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Genetic and peripheral markers of the oxytocin system and parental care jointly support the cross-generational transmission of bonding across three generations

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ABSTRACT

Background: Human and animal research indicates that oxytocin (OT) plays a key role in the cross-generational transmission of parental bonding, and human studies suggest that allelic variations on the oxytocin receptor gene (*OXTR*) and circulating OT levels interact with patterns of parental care to shape children's social-affiliative competencies. Yet, no study to date has tested the joint contribution of OT and parental care across three generations.

Methods: The study included 345 participants comprising 115 family lines of grandmothers, mothers, and their infants. Salivary OT and allelic variations on the OXTR (rs53576 and rs2254298) and *CD38* (rs3796863) single nucleotide polymorphisms (SNPs), which have been previously associated with parental bonding, were assessed in all participants. Parental care was measured from grandmothers to mothers and from mothers to their infants.

Results: Mothers receiving parenting characterized by high overprotection from grandmothers showed more rejection toward their infants only when carrying the G allele on the OXTRrs53576 (AG/GG). These mothers of highly overprotective grandmothers also had lower oxytocin levels. Infants who were OXTRrs2254298 A carriers (AA/AG) and whose mothers reported more rejection toward their infants had higher oxytocin levels. Grandmothers receiving higher overprotection from great-grandmothers showed poorer parenting style compared to grandmothers experiencing lower parental overprotection only when carrying the OXTRrs2254298 GG genotype.

Conclusions: Our findings are the first to demonstrate how genetic and peripheral markers on the oxytocin system interact with experienced parenting to shape bonding across three generations. Results have important implications for specifying the biological and behavioral determinants associated with the continuity of adaptive versus maladaptive patterns of attachment across generations.

1. Introduction

Studies have repeatedly shown that oxytocin (OT), a nine-amino-acid neuropeptide synthesized in the hypothalamus, plays a critical role in human social affiliations and in the formation of attachment bonds, particularly the parent-infant bond (Feldman et al., 2011, 2007; Gordon et al., 2010). Children experiencing high-quality caregiving from their parents, as measured by direct observations of sensitive parenting that is characterized by enhanced positive affect, social gaze, affectionate touch, and warm vocalizations, showed higher levels of peripheral OT and better social competencies (Feldman et al., 2013; Weisman et al., 2015). In parallel, parents who reported receiving more optimal caregiving from their own parents (grandparents) had higher plasma OT, expressed more sensitive parenting, and provided more touch to their

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infants (Feldman et al., 2012), suggesting that the intergenerational transmission of parental care is at least partially mediated by the OT system (Feldman et al., 2010c). Moreover, genetic polymorphisms on oxytocin pathway genes, including the oxytocin receptor gene (*OXTR*) and *CD38*, which mediates OT release from the hypothalamus through the posterior pituitary into the bloodstream (Feldman et al., 2016), have been associated with both parenting quality (Feldman et al., 2012) and peripheral OT levels in plasma and saliva (Feldman et al., 2012; Weisman et al., 2013). These findings accord with research in animal models which indicates that parenting is both genetically and epigenetically is modulated by the OT system (Maud et al., 2018; Shahrokh et al., 2010). Still, to date, no study has tracked peripheral and genetic biomarkers of the OT system across three generations as related to patterns of parental care.

Genetic association studies describe relationships between allelic variations on multiple OT-pathway genes and parental behavior as well as interactions between genes and parenting behavior in shaping the cross-generational transmission of attachment (Feldman et al., 2016). For instance, mothers homozygous for the *OXTR* rs53576 G allele were found to show more sensitivity during interactions with their infants (Bakermans-Kranenburg and van Ijzendoorn, 2008). Similarly, a study of 323 mothers, fathers, and nonparents showed that alleles associated with greater propensity for psychiatric disorders and maladaptation (termed by some models as "risk alleles") on the *OXTR* (rs2254298, rs1042778) and *CD38* (rs3796863) genes were related to less parental touch and the interaction of low plasma OT and such alleles on the *CD38* gene predicted shorter episodes of parent-infant mutual gaze. Following parents and their first-born infants from birth to 3 years, it was found that parents' interactive synchrony at 1 and 6 months and mothers' *CD38* SNPs predicted children's social reciprocity during interactions with their best friend at 3 years, suggesting that the transmission of bonding from the parent-child attachment to the child's next attachment with close friends is underpinned by the joint contribution of genetic variations on the OT system and parenting behavior (Feldman et al., 2013). A longitudinal study assessing continuity from parent-child to romantic attachment from 12 months to 26 years showed that attachment continuity was moderated by the child's *OXTR* rs53576 polymorphisms; only among GG homozygous attachment security in infancy predicted romantic attachment in adulthood (Raby et al., 2013). Studies on the cross-generation transmission of peripheral OT have similarly shown that parental OT predicts the child's OT both concurrently and longitudinally as mediated by sensitive parenting (Feldman et al., 2013, 2010b). Overall, these studies indicate that the transmission between the various human affiliative bonds, from parents to close friends, from parents to romantic partners, or from parenting received in childhood to caregiving provided to one's own children, is at least partly supported by allelic variations on OT-pathway genes, peripheral levels of OT in plasma or saliva, and sensitive parental care and stronger parent-infant bond. These findings lend support to our central hypothesis; that the cross-generational transmission of bonding across three generations would similarly be underpinned by the interaction of genes and parental behavior (Feldman et al., 2016).

Two main conceptual models consider the interaction between allelic variations on key genes and early environmental experiences in shaping developmental or mental health outcomes: the diathesis-stress and differential susceptibility models, with more recent studies advocating the differential susceptibility viewpoint (Belsky and Pluess, 2009; Belsky and van, 2017; Luijk et al., 2011; van Ijzendoorn et al., 2012). Within this model, "risk" alleles are regarded as those conferring greater plasticity and greater openness to environmental inputs, not necessarily greater risk. Such "plasticity" genes turn into vulnerability factors only when the individual is exposed to environmental adversity, particularly when the adversity occurs early in life. To date, however, very little work within the differential susceptibility model addressed genetic variability on OT-pathway genes. In the current study, we consider "risk allele" within the frame of the differential susceptibility model wherein such alleles are thought to confer greater risk for bonding only when combined with less optimal patterns of caregiving. As such, our study is the first to test the "differential susceptibility" model across three generations and measure how such "plasticity" alleles interact with rejecting or overprotective parenting a girl experiences in early childhood shape the type of caregiving she will eventually provide to her own infant in the next generation, whether from grandmother to mother or from mother to infant.

