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Association between the oxytocin receptor (*OXTR*) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition

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Evidence both from animal and human studies suggests that common polymorphisms in the oxytocin receptor (*OXTR*) gene are likely candidates to confer risk for autism spectrum disorders (ASD). In lower mammals, oxytocin is important in a wide range of social behaviors, and recent human studies have shown that administration of oxytocin modulates behavior in both clinical and non-clinical groups. Additionally, two linkage studies and two recent association investigations also underscore a possible role for the *OXTR* gene in predisposing to ASD. We undertook a comprehensive study of all 18 tagged SNPs across the entire *OXTR* gene region identified using HapMap data and the Haploview algorithm. Altogether 152 subjects diagnosed with ASDs (that is, DSM IV autistic disorder or pervasive developmental disorder—NOS) from 133 families were genotyped (parents and affected siblings). Both individual SNPs and haplotypes were tested for association using family-based association tests as provided in the UNPHASED set of programs. Significant association with single SNPs and haplotypes (global *P*-values < 0.05, following permutation test adjustment) were observed with ASD. Association was also observed with IQ and the Vineland Adaptive Behavior Scales (VABS). In particular, a five-locus haplotype block (rs237897-rs13316193-rs237889-rs2254298-rs2268494) was significantly associated with ASD (nominal global *P* = 0.000019; adjusted global *P* = 0.009) and a single haplotype (carried by 7% of the population) within that block showed highly significant association (*P* = 0.00005). This is the third association study, in a third ethnic group, showing that SNPs and haplotypes in the *OXTR* gene confer risk for ASD. The current investigation also shows association with IQ and total VABS scores (as well as the communication, daily living skills and socialization subdomains), suggesting that this gene shapes both cognition and daily living skills that may cross diagnostic boundaries.
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Introduction

A large body of evidence shows that both oxytocin (OXT) and arginine vasopressin (AVP) peptides markedly modulate behavior and cognition across species.[1] Interestingly, one facet of social memory, recognition of a familiar conspecific, is modulated by both OXT and AVP in lower mammals.[2] This facet of social cognition may be especially relevant to autism spectrum disorders (ASDs). The development of an OXT knockout mouse and the deficits observed in its behavior underlines the role of this hormone in particularly molding social memory.[3] Moreover, the absence of exposure to OXT during development was associated with abnormalities in the development of emotional behavior.[4] The role of OXT and AVP in shaping social communication in non-human species resonates with the core deficits in ASD, especially impairment in communication skills and social interactions.

In humans and other mammals, OXT and AVP are both nonapeptides synthesized in the hypothalamus that are released into the bloodstream via axon terminals in the posterior pituitary or neurohypophysis. These two peptides are closely related structurally, differing at only two amino acids and have a common evolutionary origin.[5] Two genome-wide scans highlight the 3p25 region, containing the *OXTR* gene, as a putative linkage site for ASD.[6,7] Recently Wu *et al.*[8] genotyped four single-nucleotide

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polymorphisms (SNPs) in *OXTR* in 195 Chinese Han trios and demonstrated association with ASD with both individual SNPs and haplotypes. Jacob *et al*.[9] genotyped two *OXTR* SNPs and observed significant association with a single SNP (rs2254298) in Caucasian children and adolescents with autism.

As noted in a review by Insel *et al*.,[10] OXT receptors are remarkably plastic, induced several fold after gonadal steroid administration and expressed in different brain regions even in closely related species. Moreover, OXT and AVP receptors are developmentally regulated and expressed more in the immature brain, and both peptides have been shown to have effects on neural development. OXT receptors are distributed in various brain areas[11] that are associated with social behavior, including reproductive and parenting behaviors, affiliation and attachment, social memory and reactivity to social stress in non-human mammals.[12,13] Recent investigations extend the role of OXT in social cognition to healthy humans[14–17] as well as to subjects with ASD.[18–22] A recent study shows that intranasal OXT in human modulates amygdala responses to facial expressions irrespective of their valence.[23]

