Molecular Features of Therapeutic Response to Oxytocin in Autism Spectrum Disorder

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Background

Prevalence of Autism Spectrum Disorder

The 2018 CDC report determined that 1 in 59 (~1.7%) of the children born in the United States will be diagnosed with autism spectrum disorder (ASD).

Limitations of Current Interventions

Deficits in social motivation and engagement are core clinical features of ASD yet there are no FDA-approved pharmaceuticals to treat these deficits. Oxytocin, an endogenously expressed neuropeptide with demonstrated pro-social effects, is currently being evaluated to address this need through the NIH-funded phase-2 clinical trial "Study of Oxytocin in Autism and Reciprocal Social Behavior (SOARS-B)[clintrials.gov - NCT01944046]. This study is unique because of its size (n=290), it includes males and females (ages 3-17), and high functioning (HF) and low functioning (LF) individuals defined by verbal status and IQ. Up to 48 IUs of oxytocin was delivered via a twice-daily intranasal spray for up to 48 weeks and clinical response was evaluated using a number of behavioral metrics including the ABC-SW subscale and the Vineland 2 adaptive behavior composite

Rationale for Molecular Profiling

While previous studies have determined that treatment with oxytocin is well-tolerated, its efficacy for improving deficits in social behavior is less clear. Prosocial effects appear to be sensitive to endogenous levels of circulating oxytocin, indicating that baseline molecular testing could be used to identify those who would benefit from oxytocin treatment. SOARS-B is both the largest and longest-running of such studies to-date, and we aim to expand the repertoire of predictive biomarkers by integrating the four following datasets with clinical outcomes.

- Gene Expression
- DNA Methylation
- Copy Number Variants (CNVs)
- Single Nucleotide Polymorphisms (SNPs)

