

ORIGINAL ARTICLE

Association of the oxytocin receptor (*OXTR*) gene polymorphisms with autism spectrum disorder (ASD) in the Japanese population

Xiaoxi Liu¹, Yoshiya Kawamura², Takafumi Shimada², Takeshi Otowa², Shinko Koishi², Toshiro Sugiyama³, Hisami Nishida⁴, Ohiko Hashimoto⁵, Ryoichi Nakagami², Mamoru Tochigi², Tadashi Umekage², Yukiko Kano³, Taku Miyagawa¹, Nobumasa Kato⁶, Katsushi Tokunaga¹ and Tsukasa Sasaki⁷

The oxytocin receptor (*OXTR*) gene, which is located on chromosome 3p25.3, has been implicated as a candidate gene for susceptibility of autism spectrum disorder (ASD). Positive associations between *OXTR* and ASD have been reported in earlier studies. However, the results were inconsistent and demand further studies. In this study, we investigated the associations between *OXTR* and ASD in a Japanese population by analyzing 11 single-nucleotide polymorphisms (SNPs) using both family-based association test (FBAT) and population-based case-control test. No significant signal was detected in the FBAT test. However, significant differences were observed in allelic frequencies of four SNPs, including rs2254298 between patients and controls. The risk allele of rs2254298 was 'A', which was consistent with the previous study in Chinese, and not with the observations in Caucasian. The difference in the risk allele of this SNP in previous studies might be attributable to an ethnic difference in the linkage disequilibrium structure between the Asians and Caucasians. In addition, haplotype analysis exhibits a significant association between a five-SNP haplotype and ASD, including rs22542898. In conclusion, our study might support that *OXTR* has a significant role in conferring the risk of ASD in the Japanese population.

Journal of Human Genetics (2010) 55, 137–141; doi:10.1038/jhg.2009.140; published online 22 January 2010

Keywords: ASD; case-control; family-based association; *OXTR* gene; SNP

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopment disorder characterized by impairment in social interaction and communication, as well as restricted and stereotyped behaviors and interests.^{1,2} In its broad definition, it affects 1 in every 152 children^{3,4} and the ratio between affected male and female is 4:1. Owing to its early onset (with usual appearance of the symptoms before the age of 3 years) and lack of effective therapeutic strategy, ASD causes huge emotional and financial burden to the patients, their families and the society.

Twin studies show that the concordance rate of ASD in monozygotic twins is 70–90%, but only 0–10% in dizygotic twins.⁵ Family studies indicate that the prevalence of ASD in siblings of the affected individuals is 50–100 times higher than the general population.⁶ Lines of compelling evidence have firmly established that ASD has a strong genetic component; however, the underlying mechanism remains elusive, which might be due to the genetic heterogeneity and complexity.

In the past decades genome-wide linkage studies and genetic association studies have been performed to identify susceptibility

genes for ASD, in which a number of potential candidate genes have been implicated.^{7–10} However, all known genetic variations can only account for 10–20% of the ASD patients.¹¹

Several hypotheses have been proposed to explain the causes of ASD.^{12–14} In these hypotheses, the oxytocin and vasopressin systems are receiving increasing attention because of their special roles in regulating a range of social behaviors, including social recognition, pair bonding and maternal care, which were observed in animal studies.^{2,15} Mice lacking the oxytocin gene fail to develop social memory.¹⁴ *OXTR* knockout mice showed several aggravations in social behaviors including mother-offspring interaction.¹⁶ In human, autistic children tended to have a lower plasma oxytocin level compared with healthy individuals.¹⁷ Oxytocin infusion might reduce repetitive behaviors in autistic patients.¹⁸ In addition, the oxytocin receptor (*OXTR*) gene is located at 3p25.3, in the chromosomal region, which has been suggested for autism in a genome-wide linkage study.¹⁹ On the basis of these findings, it is reasonable to postulate that the dysfunction of oxytocin system may be associated