The human *OXTR* is a 389-amino-acid polypeptide with seven transmembrane domains and belongs to the class I G protein-coupled receptor (GPCR) family.[24] *OXTR* is located on chromosome 3p25 and spans ~19 kbp and contains three introns and four exons.[25] Several dozen SNPs have been identified within the gene by the National Center for Biotechnology Information database (dbSNP). To further explore the role of the *OXTR* gene in contributing risk to ASD, we undertook a comprehensive study using all 18 tagged SNPs across the entire *OXTR* gene region identified using HapMap data and the Haploview algorithm.[26] Altogether 152 subjects diagnosed with ASD from 133 families were genotyped and both individual SNPs and haplotypes were tested for association with ASD. Moreover to better understand how variants in the OXTR mediate risk for ASD, we examined association between *OXTR* with intelligence quotient (IQ) and VABS (Vineland Adaptive Behavioral Scales) scores.[27]

Methods

Participants

Altogether 152 subjects with ASDs (128 men, 24 women) and their parents (133 mothers and 133 fathers) and unaffected siblings (146) were recruited. Nineteen of the families had two children diagnosed with ASD. Subjects were diagnosed with DSM IV autistic disorder ($N = 131$) or pervasive developmental disorder-not otherwise specified (PDD-NOS; $N = 21$). Families were recruited through treatment centers, special schools, the Israeli National Organization for Children with ASD and by acquaintance with other families who participated. Subjects were between the ages of 2 years and a month to 33 years and 8 months. All subjects tested negatively for Fragile-X using a PCR procedure with an ABI 310 DNA analyzer.[28–30] In addition, none of the subjects was known (according to parents' reports) to have any other chromosomal abnormality or tuberous sclerosis.

Many of the subjects had a previous diagnosis within the ASD spectrum made by independent clinicians. However, for the purpose of this study, two trained clinicians confirmed the subjects' diagnosis of DSM IV autistic disorder or PDD-NOS. All subjects were diagnosed using the ASD Diagnostic Interview—Revised (ADI-R),[31] and for 78 of them the ASD Diagnostic Observation Scale—Generic (ADOS-G)[32] was also available.

The ADI-R is a standardized, semi-structured interview based on the ICD-10 definition of ASD (World Health Organization, 1993). Three areas of child functioning are assessed by this parent interview: communication and language; reciprocal social interaction; and repetitive, restrictive and stereotyped behavior. To receive a diagnosis of autistic disorder, one must meet the cutoff criteria for ASD on each of the three aforementioned areas and show evidence for developmental abnormality before the age of 36 months. To receive a diagnosis of PDD-NOS, one must meet the cutoff criteria for ASD for social interaction and for only one of the two other areas as proposed by Risi *et al*.[33]

The ADOS-G is a semi-structured, standardized assessment used for diagnosing children with ASDs. It is an assessment of the child's social interaction, communication, play and imaginative use of materials, through an interaction with a trained professional. Diagnostic algorithms are provided for ASD and for PDD-NOS. In the case of a discrepancy between the two diagnostic measures, one yielding a diagnosis of autistic disorder and the other of PDD-NOS, the final diagnosis was that of PDD-NOS, as proposed by Risi *et al*.[33]

In addition to the ADI-R, parents were also interviewed using the Vineland Adaptive Behavior Scales—Interview edition.[34] The VABS[27] is a structured interview administered to caregiver/s to assess the child's daily living skills on three domains: communication (receptive, expressive and written), daily living skills (personal, domestic and community), and socialization (interpersonal relationships, play and leisure time, and coping skills). In addition to the three domain scores, a total score reflecting overall functioning was also computed. The average total VABS scores were 52.21 (s.d. = 19.88). The VABS has excellent levels of split-half, inter-rater and test–retest reliability for each domain used in the current work.[27]

The subjects' level of functioning was assessed using standard intelligence measures selected according to the subjects' age and abilities (that is, Wechsler Intelligence Scale for Children;[35] Kaufman Assessment Battery for Children;[36] Bayley Scales of Infant Development;[37] Merrill–Palmer Scale of Mental Tests;[38] Mullen Scales of Early Learning;[39] Cattel Measurement of Intelligence of Infants and Young

Children;[40] Leiter International Performance Scale;[41] or Stanford–Binet Intelligence Scale[42]). As some tests (for example, Mullen and Leiter) yield mental ages rather than IQ, mental age was transformed to an IQ estimate using the basic IQ formula: IQ = (Mental Age/Chronological Age)*100MA. Average IQ scores were 68.7 (s.d. = 29.1).