Research in animal models provides support for our three-generation transmission hypothesis and show that maternal care affects neural development in the next generation, which shapes maternal care for the following generation (Champagne and Meaney, 2001). However, three-generation studies that investigate the intergenerational continuity of parental bonding from grandmothers to their female offspring (i.e. mother) and its effect on parental bonding from mother to infant as related to the OT system have not yet been conducted in humans. Such studies provide a unique opportunity to specify factors related to the parent-infant bond that may continue to impact families across generations. A three-generation study can also help tease apart factors related to OT-pathway genes, which tap biological factors that have some stability across generations, from factors that involve behavioral patterns of caregiving which are more reactive to situational variations or historical context and may be more amenable to intervention.

Cultural studies of the OT system have shown that the effects of allelic variations on the *OXTR* and CD38 genes on physiological functions and social competencies differ in Asian cultures (Bakermans-Kranenburg and van Ijzendoorn, 2008; Feldman et al., 2016; Mileva-Seitz et al., 2013). We thus used a three-generational sample from Japan to further validate the links between OT-related genes and peripheral OT levels with parenting patterns in an Asian culture and to tap into the broader issue of social transmission within a distinct cultural context. Among the unique features of parenting in Asian cultures, particularly in Japan, is parental overprotection. Parental overprotection is a common practice that is accepted as a social norm and is described as a key risk factor for the high rates of school refusal in Japan (Crystal DS, 1994). Overprotection can lead to poor development of autonomy of children and a recent study showed that the mother's OT level is mediated by maternal overprotection in a Japanese sample (Miura and Fujiwara, 2015). Since peripheral OT levels are associated with polymorphisms on the *OXTR* and *CD38* genes, it is possible that such links are related to the experience of parental overprotection that is transferred from grandmothers to mothers and impacts the mother's capacity to bond to her infant.

The goal of the current three-generation study was to test the interactive effects of genetic and peripheral biomarkers on the OT system and parental care on the cross-generational transmission of bonding. We hypothesized that maternal bonding to their infant (from mothers to infants) would be associated with (a) the experience of parenting from grandmothers to mothers, (b) peripheral levels of OT (c) allelic variations on OXTR (rs53576 and rs2254298) and *CD38* (rs3796863), and (d) the interaction between experienced parental care from grandmothers and polymorphisms on the OXTR (rs53576 and rs2254298) and *CD38* (rs3796863) genes. In addition, we expected that OT levels in mothers and infants would be associated with parenting, the parent's OT level (i.e. OT levels of grandmothers and mothers), genetic variability on OT-pathway genes including OXTR (rs53576 and rs2254298) and *CD38* (rs3796863) genes, and their interaction. Specific paths tested in the current study are presented in Fig. 1 and the implications of the significant pathways within the model are further elaborated in the Discussion.

2. Methods

2.1. Participants

The study included 345 participants comprising 115 family lines of

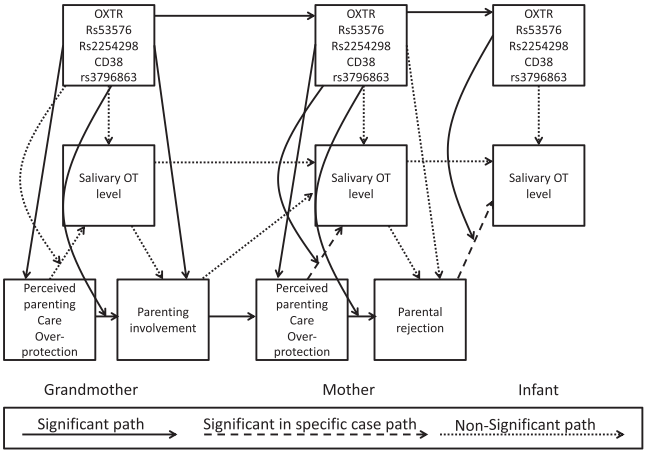

Fig. 1. An overall model of the finding linking grandmother, mother, and infant OXTR related gene, OT level, perceived parenting, and parenting.
*Arrows from genotype directed to other arrows means interaction of genotype and the association between the arrows.

mothers, their infants aged 3 to 10 months (infants), and the maternal grandmothers. We selected to focus on infants at this age as this period chart the time of non-verbal social communication in humans; from the beginning of face-to-face interaction and affect synchrony at 3 months to the time when infants begin to use language at 9–10 months (Feldman, 2007). This period is considered the sensitive period for the development of the biological and behavioral aspects of the maternal-infant attachment system (Feldman, 2015). Mothers' age averaged 33.39 (SD = 5.22, range = 21–44 years). Mothers were of middle-class background; 84.3% had some or full college degree and 67.2% earned more than 400 million yen (100 yen is equivalent to approximately 1 USD) annually, which is above average annual income in Japan, and 99.1% of mothers were married to the infant's father. Infants' mean age was 5.46 months (SD = 1.50), 50.4% (N = 58) were females, and 54.8% (N = 63) were firstborn. All infants were healthy since birth and 6.1% (N = 7) were low birthweight. Grandmothers' age was 62.01 (SD = 5.65, range = 41–73 years) and 54% had some or full college education. All participants were Japanese, 46.1% were from Tokyo (urban area) and 53.9% were from Okinawa prefecture (suburban area). Families were recruited during the three-month infant health checkup. A research coordinator (MO, KS) approached mothers and asked them to participate in a research lab where the study took place. The study was approved by the ethics committee of National Center for Child Health and Development (No. 651) and all participants signed an informed consent.

2.2. Procedure

All participants, including grandmothers, mothers, and infants, visited the research lab or a public center which was the closest to their home, between late morning and early afternoon hours (1000–1600 h). Participants were asked to refrain from eating 30 min before arrival. Questionnaires were mailed to mothers and grandmothers separately prior to the visit and the completed questionnaire were collected during the visit. After arrival, salivary OT was collected using Salivette®, and specimen for DNA genotyping was collected using Oragene®. OT collection was set to a time between feeding and was not conducted within the half-hour before or after breastfeeding. Among mothers, exclusive breastfeeding occurred in 51.3% of the cases, mixed feeding in 35.4%, and exclusive bottle-feeding mothers in 13.3% the mothers. Maternal oxytocin levels did not differ by breastfeeding status (p = 0.29).

2.3. OT collection and analysis

Saliva was collected using Sallivettes (Sarstedt, Rommelsdorft, Germany). Mothers and grandmothers were asked to chew a roll of cotton for 1–2 min and to put a similar roll in their infant's mouth for the researcher to collect saliva. Salivettes were kept ice-chilled for up to 1 day before centrifuged at 4 °C at 1500 *g* for 15 min. The liquid samples were stored at −80 °C. To concentrate the samples by 3 or 4 times, the liquid samples were lyophilized overnight and kept in −20 °C until assayed. The dry samples were reconstructed in the assay buffer immediately before analysis, consistent with previous research (Feldman et al., 2007). Determination of OT was performed by commercial OT enzyme-linked immunosorbent assay kit (Assay Design, Ann Arbor, Michigan) as described previously (Feldman et al., 2010a, 2007). Measurements were performed in duplicate and concentrations of samples were calculated by using MATLAB-7 (The MathWorks, Inc. Natick, MA) according to relevant standard curves. The intra-assay coefficients were 15.5%, and the inter-assay coefficients were 6.4%.

