Family-Based Association Analyses of Alcohol Dependence Phenotypes Across *DRD2* and Neighboring Gene *ANKK1*

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Background: There is an extensive and inconsistent literature on the association of the dopamine D2 receptor gene (*DRD2*) with alcohol dependence. Conflicting results have been attributed to differences in the severity of the alcohol dependence phenotype across studies, failure to exclude related disorders from comparison groups, and artifacts of population-stratification. Recently the genetic polymorphism most widely analyzed in *DRD2*, Taq1A, has been discovered to reside in a neighboring gene, ankyrin repeat and kinase domain containing 1 (*ANKK1*), located 10 kb downstream from *DRD2*.

Methods: To more carefully characterize evidence for association across this region, we genotyped 26 single nucleotide polymorphisms (SNPs) spanning *DRD2* and *ANKK1* in a sample of 219 Caucasian families (n = 1,923) from the Collaborative Study on the Genetics of Alcoholism (COGA), making this the most extensive analysis to date of association between this region and alcohol dependence. We used family-based analyses robust to population-stratification, and we made use of rich phenotypic data to analyze alcohol dependence and subtypes hypothesized in the literature to be more directly influenced by *DRD2*.

Results: We found that the evidence for association is strongest in the 5′ linkage disequilibrium block of *ANKK1* (that does not contain Taq1A), with weak evidence of association with a small number of SNPs in *DRD2*. The association in *ANKK1* is strongest among the subsets of alcoholics with medical complications and with antisocial personality disorder.

Conclusions: More extensive genotyping across *DRD2* and *ANKK1* suggests that the association with alcohol dependence observed in this region may be due to genetic variants in the *ANKK1* gene. *ANKK1* is involved in signal transduction pathways and is a plausible biological candidate for involvement in addictive disorders.

Key Words: *DRD2*, *ANKK1*, Genetics, Alcohol Dependence, Association Analyses.

THERE IS AN EXTENSIVE and controversial literature examining possible association between the dopamine D2 receptor gene (*DRD2*) and alcohol dependence. *DRD2* is considered a good candidate gene for alcohol dependence risk, because it is thought that the rewarding effects of alcohol are mediated through the mesolimbic dopamine system (Wise and Rompre, 1989). An association between *DRD2* and alcoholism was originally reported by Blum et al. (1990) when an increased frequency of the Taq1A1 restriction fragment length polymorphism was observed in postmortem brain tissue from severe alcoholics as compared to nonalcoholic controls (Blum et al., 1991). The association between alcoholism and polymorphisms in *DRD2* has been replicated by several groups (Amadeo et al., 1993; Blum et al., 1991; Comings et al., 1991; Foley et al., 2004; Hietala et al., 1997; Higuchi et al., 1994; Ishiguro et al., 1998; Konishi et al., 2004; Kono et al., 1997; Neiswanger et al., 1995; Noble, 2003; Noble et al., 1994; Parsian et al., 1991), but many other studies have failed to replicate an association between *DRD2* and alcohol dependence (Arinami et al., 1993;

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Bolos et al., 1990; Chen et al., 1996, 1997, 2001; Cook et al., 1992; Cruz et al., 1995; Edenberg et al., 1998; Gelernter and Kranzler, 1999; Gelernter et al., 1991; Goldman et al., 1992, 1997; Lee et al., 1999; Lobos and Todd, 1998; Lu et al., 1996; Parsian et al., 2000; Sander et al., 1995, 1999; Schwab et al., 1991; Suarez et al., 1994; Turner et al., 1992; Waldman et al., 1999). Thus, the purported association remains controversial.

Three explanations have been put forth to account for the inconsistency in the literature: differences in severity of alcohol dependence across studies, failure to exclude related conditions from control groups, and population-stratification. It has been suggested that the association between *DRD2* and alcohol dependence is limited primarily to "severe" alcoholics (Blum et al., 1991; Lawford et al., 1997; Noble et al., 2000); however, it has been difficult to empirically evaluate this hypothesis, as severity has been determined using different criteria across different studies. Some of the severity subtypes that have been most widely examined are comorbid antisocial personality disorder (ASPD) (Bolos et al., 1990; Gelernter et al., 1991; Hill et al., 1999; Matsushita et al., 2001; Ponce et al., 2003), presence of withdrawal symptoms (Chen et al., 1997; Finckh et al., 1997; Gelernter et al., 1991; Ishiguro et al., 1998; Matsushita et al., 2001; Turner et al., 1992), and resulting medical complications (Blum et al., 1990, 1991; Chen et al., 1997; Gorwood et al., 2000; Lawford et al., 1997; Noble, 2000a; Parsian et al., 2000; Turner et al., 1992); however, results have been inconsistent across studies. The phenotype of resulting medical complications has received the most consistent support (Blum et al., 1990, 1991; Lawford et al., 1997; Noble, 2000b), yet interpretation of this measure of alcoholism severity is problematic because medical complications could have a separate genetic basis depending on the organ system involved and/or reflect the duration of alcoholism rather than its severity (Gelernter et al., 1993).

Another problem with interpretation of the literature is the suggestion that *DRD2* may contribute more broadly to a "reward deficiency syndrome," a collection of addictive, impulsive, or compulsive behaviors, including alcoholism, polysubstance abuse, smoking, obesity, attention-deficit disorder, and gambling (Blum et al., 1996; Noble, 2003). The hypothesis is that individuals with genetic variants causing impairments in the dopamine reward pathway may abuse multiple substances and engage in diverse experiences that are emotionally heightening in order to enhance stimulation of an impaired pathway (Comings and Blum, 2000). Therefore, it has been suggested that the failure to exclude related phenotypes from control groups in tests for association with alcohol dependence could have contributed to negative reports (Noble et al., 2000).

The other major explanation put forth to account for inconsistent results is population-stratification, referring to the possibility that cases and controls may differ on variables other than disease status (e.g., ethnicity), and accordingly, differences in allele frequencies between the groups may be attributable to these third variables, rather than to a true association between genotype and disease status. This is particularly problematic in relation to *DRD2*, as the frequency of the Taq1A1 allele differs substantially across populations (Goldman et al., 1993; Kidd et al., 1998) and much of the literature on *DRD2* is based on case-control studies. Family studies avoid this potential confound, and therefore are noteworthy: none of the 5 published family-based studies found association between the *DRD2* locus and alcoholism (Blomqvist et al., 2000; Bolos et al., 1990; Edenberg et al., 1998; Neiswanger et al., 1995; Parsian et al., 1991); however, some family studies have found evidence of linkage (Hill et al., 1999), which may suggest the involvement of a nearby variant in the region. A related problem is that differences in the extent of linkage disequilibrium (LD) between the Taq1A allele and other polymorphisms in *DRD2* have been documented across populations (Goldman et al., 1993; Kidd et al., 1998). In the likely event that the Taq1A polymorphism is not the functional variant itself, but is in LD with the functional variant, differing LD structures between Taq1A and other genetic polymorphisms in the region would influence the ability to detect association across studies as well.