Genotyping

DNA was extracted by Master Pure kit (Epicentre, Madison, WI, USA).

Single-nucleotide polymorphisms were identified by searching through the dbSNP public database (http://www.ncbi.nlm.nih.gov/SNP/). Altogether 18 tagged SNPs were identified across the gene region (19.21 kbp) by Haploview (http://www.broad.mit.edu/mpg/haploview/).[26] In our sample, a single SNP was not in Hardy–Weinberg equilibrium and one SNP was in complete LD with the other SNPs. Therefore, 16/18 tagged SNPs were included in the association test. The location of the SNPs assayed and their location along the OXTR genomic region and their relationship to the exon/intron boundaries of the gene are shown in Table 1 and Figure 1.

Genotyping of all SNPs was performed using the SNaPshot Method (Applied BioSystems, Foster City, CA, USA). This method relies upon the extension of a primer immediately adjacent to the SNP using fluorescently labeled ddNTPs. The fluorescently labeled extension primers can then be visualized by electrophoresis on a capillary ABI PRISM 310 automated sequencer. Amplification of the *OXTR* SNPs regions was amplified using the pairs of primers as described in Table 2.

PCR cycling conditions in the SNaPshot Method were as follows: samples were initially heated at 94°C for 5 min followed by 35 cycles of 94°C (30 s), 55°C (30 s) 72°C (90s) and a final extension step of 72°C for 5 min. After the first PCR cycle, the PCR product was cleaned with ExoSAP for 37°C for 30 min and then at 80°C for 15 min. The conditions for the second PCR were as follows: 96°C (10 s), 50°C (5 s) and 60°C (30 s) for 25 cycles. The second PCR product was cleaned using shrimp alkaline phosphatase (SAP) initially at 37°C for 1 h followed by 72°C for 15 min.

The extension primers for SNaPshot Method are shown in Table 2. In the case of Mendelian error, the genotyping for the entire family was rechecked and if the error persisted the genotyping for that SNP was not included in the analysis. The overall Mendelian error rate following our double-check procedure was $< 0.5\%$.

Statistical methods

We used the logistic-based variant of the transmission disequilibrium test (TDT) the so-called ETDT[43] to assess association (and linkage) without confounding effect of population stratification. The TDT, in its simplest version, compares, for one allele, the number of times this allele is transmitted to the number of times in which this allele is not transmitted to an affected offspring. Note that only heterozygous parents are informative. This approach can be extended to haplotypes. The various tests are implemented in the latest version (3.0.9) of UNPHASED (http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/). UNPHASED[44] is a suite of programs for association analysis of multilocus haplotypes from unphased genotype data.

Correction for multiple testing

Common methods used for controlling are the Bonferroni correction or more refined variations of the method. Applying the Bonferroni correction, which is a single-step procedure applied to a multiple-testing scenario with correlated hypotheses (for example, association analysis of multiple markers and gene–gene interaction), leads to conservative results. Thus, multistep procedures were developed to achieve higher power for correlated tests while controlling the family-wise error rate. We used the permutation test option as provided in UNPHASED to avoid spurious results and correct for multiple testing. Permutation test correction was performed using 1000 iterations (random permutations) and applied to correction of global *P*-values (the *P*-value generated by UNPHASED as an overall test of each block of haplotypes). In nuclear families, the permutations are generated by randomizing the transmission status of the parental haplotypes. In unrelated subjects, the trait values are randomly shuffled between subjects. The randomization is held constant over all analyses specified by the disease-, trait-, marker- and window options, as well as any tests selected by the individual, test-confounders and test-modifiers options. In each permutation, the minimum *P*-value is compared to the minimum *P*-value over all the analyses in the original data. This allows for multiple-testing corrections over all tests performed in a run. This procedure corrects for multiple testing but accounts for correlation between markers, hence is less conservative than a Bonferroni correction, which is appropriate for independent tests such as unlinked markers.

