6q25.1, 7p14.1) with the strongest association detected for rs55768019 ($P = 7.58 \times 10^{-7}$). This first GWAS of AD using the AUDIT measure found results consistent with previous genetic studies using DSM diagnosis: concordance in heritability

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*Correspondence to:
Hamdi Mbarek, Department of Biological Psychology, VU University Amsterdam, Van der Boechorststraat 1, 1081 BT, Amsterdam, The Netherlands.
E-mail: h.mbarek@vu.nl
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estimates and direction of SNPs effect and overlap with top hits from previous GWAS. Thus, the use of appropriate questionnaires may represent cost-effective strategies to phenotype samples in large-scale biobanks or other population-based datasets.

Key words: cohol dependence; heritability; GWAS

INTRODUCTION

Alcohol dependence (AD) is a common and debilitating disorder, that ranks among the leading causes of the global burden of disease [World Health Organization, 2010]. It may result in serious medical, legal, social and psychiatric problems and influences many facets of society. Clarifying the etiology of AD is therefore of great importance.

Epidemiological studies within Europe estimate higher 12-month prevalence rates for AD in men (ranging from 0.4% to 14.5%) than women (ranging from 0.1% to 4.2%). Lifetime prevalences rates are higher, with a maximum of 22.2% in men and 6.5% in women [Rehm et al., 2005]. Comparable rates were seen in the USA for lifetime AD in men (20.1%) and in women (8.2%) [Kessler et al., 1994].

AD is a complex trait in which both genetic and environmental factors affect susceptibility. Family and twin studies have consistently demonstrated a substantial genetic contribution to its risk [Heath et al., 1997; de Moor et al., 2011] with heritability estimates ranging from 40% to 60% [Prescott and Kendler, 1999; Enoch and Goldman, 2002]. The magnitude of the heritability estimates was found to be similar in men and women but the presence or absence of qualitative sex differences (i.e., different genes operating in men and women) is unclear. One twin study concluded that men and women have partially overlapping genetic risk factors for alcoholism [Prescott et al., 1999] while in another twin study genetic risk factors in men and women were the same [Heath et al., 1997].

In an effort to uncover specific genomic influences on AD, a variety of study designs have been employed. From the early genetic linkage analysis [Long et al., 1998; Williams et al., 1999; Saccone et al., 2000] and candidate gene association [Macgregor et al., 2009; van Beek et al., 2010] to the most recent genome-wide association studies (GWAS), several risk loci have been reported for AD in European, African Americans, Australian and Asian-ancestry populations, although the overlap of the top genetic signals across studies has been limited [Rietschel and Treutlein, 2013]. Still, with the advent of GWAS, the number of candidate loci affecting AD has increased. The best known and replicated risk alleles map to alcohol metabolizing enzyme genes, especially *ADH1B* [Li et al., 2011], *ADH1C* [Li et al., 2012a], and *ADH4* [Luo et al., 2006] on chromosome 4 and *ALDH2* on chromosome 12 [Li et al., 2012b]. Several alcohol phenotypes have been analyzed. The most commonly studied phenotype is the dichotomous classification into cases and controls for AD disorder based on DSM-IV criteria for SNPs in the following regions were identified: 2q35 [Treutlein et al., 2009], 11p15 [Edenberg et al., 2010], 6q12, [Zuo et al., 2011], 1p35 [Zuo et al., 2013], 4q22-23 and 2p16 [Gelernter et al., 2014]. In addition, studies that defined AD as a quantitative trait derived from the DSM-IV diagnosis reported SNPs in these regions: 3q21 and 6p21 [Heath et al., 2011], 15q14 [Wang et al., 2013], 13q32 and 11p15 [Wetherill et al., 2014]. However, most of these studies did not yield genome-wide significant results, except for the study by Gelernter et al. [2014] which identified new loci in 4q-23 and 2p16 using both the binary and ordinal AD classifications based on DSM-IV diagnosis. A detailed summary of all published GWAS for AD is presented in supplementary Table S1. A more recent study explored gene set and pathway analyses for the known loci [Biernacka et al., 2013].

Both in research and in clinical settings AD is commonly diagnosed via semi-structured psychiatric interviews. In large-scale epidemiological studies in which detailed diagnosis-based measures of alcohol use cannot be obtained, self-report measures of problem drinking may provide a less costly and time-consuming strategy to phenotype a high number of participants. As a time-efficient screening instrument, the Alcohol Use Disorder Identification Test (AUDIT) [Babor et al., 2001] has been developed to detect hazardous and harmful alcohol use. The AUDIT is one of the most accurate and time-efficient screening instruments and it has been found to provide an accurate measure of AD risk across gender, age, and cultures [Saunders et al., 1993; Allen et al., 1997]. The test contains 10 multiples choice questions. The performance of the AUDIT in detecting AD was recently evaluated in the Netherlands Study of Depression and Anxiety [Boschloo et al., 2010]. Using separate cut-off points for women ($\geq$6) and for men ($\geq$9), the AUDIT accurately detected DSM-IV AD in participants with depression/anxiety disorders and healthy controls.