Interpreting the literature on *DRD2* and alcohol dependence (and related phenotypes) is further complicated by the recent discovery that the Taq1A polymorphism that has been most extensively studied is actually located 10 kb downstream from the *DRD2* gene in a neighboring gene, ankyrin repeat and kinase domain containing 1 (*ANKK1*) (Neville et al., 2004). *ANKK1* contains a single serine/threonine protein kinase domain, and is a member of a family of proteins involved in signal transduction. The Taq1A SNP is located within an exon of *ANKK1*, causing a nonsynonymous coding change (Glu713Lys) that may affect the substrate binding specificity of the gene product. It has been hypothesized that the *ANKK1* gene may be involved in the dopaminergic reward pathway through signal transduction (Neville et al., 2004).

The present study attempts to more carefully characterize the involvement of the *DRD2* and *ANKK1* genes using data collected as part of the Collaborative Study on the Genetics of Alcoholism (COGA). This report is a substantial extension of a previous analysis of the Taq1A polymorphism in a subset of the COGA sample in which we reported no association between this polymorphism and alcohol dependence (Edenberg et al., 1998). We aimed to clarify and extend the current literature by: (1) genotyping multiple SNPs across the region containing both the *DRD2* and *ANKK1* genes; (2) conducting family-based association analyses using our extended COGA pedigrees to avoid problems associated with case-control studies; and (3) using the wealth of phenotypic data collected as part of the COGA project (a) to test for association with phenotypes related to alcohol dependence severity that have been examined in the literature and (b) to screen out related disorders from comparison groups (for discordant sibling comparisons). Specifically, this study examined DSM-IV alcohol dependence as a phenotype, and then tested for association with more severe subgroups of alcohol-dependent individuals that have been studied in the literature: alcohol dependence with comorbid ASPD, alcohol dependence with

medical complications, alcohol dependence with high symptom count, and alcohol dependence with withdrawal.

METHODS

Sample

The Collaborative Study on the Genetics of Alcoholism (COGA) is a multi-site project, in which families were collected by 6 centers across the United States: Indiana University, State University of New York Health Science Center, University of Connecticut, University of Iowa, University of California/San Diego, and Washington University, St Louis. Probands identified through inpatient or outpatient alcohol treatment programs by each of these 6 sites were invited to participate if they had a sufficiently large family (usually sibships >3 with parents available) with 2 or more members in a COGA catchment area (Reich, 1996). The institutional review boards of all participating centers approved the study. Additional details about the study have been published previously (Begleiter et al., 1995; Reich, 1996).

Because patterns of LD and allele frequencies often differ between races, we analyzed the subset of 219 Caucasian families ($n = 1{,}923$). However, we note that the results were largely unchanged in the full sample ($n = 262$ families, 2,282 individuals; available upon request from the authors).

Phenotypes

All individuals were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview (Bucholz et al., 1994; Hesselbrock et al., 1999). For this study, lifetime DSM-IV diagnoses of alcohol dependence were analyzed (American Psychiatric Association, 1994). In the Caucasian sample, 768 individuals (32%) met criteria for DSM-IV alcohol dependence. Males were over-represented among the alcohol-dependent individuals, consistent with epidemiological data, comprising 66% of the affected individuals. This gender difference was further exaggerated in the more severe alcohol dependence subgroups. Withdrawal was evaluated in the SSAGA according to DSM-IV criteria by the endorsement of 2 or more of the following somatic symptoms after stopping or reducing drinking: shakes (hands trembling), inability to sleep, anxiety or depression, sweating or racing heart beat, nausea/vomiting, hallucinations, or grand mal seizures (Bucholz et al., 1994). The question specified that these symptoms should be "more intense than the usual hangover," resulting after "cut(ting) down, stop(ping) or go(ing) without drinking after drinking steadily for some time." In addition, the endorsement of "DTs" was included. Of the 768 alcohol-dependent individuals, 376 (49%; 72% male) met criteria for withdrawal. Previous studies have illustrated that alcohol dependence with withdrawal is associated with a greater number of alcohol, drug, and depressive problems (Schuckit et al., 2003). Medical complications were assessed by an item in the SSAGA that asked if they had ever experienced any of the following health problems as a result of prolonged use of alcohol: liver disease or yellow jaundice, stomach disease or vomiting blood, pancreatitis, or cardiomyopathy. Individuals with a positive endorsement of any of these items were coded as having experienced medical complications; 130 (17%; 82% male) of the alcohol-dependent individuals in the sample reported at least 1 medical complication. Medical complications were significantly correlated with severity of the dependence phenotype: just over 80% of individuals who reported medical complications met criteria for 6 or 7 of the DSM-IV alcohol dependence criteria, compared to only 17% of the sample who did not report any medical complications. To attempt to tease apart medical complications from alcohol dependence severity, we also analyzed the subgroup of alcohol-dependent individuals who reported 6 or 7 symptoms ($n = 363$; 74% male), in an effort to clarify this unresolved issue, since the converse was not true (i.e., only 29% of individuals with a high symptom count also reported medical complications). Finally, ASPD was diagnosed in the SSAGA using DSM-IIIR criteria (as DSM-IV was under development at the time that interviewing began). Of the alcohol-dependent individuals, 163 (21%; 86% male) also met criteria for ASPD. Previous studies have demonstrated that alcohol dependence with ASPD may be a particularly severe form of the disorder (Hesselbrock and Hesselbrock, 1994). Table 1 shows the complete distribution of overlap across the alcohol-dependent severity subgroups. The greatest overlap was between the groups of alcohol-dependent individuals meeting criteria for withdrawal, and alcohol-dependent individuals with high symptom (6 or 7) counts.

SNP Analyses

Publicly available databases, dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) and HapMap (http://www.hapmap.org) were used to identify SNPs within and flanking the *DRD2/ANKK1* genes. Genotyping was done with a modified single nucleotide extension reaction, with allele detection by mass spectroscopy (Sequenom MassArray system; Sequenom, San Diego, CA). All genotypic data were checked for Mendelian inheritance of marker alleles with the USERM13 (Boehnke, 1991) option of the MENDEL linkage computer programs, which was then used to estimate marker allele frequencies. Chi-square tests were used to ensure that all SNPs were in Hardy–Weinberg equilibrium.

We genotyped 29 SNPs across the region. Three SNPs had very low heterozygosities (<0.05) and were dropped, leaving a total of 26 SNPs for analyses: 16 SNPs across *DRD2* and 10 SNPs across *ANKK1*. Figure 1 shows the location of genotyped SNPs across the region. LD across the region was computed from haplotype frequencies generated by the Transmit software (Clayton, 1999) for all pairs of SNPs. This program uses family data and assumes no recombination to limit the haplotypes to those consistent with Mendelian inheritance. It then uses the expectation maximization (EM) algorithm over the consistent haplotypes to determine the haplotype frequencies.

