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CORRESPONDENCE

Association of rare *PTP4A1-PHF3-EYS* variants with alcohol dependence

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In a recent genome-wide association study (GWAS), we identified a unique novel, functional and replicable risk genomic region for alcohol dependence. This region (Chr6: 64,066,604-64,831,120) included the protein tyrosine phosphatase type IVA 1 gene (*PTP4A1*), the plant homeodomain finger protein 3 gene (*PHF3*) and part of the eyes shut homolog gene (*EYS*). It was enriched with numerous replicable common risk variants (minor allele frequency (MAF) >0.05) for alcohol dependence across African–Americans, European–Americans and European–Australians. Within 90 Mb range surrounding this region in the discovery sample, all variants with $P<10^{-4}$ were concentrated in this region. Most of these risk variants had significant *cis*-acting regulatory effects on mRNA expression. The distributions of $-\log(P)$ values for association and functional signals in this region were highly consistent across six independent populations. We thus speculated that this region might harbor a causal variant(s) for alcohol dependence.[1]

Rare variants, which may not be well-captured by a GWAS, may be of equal importance as common variants in the etiology of complex disorders. Recent studies have indicated that rare variants, which usually have strong effects and high penetrance, are involved in the genetic susceptibility to an increasing number of human diseases or traits; for example, plasma levels of high-density lipoprotein cholesterol[2] and type 1 diabetes[3] as well complex neuropsychiatric disorders such as autism.[4–6] However, the associations between rare variants within the *PTP4A1-PHF3-EYS* region and alcohol dependence have never been tested. Furthermore, alcohol dependence has high rates of comorbidity with numerous neuropsychiatric disorders, including anxiety disorders, major depression, bipolar disorders, schizophrenia, post-traumatic stress disorder and so on. The associations between rare *PTP4A1-PHF3-EYS* variants and these neuropsychiatric disorders have never been tested either.

To further explore the role of *PTP4A1-PHF3-EYS* region in alcohol dependence, in the present study, we imputed the untyped variants in this *PTP4A1-PHF3-EYS* region across 21 independent cohorts with 11 different neuropsychiatric disorders (Table 1), and then examined the associations between rare *PTP4A1-PHF3-EYS* variants ($0<$MAF <0.05 in controls) and these disorders, so that we could test whether rare *PTP4A1-PHF3-EYS* variants were associated with alcohol dependence and whether these associations were specific for alcohol dependence. The data of these disorders were all of those with neuropsychiatric and neurological disorders available for our analysis from the dbGaP database (http://www.ncbi.nlm.nih.gov/gap/).

A total of 49 268 subjects in these 21 cohorts were analyzed (Table 1), including 10 554 subjects in 3 cohorts with alcohol dependence (DSM-IV) and 38 714 subjects in other 18 non-alcoholism cohorts. The European–Australian cohort with alcohol dependence included 871 parents, 1645 affected offspring and 3922 unaffected offspring. Detailed demographic information for these samples has been published (Supplementary Table S1).[1,7–9] We used the same strategies as previously to maximize the success rate and accuracy of imputation and to stringently clean the phenotype and genotype data.[8,9] Associations between individual variants and diseases were tested using the program PLINK (for case–control data) and FBAT (for family-based data).[8,9] The experiment-wide significance levels (α) were set at 1.4×10^{-5} (in AAs) and 2.1×10^{-5} (in EAs) for these individual rare variant analyses based on the correction for the numbers of effective genetic markers ($n=175$, 115 in AAs and EAs, respectively; calculated from the entire marker set by the adjusted Bonferroni-type program SNPSpD) and the number of cohorts (that is, $n=21$). Furthermore, associations between rare variant constellations across the entire *PTP4A1-PHF3-EYS* region and diseases were tested using the Variable MAF Threshold (VT) test implemented in the program SCORE-Seq,[9] in order to explore the synthetic effects of rare variants within the entire region. In VT test, the weight was set at 1 in the regression model, while the MAF threshold varied between 0 and 0.05. Finally, a total of 197–956 single-nucleotide polymorphisms (SNPs) with $0<$MAF<0.05 (in controls) were extracted for association analysis. The MAFs and minimal *P*-values of the most significant risk SNPs, the SNP numbers with $P<0.05$ and the *P*-values for VT tests are shown in Table 1.

We found a rare variant constellation across the entire region that was significantly associated with alcohol dependence in European–Australians ($P=4.2\times10^{-3}$), and modestly associated with schizophrenia in European–Americans (in GAIN data: $P=0.033$), but not with any other neuropsychiatric disease examined ($P>0.10$). Additionally, single-point association analysis showed that 0–108 individual SNPs were nominally associated with these 11 diseases ($P<0.05$), but none of them were significant after experiment-wide correction (Table 1).

