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Short Communication

Dysregulation of the oxytocin receptor gene in Williams syndrome

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ABSTRACT

Williams syndrome (WS) is caused by a microdeletion of chromosome 7q11.23, and is characterized by various physical and cognitive symptoms. In particular, WS is characterized by hypersocial (overfriendly) behavior; WS has gained attention as aspects of the WS phenotype contrast with those of autism spectrum disorder (ASD). The oxytocin receptor gene (*OXTR*) contributes to social phenotypes in relation to regulation of oxytocin (OXT) secretion. Additionally, mounting evidence has recently shown that DNA methylation of *OXTR* is associated with human social behavior. However, the role of *OXTR* in WS remains unclear. This study investigated the regulation of *OXTR* in WS. We examined the gene expression levels in blood from WS patients and controls, and then analyzed the methylation levels in two independent cohorts. We showed that *OXTR* was down-regulated and hypermethylated in WS patients compared to controls. Our findings may provide an insight into *OXTR* in mediating complex social phenotypes in WS.

1. Introduction

Williams syndrome (WS) is a genetic disorder caused by heterozygous deletions in chromosome 7q11.23, and displays multiple symptoms, such as cardiovascular disease, endocrine disturbance, and intellectual disabilities (Pober, 2010). Notably, the most prominent feature of WS is hypersociability, commonly described as “cocktail party personality”, that is, an overfriendly, talkative personality, with little fear of strangers (Pober, 2010). Aspects of this social behavior seem to be the opposite of autism spectrum disorder (ASD), which is characterized by social and interpersonal dysfunction. Therefore, WS has raised increasing attention as a model for studying the neural basis of social behavior. However, the genetic basis of its phenotypes, including hypersociability, remains to be fully explained.

Oxytocin (OXT) is a neuropeptide synthesized in the paraventricular and supraoptic nucleus of the hypothalamus, and plays an important role in the regulation of complex social cognition and behavior (Meyer-Lindenberg et al., 2011). OXT is considered to exert its effects through the oxytocin receptors synthesized from the oxytocin receptor gene (*OXTR*), which is located on chromosome 3p25.3. Numerous studies have reported links between single nucleotide polymorphisms (SNPs) in *OXTR* and ASD, depression, and schizophrenia (Kraaijenvanger et al., 2018). In addition to SNPs, recent reports have suggested that epigenetic modification of *OXTR* may play a role in social behavior (Tops et al., 2019).

DNA methylation is a major epigenetic modification that is involved in regulation of gene expression. In particular, DNA methylation of *OXTR* has been shown to be associated with human social behavior and brain activity during social-cognitive processing. A recent review suggested that increased levels of *OXTR* methylation was associated with general impairments in social, cognitive, and emotional functioning (Maud et al., 2018). Furthermore, multiple studies showed that DNA methylation of *OXTR* increased in ASD (Gregory et al., 2009; Rijlaarsdam et al., 2017). Meanwhile, a recent perspective article (Haas and Smith, 2015) proposed an epigenetic model involving reduced methylation of *OXTR* in WS, based on the findings of overexpression of *OXTR* in WS in a prior transcriptome study (Henrichsen et al., 2011). However, it remains unknown whether DNA methylation of *OXTR* is associated with WS.

The aim of the current study was to examine whether *OXTR* is

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dysregulated in WS. To this end, we investigated the levels of *OXTR* expression in the blood from WS patients and controls, and then examined the methylation levels obtained from two independent cohorts.

2. Materials and methods

2.1. Participants

We used three datasets in this study. The expression set for gene expression analysis consisted of 15 patients with WS and 15 age–-sex–race balanced Japanese controls. WS patients were recruited from Kyoto University, Osaka City General Hospital, and Todaiji Ryoiku Hospital for Children. The diagnosis of WS was confirmed by fluorescent in situ hybridization and the degree of social function was evaluated by using the Social Responsiveness Scale (SRS-2) (Bruni et al., 2014). For DNA methylation analysis, the methylation sets A and B were used. Methylation set A consisted of 34 patients with WS and 34 age–sex–race balanced Japanese controls (GSE119778). Methylation set B consisted of 20 pediatric patients with WS and 15 age-balanced controls of European ancestry (GSE66552) (Strong et al., 2015). The controls in the expression set and methylation set A had no history of psychiatric illness or current physical illness, and were recruited from the general community through advertising. All Japanese participants were non-smokers and had not received any psychotropic medication for at least 3 months before collection of blood samples. Demographic and clinical characteristics are shown in Supplementary Table 1. This study was approved by the ethics committees of each participating institution, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and/or their parents.