2.4. Genotyping collection and analysis

DNA was extracted from 2 ml of passive drawer saliva samples using Oragnere® saliva sampling kit (DNA Genotek Inc., Ottawa, Canada) following the manufacturer's instruction. Genotyping of variants (OXTRrs53576 and rs2254298 and CD38rs3796863) were done by the TaqMan™ allele-specific amplification method (Life Technologies, Carlsbad, CA) on an ABI PRISM 7900 H T Sequence Detection System (Life Technologies). PCR was performed on a 384-well format, and automatic allele calling was performed using ABI PRISM 7900 H T data collection and analysis software, version 2.4 (Life Technologies). All genotype frequencies of OXTR and CD38 SNPs were in Hardy-Weinberg equilibrium, except for grandmother's OXTR rs53576. Consistent with prior research, we considered the *OXTR* rs53576 AA variant, the *OXTR*rs2254298 GG variant, and the CD38 rs3796863 CC variant as risk genotype, as those have been associated with greater psychiatric risk and less adaptive parenting (Bakermans-Kranenburg and van Ijzendoorn, 2008; Feldman et al., 2015), and, according with the differential susceptibility model, as "plasticity" allele, although the term "risk alleles" is inconclusive as other studies identified other alleles as more risky (Bradley et al., 2011). Distributions of alleles in the current sample were shown in Table 1.

2.5. Parenting behaviors

The following self-report measures were used:

2.5.1. Experienced caregiving

The experience of being cared by one's own parent during the first years of life was assessed with the Parental Bonding Instrument (PBI), a self-report measure for adults on perceived experience of parenting, with good reliability and validity pertaining to the first 16 years of life (Parker, 1989). We used the validated Japanese version (Ogawa, 1991) in this study. The PBI yields two measures of parenting - care and overprotection. Grandmothers reported their experience of parenting from great grandmothers and mothers reported their experienced parenting from grandmothers.

We used two different instruments to assess caregiving patterns, one retrospective and one current. The Alabama Parenting Questionnaire (APQ) assesses adults' retrospective accounts of the caregiving received in their childhood and was used to measure grandmothers' parenting to mothers, whereas the Mother-Infant Bonding Scale (MIBS) evaluates current bonding and was used to assess the mother's current parenting to her infant.

2.5.2. Parenting styles

Grandmother's parenting style during the mother's childhood was

Table 1
Distribution of OT, perceived parenting, mother-infant bonding scales, and OXTR related gene polymorphism.

			N	Mean or %	SD	Min	Max
Mother	Salivary OT		106	33.07	19.22	11.10	110.86
	PBI-care		115	29.74	6.31	6	36
	PBI-overprotection		115	9.43	7.27	0	34
	Mother-infant bonding scale		115	1.21	1.61	0	9
	OXTR rs53576	AA	52	45.2			
		AG/GG	63	54.8			
	OXTR rs2254298	GG	52	45.6			
		AA/AG	62	54.4			
	CD38 rs3796863	CC	40	35.1			
		AA/AC	74	64.9			
Infant	Salivary OT		90	11.14	10.29	0.37	59.82
	OXTR rs53576	AA	35	31.8			
		AG/GG	75	68.2			
	OXTR rs2254298	GG	60	54.1			
		AA/AG	51	45.9			
	CD38 rs3796863	CC	47	42.3			
		AA/AC	64	57.7			
Grandmother	Salivary OT		99	51.96	41.77	8.36	307.97
	PBI-care		115	28.64	6.18	6	36
	PBI-overprotection		115	8.84	6.13	0	27
	APQ, involvement		115	4.11	0.54	2.11	5
	OXTR rs53576	AA	61	53.5			
		AG/GG	53	46.5			
	OXTR rs2254298	GG	58	51.3			
		AA/AG	55	48.7			
	CD38 rs3796863	CC	47	42.0			
		AA/AC	65	58.0			

assessed by the Alabama Parenting Questionnaire (APQ) (Frick, 1991) in Japanese. The APQ consists of 42 questions on a 5-point Lickert scale, yielding five subscales; involvement, positive parenting, poor monitoring, inconsistent care, and corporal punishment. Here we used the involvement scale that includes 10 items as a proxy for adequate caregiving (Cronbach's alpha = 0.79).

2.5.3. Parental rejection

Mothers reported on the experience of rejection for their infants using the Mother-Infant Bonding Scale (MIBS) (Yoshida et al., 2012), which was validated in Japanese setting. The MIBS assesses the parent's feelings of rejection, anger, and lack of affection for their infants (higher score denotes lower parental bonding, rejection, and poor emotional involvement (Figueiredo and Costa, 2009)). The MIBS is comprised of 10 items, with 4-Lickert scales, ranging 0–30, and shows moderate internal validity (Cronbach's alpha = 0.60).

2.6. Statistical analysis

We measured associations between genes, OT levels, and parenting within each individual and across generations using regression models. Next, several interaction effects were measured. We assessed interactions of *OXTR* or *CD38* polymorphisms and parenting styles among grandmother and mother for OT levels as outcome, and for parental rejection as an outcome among mother. Consistent with prior research on the interaction of oxytocin and parenting measures (Feldman et al., 2010b), we used the median split for parenting variables. However, for a fuller analysis, we also checked interaction effects using the parenting style variables as continuous variable and with including OT outliers. The number of outliers for the self-report measures is presented in Footnote[1] Outliers for the Further, interaction effect of *OXTR* or *CD38* polymorphism of mother and parenting style of grandmother for maternal OT level as an outcome was also measured. Finally, interaction of infant *OXTR or CD38* polymorphisms and maternal rejection on infants' OT level of infants as an outcome was also assessed. To determine whether a significant interaction effect can be indicative of the differential susceptibility model, we followed the recommendations of Roisman et al (2012) and applied two indices of plasticity that can indicate that the interaction is consistent with the differential susceptibility model; the regions of significance (RoS) test and the proportion of the interaction (PoI) index, using the revised criteria for differential susceptibility for PoI (0.2-0.8) which has been recommended by Del Giudice (2017). These measures have been recommended as indices that test whether an interaction effect can be taken to support the differential susceptibility hypothesis. Finally, in addition to dichotomous analysis, following the procedures demonstrated in previous studies (Bradley et al., 2011; Ludmer et al., 2015), *OXTR* or *CD38* polymorphism were treated as continuous variable (i.e. 0, 1, 2, higher score denoted greater risk) to assess interaction effects.