¹Department of Human Genetics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; ²Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; ³Department of Child Psychiatry, University of Tokyo Hospital, Tokyo, Japan; ⁴Asunaro Hospital for Child and Adolescent Psychiatry, Tsu, Japan; ⁵Department of Occupational Therapy, Faculty of Nursing and Rehabilitation, Aino University, Ibaraki, Japan; ⁶Department of Psychiatry, Graduate School of Medicine, University of Showa, Tokyo, Japan and ⁷Office for Mental Health Support, University of Tokyo, Tokyo, Japan
Correspondence: Dr T Sasaki, Office for Mental Health Support, the University of Tokyo, Hongo 7-3-1, Bunkyo, Tokyo 113-8655, Japan.
E-mail: psytokyo@yahoo.co.jp

Received 20 October 2009; revised 3 December 2009; accepted 16 December 2009; published online 22 January 2010

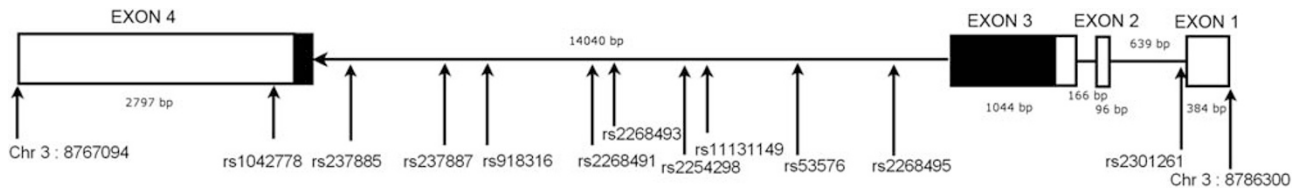


Figure 1 Schematic diagram of the 11 SNPs of the *OXTR* genotyped in the current study. Each block indicates an exon. White and black blocks show untranslated region and coding regions, respectively.

with susceptibility to autism. To date, Chinese and Caucasian studies observed significant genetic associations between *OXTR* and ASD.^{20–22} Among them, Wu *et al.*²⁰ were the first to study the genetic association of single-nucleotide polymorphisms (SNPs) of *OXTR* in autism. They investigated four SNPs in Han-Chinese ASD trios and found that 'A' alleles of rs53576 and rs2254298 were significantly overtransmitted. Two Caucasian studies^{21,22} also found the association between *OXTR* and ASD, but the detail of the results was inconsistent compared with the Chinese result. Jacob *et al.*²¹ investigated the two SNPs (rs53576 and rs2254298), which were suggested in the Chinese study, and found that the 'A' allele of rs2254298 was undertransmitted, not overtransmitted. No association of rs53576 was observed. Lerer *et al.*²² found an association of a haplotype comprising rs2254298 and four other SNPs (rs237897–rs13316193–rs237889–rs2254298–rs2268494) with ASD, but as a single SNP, rs2254298 was not associated with ASD (Lerer *et al.*²²). Yrigollen *et al.*²³ found that rs2268490, which was in the same haploblock of rs2254298, was associated with ASD.

These results require further studies to clarify the role of the *OXTR* polymorphisms in ASD. In this study, we conducted family-based association test (FBAT) and population-based case–control test in Japanese subjects with ASD.

