

Association between common alcohol dehydrogenase gene (*ADH*) variants and schizophrenia and autism

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Abstract Humans express at least seven alcohol dehydrogenase (*ADH*) isoforms that are encoded by *ADH* gene cluster (*ADH7–ADH1C–ADH1B–ADH1A–ADH6–ADH4–ADH5*) at chromosome 4. *ADH*s are key catabolic enzymes for retinol and ethanol. The functional *ADH* variants (mostly rare) have been implicated in alcoholism risk. In addition to catalyzing the oxidation of retinol and ethanol, *ADH*s may be involved in the metabolic pathways of several neurotransmitters that are implicated in the neurobiology of neuropsychiatric disorders. In the present study, we comprehensively examined the associations between common *ADH* variants [minor allele frequency (MAF) >0.05] and 11 neuropsychiatric and neurological disorders. A total of 50,063 subjects in 25 independent cohorts were analyzed. The entire *ADH* gene cluster was imputed across these 25 cohorts using the same reference panels. Association analyses were conducted, adjusting for multiple comparisons. We found 28 and 15 single nucleotide poly-

morphisms (SNPs), respectively, that were significantly associated with schizophrenia in African-Americans and autism in European-Americans after correction by false discovery rate (FDR) ($q < 0.05$); and 19 and 6 SNPs, respectively, that were significantly associated with these two disorders after region-wide correction by SNPSpD ($8.9 \times 10^{-5} \leq p \leq 0.0003$ and $2.4 \times 10^{-5} \leq p \leq 0.0003$, respectively). No variants were significantly associated with the other nine neuropsychiatric disorders, including alcohol dependence. We concluded that common *ADH* variants conferred risk for both schizophrenia in African-Americans and autism in European-Americans.

Introduction

Humans express at least seven alcohol dehydrogenase (*ADH*) isoforms, each with slightly different properties

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(Luo et al. 2008). ADHs are expressed predominantly in the liver, the upper digestive tract (from mouth to stomach), and kidney, and partly in the brain (Yoshida et al. 1998). Particularly, because ADHs are key catabolic enzymes for ethanol, *ADH* variants have been implicated in the risk for alcohol dependence by previous studies [reviewed by (Luo et al. 2006)]. However, in addition to catalyzing the oxidation of retinol and ethanol, ADHs may be involved in the metabolic pathways of several neurotransmitters including serotonin, epinephrine, norepinephrine, and dopamine (Holmes 1994; Svensson et al. 1999). The functions of ADHs in the metabolism of these monoamines suggest their potential roles in the etiology of other neuropsychiatric disorders.

ADH isoforms are encoded by *ADH7–ADH1C–ADH1B–ADH1A–ADH6–ADH4–ADH5* gene cluster at chromosome 4. It has been widely reported by candidate gene studies that at least four functional *ADH* gene variants, i.e., rs1229984 (*ADH2**2; Arg48His), rs2066702 (*ADH2**3; Arg370Cys), rs1693482 (*ADH3**2; Arg272Gln), and rs698 (*ADH3**2; Ile350Va), significantly affect the risk for alcohol dependence [reviewed by (Luo et al. 2006)]. These variants are rare in most populations, e.g., in Europeans (minor allele frequency (MAF)_{rs2066702} = 0.000 and MAF_{rs1229984} = 0.008) and Africans (MAF_{rs1229984} = 0.000, MAF_{rs1693482} = 0.052, and MAF_{rs698} = 0.042). In one of our previous studies, we also found that the rare variant constellation across the entire *ADH* cluster was associated with alcohol dependence in European-Americans, European-Australians, and African-Americans (Zuo et al. 2013b). So far, numerous genome-wide association studies (GWASs) of alcohol dependence using common variants as markers have also been performed; however, only one GWAS identified one common *ADH* variant (rs1789891; MAF = 0.192) that was associated with alcohol dependence at the genome-wide significance level ($p = 1.3 \times 10^{-8}$; OR = 1.46; $\alpha = 5 \times 10^{-8}$) (Frank et al. 2012). This leads to a hypothesis that common *ADH* variants might be associated with other diseases rather than alcohol dependence only. For example, one candidate gene study reported that common variants at *ADH7* were associated with Parkinson's disease (Buervenich et al. 2000). To further test this hypothesis, in the present study, we comprehensively examined the associations between common *ADH* variants (MAF >0.05 in both cases and controls) and 11 neuropsychiatric and neurological disorders including schizophrenia, autism, attention deficit hyperactivity disorder (ADHD), alcoholism, major depression, bipolar disorder, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), early onset stroke, ischemic stroke, and Parkinson's disease in subjects of European or African descent.

