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Animal behaviour

The Williams syndrome prosociality gene *GTF2I* mediates oxytocin reactivity and social anxiety in a healthy population

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The neurohormone oxytocin plays a central role in human social behaviour and cognition, and oxytocin dysregulation may contribute to psychiatric disorders. However, genetic factors influencing individual variation in the oxytocinergic system remain poorly understood. We genotyped 169 healthy adults for a functional polymorphism in *GTF2I* (*general transcription factor II-I*), a gene associated with high prosociality and reduced social anxiety in Williams syndrome, a condition reported to involve high oxytocin levels and reactivity. Participants' salivary oxytocin levels were measured before and after watching a validated empathy-inducing video. Oxytocin reactivity, defined as pre- to post-video percentage change in salivary oxytocin, varied substantially and significantly between individuals with different *GTF2I* genotypes, with, additionally, a trend towards an interaction between genotype and sex. Individuals with more oxytocin-reactive genotypes also reported significantly lower social anxiety. These findings suggest a model whereby *GTF2I* has a continuum of effects on human sociality, from the extreme social phenotypes and oxytocin dysregulation associated with gene deletion in Williams syndrome, to individual differences in oxytocin reactivity and sociality associated with common polymorphisms in healthy populations.

1. Introduction

The neurohormone oxytocin plays central roles in human social cognition, emotionality and behaviour [1–3]. Moreover, dysregulated oxytocin levels have been reported in psychiatric disorders including major depression [4] and autism [5]. Despite intense interest in social effects of oxytocin, the genetic mechanisms regulating individual variation in oxytocin are poorly understood.

Genetic disorders characterized by atypical social behaviour may provide useful insights into genes influencing the human oxytocinergic system. Williams syndrome, a neurodevelopmental disorder caused by hemizygous deletion of approximately 25 genes at chromosomal region 7q.11.23, is characterized by high prosociality and low social anxiety, which may be linked with the increased oxytocin levels and reactivity reported in this condition [6,7]. High prosociality in Williams syndrome, and in mouse models of this syndrome, has been linked with reduced expression of the gene *general transcription factor II-I* (*GTF2I*) [8,9], which is located within the 7q11.23 Williams syndrome deletion region. Single nucleotide polymorphisms (SNPs) of this gene are also associated with autism risk [10] and, among healthy populations, with variation in social anxiety, autistic-like traits, threat-related amygdala activity, and extraversion [11–13]. These latter findings provide evidence of roles for *GTF2I* in sociality and anxiety among healthy humans, which notably resemble the roles of oxytocin itself [7,11,14].

Convergent lines of evidence thus suggest that the associations of *GTF2I* with social behaviour—in Williams syndrome and in healthy populations—may be mediated by effects of *GTF2I* genetic variation on oxytocin. We tested this hypothesis using data on salivary oxytocin levels collected before and after experimental empathy induction, data on self-reported social anxiety, and data from genotyping of rs13227433, the *GTF2I* SNP previously associated with social anxiety, extraversion and amygdala reactivity in healthy populations.

2. Material and methods

(a) Study population

Healthy participants were recruited from a Canadian university (92 females, 77 males). The study population was of mixed ethnicity (42% East Asian, 25% Caucasian, 22% South Asian, 11% other or mixed ethnicity) with an average age of 20.3 ± 2.2 years. No participants reported having children. No female participants reported being pregnant, and mean oxytocin levels and reactivity did not differ with use of hormonal contraception or stage of menstrual cycle ($p > 0.24$ for all tests, electronic supplementary material, tables S1 and S2).

(b) Experimental design

Saliva samples were collected before and after participants watched a validated empathy-inducing video of a child with terminal cancer [15]. Oxytocin was quantified in both samples, and reactivity was calculated as pre-video to post-video percentage change in salivary oxytocin. As part of the experiment, participants also completed the Schizotypal Personality Questionnaire-Brief Revised [16,17], which includes a four-item excessive social anxiety subscale. Each high-anxiety item endorsed ('agree' or 'strongly agree') was scored 1; social anxiety scores thus ranged from 0 to 4 with higher scores indicating greater social anxiety.

