Different Phenotypic and Genotypic Presentations in Alcohol Dependence: Age at Onset Matters*

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ABSTRACT. Objective: Several theoretical typology models have been proposed to classify alcoholism into more homogeneous subtypes using various criteria, for which age at onset of alcohol dependence is shared across many models. We investigated the evidence for the distinction between early- versus late-onset alcoholism by examining relevant phenotypic and genotypic variables. **Method:** Data are from 1,248 individuals with alcohol dependence, who were interviewed to collect detailed clinical information. Early versus late onset of alcohol dependence was defined by the age at onset of 22 years. Odds ratio (OR) and Cohen's *d* were calculated as effect size for comparisons of clinical features between the two groups. We adjusted interviewed age and gender in logistic regression models. Case-control genetic analyses were conducted for the association between *HTR1B, SLC6A4, DRD2,* and *OPRμ1* genes and subgroups of alcohol dependence using a sample of 530 controls screened for alcohol problems. **Results:** Early-onset alcoholism exhibited significantly ($p < .01$) different clinical characteristics from late-onset alcoholism, including higher severity in alcohol dependence symptoms ($d = 0.22$) and maximum drinking quantity within 24 hours ($d = 0.40$), more rapid progression from regular drinking to meet alcohol dependence diagnosis ($d = 1.73$), higher expectancies for alcohol ($d = 0.22–0.47$), more comorbidity with externalizing disorders (ORs = 2.8–2.9), and greater prevalence of family alcohol use problems ($d = 0.26–0.43$). In addition, markers in the *HTR1B* and *OPRμ1* genes showed genetic associations with subgroups of alcohol dependence (ORs = 1.5–2.4). **Conclusions:** Our findings support that subgroups of alcohol dependence defined by onset age have phenotypic and genetic differences. The early-onset subgroup had more severe features for almost every aspect we examined. Coupled with genetic association findings, age at onset of alcohol dependence may serve as a simple but important clinical marker with implications for future etiological research and intervention. (*J. Stud. Alcohol Drugs, 72,* 752–762, 2011)

ALCOHOL DEPENDENCE (AD) is a complex disorder with a wide variety of phenotypic manifestations in disease progression and prognoses, comorbidity, symptoms presentation, and severity. Twin studies have shown that the heritability of AD ranges from 51% to 64% (Prescott et al., 2006). Patients who exhibit different clinical characteristics and symptoms may have different etiology and neurobiological pathways to develop alcoholism (Cloninger, 1987; Leggio and Addolorato, 2008). To better understand the neurobiological mechanisms underlying AD and to improve treatment response, some have advocated for classifying alcoholics with heterogeneous clinical presentations into more specific subtypes (Babor et al., 1992; Cardoso et al., 2006; Leggio et al., 2009; Pombo and Lesch, 2009).

Previously, several theoretical typology models were proposed to subdivide alcoholic individuals into binary or categorical classification, such as Type I and Type II alcoholism (Cloninger, 1987), Type A and Type B (Babor et al., 1992), or a four-group or five-group typology model (Cardoso et al., 2006; Lesch et al., 1988). The Type I and Type II classification by Cloninger and colleagues, which is based on a proposed neurobiological mechanism, is among the most widely applied classification schemes for alcoholism (Pombo and Lesch, 2009). Conceptually, Type I and Type II alcoholics differ in many aspects, including disease course, magnitude of inheritance, and personality traits (Johnson, 2000; Kiefer and Mann, 2005). Type I alcoholics are more strongly influenced by social milieu and exhibit alcohol problems at a later age. They tend to use alcohol for its anti-anxiety effects and have high scores in harm-avoidance personality traits. Type II alcoholics are male predominant, have high genetic diathesis, and exhibit alcohol problems at an early age. They use alcohol for its euphoric effects and are characterized by high novelty seeking and low harm-avoidance personal-

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ity traits (Cloninger, 1987). In addition, Type I and Type II alcoholics respond differently to pharmacological therapy (Johnson, 2000; Kiefer and Mann, 2005). The two subtypes are hypothesized to correspond to different neurobiological pathways and may exhibit genetic heterogeneity.

The Type I and Type II typology models had been modified to introduce several other classification schemes by von Knorring et al. (1985), Gilligan et al. (1988), and Sullivan et al. (1990), who differentiated the Type I and Type II alcoholics by various criteria. Von Knorring and colleagues (1985) defined Type I and Type II alcoholics by age at onset of alcohol problems, help-seeking behaviors, and frequency of social-related problems. Type I alcoholics, with fewer social-related problems, have drinking problems after age 25 years and reported the first treatment attempt after age 30 years. In contrast, Type II alcoholics have more social-related problems, exhibit early drinking problems (<25 years), and report earlier treatment attempt (<30 years). Sullivan and colleagues (1990) added family history as another criterion on the von Knorring et al. (1985) model, and the Type II alcoholics have a strong family history of alcohol use problems. Gilligan and colleagues (1988) mainly used onset age, drinking problems, and social-related problems to differentiate the two types of alcoholism. For different binary typology models, a variety of characteristics were detailed for each subtype. The variations across proposed subtypes make comparison of findings across studies, clinical application, and validation of others' findings more difficult. Some individuals who do not follow typical description in typology models may fail to be clearly assigned to subgroups and leave a certain proportion of unclassified cases. Among these typologies, one unifying feature is age at onset of alcohol use problems. A recent review article also emphasized the use of simple classification such as onset age to subtype AD in clinical practice for its potential utility, including exploring genetic influences and the response to pharmacotherapy (Leggio et al., 2009).