3. Results

3.1. Demographics, OT distribution, and OXTR and CD38 polymorphisms

Descriptive statistics for all study variables in grandmothers, mothers, and infants are presented in Table 1. Mothers' salivary OT distribution was skewed with a long right tail (Kurtosis: 7.73, Skewness: 2.00). Once outliers 3 standard deviation above the mean (> 70 pg/ml) were excluded, as is customary in hormonal studies and due to the fact that such values may not be valid (n = 5), parameters improved (Kurtosis: 3.21, Skewness: 0.88). Maternal OT was unrelated to demographic variables including age, education, height, weight, smoking, mode of delivery, breastfeeding, and time since birth (final sample for maternal OT analysis = 106). Infant salivary OT distribution was also skewed (Kurtosis: 11.07, Skewness: 2.53) and thus outliers (> 42 pg/ml) were excluded (n = 2; Kurtosis: 6.18, Skewness: 1.59). Infant OT was unrelated to infant age or sex (final sample for infants' OT analysis = 90). Grandmothers' salivary OT distribution was also skewed (Kurtosis: 17.93, Skewness: 3.29), thus, outliers (> 175 pg/ml) were excluded (n = 2; Kurtosis: 6.09, Skewness: 1.55). Grandmothers' OT

[1] Outliers were considered values 3SD above or below the mean: MIBS: 2 outliers, maternal PBI care, 1 outliers,Maternal PBI overprotection, 2 outliers; grandmothers' APQ involvement, 1 outlier, grandmothers' PBI care, 1 outlier, grandmothers' PBI overprotection, no outlier.

Table 2
Correlation between mother, infant, and grandmother's variables.

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
(1)	Mother	Salivary OT (log)	1.00																
(2)		PBI-care	0.14	1.00															
(3)		PBI-overprotection	−0.15	**−0.47**	1.00														
(4)		MIBS, parental rejection	0.04	**−0.21**	**0.27**	1.00													
(5)		OXTR rs53576	−0.10	−0.10	−0.04	−0.05	1.00												
(6)		OXTR rs2254298	0.07	**−0.20**	0.14	0.06	**−0.31**	1.00											
(7)		CD38 rs3796863	−0.04	0.10	−0.03	0.07	−0.04	−0.01	1.00										
(8)	Infant	Salivary OT (log)	0.03	0.04	0.05	0.13	−0.10	0.01	−0.04	1.00									
(9)		OXTR rs53576	0.06	−0.01	0.04	0.08	**0.42**	−0.19	−0.03	0.10	1.00								
(10)		OXTR rs2254298	−0.05	−0.09	0.10	−0.02	−0.01	**0.50**	−0.08	0.03	**−0.40**	1.00							
(11)		CD38 rs3796863	−0.07	0.005	0.01	0.07	0.08	−0.01	**0.46**	−0.02	0.09	−0.12	1.00						
(12)	Grandmother	Salivary OT (log)	0.13	−0.07	−0.04	0.10	−0.01	0.005	−0.08	−0.03	0.10	−0.02	0.05	1.00					
(13)		PBI-care	0.16	**0.27**	−0.04	−0.07	0.02	0.07	−0.12	−0.20	0.16	−0.02	−0.08	0.07	1.00				
(14)		PBI-overprotection	−0.17	**−0.32**	0.15	0.04	−0.17	0.07	**0.18**	0.003	**−0.21**	−0.01	0.14	−0.04	**−0.68**	1.00			
(15)		APQ, involvement	0.04	**0.44**	−0.17	−0.17	−0.14	−0.002	0.06	0.07	−0.07	0.05	0.17	−0.0002	**0.28**	**−0.29**	1.00		
(16)		OXTR rs53576	−0.08	**−0.22**	0.06	0.08	**0.52**	−0.06	−0.07	0.06	**0.27**	0.08	0.05	−0.03	**−0.20**	−0.01	**−0.19**	1.00	
(17)		OXTR rs2254298	0.03	−0.01	0.12	−0.005	**−0.21**	**0.43**	0.04	0.08	−0.09	**0.32**	0.07	0.10	0.01	0.04	0.05	**−0.24**	1.00
(18)		CD38 rs3796863	0.18	0.15	−0.08	−0.07	0.11	−0.05	**0.48**	0.08	0.03	−0.02	**0.29**	0.005	0.01	−0.03	0.16	0.01	0.03

Note: number of risk alleles for each OXTR related gene polymorphism were counted as follows: OXTR rs53576 (AA:2, AG:1, GG:0), OXTR rs2254298 (GG:2, AG:1, AA:0), CD38 rs3796863 (CC:2, AC:1, AA:0). Bold signifies $p < 0.05$.

level was unrelated to age, education, height, weight, and smoking (final sample for grandmothers' OT analysis = 99). All OT values were log-transformed prior to statistical analysis. The distributions of risk alleles were 35.1%–45.6% for mothers, 31.8%–54.1% for infants, and 42.0%–53.5% for grandmothers.

3.2. Relations among parenting and genetic polymorphisms within generation

Correlations among study variables, including salivary OT levels, experienced parenting (PBI scales), parental rejection (MIBS for mother), parenting style (APQ involvement for grandmother), and genetic risk alleles as continuous variables within each generation are presented in Table 2.

Mothers: Experienced parenting was inversely correlated with parental rejection (r_{care} = -0.21, p = 0.028; $r_{overprotection}$ = 0.27, p = 0.004). Mothers carrying the *OXTR* rs2254298 GG risk genotype reported receiving significantly lower care from grandmothers (mean [M] = 28.1, standard deviation [SD] = 7.38) compared to those carrying the low-risk genotype (M = 31.0, SD = 4.93), [F(1, 112) = 6.41, p = 0.013]. We also found that continuously-measured risk alleles on *OXTR* rs53576 and rs2254298 were significantly correlated (r = -0.31, p = 0.0007). Contrary to our expectation, maternal OT levels were unrelated to maternal *OXTR* or *CD38* polymorphism (all $p > 0.18$ by ANOVA).

Grandmothers: similar correlation was found between experienced parenting and parenting style (r_{care} = -0.28, p = 0.002; $r_{overprotection}$ = 0.29, p = 0.001). Grandmothers carrying the OXTR rs53576 AA risk genotype experienced lower parental care from their parents (i.e. great-grandmothers) (M = 27.5, SD = 5.93) compared to grandmothers carrying the G allele (M = 29.9, SD = 6.32) [F(1, 112) = 4.15, p = 0.044]. We found no associations between OT levels and allelic variability on the *OXTR* or *CD38* genes and continuously-measured risk alleles on *OXTR* rs53576 and rs2254298 were significantly correlated (r = -0.24, p = 0.011). Overall, grandmother's OT level was not associated with grandmother's *OXTR* or *CD38* genes polymorphism (all $p > 0.53$ by ANOVA).