Materials and methods

Subjects

A total of 50,063 subjects in 25 independent cohorts with 11 different neuropsychiatric and neurological disorders were analyzed. They included case–control and family-based samples, genotyped on Illumina, Affymetrix, or PERLEGEN microarray platforms. All subjects gave informed consent. Diagnoses, ethnicities, study designs, sample sizes, and dataset names for these cohorts are shown in Table 1. More detailed demographics data of these cohorts were published previously (Stefansson et al. 2009; Anney et al. 2010; Zuo et al. 2011, 2012, 2013a, b).

The African-American schizophrenia cohort came from the GAIN dataset (dbGaP access number: phs000021.v3.p2), including 1,195 cases with schizophrenia and 954 controls. The subjects were genotyped on AFFYMETRIX AFFY_6.0 platform. All subjects were at least 18 years old. The cases included 746 males (41.9 ± 10.8 years) and 449 females (43.0 ± 9.8 years); and the controls included 362 males (46.2 ± 13.7 years) and 592 females (45.0 ± 12.9 years). Affected subjects met lifetime DSM-IV criteria for schizophrenia (American Psychiatric Association 1994). Cases were excluded if they had worse than mild mental retardation, or if their psychotic illness was judged to be secondary to substance use or a neurological disorder. Controls were excluded if they did not deny all of the following psychosis screening questions: treatment for or diagnosis of schizophrenia or schizoaffective disorder; treatment for or diagnosis of bipolar disorder or manic-depression; treatment for or diagnosis of psychotic symptoms such as auditory hallucinations or persecutory delusions.

The Autism cohort came from the AGP dataset (dbGaP access number: phs000267.v1.p1). A total of 1,366 families (trios) contained 4,075 European-American subjects including 1,330 probands with autism. The probands consisted of 1,121 males (7.2 ± 3.2 years) and 209 females (7.1 ± 3.0 years). Affected subjects were diagnosed using the Autism Diagnostic Interview-Revised (ADI-R) and Autism Diagnostic Observation Schedule (ADOS) instruments, and met DSM-IV criteria for autism (American Psychiatric Association 1994). Cases with known karyotypic abnormalities, fragile X mutations, or other genetic disorders were excluded. The subjects were genotyped on ILLUMINA_Human_1 M platform.

Imputation

To make the genetic marker sets highly consistent across the different samples, we imputed the missing single

Table 1 Associations between *ADH* gene cluster and different neuropsychiatric or neurological disorders