To summarize different clinical features that cluster within early- or late-onset AD subgroups according to previous typology models, alcohol-dependent patients who had early onset usually were male, were heavy drinkers, and had high familial alcohol use problems and more antisocial behavioral problems. Men and women were equally affected with late-onset AD. Also, those with late-onset AD tended to exhibit less severe symptoms and psychopathologies and generally respond better to treatment. Among these clinical characteristics, one important feature is the comorbidity with antisocial personality disorder (ASPD) or conduct disorder (CD) in patients with AD. Prior studies found that alcohol-dependent patients with ASPD had earlier onset age and progressed faster to problematic drinking than those without ASPD (Hesselbrock et al., 1985; Schuckit, 1973). However, in families aggregated with multiple AD cases, early-onset AD occurred without the necessity of ASPD being present (Hill, 1992). Using a simple cutoff of early- versus late-onset AD, we first aimed to evaluate empirical evidence for the differences in a wide variety of clinical features, including comorbidity patterns, personality, disease severity and progression, family history of alcohol use problems, and alcohol expectancies.

Prior typology models also linked to different underlying genetic mechanisms of AD. For instance, Cloninger et al. (1987) proposed a deficit in dopaminergic neurotransmission in Type I alcoholics, whereas Type II alcohol-dependent individuals have a deficit in serotonergic neurotransmission. Several genetic studies investigated associations between candidate genes in these neurotransmitter systems and AD or alcohol-related traits. Hallikainen et al. (1999) found that the serotonin transporter gene, *SLC6A4,* was associated with an increased risk for AD with early onset, impulsivity, habitually violent behaviors, and ASPD. Hasegawa et al. (2002) reported that the frequency of *HTR1B* C allele was higher in antisocial alcoholics, who exhibited early-onset AD and had a family history of alcoholism. One meta-analysis suggested that the existence of 5-hydroxytryptamine abnormalities among alcoholics was characterized particularly as either early-onset, more severe subtype, or comorbid with other psychiatric conditions (Feinn et al., 2005). Another widely studied candidate gene with AD was *DRD2,* a dopamine receptor gene, with somewhat inconsistent results. Stronger effects were reported with severe alcoholics in a review article that included 11 association studies (Noble, 2000) and was reported in a linkage study (Hill et al., 1999). Connor et al. (2002) found that *DRD2* gene was associated ($p = .0001$) with early-onset problematic drinking. However, negative findings were reported for *DRD2* with age at onset of AD or sensation-seeking personality trait (Derringer et al., 2010; Hack et al., 2011). The μ-opioid receptor gene (*OPRμ1*) was also studied in Type I and Type II alcoholism. Kiefer and colleagues (2008) found that naltrexone, which acts on μ-opioid receptors, is effective in Type II alcoholics only. However, another study suggested that *OPRμ1* is associated with both Type I and Type II alcoholics (Bart et al., 2005). Thus, previous studies have indicated the aforementioned candidate genes for AD, and those gene effects might vary in early-onset or severe subtypes of alcoholic patients. Therefore, we chose to test genetic effects in the binary subtypes of alcoholism using onset age for four genes—*HTR1B, SLC6A4, DRD2,* and *OPRμ1*—in the current study.

The present study aimed to evaluate the differences in clinical features and genetic variation among alcohol-dependent patients subtyped by onset age, which may result from different underlying biological mechanisms. Data were used from 1,248 individuals from 591 alcoholic families participating in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD; Prescott et al., 2005) and 530 controls without a history of alcoholism. In this culturally and genetically homogeneous White sample, we attempted to test for a series of clinical characteristics in early- vs. late-onset

AD subgroups. The second goal was to conduct genetic association analysis for a number of markers in four candidate genes—*HTR1B, SLC6A4, DRD2,* and *OPRμ1*—to test for associations in the two AD subgroups.

Method

Subjects

There were 1,248 participants in the IASPSAD who were recruited in Ireland and Northern Ireland between 1998 and 2002. We excluded 16 individuals who did not provide age-at-onset information about AD. In total, 1,232 participants (65% male, average interview age of 42 years) remained in the phenotypic analyses. Details of the study design, sample ascertainment, and clinical characteristics of this sample were described elsewhere (Prescott et al., 2005). In brief, ascertainment of probands was mainly conducted in community alcoholism treatment facilities and in public and private hospitals. Probands were eligible for study inclusion if they met *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV; American Psychiatric Association, 1994), criteria for AD and if all four grandparents had been born in Ireland, Northern Ireland, Scotland, Wales, or England. After a prospective family was identified through probands, parents and potentially affected siblings whom the probands provided permission to contact were recruited. All participants provided informed consent.

In conducting a case–control genetic association study, we also recruited controls who were screened for heavy drinking or problematic alcohol use in Northern Ireland and Ireland (Kuo et al., 2008). Only one alcohol-dependent patient was selected from each family to form an independent AD case group in the case–control design. Samples were selected based on a high yield of high-quality DNA for genotyping. We included 530 controls and 575 independent AD cases (399 probands and 176 siblings) from the IASPSAD families for genetic analyses.

