

***GABBR1* and *SLC6A1*, two genes involved in modulation of GABA synaptic transmission influence risk for alcoholism; results from three ethnically diverse populations**

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SUPPLEMENTARY MATERIALS

Genotyping method: *SLC6A1* promoter indel polymorphism

TABLE S1: *GABBR1* rs29220 minor allele frequencies and Hardy-Weinberg p values in the 1000 Genomes population samples and our Plains Indian sample

FIGURE S1: *GABBR1* haplotype block structure in African Americans and Finnish Caucasians

FIGURE S2: *GABBR1* haplotype frequencies in African American and Finnish Caucasian men

FIGURE S3: Typical TaqMan genotype discrimination plot for *GABBR1* rs29220: African American samples: cases and controls combined

FIGURE S4: Typical TaqMan genotype discrimination plot for *GABBR1* rs29220: Finnish Caucasian samples: cases and controls combined

FIGURE S5: HapMap Caucasians: *GABBR1* rs29220 is in LD with two intronic *HLA-G* SNPs but is not in LD with SNPs in the 3' UTR, the likely location of balanced selection

Genotyping method for *SLC6A1* promoter indel polymorphism

A 21-bp insertion/deletion polymorphism in the *SLC6A1* promoter region that creates a second tandem copy of the sequence has been shown to create an enhancer element, thereby increasing gene expression (Hirunsatit et al, 2007, 2009).

The functional promoter polymorphism was amplified from genomic DNA from the AA sample using the primer sequences: Forward 5'- (AAGGACAGGCCAACGGAAGCA)-3' and Reverse-5'(CTCTTG CAGCTTCGCCGCCAG)-3'. Owing to the high GC content in the VNTR region, amplification was performed using Invitrogen's PlatinumTaq (1.25 units), 1X Optimized buffer A and 1X PCR Enhancer, according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA), with 5 μ M of each primer and 2.5 mM dNTPs in a total reaction volume of 20 μ l. Amplifications were performed on a Perkin-Elmer 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) with one cycle at 95°C for 2 min followed by 35 cycles of 95°C for 30 s, 70°C for 30 s, 72°C for 30 s, and a final 10 min extension at 72°C. The forward primer was labeled with the fluorescent dye 6-FAM, amplicons were visualized with GeneScan-500 LIZ Size Standard (Applied Biosystems, Foster City, CA, USA) and analyzed on an ABI 3730 capillary sequencer. Allele sizes (allele1, 375 bp; allele2, 396 bp) were determined using GeneMapper v4.0 (Applied Biosystems, Foster City, CA, USA).