MATERIALS AND METHODS

Subjects

Two sample sets were included in this study. A set consists of 217 families including 223 affected individuals (189 males and 34 females, age=18.35 ± 9.5 years (mean ± s.d.)) with their parents. Among the 223, 207 were diagnosed with autistic disorder, 3 with Asperger's disorder and 13 with pervasive developmental disorder not otherwise specified. Another set comprised 65 unrelated autistic disorder cases without parents' sample (57 males and 8 females, age=25.20 ± 4.8 years (mean ± s.d.)). The 282 ASD samples for case–control association study are the combination of 217 independent cases from the family samples and 65 unrelated samples. The diagnoses were made by two or more senior child psychiatrists through interviews and reviews of clinical records, according to the DSM-IV criteria (American Psychiatric Association, 1994). Controls consisted of 440 unrelated healthy Japanese subjects (272 males and 168 females, age=40.9 ± 9.7 years (mean ± s.d.)). The cases were recruited from the outpatient clinics of the Departments of Psychiatry, Tokyo University Hospital and Tokai University Hospital, and seven day care facilities for subjects with developmental disorders. The hospitals and facilities were located around Tokyo, Nagoya and Mie. The controls were recruited around Tokyo, without any psychiatric disorder disturbing their work function. The Mini-International Neuropsychiatric Interview²⁴ and other questions were administered in the recruitment of controls to exclude those who had present or lifetime history of mental disorders. All the patients and controls were ethnically Japanese, with no parents or grandparents of ethnicity other than Japanese. Objective of the study was clearly explained, and written informed consent was obtained from all control subjects or parents of the affected individuals. The consent was also obtained from the affected individuals when they were able to follow the explanation. The study was approved by the Ethical Committee of the Faculty of Medicine, the University of Tokyo.

Genotyping and selection of the SNPs

Genomic DNA was extracted from the peripheral blood using the standard phenol–chloroform method for the family samples and unrelated case samples. For the control samples, genomic DNA was isolated from whole blood by using Wizard Genomic DNA Purification Kit.²⁵

On the basis of the genotype data in the Japanese population from the HapMap Project, we selected pair-wise tag-SNPs with minor allele frequencies >0.05 and a threshold of $r^2=0.8$ in the *OXTR* region including promoter and other areas, using the Tagger program implemented in Haploview. Intron 3 was the most densely mapped as shown in Figure 1. In addition, we studied three other SNPs, rs2268493, rs53576 and rs1042778. These were reported to be associated with ASD in the previous studies.^{20–23} Thus, a total of 12 SNPs were selected. All SNPs were genotyped by using TaqMan genotyping platform. The TaqMan probes were ordered from the Assays on Demand system of the Applied Biosystems (Applied Biosystems, Foster City, CA, USA). Genotyping was performed in 5- μ l system containing 2.5 μ l of TaqMan Universal PCR Master mix, 0.25 μ l of 20 \times TaqMan probe and 1 μ l genomic DNA using Roche LightCycler 480 II (Roche Diagnostics, Tokyo, Japan). Allele calling was performed using LightCycler CW 1.5 software (Roche Diagnostics).

Statistical analysis

Hardy–Weinberg equilibrium was tested in control subjects and parent subjects in family samples by χ^2 -test using Haploview software ver 4.1.^{26,27} FBATs were conducted using FBAT ver 1.7.2.^{28,29} Case–control association analyses were performed by standard χ^2 test using the PLINK program.³⁰ Odds ratio for each allele and the 95% confidence intervals were also calculated. To correct the multiple testing, Bonferroni correction was implemented based on the number of SNPs analyzed in the *OXTR*. We set the critical *P*-value for a positive association at 0.0045.

Pairwise linkage disequilibrium (LD) was derived from genotyping data of control samples using the confidence intervals method (the Gabriel method) provided by HaploView. The same software was used to estimate haplotype frequency and haplotype *P*-value. The empirical *P*-values were generated on the basis of 10 000 permutation tests. The global *P*-value for each haploblock was calculated by the UNPHASED software (<http://www.mrc-bsu.cam.ac.uk/personal/frank/software/unphased>).

RESULTS

Genotype distribution of one SNP (rs2268490) was significantly deviated from Hardy–Weinberg equilibrium in control subjects (*P*-value <0.001), and this SNP was excluded from the analysis. No significant deviation from Hardy–Weinberg equilibrium was observed in parents of the family samples for any SNPs. We performed the Mendelian inheritance check for the family samples. Two families showed inconsistency and were also excluded.

Single-marker association analysis

The FBAT did not reach the statistical significance (*P*-value <0.05) in this study (Table 1). The results of SNP analyses based on case–control test are summarized in Table 2. Statistically significant association with ASD was observed in four SNPs, including rs237887, rs2268491, rs2254298 and rs2268495 (*P*=0.023, 0.004, 0.001 and 0.032, respectively). *P*-value for rs53576 was marginally 5% (*P*=0.053). Two SNPs

(rs2268491, rs2254298) remained significant after Bonferroni correction for the multiple testing.

