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Associations between the oxytocin receptor gene (*OXTR*) and affect, loneliness and intelligence in normal subjects ☆

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ABSTRACT

Associations of oxytocin receptor gene (*OXTR*) variants and autism spectrum disorders (ASD) have been reported in earlier studies; in one of the studies associations with IQ and daily living skills were found additionally. Variations of the oxytocin receptor gene might also regulate affect, attachment and separation beyond the diagnostic borders of autism. We tested hypotheses of associations between positive and negative affects and social and emotional loneliness (285 adults), IQ (117 adolescents) and polymorphisms of the oxytocin receptor gene (*OXTR* rs53576, rs2254298 and rs2228485) in normal subjects. Individuals with the oxytocin *OXTR* rs53576 A/A genotype showed lower positive affect scores ($F = 5.532$, df = 1; $p = 0.019$). This effect was restricted to males ($F = 13.098$, df = 1; $p = 0.00047$). Haplotypes constructed with the three markers were associated with positive affect ($p = 0.0012$), negative affect ($p < 0.0001$) and emotional loneliness ($p < 0.0001$). Non-verbal intelligence was significantly reduced in rs53576 A/A adolescents ($T = 2.247$, $p = 0.027$). Our findings support a role for the oxytocin receptor haplotypes in the generation of affectivity, emotional loneliness and IQ.

1. Introduction

Cumulative evidence suggests, that oxytocin is important for the psychophysiological *calm and connection system*, which is crucial for the regulation of well-being and socialization (Uvnas-Moberg et al., 2005). Disruption of social behaviour, interaction and communication is a key feature of autism. Experimental studies suggest a role for oxytocin in the pathophysiology of autism because oxytocin administration in patients with autism spectrum disorders led to improvement of autism-specific symptoms such as repetitive behaviours and social cognition (Hollander et al., 2007, 2003). Autism is a highly heritable disorder (Pickles et al., 1995; Wu et al., 2005) and the 3p25 region, which harbours the oxytocin receptor gene, was identified as region linked to autism spectrum disorder in two genome-wide scans (Lauritsen et al., 2006; McCauley et al., 2005). Wu et al. (2005) reported associations of the *OXTR* rs53576 A-allele and *OXTR* rs2254298 A-allele with autism in a family-based association study. Haplotypes with up to four markers particularly including rs53576 were also associated with autism. Haplotypes involving *OXTR* rs2228485 showed an excess transmission from parents to affected offspring. In another study Jacob et al. (2007) reported an association with the *OXTR* rs2254298 polymorphism as well, but in contrast to Wu et al. (2005) the G-allele was overtransmitted. Yrigollen et al. (2008) found associations with both multivariate and univariate phenotypes of autism spectrum disorder (ASD) with rs2268493, a SNP located in the same linkage disequilibrium block as rs2254298. In another association study Lerer et al. (2008) observed associations between an *OXTR* five-locus haplotype block involving rs2254298 with autism spectrum disorder (ASD; G overtransmitted) but not with

Abbreviations: ASD, autism spectrum disorder; bp, base pairs; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders; EDTA, ethylenediaminetetraacetic acid; HAWIE, Hamburg–Wechsler-Intelligence Test (Adults); HAWIK, Hamburg–Wechsler-Intelligence Test (Children); IQ, intelligence quotient; MANOVA, Multivariate analysis of variance; *OXTR*, Oxytocin receptor gene; PANAS, Positive and Negative Affect Scale; *UCLA loneliness* scale, University of California, Los Angeles *loneliness* scale.

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Table 1
Affect (PANAS) and loneliness (*UCLA loneliness* scale) scores by *OXTR* rs53576, rs2254298 and rs2228485 genotypes in 289 adult subjects from the Greifswald family study.