The present paper aimed to determine the genetic underpinning of AD-AUDIT, assessed by applying the above-mentioned cut-offs to the AUDIT score, in two large cohorts of European ancestry, that is the Netherlands Twin Register (NTR) and the Netherlands Study of Depression and Anxiety (NESDA). First, heritability estimates of AD-AUDIT were calculated in the twin sample using structural equation modeling. Next, we sought to identify specific variants associated with AD-AUDIT by a genome-wide association study (GWAS) based on data from NTR and NESDA participants. Finally, we estimated the additive genetic variance explained by common SNPs for AD-AUDIT.

MATERIALS AND METHODS

Study Population

Participants were part of the Netherlands Twin Register (NTR) or the Netherlands Study of Depression and Anxiety (NESDA). The NTR longitudinally follows adolescent and adult twins and their relatives (parents, siblings, spouses, and adult offspring; [Boomsma et al., 2006; Willemsen et al., 2013]. At the time of data analysis, data on alcohol use were available for 26,943 individuals. Participants were only selected if, in at least one of the surveys in which they completed, they reported that they ever drank alcohol (3,380 participants were excluded because they reported they never drank alcohol and 1,401 participants because of invalid or missing data on alcohol consumption). In this paper we analyze data from 7,694 twin pairs and from 5,466 subjects who were genotyped. Mean age was 43.34 (SD = 15.67) years and 65.2% were women.

NESDA is a longitudinal study focusing on the course and consequences of depression and anxiety disorders. Subjects for NESDA were recruited from three sources, namely the general population, mental health organizations and general practices [Penninx et al., 2008]. Among the 2,981 participants enrolled at baseline, we selected 2,376 participants with available genotype and phenotype data for the present analyses. Mean age was 42.06 (SD = 13.06) years and the proportion of females was 67.5%.

Measurement of Alcohol Dependence

The presence of Alcohol Dependence was ascertained in both cohorts by using the Alcohol Use Disorders Identification Test (AUDIT) developed by the World Health Organization for use in primary care settings to identify signs of hazardous or harmful drinking in the past year [Saunders et al., 1993; Babor et al., 2001]. Previous studies in general population samples from different countries consistently identified a two factor structure with one factor measuring alcohol consumption and the other factor measuring alcohol-related problems [Bergman and Kallmen, 2002; Lima et al., 2005; Shevlin and Smith, 2007]. The AUDIT includes these dimensions (with questions 4–6 reflecting alcohol dependence, questions 7–10 reflecting alcohol-related harm and questions 1–3 reflecting hazardous consumption levels) but is usually analyzed as a sum score. Recently, Boschloo et al. [2010] studied the performance of the AUDIT in detecting AD as measured by DSM-IV diagnosis obtained via psychiatric interview in our NESDA sample and showed that the AUDIT accurately detected DSM-IV AD in both depressed/anxious patients and healthy controls. The best performance of the AUDIT was obtained when using the cut-off point of $\geq$9 for men (patients: Area Under the Curve [AUC] 0.89, sensitivity 0.84, specificity 0.83; controls: AUC 0.89, sensitivity 0.80, specificity 0.85) and of $\geq$6 for women (patients: AUC 0.88, sensitivity 0.85, specificity 0.80; controls: AUC 0.94, sensitivity 1.00, specificity 0.81).

Using the above cut-offs (males $\geq$9 and females $\geq$6) the AUDIT case-control status was determined in survey 8 data for NTR and in baseline assessment for NESDA. In NESDA, 165 of 1,876 potential AD-AUDIT controls did receive a DSM lifetime diagnosis of alcohol dependence and were excluded. The DSM diagnosis was available only for the NESDA cohort, in which 15.9% of participants are diagnosed reported lifetime AD (36.7% of the cases for the present analyses). In order to extend the number of controls, NTR controls were added (N = 582) based on surveys 2–7 data if they consistently scored low (=0) on the Cutting down, Annoyance by criticism, Guilty feeling, and Eye-opener (CAGE) test measuring drinking behaviors and consequences [Ewing, 1984] and scored low on alcohol consumption (frequency: less than one time per week, and quantity: less than one glass per week). Thus, the sample included 665 cases and 1,711 controls for NESDA and 709 cases 4,757 controls for NTR (Table I).

DNA Sampling and Genotyping

A detailed description of DNA sampling and genotyping is included in the supplementary information.

