Sensitive Parenting Is Associated with Plasma Oxytocin and Polymorphisms in the *OXTR* and *CD38* Genes

Ruth Feldman, Orna Zagoory-Sharon, Omri Weisman, Inna Schneiderman, Ilanit Gordon, Rina Maoz, Idan Shalev, and Richard P. Ebstein

Background: Research in mammals has demonstrated the involvement of oxytocin (OT) in social bond formation; yet, its role in human bonding remains unclear. Plasma OT has been used as a proxy for central activity and studies indicate its association with human affiliative behaviors. Molecular genetic studies also reveal a role for OT neuropathways in shaping the social brain. However, the links between peripheral OT, genetic markers, and their combined contribution to human parenting are unknown.

Methods: Participants included 352 individuals: 272 mothers and fathers and their 4- to 6-month-old-infants and 80 nonparents. Plasma OT was assayed from adults who were genotyped for oxytocin receptor (*OXTR*) and *CD38* risk alleles associated with social dysfunctions. *CD38* is an ectoenzyme that mediates the release of brain OT. Parent-infant interactions were microcoded for parental touch and gaze synchrony and participants reported on parental care in childhood.

Results: *OXTR* (rs2254298 and rs1042778) and *CD38* (rs3796863) risk alleles were each associated with lower plasma OT. Reduced plasma OT and both *OXTR* and *CD38* risk alleles were related to less parental touch. The interaction of high plasma OT and low-risk *CD38* alleles predicted longer durations of parent-infant gaze synchrony. Parents reporting greater parental care showed higher plasma OT, low-risk *CD38* alleles, and more touch toward their infants.

Conclusions: Results indicate that peripheral and genetic markers of the extended OT pathway are interrelated and underpin core behaviors associated with human parenting and social engagement. These findings may have important implications for understanding neuropsychiatric disorders marked by early social dysfunctions.

Key Words: *CD38*, human social affiliation, oxytocin, oxytocin receptor (*OXTR*), parental touch, parent-infant bonding, parent-infant synchrony

Oxytocin (OT)—a nine amino-acid neuropeptide synthesized in the hypothalamus—has long been associated with parturition and nursing, whereas recent research has underscored its central role in affiliative behaviors including the development of parenting (1–5). Studies across species point to the involvement of OT in the initiation and expression of maternal behavior, the species-typical postpartum repertoire through which mammalian mothers bond with their young. Oxytocin-mediated maternal behaviors include the licking and grooming and arched-back nursing of rat dams (6), the sheep's olfactory-based recognition of her ewe (7), and the grooming and contact of Rhesus macaques (8). These maternal behaviors appear to partially correlate with both peripheral measures of OT in plasma or urine (8) and the expression of oxytocin receptors (*OXTR*) in the brain (1,9). Oxytocin has also been implicated in the transmission of parenting style across generations (10). Female rats exposed to greater maternal care in infancy exhibited more widespread expression of *OXTR* in their brains and expressed more maternal behavior toward their infants. Overall, these studies reveal the crucial role of OT neuropathways for the formation of the unique maternal-infant bond characteristic of mammals. Evidence further suggests that parenting behavior is transmitted across generations underpinned by regional-specific patterns of receptor localization in the brain (11). These neuropathways are sensitive to social influences and are shaped in early infancy through the provision of parenting behavior and sculpting of the epigenome in rodents (12,13) and possibly in humans (14).

Although OT neuropathways and their receptors are implicated in parent-infant bonding across species, human research has generally been constrained by the inability to directly assess brain oxytocinergic pathways at the neurochemical level. Thus, human studies have largely been limited to peripheral measures that are considered proxies for brain activity. One widely used measure of central oxytocinergic function is plasma OT, despite the poorly understood relationship between peripheral measures and brain neuropeptide bioactivity. Recent studies have demonstrated associations between peripheral OT and aspects of human parental care, particularly parental touch (3,4,15–20). Consistent with findings in other mammals (15), patterns of human parental touch are associated with peripheral OT changes. For example, mothers who provided high levels of affectionate contact showed OT increase following mother-infant interaction, but such an increase was not observed among mothers displaying minimal contact (17). Interestingly, the oxytocinergic system that supports bond formation in mammals functions as a biobehavioral feedback loop; maternal-infant touch and contact increase the expression of OT (9), while the administration of OT leads to the induction of maternal behavior (21). Notably, associations between peripheral OT and parenting were also found for fathers (3,17), suggesting that OT neuropathways may be activated through the provision of paternal care.

Neurogenetics, in addition to peripheral measures, is a powerful complementary strategy toward understanding the role of OT in human parenting. Different parenting styles can be stratified by polymorphisms in genes encoding elements of neuropeptide brain pathways. Such associations between specific oxytocinergic polymorphisms and behavioral phenotypes may provide provisional

From the Department of Psychology and the Gonda Brain Sciences Center (RF, OZ-S, OW, ISc, IG), Bar-Ilan University, Ramat-Gan; Department of Human Genetics (RM, ISh), Hadassah-Hebrew University of Jerusalem, Jerusalem, Israel; and Department of Psychology (RPE), National University of Singapore, Singapore.

Address correspondence to Ruth Feldman, Ph.D., Bar-Ilan University, Department of Psychology and the Gonda Brain Sciences Center, Ramat-Gan 52900, Israel; E-mail: feldman@mail.biu.ac.il.

Received Jul 22, 2011; revised Dec 1, 2011; accepted Dec 20, 2011.

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.12.025

evidence for the involvement of OT in parent-infant bonding. Indeed, several recent studies pointed to the associations between parenting-related phenomena and *OXTR* (22–25). In addition, variations in *OXTR* have been associated with social (26–29) and emotional processes (30–33), as well as with susceptibility to disorders characterized by dysfunction of social relationships, especially autism spectrum disorders (ASD) (34–38). In particular, *OXTR* tagging single nucleotide polymorphisms (SNPs) rs2254298 (34–36,38) and rs1042778 (27,34) have been linked with phenotypes resonating with social cognition, such as empathy, and disorders of social functioning, including ASD and major depressive disorder (rs2254298 G allele) (28). An imaging study showed that subjects with the *OXTR* rs2254298 G versus A allele exhibited greater gray matter volume (39), and homozygosity for the G allele indicated smaller volumes of both left and right amygdala (39).

