PG-D-16-00067R1

Genetic variation in *GABRβ1* and the risk of developing alcohol dependence

Running head: *GABRβ1* and alcohol dependence risk.

William A McCabe [1,2], **Michael J Way** [1,2], **Kush Ruparelia**[1,2], **Susanne Knapp**[3], **M Adam Ali**[1,2], **Quentin M Anstee**[4], **Howard C Thomas**[3], **Andrew McQuillin**[2], **and Marsha Y Morgan**[1]

[1] UCL Institute for Liver & Digestive Health, University College London, London, NW3 2PF, UK

[2] Molecular Psychiatry Laboratory, Division of Psychiatry, University College London, WC1E 6DE, UK

[3] Department of Gastroenterology & Hepatology, Imperial College, St Mary's Hospital Campus, Praed Street, London W2 1NY, UK

[4] Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

Author and details for correspondence:

Dr Marsha Y Morgan
UCL Institute for Liver & Digestive Health,
Division of Medicine, Royal Free Campus,
University College London
Rowland Hill Street,
London NW3 2PF
Telephone: +44 (0)207 433 2873
E-mail: marsha.morgan@ucl.ac.uk

Abstract

Background: **Associations between the γ-aminobutyric acid type-A receptors ($GABA_A$) and alcohol dependence risk have been reported, although the receptor subunit driving the association is unclear. Recent work in mice has highlighted a possible role for variants in the *Gabr* β1 subunit (*Gabrβ1*) in alcohol dependence risk, although this gene does not contain any common non-synonymous variants in man. However, the $GABA_A$ receptor is a hetero-pentamer so multiple potential variants within the gene complex could generate the alcohol dependence phenotype.**

Methods: **The association between *GABRβ1* variants and alcohol dependence risk was explored in a British and Irish population of alcohol dependent cases (n=450) and ancestrally-matched, controls screened to exclude current or historical alcohol misuse (n=555). Twelve common single nucleotide polymorphisms (SNPs), and a rare non-synonymous variant, rs41311286, were directly genotyped; imputation was then performed across the whole gene.**

Results: **No allelic association was observed between alcohol dependence risk and any of the directly genotyped or imputed SNPs. However, post-hoc testing for genotypic association identified five common intronic SNPs which showed modest evidence for association after correction for multiple testing; two, rs76112682 and rs141719901, were in complete linkage disequilibrium ($P_{corrected}$=0.02, OR [95% CI] = 5.9 [1.7-2.06]).**

Conclusion: **These findings provide limited support for an association between *GABRβ1* and the risk of developing alcohol dependence; further testing in expanded cohorts may be warranted.**

Keywords: Alcohol dependence, British, GABA receptor, *GABRB1*, Genetic association, imputation analysis, Irish, linkage disequilibrium

Introduction

Alcohol dependence is a complex disorder which contributes substantially to the global burden of disability and death (World Health Organisation, 2014). Genetic factors undoubtedly play a role in the development of this condition but the identification of the exact risk variants has proven difficult (Stickel *et al.,* 2016).

Alcohol interacts with several neurotransmitter membrane receptors and ion channels (Enoch, 2008); it specifically alters the ratio of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) to the excitatory neurotransmitter glutamate, both of which bind to GABA receptors in the brain. Thus, genetic variations in the genes encoding key GABA receptors may influence the effects of alcohol on the brain and hence the risk for developing alcohol dependence (Enoch, 2003; Enoch, 2008). The $GABA_A$ receptor is a hetero-pentamer consisting of five subunits; thus several different genes encode the subunit proteins which make up the complex (Kumar *et al.,* 2009; Kelm *et al*., 2010; Stephens *et al.,* 2016).