		Mean	SDEV	*N*	*F*	*p*	*F*	*p*	*F*	*p*
rs53576							AA vs G		GG vs A	
Social loneliness	AA	2.42	0.35	37	1.458	0.234	2.926	**0.088**	0.409	0.523
	AG	2.33	0.30	114						
	GG	2.33	0.30	138						
Emotional loneliness	AA	1.71	0.57	37	0.071	0.931	0.134	0.714	0.049	0.825
	AG	1.68	0.55	114						
	GG	1.67	0.62	138						
PANAS negative	AA	2.54	0.58	37	0.453	0.636	0.160	0.689	0.907	0.342
	AG	2.54	0.47	114						
	GG	2.48	0.54	138						
PANAS positive	AA	3.55	0.51	37	3.126	**0.045**	5.532	**0.019**	0.005	0.946
	AG	3.74	0.41	114						
	GG	3.70	0.39	138						
rs2254298							AA vs G		GG vs A	
Social loneliness	AA	2.25	0.00	2	0.359	0.699	0.170	0.681	0.653	0.420
	GA	2.31	0.29	43						
	GG	2.35	0.31	242						
Emotional loneliness	AA	1.13	0.18	2	0.923	0.398	1.829	0.177	0.013	0.911
	GA	1.70	0.55	43						
	GG	1.68	0.59	242						
PANAS negative	AA	2.10	0.42	2	0.659	0.518	1.250	0.264	0.232	0.631
	GA	2.49	0.42	43						
	GG	2.52	0.54	242						
PANAS positive	AA	3.35	0.07	2	2.641	**0.073**	1.381	0.241	2.892	**0.090**
	GA	3.81	0.42	43						
	GG	3.68	0.42	242						
rs2228485							TT vs C		CC vs T	
Social loneliness	TT	2.36	0.31	144	0.419	0.658	0.818	0.367	0.027	0.869
	CT	2.32	0.31	110						
	CC	2.36	0.29	28						
Emotional loneliness	TT	1.66	0.60	144	0.419	0.308	0.128	0.720	2.346	0.127
	CT	1.65	0.57	110						
	CC	1.83	0.57	28						
PANAS negative	TT	2.53	0.54	144	0.314	0.731	0.595	0.441	0.007	0.933
	CT	2.48	0.51	110						
	CC	2.50	0.45	28						
PANAS positive	TT	3.67	0.41	144	1.049	0.352	2.012	0.157	0.037	0.847
	CT	3.74	0.44	110						
	CC	3.72	0.35	28						

rs2254298 alone. rs2254298 alone was however associated with Vineland Adaptive Behavior Scale (VABS) daily living skills and communication subdomain scores with G being the risk allele. With the VABS a caregiver assesses a child's daily living skills. *OXTR* rs4686301 and rs1042778, but not rs2254298 was also associated with intelligence (IQ) as measured with standard intelligence tests.

Based on their findings Lerer et al. (2008) suggested that *OXTR* shapes ASD-related phenotypes such as social activities and intelligence beyond diagnostic boundaries.

Other possible phenotypes might include affect regulation, social interactions and loneliness, which have frequently been found in individuals with autism (Bieberich and Morgan, 2004; Whalen et al., 2006). Oxytocin administration and social support increase calmness and reduce anxiety and stress-response (Heinrichs et al., 2003) and oxytocin administration in humans increased trust (Kosfeld et al., 2005). Function of the human amygdala, a key structure for emotion regulation, is strongly modulated by intranasal application of oxytocin (Domes et al., 2007a; Kirsch et al., 2005). Oxytocin is also involved in the modulation of learning, memory and intelligence in animal as well as human studies (de Wied et al., 1993; Lerer et al., 2008).

Furthermore oxytocin is involved in the regulation of social bonding and separation not only in subjects with autism (Panksepp, 2003b). Mind reading (making sense of or predict another person's behaviour), a key ability required for social interaction, is increased after intranasal administration of oxytocin in normal subjects (Domes et al., 2007b). Separation, the disruption of social bonds, is a painful experience which might be buffered by oxytocin (Panksepp, 2003b). Accordingly Meinlschmidt and Heim (2007) found stress system reactivity to be attenuated after application of oxytocin in subjects with early parental separation. There is good evidence that loneliness is the human equivalent of separation distress in animals (Panksepp, 2003a) and so Panksepp et al. (1997) hypothesized that an orally effective ligand for oxytocin receptors should prove to be a powerful alleviator of loneliness in humans.

Based on those results we attempted to explore possible associations between *OXTR* rs53576, rs2254298, rs2228485 and positive/negative affect, different degrees of loneliness and intelligence in normal subjects.