Phenotype measures

Clinical features of probands and affected siblings were assessed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview version II (Bucholz et al., 1994) modified to reduce assessment time by omitting items that assessed age at onset of each symptom. Age at onset of AD was collected in the SSAGA interview. Theoretical typology models in the literature often defined early onset of AD as being before 25 years of age (Gilligan et al., 1988; Sullivan et al., 1990; von Knorring et al., 1985). However, a recent epidemiological study in the National Epidemiologic Survey on Alcohol and Related Conditions obtained a best-fitting, two-group model using admixture analysis to empirically derive subgroups of AD with a cutoff age at 22 years (Le Strat et al., 2010). We adopted the age of 22 to define our alcohol-dependent patients into early-onset alcoholism (EOA; age at onset ≤ 22 years) and late-onset alcoholism (LOA; age at onset > 22 years) groups. According to this criterion, there were 528 and 704 AD patients in the EOA and LOA groups, respectively. A modified version of the Structured Clinical Interview for DSM-III-R (Spitzer et al., 1992) was used to assess comorbid conditions, including major depressive disorder, ASPD, and CD. All interviews were conducted by clinically trained research interviewers, most of whom had extensive clinical experience with alcoholism.

A series of clinical characteristics were measured in the present study, including alcohol-related traits, family history of alcohol use problems, personality traits, alcohol expectancy, and lifetime history of other comorbid conditions. Three alcohol-related traits were assessed and analyzed in the present study: maximum drinking within 24 hours, AD symptom severity, and progression of alcohol use problems (measured as the duration between the age at beginning regular drinking and the age at onset of AD). Maximum drinking within 24 hours was measured by asking about the largest number of drinks an individual consumed in 24 hours, and consumed volume was converted into standard drinks (i.e., 12 oz. of beer, 4 oz. of wine, or 1.5 oz. of distilled spirits). Severity of AD has been defined using different criteria across studies without consensus. We defined the AD symptom severity using total number of alcohol criteria endorsed. This variable was determined by the sum of alcohol diagnostic items in the SSAGA interview questions regarding the number and frequency of AD symptoms, which was similarly used in the Collaborative Study on the Genetics of Alcoholism (McCutcheon et al., 2009). The total score for symptom severity could thus range from 0 to 35.

Family history of alcohol use problems was assessed by the interviewed subjects to report alcohol use problems among parents and siblings (offspring were not considered because many were too young to have passed through the risk period for developing AD) using the Family History–Research Diagnostic Criteria probe structure. For details of the assessment and results of validity and reliability of diagnosis of alcoholism based on family history reports, please refer to Prescott et al. (2005). In brief, we found no effects of informant sex or informant status (proband versus other sibling) for classification of alcohol use diagnoses. The accuracy of parental AD based on reports by offspring and sibling AD based on reports by their siblings was good, and both the positive and negative prediction values ranged from 80% to 95%. In the current analysis, we combined all available answers to record alcohol use problems for each family member. For instance, the presence of the father's drinking problem was determined by any positive report from index subjects, siblings, and parents. Given that parents and siblings have the same kinship coefficient with index probands, the magnitude of family loading of alcohol use

problems was calculated by the proportion of the presence of alcohol use problems among family members. For instance, if there was one parent and two of the three siblings had drinking problems, the index was calculated as 3/5. To evaluate whether family loadings of alcohol use problems are different between male and female alcoholics, this measure was also calculated by genders. We used the 17-item Alcohol Expectancy Questionnaire (Brown et al., 1987) with four subscales: sexual (4 items), positive affect (6 items), negative affect (4 items), and aggression (3 items). We also measured personality traits, including neuroticism and extraversion assessed by the short form of the Eysenck Personality Questionnaire (Eysenck and Eysenck, 1992). Novelty seeking was assessed by the 18-item version from Cloninger's Tridimensional Personality Questionnaire (Cloninger, 1987). For alcohol expectancy and personality questionnaires, individuals who had more than half of the missing items in a specific scale were treated as missing in the analysis; otherwise, we used the mean score from items answered to rescale to a sum score based on total items in each scale.

Genotyping

DNA was extracted using standard techniques. Genotypes for a total of 50 single nucleotide polymorphisms (SNPs) in the *HTR1B* (n = 3), *SLC6A4* (n = 11), *DRD2* (n = 14), and *OPRμ1* (n = 22) genes were obtained as part of a large candidate gene study using an Illumina custom genotyping array designed in Dr. David Goldman's Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism. Detailed information about array design, genotyping, and SNP selection is described elsewhere (Hodgkinson et al., 2008). In brief, for each gene selected, a genomic region including 5 kb upstream and 1 kb downstream was retrieved from the National Center for Biotechnology Information Human Genome Build 35.1, and minimum index SNPs that represented maximum haplotype information for each gene were selected. The performance of the initially selected SNP set was validated by the manufacturer and replacements made where necessary. All genotyping was conducted in Dr. Goldman's laboratory.

Genotyping was performed using the Illumina GoldenGate genotyping protocols on 96-well format Sentrix arrays, and 500 ng of sample DNA were used per assay. All pre–polymerase chain reaction processing was performed using a Tecan liquid handling robot running Illumina protocols. Arrays were imaged using an Illumina Beadstation GX500 and the data analyzed using GenCall v6.2.0.4 and GTS Reports software v5.1.2.0 (Illumina).

Statistical analyses

To compare the EOA and the LOA subgroups, we used chi-square tests (two-tailed) to examine categorical variables (e.g., gender and comorbidities) and t tests for continuous variables (e.g., personality traits, progression, and severity). Odds ratio (OR) and Cohen's d were used to calculate the effect sizes for comparisons between the two subgroups for binary and continuous variables. The small, medium, and large effect sizes for Cohen's d are defined as ≥0.2, ≥0.5, and ≥0.8, respectively. Considering potential influences of gender and interviewed age on the above comparisons, we further applied logistic regression models to obtain covariate adjusted p values. All analyses were conducted using SPSS 15.0 (SPSS Inc., Chicago, IL). Because many clinical variables were examined, we set $p < .01$ as the threshold for statistical significance in comparisons of clinical features (we note in Table 1 that, with a more rigorous Bonferroni correction, a p value of .0025 can be used to claim significance).