Previous studies have identified an association between GABA receptor genes and alcohol dependence risk, but with attribution to different receptor subunits. Risk association with microsatellite markers corresponding to introns in the *GABA* β1 subunit (*GABRβ1*) have been reported and replicated in several populations (Long *et al.,* 1998; Parsian and Zhang 1999; Song *et al.,* 2003; Zhang *et al.,* 2005; Reck *et al.*, 2005) but not necessarily all (Drgon *et al.*, 2006; Couvalt *et al.*, 2008; Mathews *et al.*, 2007; Lydall *et al.*, 2011) and no significant associations have been identified with genes in the GABA receptor cluster in genome–wide association studies (GWAS) of alcohol dependence risk (Stickel *et al*, 2016).

Interest in the role of *GABRβ1* in mediating alcohol dependence risk in man was revived recently following identification of two functional mutations in murine *Gabrβ1* which are

associated with the development of a strong motivation to consume alcohol in mouse strains which are normally highly alcohol averse (Anstee *et al.,* 2013). These mutations correspond to L285R and P228H, neither of which has a common human homologue: L285R corresponds to human residue 310 and only one person with variation in this residue has been identified amongst the 60,706 individuals in the Exome Aggregation Consortium (ExAC) data (Lek *et al.*, 2016); P228H corresponds to human residue 253 -- no variants affecting this residue have been identified in the ExAC data (Lek *et al.*, 2016).

There are, in fact, no common (>1% minor allele frequency) non-synonymous variants in *GABRβ1.* In European populations, rs41311286 (His421Gln), the variant most frequently observed, has a minor allele frequency of only 0.4% (Lek *et al*., 2016). Nevertheless, *GABRβ1* is comprised of over 99.7% intronic DNA, which may contain genetic variations which could for example, influence mRNA stability and transcriptional regulation and hence have functional effects (Shabalina *et al*., 2013). Thus, the primary aim of this study was to investigate the association between variants in *GABRβ1* and alcohol dependence risk, by conducting a two-stage case-control study comprising of a primary allelic analysis of selected tagging SNPs followed by an imputation analysis extending the full length of the gene.

Methods

Study populations

Genomic DNA was available from 450 cases (mean [range] age: 52.0[20-81] yr: 47% men) and 555 controls (mean age: 38.2[18-87] yr: 54% men). The cases comprised of individuals diagnosed as alcohol dependent using the *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition* (DSM-IV; American Psychiatric Association, 1994) criteria. All were of English, Scottish, Welsh or Irish descent with a maximum of one grandparent of white European origin. The controls comprised ancestrally-matched individuals none of whom had a past or current history of alcohol misuse. None of the included individuals was related.

SNP selection and genotyping

Thirteen single nucleotide polymorphisms (SNPs) were genotyped across *GABRβ1* using the KBiosciences Competitive Allele Specific PCR (KASPar) genotyping platform (LGC Genomics, Hoddesdon, UK). The selection included: (i) seven tagging SNPs around an intronic tetra-nucleotide microsatellite marker, which had previously shown evidence for variation in humans (Dean *et al.,* 1991); (ii) five additional tagging SNPs which, according to the HapMap project data, were predicted to provide additional mapping information across the gene, and (iii) rs413211286, the only non-synonymous SNP identified in the 1000 genomes European population (Abecasis *et al*., 2012) (Supplementary Fig. 1). Amplification and detection was undertaken using a LightCycler® 480 Real-Time PCR machine (Roche Molecular Diagnostics, Burgess Hill, UK).

Genotype calling was performed automatically by built-in Roche software with some manual editing. Approximately 12% of samples, randomly selected *a priori*, were genotyped in duplicate. Samples with conflicting calls (<0.05% of total) were excluded from further analyses.

Imputation

Imputation was performed using IMPUTE2 v2.3.2 using phase 3 integrated reference haplotypes from the 1000 genomes sequencing project (Haplotype release date October 2014) (www.1000genomes.org/) as a reference panel (Abecasis *et al.,* 2012). The imputation was performed over the (hg19) region chr4:46,990,000-47,500,000 using recommended settings (Marchini *et al.,* 2007).