LD structure

The standardized measure of LD denoted as r^2 was calculated for all pairs of SNPs. Two haploblocks were suggested according to confidence intervals method (Figure 2). LD block 1 consisted of two SNPs (rs237885, rs237887), which correspond to exon 4 untranslated region, and exon4-intron 3 boundary region. Block 2 that spans about 4.7 kb comprises five SNPs located in intron 3. Of these five, two SNPs (rs2268491 and rs2254298), which were significantly associated with ASD in this study, are in perfect LD ($r^2 > 0.95$). Two unassociated SNPs (rs2268493 and rs11131149) were in low LD with the two associated SNPs, but they were in perfect LD with each other.

We then carried out haplotype analysis within these two haploblocks and conducted permutation test. For haplotype analysis positive association was noted in both haploblocks (Table 3). Two out of three haplotypes in the block 1 accounted for 85% in controls and showed nominal association with ASD with $P=0.029$ and 0.042 , respectively. One of the five-SNP haplotypes in the block 2, which carries 'A' allele of rs2254298, showed a significant association with ASD ($P=0.004$). The global P -value for the two haploblocks is 0.0093 and 0.00187 , respectively. By permutation test, a significant association with ASD was observed for the five-SNP haplotype in the block 2, but not for the haplotypes in the block 1.

DISCUSSION

The case-control analysis of this study generated significant associations between SNPs in OXTR and ASD in the Japanese population, whereas family-based analysis did not provide a support for the association. In the case-controls analysis, 4 SNPs out of 11 were significantly associated with ASD at 5% or stronger level of

significance. The most significantly associated SNP was rs2254298 with 'A' as the risk allele. Haplotype analysis also provided a support for the association of the five-SNP haplotype in intron 3, containing rs2254298, with ASD. These results may suggest the potential role of OXTR in ASD, as implicated in the previous studies.²⁰⁻²²

In contrast to the case-control analysis, we did not obtain a support for the association in the family-based analysis. This could be due to the insufficient power with limited numbers of informative families. Regarding the two interesting SNPs (rs2254298 and rs53576), 'A' alleles were preferentially transmitted to the affected individuals ($Z=0.862$ in 124 informative families for rs2254298 and $Z=0.931$ in 128 informative families for rs53576). This could be consistent with the

Table 2 Allele frequencies of 11 OXTR SNPs in 280 unrelated ASD cases and 440 controls

SNPs (minor/major alleles)	MAF (case)	MAF (controls)	P-value	OR (95% CI)
1 rs1042778 (T/G)	0.098	0.097	0.931	1.016 (0.709-1.456)
2 rs237885 (G/T)	0.247	0.292	0.068	0.799 (0.627-1.017)
3 rs237887 (A/G)	0.378	0.439	0.023	0.776 (0.624-0.966)
4 rs918316 (C/T)	0.204	0.222	0.424	0.899 (0.693-1.167)
5 rs2268491 (T/C)	0.325	0.255	0.004	1.405 (1.112-1.775)
6 rs2268493 (C/T)	0.152	0.160	0.694	0.942 (0.701-1.266)
7 rs2254298 (A/G)	0.335	0.256	0.001	1.459 (1.156-1.841)
8 rs11131149 (A/G)	0.165	0.172	0.743	0.953 (0.714-1.271)
9 rs53576 (G/A)	0.333	0.383	0.053	0.802 (0.642-1.003)
10 rs2268495 (A/G)	0.186	0.235	0.032	0.747 (0.572-0.976)
11 rs2301261 (A/G)	0.115	0.085	0.065	1.395 (0.979-1.988)

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; OXTR, oxytocin receptor.