Statistical Analyses

Multiplex families of alcoholics were used in tests of association between each of the SNPs and each of the phenotypes studied,

Table 1. Overlap Between Subgroups of Alcohol-Dependent Individuals With Severity-Related Conditions

	Medical complications ($n = 130$)	ASPD ($n = 163$)	Withdrawal ($n = 376$)	High symptom count ($n = 363$)
Medical complications	–	50 (30.7%)	111 (29.5%)	106 (29.2%)
ASPD	50 (38.5%)	–	115 (30.6%)	118 (32.5%)
Withdrawal	111 (85.4%)	115 (70.6%)	–	297 (81.8%)
High symptom count	106 (81.5%)	118 (72.4%)	297 (79.0%)	–

Number of individuals, with column percentages in parentheses, provided in each cell.
ASPD, Antisocial personality disorder.

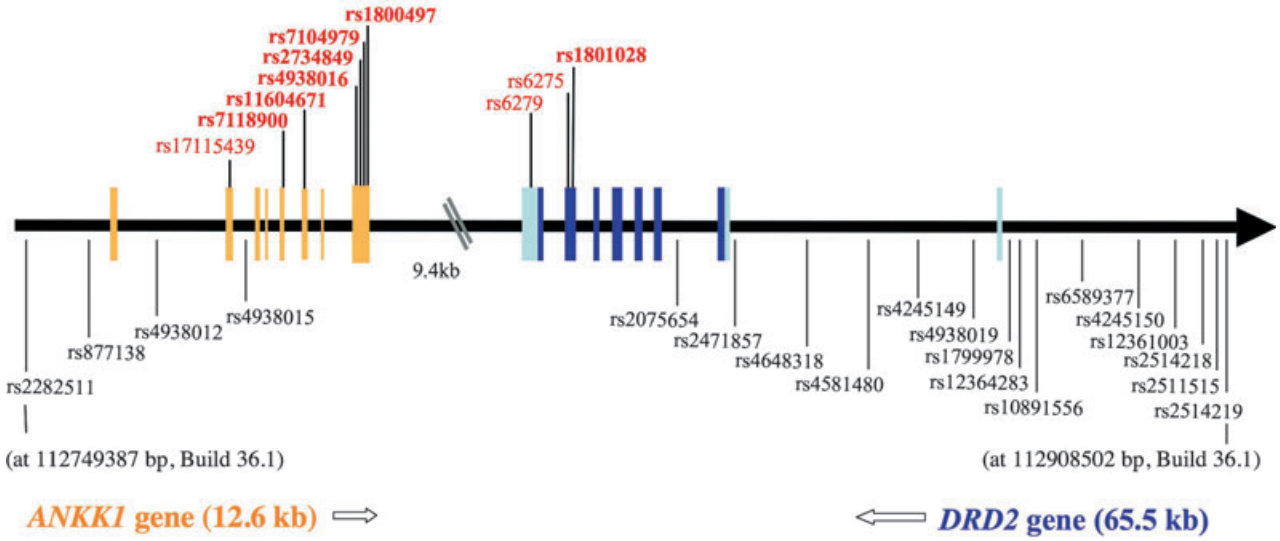

Fig. 1. Location of genotyped SNPs across the region. Exons for *DRD2* shown in dark blue; exons for *ANKK1* shown in orange. The light blue boxes represent 5′ and 3′ UTRs; the red SNPs are coding SNPs.

using the Pedigree Disequilibrium Test (PDT) (Martin et al., 2000). The PDT uses all available trios in a family (2 parents plus child genotyped) as well as discordant siblings. This test produces 2 statistics: the "PDT-ave," which averages the association statistic over all families, and the "PDT-sum," which gives greater weight to larger families with more informative trios and discordant siblings (Martin et al., 2000). We expect genetic heterogeneity in our sample whereby not every gene will necessarily have a detectable impact on alcohol dependence/related phenotypes in all families in the sample. By chance, depending on whether larger or smaller families are associated with the gene under study, this will determine whether association would be detected using the sum or average statistic (i.e., if larger families happen to be associated, the sum statistic will be more significant; on the other hand, if larger families are not associated with the gene they would mask any potential association in the sum statistic due to the heavier weighting of these families). Accordingly, we computed both statistics because we expect genetic heterogeneity and it is impossible to know a priori what subset of families will show association.

To the extent that the *DRD2* gene may be related to broad disinhibitory phenotypes, special care must be utilized when classifying "unaffecteds" (Noble, 2003). Accordingly, across all analyses, we removed from the unaffected group (and coded as unknown) individuals meeting criteria for DSM-IIIR illicit drug dependence ($n = 130$) or ASPD ($n = 31$). In addition, a small number of individuals ($n = 73$) were removed from the unaffected group because they endorsed 3 or more symptoms of alcohol dependence, but did not meet criteria for a diagnosis due to the requirement of clustering in time.

RESULTS

Figure 2 shows the LD across the region. From the D′ measure, the SNPs in the region appear to fall into 3 LD blocks: rs2282511–rs4938015, rs7118900–rs4648318, and rs4581480–rs2511515. The r^2 measure reveals very high correlation among SNPs in the first block, but only moderate correlation in the remaining blocks.

Table 2 presents results for all genotyped SNPs in *DRD2* and *ANKK1* with each of the phenotypes analyzed. Across all phenotypes, *p*-values were generally more significant using the PDT-sum versus average statistic, suggesting some heterogeneity in the sample with larger families contributing more to the signal. However, the PDT-average statistics yielded results in the same direction, indicating that it was not the case that only a few large COGA families contributed to the signal. When analyzing DSM-IV alcohol dependence, 3 of the 10 SNPs in *ANKK1* and 1 of the 16 SNPs in *DRD2* yielded *p*-values < 0.05. With the more severe alcohol dependence phenotypes, alcohol dependence with resultant medical complications, and alcohol dependence with comorbid ASPD, the evidence for association with *ANKK1* increased despite a reduction in sample size. For alcohol dependence with medical complications, 6 SNPs in *ANKK1* were significant at $p < 0.05$, with 3 SNPs significant at $p < 0.01$. For alcohol dependence with ASPD 9 of the 10 SNPs genotyped in *ANKK1* were significant at $p < 0.05$. With this comorbid phenotype 5 SNPs in *DRD2* also yielded *p*-values < 0.05. The association observed with alcohol dependence with a high number of symptoms was not as strong as that observed with the subgroup with medical complications. In addition, severity as defined by alcohol dependence with withdrawal did not yield more significant evidence for association.

