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Common Variants in HLA-DRA Gene are Associated with Alcohol Dependence in Two Caucasian Samples

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Abstract HLA-DRA gene polymorphisms might play an important role in alcohol dependence (AD). We examined genetic associations of 29 single-nucleotide polymorphisms (SNPs) within the HLA-DRA gene with AD using two Caucasian samples—the Collaborative Study on the Genetics of Alcoholism (COGA) sample (660 AD cases and 400 controls) and the Study of Addiction: Genetics and Environment (SAGE) sample (623 cases and 1,016 controls). Logistic regression analysis using PLINK showed that 16 SNPs were associated with AD in the COGA sample and 13 SNPs were associated with AD in the SAGE sample ($p<0.05$). The best novel signal was SNP rs2239803 associated with AD in both samples ($p=0.000817$ for the COGA sample and $p=0.0026$ for the SAGE sample, respectively) while one flanking SNP rs4935356 also showed strong association in both samples ($p=0.00219$ and 0.0026 for the COGA and SAGE samples, respectively). Furthermore, these two SNPs revealed stronger associations in meta-analysis of these two samples ($p=8.97\times10^{-6}$ and 2.02×10^{-5} for rs2239803 and rs4935356, respectively). In addition, the G-A haplotype from these two SNPs revealed a significant association with AD in both the COGA and SAGE samples ($p=0.0007$ and 0.0019, respectively). These findings highlight the novel associations with HLA-DRA that may play an important role in the etiology of AD.

Keywords Alcohol dependence · HLA-DRA · Single-nucleotide polymorphism · Association · Meta-analysis · Haplotype

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Introduction

The pathology of alcohol dependence (AD) is complex and reflects dysfunctions in various organs in human body. Harmful use of alcohol could affect human's brain, behavior, and immune system, and results in more than 60 different diseases (WHO 2010). Alcohol is the third leading risk factor globally for disease burden, while harmful use leads to 2.5 million deaths worldwide every year (WHO 2010). Besides acting as a major risk factor for cardiac disease (Dolara and Marascio 1982; Ettinger et al. 1978), cancer (Boffetta and Hashibe 2006; Druesne-Pecollo et al. 2009; Longnecker 1995), and liver disease (Kuller et al. 1978; Menon et al. 2001), AD could damage the brain through many mechanisms such as alcohol-induced brain dysfunctions and cognitive impairment as showed in research in human and also in animal models (Lovinger 1997; Savage et al. 2000). It is also reported that AD frequently coexists with drug abuse, nicotine dependence, and mood and anxiety disorders (Grant et al. 2004a, b; Regier et al. 1990). Furthermore, the high prevalence of comorbidity of depression in AD suggested that the effect of major depression may account for some immune changes associated with AD (Kessler et al. 1997; Penick et al. 1994; Regier et al. 1990). Recent studies have also demonstrated that innate immune system is an essential physiological link between AD and

major depressive disorders (Schleifer et al. 2006). In summary, AD could affect the brain function and cause significant changes in human's immune system (Irwin and Miller 2007); however, possible mechanisms involved in AD affecting brain, behavior, and immunity are still unknown (Cui et al. 2011).

