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Test of Association Between 10 SNPs in the Oxytocin Receptor Gene and Conduct Disorder

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Abstract

Animal and human studies have implicated oxytocin (OXT) in affiliative and prosocial behaviors. We tested whether genetic variation in the OXT receptor (OXTR) gene is associated with conduct disorder (CD).

Methods—Utilizing a family-based sample of adolescent probands recruited from an adolescent substance abuse treatment program, control probands and their families (total sample n=1,750), we conducted three tests of association with CD and 10 SNPs (single nucleotide polymorphisms) in the OXTR gene: (1) family-based comparison utilizing the entire sample; (2) within-Whites, case-control comparison of adolescent patients with CD and controls without CD; and (3) within-Whites case-control comparison of parents of patients and parents of controls.

Results—Family-based association tests failed to show significant results (no results $p<0.05$). While strictly correcting for the number of tests ($\alpha=0.002$), adolescent patients with CD did not differ significantly from adolescent controls in genotype frequency for the OXTR SNPs tested; similarly, comparison of OXTR genotype frequencies for parents failed to differentiate patient and control family type, except a trend association for rs237889 ($p=0.004$).

Conclusions—In this sample, 10 SNPs in the OXTR gene were not significantly associated with CD.

Keywords

antisocial; delinquency; callousness; genetics

Introduction

Conduct disorder (CD) is a common, moderately heritable (Rhee & Waldman, 2002), mental disorder characterized by violation of the basic rights of others or societal norms or rules, and may be viewed, in part, as a deficit in normative social development. Low levels of social competence, broadly defined as compliance with peers, positive affect, the ability to engage in prosocial interactions and correctly read and interpret social situations, is a risk factor for conduct problems (Brotman et al., 2005; Drugli et al., 2007). CD youth have more difficulty interpreting the emotional states of others, such as facial expressions (Fairchild et

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al., 2009), are more likely to interpret the intentions of others in neutral or ambiguous social situations as hostile or aggressive (Crick & Dodge, 1994), and sometimes exhibit anger, frustration and poor self regulation of attention and behavior (Caspi et al., 1996; Campbell et al., 1992). Such emotional dysregulation often occurs around disappointments and enforced limits to youths' behavior, but seemingly contradictorily, such youths are often less emotionally reactive to misfortune or pain of others (Decety & Meyer, 2008). Thus many problems that CD youths face arise within a social context and implicate low levels of social competence.

Guttman-Steinmetz & Crowell (2006) proposed the following theory underlying the intergenerational transmission of externalizing behaviors, such as CD: parental low sensitivity, unavailability, poor supervision, and low responsiveness lead to both the child being exposed to environmental dangers and insecure attachment in the child. Such an insecure attachment is believed to be related to externalizing behaviors and to impact the child's growth in developmental domains such as emotion regulation, self control, and compliance. Though causality of these relationships is difficult to assure, some empirical data support this model. Low maternal responsiveness early in life predicts conduct problems (Kochanska et al., 2008) in childhood (Shaw et al., 1998) and adolescence (Olson et al., 2000). CD youth are also more likely to demonstrate an insecure attachment (Keller et al., 2005); insecure attachment, especially avoidant, has been linked with scape-goating or victimizing play partners, displacement of anger and frustration onto others (Guttman-Steinmetz & Crowell, 2006) and development of antisocial outcomes (Kochanska et al., 2009).

Here we posit that many of the domains of social dysfunction seen with CD youth and their families are similarly shown in studies of oxytocin (OXT). Animal work, especially with rodents, supports that OXT plays an integral role in maternal care, mother-infant bonding, the offspring's response to the mother and social bonding between mating pairs (see Lee et al., 2009 and Ross & Young, 2009 for recent reviews). Studies of humans have been more limited, but further support a role of OXT in affiliative behaviors (Feldman et al., 2007; Bakermans-Kranenburg & van Ijzendoorn, 2008; Zak et al., 2005; Ditzen et al., 2009; Domes et al., 2007; Zak et al., 2007; Theodoridou et al., 2009).