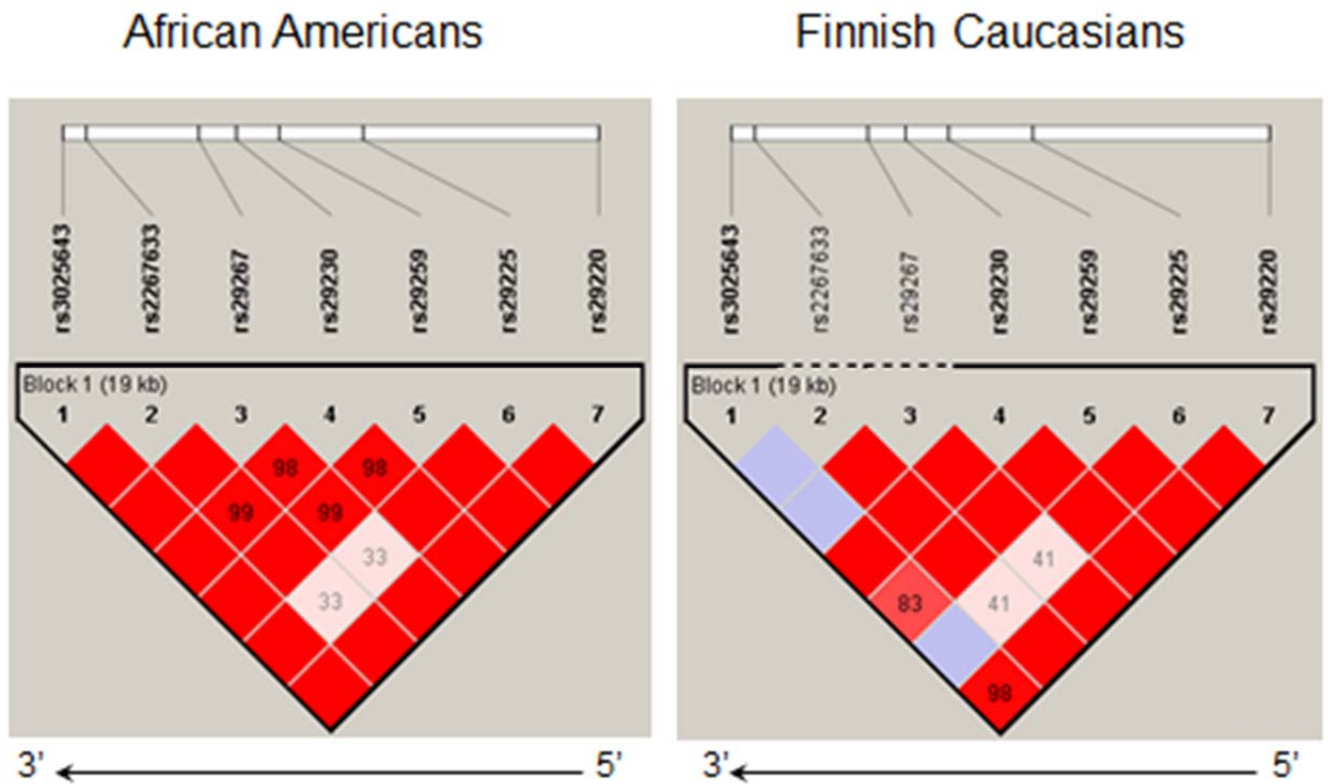
TABLE S1

GABBR1 rs29220 minor allele frequencies and Hardy-Weinberg p values in the 1000 Genomes population samples and our Plains Indian sample

1000 Genome Population Description		N	HWE P value	rs29220 MAF
	AFRICAN			
YRI	Yoruba in Ibadan, Nigeria	108	0.15	0.33
ESN	Esan in Nigeria	99	0.64	0.32
GWD	Gambian in Western Division, The Gambia	113	0.24	0.34
LWK	Luhya in Webuye, Kenya	99	0.65	0.35
MSL	Mende in Sierra Leone	81	0.16	0.31
ACB	ACB - African Caribbean in Barbados	65	0.65	0.37
ASW	African Ancestry in Southwest USA	61	0.28	0.36
	SOUTHEASTERN ASIAN			
CHS	Southern Han Chinese, China	105	0.28	0.43
CHB	Han Chinese in Beijing, China	103	0.17	0.40
CDX	Chinese Dai in Xishuangbanna, China	94	0.24	0.39
JPT	Japanese in Tokyo, Japan	103	0.99	0.37
KHV	Kinh in Ho Chi Minh City, Vietnam	99	0.63	0.40
	SOUTH ASIAN			
BEB	Bengali in Bangladesh	84	0.01	0.38
PJL	Punjabi in Lahore, Pakistan	93	0.88	0.42
ITU	Indian Telugu in the UK	100	0.80	0.41
STU	Sri Lankan Tamil in the UK	96	0.65	0.47
GIH	Gujarati Indian in Houston, TX, USA	103	0.98	0.31
	CAUCASIAN			
FIN	Finnish in Finland	99	0.96	0.44
GBR	British in England and Scotland	91	0.34	0.20
IBS	Iberian populations in Spain	107	0.67	0.27
TSI	Toscani in Italy	107	0.16	0.27
CEU	Northern / Western European in Utah, USA	99	0.85	0.23
	AMERICAS			
PEL	Peruvian in Lima, Peru	79	0.71	0.21
CLM	Colombian in Medellin, Colombia	93	0.90	0.40
MXL	Mexican Ancestry in Los Angeles, CA, USA	64	0.69	0.36
PUR	Puerto Rican in Puerto Rico	104	0.72	0.32
LNG PI	LNG Plains Indians, Oklahoma, USA			0.39

FIGURE S1

***GABBR1* haplotype block structure in African Americans and Finnish Caucasians**



The numbers in the squares refer to pairwise linkage disequilibrium measured as D' . Haplotype blocks were defined using a setting of average pairwise D' within-block of ≥ 0.80 . The direction of gene transcription is from right to left.