2.2. Gene expression analysis

Total RNA was extracted from the peripheral blood samples using Paxgene Blood RNA kit (Qiagen, Tokyo, Japan). RNA quality was confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Tokyo, Japan). Reverse transcription of total RNA was performed with a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Yokohama, Japan). *OXTR* expression level was measured by Taqman Gene Expression Assays using the ABI 7900 H T Fast Real-Time PCR System (Thermo Fisher Scientific), and normalized relative to *GAPDH* expression using the ddCt method. All primers are listed in Supplementary Table 2.

2.3. DNA methylation analysis

Two independent DNA methylation datasets were used (Supplementary Table 1). Methylation sets A and B consisted of blood DNA methylation data from the Illumina 450 K array. Methylation set A is our generated dataset (GSE119778), which has been submitted for publication elsewhere. Methylation set B, which is publicly available (GSE66552) (Strong et al., 2015), was downloaded from the Gene Expression Omnibus (GEO) database and analyzed by using the GEO2R online tool.

2.4. Blood-brain correlations of the significant CpG sites

The Blood Brain DNA Methylation Comparison Tool (https://epigenetics.essex.ac.uk/bloodbrain/) was used for comparison of methylation status between blood and brain tissue samples (Hannon et al., 2015). This tool includes methylation data in matched blood and four different brain region (prefrontal cortex, entorhinal cortex, superior temporal gyrus, and cerebellum) samples obtained from 71 to 75 individuals. Therefore, it is possible to estimate the degree of brain DNA methylation from blood DNA methylation. Correlations were assessed using the Pearson's correlation coefficient.

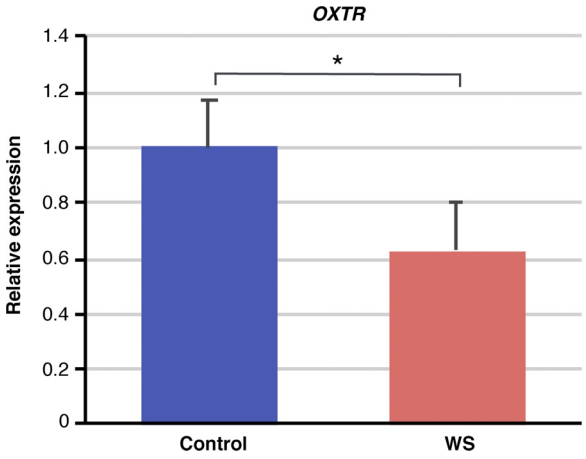

Fig. 1. Gene expression levels of *OXTR*.
The gene expression level was normalized to that of the internal control glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). The Mann-Whitney U-test was used for the statistical analysis. Asterisk indicates $p < 0.05$.

2.5. Statistical analysis

All statistical analyses were performed with R. Statistical differences in gene expression levels between two groups were carried out using Mann–Whitney U-tests. Significant differences in methylation levels between two groups were assessed with the false discovery rate (FDR) using the Benjamini–Hochberg method.

3. Results

3.1. OXTR expression

We sought to determine the levels of *OXTR* expression in WS patients and controls using the expression set, which consists of high-quality RNA with an RNA integrity number (RIN) over 8. RIN scores did not significantly differ between the two groups (Supplementary Table 1). Notably, we found that WS patients showed significantly lower expression of *OXTR* compared to controls ($p < 0.05$; Fig. 1). We then examined the correlation between *OXTR* expression and social function of WS patients. SRS-2 were used to evaluate the social function of WS, and showed total T-scores of 63.3, which implies mild to moderate impairment. However, there was no significant correlation between the expression levels of *OXTR* and total SRS T- scores (Supplementary Fig. 1). Taken together, these results highlighted that *OXTR* expression was down-regulated in WS compared to controls, although no clear correlation between expression levels and severity of social function was found.

3.2. OXTR methylation

DNA methylation in gene promoter regions typically acts to repress gene expression. Thus, to examine the effects of DNA methylation on the gene expression of *OXTR*, we compared the methylation of 17 CpG sites of *OXTR* in WS patients and controls using methylation set A. We found that WS patients had significantly higher methylation at three CpG sites (cg25140571, cg00247334, and cg17036624) around the transcriptional start site (TSS) than did the controls (FDR < 0.05; Fig. 2 and Supplementary Table 3). Next, we sought to confirm these results using an independent cohort, methylation set B. This cohort has different backgrounds compared to the cohort in methylation set A, such as age and races. Similar to set A, we found that WS patients had significantly higher levels of methylation at those CpG sites than did the controls (FDR < 0.05; Supplementary Table 4). It was important to establish whether the methylation we observed in the blood was