Because *p*-values are necessarily influenced by sample size, Table 3 presents the overtransmission ratio and frequencies of the overtransmitted allele in affected and unaffected members of discordant sibling pairs to give an indication of effect size. These were calculated for the SNP rs4938012, the most significant SNP in the block of SNPs in high LD at the 5′ end of *ANKK1* that yielded evidence of association. The over-transmission ratio is calculated by dividing the number of times a particular allele was transmitted to affected indi-

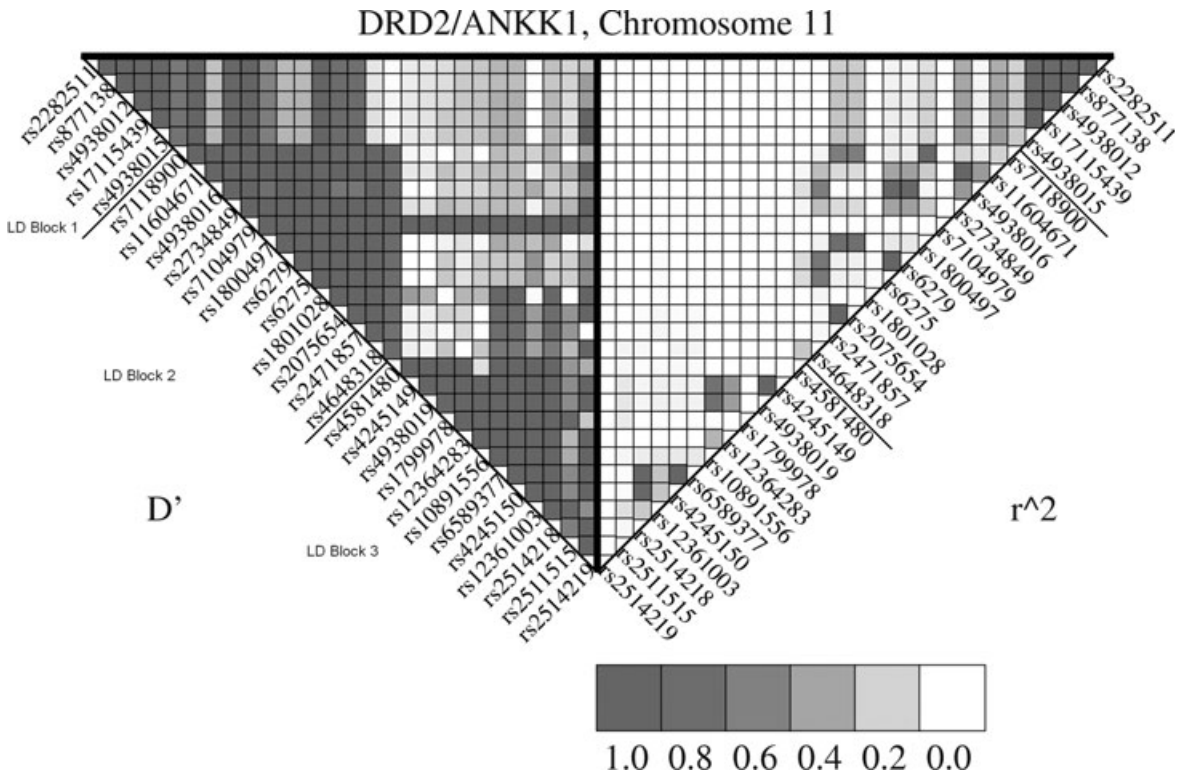

Fig. 2. Linkage disequilibrium across the region.

Table 2. *p*-Values From Family-Based Association Tests

Marker	Gene	LD block	Chromosomal position[a]	Heterozygosity	AD		AD + Med compl		AD + ASPD		AD + Withdrawal		AD + 6, 7 Symptoms	
					Sum	Average	Sum	Average	Sum	Average	Sum	Average	Sum	Average
rs2282511	*ANKK1*	1	112749387	0.47	0.06	0.25	**0.009**	**0.02**	**0.03**	0.10	0.06	0.23	0.15	0.25
rs877138	*ANKK1*	1	112761718	0.46	0.08	0.30	**0.03**	0.13	**0.02**	0.23	0.07	0.21	**0.05**	0.14
rs4938012	*ANKK1*	1	112764864	0.45	**0.03**	**0.03**	**0.008**	**0.01**	**0.01**	**0.04**	**0.04**	**0.05**	**0.02**	**0.03**
rs17115439	*ANKK1*	1	112769482	0.45	**0.04**	0.10	**0.01**	**0.02**	**0.01**	0.06	**0.04**	0.06	**0.03**	**0.05**
rs4938015	*ANKK1*	1	112769854	0.45	0.06	0.15	**0.02**	0.07	**0.02**	0.22	0.09	0.20	0.06	0.08
rs7118900	*ANKK1*	2	112772031	0.31	0.20	0.83	0.72	0.73	**0.02**	0.08	0.06	0.57	0.40	0.95
rs11604671	*ANKK1*	2	112773269	0.51	0.51	0.79	0.07	0.06	**0.03**	0.12	0.38	0.60	0.37	0.31
rs4938016	*ANKK1*	2	112775225	0.45	**0.03**	**0.03**	0.06	**0.04**	0.50	0.29	0.77	0.63	0.52	0.25
rs2734849	*ANKK1*	2	112775370	0.50	0.29	0.71	**0.02**	**0.03**	**0.04**	0.12	0.37	0.75	0.20	0.30
rs1800497[b]	*ANKK1*	2	112776038	0.32	0.41	0.83	0.49	0.56	**0.03**	**0.04**	0.10	0.72	0.53	0.75
rs6279	*DRD2*	2	112786283	0.46	0.43	0.32	0.12	0.10	0.53	0.25	0.89	0.58	0.48	0.14
rs6275	*DRD2*	2	112788687	0.45	0.37	0.20	0.12	0.13	0.29	0.10	0.77	0.41	0.19	**0.03**
rs2075654	*DRD2*	2	112794276	0.27	0.57	0.65	0.19	0.24	**0.01**	0.06	0.11	0.57	0.17	0.64
rs2471857	*DRD2*	2	112803549	0.28	0.60	0.70	0.29	0.47	**0.01**	0.06	0.13	0.58	0.17	0.54
rs4648318	*DRD2*	2	112818599	0.40	0.92	0.37	0.56	0.11	1.00	0.34	0.57	0.97	0.54	0.17
rs4581480	*DRD2*	3	112829684	0.18	0.98	0.93	0.56	0.80	0.38	0.59	0.61	0.54	0.92	0.43
rs4245149	*DRD2*	3	112843567	0.26	0.71	0.66	0.55	0.74	**0.02**	**0.04**	0.82	0.49	0.70	0.84
rs4938019	*DRD2*	3	112846601	0.26	0.71	0.38	0.29	0.66	**0.03**	0.06	0.90	0.97	0.60	0.39
rs1799978	*DRD2*	3	112851561	0.09	**0.02**	0.17	1.00	0.78	0.32	0.35	**0.04**	0.07	0.46	0.27
rs12364283	*DRD2*	3	112852165	0.14	0.90	0.30	0.53	0.48	0.06	0.14	0.61	0.53	0.15	0.44
rs10891556	*DRD2*	3	112857971	0.32	0.33	0.11	0.94	0.48	0.08	0.16	0.83	0.33	0.69	0.73
rs6589377	*DRD2*	3	112860946	0.45	0.68	0.84	0.46	0.17	0.55	0.75	0.96	0.55	0.36	0.62
rs4245150	*DRD2*	3	112869857	0.45	0.65	0.82	0.54	0.52	0.70	0.70	0.85	0.97	0.60	0.81
rs12361003	*DRD2*	3	112886028	0.47	0.40	0.45	0.17	0.40	**0.03**	0.14	0.10	0.28	0.28	0.92
rs2514218	*DRD2*	3	112898204	0.44	0.49	0.58	0.39	0.26	0.80	0.56	0.80	0.89	0.47	0.92
rs2511515	*DRD2*	3	112902865	0.43	0.92	0.79	0.61	0.54	0.18	0.28	0.59	0.73	1.00	0.94

