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Test of association between GABRA2 (SNP rs279871) and adolescent conduct/alcohol use disorders utilizing a sample of clinic referred youth with serious substance and conduct problems, controls and available first degree relatives

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ABSTRACT

Recent findings have linked the GABRA2 gene with antisocial personality disorder and alcohol dependence (AD) in adults and conduct disorder (CD), but not AD symptoms, in children and adolescents. We sought to replicate previous findings and test for an association between a single nucleotide polymorphism (SNP) in the GABRA2 gene (rs279871) and CD among adolescents.

Methods: Adolescent patients ($n = 371$), 13–18 years old, were recruited from a university substance abuse treatment program. Patient siblings ($n = 245$), parents of patients ($n = 355$), adolescent controls ($n = 185$), siblings of controls ($n = 163$) and parents of controls ($n = 263$) were included in these analyses (total sample $n = 1582$). Case-control (using only Caucasian and Hispanic probands) and family-based association tests were completed to test for association between rs279871 and several *a priori* CD and AD phenotypes.

Results: For case-control association tests, rs279871 was significantly associated with CD ($p = 0.02$) but not AD phenotypes; the result did not survive strict correction for multiple testing. All family-based association tests were non-significant (CD $p = 0.48$; CD symptom count age corrected within sex $p = 0.91$; AD $p = 0.84$; alcohol use disorder $p = 0.52$).

Conclusions: Consistent with previous findings, the results do not support the association between GABRA2 SNP rs279871 and AD in adolescents. Our results also do not support an association between rs279871 and CD; the study limitations are reviewed.

1. Introduction

Results from the Collaborative Study on the Genetics of Alcoholism (COGA) demonstrated highly significant associations between alcohol dependence (AD) and multiple single nucleotide polymorphisms (SNPs) and three-SNP-haplotypes in the GABRA2 gene (Edenberg et al., 2004), a gene which encodes the GABA A receptor alpha 2 subunit. These results were quickly replicated by two other groups (Covault et al., 2004; Lappalainen et al., 2005).

Though very encouraging, there have also been some inconsistencies. While some studies have suggested that excluding individuals with comorbid major depression and/or cocaine and opioid dependence improves the association (Covault et al., 2004), others have found the association to be strongest in those with alcohol or illicit drug dependence (Agrawal et al., 2006). In addition, there has been a lack of consistency for association on a SNP by SNP level (Covault et al., 2004; Lappalainen et al., 2005) and in the directionality of association by haplotype (Covault et al., 2004; Soyka et al., 2008).

Dick et al. (2006) extended the work on GABRA2 by utilizing children and adolescents recruited from both alcohol dependent COGA patient families and control families obtained from community sources (i.e. dental clinics). Results suggested that individuals carrying at least one A allele of the SNP rs279871, which was significantly over-transmitted to alcohol dependent subjects in the adult COGA sample, were twice as likely to meet the three symptom threshold of DSM-III-R for conduct disorder (CD) (OR 2.0; 95%CI = 1.02–3.90). We sought to replicate these findings in families of youth with serious substance and behavior problems and adolescent control families.

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2. Methods

2.1. Sample

Adolescent patients (ages 13–18) were recruited from a university substance abuse treatment program ($n = 371$). Close age siblings were also invited to participate, as were parents of patients. Two hundred and forty five full siblings of patients gave written informed consent, completed diagnostic instruments for CD/AD and provided a DNA sample. Three hundred and fifty five parents of patients (113 fathers and 242 mothers) provided a DNA sample which was genotyped for rs279871.

Control adolescents were recruited through a marketing research company to be similar to patients in terms of age, race, sex and zip code of residence ($n = 185$). At initial recruitment controls were not excluded for conduct or substance use disorders. Close age siblings were also invited to participate, as were parents of controls. One hundred and sixty three siblings of controls gave written informed consent, completed diagnostic instruments for CD/AD and provided a DNA sample. Two hundred and sixty three parents of controls (105 fathers and 158 mothers) provided DNA which was genotyped for rs279871.

2.2. Assessments

CD was assessed among adolescents utilizing the Diagnostic Interview Schedule for Children (DISC), which has shown discriminative validity in the study population (Crowley et al., 2001). Early study participants were evaluated with DISC2.3, which assesses DSM-III-R diagnoses. Once available, DISC-IV was utilized; DISC-IV was locally modified to allow assessment of DSM-III-R criteria, allowing combined analyses of III-R diagnoses across study years (Schulz-Heik et al., 2008).

Adult siblings (over 18) were assessed utilizing the Diagnostic Interview Schedule (DIS) (Robins et al., 1981). Similar to the DISC, the study began with the III-R version and a locally modified version IV (which assessed III-R diagnoses) was used once available. One CD III-R criterion was removed from the DIS-III-R at the beginning of the study (forced sex) and in accordance with the DSM-III-R, breaking and entering was not included as a CD criterion under the antisocial personality disorder diagnosis; thus adult siblings assessed with the version III-R of the DIS ($n = 41$; 36 patient siblings and 5 control siblings) were not asked about breaking and entering or forced sex. Those siblings were excluded from analyses utilizing CD symptom count. Of those 41 adult siblings, 21 had determinable CD diagnoses (defined here as at least three lifetime CD DSM-III-R criteria) because they had scores of 0 or ≥ 3 (i.e. the missing two items whether endorsed or not would not make those with a score of 0 meet the symptom count threshold). Adult siblings assessed with version III-R of the DIS with determinable CD diagnoses (21/41) were included in analyses utilizing CD diagnosis.