TABLE I. Characteristics of the Study Sample

	GWAS	
Description	**AUDIT cases[a]**	**AUDIT controls[b]**
Number of subjects	1,374	6,468
Mean age in years (sd)	41.1 (14.6)	43.3 (15)
Women (%)	62.4	66.6
Alcohol drinking frequency (more than once a week) (%)	79.6	50.4

[a]AUDIT cases: AUDIT score >= 9 for men and >= 6 for women.
[b]AUDIT controls: AUDIT score <9 for men and <6 for women and also if they consistently score low(=0) on CAGE.

Statistical Analysis

Heritability. The influence of genetic factors on the AD-AUDIT was investigated through structural equation modeling of data collected in MZ and DZ twin pairs [Boomsma, 2013]. For the dichotomous AD trait as defined above, a threshold model was applied in which the trait is assumed to have an underlying continuous liability with a standard normal distribution with zero mean and unit variance. A threshold divides this normal distribution into discrete categories. Different thresholds were estimated for men and women. A regression of the z-score of age was modeled as a fixed effect on the threshold. A detailed description of heritability analysis is included in the supplementary information.

GWAS. The GWAS for AD-AUDIT was conducted using logistic regression under an additive genetic model with adjustment for sex, age, age-squared, and principal components of genetic ancestry. Principal components (PC's) of genetic ancestry were inferred using EIGENSOFT [Patterson et al., 2006]. The first three PC's were retained and used as covariates to correct for the Dutch population substructure in the downstream association analysis [Abdellaoui et al., 2013], and sample specific covariates. The genomic control (GC) inflation factor was calculated and a quantile—quantile (Q-Q) plot was generated to visualize the distribution of the test statistics. Because the GWAS data included family members we added the—family option in the analysis, which takes the familial structure of the data into account using a sandwich estimator. Imputed SNPs were analyzed using PLINK software [Purcell et al., 2007] and genotype imputation uncertainty was accounted for by using allelic dosage. Details for the genotype imputation and quality control are included in the supplementary information. SNPs with values of $P < 5\times10^{-8}$ were declared genome-wide significant.

SNP-heritability. The amount of variance in liability to AD-AUDIT explained by the joint effect of all SNPs (SNP heritability, h^2_{SNP}) was estimated using the Restricted maximum likelihood analysis implemented in the software tool genome-wide complex trait analysis (GCTA) [Yang et al., 2011]. A genetic-relationship-matrix (GRM) is calculated and h^2_{SNP} is estimated in a linear mixed model in which the measure of genetic similarity is included as a random effect to predict the phenotype. Analyses were

TABLE II. Twins Correlation Estimates

	Full model	Best model
R mzm	0.557 (0.377–0 .70)	0.599 (0.515–0.674)
R mzf	0.631 (0.107–0.639)	
R dzm	0.409 (0.518–0.70)	0.412 (0.289–0.524)
R dzf	0.402 (0.227–0.558)	
R dos	0.434 (0.207–0.628)	

The correlations within: monozygotic male (mzm), dizygotic male (dzm), monozygotic female (mzf), dizygotic female (dzf), and dizygotic opposite sex (dos) twin pairs.

adjusted for covariates included in the GWA analyses and related individuals (threshold = 0.025) were excluded (N = 2,737). A detailed description of the GRM employed is included in the supplementary information.

SNP effect concordance analysis. Using SECA software [Nyholt, 2014] we tested whether the direction of SNP effects were positively correlated between our GWAS results and GWAS data including 2,669 DSM-IV based diagnosed AD cases, and 2,002 controls of European American ancestry from the study by Gelernter et al. [2014]. In the two datasets SNP subsets were generated utilizing 12 P-value thresholds ranging from 0.01 to 1, the proportion of the total (144) combination of subsets where the SNP effects are nominally correlated and the relative Fisher's exact test *P*-value are calculated. The significance of observing such proportion of subsets with correlated effects is evaluated comparing it to those under the null-hypothesis obtained via 10,000 permutations.

RESULTS

Heritability

Estimating the heritability with twin models. To estimate the heritability of AD-AUDIT, data from 7,694 MZ and DZ twin pairs were analysed. The within MZ twin pairs correlation (MZ correlation) of 0.60 (CI: 0.51–0.67) was quite high and substantially larger than the DZ correlation of 0.41 (CI: 0.29–0.52) suggesting an influence of genetic factors. Since the DZ correlation is more than half the MZ correlation, shared environmental factors may also play a role (Table II). MZ and DZ correlations did not differ significantly between men and women. The baseline model indicated a significant effect of age (estimated for men 0.17 and for women 0.15), which could be constrained to be equal in men and women (df = 2, chi**2 = 3.8, $P = 0.15$). The threshold in men and women did not differ (df = 3, chi**2 = 0.5, $P = 0.69$) and was estimated at 0.98, indicating a prevalence of 16.3% for AD-AUDIT. The heritability of AD-AUDIT in the ACE model (see methods) is 38% (8–65%), with the influence of shared environmental factors estimated at 22% (0–33%). As expected, since the 95% CI includes zero, the C component could be dropped ($P = 0.064$), while removing A from the model led to a significant deterioration of model fit ($P = 0.014$) (Table III). The best fitting model is an AE model with the heritability estimated at 60% (55–69%) and unique environmental factors explaining the remaining 40% (33–49%) of the variance.