AD (*n* = 768 affected), alcohol dependence; AD + Med Compl (*n* = 130 affected), alcohol dependence with medical complications; AD + ASPD (*n* = 163), alcohol dependence with comorbid antisocial personality disorder; AD + Withdrawal (*n* = 376), alcohol dependence with withdrawal; AD + 6, 7 Symptoms (*n* = 363 affected), alcohol-dependent individuals with 6 or 7 symptoms.

[a]According to NCBI genome build 36.2, dbSNP 126.

[b]Boldface SNP is the TaqIA marker frequently genotyped in the *DRD2* literature.

p-Values ≤0.05 are highlighted in bold, *p*-values are uncorrected for multiple testing.

Table 3. Comparison of Effect Sizes Across Phenotypes at rs4938012

	AD	AD + Med Compl	AD + ASPD	AD + Withdrawal	AD + 6, 7 Symptoms
Overtransmission ratio	1.09	1.36	1.2	1.11	1.11
Frequency in affected sibs	70%	72%	74%	69%	70%
Frequency in unaffected sibs	66%	64%	61%	63%	62%

G allele is overtransmitted/more frequent in affected individuals.

AD, Alcohol dependence; AD + Med Compl, alcohol dependence with medical complications; AD + ASPD, alcohol dependence with comorbid antisocial personality disorder; AD + Withdrawal, alcohol dependence with withdrawal; AD + 6, 7 Symptoms, alcohol-dependent individuals with 6 or 7 symptoms.

viduals, across all families, with the number of times it was not transmitted. In the absence of association, we expect these numbers to be equal, yielding a ratio of 1.0. Ratios higher than 1.0 indicate how much more frequently the allele was transmitted to affected individuals. The overtransmission ratio for DSM-IV alcohol dependence was 1.09 with a 4% frequency difference between discordant siblings. The phenotypes alcohol dependence with medical complications and alcohol dependence with comorbid ASPD were the only 2 severity indices that yielded an increase in the overtransmission ratio, an increase in the frequency among affected siblings, and a decrease in frequency among unaffected siblings from discordant pairs. The subgroup of alcohol-dependent individuals with medical complications had the highest overtransmission ratio (1.36), with an 8% increase in frequency of the allele in the affected group compared to the unaffected group, while the subgroup of alcohol-dependent individuals with ASPD showed the greatest difference between the frequency of the overtransmitted allele in affected compared to unaffected siblings (13% difference).

DISCUSSION

By genotyping SNPs across the region containing *ANKK1* and *DRD2*, by conducting family-based analyses, and by carefully characterizing the alcohol dependence phenotype, this study provides the most extensive investigation of the *DRD2* region with alcohol dependence to date. Interestingly, our analyses yield evidence of association, as indicated by the overtransmission of particular alleles to affected individuals within a family, and an increased frequency of the overtransmitted allele in affected as compared to unaffected siblings, across multiple SNPs in this region. However, the evidence for association is most strongly concentrated in SNPs in the 5′ block of the *ANKK1* gene.

These results closely parallel a recent paper examining association between *ANKK1* and *DRD2,* as well as 2 other nearby genes, *NCAM1* and *TTC12,* with respect to nicotine dependence (Gelernter et al., 2006). The LD structure across the region containing *ANKK1* and *DRD2* in the Gelernter sample closely matches that found in the COGA sample, and, similar to our finding, association was observed with SNPs in the block containing *ANKK1/TTC12*, with only weak evidence of association in *DRD2*. The most significant SNP associated with nicotine dependence in the Gelernter paper, rs4938012, was also the most significant SNP with alcohol dependence in our analyses. Unfortunately, we do not have nicotine dependence diagnoses for the COGA sample. We do have a measure of habitual smoking, as measured by interview report of smoking at least 1 pack a day for 6 months or longer, but there was no evidence of association with this phenotype in the COGA sample (this phenotype may more closely resemble another phenotype analyzed in the Gelernter paper, Fagerstrom Test for Nicotine Dependence Scores, which did not yield strong evidence for association in their sample). The sample analyzed in the Gelernter report was ascertained based on sibling pairs affected with cocaine or opioid dependence, and 40% of the sample was affected with alcohol dependence (Gelernter et al., 2006); thus, it is unclear to what extent their results may have been influenced by alcohol dependence in the sample.

Supporting previous findings in the literature, the effect found here does appear to be stronger in subgroups of more severe alcohol-dependent individuals (Noble et al., 2000). Specifically, we found stronger evidence of association with alcohol dependence with medical complications and alcohol dependence with comorbid ASPD. The findings with medical complications extend the current literature in which this phenotype has received fairly consistent support (Blum et al., 1990, 1991; Lawford et al., 1997; Noble, 2000b). However, despite the fact that experiencing medical complications is correlated with meeting criteria for a higher number of dependence criteria, our analyses of the subgroup of alcohol-dependent individuals meeting 6 or 7 dependence criteria suggest that simply having a high symptom count does not account for the association. In addition, there was not increased evidence of association with the subgroup of alcohol-dependent individuals with withdrawal symptoms. These analyses suggest that the association with gene(s) in this region may be more specific to alcohol dependence characterized by medical complications and alcohol dependence with comorbid ASPD, rather than broadly applying to "more severe" cases of alcohol dependence.