Most sig. SNP	Location	Affected		Unaffected		Minimal <i>p</i> value	SNP # (total)	SNP # (<i>p</i> < 0.05)	SNP # (<i>p</i> < α)	SNP # (<i>q</i> < 0.05)	Ethnicity	Human disease	Dataset
		<i>N</i>	MAF	<i>N</i>	MAF								
rs1789916	Intron 3 of ADH1C	1,195	0.114	954	0.158	8.9×10^{-5}	632	50	19	28	AA (CC)	Schizophrenia	GAIN
rs284781	Between ADH1C and ADH7	1,351	0.068	1378	0.090	0.0029	630	7	0	0	EA (CC)	Schizophrenia	GAIN
rs6811453	3'UTR of ADH1A	1,437	0.334	1347	0.289	0.0108	554	25	0	0	EA (CC)	Schizophrenia	MGS_nonGAIN
rs6532803	Between ADH4 and ADH6	98	0.136	20	0.625	0.0049	459	43	0	0	AA (CC)	Schizophrenia	MGS_nonGAIN
rs62323588	Between ADH4 and ADH5	1,331	0.107	2745	0.116	2.4×10^{-5}	921	141	6	15	EA (Fam)	Autism	AGP
rs10516441	Between ADH1C and ADH7	924	0.062	1833	0.051	0.0048	1028	7	0	0	CA (Fam)	ADHD	IMAGE
rs904092	5' flanking to ADH1A	681	0.181	508	0.243	0.0005	916	26	0	0	AA (CC)	Alcoholism	SAGE + COGA
rs1583977	Between ADH1C and ADH7	1,409	0.113	1518	0.093	0.0010	965	13	0	0	EA (CC)	Alcoholism	SAGE + COGA
rs1442486	5'UTR of ADH7	1,645	0.424	3928	0.446	0.0019	940	14	0	0	EAu (Fam)	Alcoholism	OZ-ALC
rs1154415	Intron 4 of ADH5	1,754	0.408	1800	0.446	0.0030	1027	120	0	0	CA (CC)	Major depression	PRSC
rs4147547	Intron 7 of ADH6	368	0.346	1034	0.234	0.0046	623	64	0	0	EA (CC)	Bipolar disorder	BDO + GRU
rs1693435	Between ADH1B and ADH1C	653	0.048	1034	0.105	0.0118	620	1	0	0	EA (CC)	Bipolar disorder	BARD + GRU
rs2851012	3'UTR of ADH7	141	0.158	671	0.244	0.0230	606	4	0	0	AA (CC)	Bipolar disorder	BARD + GRU
rs10014818	3'UTR of ADH6	2,320	0.084	2244	0.082	0.0048	959	19	0	0	CA (Fam)	Alzheimer's disease	LOAD × 4
rs1229981	5'UTR of ADH1B	806	0.132	782	0.087	0.0118	629	12	0	0	EA (CC)	Alzheimer's disease	GenADA
rs1154455	Intron 8 of ADH7	261	0.587	246	0.388	0.0017	893	61	0	0	CA (CC)	ALS	GRU
rs1154479	5'UTR of ADH7	372	0.381	430	0.292	0.0012	969	264	0	0	EA (CC)	Early Onset stroke	GEOS × 3
rs1497378	Between ADH1A and ADH6	309	0.139	290	0.207	0.0013	880	45	0	0	AA (CC)	Early onset stroke	GEOS × 3
rs2298754	Intron 8 of ADH1C	219	0.135	266	0.070	0.0050	916	26	0	0	CA (CC)	Ischemic stroke	ISGS
rs4699701	Intron 4 of ADH5	2,000	0.094	1,986	0.076	0.0043	959	85	0	0	CA (CC)	Parkinson's disease	NGRC
rs2718682	5' flanking to ADH7	900	0.294	867	0.334	0.0113	933	7	0	0	CA (CC)	Parkinson's disease	PDRD + GRU
rs67631357	Between ADH1B and ADH1C	677	0.273	538	0.216	0.0016	953	32	0	0	CA (CC)	Parkinson's disease	pd_v3_550v3
rs2584462	Between ADH1C and ADH7	263	0.091	263	0.238	0.0264	944	5	0	0	CA (CC)	Parkinson's disease	pd_v3_300v1
rs7655945	5'UTR of ADH6	263	0.276	263	0.210	0.0702	771	0	0	0	CA (CC)	Parkinson's disease	pd_v3_250 s
rs146919815	5'UTR of ADH7	940	0.122	801	0.167	0.0381	947	10	0	0	CA (CC)	Parkinson's disease	Ing_coriell_pd_v3

Only the most significant risk markers are listed. The significance level (α) is corrected for the numbers of effective genetic markers (calculated by SNPSpD) [GenADA: Li et al. 2008; Filippini et al. 2009; AGP: The AGP Consortium 2007, 2010a, b]