2. Methods

2.1. Population

Participants derive from the population-based representative Study of Health in Pomerania (SHIP-I; $n = 4310$; age between 20 and 79; final response of 68.8%), Germany (Grabe et al., 2005; John et al., 2001). A smaller family sample (adult probands living in families and children aged between 11 and 18) was recruited from SHIP for subsamples to investigate pathways to addiction (*Greifswald family study*). The Greifswald family study was initially designed to investigate pathways to addiction (Barnow et al., 2007). For this purpose index probands with alcohol dependence/abuse and normal controls were selected. To avoid bias only control probands without alcohol dependence/abuse (DSM-IV), of which DNA and phenotype data were available, were included

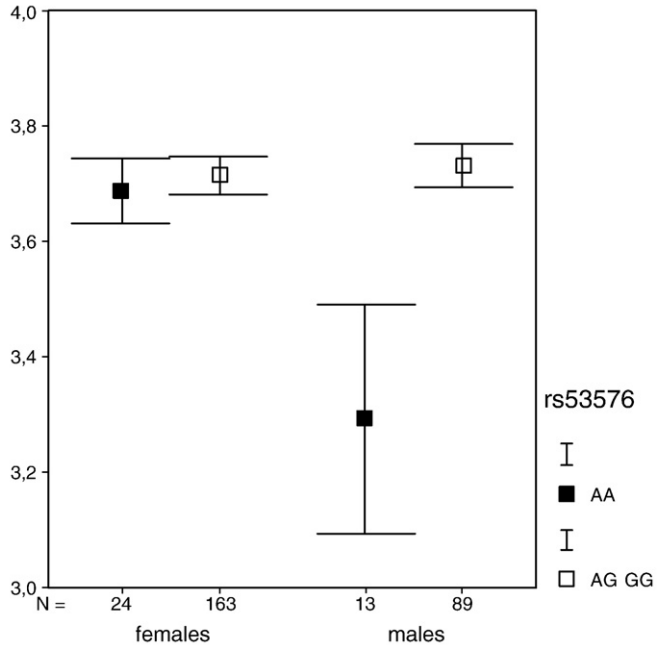

Fig. 1. Positive affect (PANAS) scores (mean ± SEM) by *OXTR* rs53576 genotypes and sex in 289 adult subjects from the Greifswald family study.

into this study. Two subsamples were analysed separately: (1) a group of adults ($n = 289$; 102 males, 187 females; 41.69 ± 7.21 years) and (2) a group of unrelated adolescents ($n = 117$; 59 males, 58 females; 15.14 ± 2.05 years). In families with sisters only the first-born child was included into the analyses of the adolescent sample. Written informed consent was obtained from all probands. The study was approved by the ethics committee of the University of Greifswald.

2.2. Assessment instruments

Affect was assessed with the *Positive and Negative Affect Schedule* (PANAS). Positive (interested, excited, strong, enthusiastic, proud, alert, inspired, determined, attentive, and active) and negative affects (distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid) within the last 4 weeks are rated (Watson et al., 1988). The *UCLA loneliness* scale was developed to assess subjective feelings of social loneliness (being part of a group) and emotional loneliness (feelings of closeness to others; (Green et al., 2001). In adolescents intelligence was tested with HAWIK (Tewes, 1985) (aged 11–15) and with HAWIE for those aged 16–18 (Tewes, 1991); combined values for all adolescents (verbal and non-verbal intelligence) were used.

2.3. Genotype analyses

DNA was isolated from 7.5 ml EDTA-anticoagulated blood with a standard procedure (Miller et al., 1988) or from saliva specimens according to the DNA-by-mail approach as described by Freeman et al. (1997). The rs53576 polymorphism was analysed essentially as described by Wu et al. (2005). A 340 bp genomic fragment harbouring the rs53576 polymorphism PCR-amplified with the primers 5′-GCCCACCATGCTCTCCACATC-3′ and 5′-GCTGGACTCAGGAGGAATAGG-GAC-3′. Restriction fragment length analysis with the enzyme BamHI resulted in two fragments of 110 and 230 bp for the A-allele. The G allele remained undigested. The rs2254298 and the rs2228485 polymorphism were also analysed as described by Wu et al. (2005).

Table 2
Frequencies of 3-marker haplotypes.

Haplotype		
Nr.	rs53576/rs2254298/rs2228485	Frequencies
1	AAC	0.0005
2	AAT	0.0005
3	AGC	0.1850
4	AGT	0.1120
5	GAC	0.0680
6	GAT	0.0120
7	GGC	0.4630
8	GGT	0.1590

For 2 subjects genotyping of rs2254298 and for 7 genotyping of rs2228485 were not successful.