FIGURE S2

***GABBR1* haplotype frequencies in African American and Finnish Caucasian men**

African American men							rs29220	
H1	1	1	1	1	1	1	1	0.38
H2	1	1	1	1	1	1	2	0.35
H3	2	1	1	1	1	1	1	0.10
H5	1	1	1	2	2	2	1	0.06
H6	1	2	2	2	2	2	1	0.04
H4	1	2	2	2	2	1	1	0.07

Finnish Caucasian men							rs29220	
H1	1	1	1	1	1	1	1	0.20
H2	1	1	1	1	1	1	2	0.42
H3	2	1	1	1	1	1	1	0.29
H5	1	1	1	2	2	2	1	0.04
H6	1	2	2	2	2	2	1	0.02
H4	1	2	2	2	2	1	1	0.03

The haplotype block extends from the intergenic SNP rs3025643 to the intronic SNP rs29220. The order of SNPs is given in Table 1. The 6 haplotypes account for 99% of the haplotype diversity and are arranged in a cladistics grouping. Alleles 1 and 2 are located on opposite DNA strands.

FIGURE S3

Typical TaqMan genotype discrimination plot for *GABBR1* rs29220: African American samples: cases and controls combined

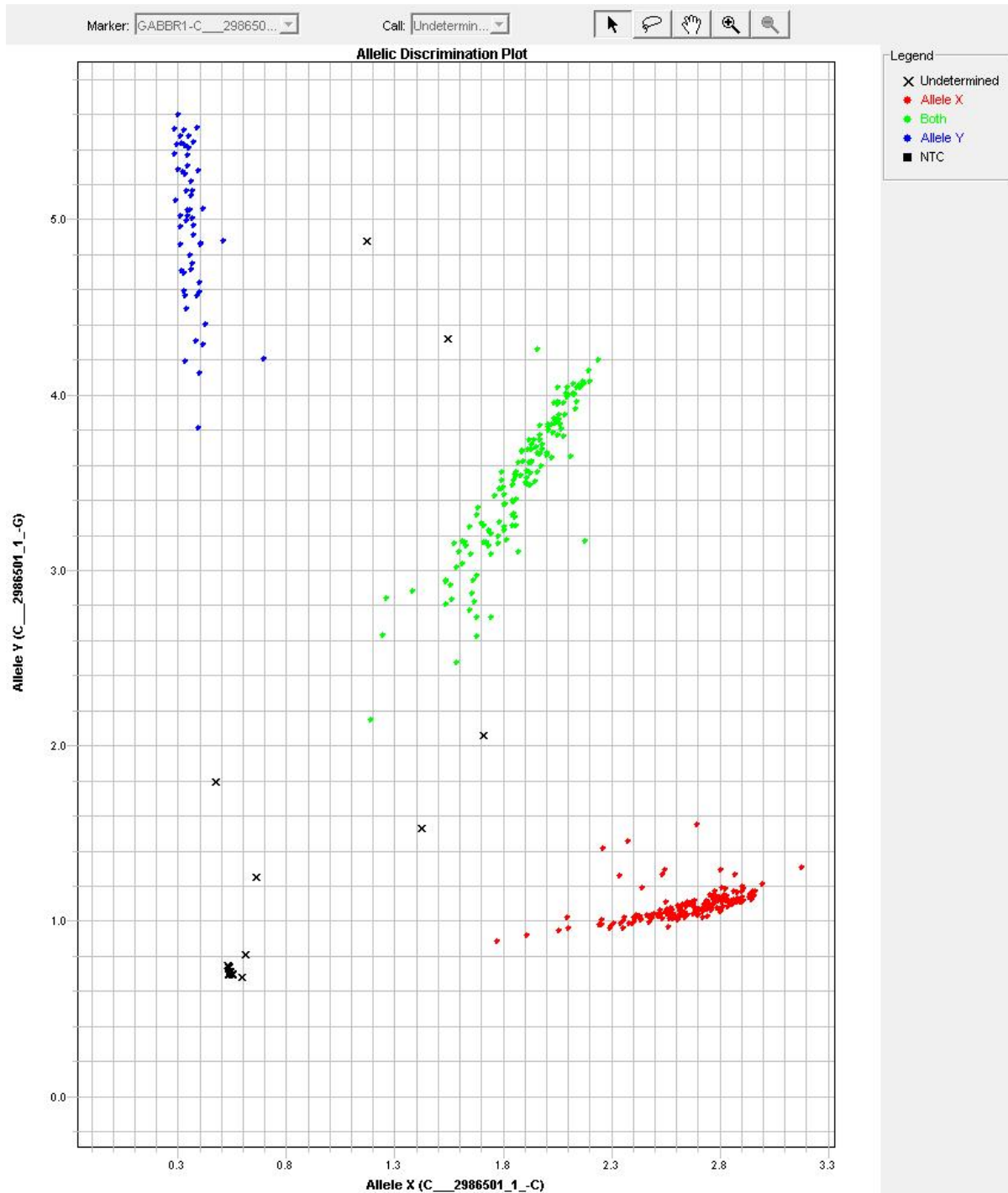


FIGURE S4

Typical TaqMan genotype discrimination plot for *GABBR1* rs292220: Finnish Caucasian samples: cases and controls combined