It should be noted that while this multiple-testing correction adjusts for the number of markers tested, it only adjusts the global *P*-value of the particular haplotype block tested within any window size. The permutation test we used does not correct for the overall number of haplotype blocks examined across all window sizes tested. Nevertheless, it should be considered that all haplotypes represented in sliding windows covering a given region are likely to be highly correlated with each other and are therefore unlikely to greatly inflate the number of independent tests undertaken in this study. Nevertheless, our results should hence be interpreted cautiously.

Results

As shown in Table 1, single SNP analysis revealed two SNPs (rs2268494 intron 3 and rs1042778 exon 4

Table 1 Position of tagged SNPs sites and their relationship to the exon–intron boundaries of the *OXTR* gene

rs no.	*SNP*	*Position*	*HWE parents*	*HWE probands*	*Location*		*Size*	*Function*	P-*value ASD*	P-*value IQ*	P-*value VABS*	P-*value communication*	P-*value daily living skills*	P-*value social*
					Exon 1	8785917–8786300	384	Non-coding						
rs4564970	C/G	8785408	1.0	0.929	Intron 1	8785278–8785916	639		0.3270	0.5218	0.4032	0.083	0.035	0.309
					Exon 2	8785182–8785277	96	Non-coding						
					Intron 2	8785016–8785181	166							
					Exon 3	8785040–8785181	122	5′-UTR						
						8785016–8785039	922	Coding						
rs237897	A/G	8783285	0.315	0.484					0.9237	0.6427	0.3016	0.984	0.885	0.825
rs13316193	C/T	8777743	0.116	0.353					0.5445	0.7846	0.7014	0.568	0.017	0.998
rs237889	C/T	8777483	0.691	0.453					1.0000	0.4905	0.4424	0.728	0.372	0.508
rs2254298	A/G	8777228	0.21	0.113					0.6547	0.7673	0.8731	0.023	0.029	0.125
rs2268494	A/T	8777046	1.0	0.785					0.0117 (0.038)*	0.1274	0.2124	0.640	0.605	0.879
rs4686301	C/T	8773586	0.972	0.117	Intron 3	8769911–8783951	14 040		0.0971	0.0029	0.0455	0.041	0.088	0.125
rs9840864	C/T	8773477	0.848	0.121					0.6381	0.8513	0.4988	0.102	0.072	0.249
rs237888	C/T	8772095	1.0	1.0					0.1615	0.4517	0.1742	0.283	0.049	0.083
rs2268490	C/T	8772085	0.775	0.103					0.2695	0.5388	0.3149	0.022	0.054	0.109
rs237887	A/G	8772042	0.978	0.516					0.7797	0.3906	0.3638	0.038	0.020	0.295
rs237885	G/T	8770543	0.213	0.462					0.7150	0.3904	0.7538	0.486	0.344	0.900
rs2139184	A/C	8770494	1.0	1.0					0.4142	0.8323	0.4875	0.368	0.221	0.575
					Exon 4	8769663–8769910	248	Coding						
rs1042778	G/T	8769545	0.18	0.114		8767114–8769662	2549	3′-UTR	0.014 (0.0091)*	0.0139	0.3549	0.742	0.415	0.646
rs6770632	A/C	8768724	0.195	0.215					0.0801	0.0893	0.0267	0.002	0.021	0.021
rs11131145/ rs9872310	A/G	8768381	0.103	0.217					0.3711	0.7309	0.7091	0.270	0.270	0.215

Abbreviations: ASD, autism spectrum disorder; HWE, Hardy–Weinberg equilibrium; IQ, intelligence quotient; OXT, oxytocin; OXTR, oxytocin receptor; SNP, single-nucleotide polymorphism; VABS, Vineland Adaptive Behavior Scales.
The significance levels for association with ASD, IQ and VABS scores are shown for SNPs.
**P*-values in parentheses for association values with ASD indicate adjusted value using IQ as a confounding factor in the UNPHASED program.