Genetic association analyses were conducted using PLINK (Purcell et al., 2007) in subgroups of AD compared with controls at both single marker and haplotype levels. We used Haploview (Barrett et al., 2005) to calculate the strength of linkage disequilibrium among markers and to define haplotype blocks using the default method in Haploview by Gabriel et al. (2002). Hardy–Weinberg equilibrium was tested in controls for each marker, and any marker with p value less than .001 was excluded from analysis. ORs were estimated to measure the effect size of allelic genetic associations while adjusted for gender in logistic regression models. To address potential multiple testing issue, we reported empirical p values that were calculated using 5,000 permutations for all markers. We then applied a method to account for false discovery rate (FDR) developed by Benjamini and Hochberg (1995). In general, the permutation p values are in concordance with the nominal p values; thus, permutation p values were displayed, and $p \leq .01$ is indicated in bold in Tables 2 and 3.

Results

Clinical features

Among 1,232 alcohol-dependent patients, 528 (42.9%) had AD onset before age 22 years and formed the EOA group. By definition, the age at onset of AD was younger ($p < .001$) in the EOA group (M = 18.7, SD = 2.2) than in the LOA group (M = 31.9, SD = 8.0). Results of the demographic and clinical features related to drinking behaviors are shown in Table 1. Men were more predominant in the EOA group (71.8%) compared with the LOA group (59.7%), with an OR of 1.7. There was no gender difference in terms of onset and interviewed age in either the EOA or LOA group. Patterns of drinking behaviors were dissimilar between the two subgroups. Two features, regular drinking age and progression years, had large effect size with Cohen's $d > 0.8$, with the EOA group starting to drink alcohol regularly at an early age (15.6 years). The EOA group had a more rapid

TABLE 1. Demographic and clinical features relevant to drinking behaviors in subgroups of alcoholism

Variable	*n*	EOA (≤22) (*n* = 528)	LOA (>22) (*n* = 704)	Effect size[a]	*p*	Adjusted *p*[b]
Male, *n* (%)	799	379 (71.8)	420 (59.7)	1.72	<.001	
Interviewed age, *M* (*SD*)						
Males	799	37.5 (9.4)	45.7 (9.8)	0.85	<.001	
Females	433	35.9 (8.5)	45.2 (8.0)	1.13	<.001	
Social class	1,232	3.9 (1.5)	3.7 (1.5)	0.13	.001	.273
Drinking behaviors, *M* (*SD*)						
Maximum drinking in 24 hours	1,229	42.8 (19.9)	35.2 (18.0)	0.40	<.001	<.001
Severity	1,232	23.9 (7.8)	22.2 (7.4)	0.22	<.001	.009
Regular drinking age	1,232	15.6 (2.1)	19.9 (5.9)	0.97	<.001	<.001
Progression years	1,232	3.1 (2.2)	12.0 (6.9)	1.73	<.001	<.001
Personality traits, *M* (*SD*)						
Novelty seeking	1,152	10.6 (4.0)	9.7 (4.0)	0.23	<.001	.923
Extraversion	1,153	4.6 (2.7)	4.6 (2.6)	0.00	.952	.571
Neuroticism	1,152	8.1 (3.4)	7.5 (3.2)	0.18	.002	.188
Alcohol expectancy, *M* (*SD*)						
Positive affection	1,152	24.0 (6.3)	23.4 (6.6)	0.09	.123	.415
Negative affection	1,152	18.0 (4.3)	17.0 (4.6)	0.22	<.001	.001
Aggression	1,152	10.5 (3.6)	8.8 (3.6)	0.47	<.001	<.001
Sexual	1,151	15.5 (4.8)	14.0 (5.0)	0.31	<.001	.028
Comorbidity, *n* (%)						
Depression	1,210	395 (76.1)	473 (68.5)	1.47	.004	.164
ASPD with CD	1,232	198 (37.5)	120 (17.0)	2.94	<.001	<.001
ASPD or CD	1,232	287 (54.4)	210 (29.8)	2.78	<.001	<.001
Parental drinking problems, *n* (%)						
Mother	1,070	138 (29.8)	128 (21.1)	1.59	<.001	<.001
Father	1,058	296 (64.6)	361 (60.2)	1.21	.138	.558
Drinking problems in nuclear family, *M* (*SD*)						
All members	1,054	0.53 (0.23)	0.47 (0.23)	0.26	<.001	<.001
Male proband	686	0.49 (0.22)	0.44 (0.22)	0.23	<.001	.001
Female proband	368	0.62 (0.23)	0.52 (0.23)	0.43	<.001	<.001

Notes: EOA = early-onset alcoholism (≤22 years); LOA = late-onset alcoholism (>22 years); ASPD = antisocial personality disorder; CD = conduct disorder. [a]Effect size calculated using Cohen's *d* for continuous variables and odds ratio for binary variables; [b]*p* values adjusted for gender and interviewed age.

progression from regular drinking to developing AD diagnosis (about 3 years), whereas the progression in the LOA subgroup took nearly 12 years. After adjusting for gender and interviewed age in the models, the EOA group still had more problematic drinking behaviors than the LOA group (all *p*s < .01). The EOA group consumed a higher maximum amount of alcohol within 24 hours and exhibited higher symptom severity than in the LOA subgroup (Table 1).