Infants: We also found that continuously-measured risk alleles on *OXTR* rs53576 and rs2254298 were significantly correlated (r = -0.40, $p < 0.0001$). As with mothers and grandmothers, infants' OT level was unrelated to infant *OXTR* and *CD38* genes polymorphism (both $p > 0.24$ by ANOVA).

3.3. Intergenerational transmission of parenting and OT

Correlation among cross-generational variables, including salivary OT levels, PBI scales, MIBS for mother, APQ involvement for grandmother, and genetic risk alleles measured as continuous variables appear in Table 2.

As expected, grandmother's parenting style was significantly associated with mother's experienced care (r_{care} = 0.44, $p < 0.001$) and marginally with mother's experienced overprotection ($r_{overprotection}$ = -0.17, p = 0.063), suggesting that grandmother's parenting style is accurately reflected in the mother's experienced parenting. Moreover, mother's experienced care was significantly and negatively associated with grandmother's risk alleles on *OXTR* rs53576 (r = -0.22, p = 0.018) and this may have been related to poor involvement by grandmother, as correlation of risk alleles on *OXTR* rs53576 and grandmother's parenting style showed significant correlation (r = -0.19, p = 0.047).

On the other hand, peripheral OT levels were not directly transmitted from grandmother to mother (r = 0.13, p = 0.22), mother to infant (r = 0.03, p = 0.79), and grandmother to infant (r = -0.03, p = 0.80). Similarly, grandmother's parenting style was not associated with mother's OT levels (r = 0.04, p = 0.71), and mother's parental rejection was not associated with infant's OT levels (r = 0.13, p = 0.24).

With regards to *OXTR* and *CD38* polymorphisms, the *OXTR* rs53576, rs2254298 and *CD38* rs3796863 risk genotypes in grandmother were significantly associated with the same risk genotypes of mother (chi-square = 17.75, 10.58 and 11.66, p < 0.001, p = 0.001 and 0.001, respectively). Among mothers and infants, *OXTR* rs53576, rs2254298 and *CD38* rs3796863 risk genotypes in mother were significantly associated with the risk genotypes of her infant (chi-square = 10.18, 19.94 and 9.90, p = 0.001, p < 0.001 and 0.002, respectively). Further, we observed intergenerational transmission of risk genotypes from grandmother to infant for *OXTR* rs2254298 and *CD38* rs3796863 risk genotypes (chi-square = 11.24 and 4.34, p < 0.001, p = 0.002 and 0.037, respectively), but not for OXTR rs53576 (p = 0.12). When we apply risk alleles as continuous variables, intergenerational correlation coefficient for *OXTR* rs53576, rs2254298 and CD38 rs3796863 were all > 0.42, which were statistically significant (Table 2).

3.4. Interaction effects of OT and parenting

Mothers: Interaction between mothers' experienced parenting (using median-split) and OT-pathway genes variants were investigated in relation to parental rejection (Table 3). Mothers who experienced high parental overprotection showed significantly higher parental rejection only if they carried the low risk genotype on the *OXTRrs*53576 (AG or GG) (coefficient: -0.97, p = 0.028), as compared to mothers who carried the high risk genotype on the OXTRrs2254298 (GG, coefficient: -0.98, p = 0.056) and CD38 rs3796863 (CC, coefficient: -1.21, p = 0.029). We found no interaction between experienced parental care and maternal genes variants for parental rejection (all p for difference > 0.2). When we used mothers' experienced parenting as a continuous variable, the significant interaction effect of parental overprotection and *CD38* rs3796863 remained (p for interaction term = 0.028), but the other interaction terms were non-significant (all p > 0.1).

We next examined interaction effects of maternal experienced parenting and OT-pathway genes in relation to maternal OT level (Table 3, Figs. 2 and 3). We found that mothers experiencing high parental overprotection showed lower OT levels as compared to mothers experiencing low parental over-protection only when mothers carried the risk genotype (AA) on the *OXTR* rs53576 (Salivary OT levels: 23.77 vs 31.45 pg/ml, respectively, p = 0.033 see Fig. 2), while no association between experienced parental overprotection and OT levels was found among OXTR rs53576 G carriers (p = 0.73), and the interaction term was not statistically significant (p = 0.19). Similarly, mothers with high experienced over-protection showed lower OT level only when mother was an *OXTRrs*2254298 A-allele carrier (24.64 vs 33.61 pg/ml, respectively, p = 0.023, see Fig. 3), while no significant association

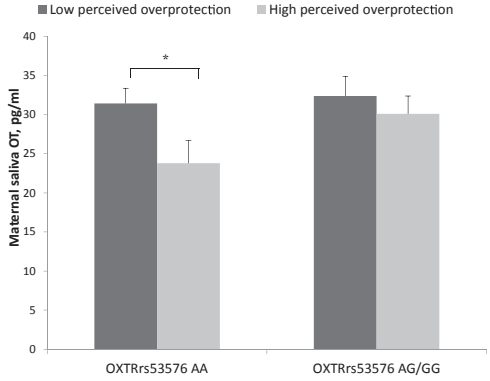

Fig. 2. Experienced parental overprotection and OT levels among mothers, stratified by maternal OXTRrs53576 gene polymorphism. *p < 0.05.

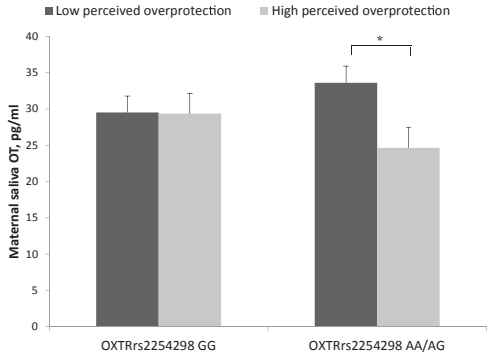

Fig. 3. Experienced parental overprotection and OT levels among mothers stratified by maternal OXTRrs2254298 gene polymorphism. *p < 0.05.

emerged if maternal *OXTRrs*2254298 was GG genotype (p = 0.91), and the interaction term was marginally significant (p = 0.083). This interaction was further tested for differential susceptibility using Roisman et al. (2012) indices. RoS for both upper (> 1.54) and lower (< -1.57) limit did not include dichotomized experienced over-protection (i.e., 0–1), and PoI index was 0.69, suggesting that this interaction fits the differential susceptibility model. As for experienced parental care, no association was found with maternal OT level, which did not differ by maternal OT-pathway genes (all p > 0.5). Even when we included OT outliers and experienced parenting as continuous variables, all the interaction terms were non-significant (all p > 0.1).