GWAS. After quality control metrics were applied (see methods), 6,464,174 autosomal SNPs were examined for the association with AD-AUDIT. The comparison between observed and expected GWAS *P*-values in the QQ plots revealed that there were many SNPs with a distinct deviation from expectation (Fig. S1). Moreover, no genomic inflation had occurred (lambda = 1.013). The results of the GWAS for AD-AUDIT are represented in a Manhattan plot (Fig. S1). The GWAS demonstrated no SNPs reaching genome wide significance. However, fifty SNPs had *P*-values $<10^{-5}$. Among these top signals, there were four chromosomal loci (4q34.1, 2p16.1, 6q25.3, 7p14.1) containing three or more SNPs within less than 70 kb of each other that showed nominal association with AD-AUDIT (Table IV). The strongest association was detected with rs55768019 ($P = 7.58 \times 10^{-7}$, OR = 0.80) in an intergenic region on chromosome 4q34.1 (Fig. S2). The second strongest signal was observed with three highly correlated SNPs in an intergenic region on chromosome 2p16.1 (rs181048070, rs145441266 and rs143998490, $P = 1.49 \times 10^{-6}$–2.07×10^{-6}, OR = 0.5). On chromosome 6q25.3 we found nine SNPs near the regulator of G-protein signaling 17 (RGS17) gene that showed suggestive evidence of association with AD-AUDIT ($P = 4.89 \times 10^{-6}$–1.05×10^{-5}, OR = 0.78–0.80) (Fig. S3). Seven SNPs mapping to the intronic region of *C7orf10* on chromosome 7p14.1 were also nominally associated with AD-AUDIT ($P = 6.01 \times 10^{-6}$–1.02×10^{-5}, OR = 0.75–0.76) (Fig. S4).

GWA results were compared to the top SNPs (*P*-value $<5 \times 10^{-8}$) from previous GWAS analyses of AD based on DSM-IV diagnosis with either dichotomous or quantitative traits (Table V and Supplementary Table S1). A significant association ($P = 1.58 \times 10^{-4}$, OR = 1.77) was observed for rs1229984 Located in *ADH1B* at 4q22-23. We also observed an association ($P = 7.5 \times 10^{-3}$, OR = 1.16) for rs7119734 Located in *DSCAML1* at 11q23.

SNP heritability. Related individuals (threshold = 0.025) from the GWAS dataset were removed in the GCTA analysis,

TABLE III. Model Parameters for the Full ACE Model and the Submodels

	−2LL	Df	versus	Delta df	Dif LL	*P*
Full ACE model	6644.245	7,690				
AE model	6647.685	7,691	1	1	3.440	0.064
CE model	6650.306	7,691	1	1	6.061	0.014
E model	6835.787	7,692	2	1	188,102	<0.001

TABLE IV. Top Ranked SNPs With *P*-value $<10^{-5}$ for Association With AD-AUDIT