Recently, the critical role of a new element—*CD38*—in oxytocinergic neural transmission has been described (40). *CD38* (nicotinamide adenine dinucleotide+-glycohydrolase/CD38 [Enzyme Commission 3.2.2.5, Enzyme Commission 3.2.2.6]) is a multifunctional molecule that combines enzymatic and receptor properties and is implicated in various physiological processes, including proliferation, differentiation, migration, adhesion, and secretion (40). Researchers used *CD38* gene knockout mice (*CD38*−/−), and found that *CD38* (adenosine diphosphate-ribosyl cyclase) mediates ryanodine-sensitive intracellular calcium mobilization and plays a key role in OT release from soma and axon terminals of hypothalamic neurons (40). Lack of *CD38* resulted in marked deficits in social behavior and reduced plasma OT. In particular, maternal behavior was dependent on *CD38* and social amnesia in male animals was evident in its absence. Spurred by these findings, two groups (41,42) have independently found associations between *CD38* SNPs and ASD. Additionally, correlations were reported between social skills in autistic individuals and *CD38* messenger RNA expression in lymphoblastoid cells (42–44). Furthermore, the *CD38* rs3796863 C allele, which is overtransmitted in ASD (41), was associated with lower *CD38* expression compared with the A allele and was present in all except one of the significant haplotypes showing genetic association with ASD in family-based design (42). These findings underscore the role of *CD38* as an indispensable regulator of OT release and as a contributing factor in disorders characterized by social deficits.

The current study addressed the involvement of OT in human parenting by investigating the relations between multiple indices of this nonapeptide system, including plasma levels and selected *OXTR* and *CD38* SNPs, with two prototypical human parental behaviors: parental touch and gaze synchrony. These core social behaviors were found in longitudinal studies to predict emotion regulation and social adaptation from infancy to adolescence (20,45). Similarly, OT administration has been shown to increase the duration of social gaze in ASD (46), suggesting the involvement of OT in these core social behaviors. We recruited a large group of mothers, fathers, and nonparents. Plasma OT was assayed and participants were genotyped for *OXTR* rs2254298 and rs1042778 and *CD38* rs3796863 SNPs. Parents were observed interacting with their infant and participants reported on parental care in their childhood. We hypothesized that *CD38* and *OXTR* risk alleles that correlate with deficits in social communication (28,31,33–36,39,41,42) would be associated with low plasma OT and less parental touch and gaze synchrony. This hypothesis is consistent with the biologically plausible notion that plasma OT partially reflects central oxytocinergic activity and individual differences in social behaviors are partly due to polymorphic genes encoding elements of nonapeptide neurotransmission. By integrating neurogenetic, hormonal, and observational techniques, we expected to shed further light on the neurobiological mechanisms underlying the formation of affiliative bonds between human parents and their infants.

Methods and Materials

Participants

352 individuals participated: 272 parents (151 mothers, 121 fathers) of 4- to 6-month-old infants and their infants, and 80 nonparents (40 male subjects). Participants were healthy with at least a high-school education, between 21 and 37 years of age, of Israeli-Jewish ethnicity, and were considered middle class. Of the parent group, 160 were married couples, mothers and fathers of 80 infants, and 112 were mothers ($n = 71$) and fathers ($n = 41$) of 112 unrelated infants. Overall, 192 infants participated: 84 girls and 108 boys. Infants were healthy singletons with birth weight >2600 grams and were 5.78 months old (SD = 1.13).

Procedure and Measures

Participants arrived at the lab in the afternoon (4:00 PM–7:00 PM) to control for diurnal variability in OT. Participants were first debriefed about the study and signed informed consent, and then blood for OT and mouthwashes for DNA were collected. Following these activities, parents engaged in parent-infant interaction. Each parent entered a large playroom with two cameras placed on adjacent walls, one focused on the parent's face and the other on the infant's face and upper body, and the two pictures were combined into a single frame. Infants sat on an infant seat, parents sat next to him/her, and instructions were: "play with your infant as you typically do." For couples, parents were videotaped sequentially and order was counter-balanced between mothers and fathers. Following the activity, all participants completed self-report measures.

Blood was drawn from antecubital veins into 9 mL chilled vacutainer tubes containing lithium heparin that was supplemented with 400 KIU of Trasylol (Trasylol–Bayer, Leverkusen, Germany) per 1 mL blood, consistent with previous research (47–51). Blood samples were kept ice-chilled for a maximum time of up to 2 hours (most samples for less) before being centrifuged at 4°C at 1000*g* for 15 minutes. For nursing mothers, OT was sampled at least an hour before and an hour after breastfeeding. Supernatants were collected and stored at −80°C until assayed. DNA was extracted from 20 mL of mouthwash using the Master Pure Kit (Epicentre, Madison, Wisconsin).

Plasma OT

Determination of OT was performed by using a 96-plate commercial OT-ELISA Kit (Assay-Design, Ann Arbor, Michigan), consistent with previous research (47–51). The immunoassay for the determination of OT is considered sensitive and reliable (52). Measurements were performed in duplicate. Samples were diluted 1:5 in the assay buffer and treated according to the kit's instructions. At the final step, the optical density of the samples and standards were measured with a wavelength of 405 and of 590 for additional correction. Sample concentrations were calculated by MatLab-7 (MathWorks, Natick, Massachusetts) according to the relevant standard curve within the range of 15 pmol/L to 1200 pmol/L. For each plate, a separate standard curve was constructed. The assay's reported intra-assay and interassay coefficients of variability were 19.1% to 12% and 5.2% to 14.5%, respectively. The intra-assay and interassay coefficients we received were <12.4% and 14.5%. The kit recognizes exclusively OT and not other peptides, such as arginine vasopressin and somatostatin.