Genetic components are important in the development of AD with an overall estimated heritability of 50–60 % for both sexes (Heath et al. 1997; Kendler et al. 1992; Prescott and Kendler 1999). Whole-genome linkage study and association study have successfully identified several chromosome region and genes that are related to AD. The most consistent findings involved in alcohol dehydrogenase gene cluster include ADH1B and ADH1C genes (Ehlers et al. 2004; Long et al. 1998; Prescott et al. 2006; Reich et al. 1998) and Gamma-amino butyric acid receptor genes (GABRG1 (Covault et al. 2008; Ray and Hutchison 2009), GABRA2(Covault et al. 2008; Edenberg et al. 2004), GABRA4, and GABRB1 cluster at chromosome 4p region (Covault et al. 2004, 2008; Edenberg et al. 2004; Fehr et al. 2006; Lappalainen et al. 2005; Loh et al. 1999; Long et al. 1998; Ray and Hutchison 2009; Reich et al. 1998; Soyka et al. 2008). More recently, several genome-wide association (GWA) studies have been conducted on AD (Bierut et al. 2010; Edenberg et al. 2010; Treutlein et al. 2009; Treutlein and Rietschel 2011; Wang et al. 2011) and identified several novel genetic loci. Bierut et al (2010) identified 15 single-nucleotide polymorphisms (SNPs) associated with AD ($p<10^{-5}$), and especially replicated GABRA2 from the Study of Addiction: Genetics and Environment (SAGE). Edenberg et al. (2010) identified 11 SNPs associated with AD ($p<10^{-5}$), and a cluster of genes on chromosome 11 (SLC22A18, PHLDA2, NAP1L4, SNORA54, CARS, and OSBPL5) showed strong associations with AD. Wang et al. (2011) reported results from a meta-analysis of two GWA studies with 1,283 cases and 1,416 controls in Caucasian populations, and three new loci (KIAA0040, THSD7B, and NRD1) have been identified. Although GWA study is a powerful tool to clarify the genes underlying complex diseases such as AD, few results have been replicated (Bierut et al. 2010; Lind et al. 2010; Treutlein et al. 2009; Treutlein and Rietschel 2011). True associations may also have been overlooked because of the stringent significant threshold by GWA guidelines ($p<10^{-7}$).

The HLA-DRA gene at 6p21.3 is one of the HLA class II alpha chain paralogues, and it plays a central role in the immune system by presenting peptides derived from extracellular proteins. Strominger (1986) and Auffray and Strominger (1986) provided a very comprehensive discussion of the molecular structure and useful map of the HLA gene cluster and also presented a hypothesis for its association with autoimmune disease that depends on somatic mutation in the immune system. HLA-DRA has been claimed to have influences on alcohol-induced liver disease (Kiernan et al. 1980; Marbet et al. 1988). More recently, HLA-DRA has been reported in a number of studies to be associated with multiple neurological disorders, such as bipolar disorder (Nakatani et al. 2006), neurodegeneration disorder and Parkinson's disease (Hamza et al. 2010; Puschmann et al. 2011), and multiple sclerosis (International Multiple Sclerosis Genetics et al. 2007). Cancer may be also involved (Rangel et al. 2004). Incorporating biologically relevant information, we hypothesized that HLA-DRA might be a novel target candidate gene region associated with AD. A total of 29 SNPs in HLA-DRA gene were assessed for their associations with AD among 1,639 people (623 cases and 1,016 controls) from SAGE sample and 1,060 people (660 cases and 400 controls) from the Collaborative Study on the Genetics of Alcoholism (COGA) sample.

Materials and Methods

Samples

The COGA Sample

The COGA Case–Control Study is a case–control GWA study of alcoholism, in which the subjects have been drawn from the COGA (Edenberg et al. 2010). In the present study, we chose 1,060 Caucasian (non-Hispanic) individuals (660 cases and 400 controls). Phenotypes include AD as a binary trait according to the DSM-IV diagnosis.

The SAGE Sample

The SAGE is a comprehensive GWA study using approximately 4,000 unrelated subjects of European and African-American descent. AD cases include 1,944 subjects with the primary phenotype having been DSM-IV AD (Bierut et al. 2010). Controls comprise 1,965 subjects who have used alcohol but have never been addicted to alcohol or other illicit substances. Based on our previous GWA meta-analysis study on AD (Wang et al. 2011), we used 1,639 Caucasian individuals (623 cases and 1,016 controls) from the SAGE sample. Among the total 2,699 study participants that we selected in our study, 1,639 Caucasian individuals (623 cases and 1,016 controls) were from the SAGE sample, while the other 1,060 subjects (660 cases and 400 controls) were from the COGA sample. The mean age is 35.8 years (35.1 for cases and 36.2 for controls) for the SAGE sample and 40.8 years (38.6 for cases and 44.3 for controls) for the COGA sample (Table 1).