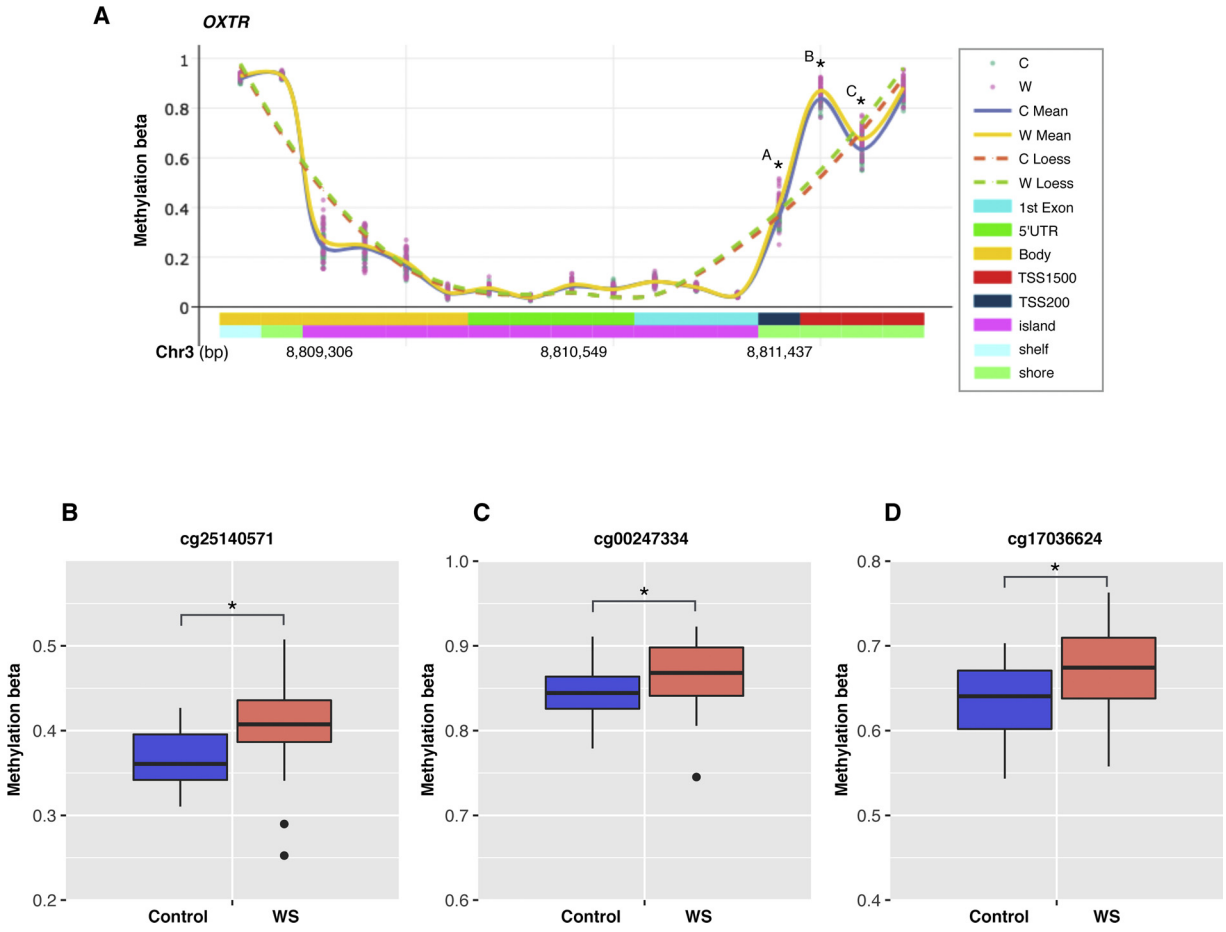

Fig. 2. Methylation levels of CpG sites in *OXTR*.
(A) Scatter plots showing *OXTR* methylation in Williams syndrome patients and controls. Data points are methylation levels for each probe in individual samples based on GSE119778. (B) cg25140571, (C) cg00247334, (D) cg17036624. The false discovery rate (FDR) was estimated using the Benjamini–Hochberg approach. Asterisks indicate FDR < 0.05. The line within the box represents the median. C, Control; W, Williams syndrome. 5′UTR, 5′ untranslated region; Body, gene body; TSS1500, 200 − 1500 bases upstream from the transcriptional start site; TSS200, 0 − 200 bases upstream from the transcriptional start site; island, CpG island; shore, up to 2 kb from CpG island; shelf, 2 − 4 kb from CpG island.

correlated with the methylation status in the brain. Due to the difficulty of obtaining brain samples, we used the Blood Brain DNA Methylation Comparison Tool for this investigation. Three CpG sites (cg25140571, cg00247334, and cg17036624) were significantly correlated with the superior temporal gyrus (Supplementary Table 5). In particular, cg25140571 was also strongly associated with the prefrontal cortex. Taken together, our findings highlighted that three CpG sites around the TSS of *OXTR* were hyper-methylated in WS patients compared to controls, independent of age and race.

