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No association between oxytocin or prolactin gene variants and childhood-onset mood disorders

John S. Strauss¹, Natalie L. Freeman¹, Sajid A. Shaikh¹, Ágnes Vetró², Enikő Kiss², Krisztina Kapornai², Gabriella Daróczy², Timea Rimay², Viola Osváth Kothencné², Edit Dombóvári², Emília Kaczvinszk², Zsuzsa Tamás³, Ildikó Baji³, Márta Besny³, Julia Gáboros³, Vincenzo DeLuca¹, Charles J. George⁴, Emma Dempster⁵, Cathy L. Barr⁶, Maria Kovacs^{4,7}, and James L. Kennedy¹ the International Consortium for Childhood-Onset Mood Disorders

¹Centre for Addiction and Mental Health, University of Toronto, Toronto, ON, Canada ²Szeged University Medical Faculty, Department for Child & Adolescent Psychiatry, Szeged, Hungary

³Vadaskert Hospital, Budapest, Hungary ⁴Western Psychiatric Institute and Clinic, University of Pittsburgh Medical Center, Pittsburgh, PA, USA ⁵Institute of Psychiatry, London, England ⁶The Toronto Western Research Institute, Division of Cell and Molecular Biology, Toronto, ON, Canada

⁷Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Abstract

Background—Oxytocin (OXT) and prolactin (PRL) are neuropeptide hormones that interact with the serotonin system and are involved in the stress response and social affiliation. In human studies, serum OXT and PRL levels have been associated with depression and related phenotypes. Our purpose was to determine if single nucleotide polymorphisms (SNPs) at the loci for *OXT*, *PRL* and their receptors, *OXTR* and *PRLR*, were associated with childhood-onset mood disorders (COMD).

Methods—Using 678 families in a family-based association design, we genotyped sixteen SNPs at *OXT*, *PRL*, *OXTR* and *PRLR* to test for association with COMD.

Results—No significant associations were found for SNPs in the *OXTR*, *PRL*, or *PRLR* genes. Two of three SNPs 3' of the *OXT* gene were associated with COMD ($p \leq 0.02$), significant after spectral decomposition, but were not significant after additionally correcting for the number of genes tested. Supplementary analyses of parent-of-origin and proband sex effects for *OXT* SNPs by Fisher's Exact test were not significant after Bonferroni correction.

Conclusions—We have examined sixteen *OXT* and *PRL* system gene variants, with no evidence of statistically significant association after correction for multiple tests.

Keywords

oxytocin; prolactin; mood disorder; single nucleotide polymorphism (SNP)

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Corresponding author: John Strauss, Centre for Addiction and Mental Health, 250 College St., Toronto, ON, M5T 1R8, Canada, john_strauss@camh.net, F: 416.979.4666.

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Introduction

Twin and adoption studies of depressive symptoms in juvenile populations have indicated that genetic factors contribute to the etiology of depressive symptoms in childhood (Warner et al., 1995; Kovacs et al., 1997; Mitchell et al., 1989; Mufson et al., 1992; Happonen et al., 2002; Hudziak et al., 2000; Thapar and McGuffin, 1994), yet molecular genetic studies of depression-related phenotypes in pediatric samples are relatively few in number, with many focused on serotonin system genes. Genetic association studies of variants in the serotonin transporter (SLC6A4) and serotonin receptor (e.g. 5HT1A, 1B, 2A) genes have found weak, inconsistent associations with depression (Lemondé et al., 2003; Parsey et al., 2006; Huang et al., 2004; Caspi et al., 2003; Lotrich and Pollock, 2004; Surtees et al., 2006; McMahon et al., 2006). This suggests that genes outside of the serotonergic system, may also be important in the etiology of depressive disorders such as COMD.

Oxytocin (OXT) and prolactin (PRL) are neuropeptide hormones that have been shown to be associated with antidepressant activity, to interact with the serotonin system and to be involved in the stress response and the formation of social bonds. Oxytocin (OXT) has been proposed to balance the suppression of the serotonergic system by the HPA following stressful events (Legros, 2001) and reduce anxiety (Domes et al., 2007). Evidence from animal and human investigation supports the notion of a relationship between oxytocin and serotonin systems (Emiliano et al., 2007; Lee et al., 2003; Amico et al., 2004). Published studies suggest that stress and fear induce compensatory (Windle et al., 2004; Ebner et al., 2005; Kirsch et al., 2005) changes in OXT expression.