2.4. Data analysis

Intercorrelated affect and loneliness scales were tested for group differences with MANOVA for each polymorphism separately, intelligence scales with *t*-test. A nominal *p* value <0.05 was regarded as significant; given the exploratory nature of the study no correction for multiple testing was carried out. Data were analysed with SPSS for Windows (version 11.5) and SPSS SamplePower, SPSS Inc., Chicago, IL, USA).

2.5. Haplotype analysis

Haplotype-frequencies were estimated with HAPLOVIEW 4.1. Differences in genotype specific values (social and emotional loneliness, positive and negative affects) were estimated and tested with linear models, adjusted for age and sex. *p* values for the influence of the markers tested and for the differences between the three genotypes were tested and adjusted according to Tukey.

3. Results

Genotype frequencies for rs53576, rs2254298 and rs2228485 in the adult and adolescent sample were in accordance with the Hardy–Weinberg-equilibrium ($p > 0.05$). No sex differences could be detected for rs53576 ($\chi^2 = 1.120$; df = 2, $p = 0.571$), rs2254298 ($\chi^2 = 3.405$; df = 2, $p = 0.182$) and rs2228485 ($\chi^2 = 1.497$; df = 2, $p = 0.473$) in the adult sample, due to low sample size no gender-specific calculations were performed for the adolescent sample. We counted allele frequencies of A = 0.33 and G = 0.67 for rs53576 in our study (adult sample), whereas in the sample of Wu et al. (2005) the A-allele is more frequent: A = 0.66 and G = 0.34. However in an Afroamerican (A = 0.22; G = 0.78) and two Caucasian samples allele frequencies were similar to ours (A = 0.33; G = 0.67; HapMap).

3.1. Adult sample

The *OXTR* rs53576 A/A genotype was significantly associated with lower values for positive affect (PANAS, Table 1). The influence of *OXTR* rs53576 on positive affect was restricted to males (Fig. 1; AA vs G; $F = 13.098$, df = 1; $p = 0.00047$; power = 95%; comparison of all three genotypes: $F = 6.569$, df = 2; $p = 0.002$). For social loneliness a

Table 3
p values for associations in adults: 3-marker haplotypes.

	p
Emotional loneliness	<0.0001
Social loneliness	0.2771
Negative affect	<0.0001
Positive affect	0.0012

Table 4
Emotional loneliness.

Haplotypes		Mean*	*p* value for pairwise differences (Tukey)						
	Frequency	(95%-CI)	AAC	AGC	AGT	GAC	GAT	GGC	GGT
AAC	0.1%	1.85 (− 10.56 to 14.2)	—	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
AGC	18.5%	1.78 (1.68–1.89)		—	0.9859	0.6475	0.4733	0.9619	0.1972
AGT	11.2%	1.71 (1.57–1.86)			—	0.9789	0.7924	0.6311	0.9445
GAC	6.8%	1.61 (1.44–1.79)				—	0.9836	0.1506	1.0000
GAT	1.2%	1.47 (1.16–1.77)					—	0.2154	0.9527
GGC	46.3%	1.84 (1.80–1.88)						—	< .0001
GGT	15.9%	1.63 (1.56–1.70)							—

*Adjusted for age and sex.

trend for higher values was found in rs53576 homozygote A/A probands (AA vs G-bearing genotypes: power = 40%).

Further secondary analyses of rs53576 yielded differences on a trend level, but only in the male group and not in the female or total sample (male subjects, rs53576, A-bearing genotypes vs G/G: higher values for negative affect: $F = 2.783$, df = 1; $p = 0.098$; rs53576 A/A: higher scores for social loneliness: $F = 3.338$, df = 1; $p = 0.071$).

OXTR rs2254298 A/A subjects showed lower values for positive affect (trend; Table 1). No influences of the *OXTR* rs2228485 genotype on the affect or loneliness were found in the total group, however in the male group individuals carrying the T/T genotype showed lower values for positive affect on a trend level (T/T $n = 54$: 3.61 ± 0.50; C* $n = 47$: 3.75 ± 0.34; $F = 2.797$; df = 1; $p = 0.098$) and C/C individuals showed higher values for emotional loneliness (C/C $n = 12$: 1.90 ± 0.43; T* $n = 89$: 1.61 ± 0.54; $F = 3.086$, df = 1, $p = 0.082$).