Although novelty seeking and neuroticism personality scores were higher in the EOA subgroup than in the LOA subgroup, none of the differences remained significant after adjusting for gender and interviewed age. In terms of alcohol expectancy, the EOA group had higher scores for negative affect and aggression. The EOA subgroup (76.1%) had slightly higher comorbidity proportion with major depressive disorder than the LOA subgroup (68.5%). Comorbidity with ASPD and/or CD was significantly more frequent ($p < .001$) in the EOA subgroup than the LOA subgroup, with adjusted ORs ranging from 1.7 to 1.8.

Consistent with our expectation, the EOA group had higher family loading of alcohol use problems than the LOA group. A large proportion (>60%) of fathers had drinking problems in both subgroups, but a significantly higher proportion ($p < .001$) of maternal alcohol use problems were observed in the EOA subgroup than in the LOA subgroup. In addition, alcoholics with early-onset age showed a higher proportion of family history of drinking problems than those with late-onset age, and the magnitude of such difference was higher when restricted to female alcoholics ($p < .001$). Especially in the EOA group, family loading of alcohol use problems among relatives of female probands was approximately 20% higher than that among relatives of male probands. We also tested potential interaction effects among gender, onset age, and family loading of alcohol use problems. Using regression models with covariates adjusted, early age at onset group and female gender showed significant interaction to predict family history of drinking problems ($p < .001$). We also found significant interaction of gender and family history to predict early age at onset of AD ($p < .001$), with the direction of female probands with family history exhibiting early AD onset. In sum, the EOA subgroup was characterized by more men, higher maximum drinking amount and severity, rapid progression, higher negative and aggression alcohol expectancy, higher proportion of family history of alcohol use problems, and more frequently comorbid with ASPD/CD than the LOA subgroup.

TABLE 2. Single marker associations for the four genes with subgroups of alcoholism

		Control (n = 530)	EOA (n = 236)			LOA (n = 336)		
Gene	Marker	$F_{Control}$	F_{Case}	OR	p^a	F_{Case}	OR	p^a
DRD2	rs2242592	0.258	0.288	1.17	.23	0.295	1.21	.10
	rs6279	0.267	0.290	1.12	.38	0.298	1.17	.16
	rs2587548	0.376	0.410	1.15	.23	0.398	1.10	.38
	rs1076563	0.380	0.410	1.13	.28	0.394	1.06	.61
	rs1079596	0.169	0.172	1.03	.88	0.855	1.19	.22
	rs1125394	0.165	0.178	1.10	.55	0.857	1.18	.24
	rs2471857	0.171	0.172	1.01	.94	0.857	1.23	.14
	rs4648318	0.226	0.249	1.14	.36	0.252	1.16	.22
	rs4274224	0.496	0.539	1.19	.13	0.541	1.20	.07
	rs4581480	0.877	0.901	1.28	.19	0.896	1.22	.24
	rs4648317	0.142	0.147	1.04	.81	0.164	1.19	.24
	rs4350392	0.831	0.850	1.15	.41	0.833	1.02	.95
	rs1799978	0.044	0.050	1.15	.59	0.046	1.05	.90
	rs12364283	0.075	0.082	1.10	.68	0.088	1.20	.31
HTR1B	rs2000292	0.735	0.741	1.03	.85	0.278	1.07	.57
	rs130060	0.951	0.980	2.44	**.01**	0.971	1.72	.05
	rs1213366	0.437	0.446	1.04	.78	0.456	1.08	.45
OPRμ1	rs1799971	0.883	0.895	1.12	.54	0.898	1.16	.38
	rs510769	0.265	0.277	1.06	.66	0.758	1.14	.30
	rs3778151	0.164	0.176	1.09	.60	0.837	1.01	.95
	rs483021	0.947	0.948	1.02	1.00	0.961	1.35	.24
	rs660756	0.644	0.654	1.05	.73	0.680	1.18	.13
	rs1067684	0.095	0.107	1.15	.46	0.913	1.10	.61
	rs558025	0.731	0.767	1.22	.14	0.764	1.19	.14
	rs10485058	0.131	0.144	1.11	.51	0.171	1.36	.03
	rs548339	0.637	0.657	1.09	.49	0.679	1.20	.08
	rs569284	0.055	0.058	1.07	.81	0.962	1.45	.13
	rs2236256	0.531	0.541	1.04	.74	0.502	1.14	.21
	rs1918760	0.625	0.633	1.03	.82	0.388	1.06	.61
	rs2281617	0.871	0.88	1.09	.67	0.150	1.19	.25
	rs6941251	0.572	0.608	1.16	.21	0.590	1.08	.48
	rs1998220	0.575	0.599	1.10	.40	0.587	1.05	.65
	rs9322451	0.82	0.837	1.12	.46	0.824	1.02	.90
	rs9479791	0.878	0.897	1.20	.34	0.889	1.11	.54
	rs790266	0.537	0.58	1.19	.13	0.565	1.12	.27
	rs2272381	0.843	0.877	1.33	.10	0.892	1.54	**.005**
	rs9371781	0.304	0.309	1.02	.86	0.339	1.18	.13
	rs6935927	0.573	0.594	1.09	.46	0.575	1.01	.96
	rs4314511	0.834	0.863	1.25	.17	0.847	1.10	.50
SLC6A4	rs3813034	0.602	0.621	1.08	.53	0.402	1.02	.88
	rs1042173	0.599	0.622	1.10	.42	0.402	1.00	1.00
	rs3794808	0.612	0.626	1.06	.64	0.616	1.02	.92
	rs140701	0.62	0.623	1.01	.95	0.382	1.01	.96
	rs4583306	0.609	0.62	1.04	.73	0.615	1.02	.84
	rs2020942	0.399	0.407	1.03	.78	0.402	1.01	.92
	rs6355	0.193	0.302	1.58	.19	0.988	1.61	.33
	rs2066713	0.393	0.399	1.03	.82	0.398	1.02	.84
	rs16965628	0.061	0.067	1.10	.73	0.944	1.10	.68
	rs2020933	0.049	0.054	1.11	.70	0.956	1.11	.72
	rs2020930	0.036	0.043	1.23	.47	0.973	1.32	.40