SNP	Chr band	Position (hg19)	Nearest gene (bp)	Location	Coded allele/other allele	Frequency of coded allele	*P*	OR (SE)
rs55768019	4q34.1	175027516	HPGD (383 kb)	Intergenic	A/G	0.58	7.58×10^{-7}	0.80 (0.04)
rs2253612	2q33.1	201568433	AOX2P	Intron	T/C	0.91	8.25×10^{-7}	0.70 (0.07)
rs62338789	4q34.1	175026722	HPGD (383 kb)	Intergenic	C/A	0.58	8.81×10^{-7}	0.80 (0.04)
rs56286907	4q34.1	175027437	HPGD (383 kb)	Intergenic	C/T	0.58	9.20×10^{-7}	0.80 (0.04)
rs5009515	4q34.1	175027304	HPGD (383 kb)	Intergenic	C/T	0.58	9.63×10^{-7}	0.80 (0.04)
rs35498779	4q34.1	175027264	HPGD (383 kb)	Intergenic	G/T	0.58	9.75×10^{-7}	0.80 (0.04)
rs5009512	4q34.1	175027171	HPGD (383 kb)	Intergenic	G/C	0.58	9.78×10^{-7}	0.80 (0.04)
rs5009511	4q34.1	175027160	HPGD (383 kb)	Intergenic	G/A	0.58	9.86×10^{-7}	0.80 (0.04)
rs5009514	4q34.1	175027221	HPGD (383kb)	Intergenic	C/T	0.58	9.96×10^{-7}	0.8068 (0.04)
rs5009513	4q34.1	175027192	HPGD (383 kb)	Intergenic	C/A	0.58	1.02×10^{-6}	0.80 (0.04)
rs5009510	4q34.1	175027066	HPGD (383 kb)	Intergenic	T/C	0.58	1.04×10^{-6}	0.80 (0.04)
rs5009509	4q34.1	175026907	HPGD (383 kb)	Intergenic	A/G	0.58	1.04×10^{-6}	0.80 (0.04)
rs17060170	4q34.1	175026757	HPGD (383 kb)	Intergenic	A/G	0.58	1.04×10^{-6}	0.80 (0.04)
rs41475450	4q34.1	175026876	HPGD (383 kb)	Intergenic	G/A	0.58	1.04×10^{-6}	0.80 (0.04)
rs74745534	6p12.3	49556793	RHAG (48 kb)	Intergenic	C/T	0.98	1.40×10^{-6}	0.40 (0.18)
rs181048070	2p16.1	59543376	LOC101927285 (264 kb)	Intergenic	G/A	0.98	1.49×10^{-6}	0.50 (0.14)
rs117557854	11q22.1	100139171	CNTN5	Intron	G/A	0.98	1.55×10^{-6}	0.48 (0.15)
rs75433892	18p11.22	11712922	GNAL	Intron	G/A	0.99	1.56×10^{-6}	0.27 (0.27)
rs145441266	2p16.1	59523997	LOC101927285 (264 kb)	Intergenic	G/A	0.98	2.05×10^{-6}	0.50 (0.14)
rs143998490	2p16.1	59521976	LOC101927285 (264 kb)	Intergenic	T/G	0.98	2.07×10^{-6}	0.50 (0.14)
rs2540053	2q33.1	201567968	AOX2P	Intron	A/G	0.91	3.52×10^{-6}	0.72 (0.07)
rs2400954	12q21.31	81932981	PPFIA2	Intron	T/C	0.63	3.90×10^{-6}	0.81 (0.04)
rs79978308	21q22.2	41020545	B3GALT5	Promoter	C/T	0.97	4.69×10^{-6}	0.56 (0.12)
rs7199896	16q21	58372984	PRSS54 (44 kb)	Intergenic	A/T	0.94	4.87×10^{-6}	0.63 (0.09)
rs10649162	6q25.3	153620555	RGS17 (168 kb)	Intergenic	I/R	0.78	4.89×10^{-6}	0.79 (0.05)
rs62191099	2q37.3	239857753	FLJ43879 (931 kb)	Intergenic	G/A	0.70	5.23×10^{-6}	0.80 (0.04)
rs12632235	3q12.2	100536284	ABI3BP	Intron	C/T	0.99	5.31×10^{-6}	0.46 (0.16)
rs1479432	6q25.3	153615554	RGS17 (168 kb)	Intergenic	G/A	0.78	5.65×10^{-6}	0.79 (0.05)
rs6935546	6q25.3	153612379	RGS17 (168 kb)	Intergenic	G/A	0.78	5.77×10^{-6}	0.79 (0.05)
rs9371286	6q25.3	153611223	RGS17 (168 kb)	Intergenic	T/G	0.78	5.84×10^{-6}	0.79 (0.05)
rs17620991	7p14.1	40836521	C7orf10	Intron	G/C	0.86	6.01×10^{-6}	0.75 (0.06)
rs17688247	7p14.1	40832144	C7orf10	Intron	C/T	0.86	6.01×10^{-6}	0.75 (0.01)
rs57126985	21q21.1	16590032	NRIP1 (1525)	Intergenic	G/C	0.97	6.43×10^{-6}	0.57 (0.12)
rs2127305	6q25.3	153625746	RGS17 (168 kb)	Intergenic	A/T	0.78	6.43×10^{-6}	0.79 (0.05)
rs2169976	6q25.3	153625452	RGS17 (168 kb)	Intergenic	G/A	0.78	6.49×10^{-6}	0.79 (0.05)
rs2400955	12q21.31	81933208	PPFIA2	Intron	C/T	0.68	6.88×10^{-6}	0.80 (0.04)
rs1871401	6q25.3	153628192	RGS17 (168 kb)	Intergenic	T/C	0.78	6.98×10^{-6}	0.79 (0.05)
rs34994199	7p14.1	40827651	C7orf10	Intron	C/A	0.86	7.46×10^{-6}	0.75 (0.06)
rs66651482	7p14.1	40851242	C7orf10	Intron	G/T	0.86	8.33×10^{-6}	0.76 (0.06)
rs67648979	7p14.1	40851735	C7orf10	Intron	T/C	0.86	8.34×10^{-6}	0.76 (0.06)

(*Continued*)

TABLE IV. (Continued)