N sample size, *MAF* minor allele frequency, *CC* case-control sample, *Fam* family sample, *EA* European-American, *EAu* African-American, *AA* African-American, *CA* Caucasian, *ADHD* attention deficit hyperactivity disorder, *ALS* amyotrophic lateral sclerosis

nucleotide polymorphisms (SNPs) across the entire *ADH* gene cluster (Chr4: 100204900–100631900) in all samples of 25 cohorts using the same reference panels (i.e., 1,000 genome project and HapMap 3 panels). We used the programs IMPUTE2 (Howie et al. 2009) and BEAGLE (Browning and Browning 2009) for imputation, with the reference CEU panel for the samples of European descent and the reference YRI panel for the samples of African descent. We maximized the success rate and accuracy of imputation and minimized the false-positives during the imputation process. Only the genotypes that were consistently imputed from the two independent reference panels (i.e., 1,000 genome project and HapMap 3 panels) and the genotypes that were consistently imputed by both IMPUTE2 and BEAGLE were selected for analysis. The uncertainty rate of inference for missing genotypes was controlled at <1 %. Furthermore, only the SNPs that had similar MAFs (with frequency difference <2 % within the same ethnicity) in the healthy controls across different cohorts and HapMap database were selected for analysis. After this strict selection, we were highly confident with the quality of these imputed genotype data. Checking the imputed genotypes in all of our four family-based cohorts, we did not find any one individual with more than 0.1 % Mendelian inconsistency (considering all SNPs tested) or any one SNP with more than 0.1 % Mendelian inconsistency (considering all individuals tested).

Data cleaning

We stringently cleaned the phenotype data [detailed previously (Zuo et al. 2012)] and then the imputed genotype data. Subjects with poor genotypic data, allele discordance, sample relatedness, a mismatch between self-identified and genetically inferred ethnicity, or a missing genotype call rate ≥ 2 % across all SNPs were filtered out. Furthermore, we filtered out the monomorphic SNPs and the SNPs with allele discordance, Mendelian errors (in family samples), an overall missing genotype call rate ≥ 2 %, MAFs <0.05 in either cases or controls, or Hardy–Weinberg Equilibrium (HWE) ($p < 10^{-4}$) within controls. We also filtered out the SNPs with MAF differences ≥ 2 % or missing rate differences ≥ 2 % between two samples that had the same phenotype and microarray platform. The cleaned sample sizes and cleaned SNP numbers are shown in Table 1.

Association test

For case–control cohorts, the allele frequencies were compared between cases and controls using logistic regression analysis as implemented in the program PLINK (Purcell et al. 2007). Diagnosis served as the dependent variable, alleles served as the independent variables, and ancestry

proportions (to control for admixture effects) (Zuo et al. 2012), sex, and age served as covariates. The ancestry proportions for each individual were estimated using the program STRUCTURE (Pritchard et al. 2000). For those non-alcoholism cohorts, alcohol drinking behavior, if available, was also included as a covariate. Furthermore, for family cohorts, we used DFAM as implemented in PLINK to test associations (as effective as the program FBAT). The $-\log(p)$ value distribution is shown in Fig. 1. The MAFs and minimal p values of the most significant risk SNPs are shown in Table 1. The statistically significant risk SNPs associated with diseases ($p < \alpha$) are shown in Table 2. Finally, we did bioinformatic analysis of these significant risk SNPs to explore their potential functions using the UCSC Genome Browser including ENCODE data (<http://genome.ucsc.edu>).

Correction for multiple testing

The experiment-wide significance level (α) was corrected for the number of effective markers that were calculated from the entire marker set by the program SNPSpD. SNPSpD is based on an adjusted Bonferroni correction