Statistical Analysis

There are 29 SNPs within HLA-DRA gene available in both samples. Hardy–Weinberg equilibrium was tested for all the

Table 1 Descriptive characteristics of cases and controls

Variable	Cases		Controls	
	SAGE	COGA	SAGE	COGA
Number of participants	623	660	1016	400
Gender, *n* (%)				
Male	329 (53)	465 (70)	306 (30)	107 (27)
Female	294 (47)	195 (30)	710 (70)	293 (73)
Age	35.1±7.7	38.6±10.7	36.2±7.6	44.3±11.6
Range	18–61	18–72	18–65	18–73

SNPs using the controls by using Haploview software (Barrett et al. 2005). Then, minor allele frequency (MAF) was determined for each SNP and the linkage disequilibrium (LD) structure was constructed using Haploview. Logistic regression analysis of AD as a binary trait, adjusted for age and sex, was performed for the COGA and SAGE samples using PLINK v1.07 (Purcell et al. 2007). The asymptotic p values for this test were observed while the odds ratio (OR) and its standard error were estimated. For logistic regression, the additive model was applied. Empirical p values were generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK. Pointwise estimates of an individual SNP's significance values (empirical pointwise p values) were calculated. Results from the two GWA analyses were directly meta-analyzed by combining the separate results of the COGA and SAGE samples (OR and standard error of OR) into one meta-analysis of overall effects. For meta-analysis of two datasets, the basic meta-analysis function in PLINK was applied. Fixed-effect meta-analysis p value and fixed-effect OR were estimated. The between-study heterogeneity was tested by the χ^2-based Cochrane's Q statistic. Haplotype analysis was performed for the COGA and SAGE samples separately using UNPHASED version 3.1.4 (Dudbridge 2008).

Results

All SNPs are in Hardy–Weinberg equilibrium in the controls. The results of the top 10 SNPs were summarized in Table 2. A more comprehensive list of SNPs (total 29 SNPs) was presented in the Supplementary Table S1. Thirteen SNPs showed associations in the SAGE sample and 16 SNPs showed associations in the COGA sample ($p<0.05$). Single marker analysis showed that SNP rs2239803 was most significantly associated with AD in the COGA sample ($p=0.0008171$); moreover, it was also the most significant marker that replicated in the SAGE sample with $p=0.002566$. In addition, for the top 10 SNPs (rs2239803, rs8084, rs9268658, rs4935356, rs2239804, rs7195, rs7194, rs2213586, rs7192, and rs2213585) that we observed from COGA sample, all were well replicated and demonstrated significant results from SAGE sample ($p<0.05$).

Meta-analysis showed that those 10 SNPs were associated with AD ($p<10^{-3}$) with the peak marker at SNP rs2239803 ($p=8.97\times10^{-6}$) (Table 2). All p values based on Q statistic were larger than 0.05, which indicated that there was no heterogeneity for these SNPs between COGA and SAGE sample. The MAF for the same SNP of both datasets were identical while the association directions for the top 10 SNPs were the same for both datasets as revealed by OR values. All 10 SNPs for the COGA and SAGE samples had empirical pointwise p values $p<0.05$ using a permutation procedure (Table 2).

We identified haplotype blocks for 19 flanking SNPs using Haploview. Figure 1 shows the LD (r^2) structure. SNPs rs2239803 and rs4935356 have strong LD ($r^2=0.94$ and $D'=0.99$). Two-SNP haplotype analyses based on UNPHASED (Table 3) revealed that the G-A haplotype from rs2239803 and rs4935356 was significantly associated with AD both in the COGA and SAGE samples ($p=0.0007$ and 0.0019, respectively).