The *p*-values reported in the text are uncorrected for multiple testing. No consensus exists for the most appropriate way to handle the analysis of correlated phenotypes and correlated SNPs. The Nyholt correction has been proposed as a means of dealing with the latter concern (Nyholt, 2004), by

using information on the pairwise LD matrix for the genotyped SNPs to compute the number of "effectively independent" SNPs. Using the updated method of Li and Ji (2005), the effective number of SNPs (M_{eff}) based on the 26 SNPs genotyped in this study was 11. With $M_{eff} = 11$, the Bonferroni corrected significance threshold required across the region would be $p = 0.005$. The most significant SNPs in *ANKK1* approached this more stringent level of significance ($p = 0.008$ with rs4938012). However, rather than employing strict *p*-value cut-offs for evaluating evidence for association for a particular gene, we consider as positive a group of SNPs yielding evidence of overtransmission that correspond with the pattern of LD. In the current paper, we have 3 nominally significant SNPs ($p < 0.05$) and 3 trend level SNPs ($p < 0.10$) of 10 in *ANKK1* with the phenotype of alcohol dependence, but only 1 of 16 SNPs in *DRD2*. The single SNP in *DRD2* yielding $p < 0.05$ has low heterozygosity and is surrounded by clearly negative SNPs. Accordingly, although we cannot rule out involvement of *DRD2,* we believe our data more strongly support a risk locus in *ANKK1*. The support for this conclusion is even stronger with the phenotype of alcohol dependence with medical complications, where 6 SNPs yield $p < 0.05$ and 2 additional SNPs yield $p < 0.10$ of the 10 SNPs in *ANKK1*, and none of the SNPs in *DRD2* yield any evidence of association. Furthermore, our confidence in this conclusion is bolstered by the parallel pattern of results observed in the Gelernter et al. (2006) report of extensive analyses of genetic variation across this region. Our results are also consistent with the report that the *DRD2* Taq1A polymorphism was not associated with alcohol dependence in a subset of the sample analyzed here (Edenberg et al., 1998).

We have chosen not to run a series of haplotype analyses across the region because we believe that this would compound the problems associated with multiple testing, and that the single SNP association results compellingly suggest that the association is concentrated in the 5′ block of LD in *ANKK1*. However, there were several SNPs in the second LD block (that spanned both *ANKK1* and *DRD2*), that showed some evidence of association with the phenotype of alcohol dependence with comorbid ASPD. Accordingly, we ran a haplotype analysis with the 3 tag SNPs in block 2 indicated by Haploview Tagger (rs6275–rs11604671–rs4648318) with the phenotype of alcohol dependence with comorbid ASPD. The haplotype analysis was not significant with either sum or average PDT (*p*-values >0.10). Because there has been longstanding interest in a possible role of the Taq1A polymorphism in susceptibility to alcohol dependence, we also ran a 2 SNP haplotype, comprised of rs4938012 (the SNP showing the strongest evidence of association from block 1), and rs1800497 (the Taq1A polymorphism in the second LD block), to approximate a multi-locus model, in order to test whether genetic variation in this second, independent block of SNPs in *DRD2* contributed any independent evidence for association in addition to the evidence for association yielded by single SNPs in the first block of *ANKK1*. The transmission rates for the individual haplotypes for the 2 SNP haplotype comprised of rs4938012 and the Taq1A polymorphism indicated that both of the haplotypes containing the "1" allele at rs4938012 were undertransmitted and both haplotypes containing the "2" allele at rs4938012 were overtransmitted. Accordingly, the Taq1A polymorphism does not add any additional information over the evidence for association yielded by SNPs in the first LD block.

Thus, our data indicate that the evidence for association in our sample of families with multiple alcoholic members lies primarily in the *ANKK1* gene. *ANKK1* is a member of an extensive family of proteins involved in signal transduction and cellular responses to external stimuli. Accordingly, it is possible that *ANKK1* may be involved in dopaminergic reward processes via signal transduction. The strongest evidence for association in *ANKK1* came from SNPs across the most 5′ block in the gene. Data from the HapMap project suggest that this block of LD extends beyond *ANKK1* and into the neighboring 5′ gene, Tetratricopeptide Repeat Domain 12 (*TTC12*). Tetratricopeptide repeat domains are found in a variety of organisms including bacteria, yeast, fungi, plants, and humans, and appear to be involved in a variety of functions including protein–protein interactions. Because of the extensive LD across *ANKK1* and *TTC12* in this region, it is unclear whether variation in *TTC12* may also be involved in the detected evidence for association. Finally, we note that our findings do not rule out involvement of dopaminergic transmission in addictive processes, for which there is evidence from several lines of research, including animal studies (Crabbe and Phillips, 1998; Thanos et al., 2004), and brain imaging studies (Volkow et al., 2004).

In short, our analyses, using data from the largest family-based study of alcoholism to date (COGA), suggest that further investigation of the role of the several genes clustered in this chromosomal region is warranted. These results suggest that genes in the vicinity of *DRD2*, such as *ANKK1*, and possibly others, are involved in the risk for specific severe forms of alcohol dependence. The involvement of multiple genes in the region may also provide a possible explanation for the inconsistency in the literature surrounding *DRD2* and addictive behaviors.

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REFERENCES

Amadeo S, Abbar M, Fourcade ML, Waksman G, Leroux MG, Madex A, Selin M, Champiat J-C, Brethome A, Leclaire Y, Castelnau D, Venisse J-L, Mallet J (1993) D2 dopamine receptor gene and alcoholism. J Psychiatr Res 27:173–179.

Arinami T, Itokawa M, Komiyama T, Mitsushio H, Mori H, Mifune H, Hamaguchi H, Toru M (1993) Association between severity of alcoholism and the A1 allele of the dopamine D2 receptor gene TaqI A RFLP in Japanese. Biol Psychiatry 33:108–114.

American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders. 4th ed. American Psychiatric Association, Washington, DC.

Begleiter H, Reich T, Hesselbrock V, Porjesz B, Li TK, Schuckit M, Edenberg HJ, Rice J (1995) The collaborative study on the genetics of alcoholism. Alcohol Health Res World 19:228–236.

Blomqvist O, Gelernter J, Kranzler HR (2000) Family-based study of DRD2 alleles in alcohol and drug dependence. Am J Med Genet B Neuropsychiatr Genet 96:659–664.

Blum K, Cull JG, Braverman ER, Comings DE (1996) Reward deficiency syndrome. Am Sci 84:132–145.

Blum K, Noble EP, Sheridan PJ, Finley O, Montgomery A, Ritchie T, Ozkaragoz T, Fitch RJ, Sadlack F, Sheffield D (1991) Association of the A1 allele of the D2 dopamine receptor gene with severe alcoholism. Arch Gen Psychiatry 48:409–416.

Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB (1990) Allelic association of human dopamine D2 receptor gene in alcoholism. J Am Med Assoc 263:2055–2060.

Boehnke M (1991) Allele frequency estimation from pedigree data. Am J Hum Genet 48:22–25.

Bolos AM, Dean M, Lucase-Derse S, Ramsburg M, Brown GL, Goldman D (1990) Population and pedigree studies reveal a lack of association between the D2 receptor gene and alcoholism. JAMA 264:3156–3160.

Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI, Reich T, Schmidt I, Schuckit MA (1994) A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. J Stud Alcohol 55:149–158.