Human biomarker studies provide another line of evidence implicating oxytocin in depressive symptoms (Zetsche et al., 1996; Bell et al., 2006; Scantamburlo et al., 2007; Cyranowski et al., 2008; Costa et al., 2009).

Prolactin secretion is also dependent on the serotonergic system. Human serum PRL levels have been shown to reflect serotonergic function via D-fenfluramine challenge (Lee et al., 2003; Mann et al., 1995). Studies show that the prolactin response to D-fenfluramine and serotonergic agents is blunted in depressed individuals compared to controls (Mann et al., 1995; Heninger et al., 1984; Golden et al., 1992; Anderson et al., 1992; Papakostas et al., 2006). Several papers show stress-induced increases in PRL (Drago et al., 1990; Bodnár et al., 2004; Torner et al., 2004). The postpartum antidepressant-like effects of lactation may be mediated by the parity of the mother (Sibolboro Mezzacappa and Endicott, 2007). Plasma PRL levels in response to tryptophan challenge are associated with neuroticism, a correlate of depressive disorders (Brummett et al., 2008). Still other results support an OXT-PRL feedback loop in the hypothalamus (Liu et al., 1994; Kokay et al., 2006).

We genotyped single-nucleotide polymorphisms (SNPs) across the *OXT* and *PRL* genes and their receptors in a large Hungarian cohort of families with COMD. The present study is the first genetic analysis to examine the association between variants in these neuroendocrine genes and depression with onset in childhood.

Methods

Sample Recruitment

Detailed descriptions of the sample recruitment have been published (Liu et al., 2006; Kapornai et al., 2007; Kiss et al., 2007). Families were recruited from 23 mental health centers across Hungary. This study was institutional review board approved at all sites. Written informed consent was obtained from each parent and child.

The Interview Schedule for Children and Adolescents – Diagnostic version, a modification of the Interview Schedule for Children and Adolescents (Sherill and Kovacs 2000), was the semi-structured interview used to collect clinical data. Best estimate consensus diagnostic procedures were used. We included 678 families with at least one parent and a proband in the statistical analyses. Of all participants, 855 were affected -- probands ($n = 678$) and affected siblings ($n = 177$) all met DSM-IV criteria for either unipolar depression ($n = 838$) prior to age 14 years or bipolar disorder ($n=17$) prior to age 17 years. The mean proband age at study entry was 12.6 years [S.D. 2.32 years]. Probands were 55% male. Bipolar cases were included in the analyses since a significant proportion of unipolar children will switch as they mature (Kovacs et al., 1997, Geller et al., 2001), --less than 1% of the entire sample had a bipolar spectrum diagnosis at intake (Dempster et al., 2007), and evidence that unipolar and bipolar illnesses share inherited risk (McGuffin et al., 2003).

Laboratory

Genomic DNA was isolated from blood for each participant using a high-salt method (Lahiri and Nurnberger 1991). Four buccal swabs were also collected per individual for quality control purposes including resolution or confirmation of Mendelian inconsistencies. Single nucleotide polymorphisms (SNPs) were selected for each of the four genes based on Caucasian linkage disequilibrium (LD) plots from the International HapMap project database (www.hapmap.org) with a minimum minor allele frequency (MAF) of 20% (Table 1). SNPs were genotyped independently using 50 ng DNA in a standard Applied Biosystems (Foster City, CA) TaqMan® assay-on-demand reaction modified for a 10 μ l final volume. Amplified products were analyzed on an AB 7500 Sequence Detection System.

Statistical Analysis

Several analyses were performed using Haploview v 3.32 (Barrett et al., 2005), including Hardy-Weinberg equilibrium and Mendelian inheritance, and the transmission disequilibrium test (TDT). A corrected α significance threshold was calculated for each gene using Nyholt's (2004) spectral decomposition (SNPSpD) method. Correction by SNPSpD was followed by correction for the number of genes tested, to account for multiple comparisons. Haplotype blocks were defined using the Gabriel et al. (2002) criteria. All haplotypes with a frequency greater than 0.05 were also tested for association with COMD.