4. Discussion

The current study shows that *OXTR* was down-regulated and hypermethylated in WS patients compared to controls. In particular, three CpG sites around the TSS of *OXTR* demonstrated increased methylation levels in WS patients compared to controls, and these results were confirmed in different ethnic groups. Our results seem to be consistent with those of previous studies that showed increased methylation of *OXTR* in ASD (Gregory et al., 2009; Rijlaarsdam et al., 2017). In addition, we found that the identified hypermethylated CpG sites in WS patients were significantly correlated with the superior temporal gyrus and/or prefrontal cortex, both of which are known to mediate social function. This seems to show a similar tendency to a prior ASD study, which reported that some CpG sites within the promoter region of *OXTR* were hypermethylated in both cortex and peripheral blood (Gregory et al., 2009). These results may provide further support for the hypothesis that WS and ASD patients share common dysregulated pathways in the OXTR system.

It has been considered that WS and ASD are contrasting in their social phenotypes. However, both WS and ASD have been shown to have poor social outcomes. A recent study showed similarities in the interpersonal distance during social interactions of WS and ASD patients, based on parent-reported SRS scores (Lough et al., 2015). In addition, it appears that social behavior characteristics of WS includes a subtle spectrum of distinct socially positive and maladaptive behaviors, which differ from those seen in typical extraversion (Järvinen et al., 2013). Therefore, these findings provide support for our results, which indicate that WS and ASD are closely related though they may superficially appear different in their social phenotypes. Further, multiple brain pathways including the OXTR system might be dysregulated and contribute to social functioning in WS.

It remains unclear, particularly at the mechanistic level, how *OXTR* is hypermethylated in WS. Multiple genes related to epigenetic regulation are located within deleted chromosomal regions of WS. For instance, the BUD23 RRNA methyltransferase and ribosome maturation factor gene (*BUD23*) encodes a methyltransferase, and the NOP2/Sun RNA methyltransferase 5 gene (*NSUN5*) which encodes a cytosine-5 RNA methyltransferase. Furthermore, an epigenetic-wide association study of WS demonstrated that DNA methylation was altered throughout the genome (Strong et al., 2015). Therefore, these findings

provide a clue that the genes deleted in the 7q11.23 region could trigger epigenetic disturbance, and impacting the rest of the genome.

With regard to OXT in WS, one pioneer study showed that basal OXT levels were increased in WS patients compared to controls, and could be related to the social behavior in WS (Dai et al., 2012). Although this study had a limited sample size, it revealed that higher levels of basal OXT were correlated with increased approach to strangers, and decreased adaptive social behaviors. Unexpectedly, our findings were in the opposite direction to the dysregulation that has been reported in WS (Haas and Smith, 2015). This previous study, based on public gene expression data (Henrichsen et al., 2011), revealed increased expression of *OXTR* in WS patients compared to controls (Haas and Smith, 2015). One of the reasons for the inconsistent findings could be RNA quality and sex bias. The previous report relied on a small sample of only females and lacked RNA quality details.

There were several limitations to this study. First, the sample size was relatively small due to the rare disease presentation. Second, we showed no clear correlation between the *OXTR* expression levels and the severity of social behavior problems in WS patients. It may be inappropriate to use the SRS-2 to assess the complicated social behavior in WS, as it is designed to measure the severity of ASD with a focus on social impairment. Third, we could not examine the relationships between levels of OXT and dysregulation of *OXTR* in WS, due to the difficulty of obtaining OXT from patients under controlled conditions. Furthermore, it should be noted that methylation and expression of *OXTR* were not directly compared with data from an ASD cohort. It may also be worth noting that it remains unclear if the CpG sites identified are indeed responsible for the noted decreased expression.

In summary, to our knowledge, no previous study has reported *OXTR* dysregulation using blood samples from WS patients. We found that *OXTR* was down-regulated and hypermethylated in WS patients. Further studies are necessary to elucidate the biological underpinnings of social cognition and behavior in WS.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.104631.

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