Discussion

We conducted a candidate gene association study to identify possible SNPs within HLA-DRA gene for AD by using COGA and SAGE samples. Total 18 SNPs showed significant associations ($p<0.05$) with AD through meta-analysis (Table S1). Haplotype analysis further supported the single marker analysis results. To our knowledge, this is the first candidate gene study to investigate the associations between HLA-DRA SNP polymorphisms and AD.

The HLA-DRA gene lies in the major histocompatibility complex region that controls the immune system, growth, and reproduction at 6p21.3. It is one of the three major isotypes (HLA-DR, HLA-DP, and HLA-DQ) of HLA class II alpha chain that contains an additional octamer element originally found in the promoter and enhancer of immunoglobulin genes (Cogswell et al. 1990). In our study, single marker analysis showed that several SNPs in the HLA-DRA were associated with AD. Demirhan and Tastemir (2008) reported a significantly higher frequency of fragile sites and chromosomal aberrations at 6p21 on chronic alcohol users. Interestingly, a number of studies published during 1980s emphasized on the association of human leukocyte antigen (HLA) system with alcoholics and alcoholic liver disease (Bell and Nordhagen 1980; Gluud et al. 1980; Kiernan et al. 1980; Marbet et al. 1988). List and Gluud (1994) conducted a meta-analysis on HLA-antigens in alcoholics; however, no significant association was found between specific HLA

Table 2 Top 10 SNPs within HLA-DRA gene associated with alcohol dependence

SNP	Position	Allele[a]	Pmeta[b]	Q[c]	MAF[d]	OR[e]	P_{SAGE}[f]	EMP[g]	MAF[h]	OR[i]	P_{COGA}[j]	EMP2[k]
rs2239803	32519811	A	8.97E-06	0.45	0.49	0.81 (0.53–0.88)	2.60E-03	0.002	0.46	0.74 (0.73–1.33)	8.17E-04	0.001
rs4935356	32520366	C	2.02E-05	0.6	0.49	0.81 (0.67–0.92)	2.60E-03	0.002	0.48	0.76 (0.8–1.2)	2.19E-03	0.001
rs9268658	32518694	A	2.20E-05	0.59	0.49	0.81 (0.25–0.87)	2.80E-03	0.0029	0.48	0.76 (0.21–0.86)	2.18E-03	0.001
rs2239804	32519501	G	2.35E-05	0.62	0.49	0.81 (0.7–0.94)	2.60E-03	0.002	0.48	0.76 (0.85–1.23)	2.60E-03	0.002
rs8084	32519013	A	6.08E-04	0.23	0.42	1.15 (0.31–0.95)	5.20E-02	0.043	0.37	1.33 (0.21–0.81)	2.14E-03	0.001
rs7195	32520517	A	6.53E-04	0.35	0.38	1.17 (0.53–0.88)	3.50E-02	0.031	0.33	1.30 (0.76–1.38)	4.45E-03	0.0049
rs7194	32520458	G	8.23E-04	0.32	0.38	1.16 (0.66–0.92)	4.30E-02	0.046	0.33	1.30 (0.82–1.22)	4.45E-03	0.0049
rs2213586	32521072	T	8.23E-04	0.32	0.38	1.16 (0.59–0.9)	4.30E-02	0.046	0.33	1.30 (0.78–1.32)	4.45E-03	0.0049
rs2213585	32521128	C	8.37E-04	0.33	0.38	1.16 (0.58–0.89)	4.30E-02	0.018	0.33	1.30 (0.77–1.29)	4.58E-03	0.0049
rs7192	32519624	T	8.87E-04	0.32	0.38	1.16 (0.71–0.95)	4.60E-02	0.049	0.33	1.30 (0.82–1.18)	4.45E-03	0.0049

[a] Minor allele

[b] *p* value for the meta-analysis

[c] *p* value for Cochrane's *Q* statistic

[d] Minor allele frequency in the SAGE sample

[e] Odds ratio for the SAGE sample

[f] *p* value for the SAGE sample based on logistic regression

[g] Empirical *p* value for the SAGE sample generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK

