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Variation in the oxytocin receptor gene is associated with increased risk for anxiety, stress and depression in individuals with a history of exposure to early life stress

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ABSTRACT

Background: Oxytocin is a neuropeptide that is involved in the regulation of mood, anxiety and social biology. Genetic variation in the oxytocin receptor gene (*OXTR*) has been implicated in anxiety, depression and related stress phenotypes. It is not yet known whether *OXTR* interacts with other risk factors such as early life trauma to heighten the severity of experienced anxiety and depression.
Methods: In this study, we examined genotypes in 653 individuals and tested whether SNP variation in *OXTR* correlates with severity of features of self-reported experience on the Depression Anxiety and Stress Scale (DASS), and whether this correlation is enhanced when early life trauma is taken into account. We also assessed the effects of *OXTR* SNPs on RNA expression levels in two separate brain tissue cohorts totaling 365 samples.
Results: A significant effect of *OXTR* genotype on DASS anxiety, stress and depression scores was found and ELS events, in combination with several different *OXTR* SNPs, were significantly associated with differences in DASS scores with one SNP (rs139832701) showing significant association or a trend towards association for all three measures. Several *OXTR* SNPs were correlated with alterations in *OXTR* RNA expression and rs3831817 replicated across both sets of tissues.
Conclusions: These results support the hypothesis that the oxytocin system plays a role in the pathophysiology of mood and anxiety disorders.

1. Background

Oxytocin (OXT) is a mammalian hormone that is best known for its role in lactation, parturition and maternal behavior. It is synthesized in the hypothalamic paraventricular and supraoptic nuclei, transported to the posterior pituitary and released into the general circulation. It is also found in extra-hypothalamic brain areas. OXT has been shown to exert effects on memory (de Wied et al., 1993; Lerer et al., 2008), anxiety (Heinrichs et al., 2003) and social interaction (Kosfeld et al., 2005; Uvnas-Moberg, 1998). OXT concentrations in plasma and cerebrospinal fluid (CSF) have been reported to have an inverse relationship with measures of aggression (Lee et al., 2009), suicidality (Lee et al., 2009), and depression (Scantamburlo et al., 2007).

Of direct relevance to the current study, decreased CSF OXT concentrations were observed in women with a history of childhood abuse, particularly associated with emotional abuse (Heim et al., 2009). In contrast, higher plasma OXT levels are associated with higher social anxiety symptom severity in a cohort of patients with Generalized Social Anxiety Disorder (Hoge et al., 2008).

Oxytocin receptors (*OXTR*) are localized in hypothalamic and extra-hypothalamic brain regions including the cerebral cortex. The

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human *OXTR* gene is located at 3p25.3 and spans approximately 17 kilobases (Kb) containing 4 exons and 3 introns (Kimura et al., 1992). Previous work had demonstrated associations between the OXT receptor gene (*OXTR*) haplotypes and affectivity or depressive temperament (TEMPS-A scale) (Kawamura et al., 2010). In this study, we attempted to confirm and extend these findings, as well as to assess whether *OXTR* variants are associated with increased stress, depression and anxiety phenotypes particularly in the context of early life stress, using the Depression Anxiety Stress Scale (DASS).

2. Materials and methods

2.1. Sample

A total of 1226 participants in the BRAINnet Foundation Database www.brainnet.net, (Koslow et al., 2013) which includes the Brain Resource International Database administered for scientific purposes (Gordon, 2003; Gordon et al., 2005)) have been assessed using the DASS measure (Kemp et al., 2005; Lovibond SH, 1995), a 42-item self report designed to measure the negative emotional states of depression, anxiety and stress. The Depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia, and inertia. The Anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The Stress scale is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatient. Exclusion criteria included self-report of a personal history of physical brain injury, neurological disorder, psychiatric illness or other serious medical condition including history of sleep disorder (Kemp et al., 2005). DASS scores were treated as a continuous measure in all analyses. Individuals were assessed for the number of traumatic events they had been exposed to in early life using the Early Life Stress Questionnaire (ELSQ; (Chu et al., 2013)). The ELSQ is based on the Child Abuse and Trauma Scale, which has been shown to have strong internal consistency, test-retest reliability, and validity, as it correlates with adult outcome and psychopathology (Sanders and Becker-Lausen, 1995). The measure consists of 19 events shown to have psychological impact in childhood in previous studies (Sanders and Becker-Lausen, 1995). The number of events was counted and analysis was performed using both the number of events as a continuous measure as well as grouping events into no traumas versus one or more traumas reported. The following criteria were met during collection of this cohort: 1) the investigation was carried out in accordance with the latest version of the Declaration of Helsinki, 2) the study design was reviewed by an appropriate ethical committee, and 3) informed consent of the participants was obtained after the nature of the procedures was fully explained.