Table 1 Results of the family-based association test (FBAT) of 11 SNPs in 215 ASD families

No.	SNPs	Position	Allele	Number of families	S	E(S)	Z	P-value
1	rs1042778	Chr3: 8769545	G	57	82	85	-0.739	0.46
			T	57	36	33	0.739	0.46
2	rs237885	Chr3: 8770543	G	115	81	82	-0.167	0.868
			T	115	157	156	0.167	0.868
3	rs237887	Chr3: 8772042	A	133	115	123.5	-1.278	0.201
			G	133	161	152.5	1.278	0.201
4	rs918316	Chr3: 8773181	C	93	63	69	-1.105	0.269
			T	93	127	121	1.105	0.269
5	rs2268491	Chr3: 8775398	C	119	150	154	-0.636	0.524
			T	119	94	90	0.636	0.524
6	rs2268493	Chr3: 8775840	C	82	50	49.5	0.102	0.919
			T	82	120	120.5	-0.102	0.919
7	rs2254298	Chr3:8777228	A	124	100	94.5	0.862	0.389
			G	124	156	161.5	-0.862	0.389
8	rs11131149	Chr3: 8777852	G	88	128	127.5	0.098	0.922
			A	88	54	54.5	-0.098	0.922
9	rs53576	Chr3: 8779371	A	128	156	150	0.931	0.352
			G	128	104	110	-0.931	0.352
10	rs2268495	Chr3: 8782535	A	97	62	70	-1.403	0.161
			G	97	142	134	1.403	0.161
11	rs2301261	Chr3: 8785896	G	62	89	91.5	-0.602	0.547
			A	62	37	34.5	0.602	0.547

Abbreviations: ASD, autism spectrum disorder; E(S), expected value of S under the null hypothesis (that is, no linkage or association); S, test statistics for the observed number of transmitted alleles; SNP, single-nucleotide polymorphism; Z, FBAT Z score of multiallelic test for the allele effects. Number of families: number of informative families (that is, families with at least one heterozygous parent).

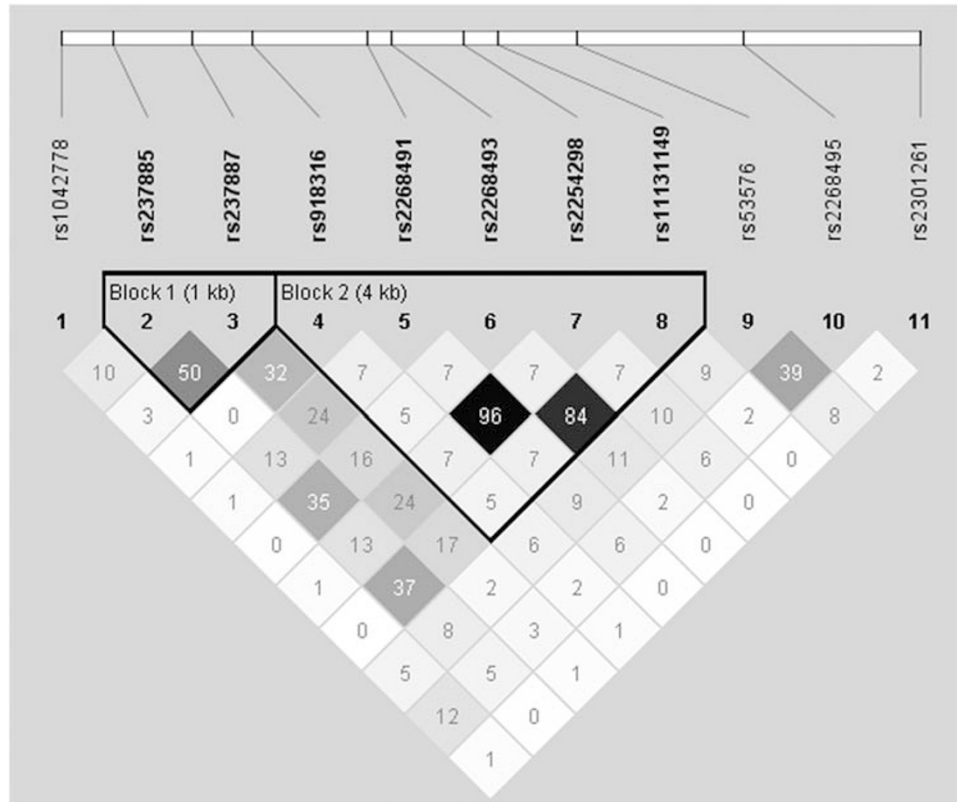


Figure 2 Pairwise LD analysis. LD between SNPs in *OXTR* was derived from genotyping data of control samples. The white bar above the SNP names represents the location of each SNP along the gene. The number within each square indicate the r^2 -values.