Coding of Parent-Infant Interaction

Interactions were microcoded on a computerized system set for .01-second frames consistent with previous research (3,53). Four categories of parent and child behaviors were microcoded, including gaze, affect, vocalization, and touch. Each was coded separately for parent and child and contained several mutually exclusive codes. Here, we used behaviors from the parent gaze, child gaze, and parent touch categories. Parent and child gaze included gaze to social partner, gaze to object, and gaze aversion (gaze away from partner but not focused on environment). Parent touch included affectionate, functional, stimulatory, and accidental touch. Two composites were computed. Gaze synchrony was indexed by conditional probability assessing episodes when parent gazed at infant given infant gazed at parent. The mean durations of gaze synchrony episodes were used. Parent touch was the sum frequencies of all forms of touch parents exhibited during play. Coding was conducted by trained coders blind to all other information. Interrater reliability was computed for 55 interactions and reliability kappas averaged .84 (range: .76–.93).

Parental Bonding Instrument

The Parental Bonding Instrument is a self-report measure for adults with good reliability and validity pertaining to the first 16 years of life and yielding two measures, care and overprotection (54).

Genotyping

DNA was extracted from 20 mL of mouthwash samples using the Master Pure Kit (Epicentre). Genotyping of the *OXTR* SNPs was performed using the SNaPshot Method (Applied BioSystems, Foster City, California) as previously described in our laboratory (34). Amplification of the *OXTR* was achieved using the following primers. Primers used in genotyping the *OXTR* SNPs are presented in Table 1.

The *CD38* SNP rs3796863 (A/G) was genotyped using high-resolution melt (HRM) analysis. Polymerase chain reactions were performed using 5 μL Thermo-Start Master Mix (Thermo-Scientific, Barrington, Illinois), 2 μL primers (2.5 μmol/L), 1 μL SYTO9 (dye; Molecular Probes, Eugene, Oregon), and 1 μL of water to total 9 μL volume and an additional 1 μL of genomic DNA. All polymerase chain reactions and HRM analyses were performed on a Rotor-Gene 3000 (Corbett Life-Science, Eight Mile Plains, QLD, Australia), using the following primers that produced a 162 base pair amplicon: F5′-GGTGCACAGACCACTTAGCA′3; R5′TCGGAAGAGAGGAAAGCAAA′3. Polymerase chain reaction conditions were as follows: activating enzyme step at 95.0°C for 15 minutes, 45 cycles of denaturation at 95.0°C for 5 seconds, reannealing at 60°C for 15 seconds, and extension at 72°C for 10 seconds. The reaction proceeded to a hold at 40°C for 2 minutes, a second hold at 82°C for 2 minutes, and then the melt procedure ramped from 82°C to 90°C raising by .1°C every 3 seconds where fluorescence was acquired. High-resolution melt distinguished between the three genotypes (AA, AG, GG) and the method was verified by comparison of a portion of HRM results with those obtained by genotyping the same samples using the SNaPShot procedure described above. All genotype frequencies of *OXTR* and *CD38* SNPs were in Hardy-Weinberg equilibrium.

Statistical Analysis

First, parameters of the plasma OT distribution were assessed. Analysis of variance was used to examine differences related to genetic variations in 1) plasma OT, and 2) parental care. To correct for multiple comparisons, alpha was set at $p = .025$. Multivariate analysis of variance was used to examine differences related to plasma OT and genetic variations in parenting behavior. Pearson correlations examined associations between study variables. Two hierarchical regressions were computed predicting variability in parental touch and gaze synchrony from study measures.

Results

OT Distribution and Links Between Genetic Variations and Peripheral OT

Mean plasma OT was 365.30 pmol/L (SD = 211.3) with a wide range (43.80 – 1422.07 pmol/L) and no difference between women (mean = 356.70 pmol/L; SD = 205.26) and men (mean = 375.49 pmol/L, SD = 218.50), [$F(1,351) = .69$, ns]. Parents had higher levels of OT (mean = 379.54 pmol/L; SD = 220.29) than nonparents (mean = 316.54 pmol/L; SD = 169.82), [$F(1,351) = 5.53$; $p = .019$].

Oxytocin distribution was clustered around the center with a long right tail (kurtosis: 28.35, SE = .18; skewness: 4.36, SE = .92) and was fit by kappa, Burr, and log-logistic distributions. Once outliers greater than three SD above the means (>1000 pmol/L) were excluded ($n = 7$; 5 parents, 2 nonparents), parameters improved substantially (kurtosis: 1.86, SE = .19; skewness: 1.28, SE = .94) and the distribution was log-normal. Consistent with previous research (14), OT values were log-transformed before statistical analyses. Plasma OT was unrelated to demographic variables, including age, education, height, weight, smoking, time of last meal, menstrual cycle phase, contraceptive intake, and among mothers to mode of delivery (vaginal vs. Caesarean section), breastfeeding, weeks since birth, or interval since breastfeeding.

The frequency of the *CD38* rs3796863, *OXTR* rs1042778, and *OXTR* rs2254298 SNPs were unrelated to gender or parental status and results are reported across gender.

Analysis of variance examined main and interaction effects of *CD38* rs3796863 CC, *OXTR* rs1042778 TT, and rs2254298 GG risk alleles on plasma OT. Results indicated that individuals homozygous for the *CD38* rs379863 CC risk allele had lower plasma OT compared with carriers of the A allele (AA or AC), [$F(1,344) = 6.82$, $p = .008$]. Participants homozygous for the rs2254298 GG risk allele had lower plasma OT compared with participants carrying the A allele (AA or AG), [$F(1,344) = 4.65$, $p = .01$]. Finally, the TT risk allele on the *OXTR* rs1042778 was associated with lower plasma OT and carriers of this allele had lower plasma OT than those carrying the G allele (GG or GT), [$F(1,344) = 6.35$, $p = .012$; Figure 1]. Table 2 presents concentrations of plasma OT for the high-risk and low-risk groups on *CD38* and *OXTR* SNPs.