We sought to test three hypotheses: (1) that family-based analyses would associate CD and OXTR genotypes; (2) that antisocial youth would differ from adolescent controls in OXTR genotype frequencies; and (3) given data supporting associations between parenting practices and CD in offspring and data on oxytocin's role in parental nurturing behaviors in rodents, we hypothesized that parents of patients would similarly show differences in OXTR genotype frequencies when compared with parents of controls.

Methods

Sample

This sample was drawn from a larger study of antisocial drug dependence (Stallings et al., 2005) and has been used in previous genetic association studies (i.e. Sakai et al., 2010). Adolescent patients were recruited from a university treatment program for youth with serious substance and behavioral problems; adolescent controls were recruited by a research marketing company to be similar to patients in terms of age, sex, race and zip code of residence; and first degree relatives were also invited to participate in the study. In the current analyses 419 adolescent patients (48.4% White, 38.7% Hispanic, 12.9% Other), 269 siblings of patients (48.3% White, 40.1% Hispanic, 11.5% Other; 37.2% with CD), 193 adolescent controls (56% White, 33.7% Hispanic and 10.4% Other), and 150 siblings of controls (62% White, 30.1% Hispanic and 9% Other; 14.7% with CD) were included.

Within-family and parent-specific analyses also include 278 parents of controls (169 mothers and 109 fathers) and 441 parents of patients (296 mothers and 145 fathers).

Diagnostic Assessment

For adolescent patients, adolescent controls and siblings 18 or younger, CD was assessed utilizing the Diagnostic Interview Schedule for Children (DISC) (Shaffer et al., 2000). Siblings older than 18 were assessed with the Diagnostic Interview Schedule (DIS) (Robins et al., 1981). Details on assessments are available in our previous publication (Sakai et al., 2010).

Genotyping

HapMap data were imported into Haploview (Barrett et al., 2005) and 17 single nucleotide polymorphisms (SNPs) in OXTR were identified using the tagger function. Unfortunately, despite varying PCR conditions and genotyping master mixes 7 of the SNP assays did not yield high quality data in the first 400 samples genotypes, so they were not genotyped in the full sample. Buccal cell DNA samples after isolation, extraction and polymerase chain reaction (PCR) (Anchordquy et al., 2003) were utilized for genotype determination. Under standard instructions, with predesigned/validated TaqMan® assays (Applied Biosystems), and utilizing an ABI PRISM 7900 Analyzer instrument, genotypes were determined for rs237884, rs1042778, rs2139184, rs237885, rs11706648, rs237887, rs4686301, rs2268491, rs2268492, and rs237889. Expected genotype frequencies, based on allele frequencies, were calculated for the 10 SNPs and tested against observed genotype frequencies for deviations from Hardy Weinberg Equilibrium (HWE) within Caucasians for these four samples separately: adolescent patients, adolescent controls, parents of patients and parents of controls. No significant deviations from HWE were seen for control probands or their parents. Violations for HWE were seen within Caucasian adolescent patients for two SNPs (rs1042778, $p=0.01$; rs237889, $p=0.01$) and within Caucasian parents of patients for one SNP (rs237889, $p<0.01$); because of this, we genotyped all samples for rs237889 a second time. Concordance rate between the two runs was greater than 99.5%. Of note, such deviation from HWE may be indicative of association and based on concordance between genotyping runs, does not appear to be related to genotyping error.