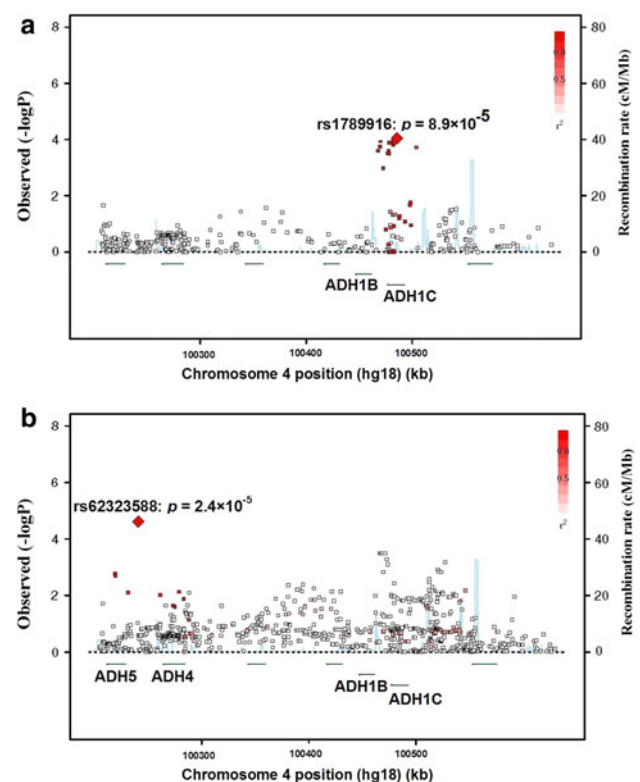


Fig. 1 Regional association plots in *ADH* cluster [left Y-axis corresponds to $-\log(p)$ value; right Y-axis corresponds to recombination rates; X-axis corresponds to genomic positions; quantitative color gradient corresponds to r^2 ; red squares represent peak SNPs. **a** Regional association plot in African-American GAIN schizophrenia sample; **b** regional association plot in European-American autism sample]

Table 2 Significant risk SNPs associated with schizophrenia and autism ($p < \alpha$)

Disease	SNP	Position	Gene	Location	OR	p value	q value	Bioinformatics
Schizophrenia	rs35348915	100467780	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.47	0.0003	0.008	
Schizophrenia	rs71612682	100467929	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.47	0.0003	0.008	CpG
Schizophrenia	rs1789892	100469681	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.47	0.0002	0.008	TFBS
Schizophrenia	rs36070167	100469884	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.49	0.0001	0.008	
Schizophrenia	rs2866152	100470090	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.48	0.0001	0.008	TFBS
Schizophrenia	rs1612735	100477030	<i>ADH1C</i>	Intron 8	1.40	0.0003	0.009	TFBS
Schizophrenia	rs1442480	100477704	<i>ADH1C</i>	Intron 8	1.41	0.0003	0.008	TFBS/CNV
Schizophrenia	rs1789900	100477740	<i>ADH1C</i>	Intron 8	1.41	0.0003	0.008	TFBS/CNV
Schizophrenia	rs1442481	100478070	<i>ADH1C</i>	Intron 8	1.40	0.0003	0.009	Conserved
Schizophrenia	rs1789901	100478392	<i>ADH1C</i>	Intron 8	1.42	0.0001	0.008	TFBS
Schizophrenia	rs1662060	100478864	<i>ADH1C</i>	Intron 8	1.43	0.0001	0.008	TFBS
Schizophrenia	rs1789904	100481431	<i>ADH1C</i>	Intron 6	1.44	0.0001	0.008	TFBS
Schizophrenia	rs1789906	100482242	<i>ADH1C</i>	Intron 6	1.43	0.0001	0.008	TFBS
Schizophrenia	rs904094	100482275	<i>ADH1C</i>	Intron 6	1.43	0.0001	0.008	
Schizophrenia	rs1789912	100482965	<i>ADH1C</i>	Intron 6	1.43	0.0001	0.008	TFBS/CpG/Conserved
Schizophrenia	rs1789916	100485719	<i>ADH1C</i>	Intron 3	1.45	8.9×10^{-5}	0.008	TFBS/CpG/CNV
Schizophrenia	rs1693427	100485850	<i>ADH1C</i>	Intron 3	1.46	8.9×10^{-5}	0.008	TFBS
Schizophrenia	rs1693428	100485954	<i>ADH1C</i>	Intron 3	1.45	0.0001	0.008	TFBS
Schizophrenia	rs1154434	100504035	<i>Intergenic</i>	11 kb to 5' of <i>ADH1C</i>	1.52	0.0002	0.008	TFBS
Autism	rs62323588	100240894	<i>Intergenic</i>	Between <i>ADH5</i> and <i>4</i>	1.50	2.4×10^{-5}	0.012	Long RNA
Autism	rs2213041	100466374	<i>Intergenic</i>	Between <i>ADH1B</i> & <i>IC</i>	1.33	0.0003	0.026	TFBS
Autism	rs1789889	100466888	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.33	0.0003	0.026	TFBS
Autism	rs2226898	100467950	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.33	0.0003	0.026	TFBS
Autism	rs1789890	100468907	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.33	0.0003	0.026	TFBS
Autism	rs1229863	100471409	<i>Intergenic</i>	Between <i>ADH1B</i> and <i>IC</i>	1.33	0.0003	0.026	Conserved