When we treated *OXTR* and *CD38* polymorphism as continuous variables to check interaction, *OXTR* rs53576 and experienced parental

Table 3
Interaction between PBI subscales and OXTR or CD38 polymorphisms on parental rejection and OT levels in mothers.

			PBI care (grandmother to mother)					PBI overprotection (grandmother to mother)				
			Low	High	β	p for difference	p for interaction	Low	High	β	p for difference	p for interaction
Parental Rejection (MIBS) (mothers to infants)	OXTR rs53576	AA	1.08	1.04	0.04	0.91	0.40	1.00	1.13	-0.13	0.76	0.16
		AG/GG	1.59	103	0.55	0.21		0.92	1.89	-0.97	**0.028**	
	OXTR	GG	1.52	1.00	0.52	0.33	0.59	0.80	1.78	-0.98	**0.056**	0.22
	rs2254298	AA/AG	1.21	1.03	0.18	0.61		1.03	1.25	-0.22	0.54	
	CD38	CC	1.61	1.00	0.61	0.27	0.56	0.79	2.00	-1.21	**0.029**	0.15
	rs3796863	AA/AC	1.27	1.03	0.24	0.52		1.03	1.31	-0.29	0.43	
Maternal OT levels (log-transformed)	OXTR rs53576	AA	3.22	3.27	-0.05	0.72	0.84	3.37	3.10	0.26	**0.033**	0.19
		AG/GG	3.35	3.36	-0.01	0.92		3.37	3.34	0.04	0.73	
	OXTR	GG	3.27	3.36	-0.08	0.52	0.53	3.30	3.31	-0.01	0.91	**0.083**
	rs2254298	AA/AG	3.33	3.30	0.03	0.80		3.42	3.13	0.29	**0.023**	
	CD38	CC	3.33	3.3	0.03	0.83	0.68	3.41	3.18	0.22	0.17	0.52
	rs3796863	AA/AC	3.29	3.33	-0.04	0.68		3.35	3.25	0.10	0.32	

Table 4
Interaction between PBI subscales and OXTR/CD38 polymorphisms on parental involvement and OT levels among grandmothers.

			PBI care (great grandmothers to grandmothers)					PBI overprotection (great grandmothers to grandmothers)				
			Low	High	β	p for difference	p for interaction	Low	High	β	p for difference	p for interaction
Parenting, involvement (APQ) (grandmothers to mothers)	OXTR rs53576	AA	3.85	4.28	-0.43	**0.004**	0.35	4.19	3.87	0.32	**0.031**	0.27
		AG/GG	4.07	4.33	-0.25	0.056		4.26	4.16	0.10	0.46	
	OXTR rs2254298	GG	3.85	4.36	-0.51	**< 0.001**	0.16	4.28	3.87	0.42	**0.005**	**0.09**
		AA/AG	4.00	4.24	0.24	0.098		4.14	4.08	0.06	0.66	
	CD38 rs3796863	CC	4.01	4.40	-0.39	**0.016**	0.90	4.27	4.11	0.16	0.34	0.50
		AA/AC	3.87	4.24	-0.37	**0.004**		4.19	3.89	0.30	**0.021**	
Grandmothers' OT level (log-transformed)	OXTR rs53576	AA	3.64	3.8	-0.16	0.34	0.86	3.71	3.68	0.03	0.86	0.49
		AG/GG	3.66	3.78	-0.12	0.48		3.81	3.62	0.19	0.25	
	OXTR rs2254298	GG	3.7	3.78	-0.08	0.59	0.63	3.75	3.73	0.02	0.91	0.47
		AA/AG	3.6	3.79	-0.19	0.30		3.76	3.59	0.18	0.31	
	CD38 rs3796863	CC	3.64	3.79	-0.15	0.41	0.99	3.77	3.63	0.13	0.48	0.81
		AA/AC	3.65	3.8	-0.15	0.31		3.76	3.68	0.07	0.61	

overprotection showed marginal interaction effect on maternal OT level ($p = 0.08$), whereas other interaction terms were non-significant ($p > 0.1$).

Grandmothers: significant interaction between experienced parenting for grandmother (i.e. from great grandmothers to grandmothers) and *OXTR* polymorphism on parenting style (i.e. involvement) of grandmother was found (Table 4, Fig. 4). Grandmothers who experienced higher parental overprotection showed significantly poorer parenting style compared to grandmothers who experienced lower parental overprotection only when grandmothers were homozygous for the risk allele on the OXTR rs2254298 (GG) (coefficient: 0.42, $p = 0.005$), whereas no association between experienced parental overprotection and parenting style was found when grandmothers had the low risk A allele on the OXTR rs2254298(AA/AG) (coefficient: 0.06, $p = 0.66$). A marginal interaction effect of OXTR rs2254298 on the association between experienced parental overprotection and parenting style (p for interaction = 0.09, see Fig. 4). This interaction was further tested for differential susceptibility (Roisman et al., 2012). RoS for both upper (> 1.43) and lower (< -2.46) limit did not include dichotomized experienced over-protection (i.e., 0–1), and PoI index was 0.37, suggesting that this interaction effect is consistent with the differential susceptibility model. OXTR rs53576 and CD38 rs3796863 did not show interaction effects for the association between experienced parental overprotection and parenting style (both p for interaction > 0.2). As for experienced parental care, high experiential parental care showed higher parenting style, regardless of OXTR/CD38 polymorphism (i.e. interaction terms were not significant). When we used grandmothers' experienced parenting as continuous variable, the interaction effect of parental overprotection and OXTR rs2254298 was marginally significant (p for interaction term = 0.06), but other interaction terms were non-significant (all $p > 0.1$). We found no interaction effect between grandmothers' experienced parenting and OXTR/CD38 polymorphism on grandmother's OT level for both using median split and continuous variable for experienced parenting (all $p > 0.1$).

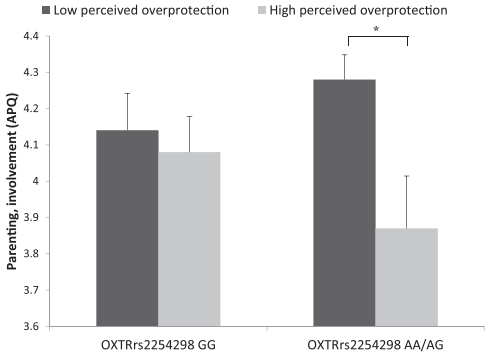

Fig. 4. Experienced parental overprotection and parenting style, involvement, among grandmothers stratified by maternal OXTRrs2254298 gene polymorphism. *p < 0.05.

When we treated OXTR and CD38 polymorphism as continuous variable to check interaction, CD38 rs3796863 and experienced parental care showed marginal interaction effect on grandmother's OT level ($p = 0.065$), and further stratification by CC, AC, and AA revealed that high experienced parental care showed higher grandmother's OT level if CD38 rs3796863 as AC genotype (data not shown). Other interaction terms were non-significant ($p > 0.1$).