Chen WJ, Chen C-H, Huang J, Hsu Y-PP, Seow S-V, Chen C-C, Cheng ATA (2001) Genetic polymorphisms of the promoter region of dopamine D2 receptor and dopamine transporter genes and alcoholism among four aboriginal groups and Han Chinese in Taiwan. Psychiatr Genet 11:187–195.

Chen C-H, Chien SH, Hwu HG (1996) Lack of association between TawI A1 allele of dopamine D2 receptor gene and alcohol-use disorders in Atayal natives of Taiwan. Am J Med Genet B Neuropsychiatr Genet 67:488–490.

Chen WJ, Lu M-L, Hsu Y-PP, Chen C-C, Yu J-M, Cheng ATA (1997) Dopamine D2 receptor gene and alcoholism among four aboriginal groups and Han in Taiwan. Am J Med Genet B Neuropsychiatr Genet 74:129–136.

Clayton D (1999) A generalization of the transmission/disequilibrium test for uncertain haplotype transmission. Am J Hum Genet 65:1170–1177.

Comings DE, Blum K (2000) Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog Brain Res 126:325–341.

Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, Knell E, Kocsis P, Baumgarten R, Kovacs BW (1991) The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA 266:1793–1800.

Cook BL, Wang ZW, Crowe RR, Hauser R, Freimer M (1992) Alcoholism and the D2 receptor gene. Alcohol Clin Exp Res 16:806–809.

Crabbe JC, Phillips TJ (1998) Genetics of alcohol and other abused drugs. Drug Alcohol Depend 51:61–71.

Cruz C, Camarena B, Mejia JM, Paez F, Eroza V, de la Fuente JR, Kershenobich D, Nicolini H (1995) The dopamine D2 receptor gene TaqI A1 polymorphism and alcoholism in a Mexican population. Arch Med Res 26:421–426.

Edenberg HJ, Foroud T, Koller DL, Goate A, Rice J, Van Eerdewegh P, Reich T, Cloninger CR, Nurnberger JI, Kowalczuk M, Wu B, Li TK, Conneally PM, Tischfield JA, Wu W, Shears S, Crowe R, Hesselbrock V, Schuckit M, Porjesz B, Begleiter H (1998) A family-based analysis of the association of the dopamine D2 receptor (DRD2) with alcoholism. Alcohol Clin Exp Res 22:505–512.

Finckh U, Rommelspacher H, Kuhn S, Dufeu P, Otto G, Heinz A, Delttling M, Giraldo-Valasquez M, Pelz J, Graf K-J, Harms H, Sander T, Schmidt LG, Rolfs A (1997) Influence of the dopamine D2 receptor (DRD2) genotype on neuroadaptive effects of alcohol and the clinical outcome of alcoholism. Pharmacogenetics 7:271–281.

Foley PF, Loh E-W, Innes DJ, Williams SM, Tannenberg AEG, Harper CG, Dodd PR (2004) Association studies of neurotransmitter gene polymorphisms in alcoholic Caucasians. Ann N Y Acad Sci 1025:39–46.

Gelernter J, Goldman D, Risch N (1993) The A1 allele at the D2 dopamine receptor gene and alcoholism. JAMA 269:1673–1677.

Gelernter J, Kranzler HR (1999) D2 dopamine receptor gene (DRD2) allele and haplotype frequencies in alcohol dependent and control subjects: no association with phenotype or severity of phenotype. Neuropsychopharmacology 20:640–649.

Gelernter J, O'Malley S, Risch N, Kranzler HR, Krystal J, Merikangas K, Kennedy JL, Kidd KK (1991) No association between an allele at the D2 dopamine receptor gene (DRD2) and alcoholism. JAMA 266:1801–1807.

Gelernter J, Yu Y, Weiss R, Brady K, Panhuysen C, Yang BZ, Kranzler HR, Farrer L (2006) Haplotype spanning TTC12 and ANKK1, flanked by the DRD2 and NCAM1 loci, is strongly associated to nicotine dependence in two distinct American populations. Hum Mol Genet 15:3498–3507.

Goldman D, Brown GL, Albaugh B, Robin R, Goodson S, Trunzo M, Akhtar L, Lucas-Derse S, Long JC, Linnoila M, Dean M (1993) DRD2 dopamine receptor genotype, linkage disquilibrium, and alcoholism in American Indians and other populations. Alcohol Clin Exp Res 17:199–204.

Goldman D, Dean M, Brown GL, Bolos AM, Tokola R, Virkkunen M, Linnoila M (1992) D2 dopamine receptor genotype and cerebrospinal fluid homovanillic acid, 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenylglycol in alcoholics in Finland and the United States. Acta Psychiatr Scand 86:351–357.

Goldman D, Urbanek M, Guenther D, Robin R, Long JC (1997) Linkage and association of a functional DRD2 variant (Ser311Cys) and DRD2 markers to alcoholism, substance abuse and schizophrenia in Southwestern American Indians. Am J Med Genet B Neuropsychiatr Genet 74:386–394.

Gorwood P, Batel P, Gouya L, Courois F, Feingold J, Ades J (2000) Reappraisal of the association between the DRD2 gene, alcoholism and addiction. Eur Psychiatry 15:90–96.

Hesselbrock M, Easton C, Bucholz KK, Schuckit M, Hesselbrock V (1999) A validity study of the SSAGA—a comparison with the SCAN. Addiction 94:1361–1370.

Hesselbrock V, Hesselbrock M (1994) Alcoholism and subtypes of antisocial personality disorder. Alcohol Alcohol 2(Suppl):479–484.

Hietala J, Pohjalainen T, Heikkila-Kallio U, West C, Salaspuro M, Syvalahti E (1997) Allelic association between D2 but not D1 dopamine receptor gene and alcoholism in Finland. Psychiatr Genet 7:19–25.

Higuchi S, Muramatsu T, Murayama M, Hayashida M (1994) Association of structural polymorphism of the dopamine D2receptor gene and alcoholism. Biochem Biophys Res Commun 204:1199–1205.

Hill SY, Zezza N, Wipprecht G, Zu J, Neiswanger K (1999) Linkage studies of D2 and D4 receptor genes and alcoholism. Am J Med Genet B Neuropsychiatr Genet 88:676–685.

Ishiguro H, Arinami T, Akazawa S, Enomoto M, Mitushio H, Fujishiro H, Tada K, Akimoto Y, Mifune H, Shioduka S, Hamaguchi H, Toru M, Shibuya H (1998) Association study between the -141C Ins/Del and TaqI A

polymorphisms of the dopamine D2 receptor gene and alcoholism. Alcohol Clin Exp Res 22:845–848.

Kidd KK, Morar B, Castiglione CM, Zhao H, Pakstis AJ, Speed WC, Bonne-Tamir B, Lu R-B, Goldman D, Lee C, Nam YS, Grandy DK, Jenkins T, Kidd JR (1998) A global survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus. Hum Genet 103:211–227.