Study Design

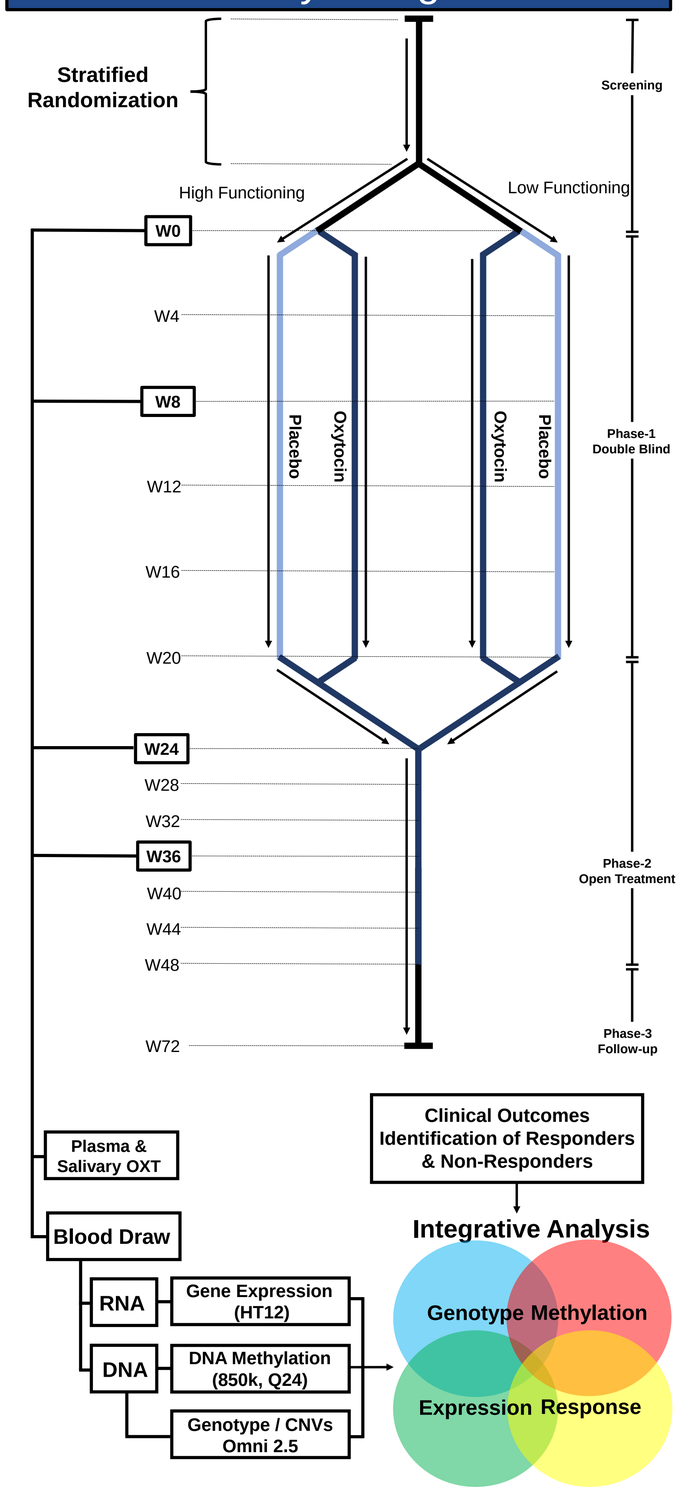

Figure 1: Graphical representation of the SOARS-B study design. Individuals with a prior diagnosis of ASD were assigned to HF or LF strata, and entered into the randomized double-blind phase where they received twice-daily doses of either oxytocin or vehicle-placebo. Blood-draws were taken during clinical visits at the highlighted time-points and sent to the Duke Biobank for DNA/RNA extraction prior to molecular profiling.

Gene Expression Data

Differential Expression Due to Oxytocin Treatment

Table 1: Summary table of gene expression data generated on the Illumina HT12 array comparing pre-oxytocin-dose samples to their most proximal post-oxytocin-dose. Significant differential expression is observed in a subset of immune-related genes which may reflect the immunomodulatory effects of oxytocin on the peripheral immune system.

Symbol	logFC	t	P-Value	Adj. P-Value	B
MAPKAP1	**0.09**	**4.87**	**1.55499E-06**	**0.04**	**4.89**
CCR2	**0.25**	**4.66**	**4.15555E-06**	**0.04**	**3.99**
SH3BP4	**0.08**	**4.66**	**4.25149E-06**	**0.04**	**3.97**
GRAMD3	**0.11**	**4.56**	**6.70306E-06**	**0.04**	**3.56**
CYBB	0.18	4.35	1.6979E-05	0.07	2.72
LOC283267	0.17	4.35	1.72058E-05	0.07	2.71
PLEKHG7	-0.11	-4.30	2.12322E-05	0.07	2.52
HIST1H2BO	-0.08	-4.28	2.31983E-05	0.07	2.44
ALG12	-0.12	-4.25	2.67165E-05	0.07	2.31
LCN1L1	-0.12	-4.14	4.2021E-05	0.07	1.90

DNA Methylation of the Oxytocin Receptor (OXTR)

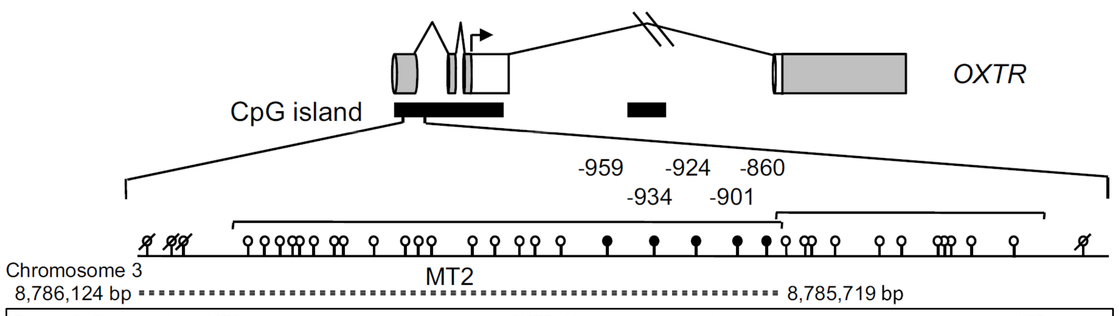

Figure 2: Scheme of the MT2 region proximal to the promotor of OXTR. Hypermethylation of this region was previously observed in ASD in both peripheral blood and post-mortem brain samples (Gregory 2009). In this study, DNA methylation in MT2 is profiled through targeted pyrosequencing of bisulfite converted DNA from all participant samples which enables us to determine the effect of baseline methylation on clinical outcomes, and any dynamic changes to methylation in response to chronic treatment with oxytocin.