SNP	Chr band	Position (hg19)	Nearest gene (bp)	Location	Coded allele/other allele	Frequency of coded allele	P	OR (SE)
rs139217154	11q22.1	100154776	CNTN5	Intron	G/A	0.98	8.48×10^{-6}	0.47 (0.16)
rs10741210	10q26.3	131984312	GLRX3	Downstream	G/A	0.58	8.63×10^{-6}	1.22 (0.04)
rs11076221	16q12.1	51961543	C16orf97 (65 kb)	Intergenic	G/A	0.94	8.79×10^{-6}	1.57 (0.1)
rs13340561	8p22	16058905	MSR1	Promoter	T/G	0.97	8.93×10^{-6}	0.59 (0.11)
rs75562159	1p36.23	7504141	CAMTA1	Intron	C/A	0.99	9.16×10^{-6}	0.37 (0.22)
rs144499073	8p22	16064119	MSR1 (13 kb)	Intergenic	C/T	0.97	9.25×10^{-6}	0.59 (0.11)
rs142530316	7p14.1	40861829	C7orf10	Intron	T/C	0.86	9.91×10^{-6}	0.76 (0.06)
rs17688571	7p14.1	40862763	C7orf10	Intron	T/C	0.86	1.02×10^{-5}	0.76 (0.06)
rs9479568	6q25.3	153559729	RGS17 (168 kb)	Intergenic	G/C	0.79	1×10^{-5}	0.79 (0.05)
rs9384096	6q25.3	153626202	RGS17 (168 kb)	Intergenic	A/G	0.78	1×10^{-5}	0.79 (0.05)

leaving 5,105 subjects. Results showed that common SNPs significantly ($P = 0.002$) capture a substantial part of the heritability of AUDIT-assessed AD. Since the h^2_{SNP} estimates may be biased by ascertainment in case-control studies (proportions of cases may differ from the population disease prevalence) h^2_{SNP} were reported on the liability scale via a linear transformation based on an estimate of the risk of the disorder (k) in the population. Using the prevalence of AUDIT-assessed AD obtained in the overall NTR cohort ($k = 0.138$) as highly representative of the Dutch population, h^2_{SNP} was 0.33 ($SE = 0.12$).

SNP effect concordance analysis. Finally, we examined the individual SNP effect direction across our GWAS and a recent AD GWAS by Gelernter et al. [2014] (using the DSM-IV definition of AD). A set of 48,361 independent SNPs overlapping across the two datasets was compared. This set was selected based on two criteria: a) the SNPs-based P-values had to be the lowest in the reference sample of Gelernter et al. [2014] and b) the SNPs had to be independent (i.e., SNPs were discarded if they showed correlation $r^2 > 0.1$ with the most significant SNP within genomic regions of 1Mb and next, within genomic regions of 10 Mb). We found a significantly enriched proportion (in 110 of the 144 possible combinations) of SNP subsets from the two GWA datasets where effects were nominally correlated, indicating a substantial concordance of SNP effects between the two studies (permuted $P = 9.99 \times 10^{-4}$, 95% CI: 5.12×10^{-5}-0.0056).

DISCUSSION

In the present study we calculated heritability estimates based on twin and SNP data and carried out a GWA analysis of AD as assessed with the AUDIT. Our study is the first to study the heritability of AD using data based on the AUDIT. The heritability estimate of 60% for AD-AUDIT is in line with the results of studies exploring the heritability of alcohol abuse, alcohol dependence and alcoholism, which report heritabilities of around 50% [Enoch and Goldman, 2001; Goldman et al., 2005]. The SNP based heritability of 33% suggests a large proportion of the twin-based heritability is explained by variations in common SNPs. However, no genome-wide significant results were found in our genome wide association study for AD. The most obvious reason is that the study had limited power. In order to detect genome-wide significant results the sample size must be larger. However, we identified four chromosomal loci (4q34.1, 2p16.1, 6q25.1, 7p14.1) enriched with SNPs that showed suggestive associations.

Discrepancies between twin-based and SNP based heritability estimates and the failure, in general, of GWAS studies to detect the specific genes involved in complex traits is known as the "missing heritability" phenomenon [Manolio et al., 2009; Sadee, 2012; Klein and Zanger, 2013; Sadee et al., 2014]. Although large consortia for substance use have obtained genome-wide significant results (like the nicotine receptor genes on chromosome 15 associated with cigarettes per day [Thorgeirsson et al., 2010], these known genetic factors explain only a small proportion of the estimated heritability (the top SNP for cigarettes per day explained 0.5% of the variation). Sources of variation that may explain the "missing heritability" are for example epistasis (dynamic gene-gene interactions), epigenetics (switching genes on and off by environmental factors), and variants

TABLE V. Association Results for the Top SNPs (*P*-value $<10^{-8}$) From Previous AD GWASs in the Present Study

