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Association of the Oxytocin Receptor Gene (*OXTR)* in Caucasian Children and Adolescents with Autism

Suma Jacob, M.D., Ph.D.[1], **Camille W. Brune, Ph.D.**[1], **C. S. Carter, Ph.D.**[2], **Bennett L. Leventhal, M.D.**[1], **Catherine Lord, Ph.D.**[3], and **Edwin H. Cook Jr., M.D.**[1]

[1] Institute for Juvenile Research, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60608, USA

[2] Brain Body Center, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60608, USA

[3] University of Michigan Autism and Communication Disorders Center (UMACC), 1111 East Catherine St., Rm. 217, Ann Arbor, MI 48109, USA

Abstract

Background—The oxytocin receptor gene (*OXTR)* has been studied in autism because of the role of oxytocin (OT*)* in social cognition. Linkage has also been demonstrated to the region of *OXTR* in a large sample. Two single nucleotide polymorphisms (SNPs) and a haplotype constructed from them in *OXTR* have been associated with autism in the Chinese Han population. We tested whether these associations replicated in a Caucasian sample with strictly defined autistic disorder.

Methods—We genotyped the two previously associated SNPs (rs2254298, rs53576) in 57 Caucasian autism trios. Probands met clinical, ADI-R, and ADOS criteria for autistic disorder.

Results—Significant association was detected at rs2254298 (p = 0.03) but not rs53576. For rs2254298, overtransmission of the G allele to probands with autistic disorder was found which contrasts with the overtransmission of A previously reported in the Chinese Han sample. In both samples, G was more frequent than A. However, in our Caucasian autism trios and the CEU Caucasian HapMap samples the frequency of A was less than that reported in the Chinese Han and Chinese in Bejing HapMap samples. The haplotype test of association did not reveal excess transmission from parents to affected offspring.

Conclusions—These findings provide support for association of OXTR with autism in a Caucasian population. Overtransmission of different alleles in different populations may be due to a different pattern of linkage disequilibrium between the marker rs2254298 and an as yet undetermined susceptibility variant in *OXTR*.

Autism is a neurodevelopmental disorder that is characterized by impairments in communication and social interaction as well as patterns of restrictive, repetitive interests and behaviors during early childhood (DSM-IV). Deficits in social interaction can include lack of social or emotional reciprocity, absence of shared enjoyment with parents and others, limited nonverbal behavior to regulate social interaction, and difficulty developing friendships. There is a male to female ratio of 4:1 or greater [1]. There is strong evidence for a complex genetic

Correspondence concerning this article should be addressed to Dr. Suma Jacob, Institute for Juvenile Research, Department of Psychiatry (M/C 747), University of Illinois at Chicago, 1747 West Roosevelt Rd, Room 155, Chicago, IL 60608, USA; e-mail: E-mail: sjacob@psych.uic.edu.

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influence of autism with estimates of concordance among monozygotic twins ranging from 64–91% and with fraternal twins or siblings from 0–9% [reviewed in 2].

There is growing interest in the role of the neurohypophyseal peptide oxytocin (OT) in the development of autism [3] because of its role in affiliation, social memory and behavior. In animal models, oxytocin has been shown to play critical roles in social processing, recognition and bonding as well as influencing stereotyped behaviors such as exaggerated grooming [3–6]. Cells mediating centrally-released OT are found distinctly in the paraventricular and supraoptic nuclei of the hypothalamus. In Ferguson et al. [5] OT knockout mice maintained olfaction and cognitive performance, but suffered deficits in social recognition which were recovered by intraventricular OT but not by vasopressin administration. Animal models have shown that altering OT early in life can produce long-lasting and sexually dimorphic changes on brain development and behavior [7].

In human studies, OT administration has been shown to increase trust [8] and amygdala activation compared to placebo in healthy males [9]. Elevated OT has been reported in obsessive compulsive disorder [10,11] and Prader-Willi syndrome [12]. Children with autism have been shown to have lower blood OT levels [13] and higher precursor OT levels [14] in comparison to typically developing children. Differences in OT peptide processing may result in inactive or less active forms of OT and potentially impact brain development or behavior associated with autism. There are also reports that treatment with OT infusions result in reduced repetitive behaviors [15] and increased retention of affective speech [16] in adults with autism and Asperger disorder.