Notes: EOA = early-onset alcoholism (≤22 years); LOA = late-onset alcoholism (>22 years); OR = odds ratio; F_{Case} = risk allele frequency for cases; $F_{Control}$ = risk allele frequency for controls.
[a]p values adjusted for gender; $p \leq .01$ indicated in **bold.**

Genotypic associations

Independent cases in each family were genotyped, including 236 early-onset alcoholics and 336 late-onset alcoholics. We listed association results of single markers and haplotypes for the four examined genes in Table 2 and Table 3, respectively. All of the association tests controlled for gender. Of the 50 markers tested, only one marker (rs130060) in the *HTR1B* gene was associated with the EOA group (OR = 2.44, p = .01), whereas one marker (rs4274224) in the *OPRμ1* gene showed association with the LOA group (OR = 1.54, p = .005). However, with FDR correction, the p value of rs4274224 increased to .25, which implied a 25% chance to be false positive. None of the markers in the *DRD2* or *SLC6A4* genes were associated with subgroups of AD.

TABLE 3. Haplotype associations for the four genes with subgroups of alcoholism

Locus	Haplotype	$F_{Control}$	EOA			LOA		
			F_{Case}	χ^2	p^a	F_{Case}	χ^2	p^a
DRD2								
Block 1 (rs2242592–rs6279)								
	22	0.257	0.288	1.55	.21	0.297	3.20	.07
	11	0.743	0.712	1.55	.21	0.703	3.20	.07
Block 2 (rs2587548–rs1076563–rs1079596–rs1125394–rs2471857–rs4648318)								
	221112	0.209	0.235	1.23	.27	0.245	2.90	.09
	222221	0.166	0.172	0.07	.78	0.145	1.37	.24
	111111	0.606	0.581	0.89	.35	0.600	0.07	.80
Block 3 (rs4648317–rs4350392–rs1799978–rs12364283)								
	2212	0.073	0.080	0.18	.67	0.089	1.29	.26
	1121	0.038	0.046	0.68	.41	0.044	0.43	.51
	2211	0.069	0.064	0.10	.75	0.075	0.24	.62
	1111	0.801	0.811	0.15	.70	0.790	0.28	.60
HTR1B								
Block 1 (rs2000292–rs130060–rs1213366)								
	112	0.430	0.439	0.08	.77	0.444	0.29	.59
	221	0.024	0.015	1.34	.25	0.018	0.67	.41
	211	0.236	0.235	0.001	.98	0.252	0.50	.48
	111	0.293	0.306	0.36	.55	0.282	0.23	.63
OPRμ1								
Block 1 (rs1799971–rs510769–rs3778151–rs483021–rs660756–rs1067684–rs558025–rs10485058–rs548339)								
	121211111	0.053	0.050	0.09	.77	0.039	1.90	.17
	122122112	0.084	0.106	1.85	.17	0.084	0.003	.96
	111121212	0.267	0.237	1.42	.23	0.232	2.55	.11
	111111111	0.222	0.241	0.62	.43	0.256	2.52	.11
	111111121	0.134	0.145	0.25	.62	0.168	3.72	.05
	211111111	0.116	0.103	0.42	.52	0.103	0.65	.42
	122111111	0.081	0.069	0.62	.43	0.078	0.04	.83
	121111111	0.043	0.049	0.28	.60	0.041	0.05	.82
Block 2 (rs1918760–rs2281617–rs6941251–rs1998220)								
	2122	0.375	0.367	0.09	.77	0.386	0.20	.65
	1122	0.053	0.028	4.35	.04	0.026	6.84	**.009**
	1211	0.132	0.121	0.34	.56	0.150	1.12	.29
	1111	0.440	0.483	2.40	.12	0.437	0.01	.91
Block 3 (rs790266–rs2272381–rs9371781–rs6935927–rs4314511)								
	11112	0.146	0.131	0.55	.46	0.151	0.0004	.98
	21221	0.274	0.273	0.01	.94	0.320	2.34	.13
	22121	0.130	0.115	0.65	.42	0.092	6.31	**.01**
	11111	0.371	0.420	3.16	.08	0.406	0.75	.39
SLC6A4								
Block 1 (rs3813034–rs1042173–rs3794808–rs140701–rs4583306–rs2020942–rs6355–rs2066713)								
	11111111	0.223	0.220	0.01	.90	0.215	0.09	.77
	22222111	0.381	0.370	0.16	.69	0.372	0.05	.82
	11111212	0.352	0.368	0.31	.57	0.360	0.21	.65
	22111212	0.025	0.011	2.70	.10	0.028	0.25	.61
Block 2 (rs16965628–rs2020933–rs2020930)								
	222	0.031	0.039	0.68	.41	0.023	0.86	.35
	111	0.938	0.935	0.06	.80	0.944	0.21	.64