Study	SNP	*P*-value in original study (OR)	Gene (chr band)	*P*-value in our study (OR)
Treutlein et al. [2009]	rs7590720	9.72×10^{-9} (0.69)	2q35	0.22 (0.94)
	rs1344694	1.69×10^{-8} (0.70)	2q35	0.28 (0.95)
Wang et al. [2011]	rs7119734	3.75×10^{-8}	DSCAML1 (11q23)	7.5×10^{-3} (1.16)
	rs2298767	6.34×10^{-9}	DSCAML1 (11q23)	0.13 (1.11)
	rs10892169	5.3×10^{-9}	DSCAML1 (11q23)	0.56 (0.96)
	rs3851576	3.62×10^{-8}	DSCAML1 (11q23)	0.35 (0.95)
Frank et al.	rs1789891	1.27×10^{-8} (1.46)	ADH1B-ADH1C (4q23)	0.07 (0.9)
Zuo et al. [2013]	rs1039630	4.7×10^{-8} (1.32)	NKAIN1-SERINC2 (1p35)	0.77 (0.98)
	rs4478858	3.1×10^{-8} (1.31)	NKAIN1-SERINC2 (1p35)	0.75 (0.98)
	rs2275436	3.8×10^{-8} (1.32)	NKAIN1-SERINC2 (1p35)	0.6 (0.97)
Park et al. [2013]	rs1229984	2.63×10^{-21} (2.35)	ADH1B (4q22-23)	1.58×10^{-4} (1.87)
	rs1442492	2.01×10^{-16} (2.22)	ADH7 (4q22-23)	0.17 (1.07)
	rs284787	1.75×10^{-15} (2.07)	ADH7 (4q22-23)	0.1 (1.08)
	rs284784	2.43×10^{-15} (2.06)	ADH1A (4q22-23)	0.019 (1.2)
	rs975833	1.12×10^{-14} (2.15)	ADH1B (4q22-23)	0.09 (0.91)
	rs2075633	6.67×10^{-14} (2.11)	ADH1B (4q22-23)	0.17 (0.93)
	rs284793	2.99×10^{-12} (1.98)	4q22-q23	0.11 (1.08)
	rs729147	3.84×10^{-11} (1.98)	4q22-q23	0.91 (1)
	rs894369	3.84×10^{-11} (1.98)	ADH7 (4q22-23)	0.91 (1)
	rs994771	4.76×10^{-11} (1.92)	ADH1A (4q22-23)	0.16 (1.06)
	rs2851300	9.88×10^{-10} (2.88)	ADH1A (4q22-23)	0.06 (0.92)
	rs4147531	1.45×10^{-9} (2.11)	ADH1A (4q22-23)	0.11 (1.07)
	rs4147532	1.6×10^{-9} (2.11)	ADH1A (4q22-23)	0.1 (1.07)
Wang et al. [2013]	rs12912251	4.5×10^{-8} (0.84)	C15orf53 (15q14)	0.85 (1)
Gelernter et al. [2014]	rs1229984	1.81×10^{-30}	ADH1B (4q22-23)	1.58×10^{-4} (1.87)
	rs2066702	1.5×10^{-13}	ADH1B (4q22-23)	NA
	rs28542574	3.62×10^{-11}	LOC100507053	NA
	rs17028615	2.88×10^{-8}	LOC100507053	NA
	rs10031423	2.01×10^{-8}	PDLIM5 (4q22-23)	0.81 (1.01)
	rs58521602	3.35×10^{-8}	METAP (4q22-23)	NA
	rs1437396	1.17×10^{-10}	MTIF2-CCDC88A (2p16.1)	0.74 (1.02)
	rs1493464	8.59×10^{-9}	5p15.33	0.55 (0.97)
	rs1856202	5.99×10^{-11}	9p13.3	0.42 (1.04)
	rs59514816	2.89×10^{-8}	DPP9 (19p13.3)	0.31 (0.94)

not covered by the common GWA chips including rare variants [Zuk et al., 2014]. However, the demands for low false positive rates very likely add to high false negative rates in GWAS dataset interpretation. For this reason we discuss several loci here that do not meet the 10^{-8} P-value threshold that protects from false positives at the cost of false negative results. Though these top-signals did not reach genome-wide significance, they may include interesting candidate regions for AD-AUDIT. The strongest association ($P = 7.58 \times 10^{-7}$) was at a locus containing a group of 13 highly correlated variants ($r^2 \geq 0.95$) in an intergenic region located on chromosome 4q34.1, less than 400 kb from *HPGD* (hydroxyprostaglandin dehydrogenase 15-(NAD). This gene encodes a member of the short-chain non-metalloenzyme alcohol dehydrogenase protein family. The encoded enzyme is responsible for the metabolism of prostaglandins, which play a role in a variety of physiologic and cellular processes such as inflammation, and has been associated with the risk of colon cancer [Thompson et al., 2013]. There are three additional candidate gene regions containing two or more SNPs showing suggestive association with AD-AUDIT. Among these are variants mapped to chromosome 2p16.1. Interestingly, a recent GWAS [Gelernter et al., 2014] has reported evidence that variation in the 2p16.1 region influences susceptibility for AD in a European and a African-American sample. This region does not contain protein coding genes so the reason why variation in this region is associated with AD remains to be determined.