Table 3 Haplotype analysis of SNPs in two LD blocks by permutation

		Estimated frequency							
(Block 1)				P-value	Permutation P-value				
<i>rs237885</i>	<i>rs237887</i>	Cases	Controls						
G	A	0.238	0.290	0.029	0.162				
T	G	0.613	0.559	0.042	0.252				
T	A	0.140	0.150	0.581	0.999				
(Block 2)		Estimated frequency							
<i>rs918316</i>	<i>rs2268491</i>	<i>rs2268493</i>	<i>rs2254298</i>	<i>rs11131149</i>	Cases	Controls	P-value	Permutation P-value	
T	T	T	A	G	0.314	0.245	0.004	0.018	
C	C	T	G	G	0.188	0.212	0.252	0.929	
T	C	C	G	A	0.149	0.155	0.739	1.000	
T	C	T	G	G	0.303	0.356	0.040	0.241	
T	C	T	G	A	0.020	0.016	0.601	1.000	

Abbreviations: LD, linkage disequilibrium; SNP, single-nucleotide polymorphism.

Chinese finding,²⁰ although the results did not reach the level of statistical significance.

Regarding the association of the intron 3 region of *OXTR*, previous studies observed controversial results.^{20–22} In a Chinese study, the 'A' allele of *rs2254298* was risk for ASD,²⁰ whereas the 'A' allele was protective for ASD in the Caucasians.²¹ In addition, a Jewish study²² found a haplotype, which contained *rs2254298* with the 'A' allele, as

protective. This study observed the association of the 'A' allele with ASD as a risk allele with the *P*-value of 0.001 in the case–control analysis, which is consistent with the Chinese investigation. This suggests that the 'A' allele of *rs2254298* may increase the risk of ASD in the Asians, but not in Caucasians. The SNP *rs2254298* itself therefore may not be a real causal variant. Another unknown variant in strong LD with this SNP might be responsible for ASD, in which the

risk allele might be in LD with 'G', not 'A', of rs2254298 in Caucasians and Jewish. Association of another SNP, rs53576, and ASD was observed in the Chinese study,²⁰ but not in the Caucasian study.²¹ This study found a weak support for the association with the same risk allele in the Chinese study ($P=0.053$, in the case-control analysis). This might suggest a role of the SNP in ASD in Asian populations, but further studies are requested for the conclusion.

A functional study of OXTR revealed that the intron 3 contains a genomic element, which may be involved in specific suppression and down regulation of the gene.³¹ In addition, we noticed there are two highly conserved sites within this haploblock by aligning homologous sequences of OXTR in human and other species using ENSEMBL database (<http://www.ensembl.org>). If the sites are functionally critical, the causal variant could be located in or close to the sites.

The boundary region of intron 3 and exon 4, which contains the block 1 in this study, may be another region of interest. SNPs including rs237885 (chr3:8770543) and rs237887 (chr3:8769545) in intron 3 and rs1042778 (chr3:8769545) in exon 4 are located in this region. Rs237887 was associated with ASD in the case-control analysis ($P=0.023$) and rs237885 showed the similar tendency ($P=0.068$) in this study. Rs1042778 was associated with ASD in the Jewish population ($P=0.014$),²² whereas the association was not observed in this study. These observations suggest that the boundary region of intron 3 and exon 4 could contain an unidentified variation, which may cause an alternative splicing event or lead to a changed composition. Three different forms of the OXTR transcripts were annotated by the Human And Vertebrate Analysis and Annotation (HAVANA) project, and the 4th exon was missing in two forms of the transcripts. OXTR mRNAs of different length (3.6 and 4.4 kb) have been also reported.³² Such kind of variations could be related with the observed association between this region and ASD.