Infants: With regard to infants, we found interaction between parental rejection and infant's OXTR gene polymorphism on infant OT level (Table 5 and Fig. 4).Infant OT level was marginally higher among infants with mothers reporting high parental rejection than low parental rejection, if infant OXTRrs2254298 was non-risk (AA/AG) genotype (Salivary OT levels: 13.18 vs 9.54 pg/ml, respectively, $p = 0.057$), and such difference was not found if infant OXTR rs2254298 was risk (GG) genotype (p for interaction = 0.09). Again, this interaction was further tested for differential plasticity (Roisman et al., 2012). RoS for both upper (> 3.48) and lower (< -0.451) limit did not include dichotomized experienced parental rejection (i.e., 0–1), and PoI index was 0.78, suggesting that this interaction effect might be explained by differential susceptibility. OXTR rs53576 and CD38 rs3796863 did not show interaction effect of parental rejection on infants' OT level (both p for interaction > 0.1). For sensitivity analysis, we included OT outliers and parental rejection as continuous variable, and all the interaction terms showed non-significant (all $p > 0.1$). When we treat OXTR and CD38 polymorphism as continuous variable to check interaction, no interaction effects were found (all $p > 0.2$).

To summarize, Fig. 1 presents significant paths, significant only in specific cases paths (i.e. interaction with OXT-related gene polymorphism), and non-significant paths linking grandmother, mother, and infant OT-related genes, peripheral OT levels, experienced parental overprotection, and parenting style. **Among grandmothers**; perception of experienced overprotection from great grandparents was associated with grandmother's *OXTR* rs53576 and parenting involvement with mother and this associations was modified by grandmother's *OXTR* rs2254298 and linked with grandmother's *OXTR* rs53576. **Among mothers**, maternal *OXTR* and *CD38* genotype was determined by grandmother's genotype. Maternal OXTR rs2254298 was associated with experienced parental care from grandmother. Furthermore, mother's experienced overprotection from grandmother was negatively associated with maternal OT level only if mothers carried the low-risk A allele on the *OXTR* rs2254298. Experienced overprotection was associated with maternal rejection of her infants, with effect modification by maternal OT-related genes. **Among infants**: infant's *OXTR* and *CD38*

Table 5
Interaction between parental rejection and OXTR/CD38 polymorphisms on OT levels among infants.

			Parental rejection (mothers to infants)				
			Low	High	β	p for difference	p for interaction
Infants' OT level (log-transformed)	OXTR rs53576	AA	2.02	2.54	−0.53	0.21	0.49
		AG/GG	1.90	2.11	−0.21	0.39	
	OXTR rs2254298	GG	2.07	2.04	0.04	0.88	**0.09**
		AA/AG	1.82	2.51	−0.68	**0.057**	
	CD38 rs3796863	CC	1.75	2.42	−0.66	**0.083**	0.13
		AA/AC	2.11	2.12	−0.01	0.96	

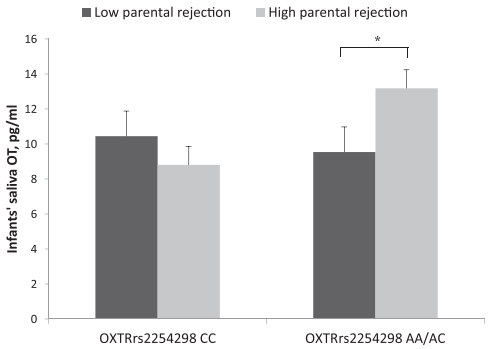

Fig. 5. Parental rejection and OT level among infants, stratified by infants' OXTRrs2254298 gene polymorphism. *p = 0.057.

genotype was determined by maternal genotype, which showed effect modification for the association between parental rejection and infant's OT level (Fig. 5).

4. Discussion

Results of the current study are the first to describe how genetic and peripheral markers on the OT system interact with parenting experiences across three generations of Japanese women; Grandmothers, mothers, and infants. We found that the intergenerational transmission of parenting, that is, the associations between the type of parenting a women received as a child and the type of parenting she provides to her child, depends, to some extent, on allelic variations on the *OXTR* and *CD38* genes. Moreover, polymorphisms on the *OXTR* or *CD38* genes interacted with the experience of parenting a woman received in each generation (grandmother from great-grandmother, mother from grandmother) and her OT levels to shape her parenting toward her child (grandmother to mother, mother to infant), as seen in Fig. 1. Specifically, we found that the experience of parental overprotection when a woman (grandmother) was growing up combined with variability on OT-pathway genes was associated with less optimal parenting. This, in turn, was transferred down across generations to shape more rejection and less optimal bonding from their daughters (mothers) to their own infants. These findings complement the cross-generational transfer of genotype on the *OXTR* and *CD38* genes from grandmothers to mothers, mothers to infants, and grandmothers to infants and while such genetic continuity is to be expected, it has not yet been shown in three generations of human mothers and children. On the backdrop of such genetic continuity we found that across generations, experienced caregiving and genes interact to shape peripheral oxytocin and parenting orientations in the next generation.

Consistent with our hypotheses, we found significant intergenerational correlations between grandmothers and mothers and between mothers and infants as well as between grandmothers and infants for genetic polymorphisms on the *OXTR* and *CD38* genes and parenting behaviors. However, contrary to our expectations, no cross-generational links were found for OT levels. This is inconsistent with previous studies, which found intergenerational continuity for OT levels between mothers and infants (Feldman et al., 2013, 2010c). Such discrepancy can be explained by cultural differences. In Japan, the larger social context is as important as the immediate family context. Thus, OT levels may be determined by other social networks to which the individual belongs, including work or social circle, and OT can also be shaped by the individual's social capital gained through social class, friendship networks, and community participation, as has been previously shown in Japan (Fujiwara et al., 2012). It is likely that multiple factors, in addition to OT-pathway genes, shape parenting behavior and their cross-generational transmission, some of which may relate to generational changes that are currently taking place in the Japanese society, changes that specifically tap the degree of overprotection and control mothers exert over their daughters.

Our findings are consistent with prior studies showing interaction effects of *OXTR* and parenting experiences on multiple outcomes. For instance, an interaction effect of *OXTR*rs53576 and child maltreatment was found to predict brain structure and function (Dannlowski et al., 2016); individuals carrying the GG genotype on the rs53576 who were maltreated as children showed decreased gray matter volume and increased amygdala activity. Consistent with this study, we also found that mothers who were G homozygous and also received high levels of parental overprotection from grandmother showed less bonding toward their infant. In contrast, our findings are inconsistent with a study showing that *OXTR* rs53576 A carriers who were maltreated showed greater social and emotional pain (Flasbeck et al., 2018). It is thus possible that the interaction between experienced parenting and *OXTR* rs53576 polymorphisms may differ by culture and further research across a variety of cultural contexts is required to tease apart factors that are universal versus those that are culture-specific in the effects of OT-pathway genes on attachment patterns.

