Piloting Treatment With Intranasal Oxytocin in Phelan-McDermid Syndrome
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Specific Aims

Gene discovery approaches, followed by functional analysis of model systems, have elucidated the neurobiology of several genetic subtypes of autism spectrum disorder (ASD) and led to important opportunities for developing disease modifying novel therapeutics. ASD can now be conceived of as having multiple distinct genetic risk loci and one example is *SHANK3*, where haploinsufficiency through deletion or mutation causes Phelan-McDermid syndrome (PMS), which is characterized by global developmental delay, motor skills deficits, delayed or absent speech, and ASD. *SHANK3* is the critical gene in this syndrome (Bonaglia et al., 2006; 2011; Durand et al., 2007) and recent studies indicate that loss of one copy of *SHANK3* causes a monogenic form of ASD with a frequency of at least 0.5% of ASD cases and up to 2% of moderate to profound intellectual disability (Leblond et al., 2014). *SHANK3* codes for a master scaffolding protein which forms a key framework in the postsynaptic density of glutamatergic synapses and plays a critical role in synaptic function (Boeckers et al, 2006). Although *SHANK3* deficiency accounts for a relatively small proportion of ASD cases, recent evidence suggests that the *SHANK3* and glutamate signaling pathways are common to multiple forms of ASD and that many different genetic causes of ASD may converge on several common pathways (Darnell et al., 2011; Sakai et al., 2011). Large-scale analyses also show that *SHANK3* and associated pathways are implicated in multiple forms of ASD, including tuberous sclerosis and Fragile X syndrome (Darnell et al., 2011; Sakai et al., 2011). Using *Shank3*-deficient mice and rats, our group has documented specific deficits in synaptic function and plasticity in glutamate signaling (Bozdagi et al, 2010). Importantly, recent data from our group demonstrate that oxytocin reverses the neurobiological deficits in both social behavior and hippocampal synaptic plasticity in *shank3*-deficient rats (Harony-Nicolas et al., unpublished; see Figure 1).

The proposed project will pilot the use of oxytocin as a novel treatment in PMS. We will use a double-blind, placebo-controlled parallel group design in 40 children with PMS to evaluate the impact of oxytocin on impairments in attention, social memory, socialization, language, and repetitive behaviors. In addition to utilizing standard clinical trial methods, a critical part of this project is to pilot *objective* outcome measures of attention, social memory, and language in this severely impaired population. Oxytocin is an FDA-approved, commercially available medication that can be compounded into an intranasal solution for passage through the blood-brain barrier (BBB). In animal models, oxytocin has been demonstrated to increase eye contact, social approach, social recognition, social memory, and to reduce stress responses (Takayangai 2005; Liu 2008, Insel 2010). Oxytocin also influences social behavior in people and increases gaze to eye regions, social cognition, social memory, positive communication, empathy, perceptions of trustworthiness, and cooperation within one's own group (Guastella 2008a, 2008b; Domes 2007; Di Simplicio 2008; Fischer-Shofty 2009; Keri 2009; Unkelbach 2008; Savaskan 2008; Rimmele 2009; Dizten 2008; Zak 2005, 2007; Theodoridou 2009; Pterovic 2008; Kosfeld 2005, Baumgartner 2008; De Dreu, 2010). Specifically in ASD, oxytocin has shown promise for modifying social behavior in mouse model systems and in affected individuals. *Importantly, oxytocin is effective in reversing phenotypic and electrophysiological changes in rat models of Phelan-McDermid syndrome (Harony-Nicolas et al., unpublished), providing additional evidence that this pathway may be a target in diverse forms of ASD.*

Aims are to:

1) Evaluate safety, tolerability, and feasibility of oxytocin vs. placebo in children with PMS targeting social withdrawal using the Aberrant Behavior Checklist – Social Withdrawal subscale (ABC-SW; Aman et al., 1985) and other secondary outcome measures of language, repetitive behavior, and global improvement.

2) Evaluate safety, tolerability, and feasibility of oxytocin vs. placebo in children with PMS targeting attention and social memory using an objective Visual Paired Comparison Task (VPC; Rose et al., 2009) with eye tracking technology.

3) Explore the feasibility of objective assessments of expressive language and social attention using Language Environment Analysis (LENA) and the Social Orienting Task (Dawson et al., 2004) respectively.

Our working hypothesis, supported by evidence in our rodent model, is that oxytocin will be safe, well tolerated, and associated with improvement on the VPC task and the ABC-SW subscale compared to placebo by positively modifying attention, social cognition, and social functioning. At the conclusion of this study, we also expect that objective measures, such as automated analysis of natural language samples (i.e., LENA) and social orienting, will successfully measure expressive language and social attention and that treatment with oxytocin will be associated with improvement on these measures. This project has the potential to further the treatment of single gene disorders associated with ASD and may shed light on pathways relevant to the treatment of ASD more broadly.

RESEARCH STRATEGY

Significance. The genetic liability to autism spectrum disorder (ASD) is now understood to be due in part to rare genetic variants where isolated gene or chromosomal abnormalities contribute a major part of the susceptibility for an affected individual. Several of the ASD genes identified to date, including *SHANK3*, are involved in the neuroligin-neurexin pathway at glutamatergic synapses (Bonaglia et al, 2011; Durand et al., 2007; Gauthier et al., 2009; Guilmatre et al., 2009; Moessner et al., 2007; Rosenfeld et al., 2010; Schaaf et al., 2011; Sebat et al., 2007). Glutamate signaling is also highly relevant to these pathways and to various forms of ASD. *SHANK3* is a protein found in glutamatergic synapses that helps form the postsynaptic density, and upon which glutamate receptors are clustered (Sheng and Kim 2000; Boeckers 2006; Kreienkamp 2008). There is now abundant evidence that *SHANK3* is a key regulator of synapse development and function and a critical protein for many synaptic processes. Loss of *SHANK3* causes Phelan-McDermid syndrome (PMS), a monogenic cause of ASD and intellectual disability (ID) responsible for between 0.5% and 2% of cases collectively. Importantly, large-scale analyses show that *SHANK3* and associated pathways are implicated in multiple forms of ASD and ID, including tuberous sclerosis and Fragile X syndrome (FXS) (Darnell et al., 2011; Sakai et al., 2011). According to the Interagency Autism Coordinating Committee 2011 Strategic Plan and subsequent updates, there is a need for translational research that takes advantage of genetic findings in order to: 1) develop animal models to understand the effects of these genetic variants on brain function, 2) inform which molecular signaling pathways are affected in ASD, and 3) discover specific targets for the development of novel therapeutics. Following this approach, our group developed *Shank3*-deficient rats and discovered that behaviorally, long-term social recognition memory and attention were impaired, with no significant change in contextual fear memory, social preference, or short-term social discrimination. Additionally, in the hippocampus and medial prefrontal cortex (mPFC), our group also found impairments in long-term potentiation (LTP) long-term depression (LTD), but not in NMDA-dependent LTD (Harony-Nicolas et al., unpublished). Treatment with oxytocin was then tested and found to reverse electrophysiological and behavioral deficits in *Shank3* heterozygous and knockout rats (see Figures 1-3). The *SHANK3* / glutamate signaling pathway is highly relevant to various forms of ASD; the link between deficits in synapse function and ASD suggest that treatment with oxytocin may have implications for ASD associated with disruptions in common underlying pathways, and this is supported by ongoing studies of oxytocin in idiopathic ASD. *This contribution will be significant because we aim to pilot a potentially disease-modifying treatment that has the potential to shed light on pathways more broadly relevant to ASD treatment.*