An interaction between the *CD38* rs3796863 and the *OXTR* rs2254298 SNPs was found indicating that individuals carrying the low-risk A alleles for the *CD38* rs3796863 and the *OXTR* rs2254298 SNPs had higher plasma OT levels compared with those carrying

Table 1. Primers Used in Genotyping the *OXTR* SNPs

	First PCR Primers	Second PCR Primer Extensions
OXTR rs1042778	F:TGGGTTCAGGGTGGTAGAAG R:AGGCTGTGCTGGCATAAGTG	$(T)_{12}$TGAAGCCACCCCAAGGAG
OXTR rs2254298	F:CCCAGAGGTCTGTGGGTGTA R:GTCAGGGAGGAGCTGTTCTG	$(T)_6$AAGAAGCCCCGCAAACTG

PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

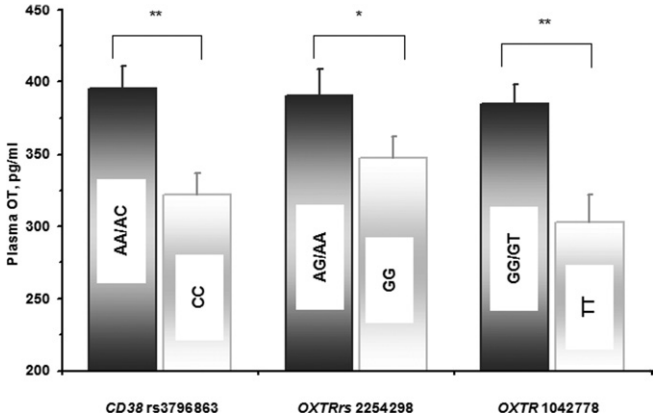

Figure 1. Effects of risk alleles in the *OXTR* and *CD38* genes on levels of plasma oxytocin. *CD38* rs3796863 = low risk (AA/AC), high risk (CC); *OXTR* rs2254298 = low risk (GG/GT), high risk (TT); *OXTR* rs1042778 = low risk (AA/AG), high risk (GG). *$p < .05$, **$p < .012$. OT, oxytocin.

either the *CD38* rs3796863 CC risk allele, the *OXTR* rs2254298 GG risk allele, or both, [$F(1,344) = 4.98$, $p = .025$].

Parenting Behavior, Plasma OT, and Genetic Variations

The frequency of parental touch correlated with plasma OT, $r = .30$, $p < .001$: parents with higher OT touched their infants more frequently (Figure 2). Similarly, parents with higher plasma OT engaged in longer episodes of gaze synchrony with their infants, $r = .143$, $p = .018$.

Multivariate analysis of variance was used to examine main and interaction effects of plasma OT (high/low groups using the median split), the *CD38* rs3796863 CC, *OXTR* rs1042778 TT, and rs2254298 GG risk alleles on parental touch and gaze synchrony. Results indicated an overall main effect for plasma OT, [$F(2,257) = 7.76$, $p = .001$, effect size [ES] = .06]; an overall main effect for *CD38* rs3796863, [$F(2,257) = 3.58$, $p = .023$, ES = .031]; an overall main effect for *OXTR* rs1042778, [$F(2,257) = 5.70$, $p = .004$, ES = .041]; and an overall interaction of plasma OT and *CD38* rs3796863, [$F(4,524) = 3.27$, $p = .039$, ES = .024]. Univariate tests are reported for each behavior.

Parental Touch. Parents with high plasma OT touched their infants more (mean = 35.05, SD = 13.18) than parents with low OT (mean = 27.68, SD = 11.53), [$F(1, 257) = 8.31$, $p = .003$]. Parents homozygous for the *CD38* rs379863 CC risk allele touched their infants less frequently (M = 28.40, SD = 13.40) than those carrying the A allele (mean = 33.12, SD = 12.27), [$F(1,257) = 6.46$, $p = .012$]. Additionally, parents homozygous for the TT risk allele on the *OXTR* rs1042778 provided less touch (mean = 26.71, SD = 12.89) than parents carrying the G allele (mean = 33.01; SD = 12.89), [$F(1,266) = 8.78$, $p = .003$; Figure 3].

Gaze Synchrony. No main effects were found for gaze synchrony but a significant interaction. Episodes of gaze synchrony were longest (mean = 9.44 sec; SE, 2.42) among parents carrying the low-risk A allele on the *CD38* rs3796863 combined with high levels of plasma OT compared with parents who either carried the *CD38* rs3796863 CC allele, had low plasma OT, or both (mean = 6.93 seconds, SE = 1.52), [$F(1,266) = 4.05$, $p = .037$; Figure 4].

Perception of Parental Care

Individuals who perceived their parents as providing more care were characterized by higher plasma OT, $r = .12$, $p = .036$, and the *CD38* rs3796863 low-risk A allele, [$F(1,351) = 5.49$, $p = .02$]. Additionally, parents reporting more parental care in their childhood provided more touch to their infants, $r = .20$, $p = .001$. No relations emerged for parental overprotection.

Regression Analysis

Two hierarchical multiple regressions were computed predicting parental touch and gaze synchrony. Variables were entered in five blocks: plasma OT, *CD38* rs3796863, *OXTR* rs1042778, *OXTR* rs2254298, and parental care. The model predicting parental touch explained 14% of the variance, [$F(5,259) = 8.45$, $p < .001$]. Independent predictors included plasma OT ($R^2 = .06$, F Change = 16.33, $p < .001$), *CD38* rs3796863 (R^2 Change = .036, F Change = 8.57, $p < .005$), *OXTR* rs1042778 (R^2 Change = .024, F Change = 6.89, $p < .013$), and parental care (R^2 Change = .013, F Change = 4.29, $p < .04$). The model predicting gaze synchrony explained 6% of the variance, [$F(5,259) = 2.74$, $p = .023$], and independent predictors were plasma OT (R^2 Change = .038, F Change = 7.24, $p < .01$) and *CD38* rs3796863 (R^2 Change = .021, F Change = 5.31, $p = .026$).

Discussion

Results of the current study demonstrate associations between peripheral levels of plasma OT and genetic variability in the OT pathway, charting links from hormonal release to its signal detection in this nonapeptide system. We found that peripheral levels of OT partially reflect OT neuropathways; that OT-mediated processes of maternal care are comparable with those observed in fathers; that prototypical parental behaviors, particularly touch and gaze synchrony (3), correlate with genetic and peripheral indices of OT; and that parents reporting more parental care in childhood have higher plasma OT and provide more touch to their infants. These findings provide evidence suggesting that processes of bond formation in humans are underpinned by OT. Results are consistent with those observed in other mammals and show that human attachment is mediated, in part, by the extended oxytocinergic system including a gene, *CD38*, essential for OT release, and the single receptor for this neuropeptide hormone, *OXTR*.

