Supporting Information

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SI Materials and Methods

Participants. Children with a diagnostic history of autism spectrum disorder (ASD) underwent a comprehensive diagnostic evaluation to determine the accuracy of the previous diagnosis based on *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR) criteria (1), which was confirmed with research diagnostic methods. These diagnostic methods included the Autism Diagnostic Interview-Revised (ADI-R) (2, 3) and the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (4, 5). The ADI-R and the ADOS-G were administered by assessors trained by a research-reliable clinician, and all assessors attained standard interrater reliability within the project. Although all participants met DSM-IV-TR criteria for ASD, expert clinical opinion and scores on the ADI-R and ADOS-G were also used to characterize children with ASD as having autism or pervasive developmental disorder-not otherwise specified (PDD-NOS). Children with ASD who met the DSM-IV-TR criteria diagnosis of autistic disorder and scored above the cutoff for autism on ADI-R and ADOS-G were categorized as having autism. Children with ASD who met the DSM-IV-TR criteria diagnosis of autistic disorder and scored above the cutoff for autism on ADI-R but in the autism spectrum range on the ADOS-G were categorized as having PDD-NOS.

Exclusion criteria included (*i*) a genetic, metabolic, or infectious etiology for ASD on the basis of medical history, neurologic history, and available laboratory testing for inborn errors of metabolism and chromosomal analysis; and (*ii*) a DSM-IV-TR diagnosis of any severe mental disorder such as schizophrenia and bipolar disorder. Participants taking psychotropic medications were included as long as their medications were stable for at least 2 wk before the blood draw. Siblings of children with ASD were required to have no evidence of ASD on the basis of behavioral scales [i.e., the Social Responsiveness Scale (SRS)], clinical evaluation, and, if needed, research diagnostic assessments. They were also required to have no present or past history of any severe neuropsychiatric disorder such as schizophrenia or bipolar disorder on the basis of a clinical psychiatric evaluation and information obtained from behavioral scales. Neurotypical control children were required to (*i*) be free of neurological disorders in the present or past on the basis of history; (*ii*) be free of psychiatric disorders in the present or past on the basis of information obtained from behavioral scales, a clinical psychiatric evaluation, and if needed, the Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Aged Children (6); (*iii*) have no historical evidence of significant difficulty during the mother's pregnancy, labor, or delivery or in the immediate neonatal period or abnormal developmental milestones based on neurological history; and (*iv*) have no sibling diagnosed with ASD.

Blood Sampling and Plasma Oxytocin Quantification. Blood samples were collected between 10:00 AM and 2:00 PM from participants to control for potential circadian rhythmicity in plasma oxytocin (OXT) concentrations (7). The average collection latency was 114 ± 5.3 s (mean ± SEM) and did not differ by group ($F_{3,127}$ = 1.2242; P = 0.3037). Fifteen milliliters of whole blood were drawn from each child's antecubital region by a trained phlebotomist using standard protocols at the Lucile Packard Children's Hospital (Stanford University, Palo Alto, CA) outpatient laboratory facility. Blood was collected into chilled EDTA-treated vacutainer tubes and placed on wet ice. Whole blood was promptly centrifuged (1,300 × g at 4 °C for 10 min) and the plasma fraction transferred and aliquotted into polypropylene tubes and flash-frozen on dry ice and stored in a −80 °C freezer.

OXT concentrations were quantified using a commercially available enzyme immunoassay (Enzo Life Sciences, Inc.). This kit is highly specific and exclusively recognizes OXT and not related peptides. Per Enzo Life Sciences literature, the cross-reactivity with vasopressin is 0.6% and the limit of assay sensitivity where the curve is no longer linear is 10 pg/mL. Based on the recommendation of the technological division of Enzo Life Sciences and published evidence (8, 9), plasma samples were extracted to remove any matrix (macromolecules in plasma) interference effects.

Sample extraction procedures were initiated by thawing plasma samples in an ice bath. Waters Sep-Pak C18 columns (Waters Corp.) were conditioned with 1 mL HPLC-grade methanol followed by 1 mL molecular biology-grade water. Each 1-mL plasma sample was drawn through the column by vacuum on a Supelco SPE vacuum manifold (Sigma-Aldrich Group). The columns were washed with 1 mL wash buffer (89:10:1, water:acetonitrile:TFA) followed by 1 mL elution buffer (80:20, acetonitrile:water). Elutes were evaporated at room temperature using compressed nitrogen and reconstituted in 225 μL assay buffer before quantification to provide a sufficient sample volume to run samples in duplicate wells (100 μL per well). The program used to calculate the final picograms-per-milliliter concentration of OXT allowed for extrapolation based on the starting sample volume. This approach has been shown to be a sound method for increasing the concentration of OXT in each well (8, 10, 11) and ensures that each sample falls within the linear portion of the standard curve when initially read.

Samples were assayed in duplicate with a tunable microplate reader (Molecular Devices) for a 96-well format according to the manufacturer's instructions. All assays were performed by a technician who had no knowledge of phenotypic, diagnostic, or genetic data. Intra- and interassay coefficients of variation were 7.4% and 9.1%, respectively.