Statistical analyses

Tests for primary allelic associations, missingness, deviation from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium were performed using PLINK version 1.9 (Purcell *et al*., 2007; Chang *et al*., 2015). The selection of SNPs for testing for association with alcohol dependence risk was restricted to directly genotyped SNPs with a minor allele frequency (MAF) >0.05 and imputed SNPs with a MAF >0.05 and an info score > 0.9. Further *post hoc* tests of association were performed using the model method and results reported on additive, recessive and dominant models of association. The model with the lowest P-value was used for each SNP; only those SNPs with a P value less than 0.05 were explored further (González *et al.,* 2008). The P value for each SNP was individually corrected for multiple testing using SNPSpD method (Nyholt, 2004). Sex differences in identified genotypic associations were explored using logistic regression with sex as a covariate.

SNP annotation

Intergenic SNPs were annotated for known and predicted regulatory elements using RegulomeDB v.1.1 (www.regulomedb.org/) and HaploReg v.4.1 (archive.broadinstitute.org/mammals/haploreg/haploreg.php). The GTEx Consortium database (www.gtexportal.org/home/) and the braineac database (www.braineac.org/) were

interrogated to identify the effects of SNPs associated with the risk for developing alcohol dependence on brain *GABRβ1* transcript expression. A regional plot of the association data was generated using LocusZoom (Prium 2010).

Ethics

United Kingdom National Health Service Multicentre Research Ethics Committee approval was granted for this study (MREC/03/11/090). Written informed consent was obtained from all subjects prior to inclusion.

Results

Genotyping accuracy

The call rates for all 13 directly genotyped SNPs were greater than 95% and they were all in Hardy Weinberg Equilibrium to P > 0.01.

Genetic association with the directly genotyped single nucleotide polymorphisms

There was no evidence of allelic association between the directly genotyped SNPs and alcohol dependence risk (Table 1). *Post hoc* testing showed evidence of genotypic association with alcohol dependence risk for three SNPs, two of which, rs2044081 ($P_{corrected}$=0.034) and rs17539445 ($P_{corrected}$=0.024) retained significance after correction for multiple testing (Supplementary Table 1, Supplemental digital content 2, *http://links.lww.com/PG/A186*).).

Imputation

Imputation returned data for 14,608 SNPs of which 12,627 were within the Ensembl accepted region for *GABRβ1 viz*. chr4:46,993,723-47,426,444. One hundred and thirty four SNPs met quality control criteria and were tested for association.

Genetic association with the imputed single nucleotide polymorphisms

There was no evidence for allelic association with alcohol dependence risk for any of the imputed SNPs (data not shown). *Post hoc* tests of genotypic association with a recessive model showed uncorrected evidence for association with alcohol dependence risk for 36 imputed SNPs, all located within intron 4 of the *GABRβ1* transcript (Supplementary Fig. 2;). Five of these SNPs retained significance after correction for multiple testing including two, rs2044081 and rs17539445 previously identified by direct genotyping (Table 2); two of the other three

SNPs, rs76112682 and rs141719901, were in complete linkage disequilibrium ($P_{corrected}$=0.02, odds ratio [95% CI] = 5.9[1.7-2.06]).

The associations with alcohol dependence risk identified by direct genotyping and by imputation were not significantly affected when sex was included as a covariate in the analyses (data not shown).

Single nucleotide polymorphism annotation

There was weak evidence from RegulomeDB v.1.1 that one SNP with evidence for association with alcohol dependence risk, rs17539445, lies in a binding site for the transcription factor Sex Determining Region Y Box 1 *(SOX1*), while a further associated SNP, rs141719901, lies in a binding site for Eomesodermin *(EOMES*) (Badis *et al.*, 2009*)*.