(c) Salivary oxytocin collection and analysis

Saliva was collected by passive drool into pre-chilled tubes and immediately frozen at −20°C. Consistent with published protocols for measuring salivary oxytocin [18–20], 0.5 ml of saliva was lyophilized overnight to concentrate the sample twofold. Measurement of oxytocin was performed in duplicate using Enzo Life Sciences enzyme-linked immunosorbent assay kit ADI-901-153 (http://www.enzolifesciences.com/ADI-901-153A/oxytocin-elisa-kit/ENZO Oxytocin ELISA). Samples from the same individual were analysed on the same plate. Plates were read at 405 nm and oxytocin concentrations were calculated from standard curves. Intra- and inter-assay coefficients of variability were less than 8% and less than 18%, respectively, for 16 plates, which were consistent with the manufacturer's normative variability ranges (12.6–13.3% and 11.9–20.9%).

Debate exists concerning measurement accuracy of oxytocin by ELISA in unextracted compared with extracted fluids, as well as the relationship between salivary and plasma oxytocin [21]. The assay used in this study has undergone rigorous testing and is highly specific to oxytocin (i.e. it does not detect vasopressin) (http://www.enzolifesciences.com/ADI-901-153A/oxytocin-elisa-kit/ENZO Oxytocin ELISA). Furthermore, salivary oxytocin has been shown to correlate positively with plasma oxytocin [22], and any possible methodological measurement effects are expected to affect all samples, rather than being genotype specific in any way.

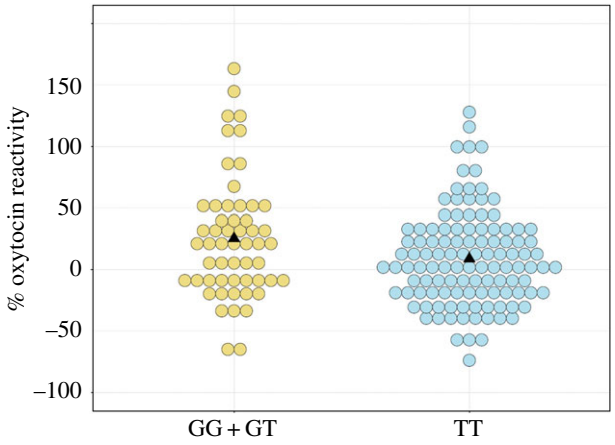

Figure 1. Dot plot indicating percentage change in salivary oxytocin for individuals with GG + GT versus TT genotypes for *GTF2I* SNP rs13227433. Each dot represents one individual. Triangles indicate the mean for each genotype group (25.4 for GG + GT, 8.8 for TT).

(d) *GTF2I* genotyping

Participants were genotyped for SNP rs13227433, which tags an approximately 73 kb haplotype that includes the promoter region of the *GTF2I* gene (electronic supplementary material, figure S1). DNA, fluorophore-labelled primers (TaqMan® SNP Genotyping Assays), and TaqMan® Master Mix were combined and run on a Roche LightCycler® 96 Real-Time PCR machine. Fluorescence data were analysed under Endpoint Genotyping with LightCycler® 96 software, v. 1.1.0.1320. Genotype frequencies did not deviate from Hardy–Weinberg equilibrium in our sampled population ($\chi^2 = 2.28$, d.f. = 1, $p = 0.13$).

(e) Statistical analysis

R (v. 3.3.1) was used to analyse all data. Mean differences were tested using *t*-tests for two-group comparisons and analyses of variance (ANOVA) for comparisons involving more than two groups. Given the rarity of the GG genotype (less than 5%), GG and GT genotypes were combined and compared with TT genotypes. Results were considered significant if $p < 0.05$.

3. Results

Salivary oxytocin increased, on average across all participants, after viewing the empathy-inducing video (paired *t*-test: $t = -2.95$, d.f. = 168, $p = 0.004$). Variation in oxytocin was analysed using a $2 \times 2 \times 4$ factorial ANOVA (genotype × sex × ethnicity). The analysis indicated a significant main effect of *GTF2I* rs13227433 genotype on oxytocin reactivity ($F = 5.5$, $p = 0.02$, mean difference: 16.6, 95% confidence intervals of difference: 2.8–30.2), with the GG + GT group showing higher reactivity (figure 1), and a trend towards an interaction between genotype and sex ($F = 3.0$, $p = 0.08$). Social anxiety was analysed using a 2×2 factorial ANOVA (genotype × sex), which also resulted in a significant main effect of genotype ($F = 4.4$, $p = 0.04$, mean difference: −0.5, 95% confidence intervals of difference: −0.03 to −0.99) with the GG + GT group self-reporting lower levels of social anxiety. Mean oxytocin reactivity and social anxiety scores for each genotype group and sex are presented in table 1. All other effects and interactions were statistically non-significant (electronic supplementary material, tables S3 and S4), including variation in baseline oxytocin levels between *GTF2I* genotype groups ($p = 0.44$, means: 101.0 pg ml^{-1} GG + GT, 109.9 pg ml^{-1} TT).