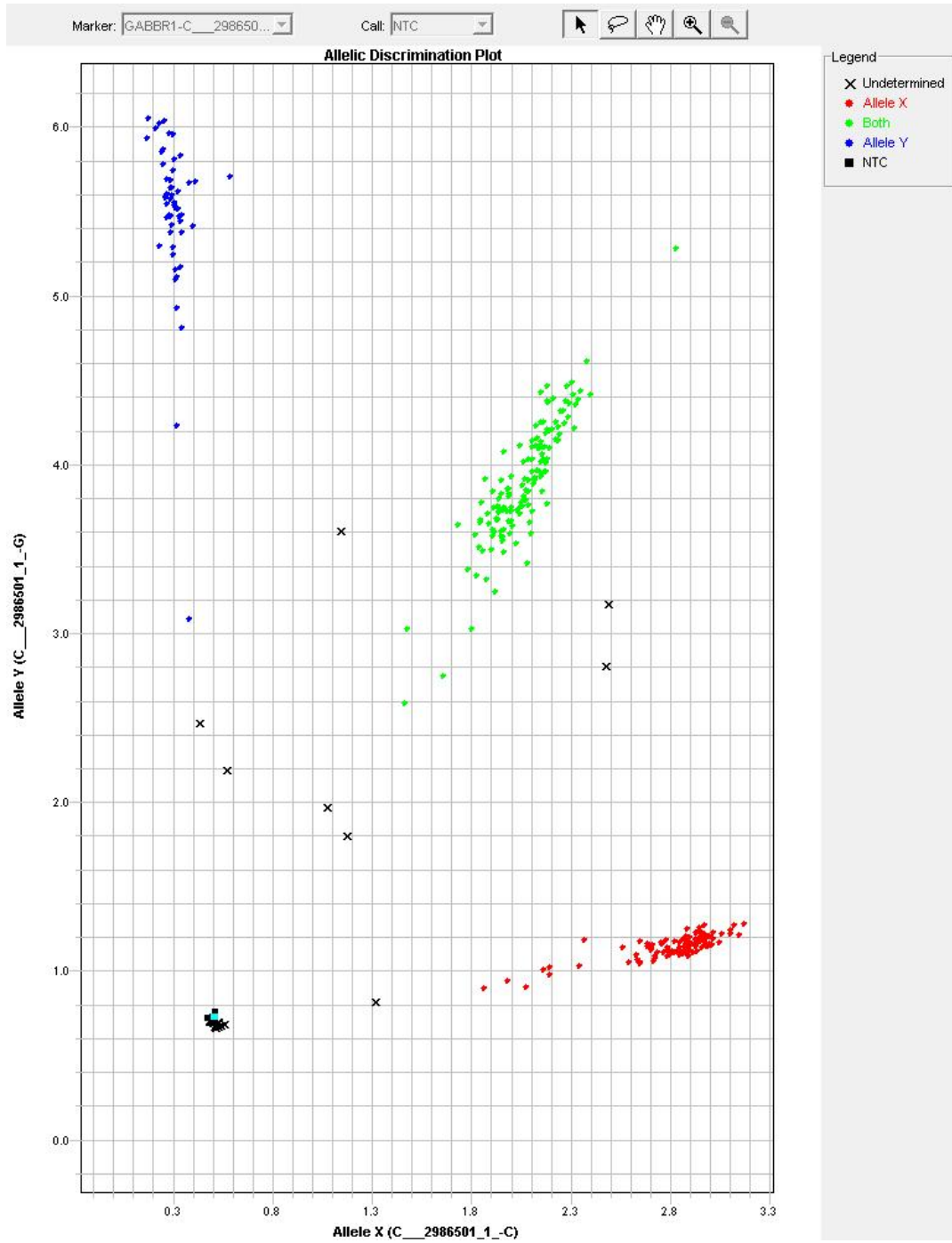


FIGURE S5

HapMap Caucasians: *GABBR1* rs29220 is in LD with two intronic *HLA-G* SNPs but is not in LD with SNPs in the 3' UTR, the likely location of balanced selection