There was no evidence from HaploReg v.4.1 that rs17539445 and rs141719901 were located in brain enhancer histone marked regions. However, both SNPs were in strong linkage disequilibrium (LD; r^2 >= 0.8) with six SNPs (rs7660967, rs73247673, rs2044081, rs5858060, rs6447543 and rs4635792) that were located in these regions.

There was no evidence from the GTEx Consortium database that either rs17539445 or rs141719901 act as a brain expression quantitative trait locus (eQTL) for the *GABRβ1* transcript. Likewise there was no evidence from the braineac database that rs17539445 is a brain eQTL for the *GABRβ1* transcript; no data were available on rs141719901 from this source.

Discussion

In the present study, direct genotyping and subsequent imputation of SNPs across *GABRβ1* provided a number of potentially interesting associations with alcohol dependence risk, including five intronic SNPs which retained significance after correcting for multiple testing. Two of these SNPs mapped genotypic information across regions of micro-satellite markers previously associated with alcohol dependence risk (Long *et al*., 1998, Parsian and Zhang 1999, Song *et al.*, 2003, Zhang *et al.,* 2005).

The *GABRβ1* gene has comparatively large introns (>100 kb) and relatively small exons (~0.1 kb). The only non-synonymous variant available for testing showed no genotypic or allelic evidence for association with alcohol dependence risk. However, this SNP is rare in European populations meaning that this study was likely underpowered to detect genetic association (Skol *et al*., 2006). Thus, the significance of any association with this SNP and alcohol dependence risk would need to be explored in much larger cohorts.

Post hoc testing identified associations between common intronic variants in *GABRβ1* and alcohol dependence risk. These associations may be primary, or reflect the high level of linkage disequilibrium across and beyond *GABRβ1*, or association signals from variants in nearby loci such as, *GABRα4,* which is directly downstream from *GABRβ1,* or *GABRα2* which is approximately 570kb further downstream from *GABRβ1*.

It is also possible that intronic and/or synonymous variants could impact on levels of protein expression (Barrett *et al*., 2012) and hence might be directly associated with alcohol dependence risk. Alcohol, taken acutely, stimulates the production of GABA and enhances $GABA_A$ receptor potentiation (Kumar *et al.*, 2009). Chronic exposure to alcohol might result in neuronal adaptation with attenuation of the GABA receptors to restore the homeostasis of

excitatory and inhibitory neurotransmission (Kumar *et al*., 2009). If, under these circumstances, intronic variants in *GABRβ1* alter the expression level or the half-life of the mRNAs this might further attenuate *GABRβ1* expression and might result in an association with disease phenotype. Furthermore, attenuation of a single subunit would impact on the subunit ratio and in turn the pentameric isoform ratios; this could also cause an overall alteration in ion channel gating, thus replicating the effect of the reported mutations in *Gabrβ1* (Anstee *et al.,* 2013). However, the fact that several of the variants are in strong linkage disequilibrium means that the effect of any one SNP is likely to be very small and it would be difficult to identify influential haplotype combinations. This might explain the contradictory evidence for association with alcohol dependence risk seen across previous studies, and may explain why the recessive model of association provided the most significant associations.

Two of the SNPs which showed modest genotypic evidence for association with alcohol dependence risk, rs17539445 and rs141719901 were predicted to lie in *SOX1* and *EOMES* transcription factor binding sites. In addition, both SNPs are in strong LD with six SNPs in brain tissue enhancer histone marked regions. Interference with transcription factor binding and/or histone binding could impact on expression levels of *GABRβ1*. However, there was no evidence, from available databases, that either SNP acts as a brain eQTL. None of the other SNPs that showed genotypic association with alcohol dependence risk were predicted to correspond to an obvious regulatory element.

In conclusion, no allelic association was detected in this study between the tested SNPs and the risk for developing alcohol dependence in a British and Irish population. However, modest genotypic associations were found with several intronic *GABRβ1* SNPs which may directly influence alcohol dependence risk or may be in linkage disequilibrium with causal risk variants

in nearby genes, including other GABA receptor subunits. Future studies should attempt to identify any linked functional variants and to better understand the effects of these intronic variants on the levels of protein expression and *GABRβ1* composition.