We found no association between OT levels and polymorphisms on *OXTR* rs53576 and rs2254298 and *CD38* rs3796863 among mothers, grandmothers, or infants. This is inconsistent with previous studies reporting associations between OT levels and *OXTR* and *CD38* polymorphisms (Feldman et al., 2013, 2012). The reasons for these findings are not fully clear but may be due to environmental factors specific to Japan, which is a collection of islands where nutrition relies mainly on fish and such diet may increase polyunsaturated fatty acid that regulate OT-related genes or peripheral OT levels (Cosenza et al., 2017). Further research is needed to elucidate the null association between OT- related genes and OT level among Japanese.

Maternal peripheral OT was low when mother had the AA genotype on the *OXTR* rs53576, which has previously been linked with greater risk and lower social competencies, and received high parental overprotection, findings consistent with a previous study in Japan (Nishizato et al., 2017). One possibility is that maternal overprotection reduces oxytocin activity when mothers had the greater risk allele on the OXTRrs53576. However, *OXTR* rs2254298 showed a different interaction effect, that is, mothers with the non-risk genotype, AA/AG, who experienced greater overprotection showed lower OT levels, suggesting that high perceived overprotection can be a trigger of dampened activity of the OT system if OXTR rs2254298 is non-risk

genotype (AA/AG) and this interaction effect may point to differential susceptibility rather than diathesis-stress. In an Israeli sample it was found that the *OXTR* rs2254298 GG genotype was associated with lower OT levels (Feldman et al., 2012) and the difference may relate to the relatively low level of parental overprotection typical of the Israeli society; hence the *OXTR* rs2254298 AA/AG carriers showed higher OT levels. On the other hand, parental overprotection is highly common in Japan due to the inter-dependent nature of the Japanese society (Markus and Kitayama, 2010) and thus culture-specific patterns of parenting may shape the link between parental styles and oxytocin biomarkers. The current study highlights the need to test the links between genetic variability and peripheral markers on the OT system in light of distinct cultural norms and habits, unique patterns of mother-daughter relationship, and the specific meaning various parenting practices, such as overprotection, carry for the culture and the family. This may be particularly notable for the construct of parental overprotection, which in individualistic cultures is associated with intrusive parenting and less optimal social outcomes, but such associations do not always hold in more collectivistic societies, as has been shown, for instance, in comparing the long-term effects of intrusive parenting on the social competence of Israeli and Palestinian children in the peer group (Feldman and Masalha, 2010). On the background of generational changes in traditional societies, such as Japan, a style that has been highly common, particularly in the rearing of daughters, may become less optimal and may be perceived less favorably in retrospect and thus, our findings regarding the experience of parental overprotection in a traditional society undergoing rapid modernization may have larger social, psychological, and educational implications.

Infants showed a different response to rejection pending their genetic makeup. Infants who received high parental rejection and had the low risk *OXTR* rs2254298 genotype showed higher OT levels, which was in line with the differential susceptibility framework. That is, infants with the low risk *OXTR* rs2254298 are susceptible to parental rejection on the one hand, but also to greater emotional involvement on the other hand, which determined infant OT. These findings may be explained by Taylor's tend-and-befriend model (Taylor et al., 2000); infants may adapt to parental rejection by enhancing their OT levels and increasing their approach orientation to the mother when their *OXTR* rs2254298 genotype is of lower risk. In contrast, when the infant carries a more risky genotype of the *OXTR* rs2254298, such strategy may not work to elevate OT levels.

Several study limitations need to be addressed in the interpretation of the findings. First, our sample size (n = 115 trios) is underpowered to investigate gene by environment interaction effects and the recommended sample size for such association studies is approximately 600 (Duncan and Keller, 2011). Still, our study presents a unique three-generation cohort and the findings should be replicated across various cultures before they can be generalized. Second, we measured parenting behavior and maternal bonding through a questionnaire and not via observations that can provide more concrete and validated measures of parent-infant bonding. Third, parenting styles were measured by different instruments in the two generations. This was due to the fact that in the grandmother-to-mother generation the measure tapped a retrospective account whereas in the mother-to-infant generation it assessed current bonding and also to the fact that we were limited to instruments that have been validated in Japanese. Still, the different constructs at the two generations (involvement and rejection) is a study limitation and future research should attempt to assess constructs that are more similar across generations. Fourth, experience of motherhood may alter the individual's perception of experienced care and thus further research investigating the perception of experienced caregiving before and after becoming a mother is needed. Finally, *OXTR* rs53576 frequency among grandmothers did not meet Hardy-Weinberg equilibrium, suggesting that selection of grandmothers might be biased. Further research is needed to test the intergenerational continuity of parenting among grandmothers who were in Hardy-Weinberg equilibrium.

Despite these limitations, our study is the first to measure intergenerational transmission of *OXTR* and OT levels using a three generation sample and to show the association between mothers' perceived parenting from grandmothers and bonding toward infants as mediated by *OXTR* rs53576 and rs2254298, and *CD38* rs3796863 genotypes. Our study provides additional indication for health providers on the importance of specific genes including *OXTR* rs53576 and rs2254298, and circulating levels of OT in shaping the cross-generational transmission of parenting. Furthermore, we highlight specific patterns of parental caregiving, particularly overprotection that may be especially important for the cross-generational transmission of healthy and maladaptive attachment patters and should become the focus of targeted interventions. Much further research is needed to probe the oxytocin system across generation. Such research should include both mothers and fathers, utilize measures of observed parenting, and assess other neurobiological and neurotransmitter systems that play a role in the development of parenting and its cross-generational transfer. It is of interest to test how the cross-generational transmission of parenting operates when mothers raise children of different ages and whether it differs when rearing preschoolers, older children, adolescents, or young adults. Finally, the cultural and historical context of the cross-generational patterns, their evolution over generations, and the neurobiological systems that sustain both stability and flexibility are exciting topics for future studies in a changing world.

5. Declaration of interest

None.

6. Author contributions

Each author declares his/her individual contribution to the article. All authors have participated in the research and/or article preparation. All authors have approved the final article.

7. Authorship

All authors have made substantial contributions to all of the following:

(1) The conception and design of the study (TF), acquisition of data (TF, MO, KS), or analysis and interpretation of data (TF, KM, EN, OM, RF).

(2) Drafting the article or revising it critically for important intellectual content (TF, OM, RF).

(3) All authors have approved the final article.

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9. Conflicts of interest

The authors report no conflict of interest.

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