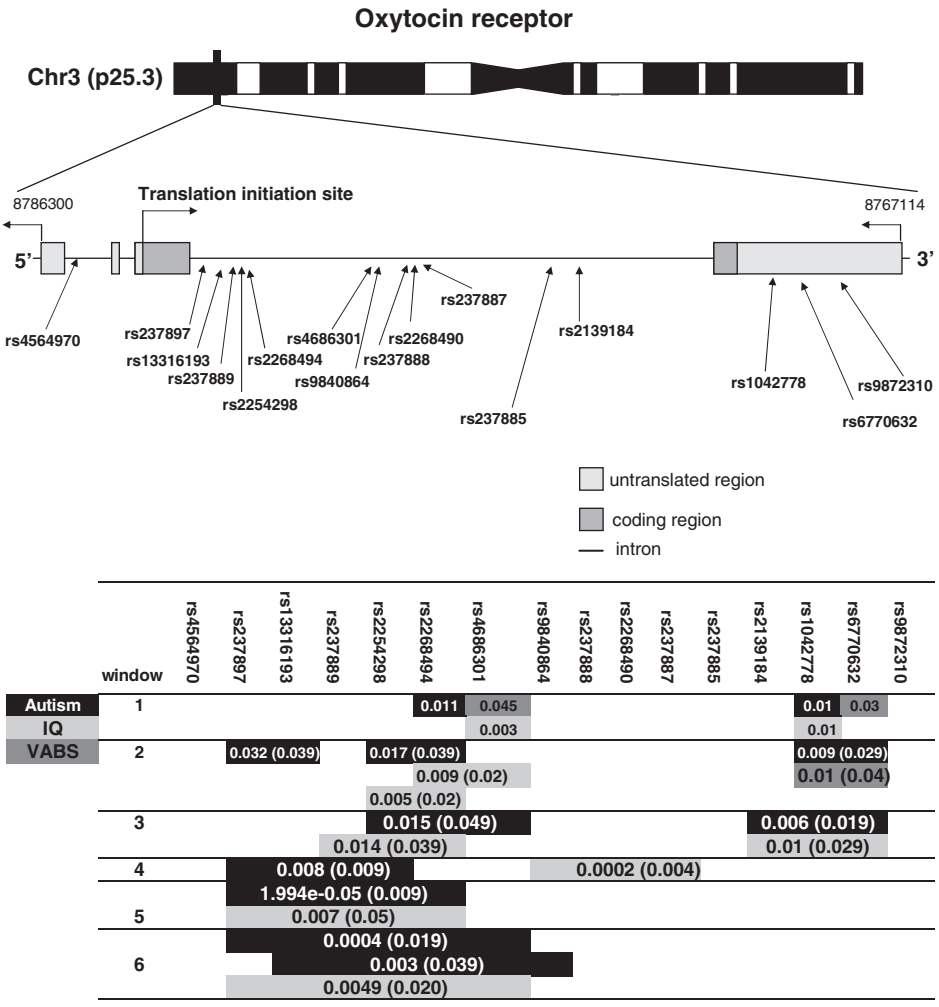

Figure 1 Top: Schematic representation of chromosome 3 and the oxytocin receptor (*OXTR*) gene with the location of the 16 tagging single-nucleotide polymorphisms (SNPs). Bottom: SNPs and haplotypes in the *OXTR* tested for association with autism spectrum disorder (ASD), intelligence quotient (IQ) and Vineland Adaptive Behavior Scales (VABS) scores. The solid bars indicate only significant associations from single SNPs (window 1) to six-locus haplotypes. Both nominal and adjusted significance levels are presented. The significance levels following the permutation test (1000 iterations) are shown in parentheses. Black blocks represent ASD, medium-shaded blocks represent VABS scores and light-shaded blocks represent IQ.

UTR) in nominal (no correction for multiple testing) association with ASD. Two SNPs were nominally associated with IQ (rs468301 intron 3 and rs1042778 exon 4) and two SNPs with total VABS scores (intron 3 UTR: rs468301 and exon 4 UTR: rs6770632). Five single SNPs showed significant association with the VABS communication subdomains, six SNPs with VABS daily living skills and one SNP with socialization (Table 1). One SNP (rs2254298) associated with VABS daily living skills and communication subdomain scores was previously identified to be associated with autism in an independent study by Jacob *et al*.[9]