We confirmed that rare variants within the *PTP4A1-PHF3-EYS* region had significant synthetic effects on alcohol dependence in European–Australians, consistent with our previous findings in a GWAS. Both studies support our conclusion that this region

Table 1 Associations between rare *PTP4A1-PHF3-EYS* variants and different neuropsychiatric disorders

										Affected		Unaffected	
Human diseases	*Ethnicity*	*Design*	*Data set name*	*SNP # (total)*	*SNP # (P < 0.05)*	*VT test P-value*	*Minimal P-value*	*Most sig. SNP*	*Gene*	N	*MAF*	N	*MAF*
Alcoholism	EAu	Fam	OZ-ALC	504	9	$\mathbf{4.2 \times 10^{-3}}$	0.006	rs79051763	*5′ to PTP4A1*	1645	0.006	1645	0.016
Alcoholism	AA	CC	SAGE + COGA	956	22	0.335	9.5×10^{-4}	rs319919	*EYS*	681	0.071	508	0.039
Alcoholism	EA	CC	SAGE + COGA	467	17	0.230	0.015	rs114282789	*EYS*	1409	0.016	1518	0.008
Schizophrenia	EA	CC	GAIN	251	26	**0.033**	0.018	rs72872860	*EYS*	1351	0.011	1378	0.019
Schizophrenia	AA	CC	GAIN	793	56	0.320	0.009	rs11755458	*EYS*	1195	0.007	954	0.017
Schizophrenia	EA	CC	MGS_nonGAIN	234	4	0.921	0.004	rs4710443	*EYS*	1437	0.019	1347	0.040
Bipolar disorder	EA	CC	BDO + GRU	197	4	0.685	0.015	rs1779781	*5′ to PTP4A1*	368	0.051	1034	0.029
Bipolar disorder	EA	CC	BARD + GRU	213	1	0.836	0.019	rs6914590	*PHF3*	653	0.009	1034	0.002
Bipolar disorder	AA	CC	BARD + GRU	618	2	0.350	0.032	rs1779776	*5′ to PTP4A1*	141	0.021	671	0.007
ADHD	CA	Fam	IMAGE	439	73	0.566	0.001	rs115865360	*EYS*	924	0.026	924	0.033
Autism	EA	Fam	AGP	488	36	0.261	1.6×10^{-4}	rs116469676	*EYS*	1330	0.004	1330	0.068
Major depression	CA	CC	PRSC	470	108	0.516	1.4×10^{-4}	rs76370578	*5′ to PTP4A1*	1805	0.040	1820	0.021
Alzheimer's disease	CA	Fam	LOAD × 4	478	7	0.825	0.005	rs116595147	*EYS*	2298	0.002	2298	0.0004
Alzheimer's disease	EA	CC	GenADA	249	0	0.987	0.126	rs74851838	*5′ to PTP4A1*	806	0.004	782	0.0007
ALS	CA	CC	GRU	554	0	0.990	0.060	rs77118852	*5′ to PTP4A1*	261	0.002	246	0.014
Early onset stroke	EA	CC	GEOS × 3	468	9	0.163	0.015	rs80059369	*EYS*	372	0.048	430	0.023
Early onset stroke	AA	CC	GEOS × 3	936	43	0.789	0.003	rs115889991	*EYS*	309	0.046	290	0.009
Ischemic stroke	CA	CC	ISGS	367	6	0.830	0.009	rs116215749	*3′ to PTP4A1*	219	0.044	266	0.013
Parkinson's disease	CA	CC	NGRC	516	9	0.819	4.0×10^{-4}	rs80059369	*EYS*	2000	0.031	1986	0.048
Parkinson's disease	CA	CC	PDRD + GRU	459	1	0.928	0.048	rs79831694	*EYS*	900	0.017	867	0.033
Parkinson's disease	CA	CC	Ing_coriell_pd	462	11	0.749	0.007	rs72875016	*EYS*	940	0.059	801	0.035

Abbreviations: AA, African–American; EA, European–American; EAu, European–Australian; CA, Caucasian; CC, case–control design; MAF, minor allele frequency; N, sample size; SNP, single-nucleotide polymorphism; VT, Variable MAF Threshold test (using SCORE-Seq).
Only the most significant risk markers are listed. The corrected significance levels (α) are set at 1.4×10^{-5} (in AAs) and 2.1×10^{-5} (in EAs) based on correction for the numbers of effective genetic markers ($n = 175$, 115 in AAs and EAs, respectively; calculated by SNPSpD) and the number of cohorts (that is, 21). *P* values < 0.05 in VT test are bold.
Data set names correspond to dbGaP. In family-based cohorts, *N* = sample size of offspring; 'affected MAF' = 'transmitted MAF', 'unaffected MAF' = 'untransmitted MAF' in offspring.

might harbor a causal variant(s) for alcohol dependence. Additionally, the rare variant constellation was also modestly associated with schizophrenia in European–Americans, but not with any other neuropsychiatric disorder examined. This genetic commonality between alcohol dependence and schizophrenia may underlie high rate of comorbidity between them. It has been reported that over one-third of patients with schizophrenia have an alcohol use disorder.[10] Our findings support us to postulate that the underlying neuropathological abnormalities of schizophrenia related to the *PTP4A1-PHF3-EYS* variants might potentially facilitate the positive reinforcing effects of alcohol use. Finally, we admit that, as a future work, the imputed genotype data in this study need to be validated by a real molecular experiment, more neuropsychiatric disorders comorbid with alcohol dependence need to be examined, and the current findings warrant replication in independent cohorts.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)