DSM-IV defined alcohol use disorders were assessed utilizing the Composite International Diagnostic Interview–Substance Abuse Module (CIDI-SAM). The reliability of previous iterations of this instrument is well documented (Cottler et al., 1989).

Study phenotypes were (1) CD (defined as meeting at least three lifetime DSM-III-R CD symptoms), (2) CD symptom count corrected for age within sex (Stallings et al., 2005), (3) DSM-IV alcohol dependence (AD) and (4) DSM-IV alcohol use disorder (AUD).

2.3. Genotyping

A predesigned/validated TaqMan® assay for allelic discrimination (Applied Biosystems) was used to determine the rs279871 SNP genotype, per instructions of the manufacturer under standard conditions using an ABI PRISM 7900 Genetic Analyzer instrument.

2.4. Data analyses

Case-control analyses were completed utilizing chi-square tests within our sample of Caucasians and Hispanics for CD, AD and AUD phenotypes. Joint analyses of patient and control families were completed using Family Based Association Test (FBAT). FBAT extends and unifies previous family-based approaches, does not require assumptions regarding distribution of the phenotype in the study sample, incorporates information from affected and unaffected subjects, and allows utilization of variable pedigree structures (Rabinowitz and Laird, 2000; Horvath et al., 2001).

3. Results

Table 1 presents demographic information for patients, siblings of patients, controls and siblings of controls. For patients and controls mean age was about 16, while for siblings of patients and controls mean age was about 17. Most patients were male (88%), as were controls (84%); about half of the siblings of patients and controls were male. About half of patients and controls were Caucasian and more than one third were Hispanic; the 'Other' category includes African Americans ($n = 16$ controls probands and $n = 36$ patient probands), American Indians ($n = 3$ control probands and $n = 13$ patient probands) and Asians ($n = 2$ patient probands). Prevalence of CD was greatest among patients (88%), followed by siblings of patients (38%), controls (16%) and siblings of controls (14%). Rates of lifetime alcohol dependence followed the same pattern.

Table 1
Demographics.

	Patient	Patient sibs	Controls	Control sibs
	($n = 371$)	($n = 245$)	($n = 185$)	($n = 163$)
Age (s.d.)	15.8 (1.20)	16.9 (4.20)	15.8 (1.52)	17.0 (4.13)
Male (%)	328 (88.4)	129 (52.7)	156 (84.3)	80 (49.1)
Race (%)				
White	178 (48.0)	116 (47.3)	102 (55.1)	98 (60.1)
Hispanic	142 (38.3)	102 (41.6)	64 (34.6)	48 (29.4)
Other	51 (13.7)	27 (11.0)	19 (10.3)	17 (10.4)
CD (%)	327 (88.1)	93 (38.0)	30 (16.2)	22 (13.5)
AD (%)	113 (30.7)	23 (9.5)	12 (6.5)	11 (6.9)
AUD (%)	272 (73.9)	80 (33.1)	30 (16.5)	30 (18.8)

CD = at least 3 DSM-III-R conduct disorder criteria (lifetime); AD = DSM-IV-defined alcohol dependence (lifetime); AUD = DSM-IV-defined alcohol use disorder (lifetime).

Table 2 presents genotype frequencies by race and family relationship, within group (i.e. control vs. patient) (top panel), case-control analyses (middle panel) and power analyses for those case-control tests (bottom panel). A-allele frequencies ranged from 0.47 to 0.79 within group (patient vs. control, race/ethnicity and family relationship) and are generally lower than that reported by Dick et al., 2006 and others (Drgon et al., 2006); however, the same general pattern (i.e. higher A-allele frequencies seen among African Americans) is seen here. Genotype frequencies for Caucasian ($p = 0.02$) and Hispanic patient probands ($p = 0.001$) and other patient mothers ($p = 0.03$) deviated from HWE but not for other subgroups (parents and probands).

In our sample of patient probands with CD and control probands without CD, race/ethnicity was significantly related to genotype ($n = 480$ excluding 2 Asian youth; $\chi^2 = 15.31$; df = 6; $p = 0.02$) and marginally related to caseness ($\chi^2 = 5.42$; df = 3; $p = 0.14$) raising concerns about population substructure; however, allele frequencies were generally similar between Caucasian and Hispanic subjects (examining patient probands with CD and control probands without CD $n = 428$; $\chi^2 = 0.58$; df = 2; $p = 0.75$). We completed case-control analyses within our sample of Caucasian and Hispanic subjects; a significant association was seen between rs279871 and CD ($p = 0.02$); other case-control analyses were non-significant. Power analyses for our case-control tests were conducted in PBAT; assumptions included: (1) population prevalence of disease = 0.1; (2) $\alpha = 0.05$; (3) an A-allele frequency of 0.53; (4) disease locus = marker locus; and, (5) a dominant model.

Table 3 presents results from family-based association tests for each of the 4 predetermined phenotypes. No tests reached statistical significance. Given that genetic heterogeneity could conceivably lead to a dampened signal in our combined sample analyses, the FBAT analyses were also completed within patient families and separately, within control families. For CD, CD symptom count, AUD and AD results were nonsignificant within-patient families (p-values were 0.43, 0.79, 0.10 and 0.70, respectively) and within control families (p-values were > 0.99, 0.38, 0.17 and 0.44, respectively).

Power analyses for our family-based tests were conducted in PBAT by identifying the family configurations and CD affection status of the study sample. Assumptions included: (1) population prevalence of disease = 0.1; (2) $\alpha = 0.05$; (3) an A-allele frequency of 0.53; and (4) disease locus = marker locus. Power is shown in Table 3 (lower half) for various odds ratios (odds ratio point estimate and