Notes: All haplotypes with frequency greater than 2% are shown in the table; haplotype "1" represents major allele, and "2" represents minor allele. EOA = early-onset alcoholism (≤22 years); LOA = late-onset alcoholism (>22 years); F_{Case} = haplotype frequency for cases; $F_{Control}$ = haplotype frequency for controls.
[a]p values adjusted for gender; $p \leq .01$ indicated in **bold.**

In haplotype analyses, the block including marker rs130060 in gene *HTR1B* did not exhibit significant association with subgroups of AD. For the *OPRμ1* gene, three blocks were defined according to the Gabriel et al. (2002) method and consisted of 18 markers in total. Marker rs4274224 was not in any of the defined blocks. One haplotype in Block 2 (consists of markers rs1918760–rs2281617–rs6941251–rs1998220) was associated with the LOA group with lower frequency in cases (2.6%) than in controls (5.3%). One haplotype in Block 3 (consists of markers rs790266–rs2272381–rs9371781–rs6935927–rs4314511) showed association with the LOA group, with lower frequency in cases (9.2%) than in controls (13.0%). After FDR correction, both p values increased to .09, which implied a 9% chance for these significant findings to be false positive.

Discussion

Typology models have been proposed to subgroup alcoholics into more homogenous groups using a variety of criteria and clinical features, in which age at onset of AD is a shared criterion that can be easily measured in clinics and can divide alcoholics into early- versus late-onset subgroups. Based on a dichotomous classification, our results showed that alcoholics with onset age earlier than 22 years are male predominant, with more severe symptom presentations and comorbidity, rapid progression to developing AD diagnosis, and more family history of alcohol use problems. These clinical characteristics conform to mainly the original and modified Cloninger's Type II alcoholism (Cloninger, 1987) and Babor's Type B alcoholism (Babor et al., 1992). One specific feature in the LOA group is its prolonged interval between regular drinking and developing AD, which is about 4 times the length compared with the EOA group. In addition, the LOA group has a later age for regular drinking (M = 19.9 years) than the EOA group. These results indicate the possibility of doing intervention for drinking problems when individuals are in their early to mid-20s, when it is not too late to take the action for prevention, at least for a subgroup of late-onset alcoholics.

Previous studies found that novelty seeking predicts early-onset alcohol abuse/dependence (Howard et al., 1997); individuals with early onset of AD may exhibit more exploratory excitability, impulsiveness, and extravagance indicated by novelty seeking, and more anxiety, irritability, and changeable mood indicated by neuroticism (Sher et al., 2000). We found that the EOA group scored higher in novelty seeking and neuroticism personality traits than the LOA group, but such differences disappeared after adjusting for gender and age at interview. To explore potential gender effect, we further stratified our analysis for personality traits by gender (data not shown) while adjusting for interviewed age. Comparing scores in the two AD subgroups, we found significantly higher novelty seeking in the EOA group only in women ($p = .003$) and significantly higher neuroticism in the EOA group only in men ($p < .001$). One previous study found that gender differences in the personality traits, such as behavioral under-control and negative emotionality, were observed in relation to adolescent alcohol problems (Martin et al., 2000). Gender of probands seemed to moderate the differences observed for certain personality traits between early- and late-onset alcoholics.

In terms of alcohol expectancy, our results showed that the EOA subgroup expects alcohol to bring more aggression and negative affect than the LOA subgroup, whereas prior studies suggested that negative expectancy was negatively correlated with excessive alcohol use (Kilbey et al., 1998; Lee et al., 1999). Note that the participants in the present study answered alcohol expectancy questions after having been diagnosed with AD because of our ascertainment criteria. Individuals who developed AD at an earlier age may have experienced more negative outcomes after prolonged alcohol use problems, and thus scored high in the negative affect subscale. High expectancy of aggression ($p < .001$) and sexuality ($p = .03$) in the EOA group was supported by a previous study showing a higher score in a questionnaire measuring sexual enhancement ($p = .021$) and aggression ($p = .012$) after consuming alcohol among alcoholics younger than age 20 than those older than age 20 (Lundahl et al., 1997). In addition, we observed high comorbidity with ASPD and/or CD (54%) in EOA, which was almost twice as much as that in LOA. This finding is consistent with reports in several early studies that antisocial alcoholism had earlier onset age, more alcoholism symptoms, and rapid course than pure alcoholics (Hesselbrock et al., 1985; Liskow et al., 1991; Schuckit, 1973).

Our results showed that EOA had higher family loading for alcohol use problems than the LOA subgroup, especially for female alcoholics. Significant interaction effects among female gender, onset age, and family loading of alcohol use problems were also observed. A female patient with EOA may indicate high familial aggregation of alcohol use problems. A family-wise intervention program may be important in such cases. If restricted to families with all siblings of early-onset versus all siblings of late-onset, the magnitude of family history of drinking problems further increased. Previous studies showed that family history was associated with early drinking behaviors. Severe alcoholics with earlier age at onset of AD had a significantly ($p = .01$) higher proportion of family history of problem drinking than the moderate alcoholic group (McGue et al., 1997). Dawson (2000) also found a positive association of family history on the risk of initiating drinking, and the effect was the strongest before age 15 and declined steadily with increasing age. This effect was weaker for men than for women. Curran et al. (1999) reported that family history is a strong predictor for number of alcohol symptoms among female alcoholics. Thus, gender serves as a potential moderator in the relationship between family history and drinking behaviors. In addition, higher family loading of alcohol use problems in female probands may indicate a threshold difference of developing AD by genders. This has been suggested by schizophrenia research that relatives of early-onset female schizophrenic probands had a higher risk of schizophrenia than relatives of early-onset male schizophrenics ($p < .01$; Sham et al., 1994). Thus, EOA versus LOA exhibited diverse clinical manifestations in many aspects, and our results provide empirical evidence for qualitative differences based on a dichotomous AD typology using age at onset.