Acknowledgements

WAM is supported by a PhD fellowship funded jointly by the Rosetrees Trust and the Royal Free Charity.

MJW is supported by a PhD fellowship funded jointly by the Hobson Charity and University College London.

QMA is the recipient of a Clinical Senior Lectureship Award from the Higher Education Funding Council for England (HEFCE).

Harriet Gordon, Jonathan Marshall and Sara Montagnese helped collect the case samples; Jacob Lawrence and the late Hugh Gurling collected the control samples. Jitinder Saini and Mikio Kajihara extracted the DNA

Authors Contributions

AM, SK, HCT and MYM conceived the research.

MYM supervised the collection and phenotypic characterized of the case samples

KR and MAA undertook the genotyping. WAM and MJW undertook the imputation analysis

WAM, AM, MJW, MYM undertook the data analyses and interpretation.

The manuscript was written by WAM and MYM and reviewed by MJW, QMA, SK and AM.

All authors reviewed and approved the submitted manuscript.

Competing Financial Interests

The authors declare that they have no competing financial interests

References:

Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, *et al* (2012). 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**:56-65.

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV — 4th ed*. Washington DC: American Psychiatric Association.

Anstee QM, Knapp S, Maguire EP, Hosie AM, Thomas P, Mortensen M, *et al* (2013). Mutations in the *GABRβ*1 gene promote alcohol consumption through increased tonic inhibition. *Nat Commun* **4**:2816.

Badis G, Berger MF, Philippakis AA, Talukder S, Gehrke AR, Jaeger S, *et al* (2009). Diversity and complexity in DNA recognition by transcription factors. *Science* **324**:1720-1723.

Barrett LW, Fletcher S, Wilton SD (2012) Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. *Cell Mol Life Sci* **69**:3613–3634.

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* doi: 10.1186/s13742-015.

Covault J, Gelernter J, Hesselbrock V, Nellissery M, Kranzler HR (2004). Allelic and haplotypic association of *GABRA2* with alcohol dependence. *Am J Med Genet B Neuropsychiatr Genet* **129B**:104–109.

Dean M, Lucas-Derse S, Bolos A, O'Brien SJ, Kirkness EF, Fraser CM, *et al* (1991). Genetic mapping of the beta 1 GABA receptor gene to human chromosome 4, using a tetranucleotide repeat polymorphism. *Am J Hum Genet* **49**:621-626.

Drgon T, D'Addario C, Uhl GR (2006). Linkage disequilibrium, haplotype and association studies of a chromosome 4 GABA receptor gene cluster: candidate gene variants for addictions. *Am J Med Genet B Neuropsychiatr Genet* **141**:854–860.

Enoch MA (2003). Pharmacogenomics of alcohol response and addiction. *Am J Pharmacogenomics* **3**:217-232.

Enoch MA (2008). The role of $GABA_A$ receptors in the development of alcoholism. *Pharmacol Biochem Behav* **90**:95–104.

González JR, Carrasco JL, Dudbridge F, Armengol L, Estivill X, Moreno V (2008). Maximizing association statistics over genetic models. *Genet Epidemiol* **32**: 246–254.

Kelm MK, Criswell HE, Breese GR (2010). Ethanol-enhanced GABA release: a focus on G protein-coupled receptors. *Brain Res Rev* **65**:113-123.

Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, *et al* (2009). The role of $GABA_A$ receptors in the acute and chronic effects of ethanol: a decade of progress. *Psychopharmacology* **205**:529-564.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, *et al* (2016). Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**:285-291.

Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, *et al* (1998). Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am J Med Genet* **81**:216–221.

Lydall, GJ, Saini J, Ruparelia K, Montagnese S, Mcquillin A, Guerrini I, *et al* (2011). Genetic association study of *GABRA2* single nucleotide polymorphisms and electroencephalography in alcohol dependence. *Neurosci Lett,* **500:** 162-166.