Results

A total of 678 families were genotyped for 16 SNPs across the *OXT*, *OXTR*, *PRL*, and *PRLR* genes. All SNPs were in Hardy-Weinberg equilibrium ($p \geq 0.11$). Seventeen families showed Mendelian errors. In eleven of these, inconsistencies were not due to sample handling errors, according to evaluation of buccal swabs taken from the same individual as the blood sample; these families were suspected of nongenetic familial relationships and removed from the analysis. The remaining six families with Mendelian errors showed a discrepancy between the blood and buccal swab samples for one member of the family. In the latter cases, the swab sample was used to genotype the discrepant individual. DNA from blood was used to re-genotype 5% of the samples at each marker: error rates were <1% and were explained by labelling/transcriptional errors.

Two of the three SNPs 3' of the *OXT* gene, showed significant results (rs2740210: allele C overtransmitted, $\chi^2 = 5.32$, $p = 0.02$; rs4813627: allele G overtransmitted, $\chi^2 = 6.01$, $p = 0.01$; see Table 2). After using SNPSpD, both were nominally significant ($M_{\text{eff}} = 2.35$, adjusted $\alpha = 0.021$), but they were not significant after further Bonferroni correction by the number of genes tested (adjusted $\alpha = 0.005$). No significant associations were found for any SNPs in the *OXTR*, *PRL*, or *PRLR* genes ($p > 0.15$, Table 2). Only one haplotype block, spanning rs1205960

and rs849886 in the *PRL* gene, was identified. None of the haplotypes were significant. (Results not shown).

We performed two additional exploratory analyses to examine parent-of-origin effects (POEs) and proband sex effects for the three SNPs downstream of the *OXT* gene, using the default TDTPHASE sub-option settings of UNPHASED v2.403 (Dudbridge et al., 2003), followed by a one-tailed Fisher's Exact test, based on the hypothesis that estrogen influences on *OXT* expression would be more relevant in transmissions to and from females. Three *OXT* SNPs revealed biased maternal transmissions of rs2740210 (allele A undertransmitted, relative risk (RR) = 0.68, CI = 0.46–1.03, likelihood ratio score (LRS) = 7.14, $p = 0.007$) and rs4813627 (allele A undertransmitted, RR = 0.71, CI = 0.49–1.04, LRS = 6.09, $p = 0.01$), while paternal transmissions were not significant; most of the paternal transmissions were in the same direction as the maternal transmissions, with no parent-of-origin effect by Fisher's Exact Test (p 's ≥ 0.12). Similarly, all three *OXT* SNPs showed biased transmission to daughters, but proband sex effects were not significant after correction for multiple tests (adjusted $\alpha=0.017$) for rs2740210 (FET, $p = 0.04$) or rs2770378 (FET, $p = 0.02$) (Table 3).

Discussion

Our results show a trend towards association of two *OXT* SNPs, rs2740210 and rs4813627, with COMD, not significant after correction for multiple testing. Supplementary analyses initially suggested proband sex effects for *OXT* SNPs rs2740210 and rs2770378, but were not significant after multiple testing correction.

OXT lies in a segmental duplication region of human chromosome 20 and shares high sequence homology in the first two exons to the arginine vasopressin (*AVP*) gene. The *OXT* and *AVP* genes lie tail-to-tail and are separated by a 10 kb intergenic region (IGR); *AVP* and *AVPR1B* genetic polymorphisms have both been significantly associated with COMD in this sample (Dempster et al., 2007, 2009). The three *OXT* SNPs genotyped in the present study reside within 2 kb of the 3' end of the *OXT* gene. Transgenic rat and mouse studies of the *OXT* and *AVP* genes have shown that the IGR contains regulatory sequences responsible for cell-specific *OXT* expression in oxytocinergic magnocellular neurons (MCNs) (Murphy and Wells, 2003).

The observation that the two *OXT* SNPs showed a nonsignificant trend for affected daughters, is of note in light of *OXT* sex effects reported in the literature (Richard and Zingg, 1990; Cushing and Carter, 2000).

To our knowledge, this is the first genetic association study of *OXT* and *PRL* variants in a pediatric mood disorder sample. Four studies have found associations between *OXTR* gene variations and autism (Wu et al., 2005; Ylisaukko-oja et al., 2005; Jacob et al., 2007; Gregory et al., 2009) and recent evidence has implicated the *OXTR* in maternal sensitive responsiveness with their 2-year-old toddlers (Bakermans-Kranenburg et al., 2008). A haplotype of three *OXTR* SNPs that we did not examine has recently been associated with negative affect and loneliness (Lucht et al., 2009). Another investigation noted an association between two *OXTR* SNPs and major depression (Costa et al., 2009); ; we studied three different loci and provided broader SNP coverage of the *OXTR*, with a childhood-onset sample.