Among the four genes examined in the current study, only two markers showed genetic associations with AD subgroups (with a 25% chance of being false-positive after an FDR correction). The *HTR1B* gene was associated with the EOA group, whereas the *OPRμ1* gene exhibited association with

the LOA group. The latter finding was consistently seen in both single marker and haplotype analyses (Tables 2 and 3). The association of *HTR1B* with EOA may be partially because of high comorbidity of ASPD or CD in this group, because more than 50% of the early-onset alcoholics were comorbid with ASPD or CD in our samples. Prior studies reported the associations of polymorphisms in the *HTR1B* gene with antisocial behavior in alcoholism or alcoholics with early-onset age and a family history of alcoholism in first-degree relatives (Hasegawa et al., 2002; Soyka et al., 2004). When we further constrain analysis to a subset of alcoholic individuals with the comorbidity of ASPD or CD, we found that the allele frequency distribution for the two markers was very similar between the subset of comorbid AD and full set of AD by EOA/LOA groups. Thus, comorbidity status with ASPD or CD did not fully account for the genetic associations we observed in the EOA group. The early- versus late-onset classification has been reported to be an effective predictor of response to treatment with certain serotonergic medications (Dahmen et al., 2005). Cloninger et al. (1987) also proposed a different response to treatment by alcoholic subtypes. Naltrexone, a drug frequently used to treat alcoholism, acts as a μ-opioid receptor that had a better efficacy in Cloninger's Type II alcoholism (Kiefer et al., 2008). Another study reported that naltrexone attenuated the subjective effects of alcohol in the family history–positive social drinkers but not in the family history–negative group (no alcoholic relatives in at least two generations; King et al., 1997). The exact explanation for the association we observed in the late-onset group with the *OPRμ1* gene was unclear. Nevertheless, the opioid system may be involved in the mechanism of AD and contributes differently in subgroups of AD by onset age.

Similar to previous typology models, we used a subjective cutoff for age at onset of AD to form early- versus late-onset groups. However, individual distinctions in terms of behaviors and symptoms may be dimensional in nature, and one may prefer to treat onset age as a continuous dimension and to see whether the relationships between factors we examined and onset age still hold. We found significant but moderate linear correlations between onset age and almost all the clinical features and alcohol-related traits (correlations between -.4 and .4, results not shown), which are consistent with the results seen in binary classification. In addition, to test whether there are linear or nonlinear effects of onset age for our examined clinical features, we added a quadratic term of onset age using regression models, aside from examining scatter plots to look at the distribution of clinical features with onset age. We found that onset age tends to have a linear effect on the majority of our examined clinical features. A special note is the progression time from regular drinking to AD diagnosis, for which the correlation is high ($r = .82$, $p < .001$). This was expected because the faster the progression duration, the earlier the development of AD.

In conclusion, there are distinct phenotypic and genetic features in early- versus late-onset AD. Following a strategy of using binary typology, our results provided empirical evidence in distinguishing the two subtypes in many aspects, including clinical characteristics and alcohol-related behaviors, alcohol expectancy, family history of alcohol use problems, and comorbidity. Gathering information for age at onset of AD is important and has clinical implications to understand environmental and genetic factors among diagnosed patients.

There are several limitations in the current study. First, most of the phenotypic data was gathered retrospectively, which makes it subject to recall and information bias. For collecting information on family history of alcohol use problems, we previously showed that the agreement among different raters (i.e., reported by index cases, siblings, or parents) had fair to good reliability ($\kappa = .75$~$.82$; Prescott et al., 2005). The average interview age for the early- and late-onset groups were 37.1 and 45.5 years; we reasoned that the majority of siblings have been through the age of risk. However, we did not have age information for all family members to perform adjustment with exact age data. We cannot rule out the possibility that some individuals may develop AD in their later age. The measures of self-reported alcohol expectancies and personality traits were all after the subjects became alcohol dependent. It is not possible to know whether these characteristics were prospectively associated with the risk to develop AD at early age. In addition, chronic alcohol use often has effects on cognitive function and affective regulation. Assessment of alcohol expectancies and personality traits cannot represent the baseline level of such measures and may be influenced by the severity of illness. Dating of symptoms and the age at onset of AD may undergo a telescoping effect that erroneously reported toward the interviewed age. We thus adjusted interviewed age and gender as covariates in all phenotypic comparisons between the two AD subgroups in an attempt to reduce the impact of such bias.

Second, participants in the current study were mainly from treatment facilities but not general population samples. There is a possible difference in treatment seeking by gender and age, and our findings in clinical patients may not be able to directly generalize to individuals with alcohol use problems from the general population.

Third, the selection of markers for each gene was based on the strategy of tagging to genotype minimum index SNPs to represent maximum haplotype information for each gene (for details, please refer to Hodgkinson et al., 2008). The markers tested in our candidate genes may not have full coverage; therefore, we might have missed some signals for those we did not genotype. In addition, among the tested markers, multiple testing is still an issue for false-positive findings, although the selected genes examined in our study have a strong a priori to be associated with AD in the literature. On the contrary, even with more than 1,000 individuals

genotyped, using the current case–control study design, the power to detect true associations for the EOA or LOA subgroups is only moderate. The current study is underpowered for small to moderate genetic effects.

Last, genetic associations cannot provide information or explanations as to how these genes function, especially how they act in EOA or LOA groups. Other study designs are required to answer these questions.

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