Conserved located at a species-conserved element, *TFBS* located at a transcription factor binding site, *CpG* located at a methylated CpG island, *CNV* located at a copy number variant (CNV), *long RNA* located at a long RNA transcript (>200 bases)

method (Li and Ji 2005). The linkage disequilibrium (LD) structures were highly similar across different phenotype groups within the same ethnicity. Approximately, 100 effective SNPs captured most information of all common SNPs across the entire *ADH* gene cluster both in subjects of European and African descents. Thus, the corrected significance level (α) was set at 0.0005. The numbers of risk SNPs that were nominally ($p < 0.05$) or significantly ($p < \alpha$) associated with phenotypes are shown in Table 1. Finally, q value for each SNP was estimated from p values within each phenotype group using the R package QVALUE (Storey and Tibshirani 2003). The numbers of risk SNPs with $q < 0.05$ and the q values for the significant risk SNPs are shown in Tables 1 and 2, respectively.

Results

Among a total of 632 common SNPs in African-American GAIN samples, 50 SNPs were nominally associated with schizophrenia ($p < 0.05$), 28 of which were significantly

associated with schizophrenia after false discovery rate (FDR) correction ($q < 0.05$). With region-wide correction for multiple testing by SNPSpD, 19 SNPs were significantly associated with schizophrenia ($8.9 \times 10^{-5} \leq p \leq 0.0003$). These 19 SNPs were in high LD with one another ($D' = 1$). Among a total of 921 common SNPs in European-Americans, 141 SNPs were nominally associated with autism ($p < 0.05$), 15 of which survived FDR correction ($q < 0.05$), and 6 of which survived region-wide SNPSpD correction ($2.4 \times 10^{-5} \leq p \leq 0.0003$) (Tables 1, 2). These six SNPs were in high LD with one another ($D' > 0.9$). After further corrected by the number of cohorts examined, these associations still remained suggestively significant. In addition, as introduced above, a recent GWAS identified a common variant (rs1789891 between *ADH1B* and *ADH1C*) that was significantly associated with alcohol dependence in the subjects of German descent (Frank et al. 2012). Interestingly, this SNP was suggestively associated with autism ($p = 0.0015$) in the present study, but not with alcohol dependence ($p > 0.05$).

Bioinformatic analysis showed that most of the significant risk SNPs ($p < \alpha$; Table 2) were located at transcription factor binding sites (TFBS). Three SNPs, i.e., rs1442481 and rs1789912 at *ADH1C* and rs1229863 between *ADH1B* and *ADH1C*, were located at species-conserved elements. Three SNPs, i.e., rs71612682 between *ADH1B* and *ADH1C* and rs1789916 and rs1789912 at *ADH1C*, were located at methylated CpG islands. rs1789900 and rs1442480 at *ADH1C* were located at a 60-bp-long copy number variant (CNV: A_16_P16787293), and rs1789916 at *ADH1C* was located at another 60-bp-long CNV (A_16_P36841645). In addition, rs62323588 between *ADH5* and *ADH4* was located at a long RNA transcript (>200 bases).

Among a total of 916 common SNPs in African-Americans, 26 SNPs were nominally associated with alcohol dependence ($p < 0.05$), some of which were suggestively associated with alcohol dependence at a non-significant trend level. The most significant one was rs904092 at 5' flanking region of *ADH1A* ($p = 0.00053$), and the second most significant one was rs2066702 (Arg370Cys; *ADH2*3*) at exon 9 of *ADH1B* ($p = 0.0015$; $f = 0.142$ in cases and 0.193 in controls). However, no SNPs survived either FDR or SNPSpD correction (Table 1). Similarly, although some SNPs were nominally associated with other neuropsychiatric and neurological disorders ($p < 0.05$), no SNPs survived either FDR or SNPSpD correction (Table 1).