[h] Minor allele frequency in the COGA sample

[i] Odds ratio for the COGA sample

[j] *p* value for the COGA sample based on logistic regression

[k] Empirical *p* value for the COGA sample generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK

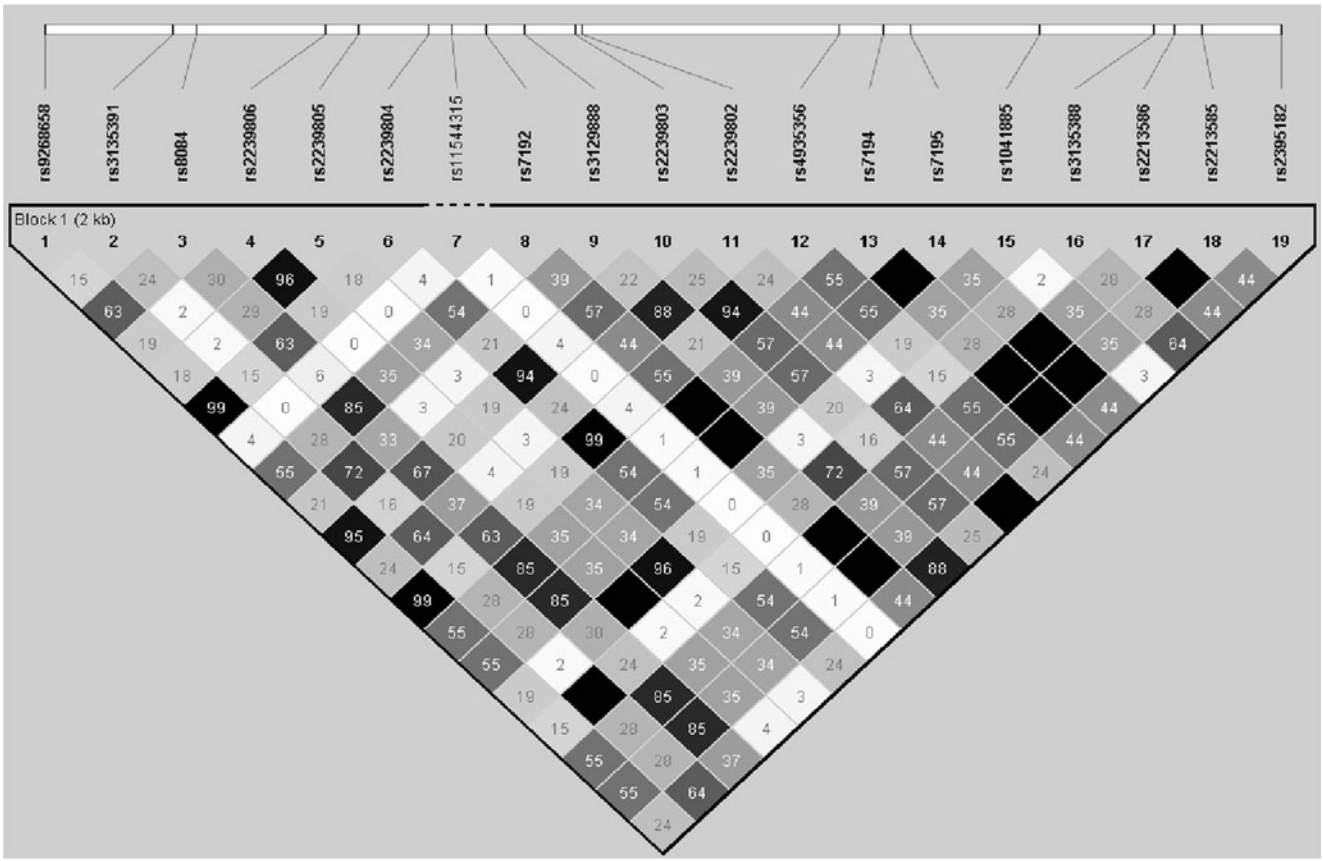

Fig. 1 Linkage disequilibrium structure of 19 SNPs within HLA-DRA. The *numbers* indicate the r^2 values between the corresponding two SNPs