Innovation. To date, the development of pharmacological treatments in ASD has mainly relied on strategies only loosely related to what is known about the neurobiology of the disorders, using etiologically heterogeneous samples, and delivering intervention broadly with mixed success. More recently, genetic discovery and model systems have led to important opportunities for developing novel, disease-modifying therapeutics. The proposed project represents a unique effort between clinical and basic science resources and draws upon the experience of clinicians with recognized expertise in PMS and ASD. This work is based on preclinical evidence that establishes proof-of-concept with oxytocin reversing electrophysiological deficits in *Shank3*-deficient rats, and on extensive clinical evidence that supplemental oxytocin can impact social behaviors in ASD. A recent example of a similar approach is our group's pilot study of insulin-like growth factor-1 (IGF-1) in PMS (Kolevzon et al., 2014) and ongoing large scale clinical trials in tuberous sclerosis and FXS. Additionally, a large-scale trial of oxytocin in ASD (SOARS-B) is currently underway. *The proposed project is innovative, in our opinion, because it is based on the combination of a previously tested drug development strategy, convincing preclinical evidence with oxytocin in a rat model system, and a safe approved drug. In addition, it is innovative in that we introduce objective measures of attention, social memory, and language in the context of experimental therapeutics.*

Approach.

Aims: Evaluate safety, tolerability, and feasibility of oxytocin vs. placebo targeting attention and social memory using the Aberrant Behavior Checklist Social Withdrawal subscale as the primary outcome measure and additional secondary and exploratory outcome measures including objective assessments of language and social attention.

Introduction. The objective of this aim is to pilot the use of oxytocin treatment in 40 patients with PMS in order to evaluate safety, tolerability, and feasibility for ASD-related deficits. We will approach this aim by employing a placebo-controlled, double-blind, parallel group design with 3 months of treatment with oxytocin or placebo followed by 3 months of open-label treatment with active drug. The rationale is that successful

completion of the proposed research will establish feasibility and contribute pilot data from the second treatment trial of PMS to the field. It may also advance knowledge about developing targeted treatments for additional monogenic causes of ASD associated with impaired synaptic development and function. It is our expectation, based on our rodent model, that the knowledge gained will provide evidence for a therapeutic that has specific and potentially disease-modifying effects on ASD associated with PMS and pave the way for more trials in other subtypes of ASD with disruptions in shared pathways. We expect to add significantly to data from ongoing trials with oxytocin in ASD.

Justification and feasibility. There has been only one controlled treatment trial in PMS to date. Our clinical experience comprehensively evaluating 45 families affected by PMS provides numerous anecdotal reports of medication trials that borrow from the broader ASD literature and target associated symptoms of attention deficit, impulsivity, hyperactivity, irritability, and repetitive behavior. Our group recently published results from a pilot trial with IGF-1 that provides preliminary evidence of safety and efficacy. Nine children with PMS aged 5-15 were enrolled in a placebo-controlled, double-blind, crossover study with three months of treatment with IGF-1 and three months of placebo separated by a four week wash-out period. IGF-1 was well tolerated without serious adverse events and was associated with significant improvement in both social impairment and restrictive behaviors, as measured by the ABC-SW subscale and the Repetitive Behavior Scale respectively. Prior to this study, a literature review revealed only two published reports of medication treatment in PMS, one case study with risperidone (Pasini et al., 2009) and one case series with intranasal insulin (Schmidt et al., 2008), both with reported improvement. Results from the case study with risperidone were optimistic but uncontrolled: an 18 year old girl with PMS was treated with risperidone 0.5 mg BID with significant improvement on the Clinical Global Impression Scale (CGI; Guy 1976) in anxiety, aggression, and insomnia after one month that was sustained after six months. In the intranasal insulin study, six children received up to 12 months of treatment with improvement based on sum of scores from a parental questionnaire in most domains, including cognitive, speech and language, and motor skills (Schmidt et al., 2009). Insulin was hypothesized in these cases to improve neuronal function by increasing central nervous system (CNS) glucose uptake and enhance synaptic plasticity via glutamatergic receptors.

Results from treatment with oxytocin in our *Shank3*-deficient rat model are extremely intriguing (see Preliminary Studies). Oxytocin has also shown promise for modifying social behavior in mouse models and in people with ASD. Oxytocin is the brain's most abundant neuropeptide. It can act as a classical neurotransmitter, a neuromodulator and a hormone with actions throughout the body (Gimple 2001, Veening 2010, Baskerville 2010). Oxytocin's half-life in the plasma is 1-2 minutes compared to ~30 minutes in the CSF. Central release of oxytocin is dependent upon CD38 and dramatically stimulates further release of oxytocin (~1000 fold) and increases the number of oxytocin-containing cells in the periventricular nucleus. Together these factors lead to long lasting oxytocin elevations throughout the brain following acute increases in CSF oxytocin. In the oxytocin receptor null mouse, a single dose of oxytocin improved social deficits, reduced aggression and reduced vulnerability to drug-induced seizures, apparently acting through the vasopressin 1A receptor. Further, repeated doses of oxytocin improved reversal learning in these mice, indicating enhanced cognitive flexibility (Sala 2011). A single dose of oxytocin also improved social deficits in *Cd38* null mice (Higashida, 2010).

In people with Fragile X syndrome, a single gene cause of ASD, oxytocin improved gaze avoidance and reduced cortisol elevations elicited by social interaction (Hall 2011). In high functioning individuals with idiopathic ASD, single doses of oxytocin enhanced attention to faces and eyes, visual and auditory affect recognition, the ability to distinguish whether others were being cooperative, and preference for interacting with receptive individuals (Andari 2010; Gustalla 2010; Hollander 2007). Oxytocin challenge has also been shown to reduce compulsive and repetitive behaviors (Hollander et al., 2003).

Sustained oxytocin treatment in autism has been the subject of limited study and results to date are mixed. One pilot study with intranasal oxytocin found improvements in measures of social cognition (Reading the Mind in the Eyes Test, Baron Cohen et al., 1997) and quality of life (World Health Organization Quality of Life Questionnaire, 1998) in a 6-week randomized, double-blind, placebo-controlled, parallel design trial of intranasal oxytocin versus placebo in 19 adults with ASD (Anagnostou et al., 2012). In another double-blind, placebo-controlled, 50 male participants with Autism or Asperger's syndrome were randomized to receive either oxytocin or placebo nasal sprays, administered twice-daily for 8 weeks (Guastella et al., 2014). Participants who received oxytocin showed no benefit following treatment on primary or secondary outcomes. However, caregivers who believed their children received oxytocin reported greater improvements compared to

caregivers who believed their child received placebo. In a 7-month, single-armed, open-label study of intranasal oxytocin in 8 male youth with ASD, six of the eight participants showed improved scores on the communication and social interaction domains of the ADOS-G, but no statistically significant improvement was seen in the Child Behavior Checklist (CBCL; Achenbach et al., 2000) or the ABC (Tachibana et al., 2013). Several other chronic treatment studies are underway in idiopathic ASD, including a large, multi-centered randomized controlled trial funded by the National Institute of Child Health and Development (PI: Sikich).