Discussion

The principal finding of the current study was that common *ADH* variants were significantly associated with the risk for schizophrenia and autism, but not other neuropsychiatric disorders, including alcohol dependence. There is growing evidence that schizophrenia and autism share genetic risk variants including SNPs and CNVs (McCarthy et al. 2009; Sebat et al. 2009; Owen et al. 2011). The present study provided new evidence in support of this shared risk.

The location of the *ADH* variants within the *ADH* gene cluster may have functional significance. All of the 19 significant risk SNPs for schizophrenia and five of the six significant risk SNPs for autism were located within or flanking *ADH1C* (i.e., in 5' flanking region of *ADH1C* or between *ADH1C* and *ADH1B*) (Table 2). These risk SNPs may have potential biological functions based on the bioinformatic analyses. It has been known that the lower functioning $\gamma\gamma$ ADH enzyme (mainly) (encoded by *ADH1C*) and $\beta\beta$ ADH enzyme (partially) (encoded by *ADH1B*) inhibit the turnover of 5-HIAL to 5-HTOL and increase 5-HIAA levels (Svensson et al. 1999). 5-HIAA is an important metabolite of serotonin. Alterations in

5-HIAA levels variably associated with schizophrenia (Wieselgren and Lindstrom 1998) and autism (Adamsen et al. 2011) have been interpreted as providing evidence of disturbances in serotonergic neurotransmission associated with these disorders (Cook and Leventhal 1996; Abi-Dargham et al. 1997; Chugani 2004). Thus, it is conceivable that *ADH1B* and *ADH1C* are involved in serotonergic dysfunction associated with these disorders. In addition, we noted that one significant risk SNP (rs62323588) for autism was located between *ADH4* and *ADH5* (Table 2). It has been known that the increased $\pi\pi$ ADH enzyme (encoded by *ADH4*) activity could lead to a very high turnover of norepinephrine aldehydes (Holmes 1994), and norepinephrine has been reported to be involved in the development of autism (Leboyer et al. 1992). These functional links may be supported, at least partially, by our current finding of the association between rs62323588 and autism.

It is also worth noting that the two top-ranked common *ADH* variants, i.e., rs904092 and rs2066702, that were suggestively associated with alcohol dependence in African-Americans at a trend level, are located in the 5' flanking region of *ADH1A* and within *ADH1B*, respectively. The functional rs2066702 (*ADH2*3*) reduces the activity of $\beta\beta$ ADH enzyme in the oxidation of ethanol, and thus may affect risk for alcohol dependence (Thomasson et al. 1995). *ADH1A* encodes $\alpha\alpha$ ADH enzyme that has similar properties to $\beta\beta$ ADH and $\gamma\gamma$ ADH and contributes to the oxidization of ethanol. Thus, *ADH1A* is also a reasonable candidate gene for alcohol dependence (Zuo et al. 2009). In view of the apparent biological functions of these ADHs, the trend-level associations between these variants and alcohol dependence may reflect the smaller effects of common variants than rare variants. Future studies with larger samples are warranted to examine whether the associations between common *ADH* variants and alcohol dependence can really reach a significant level.

In conclusion, human diseases may be caused by a constellation of rare variants (Dickson et al. 2010), common variants, or both. Our studies, including a previous work (Zuo et al. 2013b) and the present one, suggest that rare *ADH* variants are associated with alcohol dependence; common *ADH* variants were suggestively associated with alcohol dependence, but significantly associated with schizophrenia and autism. These findings may support a hypothesis that rare and common *ADH* variants play different roles in the ADH properties. The rare *ADH* variants (e.g., those four functional variants introduced above) may influence the ADH functions that are related to the ethanol metabolism, and may thus be implicated in risk for alcoholism; however, the common *ADH* variants are more likely to affect the ADH activity that is related to the monoamines' metabolic pathways, and may thus be

implicated in risk for schizophrenia, autism, and possible, more other, neuropsychiatric disorders.

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phs000196.v2.p1, phs000126.v1.p1, phs000089.v3.p2, phs000089.v3.p2, phs000089.v3.p2 and phs000089.v3.p2.

Conflict of interest There is no conflict of interest to declare.

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