Data Analyses

Linkage disequilibrium (LD) between the 10 OXTR SNPs was determined using the program Haploview (Barrett et al., 2005) (available upon request). Family-Based Association: Analyses of the entire family sample were completed utilizing the software Family Based Association Test (FBAT) (Rabinowitz et al., 2000; Horvath et al., 2001). Case-Control association tests: For seven SNPs, a significant race by genotype association was seen (for at least one of the samples – adolescent and/or parent), even when restricting the sample to Whites and Hispanics. Therefore, case control analyses were conducted within our largest racial subgroup, Whites. Correction for multiple testing, while accounting for LD between SNPs, was calculated as described by Nyholt, (2004). According to those calculations an $\alpha=0.0073$ was required to maintain a type I error rate at 5% for these ten SNPs; however, because 3 sets of calculations were completed (within-family, patient-control, and parents), we utilized an $\alpha=0.0073/3=0.0024$ as the threshold for significance in these analyses.

Results

Family-Based Association Tests: No FBAT tests reached statistical significance (p values were rs237884 p=0.21, rs1042778 p=0.79, rs237885 p=0.82, rs11706648 p=0.84, rs237887 p=0.69, rs4686301 p=0.96, rs2268491 p=0.25, rs2268492 p=0.72, rs237889 p=0.59). For

rs2139184 (p=0.06), which shows relatively low R-square values with the other SNPs studied, a trend association was seen but not after correction for multiple testing. Other association tests: Case-control analyses are presented in Table 1. Notable results included adolescent case-control results for rs2268492 (p=0.05), and patient-parent vs. control-parent results for rs237889 (p=0.004).

Discussion

We hypothesized that patients and controls would differ significantly in OXTR genotype frequencies and that parents of patients would also differ from parents of controls in this regard. The results do not strongly support this. Although an association between rs237889 genotype frequencies was seen between parents of patients vs. parents of controls, when strictly controlling for the number of statistical tests this result (p=0.004) represented only a trend association.

There are several limitations to the current study which merit attention. First, the mixed-racial composition of the study sample raises concerns regarding population substructure; genotype frequencies for 7/10 SNPs differed by race. Therefore, our association analyses within our sub-sample of Whites should provide information that is less biased by population stratification issues but at a cost to power. Second, although our family-based association tests are robust to population substructure, they are less powerful, and given our sample size provides power only to detect relatively larger effect sizes and only for some models of inheritance (i.e. for an odds ratio=2, ~86% power under an additive model but only 35% under a recessive model)(see Sakai et al., 2010). Thus, although our family-based association tests did not support association, our power estimates suggest that association with more modest effect sizes cannot be definitively ruled out. Third, although we utilized 10 SNPs, examination of HapMap data suggests that we do not have coverage of the entire OXTR gene (which spans 17kb, contains 3 introns and codes for a 389 amino acid polypeptide) (Gimpl & Fahrenholz, 2001). It is possible that association would be seen with novel mutations or other SNPs not strongly linked with the 10 SNPs examined here. Fourth, we only tested OXTR and did not test the OXT gene or other possibly related peptides. Finally, ideally we would have measured parent-child interactions and attachment and utilized a quantitative phenotype for OXTR associations in parents. Unfortunately, we ascertained patients in adolescence and did not have such assessments from their early childhood. Future studies might test for associations between specific parenting practices or types of attachment and OXTR genotypes in clinical families.

The current study adds to the very limited information on antisocial behavioral and OXTR. Although no tests of association survived strict corrections for multiple testing, based on our trend association, the extant literature on CD, OXT and social development, and a recent suggestive association between rs237889 and early-onset alcohol dependence (Edenberg et al., 2010), additional study of the oxytocin system and antisocial behavior patterns is merited.

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Table 1

Case-control Analyses Within-Whites for 10 OXTR SNPs (showing count and (%))

Section 1. A: Adolescent Patients with Conduct Disorder (at least 3 lifetime DSM-IIIR conduct disorder criteria) vs. Adolescent Controls without Conduct Disorder. ***Section 1. B:*** Parents of adolescent patients vs. parents of adolescent Controls.