There are some major limitations to the current study. First, we focused on the tag SNPs and the SNPs found associated with ASD in previous studies, but we did not study nonsynonymous SNPs annotated in the dbSNP database. It is not clear how the substitution of the amino acid affects the function of OXTR, therefore, future studies should include these SNPs. Statistical power of this study is 0.79 for the case-control study and 0.59 for family-based association study when assuming genotypic relative risk is 1.65 under dominant model and SNP frequency is 0.1 for significant level $\alpha=0.05$. Thus small effect might not be detected because of the sample size. Caution was needed to interpret this study that the controls are not age or sex matched to the case subjects. The sex imbalance in case and control subjects might be resolved by analysis confining the subjects to males considering the higher prevalence in male than in the female. The association of rs2254298 was significant after the correction of multiple testing in males ($P=0.003$, not written in the result). Finally, a caution might be noted that subjects were recruited from two areas of Japan, and the number of the case and control from the two areas were not exactly matched.

In summary, the case-control analysis of this study suggested that OXTR might have a role in the development of ASD in the Japanese population. The 'A' allele of rs2254298 may be the risk allele in Japanese, in accordance with the previous Chinese finding.²⁰ Further studies of OXTR with larger sample size and denser markers or direct sequencing may be recommended for the search of the causal variants.

ACKNOWLEDGEMENTS

This study was partly supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant #21300242).