Preliminary Studies. Our group studied both mice and rats with a targeted disruption of Shank3, in which exons coding for the ankyrin repeat domain (ARD) were deleted and expression of full-length *Shank3* was disrupted (note that a point mutation in the ARD has been described in a child with autism; Moessner et al., 2007). We examined synaptic transmission and plasticity by multiple methods and results indicate a reduction in basal neurotransmission in *Shank3* heterozygous and homozygous mice (Bozdagi et al., 2010; Yang et al., 2012) and in *Shank3*-deficient rats (homozygotes and heterozygotes) (Harony-Nicolas et al., unpublished).

Studies with a specific AMPA receptor antagonist and NMDA receptor antagonist demonstrated that the decrease in basal transmission reflected reduced AMPA receptor-mediated transmission. LTP was impaired in *Shank3* heterozygous mice, with no significant change in LTD (Bozdagi et al., 2010). In concordance with the LTP results, persistent expansion of spines was observed in control mice after theta burst pairing-induced LTP; however, only transient spine expansion was observed in *Shank3* heterozygous mice. Behaviorally, male *Shank3* heterozygotes also displayed less social sniffing and emitted fewer ultrasonic vocalizations during interactions with estrus female mice, as compared to wild-type littermate controls (Bozdagi et al., 2010).

In *Shank3* heterozygous and knockout (KO) rats, LTP was significantly impaired, with significant deficits in mGluR-dependent LTD in *Shank3* homozygous (KO) rats only, and no significant change in NMDA-dependent LTD. In concordance with the LTP results, high frequency stimulation of the hippocampus and prefrontal cortex showed impaired LTP in both *Shank3*-Het and KO rats. Behaviorally, no significant differences between WT and *Shank3*-deficient rats were found using social preference, juvenile social play, habituation-dishabituation social recognition, or short-term social discrimination. In contrast, both homozygous and heterozygous rats showed impaired long-term social recognition memory (SRM) when tested on the long-term social discrimination test (Gur et al., 2014), even as contextual fear memory (Blanchard et al., 1972) was intact. The different outcomes in two tests of hippocampal-dependent long-term memory suggest that *Shank3* deficiency selectively impairs long-term memory for social interactions. Additionally, in assessing attentional ability by applying the 5-choice serial reaction time test (Bussey et al., 2012; Mar et al., 2013), *Shank3*-deficient rats displayed lower mean accuracy levels compared to WT littermates. Taken together, these findings indicate that both attention and long-term SRM are impaired in the *Shank3*-deficient rat model.

Oxytocin has a well-studied pro-social effect on mammalian social behavior in general (Meyer-Lindenberg et al., 2011; Insel, 2010) and has been directly implicated in SRM (Bielsky et al., 2004; Choleris et al., 2009). Given this, and the potential association between oxytocin and ASD, our group examined the effect of oxytocin on social recognition impairment in the *Shank3*-deficient rats. Our data show that intracerebroventricular (ICV) injections of oxytocin reversed impairments in long-term social recognition memory seen in the *Shank3* deficient rats. We also found that oxytocin reversed the impairment in the maintenance of hippocampal LTP in *Shank3*-deficient rats, without an effect on mGluR-LTD deficit in the Shank3-KO rats, suggesting a specific mechanistic relationship between deficits in, and oxytocin-induced rescue of, social memory, attention, and hippocampal synaptic plasticity. In the proposed trial, we will be using the dose of 24 IU BID, as the dose used in the ICV injections in the rat study will not be applicable in human subjects. 24 IU BID is the typical dose of oxytocin used in clinical trials.

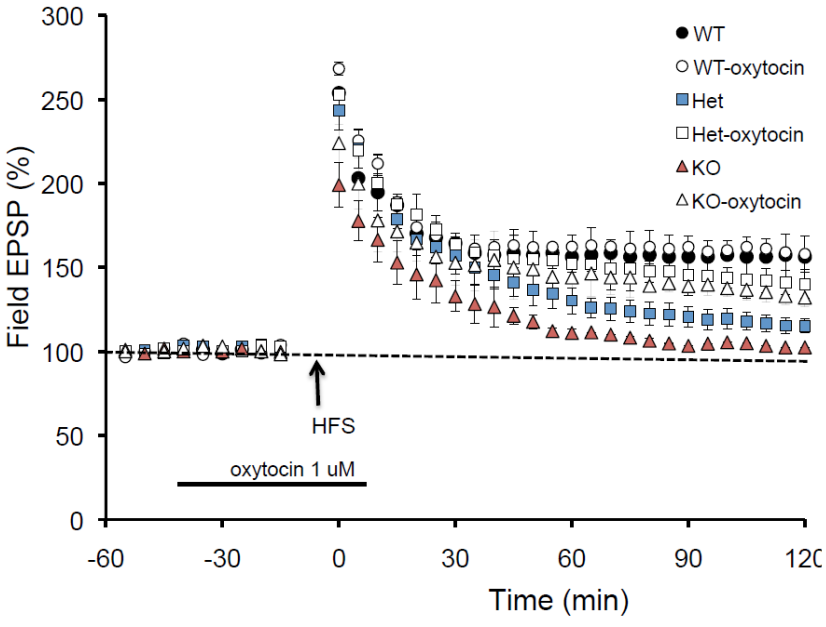


Figure 1. Reversal of electrophysiological deficits in hippocampal slices from *Shank3*-deficient rats (n=4/genotype) as compared to wild-type mice (WT) after treatment with oxytocin.

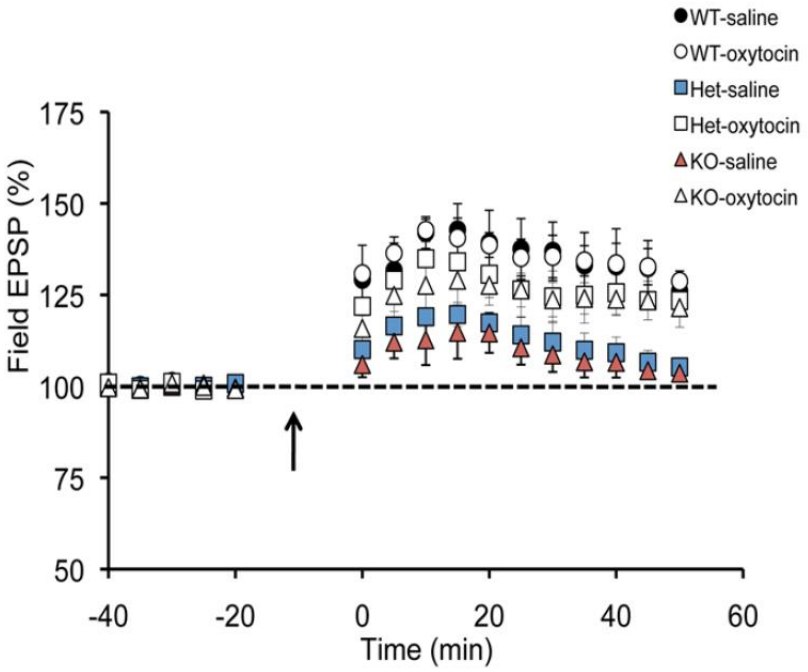
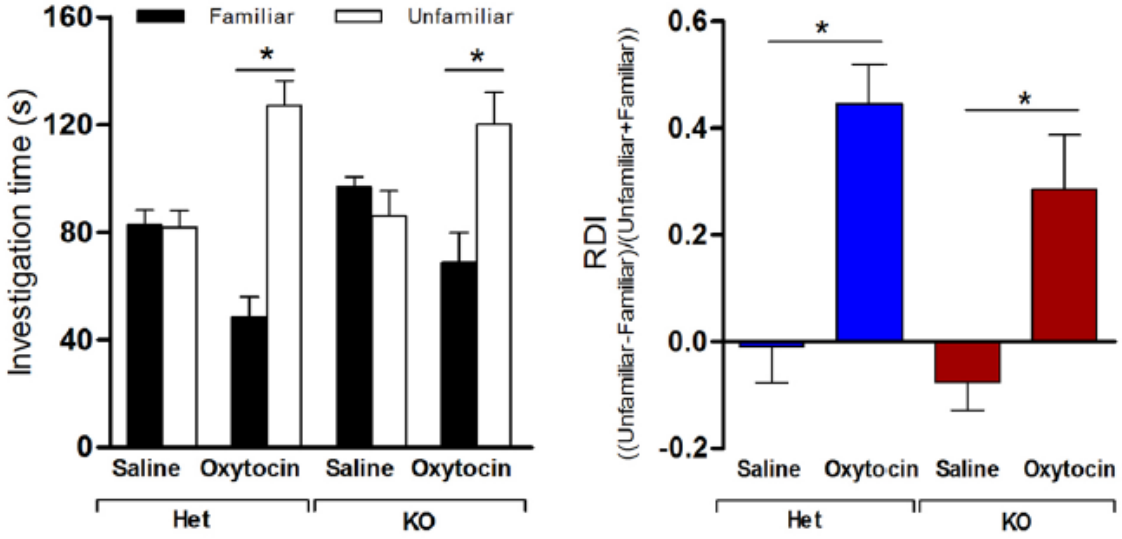