Several studies addressed the associations between peripheral OT and aspects of parent-infant bonding (55) and the current investigation adds to this literature by testing the combined contributions of peripheral and genetic markers of OT on parenting. We assessed the joint role of plasma OT and three selected SNPs in two genes, *CD38* (42) and *OXTR* (34), previously associated with deficits in social cognition, in two core phenotypes of parental bonding, parental touch (56) and gaze synchrony (57), which support children's social-emotional growth (25,51). The hypotheses that *OXTR* and *CD38* risk alleles would negatively correlate with plasma OT, parenting behavior, and memories of parental care were confirmed. Notably, we found that OT pathways also drive father-infant interactions. Although OT has been considered a maternal hor-

Table 2. Plasma OT Concentrations (in pmol/L) according to Genetic Risk

	Low Risk		High Risk		
	Mean	SE	Mean	SE	$F(1,351)$
CD38 rs3796863[a]	395.38	16.07	322.35	14.19	9.45[b]
OXTR rs1042778[c]	384.59	13.31	302.77	19.06	8.74[b]
OXTR rs2254298[d]	392.59	14.38	351.10	13.25	3.86[e]

OT, oxytocin; SE, standard error.
[a]Low risk (AA/AC), high risk (CC).
[b]$p < .01$.
[c]Low risk (GG/GT), high risk (TT).
[d]Low risk (AA/AG), high risk (GG).
[e]$p < .05$.

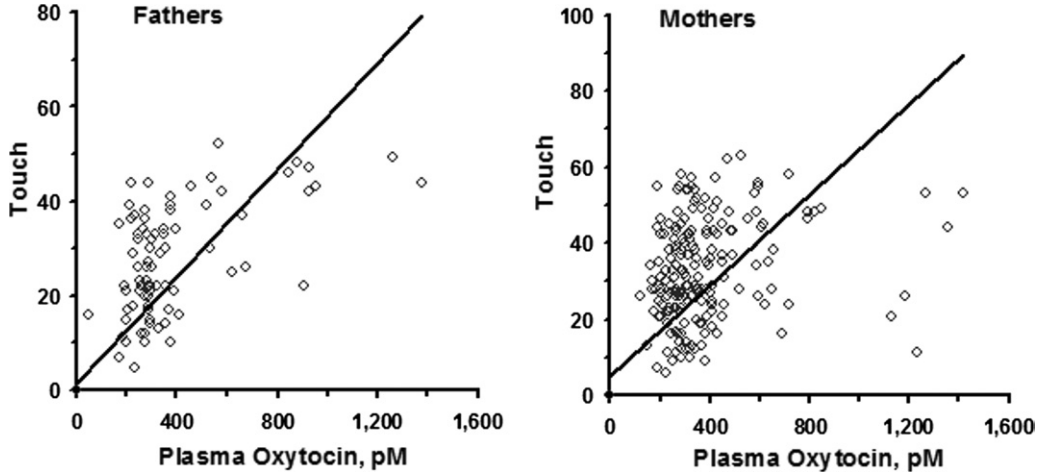

Figure 2. Correlations between plasma oxytocin and frequencies of maternal and paternal touch.

mone released at birth and lactation, no differences in plasma OT emerged between mothers and fathers, consistent with previous reports (49,51,58). Allele frequencies for the genotyped SNPs were similar in men and women and similar correlations emerged between plasma OT, *CD38* and *OXTR* SNP variants, and parenting behavior between mothers and fathers. The similar correlations found between genetic markers of OT and plasma OT levels in parents and nonparents and their links with memories of parental care may suggest that alloparenting in humans is also underpinned by oxytocinergic neural pathways, consistent with findings for voles (59), but this hypothesis requires additional research. Overall, these findings suggest that OT may support key aspects of attachment in men and women throughout life.

The findings that *OXTR* and *CD38* SNPs are associated with plasma OT strengthen the notion that plasma OT reflects oxytocinergic activity since these two receptors play an important role in the release and subsequent actions of this hormone. Of special interest are the results related to the *CD38* gene, which has recently been shown to play a critical role in regulating both the release of OT in rat brains and levels of plasma OT in rodents (40). An important question for future research concerns the relationship between plasma OT and central oxytocinergic activity and the extent to which this peripheral measure is indeed a proxy for OT effects on the social brain. Results of the two regression models indicate that plasma OT and *CD38* were each independently predictive of parenting behavior, suggesting that genetic and peripheral measures contribute independently to the development of parenting. Although further research is needed to test the unique and combined contribution of peripheral and genetic markers of the oxytocinergic system on multiple social and parenting phenotypes, the current findings suggest that peripheral measurements generate useful information regarding brain activity and affiliative behaviors.

The current findings may be important for understanding the mechanisms underlying disorders of social cognition, such as autism. *OXTR* and *CD38* risk alleles previously associated with ASD were found here to predict reduced plasma OT and less parenting behavior. Inability to maintain social gaze is a distinguishing feature of ASD and recent research shows that OT administration increased durations of social gaze in autistic individuals (60). The results that common polymorphisms in the OT neuropathway contribute to poorer social gaze already in the first stages of parent-infant bonding in a normative sample suggest that some aspects of autism pathology are on a continuum with normative social behavior (61). It is of interest that pathologies associated with disruptions to maternal-infant bonding, such as postpartum depression, also correlate with marked reduction in gaze synchrony and maternal touch

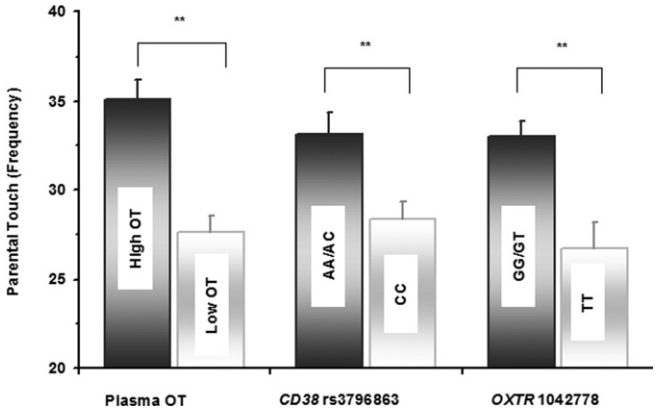

Figure 3. Effects of risk alleles in the *OXTR* and *CD38* genes on frequencies of parental touch. *CD38* rs3796863 = low risk (AA/AC), high risk (CC); *OXTR* rs2254298 = low risk (GG/GT), high risk (TT); *OXTR* rs1042778 = low risk (AA/AG), high risk (GG). $^{**}p < .01$. OT, oxytocin.