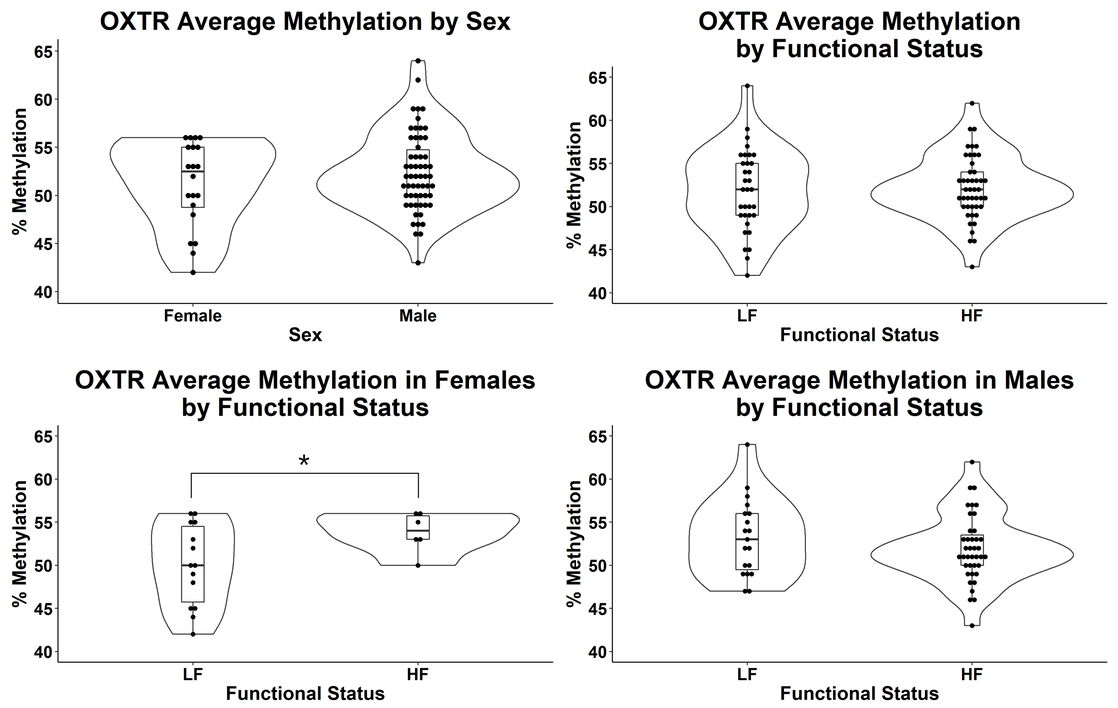

Figure 3: Violin Plots of the distribution of methylation values for all currently profiled SOARS-B participants, stratified by: **A):** All time-points from individuals divided by sex; **B)** All time-points from individuals separated by functional status; **C)** All time-points for female participants separated by functional status **(p-value = 0.026)**; **D)** All time-points from male participants separated by functional status. [Significance determined via independent two-group t-test, * indicates $p < 0.05$]

Enrichment of CNVs

	Autosome Only		With ChrX	
	Averge # CNVs	Average Cumulative Length	Averge # CNVs	Average Cumulative Length
Male	8.4	307,505	8.4	310,675
Female	9.9	415,174	10.1	425,209
HF	8.2	308,841	8.3	313,780
LF	9.0	335,122	9.1	338,360