Konishi T, Calvillo M, Leng A-S, Lin K-M, Wan Y-JY (2004) Polymorphisms of the dopamine D2 receptor, serotonin transporter, and GABA-A receptor B3 subunit genes and alcoholism in Mexican Americans. Alcohol 32:45–52.

Kono Y, Yoneda H, Sakai T, Nonomura Y, Inayama Y, Koh J, Sakai J, Inada Y, Imamichi H, Asaba H (1997) Association between early-onset alcoholism and the dopamine D2 receptor gene. Am J Med Genet B Neuropsychiatr Genet 74:179–182.

Lawford BR, Young RM, Rowell JA, Gibson JN, Feeney GFX, Ritchie TL, Syndulko K, Noble EP (1997) Association of the D2 dopamine receptor A1 allele with alcoholism: medical severity of alcoholism and type of controls. Biol Psychiatry 41:386–393.

Lee JF, Lu RB, Ko HC, Chang FM, Yin S-J, Pakstis AJ, Kidd KK (1999) No association between DRD2 locus and alcoholism after controlling the ADH and ALDH genotypes in Chinese Han population. Alcohol Clin Exp Res 23:592–599.

Li J, Ji L (2005) Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity 95:221–227.

Lobos EA, Todd RD (1998) Association analysis in an evolutionary context: cladistic analysis of the DRD2 locus to test for association with alcoholism. Am J Med Genet B Neuropsychiatr Genet 81:411–419.

Lu R-B, Ko HC, Chang FM, Castiglione CM, Schoolfield G, Pakstis AJ, Kidd JR, Kidd KK (1996) No association between alcoholism and multiple polymorphisms at the dopamine D2 receptor gene (DRD2) in three distinct Taiwanese populations. Biol Psychiatry 39:419–429.

Martin ER, Monks SA, Warren LL, Kaplan NL (2000) A test for linkage and association in general pedigrees: The Pedigree Disequilibrium Test. Am J Hum Genet 67:146–154.

Matsushita S, Muramatsu T, Murayama M, Nakane J, Higuchi S (2001) Alcoholism, ALDH2*2 allele and the A1 allele of the dopamine D2 receptor gene: an association study. Psychiatry Res 104:19–26.

Neiswanger K, Hill SY, Kaplan BB (1995) Association and linkage studies of the TAQI A1 allele at the dopamine D2 receptor gene in samples of female and male alcoholics. Am J Med Genet B Neuropsychiatr Genet 60:267–271.

Neville MJ, Johnstone EC, Walton RT (2004) Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat 23(6):540–545.

Noble EP (2000a) Addiction and its reward process through polymorphisms of the D2 dopmaine receptor gene: a review. Eur Psychiatry 15:79–89.

Noble EP (2000b) The DRD2 gene in psychiatric and neurological disorders and its phenotypes. Pharmacogenetics 1:309–333.

Noble EP (2003) D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. Am J Med Genet B Neuropsychiatr Genet 116:103–125.

Noble EP, Syndulko K, Fitch RJ, Ritchie T, Bohlman MC, Guth P, Sheridan PJ, Montgomery A, Heinzmann C, Sparkes RS (1994) D2 dopamine receptor TawI A alleles in medically ill alcoholic and nonalcoholic patients. Alcohol Alcohol 29:729–744.

Noble EP, Zhang X, Ritchie TL, Sparkes RS (2000) Haplotypes at the DRD2 locus and severe alcoholism. Am J Med Genet B Neuropsychiatr Genet 96:622–631.

Nyholt DR (2004) A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 74:765–769.

Parsian A, Cloninger CR, Zhang Z-H (2000) Functional variant in the DRD2 receptor promoter region and subtypes of alcoholism. Am J Med Genet B Neuropsychiatr Genet 96:407–411.

Parsian A, Todd RD, Devor EJ, O'Malley KL, Suarex BK, Reich T, Cloninger CR (1991) Alcoholism and alleles of the human D2 dopamine receptor locus. Arch Gen Psychiatry 48:655–663.

Ponce G, Jimenez-Arriero MA, Rubio G, Hoenicka J, Ampuero I, Ramos JA, Palomo T (2003) The A1 allele of the DRD2 gene (TaqI A polymorphisms) is associated with antisocial personality in a sample of alcohol-dependent patients. Eur Psychiatry 18:356–360.

Reich T (1996) A genomic survey of alcohol dependence and related phenotypes: results from the Collaborative Study on the Genetics of Alcoholism (COGA). Alcohol Clin Exp Res 20(Suppl 8):133A–137A.

Sander T, Harms H, Podschus J, Finckh U, Nickel B, Rolfs A, Rommelspacher H, Schmidt LG (1995) Dopamine D1, D2, and D3 receptor genes in alcohol dependence. Psychiatr Genet 5:171–176.

Sander T, Ladehoff M, Samochowiec J, Finckh U, Rommelspacher H, Schmidt LG (1999) Lack of an allelic association between polymorphisms of the dopamine D2 receptor gene and alcohol dependence in the German population. Alcohol Clin Exp Res 23:578–581.

Schuckit MA, Danko GP, Smith TL, Hesselbrock V, Kramer J, Bucholz KK (2003) A 5-year prospective evaluation of DSM-IV alcohol dependence with and without a physiological component. Alcohol Clin Exp Res 27:818–825.

Schwab S, Soyka M, Niederecker M, SAckenheil M, Scherer J, Wilderauer DB (1991) Allelic association of human dopamine D2-receptor DNA polymorphism ruled out in 45 alcoholics. Am J Hum Genet 49(Suppl):203.

Suarez BK, Parsian A, Hampe CL, Todd RD, Reich T, Cloninger CR (1994) Linkage disequilibria at the D2 dopmaine receptor locus (DRD2) in alcoholics and controls. Genomics 19:12–20.

Thanos PK, Taintor NB, Rivera SN, Umegaki H, Ilkari H, Roth G, Ingram DK, Hitzemann R, Fowler JS, Gatley SJ, Wang G-J, Volkow ND (2004) DRD2 gene transfer into the nucleus accumbens core of the alcohol preferring and nonpreferring rats attenuates alcohol drinking. Alcohol Clin Exp Res 28:720–728.

Turner E, Ewing J, Shilling P, Smith TL, Irwin M, Schuckit M, Kelsoe JR (1992) Lack of association between an RFLP near the D2 dopamine receptor gene and severe alcoholism. Biol Psychiatry 31:285–290.

Volkow ND, Fowler JS, Wang G-J, Swanson JM (2004) Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Mol Psychiatry 9:557–569.

Waldman ID, Robinson BF, Rhee SH (1999) A logistic regression extension of the transmission disequilibrium test for continuous traits: application to linkage disequilibrium between alcoholism and the candidate genes DRD2 and ADH3. Genet Epidemiol 17(Suppl 1):S379–S384.

Wise RA, Rompre P-P (1989) Brain dopamine and reward. Annu Rev Psychol 40:191–225.