Figure 2. Reversal of induced hippocampal-prefrontal LTP deficits *in vivo* from Shank3-deficient rats (n=4/genotype) as compared to wild-type mice (WT) after treatment with oxytocin.

Figure 3. Reversal of social discrimination deficits in *Shank3*-Het (n=15) and KO (n=8) on the long-term Social Discrimination (SD) test following treatment with oxytocin. **P*<0.05.



Research design. Treatment will follow a randomized, placebo-controlled parallel group design with 12 weeks in the treatment arm followed by a 12 week open label extension with oxytocin. The trial duration was selected based on what is commonly accepted as adequate in pediatric psychopharmacological trials in ASD, and our primary outcomes, the VPC and ABC-SW, are sensitive to change within this time frame (Rose et al., 2003; Aman et al., 2010).

Inclusion criteria: The proposed pilot will recruit 40 children between 5 and 17 years-old with PMS, many of whom will have already been previously evaluated as part of ongoing studies in our Center. All subjects will have a minimum raw score of 12 on the ABC-SW (see Outcome Measures section). The minimum raw score was selected by adding approximately one standard deviation to the mean ABC-SW subscale score derived from a normative sample of 601 children aged 6-17 with intellectual disability (Brown et al., 2003) and

reflects preliminary data from our group suggesting the mean ABC-SW subscale score is 11.6. Subjects will also be on stable medication and psychosocial therapy regimens for at least three months prior to enrollment.

Exclusion criteria: Cases will be excluded if any of the following are applicable: 1) allergy to oxytocin, 2) active cardiovascular disease or renal disease that is not controlled by medication, 3) are pregnant, lactating or refuse to practice contraception if sexually active, 4) have caretakers who are unable to speak English, be consistently present at visits to report on symptoms, or are otherwise judged unable to comply with the protocol by the study team; 5) comorbid conditions such that the patient is too medically compromised to participate.

Drug Administration: Participants in the study will be randomly assigned to treatment with intranasal oxytocin or placebo. The oxytocin product will use a vehicle designed to optimize nasal absorption manufactured by Mylan in Europe and marketed as Syntocinon™ under an Investigational New Drug Application (IND) from the FDA, (IND application submitted). Participants will start the trial with a dose of 12 IU BID. After the first two-week check in call, if the drug is well tolerated, the dose will be increased to 24 IU BID. Each insufflation will deliver 4 IU and three insufflations (12 IU) in each nostril will be given twice daily for a total daily dose of 48 IU. The dose of 24 IU BID was chosen because it is the most commonly used in the literature in ASD populations. This dose has been shown to be well-tolerated and caution is warranted in this severely developmentally delayed population until safety is more firmly established. Doses may be reduced by 8 IU/day if safety concerns emerge. Identical doses will be used during open treatment. Adherence to treatment will be assessed by caregiver report and weighing returned treatment bottles. Randomization will be performed by the Mount Sinai Research Pharmacy.

Outcome measures (Table 1):

Efficacy measurements will be taken at baseline, and at weeks 4, 8, and 12 of each treatment phase (double blind and open-label). Safety and tolerability will be measured every two weeks throughout the trial during monitoring visits and phone calls (see Adverse Events). All safety and efficacy measurements will be repeated four weeks post-treatment to ensure safety and explore maintenance of treatment effects. Primary and secondary outcomes will be administered by an independent evaluator who is blind to side effects to prevent the risk of bias. All evaluators will maintain a minimum of 80 percent reliability through ongoing training and routine reliability assessments.

Primary outcome: The ABC-SW subscale (Aman et al., 1985) is a parent-report rating scale used to monitor an array of behavioral features, including social withdrawal (i.e., Lethargy subscale). It was chosen as a primary outcome measure because our preliminary data suggest that it accurately reflects the phenotype and because the ABC has been well validated in both intellectually disabled and ASD patients and is currently accepted as an appropriate outcome measure within the field of pediatric psychopharmacology research (Aman et al., 2010).

Secondary outcomes: Three tasks assessing aspects of attention will be used in the proposed study.

Task 1: Attention and Social Recognition Memory will be measured using a Visual Paired Comparison paradigm (VPC) in conjunction with eye-tracking technology. Children with ASD exhibit visual processing deficits for social information, such as face recognition (Swettenham et al., 1998), but have shown enhanced perceptual processing of nonsocial information. For example, individuals with ASD show superior performance as compared to healthy controls in extracting embedded figures during a block design task (Shah and Frith 1993; Bradshaw et al., 2011). The VPC has specifically been widely used in young infants (<1y) and is therefore appropriate in children with PMS, and is also predictive of risk for ASD and cognitive outcome (Rose et al. 2001, 2009, 2011; Fagan et al., 2007). A VPC paradigm with eye-tracking technology found evidence that typically developing (TD) children show preferential recognition for social (faces) and non-social (simple objects) stimuli, but not complex block patterns (Bradshaw et al., 2010). Children with ASD, in contrast, were successful at recognizing block patterns, but showed no evidence for face recognition. The VPC task with eye-tracking has also been successfully used in Rett syndrome, another monogenic cause of ASD with evidence of synaptic deficits and severe developmental delay (Rose et al., 2013). Patients with Rett syndrome could recognize faces and patterns, though with poorer recognition than age-matched TD comparison subjects. On the VPC task, patients with Rett syndrome demonstrated less mature attention marked by a more narrowly focused gaze, with fewer and longer fixations, more uneven gaze dispersion, and shorter looking time.