Table 1. Mean oxytocin reactivity and social anxiety scores for *GTF2I* SNP rs13227433 genotype groups. Three individuals were excluded from social anxiety analyses due to incomplete questionnaire items. n.a., not applicable.

		All	GG	GT	GG + GT	TT
% oxytocin reactivity	females + males	14.2 (42.9)	32.2 (56.6)	24.0 (48.6)	25.4 (49.5)[a]	8.8 (38.4)[a]
		n = 169	*n* = 9	*n* = 46	*n* = 55	*n* = 114
mean (s.d.)	females	17.3 (47.1)	29.5 (59.8)	36.9 (50.2)	35.1 (51.8)	7.8 (41.8)
		n = 92	*n* = 8	*n* = 24	*n* = 32	*n* = 60
	males	10.5 (37.2)	53.8 (n.a.)	10.0 (43.8)	11.9 (43.7)	10.0 (34.5)
		n = 77	*n* = 1	*n* = 22	*n* = 23	*n* = 54
social anxiety	females + males	1.6 (1.5)	1.1 (1.5)	1.3 (1.4)	1.3 (1.4)[a]	1.8 (1.5)[a]
		n = 166	*n* = 9	*n* = 46	*n* = 55	*n* = 111
mean (s.d.)	females	1.5 (1.4)	1.3 (1.6)	1.0 (1.3)	1.1 (1.3)	1.8 (1.5)
		n = 90	*n* = 8	*n* = 24	*n* = 32	*n* = 58
	males	1.7 (1.6)	0 (n.a.)	1.6 (1.6)	1.6 (1.6)	1.8 (1.5)
		n = 76	*n* = 1	*n* = 22	*n* = 23	*n* = 53

[a]Mean oxytocin reactivity and social anxiety differ significantly ($p < 0.05$) between GG + GT and TT genotype groups for females + males (see the text for ANOVA results).

Given that the more oxytocin-reactive genotype group also reported lower social anxiety, a correlation analysis between oxytocin reactivity and social anxiety was performed. Self-reported social anxiety and oxytocin reactivity to the emotional video were uncorrelated (Pearson product–moment correlation = 0.007, $p = 0.93$).

4. Discussion

Our results demonstrate a relationship between oxytocin reactivity and genotypes of the SNP rs13227433, a common polymorphism in the Williams-syndrome-associated gene *GTF2I*. We further show that individuals with more oxytocin-reactive genotypes report lower levels of social anxiety. The lack of correlation between oxytocin reactivity and social anxiety suggests that multiple factors, including some not accounted for in this study, influence these variables, which is not unexpected.

Taken together, the results reported here support a model whereby common genetic variation in *GTF2I* mediates human sociality and anxiety via effects on oxytocin reactivity. Such a model is consistent with previous studies showing that variation in *GTF2I* SNPs is associated with social phenotypes in healthy populations, as described above [11–13], and it supports a hormonal basis for the effects. The mechanisms connecting *GTF2I* with oxytocin remain unknown, but may involve differential methylation of the oxytocin receptor gene *OXTR*, which has been reported among individuals with 7q11.23 deletions and duplications [23], and alternative splicing of *GTF2I* mRNA among individuals with different SNP genotypes, including those analysed here [24].

The relationship of *GTF2I* with oxytocin reactivity is relevant to Williams syndrome as it provides the first evidence that a gene subject to hemizygous deletion in this syndrome, and implicated in its characteristic high empathy and prosociality [8,9], modulates oxytocin reactivity. As such, the reported dysregulation of oxytocin levels and reactivity [7], and the high prosociality, high empathy, and low social anxiety [6] found in Williams syndrome, may arise at least in part from reduced *GTF2I* expression or activity. Further research will increase our understanding of how polymorphisms in *GTF2I*, and other oxytocin-associated genes, have contributed to the evolution of human sociality and disorders.

Ethics. Participants provided informed consent and protocols were approved by the Office of Research Ethics, Simon Fraser University (study no. 2015s0228).

Data accessibility. The dataset supporting this article has been uploaded as part of the electronic supplementary material.

Authors' contributions. T.L.P., N.V.W. and B.J.C. designed the experiment; T.L.P. conducted the experiment; T.L.P., S.R. and J.S. analysed the data; T.L.P. and B.J.C. wrote the manuscript. All authors revised and approved the manuscript and are accountable for its content.

Competing interests. We have no competing interests.

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