We next proceeded to a multimarker association analysis using a sliding window approach and examined consecutive SNPs comprising haplotypes of increasing length from 2 to 8 SNPs along the gene and tested blocks of haplotpes for association with ASD, IQ and VABS scores. Haplotype analysis, as has been suggested on theoretical grounds,[45] was more informative than single SNP analysis. A haplotype has a higher level of heterozygosity than individual single SNPs and the association study based on the haplotype may have an increased power for detecting disease association compared with SNP-based analyses. Significant haplotype association (significance levels for global P-values < 0.05 following adjustment by permutation tests) was observed with ASD as well as between the two continuous measures, IQ and total VABS scores (Figure 1). Figure 1 shows only the significant (following permutation test adjustment) haplotype blocks and their location along the *OXTR* gene (see also Table 1 for exact SNP location). VABS' subdomain haplotypes are not presented for reasons of economy of presentation of data.

Table 2 Primers used in genotyping of OXTR tagged SNPs

First PCR primers	*SNP*	*Second PCR primer extensions*
F: TTGTAATTCTAATGCACCCTCA	rs11706648	$(T)_{18}$TGCACCTGTTGGAACAAG
R: AAAGTTGAAAGATCCAAAGAGTAAA	rs237887	AGCTTTGCAATGAGGTAG
	rs2264890	CAGAAAGACACTGTTTTG
	rs237888	$(T)_{24}$ATTGTGACCAATAACTGT
F: CCCAGAGGTCTGTGGGTGTA	rs2268494	$(T)_{12}$GACCACACGGTCCCACAT
R: GTCAGGGAGGAGCTGTTCTG	rs2254298	$(T)_{6}$AAGAAGCCCCGCAAACTG
	rs237889	$(T)_{6}$GCAAAGACAGCAAGGCCA
	rs13316193	$(T)_{36}$CGTGGAGGACGGGAATGC
F: ATTGTCTCGGTGCCATTTGT	rs9872310	TCAATTGACCGTAAGTAT
R: CCATCAGAAAGAATAAAATAGGAA	rs6770632	$(T)_{12}$CCTTCTTTAATTTCTTTC
F: AGAGCTGCCTGCCAAATG	rs9840864	$(T)_{12}$CAAATGTGCAAAGGCCAG
R: GACCCCCGGAGAAGGTGCT	rs4686301	$(T)_{30}$CAGCCACAATGATGTCAG
F: AGGAGAGTGCCCAAACCTCT	rs2139184	$(T)_{6}$GCTATCACGACCATGTGC
R: GCATCTTTGGGAATCAGCTC	rs237885	$(T)_{6}$GATGCAGACATCTTGTGG
F: CCACACGTGTAAAGGCAAAC	rs2268492	GTAGCCAATGGCTGGCTA
R: AGTTGGAAGGAAGCTGCTCA		
F: AAGGGAGGGTCAAAATCAGC	rs4564970	$(T)_{18}$CCCTCAGCATATCCACCT
R: GTTAGACGGGGAAGGACCAG		
F: TGGGTTCAGGGTGGTAGAAG	rs1042778	$(T)_{12}$TGAAGCCACCCCAAGGAG
R: AGGCTGTGCTGGCATAAGTG		
F: CCTCCCCCTCAAACTTGAAT	rs237897	$(T)_{36}$TCCATAAGCCTGCCCACC
R: CCAAGGGAGAGGTGAAGACA		

Abbreviations: OXT, oxytocin; OXTR, oxytocin receptor; SNP, single-nucleotide polymorphism.

Since significant global *P*-value association was observed between haplotype blocks and ASD, we next proceeded to examine association between individual haplotypes of various lengths and ASD, IQ and VABS scores. Table 3 shows the association for individual haplotypes (within blocks) with ASD. Notably, for each significant haplotype the rs2254298 'G' SNP was included in the haplotype. This is the SNP associated with autism in the study by Jacob *et al*.[9] The longer (>3 locus) haplotypes associated with ASD were clustered close to the exon 3/intron 3 boundary (see Table 1 for locations) and showed strongest evidence for association. The five-locus haplotype A-T-T-G-A (rs237897-rs13316193-rs237889-rs2254298-rs2268494) showed highly significantly association with ASD ($P = 0.00005$). Most of the longer haplotypes associated with ASD included as their first 5′ SNP, rs237897 that was the closest tagged SNP to the exon 3/intron 3 boundary. For eight-locus haplotypes nominal, although highly significant, association was observed between ASD (Likelihood ratio $\chi^2 = 37.92$, d.f. = 15, global *P*-value = 0.00093), IQ (Likelihood ratio $\chi^2 = 52.09$, d.f. = 19, global *P*-value = 0.00006) and total VABS (Likelihood ratio $\chi^2 = 44.28$, d.f. = 19, global *P*-value = 0.0009). Permutation tests were not successfully performed >6-locus haplotypes because of the computational demand of such an analysis. The first upstream 5′ SNP in all of these haplotype groups was rs237897. Individual haplotypes and their significance levels for eight-locus haplotypes associated with ASD are shown in Table 3.