Because our group's *Shank3-deficient* rat displayed deficits in both attention and social recognition memory, we will use a VPC paradigm to assess these domains. In this paradigm, the subject is initially presented with a target for familiarization and the familiar target is then paired with a novel target. Typically, the

subject spends more time inspecting the novel target than the familiar one. Thus, recognition is indexed by a novelty score derived from the percentage of time looking at the novel stimulus vs. the familiar stimulus (Rose et al., 2001) because subjects are presumed to spend less time looking at a familiar stimulus. The VPC task also measures attention using eye tracking quantified by total looking time, gaze dispersion, and number/length of fixations on the stimulus (Rose et al., 2013). The proposed study will make use of a well-established battery of VPC paradigms (the 'Rose'), which consists of nine stimuli: five using achromatic photos of faces and four using multicolored abstract patterns. Because this paradigm includes both social and non-social stimuli, we will be able to separately examine the effect of oxytocin on social recognition as well as recognition memory and attention to non-social stimuli.

Task 2: The process of visual orienting to a new stimulus comprises three successive steps (Posner and Cohen, 1984): disengagement of attention from a previously fixated stimulus, shifting attention to a new stimulus, and engagement of the new stimulus through visual fixation. A task used to assess these measures of attention is the gap-overlap task, which we will employ in the present study. In this paradigm, three computer screens are set up side by side. A stimulus initially appears on the central screen, and once the participant orients to that stimulus, another stimulus will appear on one of the lateral screens. In the "gap" condition, the central stimulus will disappear before the peripheral stimulus appears. In the "overlap" condition, the central stimulus will remain on the screen while the peripheral stimulus appears. In both conditions, reaction times of the participant's eye movements (saccadic reaction time) from the central stimulus to the peripheral stimulus will be measured. The gap condition will be taken as a measure of shifting attention, and the overlap condition will be taken as a measure of disengaging attention.

Prospective studies using this task with dynamic, non-social stimuli have compared infants at high and low risk of being diagnosed with ASD. At 6-7 months, no differences in ability to disengage attention were seen, but by 12-14 months, the high risk cohort, and in particular those infants later diagnosed with ASD, showed longer disengage latencies in the overlap trials (Zwaigenbaum et al., 2005; Bryson et al., 2004; Elsabbagh et al., 2013). Another prospective trial, using static face and object images as stimuli, found that infants later diagnosed with ASD had longer latency times on the gap/shift trials and on the overlap/disengage trials by 7 months old (Elison et al. 2013). In a study comparing three cohorts of 5-year-old children (ASD, TD, and Down), Landry and Bryson (2004) demonstrated disengagement-specific impairment in children with ASD, who had longer reaction times for the overlap trials, though no differences were found on the gap/shift trials. Other studies found impairments in both the gap and overlap trials (van der Geest et al., 2001; Goldberg et al., 2012). The proposed study will use the gap-overlap task, modeled on the studies described, in conjunction with eye-tracking technology to assess disengagement and shifting of attention.

Task 3: Another component of attention is temporal attention, which is necessary to identify individual components in a sequence of events (Batelli et al., 2007, 2008), and which plays a role in planning and executive function. This task, especially designed to isolate temporal resolution of attention, is a modified version of the phase discrimination task used in adults (Aghdaee and Cavanagh, 2007; Battelli et al., 2007), which has also been used in neurotypical infants and subjects with Fragile X (Farzin et al., 2011; Farzin et al., 2012). The "flicker task," or the four-alternative forced-choice preferential looking paradigm, was adapted from Farzin et al., 2011, and makes use of a screen with eye-tracking technology. With this task, we aim to isolate a measure of temporal resolution, or the time interval over which the visual system is able to parse information (Farzin et al., 2012). Four squares are arranged on the screen, and cycle between black and white states, with one "target square" 180° out of sync with the other 3 "distractor" squares. That is, the target square is black when the other 3 squares are white, and vice versa. The squares cycle between black and white at set experimental frequencies of 0.2, 0.5, 1, or 2 Hz. At slower rates, the target square should be easier to individuate and identify; at faster rates, individuation of the target square becomes more difficult. If subjects are able to individuate the target square, they should look a looking preference for that square, as measured by the eye-tracker. A previous experiment in neurotypical infants and infants with Fragile X syndrome (Farzin et al., 2011), a monogenic cause of autism, showed that TD infants were better able to individuate the target square compared to FXS infants at frequencies of 0.5, 1, and 2 Hz. No difference between the groups was found at 2 Hz, and in fact neither group differed from the chance condition at that frequency. The study also found, by the target preference looking scores, that TD infants could resolve the target square at a rate of up to 1 Hz (1 phase change per second), whereas FXS infants could only do so up to 0.5 Hz (1 phase change per 2 seconds). Results from this experiment will be useful to compare with the body of work on temporal resolution of attention in FXS, as both FXS and PMS are monogenic causes of autism.

Language will be measured using the Macarthur-Bates Communicative Development Inventory (MCDI; Fenson et al., 1993; 2007) and the Vineland Adaptive Behavior Communication Domain (Sparrow, 1984); repetitive behavior with the Repetitive Behavior Scale-Revised total score (RBS-R; Bodfish et al., 2000); sensory sensitivity and sensory seeking behavior using the Sensory Profile (Dunn, 1999); Adaptive functioning using the Vineland Adaptive Behavior Composite (Sparrow, 1984); Caregiver strain using the Caregiver Strain Index (CSI; Brannan et al., 1997); global improvement will be measures using the Clinical Global Impression-Improvement and Severity Scales (CGI-S; CGI-I; Guy, 1976). All of the selected secondary measures have been previously validated as measurement tools in ASD populations and we aim to assess their utility in detecting change with oxytocin treatment in PMS.

Exploratory objective outcomes: Language Testing will be performed with a combination of standardized measures that rely on parent/caregiver report, and objective, naturalistic assessments. Dr. Helen Tager-Flusberg co-chaired a working group assembled by the National Institute on Deafness and Other Communication Disorders (NIDCD) which recommended using a combination of measures for improving language assessment and developing benchmarks of expressive language for use as treatment outcome measures (Tager-Flusberg et al., 2009). Two objective approaches can be taken with expressive natural language samples: verbatim transcription followed by coding with analysis using supporting software (e.g., Systematic Analysis of Language Transcripts; Miller & Chapman 2008) or automated technologies such as Language Environment Analysis (LENA). A recent study compared vocal production in 26 young children diagnosed mainly with Autistic Disorder to 78 typically developing controls using a digital language processor (DLP) and LENA (Warren et al., 2010). Audio processing algorithms measured the amount of vocalizations produced during a 12-hour period in the children's natural environment. Significant differences in vocal production were found between groups and this study provides proof of concept that automated measurement can capture and distinguish speech-related and non-speech vocalizations in children with language delays and ASD. In addition, LENA measurements were positively correlated with previously validated parent report measures (Warren et al., 2010), including the MCDI (Fenson et al., 1993; 2007) which we intend to use as a secondary outcome. In the proposed study, LENA sampling will occur pre- and post-treatment during the semi-structured ADOS-2 in the clinic and during a 12-hour time period in the child's naturalistic home environment. Software quantifies and analyzes language output to count the number of times a child vocalizes during a given period and then filters out vegetative sounds such as respiration and fixed signals like cries.