There were more missing fathers than missing mothers in our sample. We did not estimate missing parental genotypes. Since a missing parental genotype means the entire family is excluded from the analysis, the relatively larger number of missing fathers will reduce our power, but not influence the relative numbers of parental transmissions tested. We studied a moderately large, well-characterized sample. Statistical methods used were robust to population stratification. Corrections for multiple tests were conservative. We estimate 678 families provide a power of 0.86, given the assumptions of $\alpha=0.05$, population frequency

of 2%, additive model of inheritance, genotype relative risk of 1.4, minor allele frequency of 0.41 (mean minor allele frequency for OXT SNPs= 0.41) and complete linkage between risk and disease variants (Purcell et al., 2003).

We report evidence that was initially suggestive of possible association between two *OXT* SNPs (rs2740210 and rs4813627) and COMD. The results survived spectral decomposition, but weren't significant after correcting for the number of genes tested. *OXTR*, *PRL* and *PRLR* SNPs were not associated with COMD. In secondary analyses, the relative significances of association for two *OXT* SNPs were increased with parental and proband sex effect analyses. A trend towards proband sex effects to daughters failed to reach statistical significance after correcting for multiple tests. Considering the *OXT* SNPs are located in a putative regulatory region, our exploratory analyses by parent and proband sex are of interest as they may be relevant to epigenetic effects (Kaminsky et al., 2006).

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Members of the International Consortium for Childhood-Onset Mood Disorders: Ágnes Vetró, Eniko Kiss, Krisztina Kapornay, István Benák, Viola Kothencné Osváth, Edit Dombovári, Emília Kaczvinszky, Gabriella Daróczy, and László Mayer from Szeged University Medical Faculty, Department of Child and Adolescent Psychiatry, Szeged. Júlia Gáboros, Ildikó Baji, Zsuzsa Tamás, and Márta Besnyő, from Vadaskert Hospital, Budapest. Judit Székely, Semmelweis University I. From the Department of Pediatrics, Budapest.

Heads of the Units and Departments where the patients were collected, and collaborators: Rózsa Hasuly, MD. – Szent Rókus Hospital, Outpatient Unit of Child Psychiatry, Budapest. Ilona Riegler, MD., Márta Fohn M.D.– Heim Pál Hospital Outpatient Unit of Child Psychiatry, Budapest. Katalin Benkő, MD. – Outpatient Unit of Child Psychiatry, Szeged. Mária Mojzes, MD. – Outpatient Unit of Child Psychiatry, Baja. Róza Oláh, MD. – Kenézy Gyula Hospital Department of Child and Adolescent Psychiatry, Debrecen. Mária Károlyfalvi, MD., Gyöngyi Farkas, MD., Zsuzsa Bánk, MD.– Outpatient Unit of Child Psychiatry, Debrecen. Ferenc Dicső, MD., Dénes Kövendy MD.– Jóna András Hospital Outpatient Unit of Child Psychiatry, Nyíregyháza. Mária Gyurcsó, MD.– Petz Aladár Hospital, Outpatient Unit of Child Psychiatry, Győr. Zsuzsanna Fekete, MD., Mariann Vados, MD.- Szent György Hospital, Outpatient Unit of Child Psychiatry, Székesfehérvár. Zsuzsa Takács, MD.– Szekszárd Hospital, Outpatient Unit of Child Psychiatry, Szekszárd. Eszter Gyenge, MD., Mária Palaczky, MD., Ágnes Horváth, MD.– Outpatient Department of Child Psychiatry, Pécs. Ilona Mógör, MD. Péter Steiner M.D.– Csolnoky Ferenc Hospital, Outpatient Unit of Child Psychiatry, Veszprém. Enikő Juhász Szolnok Hospital Outpatient Unit of Child Psychiatry, Szolnok. Mária Révhelyi, MD., - Petz Aladár Hospital Department of Child and Adolescent Psychiatry, Győr. Éva Gyulai, MD.– Baja Hospital Outpatient Unit of Child Psychiatry, Baja. Katalin Bense, MD., Edina Farkas, MD.– Kecskemét Hospital Outpatient Unit of Child Psychiatry, Kecskemét. Sörfőző Zsuzsanna, MD. – Kaposi Mór Hospital, Outpatient Unit of Child Psychiatry, Kaposvár, Magdolna Gácsér- Kálmán Pándy Hospital Department for Child and Adolescent Psychiatry Gyula. Emőke Endreffy Ph.D. Szeged University Pediatric Department. Interviewers: Judit Tömöri, Tünde Horváth M., Eszter Szamosi, Laura Német. Regional research office members: Edit Sitkei, Noémi Takács, Erika Kolonics. Central research office members: Szilvia Szabó Kelemenné, Zoltán Széll, Márta Dobó, and Éva Lehoczky.