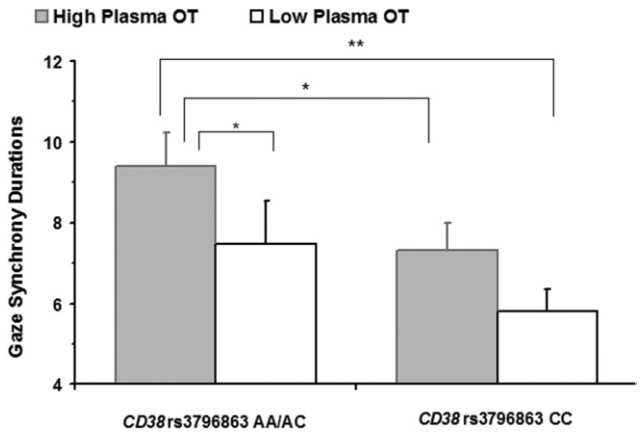

Figure 4. Interaction effects of plasma oxytocin and allelic variations on the *CD38* gene on parent-infant gaze synchrony. *CD38* rs3796863 = low risk (AA/AC), high risk (CC). $^{*}p < .05$, $^{**}p < .01$. OT, oxytocin.

(20), as well as with risk alleles on *OXTR* (34). The current findings linking these genetic markers with the first social behaviors experienced by human infants may guide genetic and other investigations of social phenotypes in nonclinical individuals. Such studies, integrating measurements of plasma OT informed by *OXTR* and *CD38* genotype, may be useful by generating potential biomarkers for the early diagnosis of neuropsychiatric disorders, especially autism.

Limitations of the study should be noted in the interpretation of the findings. First, the study is correlational and hence causal relationships are difficult to infer. Nevertheless, these results may provide a first step in that direction, as we leveraged the measurement of peripheral OT along with neurogenetic strategy toward understanding sensitive parenting. Similarly, low plasma OT is not always associated with risk and future research needs to address potential factors that moderate the links between plasma levels and social outcomes. We relied on retrospective accounts of parental care in adults with all the limitations known in such studies. Clearly, prospective longitudinal studies are required to understand the cross-generation transmission of OT and parenting in humans. To minimize multiple testing issues and focus on SNPs found to be associated with social phenotypes in the Israeli population (27,34,42), we did not include OXTR rs53576 in the current investigation. However, several studies indicate that the OXTR rs53576 SNP is associated with parenting (25), adult attachment (28), and socioemotional behaviors (29,31,62, 63), and future studies should include rs53576 among the OXTR SNPs to be genotyped.

The current study has clinical implications and may identify possible therapeutic targets. Among depressed mothers, who typically show minimal parenting behavior and low peripheral OT, interventions can aim to increase parenting behavioral repertoire, such as social gaze and touch. Importantly, the efficacy of intervention could be monitored by predicted increases in maternal plasma OT levels. Additionally, affected mothers can be stratified by *OXTR* and *CD38* polymorphisms implementing a personalized medicine approach based on genotype.

The neurobiology underlying sensitive parenting in humans remains poorly understood despite recent advances. Future research should aim at broadening our understanding of the role of this neuropeptide in human attachment processes to include other elements of the OT signaling pathway, such as leucyl/cystinyl aminopeptidase, also known as oxytocinase; the oxytocin preprotein, neurophysin; and gonadal steroid hormones, including the estrogen receptors that are targets for oxytocin in the brain. Finally, the richness of human parent-infant bonding suggests that other measures of this phenotype should be investigated toward a deeper understanding of the neurobiological basis for the human capacity to form attachment bonds throughout life.

Research at Dr. Feldman's laboratory during the study period was supported by the Israel Science Foundation (1318/08), the United States-Israel Bi-National Science Foundation (2005-273), the National Alliance for Research on Schizophrenia and Depression Foundation (independent investigator award, 2006, 2008), the Katz Family Foundation, and the Irving B. Harris Foundation. Research at Dr. Ebstein's laboratory was supported by the Israel Science Foundation founded by the Israel Academy of Sciences and Autism Speaks.

All authors report no biomedical financial interests or potential conflict of interest.