3.2. Haplotype analysis

In the haplotype analysis significant differences were found for emotional loneliness, negative and positive affects (Tables 2 and 3). Haplotype GGC (rs53576/rs2254298/rs2228485) showed significantly higher values for emotional loneliness compared with haplotype 8 (GGT) after correcting for multiple testing (Table 4). Haplotype 7 (GGC) showed higher scores for negative affect compared with haplotype 8 (GGT) and with haplotype 4 (GAC; Table 5). Positive affect scores were higher in haplotype 3 (AGT) compared with haplotypes 5–8 (GAC, GAT, GGC and GGT; Table 6). Those haplotypes all had a "G" in common for rs53576. Haplotypes with "A" in the first position (AGC and AGT) showed lower values for positive affect. Differences between haplotypes could therefore be attributed to rs53576. For social loneliness no differences could be detected (Table 7).

3.3. Adolescent sample

For non-verbal intelligence $n = 117$, and for verbal intelligence $n = 89$ subjects were included into the analyses; only values between IQ ≥ 70 or ≤ 130 were used to avoid outlier-related bias. Non-verbal intelligence was significantly reduced in rs53576 A/A subjects (A/A $n = 12$: 99.00 ± 13.95; G-bearing genotypes $n = 105$: 107.42 ± 12.11; $T = 2.247$, $p = 0.027$; power = 61%). If all subjects were included the *p* value amounted to $p = 0.011$. For verbal intelligence *OXTR* rs2254298 A-bearing genotypes showed lower scores only on a trend level (A-bearing genotypes $n = 11$: 103.45 ± 16.19; G/G $n = 78$; $T = 1.737$, $p = 0.086$). *OXTR* rs2228485 did not show any effect on intelligence.

For affect and loneliness no significant results were found for rs53576 in the adolescent sample. Social loneliness scores were lower in *OXTR* rs2254298 G/G genotypes (Table 8). *OXTR* rs2228485 TT genotypes were associated with lower scores for emotional and social loneliness.

Sample sizes were small because PANAS and loneliness scales were applied only from age 16. Secondary gender-specific analyses for affect, loneliness and intelligence scores were not meaningful for the adolescent group because sample size was too low.

4. Discussion

Our data indicate that different haplotypes of three *OXTR* receptor gene polymorphisms are associated with positive affect, negative affect and emotional loneliness scores in normal human subjects. Analyses of each polymorphisms separately showed, that particularly one genotype, the *OXTR* rs53576 A/A was associated with (1) positive affect in the adult sample and (2) intelligence deficits in a smaller sample of adolescents. These results are consistent with earlier evidence for a role of the oxytonergic system in regulation of affect, loneliness and intelligence.

Oxytocin receptor gene polymorphisms tested in this study (rs53576, rs2254298) were previously associated with autism spectrum disorders (ASD) (Wu et al., 2005). Deficits in positive affect, together with poor social interactions, have repeatedly been reported in individuals with autism (Bieberich and Morgan, 1998, 2004; Kasari et al., 1990; Whalen et al., 2006). So the association of a polymorphism, that has been previously associated with autism (Wu et al., 2005), and affect in our study population with normal subjects could be expected, as far as the genetic liability for autism is also expressed in normals (Folstein and Rutter, 1977). Oxytocin might mediate affect regulation through its dampening properties upon the HPA axis (Bartz and Hollander, 2006; Sutcliffe, 2008). Cortisol levels and HPA-axis activity could be linked with

Table 5
Negative affect.

Haplotype		Mean*	*p* value for pairwise differences (Tukey)						
	Frequency	(95%-CI)	AAC	AGC	AGT	GAC	GAT	GGC	GGT
AAC	0.1%	2.68 (− 7.67 to 13.0)	—	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
AGC	18.5%	2.64 (2.55–2.73)		—	0.9556	0.1964	0.3284	0.9451	0.1884
AGT	11.2%	2.56 (2.44–2.68)			—	0.8139	0.7250	0.4169	0.9803
GAC	6.8%	2.43 (2.29–2.57)				—	0.9971	0.0097	0.9634
GAT	1.2%	2.34 (2.09–2.60)					—	0.1139	0.8827
GGC	46.3%	2.69 (2.66–2.72)						—	< .0001
GGT	15.9%	2.51 (2.45–2.56)							—

*Adjusted for age and sex.

Table 6
Positive affect.