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Table 1

Oxytocin and prolactin system genetic marker information

Gene	SNP	Chromosome	Position	Observed heterozygosity	HWE <i>p</i> -val	% Genotyped	Minor Allele	Minor Allele Frequency
<i>OXT</i>	rs2740210	20p13	3001255	0.43	0.31	98	A	0.34
	rs2770378	20p13	3001514	0.48	0.50	97	A	0.42
	rs4813627	20p13	3003513	0.49	0.55	98	G	0.47
<i>OXT</i>	rs237885	3p25	8770543	0.49	0.11	100	T	0.44
	rs2268493	3p25	8775840	0.43	0.32	96	C	0.32
	rs237898	3p25	8783495	0.49	0.37	100	T	0.39
<i>PRL</i>	rs1205961	6p22.3	22393991	0.45	1.00	100	A	0.35
	rs1205960	6p22.3	22396139	0.37	0.46	95	T	0.25
	rs849886	6p22.3	22399346	0.48	0.62	100	T	0.47
	rs1341239	6p22.3	22412183	0.47	0.78	100	A	0.39
<i>PRL</i>	rs187490	5p13.2	35080884	0.41	0.21	100	C	0.31
	rs249522	5p13.2	35096076	0.22	0.87	98	T	0.13
	rs35614689	5p13.2	35134877	0.51	0.71	95	G	0.48
	rs4703505	5p13.2	35207778	0.40	0.74	92	A	0.27
	rs2047740	5p13.2	35239149	0.43	0.30	100	C	0.33
	rs7727306	5p13.2	35273617	0.48	0.45	100	A	0.40

Table 2
Transmission disequilibrium test results for *OXT*, *OXT*R, *PRL* and *PRL*R markers

Gene	SNP	Overtransmitted Allele	T:U	χ^2	p
<i>OXT</i>	rs2740210	C	260:210	5.32	0.02 [†]
	rs2770378	G	271:238	2.14	0.14
	rs4813627	G	299:242	6.01	0.01 [†]
<i>OXT</i> R	rs237898	A	278:269	0.15	0.70
	rs2268493	A	229:220	0.18	0.67
	rs237885	A	270:263	0.09	0.76
<i>PRL</i>	rs1341239	T	277:246	1.84	0.18
	rs849886	G	297:268	1.49	0.22
	rs1205960	G	198:193	0.06	0.80
<i>PRL</i> R	rs1205961	T	268:229	3.06	0.08
	rs7727306	A	282:279	0.02	0.90
	rs2047740	C	259:225	2.39	0.12
	rs4703505	T	184:162	1.40	0.24
	rs35614689	T	272:253	0.69	0.41
	rs249522	G	136:121	0.88	0.35
	rs187490	T	242:230	0.31	0.58

T: Transmitted, U: Untransmitted
[†] not significant at adjusted alpha = 0.005

Table 3

OXT SNP transmissions according to parent and proband sex

SNP	Paternal Transmissions					Maternal Transmissions					FET (one-tailed)
	T	U	RR	LRS	p	T	U	RR	LRS	p	
rs2740210	108	95	0.88	0.83	0.36	115	78	0.68	7.14	0.007	0.12
rs2770378	104	100	0.96	0.08	0.78	115	90	0.78	3.06	0.08	0.17
rs4813627	111	88	0.79	2.66	0.1	125	89	0.71	6.09	0.01	0.32

SNP	Transmissions to Male Proband					Transmissions to Female Proband					FET (one-tailed)
	T	U	RR	LRS	p	T	U	RR	LRS	p	
rs2740210	135	128	0.95	0.19	0.66	133	90	0.68	8.34	0.004	0.04
rs2770378	145	152	1.05	0.16	0.68	132	96	0.73	5.71	0.02	0.02
rs4813627	170	151	0.89	1.12	0.29	138	98	0.71	6.81	0.01	0.11

T: Transmitted, U: Untransmitted
RR: Relative risk
LRS: Likelihood ratio test statistic
FET: Fisher's Exact test, one-tailed p-value (adjusted $\alpha=0.017$)