phenotypes and the development of alcoholism. This inconsistency may be due to several possible explanations including small sample size in the previous studies, different study designs, and population differences between studies. In our study, we used two large samples with sufficient genotyping markers and only included Caucasian individuals. Our study suggested that HLA-DRA may be an important genetic factor associated with AD. Alcohol intoxication is well known to change behavior, induce major mood disorders, and affect the liver which is an organ with predominant innate immunity. Irwin and Miller (2007) pointed out that AD not only induces inflammation in the body and brain, but will also cause significant changes in the immune system.

HLA types are inheritable and strong candidates for the pathogenesis of autoimmune disease such as Grave's disease and type I diabetes (Donner et al. 1997), celiac disease (Michalski et al. 1996), and systemic lupus erythematosus (Rizzo et al. 2008). Results from linkage and GWA study further confirmed HLA-DRA to be associated with several different complex diseases. Barrett et al. (2009) conducted meta-analysis and identified HLA-DRA to be a major

Table 3 Haplotype analysis of HLA-DRA gene in the COGA and SAGE samples

Haplotype		COGA				SAGE			
		Case[a]	Control[b]	OR[c]	P[d]	Case	Control	OR	*p* value
rs2239803	rs4935356								
A	C	594 (46 %)	424 (54 %)	1	0.0007	544 (44 %)	999 (49 %)	1	0.0019
G	A	696 (54 %)	365 (46 %)	2.36	0.0007	700 (56 %)	1027 (51 %)	1.25	0.0019

[a] Haplotype number and frequency in cases

[b] Haplotype number and frequency in controls

[c] Odds ratio based on standard case–control haplotype analysis

[d] *p* value based on standard case–control haplotype analysis

candidate gene for type I diabetes (Barrett et al. 2009). The rs2395185 in HLA-DRA gene has been associated with asthma (Li et al. 2010; Moffatt et al. 2010) and ulcerative colitis (Allanore et al. 2011). Other studies suggested a possible association between the effect of HLA-DRA and brain dysfunction. Ward et al. (2009) pointed out that an increased HLA-DRA expression may be due to intermittent binge alcohol exposure, and its cumulative effects of multiple withdrawal episodes could push partially activated microglia to a more inflammatory level by using a rat model. The HLA region has also been shown to contain susceptibility locus for multiple sclerosis. Bennetts et al. (1999) examined the two candidate polymorphic loci HLA-DMB gene and HLA-DRA promoter region in Australian multiple sclerosis patients and suggested that there may be a complete linkage disequilibrium between the two loci. In a multistage GWA study, rs3135388 A allele in HLA-DRA has been identified and confirmed to be highly correlated with multiple sclerosis (International Multiple Sclerosis Genetics et al. 2007). A U.S. population-based study using rs3129882 in HLA-DRA as a novel locus suggested the role for the immune system in the pathogenesis of late-onset sporadic Parkinson's disease (Hamza et al. 2010). Saiki et al. (2010) also suggested a possible role of HLA region in susceptibility to Parkinson's disease in 528 cases with PD and 3,430 controls from UK. Another study showed that HLA-DRA was downregulated in bipolar and schizophrenic patient's brain by microarray and quantitative RT–PCR analyses (Nakatani et al. 2006).

In summary, to our knowledge, this is the first candidate gene study which investigates the associations between HLA-DRA SNP polymorphisms and AD. The novel associations with HLA-DRA highlight the involvement of an important biological pathway in the etiology of AD. These findings may serve as a resource for replication in other populations for future investigations on target genetic variation and AD.

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Conflict of Interest The authors declare no conflict of interest.

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