The OXTR is a G protein-coupled receptor and positively coupled to phospholipase C [17]. In mammals, OXTR are expressed at higher levels in early development [18,19] and are concentrated in brain regions that are involved in social behaviors including the olfactory bulbs, piriform cortex, amygdala, and lateral septum [5]. Compared to wild type, *OXTR* knockout mice emit fewer ultrasonic vocalizations in response to social isolation, experience deficits in social discrimination, and demonstrate more aggressive behavior [20].

Combined linkage analysis of two independent samples of 314 Finnish families demonstrated linkage in the 3p24–25 region containing the OXTR gene [21]. Four polymorphic SNPs were analyzed and two were found to be associated with autism in 195 Chinese Han trios [22]. In order to replicate these findings in a Caucasian population, we genotyped the two SNPs that were significant in the previous sample in 57 Caucasian trios and conducted family-based association analyses.

METHOD

Subjects and Assessment

After complete description of the study to the parents, written informed consent was obtained. Recruitment, assessment, and inclusion criteria were the same as that outlined in a previously described sample in which all subjects met ADI-R criteria for autism and had a best estimate diagnosis of autistic disorder by a clinical psychologist and psychiatrist [23]. Only one sibling was randomly selected from each affected sibling pair in the previous study [23] to avoid confounding linkage and association. For the current study additional inclusion criteria consisted of being Caucasian (because allele frequency differences are reported across populations and there was an insufficient sample of non-Caucasian subjects in the sample), being at least 3 years old at the time the Autism Diagnostic Interview-Revised [ADI-R, 24] was administered, having sufficient blood or DNA available, meeting Autism Diagnostic Observation Schedule (ADOS) classification for autistic disorder [25] (thereby dropping subjects in the previous sample whose ADOS classification was autistic spectrum disorder), and having complete data for ADI-R algorithm item scores (thereby including subjects recruited from the University of Chicago Developmental Disorders Clinic and excluding subjects recruited from San Diego). There were 57 probands, 45 males and 12 females, with a mean age of 6.4 years (SD=3.5).

Genotyping of SNPs

All genotyping was performed blind to clinical and demographic data and family relationships. Two SNP markers (rs2254298 [Celera ID: C_15981334_10] and rs53576 [Celera ID: C_3290335_10] were genotyped using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, www.appliedbiosystems.com). TaqMan® PCR reactions were done with Universal Master Mix Amperase® UNG, 0.25uL Taqman probe mix and 2.25uL of water for a 5uL total volume. The PCR conditions for the TaqMan® SNP Genotype Assays were: one AmpErase® step at 50.0°C for two minutes, one enzyme activation step at 95.0°C for ten minutes, and 40 alternating cycles of denaturation at 92.0°C for 15 seconds and reannealing and extension at 58.0°C for one minute. All PCR reactions were performed on a Perkin Elmer 9700 Thermocycler (Applied Biosystems, Foster City, CA). The fluorescence intensity of the final PCR product was measured using an LjL Analyst AD fluorescence microplate reader (LjL Biosystems, Sunnyvale, CA, www.moleculardevices.com) using LjL CriterionHost Software. In addition to the 57 Caucasian trios reported, one trio was excluded from the analyses because of a parent sample genotyping failure. The overall test retest-agreement performed on 16% of sample was 100%. There were no Mendelian incompatibilities for either marker.

Statistical analyses

The distributions of rs2254298 and rs53576 genotypes were tested using X^2 for Hardy-Weinberg equilibrium using the HWE program from the LINKUTIL package (http://linkage.rockefeller.edu/ott/linkutil.htm). Transmission disequilibrium tests were calculated using the TDT/S-TDT program (v. 1.1) (http://genomics.med.upenn.edu/spielman/TDT.htm) [26]. To test for association between SNPs, we used Haploview 3.32 (http://www.broad.mit.edu/mpg/haploview/) to calculate two measures of linkage disequilibrium, D′ and r^2 [27]. Haplotype association was calculated using the FBAT program version 1.7.2 (www.biostat.harvard.edu/~fbat/default.html) under “biallelic” mode [28]. Alpha was set at $p < .05$.