We also examined the role that IQ played in the observed association between ASD and OXTR. As shown in Table 1, when IQ was entered as a covariate ('confounding factor' in the UNPHASED program), significant association is nonetheless observed for the two SNPS (rs2268494 intron 3 and rs1042778) with ASD. Similarly, we also examined the five-locus haplotype association (starting with SNP rs237897) with ASD when IQ is entered as a covariate in the test. Highly significant association was nonetheless observed with IQ was a covariate (without IQ as cv: Likelihood ratio $\chi^2 = 43.32$, d.f. = 12, nominal *P*-value = 0.000019; with IQ as cv: Likelihood ratio $\chi^2 = 41.37$, d.f. = 14, nominal *P*-value = 0.00015). Therefore, it appears that the association between ASD and OXTR is not simply related to an effect of the OXTR on cognition but appears to be related at least partially to deficits in daily living skills.

Discussion

The current investigation confirms and extends the findings of Wu *et al*.[8] in a Han Chinese population and Jacob *et al*.[9] in a Caucasian population who first demonstrated provisional association between *OXTR* and ASD. Whereas the Wu *et al*.[8] genotyped only four SNPs and Jacob *et al*.[9] two SNPs, we used Haploview to identify all tagging SNPs across the gene region towards implementing a rational and cost-effective genotyping approach. Interestingly, the rs2254298 SNP associated with autism (G allele overtransmitted) in the the study by Jacob *et al*.,[9] although not by itself significantly associated with ASD in the current investigation (although G in our study is also overtransmitted), nonetheless was a core SNP in the haplotypes (and the G allele was the 'risk' allele) that

Table 3 Individual haplotypes significantly associated with DSM IV ASD

Window	*rs4564970*	*rs237897*	*rs13316193*	*rs237889*	*rs2254298*	*rs2268494*	*rs4686301*	*rs9840864*	*rs23788*	*Freq*	χ^2	P-*value*
2					**G**	**A**				0.17	6.18	**0.012**
					G	T				0.63	5.15	**0.023**
3				**T**	**G**	**A**				0.16	7.91	**0.004**
				T	G	T				0.27	6.38	**0.011**
4			**T**	**T**	**G**	**A**				0.16	7.96	**0.004**
			T	T	G	T				0.25	5.77	**0.016**
5		**A**	**T**	**T**	**G**	**A**				0.07	16.46	**4.964e−005**
		A	T	C	G	T				0.06	6.02	**0.014**
		G	T	C	G	T				0.09	6.44	**0.011**
6		**A**	**T**	**T**	**G**	**A**	**C**			0.06	7.55	**0.005**
		A	T	T	G	T	C			0.19	5.49	**0.019**
		A	T	C	G	T	T			0.05	4.08	**0.044**
		G	T	C	G	T	T			0.05	4.25	**0.039**
7		**A**	**T**	**T**	**G**	**A**	**C**	**C**		0.05	5.71	**0.016**
		A	T	T	G	T	C	G		0.16	4.07	**0.043**
		G	T	C	G	T	T	G		0.06	4.15	**0.041**
8		**A**	**T**	**T**	**G**	**A**	**C**	**C**	**T**	0.06	5.07	**0.024**
		A	T	T	G	T	C	G	T	0.16	4.24	**0.039**
		A	T	C	G	T	T	G	T	0.04	7.14	**0.007**