Marchini J, Howie B, Myers S, McVean G, Donnelly, P (2007). A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet* **39**: 906-913.

Matthews AG, Hoffman EK, Zezza N, Stiffler S, Hill SY (2007). The role of the *GABRA2* polymorphism in multiplex alcohol dependence families with minimal comorbidity: within-family association and linkage analyses. *J Stud Alcohol Drugs* **68**:625–633.

Nyholt DR (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* **74**:765-769.

Parsian A, Zhang ZH (1999). Human chromosomes 11p15 and 4p12 and alcohol dependence: possible association with the *GABRB1* gene*. Am J Med Genet* **88**:533–538.

Pruim RJ (2010). LocusZoom: Regional visualization of genome-wide association scan results. *Bioinformatics* **26**:2336-2337.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, *et al* (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**:559-575.

Reck BH, Mukhopadhyay N, Tsai HJ, Weeks DE (2005). Analysis of alcohol dependence phenotype in the COGA families using covariates to detect linkage. *BMC Genet* **6**:S143.

Shabalina SA, Spiridonov NA, Kashina A (2013). Sounds of silence: synonymous nucleotides as a key to biological regulation and complexity. *Nucleic Acids Res* **41**:2073–2094.

Skol AD, Scott LJ, Abecasis GR, Boehnke M (2006). Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* **38**:209-213.

Song J, Koller DL, Foroud T, Carr K, Zhao J, Rice J, *et al* (2003). Association of $GABA_A$ receptors and alcohol dependence and the effects of genetic imprinting. *Am J Med Genet B Neuropsychiatr Genet* **117**:39-45.

Stephens DN, King SL, Lambert JJ, Belelli D, Duka T (2016). $GABA_A$ receptor subtype involvement in addictive behaviour. *Genes Brain Behav* Aug 19. doi: 10.1111/gbb.12321. [Epub ahead of print]

Stickel F, Moreno C, Hampe J, Morgan MY (2016). The genetics of alcohol dependence and alcohol-related liver disease. *J Heptol* http://dx.doi.org/10.1016/j.jhep.2016.08.011.

World Health Organization. Global Status Report on Alcohol and Health-2014 ed. Geneva: World Health Organization; 2014. Available online at: www.who.int/substance_abuse/publications/global_alcohol_report/en/ [Accessed on 14 April 2016]

Zhang H, Zhong X, Ye Y (2005). Multivariate linkage analysis using the electrophysiological phenotypes in the COGA alcoholism data. *BMC Genetics* **30** (Suppl 1):S118.

Legends to Figures

Supplementary Figure 1. The location of the directly genotyped and imputed SNPs in *GABRB1*

The 13 directly genotyped intronic SNPs in *GABRβ1* are marked in green. The SNPs significantly associated with alcohol dependence risk are highlighted in yellow. The position of the region homologous to the sequence surrounding the *Gabrβ1* mutations reported by Anstee *et al.* (2013) are included for reference and are marked in red**.** The five SNPs in *GABRB1* with moderate genotypic association in the post hoc analysis are shown in blue.

Abbreviations: SNP: single nucleotide polymorphism

Exons have been increased in size to aid visualisation

Supplementary Figure 2. Fine mapping of the 36 imputed SNPs associated with alcohol dependence, by position on Chromosome 4.

The colour of the markers represents the degree of inter-SNP linkage with red representing the highest degree. rs76112682, which is highlighted in purple, is the most significantly associated SNP. The recombination map is plotted as a blue line graph

Table 1: Genotype counts and tests of allelic associations between the **directly genotyped** SNPs in *GABRβ1* and alcohol dependence risk.