Table 1: Outcome Measures	**Baseline**	**Wk 4**	**Wk 8**	**Wk 12**	**Wk 16 Open-label**	**Wk 20 Open-label**	**Wk 24 Open-label**	**4 weeks post-study**
Social:								
Visual Paired Comparison	X	X	X	X	X	X	X	X
Group-Overlap Task	X	X	X	X	X	X	X	X
Flicker Task	X	X	X	X	X	X	X	X
ABC-SW subscale	X	X	X	X	X	X	X	X
Social Orienting Task	X			X			X	X
Language:								
LENA	X			X			X	X
MCDI	X			X			X	X
Vineland subscales	X			X			X	X
Repetitive Behavior:								
Repetitive Behavior Scale	X	X	X	X	X	X	X	X
Other:								
Short Sensory Profile	X	X	X	X	X	X	X	X
CGI Scales	X	X	X	X	X	X	X	X
Caregiver Strain Index	X	X	X	X	X	X	X	X
Vineland Behavior Composite	X			X			X	X
Visual Analogue Scales	X	X	X	X	X	X	X	X
Visual Evoked Potential	X	X	X	X	X	X	X	X
Psychoeducational profile	X			X			X	

Social Attention measurement will also be piloted using a Social Orienting Task (Dawson et al., 2004) as an exploratory outcome. Dawson and colleagues have developed the term "social orienting impairment" which refers to the failure in children with ASD of orienting spontaneously to naturally occurring stimuli in their environment (Dawson et al., 1998). Impairments in joint attention and social orienting reflect core social deficits and specifically differentiate young children with ASD from those without ASD (Ventola et al., 2007; Dawson et

al., 2004). It has been suggested that early social attention deficits deprive children of social information input and lead to disrupted neural and behavioral development (Mundy & Neal, 2001). Failure of social orienting may represent one of the earliest and most fundamental social deficits in ASD (Dawson et al., 1998). In addition, social attention has been shown to be critical for the acquisition of verbal and gestural communication (Carpenter et al., 1998) and possibly intellectual functioning (Poon et al., 2011). The proposed study will replicate the social orienting task developed by Dawson and colleagues (2004). Orienting is defined as turning the head or eyes toward an auditory stimulus and a series of four social (e.g., calling the child"s name) and four non-social (e.g., phone ringing) stimuli will be delivered three times each. A standard and familiar testing room will be used and the presence, latency, and duration of orienting (e.g., turning the head or eyes) will be measured by trained coders through a one-way mirror and the use of video. We aim to explore the use of this measure and to assess its utility as an outcome measure sensitive to change with treatment.

Visual Evoked Potentials (VEPs) as measured by single-channel electroencephalogram (EEG) will also be piloted as an exploratory outcome. VEPs offer a noninvasive electrophysiological technique that provides quantitative and objective information by extracting the VEP from the ongoing electroencephalographic recording (EEG). VEPs assess the functional integrity of the visual pathways from the retina to the visual cortex via the optic nerve/optic radiations and are considered more effective than imaging techniques at obtaining quantitative evidence for neural dysfunction. They are thought to reflect the sum of excitatory and inhibitory postsynaptic potentials occurring on apical dendrites of pyramidal cells in superficial layers of the occipital cortex (Creutzfeldt & Kuhnt, 1973; Purpura, 1959; Eccles, 1951). Apical dendrites modulate the excitatory and inhibitory signals received by the pyramidal cells. Therefore, an examination of GABAergic (inhibitory) and glutamatergic (excitatory) activity within the brains of children with neurodevelopmental disorders may yield biomarkers for subtypes. VEPs are advantageous as they can be used on individuals of varying levels of functioning, including children who are intellectually disabled or nonverbal. VEPs have the potential to yield electrophysiological biomarkers and can be used to monitor the effects of psychopharmacological or behavioral interventions as has been demonstrated in previous literature. For the current study, VEPs will be administered using single-channel recording, which only requires three electrodes. In addition, the study will utilize short-duration stimulations that have been adapted for use in this population. There will be no task demands during VEP recordings, with the exception of attending to the stimuli presented by computer. We aim to explore the use of this measure and to assess its utility as an outcome measure sensitive to change with treatment.

Psychoeducational Profile, Third Edition (PEP-3, Pro-Ed 2005). The PEP-3 is a standardized developmental assessment that provides information about developmental level and is intended to evaluate the uneven learning strengths and weaknesses characterizing ASD and related NDDs. The PEP-3 is normed in infants and children ages 6 months through 7 years with normative samples including individuals with and without disabilities. Item level scores provide ratings for absent, emerging, and mastered skills. The first portion of the assessment is performance based, consisting of direct testing and observation of child behavior across 10 subtests. Six of the subtests measure developmental abilities (Cognitive Verbal/Preverbal, Expressive Language, Receptive Language, Fine Motor, Gross Motor, and Visual-Motor Imitation) and the other four measure maladaptive behavior (Affective Expression, Social Reciprocity, Characteristic Motor Behavior, and Characteristic Verbal Behavior). From these subtests a Communication Composite, Motor Composite and Maladaptive Behaviors Composite can be derived. The second portion of the assessment is a Caregiver Report, which offers information about developmental level and severity across Problem Behaviors, Personal Self-Care, and Adaptive Behavior. The PEP-3 yields raw, developmental age, and percentile scores for each subtest, and standard scores for communication, motor, and maladaptive behavior domains. The PEP-3 will be an exploratory outcome for this study.

RNA samples will be collected at baseline, Week 12, Week 24, and Week 28 to attempt to predict treatment response.

Adverse Events (AEs). Monitoring AEs will be conducted during scheduled and unscheduled visits using an adapted semi-structured interview, the Safety and Monitoring Uniform Report Form (SMURF) every two weeks, and extensive clinical and laboratory assessments every four weeks (see Section on the Protection of Human Subjects). AEs will be carefully documented with respect to severity, duration, management, relationship to study drug, and outcome. Severity will be graded using a scale of mild, moderate, or severe.

Data Analysis:

All statistical computing will be done using the most recent version of SAS. The analyses described below will be performed on the intent-to-treat population. All data will be explored using descriptive statistics and graphical techniques prior to any hypothesis testing. For categorical variables, we will examine frequency distributions and where appropriate contingency tables and histograms. For continuous variables, we will examine frequency distributions and, where appropriate, box-and-whisker plots. When appropriate, we will consider transformation. If necessary due to distributional considerations, we will cautiously consider a change of analysis method to a less parametric one.

Interim analysis will be completed once 20 individuals have been enrolled. Based on the results of the interim analysis, the study will either continue to recruit to 40 individuals or will end.

General Modeling: Unless otherwise specified, we will fit a mixed longitudinal model with change from baseline to each post-baseline month for a response variable, treatment (oxytocin versus placebo) as the between-subjects factor, month as a within-subjects factor, their interaction, and baseline as a covariate. We will choose between two kinds of models: (1) a random coefficients model treating month continuously with random coefficients for the intercept, slope, and slope squared, and an unstructured covariance matrix among them, and (2) an mixed model with repeated measures (MMRM) treating month categorically and examining unstructured, Toeplitz, AR(1), and compound symmetric covariance structures among months. We will choose among these candidate models using an information criterion (AIC) while remaining *blind to the significance of the treatment effect* to avoid bias in the choice of model. Distributional assumptions will be examined using residuals. These mixed models could fail to converge or encounter difficulties based on their use of asymptotics. If they do, we will attempt to simplify the models in order to eliminate the problems, and if necessary, move to analyses that assume compound symmetry, but use a Huynh-Feldt correction if compound symmetry fails.