- 1 Folstein, S. E. & Rosen-Sheidley, B. Genetics of autism: complex aetiology for a heterogeneous disorder. *Nat. Rev. Genet.* **2**, 943–955 (2001).
- 2 Ferguson, J. N., Young, L. J., Hearn, E. F., Matzuk, M. M., Insel, T. R. & Winslow, J. T. Social amnesia in mice lacking the oxytocin gene. *Nat. Genet.* **25**, 284–288 (2000).
- 3 Fombonne, E. The prevalence of autism. *Am. Med. Assoc.* **289**, 87–89 (2003).
- 4 Van Naarden Braun, K., Pettygrove, S., Daniels, J., Miller, L., Nicholas, J., Baio, J. *et al.* Evaluation of a methodology for a collaborative multiple source surveillance network for autism spectrum disorders—Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2002. *MMWR (Surveillance Summaries)* **56**, 29–40 (2007).
- 5 Ronald, A., Happé, F., Bolton, P., Butcher, L. M., Price, T. S., Wheelwright, S. *et al.* Genetic heterogeneity between the three components of the autism spectrum: a twin study. *J. Am. Acad. Child Adolesc. Psychiatry* **45**, 691 (2006).
- 6 Rutter, M., Silberg, J., O'Connor, T. & Simonoff, E. Genetics and child psychiatry: II Empirical research findings. *J. Child Psychol. Psychiatry* **40**, 19–55 (1999).
- 7 Gupta, A. R. & State, M. W. Recent advances in the genetics of autism. *Biol. Psychiatry* **61**, 429–437 (2007).
- 8 Freitag, C. M. The genetics of autistic disorders and its clinical relevance: a review of the literature. *Mol. Psychiatry* **12**, 2–22 (2006).
- 9 Veenstra-VanderWeele, J. & Cook, E. H. Molecular genetics of autism spectrum disorder. *Mol. Psychiatry* **9**, 819–832 (2004).
- 10 Vorstman, J. A., Staal, W. G., van Daalen, E., van Engeland, H., Hochstenbach, P. F. & Franke, L. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol. Psychiatry* **11**, 18–28 (2006).
- 11 Abrahams, B. S. & Geschwind, D. H. Advances in autism genetics: on the threshold of a new neurobiology. *Nat. Rev. Genet.* **9**, 341–355 (2008).
- 12 Zoghbi, H. Y. Postnatal neurodevelopmental disorders: meeting at the synapse? *Am. Assoc. Advancement Sci.* **302**, 826–830 (2003).
- 13 Geschwind, D. H. & Levitt, P. Autism spectrum disorders: developmental disconnection syndromes. *Curr. Opin. Neurobiol.* **17**, 103–111 (2007).
- 14 Hammock, E. A. D. & Young, L. J. Oxytocin, vasopressin and pair bonding: implications for autism. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **361**, 2187 (2006).
- 15 Winslow, J. T., Noble, P. L., Lyons, C. K., Sterk, S. M. & Insel, T. R. Rearing effects on cerebrospinal fluid oxytocin concentration and social buffering in rhesus monkeys. *Neuropsychopharmacology (New York, NY)* **28**, 910–918 (2003).
- 16 Takayanagi, Y., Yoshida, M., Bielsky, I. F., Ross, H. E., Kawamata, M., Onaka, T. *et al.* Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc. Natl. Acad. Sci. USA* **102**, 16096–16101 (2005).
- 17 Modahl, C., Green, L., Fein, D., Morris, M., Waterhouse, L., Feinstein, C. *et al.* Plasma oxytocin levels in autistic children. *Biol. Psychiatry* **43**, 270–277 (1998).
- 18 Hollander, E., Novotny, S., Hanratty, M., Yaffe, R., DeCaria, C. M., Aronowitz, B. R. *et al.* Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. *Neuropsychopharmacology* **28**, 193–198 (2003).
- 19 Lauritsen, M. B., Als, T. D., Dahl, H. A., Flint, T. J., Wang, A. G., Vang, M. *et al.* A genome-wide search for alleles and haplotypes associated with autism and related pervasive developmental disorders on the Faroe Islands. *Mol. Psychiatry* **11**, 37–46 (2005).
- 20 Wu, S., Jia, M., Ruan, Y., Liu, J., Guo, Y., Shuang, M. *et al.* Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. *Biol. Psychiatry* **58**, 74–77 (2005).
- 21 Jacob, S., Brune, C. W., Carter, C. S., Leventhal, B. L., Lord, C. & Cook, Jr E. H. Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. *Neurosci. Lett.* **417**, 6–9 (2007).
- 22 Lerer, E., Levi, S., Salomon, S., Darvasi, A., Yirmiya, N. & Ebstein, R. P. Association between the oxytocin receptor (OXTR) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition. *Mol. Psychiatry* **13**, 980–988 (2007).
- 23 Yrigollen, C. M., Han, S. S., Kochetkova, A., Babitz, T., Chang, J. T., Volkmar, F. R. *et al.* Genes controlling affiliative behavior as candidate genes for autism. *Biol. Psychiatry* **63**, 911–916 (2008).
- 24 Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E. *et al.* The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J. Clin. Psychiatry* **59**, 22–33 (1998).
- 25 Technical Manual: Wizard® Genomic DNA Purification Kit. *Instructions for Use of Products A1120, A1123, A1125 and A1620* (Promega Corp., Madison, MA, 2005).
- 26 Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. Vol. 21 263–265 (Oxford Univ Press, 2005).
- 27 Wigginton, J. E., Cutler, D. J. & Abecasis, G. R. A note on exact tests of Hardy-Weinberg equilibrium. *Am. J. Hum. Genet.* **76**, 887–893 (2005).
- 28 Lunetta, K. L., Faraone, S. V., Biederman, J. & Laird, N. M. Family-based tests of association and linkage that use unaffected sibs, covariates, and interactions. *Am. J. Hum. Genet.* **66**, 605–614 (2000).
- 29 Van Steen, K. & Laird, N. Family-based association tests and the FBAT-toolkit User's Manual (2004).
- 30 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
- 31 Mizumoto, Y., Kimura, T. & Iwata, R. A genomic element within the third intron of the human oxytocin receptor gene may be involved in transcriptional suppression. *Mol. Cell Endocrinol.* **135**, 129–138 (1997).
- 32 Kimura, T., Tanizawa, O., Mori, K., Brownstein, M. J. & Okayama, H. Structure and expression of a human oxytocin receptor. *Nature* **356**, 526–529 (1992).