	Section 1. A – Patient (Pt) vs. Control (Ct)				**Section 1. B – Pt parent vs. Ct Parent**			
rs237884	GG	AG	AA	P-value	GG	AG	AA	P-value
Ct	10 (10.6)	33 (35.1)	51 (54.3)	0.51	11 (6.6)	66 (39.8)	89 (53.6)	0.71
Pt	12 (6.7)	68 (38.0)	99 (55.3)		20 (8.9)	86 (38.4)	118 (52.7)	
rs1042778	TT	GT	GG	P-value	TT	GT	GG	P-value
Ct	12 (13.6)	46 (52.3)	30 (34.1)	0.17	21 (13.4)	75 (47.8)	61 (38.9)	0.81
Pt	31 (18.0)	69 (40.1)	72 (41.9)		35 (15.7)	102 (45.7)	86 (38.6)	
rs2139184	CC	AC	AA	P-value	CC	AC	AA	P-value
Ct	93 (100)	0 (0)	0 (0)	0.18[a]	163 (98.2)	3 (1.8)	0 (0)	0.99[a]
Pt	180 (96.8)	6 (3.2)	0 (0.0)		233 (97.5)	6 (2.5)	0 (0)	
rs237885	TT	GT	GG	P-value	TT	GT	GG	P-value
Ct	20 (22.2)	47 (52.2)	23 (25.6)	0.39	37 (24.2)	84 (54.9)	32 (20.9)	0.10
Pt	36 (20.6)	80 (45.7)	59 (33.7)		40 (18.1)	115 (52.0)	66 (29.9)	
rs11706648	CC	AC	AA	P-value	CC	AC	AA	P-value
Ct	10 (12.2)	32 (39.0)	40 (48.8)	0.74	21 (14.2)	59 (39.9)	68 (45.9)	0.93
Pt	21 (12.9)	71 (43.6)	71 (43.6)		27 (13.8)	82 (41.8)	87 (44.4)	
rs237887	GG	AG	AA	P-value	GG	AG	AA	P-value
Ct	18 (20.9)	42 (48.8)	26 (30.2)	0.22	35 (23.0)	71 (46.7)	46 (30.3)	0.37
Pt	31 (18.6)	67 (40.1)	69 (41.3)		39 (17.8)	102 (46.6)	78 (35.6)	
rs4686301	TT	CT	CC	P-value	TT	CT	CC	P-value
Ct	10 (11.0)	39 (42.9)	42 (46.2)	0.98	17 (10.6)	68 (42.2)	76 (47.2)	0.95

	Section 1. A – Patient (Pt) vs. Control (Ct)				Section 1. B – Pt parent vs. Ct Parent			
Pt	20 (11.5)	76 (43.7)	78 (44.8)		24 (11.4)	86 (40.8)	101 (47.9)	
rs2268491	TT	CT	CC	P-value	TT	CT	CC	P-value
Ct	4 (4.5)	19 (21.6)	65 (73.9)	0.51	2 (1.2)	33 (20.5)	126 (78.3)	0.45
Pt	4 (2.3)	34 (19.2)	139 (78.5)		6 (2.7)	38 (17.0)	179 (80.3)	
rs2268492	TT	CT	CC	P-value	TT	CT	CC	P-value
Ct	7 (8.1)	27 (31.4)	52 (60.5)	0.05 *	11 (7.0)	73 (46.2)	74 (46.8)	0.49
Pt	15 (8.7)	80 (46.5)	77 (44.8)		13 (6.2)	86 (40.8)	112 (53.1)	
rs237889	TT	CT	CC	P-value	TT	CT	CC	P-value
Ct	12 (14.5)	38 (45.8)	33 (39.8)	0.25	22 (15.1)	75 (51.4)	49(33.6)	0.004 **
Pt	23 (14.7)	55 (35.3)	78 (50.0)		41 (21.8)	63 (33.5)	84 (44.7)	

[a] Fisher's Exact Test;

* $p < 0.05$;

** $p < 0.01$.