2.2. Genotyping

Of the 1226 BRAINnet participants with DASS measures, 682 individuals had collected DNA available for genotyping. Fifteen SNPs spanning the length of *OXTR* were selected for genotyping and ten of these SNPs passed laboratory quality control (Fig. 1). Genotypes were determined using iPLEX Gold™ primer extension followed by mass spectrometry analysis on the Sequenom MassARRAY system (Sequenom, San Diego, CA) by the Australian Genome Research Facility (http://www.agrf.org.au/). Complete genotyping data was obtained for 653 individuals. The cohort of 653 individuals used for our analysis was 47% female, with an average age of 37 (range 6–87 years) and an average of 11 years of education (range 1–18 years). All cohort members were of Caucasian decent.

2.3. Quality control

Prior to evaluation of samples and SNP variants, several quality control metrics were assessed. Quality metrics for samples included: 1. Removing samples with no phenotype and 2. Removing samples with call rates across all SNPs <50%. Quality metrics for SNPs included: 1. Excluding SNPs with genotyping rates <90% across all samples, 2. Excluding SNPs that were out of Hardy–Weinberg equilibrium (HWE) and 3. Excluding SNPs with a minor allele frequency less than 5%. A total of 7 SNPs met our criteria and were used for analysis. Three SNPs were eliminated because they were out of HWE. A total of 40% of the genetic variance of *OXTR* is captured using these 7 SNPs as measured using Haploview (Barrett et al., 2005).

2.4. Imputation

To capture additional genetic variance within *OXTR*, SNPs were imputed using MaCH-Admix v2.0.198 (Liu et al., 2013) as in Corneveaux et al. (2010). The reference genome was the ALL GIANT.phase 1 release v3.20101123 (http://www.sph.umich.edu/csg/abecasis/MACH/download/1000G.2012-03-14.html) build that excludes monomorphic and singleton sites. The EUR alignment was used, which includes data from the CEPH cohorts as well as Italian, Finnish, British and Spanish samples (http://www.1000genomes.org/category/frequently-asked-questions/population) as is of a similar ethnic background to our cohorts. MaCH-Admix was run using the integrated (default) mode. Genotypes from 653 individuals were used for imputation, which comprised the full set of individuals for which there was both genotyping and full clinical data, including ELS, DASS scores, education, age and gender. Genotypes for 484 additional SNPs were imputed with an average quality score of 0.9 and a minimum quality score of 0.5. Imputed calls were imported into Haploview and Tagger was run to determine the minimal set of alleles which would tag the entire locus (Barrett, 2009; Barrett et al., 2005). The original genotyped SNPs were force-included in the models and pairwise tagging was performed. Fifty-one additional SNPs from this analysis were analyzed along with the original 7 polymorphisms. The average quality score was 0.8 for this set and the minimum quality score was 0.6. The minimum minor allele frequency was 2%. This set captured 90 of 90 alleles in the imputed set of SNPs at an $r^2 \geq 0.8$ with a max $r^2 = 0.971$.

2.5. Brain tissue

Brain tissue was obtained from National Institute on Aging Alzheimer's disease centers, the Miami Brain Bank, the Newcastle Brain Tissue Resource, the MRC London Brain Bank for Neurodegenerative Diseases, the South West Dementia Brain Bank, The Netherlands Brain Bank and the Institut de Neuropatologia, Servei Anatomia Patologica, Universitat de Barcelona. All specimens were from donors who were greater than age 65 years at age of death. Only donors clinically classified as cognitively normal at time of death were included in this analysis. Board-certified neuropathologists performed Braak and Braak (1995) staging on the based on the degree of neurofibrillary tangles and/or CERAD classification on the extent of neuritic plaques. Any samples with a history of other known neurological disease and or clinical history of stroke, cerebrovascular disease, Lewy bodies were excluded. Sample processing is given in references 17 and 19. The first set includes 177 samples and was 45% female, with an average age of 81 (range

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