Missing Data: The mixed models used to evaluate the continuous response variables are able to handle moderate amounts of missing data provided they are missing at random. We will examine the missing at random assumption by assessing baseline differences between dropouts and completers, as well as differences in response variables up to the point of premature withdrawal. If the missing at random assumption does not appear to be tenable, we will report the mixed models results but spend additional effort characterizing treatment effect at time of premature withdrawal. We will analyze each of the endpoints **using** the procedure described under general modeling and will use ABC-SW and the combined social measure as the co-primary response variables, with the primary contrasts being the difference between treatment least squares means (LSMs) at month 6 for each outcome. We will test these co-primary measures using a Bonferroni-corrected significance level of 0.025 for each.

Sample Size and Statistical Power: Power estimates are based on a sample size of 40 individuals using baseline to week 12 change scores. Our Type I error rate (α) was set at .05 and we have adequate power to detect large effects. A large effect size is deemed feasible based on the potentially disease modifying effects of oxytocin. However, our primary aim is to evaluate feasibility and detect signal of improvement.

Expected outcomes. We expect to provide evidence for the safety and feasibility of oxytocin in improving social cognition and attention in children with PMS. Further, we expect to demonstrate that oxytocin is associated with improvement on secondary outcomes of social withdrawal, language delay, and repetitive behavior, as well as on functional outcomes of global severity and caregiver strain. *If positive, results from this clinical trial will demonstrate potentially disease-modifying effects on core and associated symptoms of ASD and may inform future trials.*

Timeline and Milestones. We will require three years to complete the study: six months for study preparation and 26 months for data collection at a randomization rate of two subjects per month. Four months will be allotted for data cleaning, analysis, and manuscript preparation. This recruitment is deemed feasible based on our previous experience enrolling patients with PMS in ongoing studies at the Seaver Autism Center.

Potential problems and alternative strategies. The burden of frequent monitoring visits and the discomfort of intranasal application risks subject withdrawal; we will use the intent to treat principle so all randomized patients will be included in the data analysis. Second, concerns about recruitment feasibility must be raised with rare diseases. We have established an excellent working relationship with the national Foundation for affected families and have already evaluated 45 children, including enrolling 15 in a clinical trial with IGF-1. There has been only one treatment trial in this devastating disorder and many patients have contacted us to express interest in participating in clinical trials. Finally, the possibility that our hypotheses are not supported must be considered because the preliminary evidence is drawn from a preclinical rat model and

we do not have clear evidence for our phenotypic targets. Yet, we have selected a measurement tool (ABC) validated in developmentally delayed populations, sensitive to change with treatment, and reflective of the phenotype in our preliminary studies. Several secondary and exploratory outcomes will also be available for analysis and will provide meaningful data for future research should signal be detected elsewhere and not on the primary outcome. Standardized measures of receptive and expressive language (e.g., Vineland) that we use as part of our studies may not be sensitive to change in this intervention trial as standard error of measurement is approximately three months. For this reason, we have chosen an additional focus on measures of expressive language using natural language samples and objective measurement of language production in semi-structured and naturalistic settings.

Future directions. Future studies will refine and characterize the phenotype using functional neuroimaging, prospectively studying the natural history of the disorder, and preparing for larger scale, multi-centered clinical trials in PMS.

Protection of Human Subjects

Risks to the Subjects

Participants will undergo comprehensive medical evaluation by the study physicians. Medical history, family history, physical, routine hematology and blood chemistry (including electrolytes, liver function, and prolactin), and electrocardiography will be performed to determine eligibility for participation. Prolactin will be followed due to oxytocin's primary effects on milk let down and recent evidence that elevated prolactin levels may affect bone density and strength. Patients will be monitored for safety at weeks 4, 8, and 12 in both treatment phases, after 3 months of open-label treatment, and then again four weeks after treatment completion using physical examination, routine hematology and blood chemistry including liver profile – see Table 1 and 2. Tolerability will be monitored during visits at weeks 4, 8, and 12 in both treatment phases and every two weeks by telephone throughout the trial.

Female subjects will be assessed during each visit for pelvic and breast pain and breast discharge that may result from uterine or mammary gland contractions. Female subjects will also be provided with contact telephone numbers and instructed to call research personnel if they experience pelvic or breast pain or breast discharge. Male subjects will also be provided with these contact numbers and instructed to call if they believe they are experiencing uncomfortable or unusual effects. A urine pregnancy test will be conducted in each female subject during the initial screening evaluation and a female reproduction form will be administered in visits thereafter to assess if there are any irregularities in menstrual cycles and to assess if another pregnancy test is warranted. If the pregnancy test is positive at screening, subjects will not start test treatments and will be dropped from the study. If a female subject becomes pregnant during the course of the study, the subject will be withdrawn from the study and the subject and her caregiver will be encouraged to discuss with their obstetricians the implications of post-conception exposure to oxytocin. The requirement that male and female subjects and their partners using effective birth control during participation is stated in the consent form and will be strongly emphasized during recruitment. However, given the severe intellectual and developmental delays in patients with PMS, combined with the fact that they require 24-hour supervision, the likelihood of sexual activity is very, very low. We will systematically elicit and document treatment emergent adverse effects, their severity and the clinician's judgment about their relationship to treatment using the systematic longitudinal adverse effects instrument described in the Assessments section at every visit and during weekly phone calls. We will also tabulate all cases where a child withdrew from treatment due to adverse effects or inability to tolerate the nasal administration of oxytocin.

There is a dearth of information about the safety of sustained use of oxytocin. There have not been any published studies on child and adolescent populations using oxytocin. We will be using fairly small doses with the maximum dose of 24 IU twice daily. We will be monitoring intensively as described above.

Oxytocin has been reported to exert a large number of effects in the body and the brain (Gimpl & Fahrenholz 2001). The classic physiological effects of oxytocin in humans as well as all placental mammals are uterine contractions and milk ejection. These are conclusively known to occur only under the hormonal conditions at the end of pregnancy and postpartum when oxytocin receptors proliferate in the uterus and mammary tissue and lactogenesis occurs. Theoretically, oxytocin administration could cause uterine contractions during phases of the menstrual cycle when estrogen levels are relatively high or in women who are receiving estrogen. This, however, has never been demonstrated or reported. Intravenous (IV) administration of Oxytocin is FDA

approved for induction or facilitation of labor and postpartum contraction of the uterus. Intranasal oxytocin spray is also available in other countries to facilitate nursing in new mothers. Intranasal oxytocin was FDA approved as a lactation aid in the United States until 1995 when it was withdrawn from the US market by the company that manufactured it, Novartis. This was a business decision and the FDA has confirmed no safety concerns were involved in this decision.

A number of adverse effects have been reported in the context of these two clinical applications, primarily with IV administration of oxytocin, which include anaphylactic reaction, hypertension, hypotension, cardiac arrhythmias, nausea and vomiting, afibrinogenemia and associated bleeding and, in the context of prolonged iv administration of oxytocin, hyponatremia. There has also been at least one report of life-threatening anaphylaxis during surgery when multiple drugs were being intravenously infused simultaneously (D. Pant, et al 2009). Symptoms that may result from anaphylaxis include generalized hives, itchiness, or flushing; crampy abdominal pain, diarrhea, and vomiting; a feeling of anxiety and impending doom; swelling of the lips, tongue, throat resulting in shortness of breath, wheezes or stridor, and low oxygen; a drop in blood pressure that may result in a feeling of lightheadedness and loss of consciousness; reduced muscle tone with possible loss of bladder control; and, most seriously, coronary artery spasm may occur with subsequent myocardial infarction or cardiac dysrhythmia and possible death. There is also a report of psychosis occurring during oxytocin treatment although there are multiple ongoing pilot studies in schizophrenia that have reported reduction of psychotic symptoms.