Another marginally significant signal was observed near the regulator of G-protein signaling 17 gene (*RGS17*) located in 6q25.1, and is of particular interest as it has been linked to four substance dependence diagnoses (alcohol, cocaine, opioid or marijuana dependence) in a candidate gene study with 21 SNPs in *RGS17* and *RGS20* [Zhang et al., 2012]. *RGS17* is a member of the RGS-Rz subfamily of GTPase-activating proteins (GAP) that efficiently deactivate GalphazGTP subunits, and thereby turns off the signaling pathway of G protein-coupled receptors (GPCRs), including opioid receptors. The top signal within 6q25.1 was rs10649162 Located 168 kb away from *RGS17*. Because alcohol, cocaine, opioid or marijuana dependence are highly comorbid [Falk et al., 2008], it is possible that this region plays a role in

substance dependence in general. Finally, associations were observed across the *SUGCT* (Succinyl CoA: glutarate-CoA trasnferase) locus at 7p14.1. The functional significance of this locus is unclear and it has not been associated with substance use in previous studies (Table V).

When comparing the association results of the top SNPs from the previous GWAS by Gelernter et al. [2014] with our study, we observed a significant association for the variant that is most consistently associated with AD (rs1229984) located in *ADH1B* at 4q22-23 [Li et al., 2011; Gelernter et al., 2014]. The gene *ADH1B* codes for the alcohol dehydrogenase 1B enzyme and variations in this gene have been related to differences in alcohol consumption and dependence [Demers et al., 2014]. In the functional SNP rs1229984, arginine is changed to histidine at residue 47. The current study confirmed the association between this functional SNP and AD. We also observed an association ($P = 7.5 \times 10^{-3}$, OR = 1.16) for rs7119734 located in *DSCAML1* (Down syndrome cell adhesion molecule like 1) at 11q23. This SNP was previously linked with AD in an Australian twin-family study of alcohol use disorder [Wang et al., 2011]. Besides human studies, associations were also found in two animal studies [Morozova et al., 2012]. Two of the previous GWAS for AD [Treutlein et al., 2009; Park et al., 2013] reported significantly associated genetic variants in samples of only men. Our current sample size is relatively small to detect strong associations with AD-AUDIT in a stratified analysis, analyzing men and women separately. Nevertheless, the heritability estimates of AD-AUDIT between men and women in our data did not differ significantly. These results are in line with previous studies showing overlapping genetic risk factors for alcoholism across genders [Prescott et al., 1999], suggesting that a possible gender-genetic heterogeneity is unlikely to strongly impact on the GWAS results.

Similarly to other complex traits, our results are consistent with a predominantly polygenic genetic contribution to AD-AUDIT, reflecting the combined small effects of a large number of variants across the genome. Large samples are required to achieve the adequate statistical power to detect such effects. In epidemiological studies semi-structured psychiatric interviews do not generally not represent a feasible option to assess AD and there is a need for less costly and time consuming measures. In this study, our approach to identifying genetic risk factors for alcohol dependence focused on the use of the AUDIT, a brief self-report measure that allows for large-scale data collection. The consistency of some of our findings with results from previous genetic studies using DSM based definition of AD (concordance in heritability estimates and direction of SNP effects, as well as overlap with top hits from GWAS) suggests that the use of appropriate questionnaires may represent a cost-effective strategy to phenotype samples in large-scale biobanks or other population-based datasets. The lack of power due to the relatively small sample size may represent a major limitation of the current GWA study, as none of our top SNPs reached genome-wide significance. However, the twin and SNP-based heritability analyses were powered sufficiently to confirm previously reported heritability estimates from twin studies and, for the first time to show using a GCTA approach how much variance is explained by all SNPs tested in the GWAS.

In summary, this is the first GWAS of AD using the AUDIT measure. We found several suggestive regions associated with AD-AUDIT both located in known candidate regions for AD and novel candidate regions. Our heritability estimates based on twin data confirmed previous reports and we showed that a substantial part of this heritability is captured by common SNPs. Further studies in larger samples are needed to dissect the genetic underpinnings of AD. In this effort, the use of appropriate questionnaires like the AUDIT may represent a cost-effective strategy to phenotype samples in large-scale biobanks or population-based datasets.

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SUPPORTING INFORMATION

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