Abbreviation: ASD, autism spectrum disorder.
Window 2 represents two-locus haplotypes and window 8 represents eight-locus haplotypes.
Bold *P*-values are significant ($P < 0.05$).

showed significant association with ASD (Table 3). Additionally, this SNP was significantly associated with the VABS subdomain daily living skills scores. A single SNP, rs4564970, located in the 5′-flanking region in the vicinity of a number of transcription factors, was not associated with ASD. Sliding-window haplotype analysis by maximizing the genetic information extracted from the 16 genotyped tagged SNPS showed significant association between haplotypes and both ASD as well as IQ and VABS scores. The association between *OXTR* haplotypes and IQ and VABS scores suggests the notion that these aspects of cognition and daily living skills, respectively, contribute to the association between this gene and ASD. It should be noted, however, that the associations between *OXTR* haplotypes with IQ and the VABS are not necessarily specific to ASD and future research should address this issue by also examining other diagnostic groups such as mental retardation. Notably, the association demonstrated here between the *OXTR* gene and continuous measures including IQ and VABS scores suggests a molecular genetic validation to recent pharmacological manipulations using OXT in both syndromic[18,19,46] and non-syndromic populations.[14–16,47]

As shown in Figure 1 and Table 1, the *OXTR* gene spans 19 kb and contains three introns and four exons. Exons 1 and 2 correspond to the 5′-non-coding region and contain a number of putative transcription factor sites. Exons 3 and 4 encode the amino acids, see review.[24] Intron 3, which is the largest at 12 kb, separates the coding region immediately after the putative transmembrane domain 6. Exon 4 contains the sequence encoding the seventh transmembrane domain, the COOH terminus, and the entire 3′-non-coding region, including the polyadenylation signals. Interestingly, the longer haplotypes showing significant association with DSM IV ASD tended to cluster in the 5′-flanking region of the gene at the exon 3 coding region–intron 3 boundary. It is tempting to speculate that these SNPs/haplotypes may be in linkage disequilibrium with a critical splice site polymorphism or enchancer element in this region. Moreover, Mizumoto *et al.*[48] reported that a genomic element within the third intron of the human oxytocin receptor gene may be involved in transcriptional suppression of the gene suggesting that some of the intronic SNPs that we observed in association with ASD may alter methylation sites that regulate gene expression.

The association observed between *OXTR* haplotypes and ASD, IQ and total VABS scores (as well subdomains) are consistent with a role for OXT in cognition and social communication in both humans and other species. de Wied[49] pioneered animal studies showing that OXT modulates behavior, learning and memory. These early studies have been substantiated by a large body of experimental findings showing that OXT enhances social interactions such as pair bonding[12,13] and maternal behavior[50] and inhibits HPA-induced stress responses.[47]

A function for OXT in conferring risk ASD is further strengthened by the studies of Hollander *et al.*,[18,19] who have presented evidence that OXT infusions administered to participants (fulfilling DSM criteria for autistic disorder or Asperger's syndrome) might facilitate social information processing as well as reduce repetitive behavior. In a recent study with evident inferences for ASD research, Domes *et al.*[16] showed that intranasally administered OXT improved Reading the Mind in the Eyes Test[51] that examines the ability to infer the mental state of others from social cues of the eye region. Interestingly, despite individual variability and overlapping group distributions midday plasma OXT were significantly lower in subjects with DSM IV autistic disorder than the normal group.[21,22] Indeed, several reviews have underscored the theoretical basis for this neuropeptides involvement in contributing to DSM IV ASD.[10,21,52–54]

In summary, a role for OXT in conferring risk for DSM IV ASD is suggested by two genome-wide scans of ASD,[6,7] the role of this nonapeptide in molding social memory and communication in non-human species,[3] recent human studies showing a role of OXT in social cognition in both non-syndromic[14–17] and syndromic[18–22] subjects and the current family-based association study that replicates and extends a previous study in a Han Chinese population[8] and a Caucasian population.[9] Importantly, the potential of intranasal administration of this and related peptides as suggested by Born *et al.*[55] coupled with a pharmacogenetic approach, made feasible by the current investigation, offers a worthwhile strategy to explore a novel therapeutic window into the treatment of some of the intractable core social deficits associated with the ASD syndrome.

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