SNP	Position	Group	n	Minor Allele	Genotype Counts			MAF	Chi-square	OR (95% CI)	Significance P	Significance P corrected
					GG	GT	TT					
rs11735112	47031755	Cases	435	G	109	207	119	0.49	0.63	1.08 (0.90-1.29)	0.43	1.00
		Controls	543		119	273	151	0.47				
					TT	TC	CC					
rs6447540	47208869	Cases	440	T	4	51	385	0.07	1.45	1.26 (0.87-1.82)	0.23	1.00
		Controls	554		1	58	495	0.05				
					CC	CG	GG					
rs4695199	47210752	Cases	449	C	4	67	378	0.08	2.47	1.31 (0.94-1.83)	0.12	1.00
		Controls	553		1	70	482	0.07				
					TT	TC	CC					
rs2044081	47216323	Cases	448	T	16	111	321	0.16	2.15	1.20 (0.94-1.54)	0.14	1.00
		Controls	554		5	141	408	0.14				
					CC	CT	TT					
rs1372497	47220321	Cases	444	C	68	181	195	0.36	0.80	1.09 (0.90-1.31)	0.37	1.00
		Controls	552		63	247	242	0.34				
					GG	GT	TT					
rs17539445	47229399	Cases	444	G	15	115	314	0.16	2.44	1.22 (0.95-1.56)	0.12	1.00
		Controls	550		4	144	402	0.14				
					GG	GA	AA					
rs7672100	47263573	Cases	432	G	34	169	229	0.27	0.02	1.02 (0.83-1.24)	0.88	1.00
		Controls	549		43	212	294	0.27				
					TT	TA	AA					
rs12651232	47272719	Cases	437	T	8	91	338	0.12	1.59	0.84 (0.65-1.10)	0.21	1.00
		Controls	550		10	136	404	0.14				
					GG	GT	TT					

rs4586906	47276286	Cases	445	G	77	193	175	0.39	0.25	1.05 (0.87-1.26)	0.62	1.00
		Controls	554		81	258	215	0.38				
					CC	CG	GG					
rs13127214	47278539	Cases	448	C	6	84	358	0.11	0.55	1.12 (0.83-1.49)	0.46	1.00
		Controls	551		1	105	445	0.10				
					GG	GC	CC					
rs4695209	47283017	Cases	441	G	0	57	384	0.06	0.01	1.02 (0.71-1.47)	0.91	1.00
		Controls	552		3	64	485	0.06				
					GG	GT	TT					
rs2078610	47287037	Cases	441	G	63	229	149	0.40	0.25	1.05 (0.87-1.26)	0.62	1.00
		Controls	548		96	237	215	0.39				
					GG	GC	CC					
rs41311286	47425856	Cases	437	G	0	3	434	0.003	0.15	0.75 (0.18-3.17)	0.70	1.00
		Controls	550		0	5	545	0.005				

Abbreviations: SNP: Single Nucleotide Polymorphism; MAF: Minor Allele Frequency; OR: Odds Ratio; CI: Confidence Interval;

Table 2: Estimated genotype counts and tests of genotypic association for **imputed SNPs** in *GABRβ1* with alcohol dependence risk

SNP	Group	n	Minor Allele	Genotype Counts			MAF	OR (95% CI)	Significance	
									P	$P_{corrected}$
rs2044081*				TT	TC	CC				
	Cases	448	T	16	111	321	0.16	4.1 (1.5-11.2)	$3.36x10^{-3}$	$3.89x10^{-2}$
	Controls	554		5	141	408	0.14			
rs17539445*				GG	GT	TT				
	Cases	444	G	15	115	314	0.16	4.8 (1.6-14.5)	$2.41x10^{-3}$	$2.78x10^{-2}$
	Controls	550		4	144	402	0.14			
rs76112682†				TT	TC	CC				
	Cases	441	T	14	103	324	0.15	5.9 (1.7-20.6)	$1.74x10^{-3}$	$2.01x10^{-2}$
	Controls	541		3	127	411	0.12			
rs141719901†				AA	AAG	AGAG				
	Cases	441	A	14	103	314	0.15	5.9 (1.7-20.6)	$1.74x10^{-3}$	$2.01x10^{-2}$
	Controls	541		3	127	411	0.12			
rs3114087				AA	AG	GG				
	Cases	427	A	13	90	324	0.14	5.5 (1.5-19.3)	$3.11x10^{-3}$	$3.60x10^{-2}$
	Controls	526		3	114	409	0.11			