Haplotype		Mean*	*p* value for pairwise differences (Tukey)						
	Frequency	(95%-CI)	AAC	AGC	AGT	GAC	GAT	GGC	GGT
AAC	0.1%	3.70 (− 4.26 to 11.6)	—	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
AGC	18.5%	3.65 (3.58—3.72)		—	0.4884	0.0642	0.4336	0.9505	0.8531
AGT	11.2%	3.54 (3.44—3.63)			—	0.0008	0.0557	0.0388	0.0225
GAC	6.8%	3.84 (3.73—3.95)				—	1.0000	0.1144	0.2733
GAT	1.2%	3.86 (3.66—4.06)					—	0.6176	0.7319
GGC	46.3%	3.69 (3.66—3.71)						—	0.9956
GGT	15.9%	3.70 (3.66—3.75)							—

*Adjusted for age and sex.

positive and negative moods as measured with the PANAS in two studies (Kuehner et al., 2007; Simpson et al., 2008). It would be interesting to study the relationships between oxytocin, HPA axis and affect regulation to further elucidate a relationship between oxytocin and affect.

The effect of *OXTR* rs53576 A/A on positive affect was restricted to male subjects. Autism is more frequent in males and oxytocin binding cites differ between male and female rats in the brain (Uhl-Bronner et al., 2005). Carter (2007) speculated that protective processes mediated by the sexually dimorphic neuropeptide oxytocin (OT) might explain the lower prevalence of ASD in females. Apart from genetic or epigenetic mechanisms the mutual regulation of oxytocin and reelin, a developmentally relevant growth factor, might cause the sex differences in ASD features (Carter, 2007) and thus other phenotypes influenced by oxytocin.

Higher values for *social loneliness* were associated with the rs53576 A/A genotype on a trend level. Lack of a significant association might be due to low sample size (power 44.0%). A recently published study supports a role of rs53576 in social interaction: parents with serotonergic (5-HTT ss) and oxytonergic *OXTR* rs53576 (AA/AG) genotypes showed lower levels of sensitive responsiveness to their toddlers (Bakermans-Kranenburg and van Ijzendoorn, 2008). In contrast no effect for social loneliness was found in the haplotype analysis, but the most frequent haplotype (GGC; $f = 46.3\%$) showed higher values for emotional loneliness compared with GGT. Although theories concerning oxytocin and separation and loneliness suggest a role for *OXTR* in loneliness a larger sample is needed to explore relationships between *OXTR* polymorphisms and different loneliness phenotypes.

The association of rs53576 with non-verbal IQ might corroborate the assumption, that the effect on intelligence is not specific for autism (Lerer et al., 2008). Lack of a significant association with verbal intelligence and of any associations involving rs2228485 cannot be interpreted due to low sample size. The effect of *OXTR* rs2254298 on verbal intelligence ($p = 0.086$) might have reached significant in a larger sample.

The association between rs53576 and positive affect in the adult sample should have been replicated in the adolescent group; again sample size in the adolescent group was too small to draw definite conclusions. For *OXTR* rs2254298 positive associations with ASD were reported in three different studies (Wu et al., 2005; Jacob et al., 2007; Lerer et al., 2008), we did not find significance but only results on a trend level with rs2254298. Lack of significance might be attributed to lack of statistical power.

No functional roles for OXTR rs53576, rs2254298 and rs2228485 have been described yet. The polymorphisms might be rather in linkage disequilibrium with a not yet detected functional polymorphism (Wu et al., 2005). *OXTR* rs2228485, which is located in the promoter, did not play a major role, neither in the study of Wu et al. (2005) nor in ours. Furthermore according to HapMap allele frequencies were markedly different across study populations. Different risk alleles have been described across studies reporting associations with autism, ASD and ASD-related phenotypes (Jacob et al., 2007; Lerer et al., 2008; Wu et al., 2005). Jacob et al. (2007) assumed false positive results or phenotypic heterogeneity as potential causes for the transmission of different alleles reported. Transmission of different alleles might occur when alleles are located on different haplotypes with an unidentified susceptibility variant in the oxytocin gene (Jacob et al., 2007). Lin et al. (2007) posit, that flip-flop associations (replicating a previously reported disease-marker association but with the risk allele reversed from the previous report) do not rule out replications and that population variation in interlocus correlation might be causal. Population stratification in studies such as ours can lead to false positive associations (Lander and Schork, 1994). However the study population is of regional descent and problems of population admixture are minimal in this rural area in the north east of Germany.