RESULTS

Table 1 reports allele and genotype frequencies of the sample and the TDT for each marker. Genotype distributions for parents and probands were consistent with Hardy-Weinberg equilibrium for rs2254298 (parents: $X^2 = 0.94$, $df = 1$, $p = 0.34$; probands: $X^2 = 0.53$, $p = 0.47$) and rs53576 (parents: $X^2 = 0.07$, $p = 0.79$; probands: $X^2 = 0.42$, $p = 0.52$).

Preferential transmission of G over A occurred at rs2254298 (see Table 1). No significant association was found for rs53576. The markers showed weak LD with each other (D′ = 0.53, $r^2 = 0.01$). The haplotype test of association did not reveal excess transmission from parents to affected offspring for the haplotypes ($p > 0.10$) (Table 2).

DISCUSSION

Since there is growing evidence that OXTR may mediate genetic vulnerability to autism [3, 29], we tested two SNPs that were found to be associated with autism in the Chinese Han population [22]. The goal was to replicate these findings in a Caucasian population using standardized diagnostic criteria. The TDT revealed significant transmission disequilibrium for rs2254298, one of the two SNPs that were significant in the Chinese Han sample. In contrast to the Chinese Han population, the G allele was overtransmitted in this population. Allele frequencies differ markedly at this marker in Caucasian and Chinese populations (Table 3). Both the current study and Wu et al. [22] reported allele frequencies representative of their population in the HapMap (http://www.hapmap.org) with the Caucasian samples having a greater frequency of the G allele than the Chinese samples (Table 3).

Although the transmission of different alleles may occur because both studies are false positives or because of phenotypic heterogeneity, the transmission of different marker alleles is also consistent with the alleles being on different haplotypes with an as yet unidentified susceptibility variant in *OXTR*. This is more likely given that the minor allele frequencies at rs2259248 and other SNPs in *OXTR* differ between the 2 populations. Future research should attempt to replicate these preliminary findings across populations using larger samples. Extensive genotyping, resequencing and family-based association testing in larger samples across populations will be necessary to understand the possible role of variants in OXTR in autism susceptibility.

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Table 1

Allele and Genotype Frequencies of SNPs in Pedigrees

SNP	Sample	Allele Distribution		Genotype Distribution			TDT		
		G	A	GG	AG	AA	t(G)/t(A)	χ^2	*p*
rs2254298	Parents	196	32	83	30	1	---	---	---
	Autistic probands	104	10	47	10	0	21/9	4.80	0.03
rs53576	Parents	166	62	61	44	9	---	---	
	Autistic probands	84	30	30	24	3	23/21	0.09	0.76

SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test; t(G)/t(A), number of transmitted G and A alleles from heterozygous parents

Table 2

Genetic Association between Haplotypes and Autism

Allele rs2254298	**Allele rs53576**	**S**	**E(S)**	***Z***	***p*[a]**
G	G	50.250	45.250	1.347	0.178
G	A	30.750	29.750	0.291	0.771
A	G	8.750	12.750	−1.629	0.103
A	A	0			

S, test statistics for the observed number of transmitted alleles; E(S), expected value of S under the null hypothesis (i.e., no linkage or association).

[a] *p*, two-tailed.

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Table 3

Parental allele frequencies for *rs2254298* in autism association studies and the HapMap

	Caucasian		Chinese	
Allele	**Current Study**	**HapMap**[a]	**Wu et al. (2005)**	**HapMap**[b]
A	0.140	0.068	0.315	0.289
G	0.860	0.932	0.685	0.711

[a]CEU - CEPH, Utah residents with ancestry from northern and western Europe

[b]CHB - Han Chinese in Beijing, China