Abbreviations: SNP: Single Nucleotide Polymorphism; MAF: Minor Allele Frequency; OR: Odds Ratio; CI: Confidence Interval

*SNPs also directly genotyped

†SNPs in complete linkage disequilibrium

Supplementary Table 1: Genotypic association between the **directly genotyped** SNPs in *GABRβ1* and alcohol dependence risk

SNP (Model)	Group	n	Minor Allele	Genotype Counts			MAF	OR (95% CI)	Significance P	Significance P corrected
				GG	GT	TT				
rs11735112 (Recessive)	Cases	435	G	109	207	119	0.49	0.84 (0.62-1.13)	0.25	1.00
	Controls	543		119	273	151	0.47			
				TT	TC	CC				
rs6447540 (Recessive)	Cases	440	T	4	51	385	0.07	0.20 (0.02-1.77)	0.10	1.00
	Controls	554		1	58	495	0.05			
				CC	CG	GG				
rs4695199 (Recessive)	Cases	449	C	4	67	378	0.08	0.20 (0.02-1.81)	0.11	1.00
	Controls	553		1	70	482	0.07			
				TT	TC	CC				
rs2044081 (Recessive)	Cases	448	T	16	111	321	0.16	0.24 (0.09-0.68)	$3.36X10^{-3}$	$3.36x10^{-2}$
	Controls	554		5	141	408	0.14			
				CC	CT	TT				
rs1372497 (Recessive)	Cases	444	C	68	181	195	0.36	0.71 (0.49-1.03)	0.07	0.70
	Controls	552		63	247	242	0.34			
				GG	GT	TT				
rs17539445 (Recessive)	Cases	444	G	15	115	314	0.16	0.21 (0.07-0.64)	$2.41X10^{-3}$	$2.41x10^{-2}$
	Controls	550		4	144	402	0.14			
				GG	GA	AA				
rs7672100 (Dominant)	Cases	432	G	34	169	229	0.27	0.98 (0.76-1.16)	0.87	1.00
	Controls	549		43	212	294	0.27			
				TT	TA	AA				
rs12651232 (Dominant)	Cases	437	T	8	91	338	0.12	1.23 (0.92-1.65)	0.15	1.00
	Controls	550		10	136	404	0.14			
				GG	GT	TT				

rs4586906 (Recessive)	Cases	445	G	77	193	175	0.39	0.82 (0.58-1.15)	0.25	1.00
	Controls	554		81	258	215	0.38			
				CC	CG	GG				
rs13127214 (Recessive)	Cases	448	C	6	84	358	0.11	0.13 (0.02-1.12)	0.03	0.29
	Controls	551		1	105	445	0.10			
				GG	GC	CC				
rs4695209* (Recessive)	Cases	441	G	0	57	384	0.06	0.98 (0.98-1.02)	0.92	1.00
	Controls	552		3	64	485	0.06			
				GG	GT	TT				
rs2078610 (Dominant))	Cases	441	G	63	229	149	0.40	0.79 (0.61-1.03)	0.07	0.77
	Controls	548		96	237	215	0.39			
				GG	GC	CC				
rs41311286* (Recessive)	Cases	437	G	0	3	434	0.003	1.33 (0.32-5.56)	0.97	1.00
	Controls	550		0	5	545	0.005			

Abbreviations: SNP: Single Nucleotide Polymorphism; MAF: Minor Allele Frequency; OR: Odds Ratio; CI: Confidence Interval;
Significantly associated SNPs are annotated in bold type