***OXT* Receptor Genotyping.** The OXT receptor gene *OXTR* SNP rs2254298 was amplified using the primers 3′-GCCCACCA-TGCTCTCCACATC and 5′-GCTGGACTCAGGAGGAAT-AGGGAC. The PCR reactions were carried out in a final volume of 15 mL consisting of 50 ng genomic DNA, 50 ng each of sense and antisense primers, 7.5 mL Taq PCR Master mix (Qiagen; catalog no. 201445), and 10% (vol/vol) DMSO. The PCR conditions included an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 45 s, and extension at 72 °C for 1 min, with a final extension of 10 min at 72 °C. The PCR product of 340 bp was digested with BamHI (New England Biolabs; catalog no. R0136L) at 37 °C for 3 h. The G allele was 340 bp and the A allele yielded 110 and 230 bp. For rs53576 genotyping primers 5′-TGA AAG CAG AGG TTG TGT GGA CAG G-3′ and 5′-AAC GCC CAC CCC AGT TTC TTC-3′ and the same PCR conditions were used; 7.5 μL 307 bp PCR products were digested at 65 °C for 3 h with 5 U of the restriction enzyme BsrI (New England Biolabs; catalog no. R0527L). The products were electrophoresed through 10% polyacrylamide gel (19:1, acrylamide: bis-acrylamide) at 150 V for 40 min. A 10-bp marker was used to measure fragment size. The A allele yielded 164, 136, and 8 bp. The G allele yielded 164, 101, 34, and 8 bp. All genotypes were scored by technicians who had no knowledge of phenotypic or diagnostic data.

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Table S1. Participant characteristics

		Sex		Ethnicity					
Participants	*N*	Female	Male	Caucasian	Asian	Other	Age, y*	Full-scale IQ*	Blood collection time, min; ns
ASD									
Autism	47	9	38	25	13	9	$7.60 \pm 0.37^{a,b}$	71.58 ± 3.51^{a}	12:41 PM ± 11.29
PDD-NOS	32	8	24	22	4	6	9.10 ± 0.41^{a}	96.63 ± 4.18^{b}	12:09 PM ± 13.59
Sibling	52	23	29	25	18	9	$7.79 \pm 0.41^{a,b}$	108.08 ± 1.84^{c}	12:34 PM ± 9.32
Control	62	22	40	45	6	11	7.11 ± 0.37^{b}	115.34 ± 1.21^{c}	12:29 PM ± 6.93

χ^2 was used to test whether the distribution of individuals to different groups differed by sex and by ethnicity. Overall, weak significant effects ($0.05 > P > 0.01$) where found for each. However, post hoc tests failed to find any group that showed a significant difference from expected. ns, not significant. For age, full-scale IQ, and blood collection time, differences between groups were tested with a simple general linear model. The values are expressed in mean ± SEM. $^*P < 0.05$. Values with the same superscript within the same column of the table do not differ according to Tukey's post hoc test.

Table S2. Participant allele frequencies of *OXTR* genotypes rs2254298 and rs53576 by group

		rs2254298			rs53576		
Participants	*N*	GG	AG	AA	GG	AG	AA
ASD							
Autism	47	*n* = 33 (70%)	*n* = 12 (26%)	*n* = 2 (4%)	*n* = 13 (28%)	*n* = 28 (59%)	*n* = 6 (13%)
PDD-NOS	32	*n* = 21 (66%)	*n* = 9 (28%)	*n* = 2 (6%)	*n* = 11 (34%)	*n* = 14 (44%)	*n* = 7 (22%)
Sibling	52	*n* = 33 (63%)	*n* = 15 (29%)	*n* = 4 (8%)	*n* = 22 (42%)	*n* = 19 (37%)	*n* = 11 (21%)
Control	62	*n* = 40 (64%)	*n* = 21 (34%)	*n* = 1 (2%)	*n* = 24 (39%)	*n* = 27 (43%)	*n* = 11 (18%)

Allelic frequencies for both *OXTR* SNPs were in Hardy–Weinberg equilibrium (*Materials and Methods,* OXTR *Genotyping*).

Table S3. Participant allele frequencies of *OXTR* genotypes rs2254298 and rs53576 by ethnicity

		rs2254298			rs53576		
Participants	*N*	GG	AG	AA	GG	AG	AA
Caucasian	117	*n* = 79 (67%)	*n* = 36 (31%)	*n* = 2 (2%)	*n* = 47 (40%)	*n* = 54 (46%)	*n* = 16 (14%)
Asian	41	*n* = 26 (63%)	*n* = 9 (22%)	*n* = 6 (15%)	*n* = 14 (34%)	*n* = 11 (27%)	*n* = 16 (39%)
Other	35	*n* = 22 (63%)	*n* = 12 (34%)	*n* = 1 (3%)	*n* = 9 (26%)	*n* = 23 (66%)	*n* = 3 (8%)

Fisher's exact test was used to test whether allele frequencies for each *OXTR* genotype differed by ethnicity as reported in other studies. Significant effects were found for both rs2254298 ($P = 0.0318$) and rs53576 ($P = 0.0008$). However, ethnicity did not significantly influence any experimental outcomes, consistent with multiple other *OXTR* genotype studies using mixed ethnic samples.