5. Conclusions

Our findings corroborate the assumption that *OXTR* polymorphisms might not only influence symptomatology in patients with ASD but also psychological dimensions in normals. The associations between oxytocin receptor polymorphisms or haplotypes and affect, loneliness and intelligence are in accordance with the results of the experimental and imaging studies suggesting an influence of the oxytonergic system on affect regulation, social interaction and cognition. Conclusions of our results should be drawn with caution because of the small sample size; replication in different populations with higher sample size and endophenotypes such as mind reading is warranted.

Table 7
Social loneliness.

Haplotype		Mean[a] (95%-CI)	*p* value for pairwise differences (Tukey)						
	Frequency		AAC	AGC	AGT	GAC	GAT	GGC	GGT
AAC	0.1%	2.36 (−3.70 to 8.43)	–	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
AGC	18.5%	2.40 (2.35–2.45)		–	0.9997	0.9782	0.9721	0.9540	0.4077
AGT	11.2%	2.42 (2.35–2.49)			–	0.9288	0.9326	0.8798	0.3606
GAC	6.8%	2.36 (2.27–2.44)				–	0.9999	0.9999	0.9994
GAT	1.2%	2.33 (2.18–2.48)					–	0.9973	1.0000
GGC	46.3%	2.37 (2.35–2.39)						–	0.5429
GGT	15.9%	2.34 (2.30–2.37)							–

[a] Adjusted for age and sex.

Table 8
Loneliness (*UCLA loneliness* scale) and affect (PANAS) scores by *OXTR* rs53576, rs2254298 and rs2228485 genotypes in 73 adolescents in the adolescent group.

rs53576		Mean	SDEV	*N*	*F*	*p*	AA vs G		GG vs A	
Social loneliness	AA	2.25	0.59	5	0.464	0.631	0.807	0.807	0.411	0.524
	AG	2.39	0.35	24						
	GG	2.42	0.38	44						
Emotional loneliness	AA	1.40	0.34	5	0.989	0.377	1.778	0.187	0.780	0.380
	AG	1.78	0.63	24						
	GG	1.87	0.77	44						
PANAS negative	AA	2.46	0.54	5	0.731	0.485	0.337	0.563	0.650	0.423
	AG	2.69	0.47	24						
	GG	2.55	0.54	44						
PANAS positive	AA	3.66	0.52	5	0.012	0.988	0.025	0.875	0.001	0.975
	AG	3.70	0.46	24						
	GG	3.69	0.47	44						
rs2254298		Mean	SDEV	*N*	*F*	*p*	AA vs G		GG vs A	
Social loneliness[a]	AA	2.25		1	4.725	**0.012**	0.151	0.699	7.947	**0.006**
	GA	2.70	0.46	11						
	GG	2.34	0.34	61						
Emotional loneliness	AA	1.50		1	0.672	0.514	0.187	0.667	0.856	0.358
	GA	2.02	0.79	11						
	GG	1.77	0.70	61						
PANAS negative	AA	1.40		1	2.866	**0.064**	5.788	**0.019**[a]	0.224	0.638
	GA	2.63	0.39	11						
	GG	2.60	0.52	61						
PANAS positive	AA	4.00		1	0.826	0.442	0.443	0.508	0.780	0.380
	GA	3.55	0.48	11						
	GG	3.71	0.46	61						
rs2228485		Mean	SDEV	*N*	*F*	*p*	TT vs C		CC vs T	
Social loneliness	TT	2.30	0.37	32	2.038	0.138	3.957	0.051	0.022	0.882
	CT	2.48	0.38	33						
	CC	2.42	0.38	6						
Emotional loneliness	TT	1.61	0.57	32	3.179	0.048	3.691	0.059	0.961	0.330
	CT	2.00	0.76	33						
	CC	1.51	0.79	6						
PANAS negative	TT	2.49	0.43	32	2.332	0.105	2.593	0.112	0.821	0.368
	CT	2.74	0.53	33						
	CC	2.42	0.75	6						
PANAS positive	TT	3.63	0.45	32	0.576	0.565	1.162	0.285	0.045	0.834
	CT	3.75	0.44	33						
	CC	3.73	0.74	6						

[a] Please note: only 1 subject in the AA group.

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