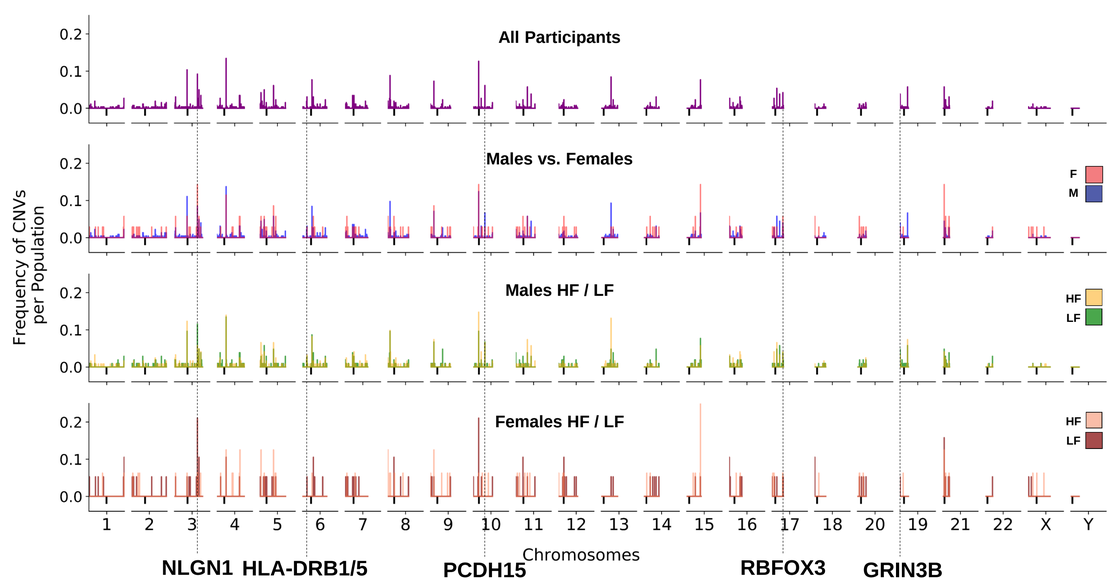

Figure 4: Summary plot of CNV enrichment (both deletions and duplications, CNVs > 10kb in size) within our study population stratified by sex and functional status. Peaks represent the frequency of observing a CNV at the given genomic location within a 100kb window in the specified clinical population. Preliminary analysis identifies 55 genes which fall within these regions, several of which are associated with neurological development or neurodegenerative disease (highlighted above). Additionally, NLGN1, HLA-DRB1, and PCDH15 have been previously implicated in the etiology of ASD.

Discussion

These data represent the multiple modalities that will be utilized to identify clinically-relevant biomarkers in the context of oxytocin treatment. Presently, only broad characterizations of our study population are possible. Once SOARS-B concludes its primary analysis, information on plasma levels of oxytocin, responder status, and specific behavioral subscales will be incorporated into our own analysis.

Gene Expression

Only broad comparisons between treatment status are presently available. We identified a slight but significant and positive change in gene expression in immune-related genes which may be a consequence of oxytocin's immunomodulatory activity. It is important to note that since our first available time-point is 8 weeks after the start of treatment, any transient changes following initial dosing will be difficult to detect. Once responders are identified, similar comparisons can be made between individuals who showed improvement and those who did not during oxytocin treatment. Additionally, we can determine if baseline expression profiles predict therapeutic response.

DNA Methylation

There is a high degree of variability in the extent of OXTR methylation within our study population. In females, hypomethylation of OXTR is significantly enriched in LF individuals. While this seems to conflict with prior assumptions about the role of OXTR hypermethylation in ASD, it is important to note the low number of female participants (14 HF, 6 LF), as well as the possibility that the standards for defining functional status (IQ and verbal status) may not be as closely linked to OXTR methylation as other social metrics.

Copy Number Variants

Regional enrichment of copy number variants can be observed at multiple locations across the genome. These enriched regions encompass 55 genes, several of which have been previously implicated in the etiology of ASD. Variation in these CNVs shows both sex- and functional-specificity at certain regions, which will be incorporated into our analysis when identifying markers of response.

Future Directions

Single Nucleotide Polymorphisms

While this study is not powered to perform a GWAS, the Omni 2.5 platform enables us to determine the impact of previously identified risk variants in the novel context of oxytocin response. Identifying enrichment of specific SNPs in the oxytocin signaling pathway which predict clinical response will improve therapeutic targeting and provide insight into the mechanisms of response.

Methylome-wide profiling

To expand upon our understanding of how epigenetic regulation mediates oxytocin response, we will utilize an array-based platform to characterize genome-wide patterns of DNA methylation. This will enable the integration of gene expression data with a broader range of epigenetic regulatory markers which could be used independently to find predictive biomarkers of response.

Acknowledgements

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