1. Ross HE, Young LJ (2009): Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front Neuroendocrinol* 30: 534–547.
2. Neumann ID (2008): Brain oxytocin: A key regulator of emotional and social behaviours in both females and males. *J Neuroendocrinol* 20:858–865.
3. Feldman R, Gordon I, Zagoory-Sharon O (2011): Maternal and paternal plasma, salivary, and urinary oxytocin and parent-infant synchrony: Considering stress and affiliation components of human bonding. *Dev Sci* 14:752–761.
4. Gordon I, Zagoory-Sharon O, Leckman JF, Feldman R (2010): Oxytocin and the development of parenting in humans. *Biol Psychiatry* 101:679–684.
5. Feldman R, Weller A, Zagoory-Sharon O, Levine A (2007): Evidence for a neuroendocrinological foundation of human affiliation: Plasma oxytocin levels across pregnancy and the postpartum period predict mother-infant bonding. *Psychol Sci* 18:965–970.
6. Zhang TY, Meaney MJ (2010): Epigenetics and the environmental regulation of the genome and its function. *Annu Rev Psychol* 61:439–466, C1–3.
7. Kendrick KM, Da Costa AP, Broad KD, Ohkura S, Guevara R, Levy F, Keverne EB (1997): Neural control of maternal behaviour and olfactory recognition of offspring. *Brain Res Bull* 44:383–395.
8. Maestripieri D, Hoffman CL, Anderson GM, Carter CS, Higley JD (2009): Mother-infant interactions in free-ranging rhesus macaques: Relationships between physiological and behavioral variables. *Physiol Behav* 96:613–619.
9. Francis DD, Young LJ, Meaney MJ, Insel TR (2002): Naturally occurring differences in maternal care are associated with the expression of oxytocin and vasopressin (V1a) receptors: Gender differences. *J Neuroendocrinol* 14:349–353.
10. Meaney MJ (2001): Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci* 24:1161–1192.
11. Champagne FA (2008): Epigenetic mechanisms and the transgenerational effects of maternal care. *Front Neuroendocrinol* 29:386–397.
12. Champagne FA, Curley JP (2009): Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neurosci Biobehav Rev* 33:593–600.
13. Caldji C, Hellstrom IC, Zhang TY, Diorio J, Meaney MJ (2011): Environmental regulation of the neural epigenome. *FEBS Lett* 585:2049–2058.
14. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonte B, Szyf M, *et al.* (2009): Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* 12:342–348.
15. Strathearn L, Fonagy P, Amico J, Montague PR (2009): Adult attachment predicts maternal brain and oxytocin response to infant cues. *Neuropsychopharmacology* 34:2655–2666.
16. Carter CS, Altemus M, Chrousos GP (2001): Neuroendocrine and emotional changes in the post-partum period. *Prog Brain Res* 133:241–249.
17. Feldman R, Gordon I, Schneiderman I, Weisman O, Zagoory-Sharon O (2010): Natural variations in maternal and paternal care are associated with systematic changes in oxytocin following parent-infant contact. *Psychoneuroendocrinology* 35:1133–1141.
18. Gordon I, Zagoory-Sharon O, Leckman JF, Feldman R (2010): Prolactin, oxytocin, and the development of paternal behavior across the first six months of fatherhood. *Horm Behav* 58:513–518.
19. Levine A, Zagoory-Sharon O, Feldman R, Weller A (2007): Oxytocin during pregnancy and early postpartum: Individual patterns and maternal-fetal attachment. *Peptides* 28:1162–1169.
20. Feldman R (2007): Parent-infant synchrony and the construction of shared timing, physiological precursors, developmental outcomes, and risk conditions. *J Child Psychol Psychiatry* 48:329–354.
21. Pedersen CA, Ascher JA, Monroe YL, Prange AJ Jr (1982): Oxytocin induces maternal behavior in virgin female rats. *Science* 216:648–650.
22. Skuse DH, Gallagher L (2011): Genetic influences on social cognition. *Pediatr Res* 69:85R–91R.
23. Riem MM, Bakermans-Kranenburg MJ, Pieper S, Tops M, Boksem MA, Vermeiren RR, *et al.* (2011): Oxytocin modulates amygdala, insula, and inferior frontal gyrus responses to infant crying: A randomized controlled trial. *Biol Psychiatry* 70:291–297.
24. Riem MM, Pieper S, Out D, Bakermans-Kranenburg MJ, van Ijzendoorn MH (2011): Oxytocin receptor gene and depressive symptoms associated with physiological reactivity to infant crying. *Soc Cogn Affect Neurosci* 6:294–300.

25. Bakermans-Kranenburg MJ, van Ijzendoorn MH (2008): Oxytocin receptor (OXTR) and serotonin transporter (5-HTT) genes associated with observed parenting. *Soc Cogn Affect Neurosci* 3:128–134.
26. Apicella CL, Cesarini D, Johannesson M, Dawes CT, Lichtenstein P, Wallace B, *et al.* (2010): No association between oxytocin receptor (OXTR) gene polymorphisms and experimentally elicited social preferences. *PLoS One* 5:e11153.
27. Israel S, Lerer E, Shalev I, Uzefovsky F, Riebold M, Laiba E, *et al.* (2009): The oxytocin receptor (OXTR) contributes to prosocial fund allocations in the dictator game and the social value orientations task. *PLoS One* 4:e5535.
28. Costa B, Pini S, Gabelloni P, Abelli M, Lari L, Cardini A, *et al.* (2009): Oxytocin receptor polymorphisms and adult attachment style in patients with depression. *Psychoneuroendocrinology* 34:1506–1514.
29. Kim HS, Sherman DK, Sasaki JY, Xu J, Chu TQ, Ryu C, *et al.* (2010): Culture, distress, and oxytocin receptor polymorphism (OXTR) interact to influence emotional support seeking. *Proc Natl Acad Sci U S A* 107:15717–15721.
30. Park J, Willmott M, Vetuz G, Toye C, Kirley A, Hawi Z, *et al.* (2010): Evidence that genetic variation in the oxytocin receptor (OXTR) gene influences social cognition in ADHD. *Prog Neuropsychopharmacol Biol Psychiatry* 34:697–702.
31. Lucht MJ, Barnow S, Sonnenfeld C, Rosenberger A, Grabe HJ, Schroeder W, *et al.* (2009): Associations between the oxytocin receptor gene (OXTR) and affect, loneliness and intelligence in normal subjects. *Prog Neuropsychopharmacol Biol Psychiatry* 33:860–866.
32. Rodrigues SM, Saslow LR, Garcia N, John OP, Keltner D (2009): Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proc Natl Acad Sci U S A* 106:21437–21441.
33. Thompson RJ, Parker KJ, Hallmayer JF, Waugh CE, Gotlib IH (2011): Oxytocin receptor gene polymorphism (rs2254298) interacts with familial risk for psychopathology to predict symptoms of depression and anxiety in adolescent girls. *Psychoneuroendocrinology* 36:144–147.
34. Lerer E, Levi S, Salomon S, Darvasi A, Yirmiya N, Ebstein RP (2008): Association between the oxytocin receptor (OXTR) gene and autism: Relationship to Vineland Adaptive Behavior Scales and cognition. *Mol Psychiatry* 13:980–988.
35. Jacob S, Brune CW, Carter CS, Leventhal BL, Lord C, Cook EH Jr (2007): Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. *Neurosci Lett* 417:6–9.
36. Wu S, Jia M, Ruan Y, Liu J, Guo Y, Shuang M, *et al.* (2005): Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. *Biol Psychiatry* 58:74–77.
37. Wermter AK, Kamp-Becker I, Hesse P, Schulte-Korne G, Strauch K, Remschmidt H (2010): Evidence for the involvement of genetic variation in the oxytocin receptor gene (OXTR) in the etiology of autistic disorders on high-functioning level. *Am J Med Genet B Neuropsychiatr Genet* 153B: 629–639.
38. Liu X, Kawamura Y, Shimada T, Otowa T, Koishi S, Sugiyama T, *et al.* (2010): Association of the oxytocin receptor (OXTR) gene polymorphisms with autism spectrum disorder (ASD) in the Japanese population. *J Hum Genet* 55:137–141.
39. Furman DJ, Chen MC, Gotlib IH (2011): Variant in oxytocin receptor gene is associated with amygdala volume. *Psychoneuroendocrinology* 36: 891–897.
40. Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O, *et al.* (2007): CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature* 446:41–45.
41. Munesue T, Yokoyama S, Nakamura K, Anitha A, Yamada K, Hayashi K, *et al.* (2010): Two genetic variants of CD38 in subjects with autism spectrum disorder and controls. *Neurosci Res* 67:181–191.
42. Lerer E, Levi S, Israel S, Yaari M, Nemanov L, Mankuta D, *et al.* (2010): Low CD38 expression in lymphoblastoid cells and haplotypes are both associated with autism in a family-based study. *Autism Res* 3:293–302.
43. Riebold M, Mankuta D, Lerer E, Israel S, Zhong S, Nemanov L, *et al.* (2011): All-trans retinoic acid upregulates reduced CD38 transcription in lymphoblastoid cell lines from autism spectrum disorder. *Mol Med* 17:799–806.
44. Ebstein RP, Mankuta D, Yirmiya N, Malavasi F (2011): Are retinoids potential therapeutic agents in disorders of social cognition including autism? *FEBS Lett* 585:1529–1536.
45. Feldman R (2011): Maternal touch and the developing infant. In: Hertenstein MJ, Weiss SJ, editors. *The Handbook of Touch: Neuroscience, Behavioral, and Health Perspectives*. New York: Springer, 373–408.
46. Andari E, Duhamel JR, Zalla T, Herbrecht E, Leboyer M, Sirigu A (2010): Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proc Natl Acad Sci U S A* 107:4389–4394.
47. Carter CS, Pournajafi-Nazarloo H, Kramer KM, Ziegler TE, White-Traut R, Bello D, Schwertz D (2007): Oxytocin: Behavioral associations and potential as a salivary biomarker. *Ann N Y Acad Sci* 1098:312–322.
48. Gordon I, Zagoory-Sharon O, Schneiderman I, Leckman JF, Weller A, Feldman R (2008): Oxytocin and cortisol in romantically unattached young adults: Associations with bonding and psychological distress. *Psychophysiology* 45:349–352.
49. Taylor SE, Saphire-Bernstein S, Seeman TE (2010): Are plasma oxytocin in women and plasma vasopressin in men biomarkers of distressed pair-bond relationships? *Psychol Sci* 21:3–7.
50. Rubin LH, Carter CS, Drogos L, Pournajafi-Nazarloo H, Sweeney JA, Maki PM (2010): Peripheral oxytocin is associated with reduced symptom severity in schizophrenia. *Schizophr Res* 124:13–21.
51. Keri S, Kiss I (2011): Oxytocin response in a trust game and habituation of arousal. *Physiol Behav* 102:221–224.
52. Kramer KM, Cushing BS, Carter CS, Wu J, Ottinger MA (2004): Sex and species differences in plasma oxytocin using an enzyme immunoassay. *Can J Zoology* 82:1194–1200.
53. Feldman R (2010): The relational basis of adolescent adjustment: Trajectories of mother–child interactive behaviors from infancy to adolescence shape adolescents' adaptation. *Attach Hum Dev* 12:173–192.
54. Parker G, Tupling H, Brown L (1979): A parental bonding instrument. *Br J Med Psychol* 52:1–10.
55. Galbally M, Lewis AJ, Ijzendoorn M, Permezel M (2011): The role of oxytocin in mother-infant relations: A systematic review of human studies. *Harv Rev Psychiatry* 19:1–14.
56. Dunbar RI (2008): The social role of touch in humans and primates: Behavioural function and neurobiological mechanisms. *Neurosci Biobehav Rev* 34:260–268.
57. Collis GM, Schaffer HR (1975): Synchronization of visual attention in mother-infant pairs. *J Child Psychol Psychiatry* 16:315–320.
58. Hoge EA, Pollack MH, Kaufman RE, Zak PJ, Simon NM (2008): Oxytocin levels in social anxiety disorder. *CNS Neurosci Ther* 14:165–170.
59. Bales KL, Kim AJ, Lewis-Reese AD, Sue Carter C (2004): Both oxytocin and vasopressin may influence alloparental behavior in male prairie voles. *Horm Behav* 45:354–361.
60. Hall SS, Lightbody AA, McCarthy BE, Parker KJ, Reiss AL (2011): Effects of intranasal oxytocin on social anxiety in males with fragile X syndrome [published online ahead of print August 20]. *Psychoneuroendocrinology*.
61. Constantino JN, Todd RD (2003): Autistic traits in the general population: A twin study. *Arch Gen Psychiatry* 60:524–530.
62. Saphire-Bernstein S, Way BM, Kim HS, Sherman DK, Taylor SE (2011): Oxytocin receptor gene (OXTR) is related to psychological resources. *Proc Natl Acad Sci U S A* 108:15118–15122.
63. Tost H, Kolachana B, Hakimi S, Lemaitre H, Verchinski BA, Mattay VS, *et al.* (2010): A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. *Proc Natl Acad Sci U S A* 107:13936–13941.