It should be noted, however, that during a large number of research studies conducted over several decades that collectively enrolled several hundred to a few thousand subjects (mostly men) who received a single high dose of oxytocin IV or intranasally (which produces very high blood concentrations), no medical complications were reported except for onset of psychotic symptoms in one subject (Ansseau et al., 1987). The three adult studies of sustained oxytocin treatment conducted to date with doses ranging from 80 IU 4x/day for 7 days to 54 IU daily for 6 weeks have not reported any significant side effects associated with oxytocin.

The potential stress, embarrassment and/or boredom associated with the psychological and behavioral assessments are considered to be minimal. Participants will be informed throughout the study that they may opt not to answer any question that they do not want to answer. Breaks will be taken as needed. There are risks to confidentiality with participation in any study of this kind; investigators will have access to subjects' medical records and protected health information.
During laboratory blood draws the risks involved are pain, bruising, and rarely, infection at the location where the blood was taken.

An internal Safety Monitoring Board will be convened and have primary responsibility for developing the oversight necessary to promptly identify and act upon any adverse events. The Safety Monitoring Board will be made up of 3 physicians who will meet routinely every year and immediately should a severe adverse event occur.

Adequacy of Protection Against Risks
Recruitment and Informed Consent:
The investigator will describe the protocol to potential subjects' parents/guardians in person, although general information and assessment for eligibility can be carried out by phone if necessary. The Informed Consent may be read to the subjects' parent/guardians, but, in any event, the investigator or designee shall give the subjects' parents/guardians ample opportunity to inquire about details of the study and ask any questions before dating and signing the Informed Consent Form. The Informed Consent will be created with a level of language fully comprehensible to the prospective subjects' parents/guardians. Informed consent will be documented by the use of a written consent form approved by the IRB and signed and dated by the subjects' parents/guardians and by the person who conducted the informed consent understood. Each subject's signed informed consent form will be kept on file by the investigator for possible inspection by regulatory authorities. The parents/guardians will receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects' parents/guardians, and will receive copies of any signed and dated consent form updates. Any amendments to the written information will be provided to parents/guardians.

Ethics and Regulatory Considerations: The study will be conducted according to Good Clinical Practice (GCP), the 1996 Declaration of Helsinki, and local rules and regulations of the United States.

Confidentiality of Source Documents and Study Data:
A subject identification code will be used in lieu of the subject's name on all study data compiled and delivered to the secure database. All source documents and study data will be kept confidential, in accordance with all requirements of the laws.

Adverse Events
Definition of an Adverse Event
An adverse event (AE) will be defined as any untoward medical occurrence in a study subject, temporally associated with the use of the experimental medication, whether or not considered related to the medication. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the experimental medication. A serious adverse event (SAE) will be defined as an AE that meets any of the following criteria:

- results in death;
- is life threatening;
- requires inpatient hospitalization;
- results in a persistent or significant disability/incapacity;
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above.

Adverse Events Reporting
The Principal Investigator (PI) will be responsible for the detection and documentation of events meeting the criteria and definition of an adverse event or serious adverse event, as provided in this protocol. During the study, when there is a safety evaluation, the investigator or team member will be responsible for reporting adverse events and serious adverse events. Each subject's parents/guardians will be instructed to contact the investigator immediately should the subject manifest any signs or symptoms they perceive as serious.

Clinical laboratory parameters and other abnormal assessments qualifying as adverse events and serious adverse events
Abnormal laboratory findings or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

Medically attended visits
For each AE the subject experiences, the subject's parents/guardians will be asked if they received medical attention defined as hospitalization, an emergency room visit or a visit to or form medical personnel (medical doctor/doctor of osteopathy or nurse practitioner) for any reason. This information will be recorded in the CRF.

Lack of Efficacy
Lack of efficacy per se will not be reported as an AE. The signs and symptoms or clinical sequelae resulting from lack of efficacy will be reported if they fulfill the AE or SAE definition.

Time Period, Frequency, and Method of Detecting AEs
All AEs occurring from the initiation of therapy until 4 weeks following its completion will be recorded on the Adverse Event form in the subject's CRF, irrespective of severity or whether or not they are considered medication-related. Onset of chronic illness (e.g. autoimmune disorders, asthma, type 1 diabetes and allergies) and conditions prompting emergency room (ER) visits or physician office visits that are not related to well-child

care, injury, or common acute illnesses (e.g., upper respiratory tract infection, otitis media, pharyngitis, and gastroenteritis) will be reported during the entire study period. The investigator will inquire about the occurrence of AEs at every visit/contact during the study and throughout the follow-up phase as appropriate. All AEs either observed by the investigator or a clinical collaborator or reported by the subject's parent/guardian spontaneously or in response to a direct question will be evaluated by the investigator. AEs not previously documented in the study will be recorded in the Adverse Event form within the subject's CRF. The nature of each event, date and time (where appropriate) of onset, outcome, intensity and relationship to drug administration should be established. Details of any corrective treatment should be recorded on the appropriate page of the CRF.

When an AE/SAE occurs, it will be the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostic reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE on the CRF or SAE Report Form as applicable. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information.

Follow-up of Adverse Events and Serious Adverse Events and Assessment of Outcome:
After the initial AE/SAE report, the investigator will actively follow each subject and provide further information on the subject's condition. All AEs and SAEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts. Investigators will follow-up subjects with SAEs or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, the event is otherwise explained, or the subject is lost to follow-up. In the case of other non-serious AEs, until they complete the study or they are lost to follow-up. Clinically significant laboratory abnormalities will be followed until they have returned to normal, or a satisfactory explanation has been provided. The investigator may perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE.

Regulatory Reporting Requirements for Serious Adverse Events:
All safety data will be reported to Safety Monitoring Board and IRB every six months, or, in the case of any major safety concern or question, immediately. If any study stopping condition occurs, this will be reported immediately, and the study will be halted, pending review by these agencies. The investigator has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards safety of other subjects are met. The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the IRB.

Safety Measures
Patients will be monitored for safety at weeks 4, 8, and 12 in both treatment phases and then again four weeks after treatment completion using physical examination (including anthropometric measurements, otolaryngological exam, vital signs, routine hematology and blood chemistry including electrolytes, liver function, and prolactin) – see Table 2. In addition, a neurological exam will be performed at baseline, Week 12 and Week 24.Tolerability will be monitored during visits at weeks 4, 8, and 12 in both treatment phases. There are a number of tertiary outcomes designed to monitor the safety of oxytocin (see Tables 1 and 2). For each of the safety measures, the Safety Monitoring Board will establish a priori criteria for termination of the intervention (see Stopping Conditions).

Monitoring for AEs will be conducted during scheduled and unscheduled visits per clinical and laboratory assessments. If a subject develops significant neurological signs and symptoms (e.g. cerebrovascular event), they will be seen immediately for a comprehensive evaluation, appropriate treatment, and removal from active participation in the study. We will also apply criteria for attribution of AEs by means of the following descriptors and codes.

- unrelated to treatment 1
- unlikely related to treatment 2
- possibly related to treatment 3
- probably related to treatment 4
- definitely related to treatment 5

Stopping Conditions for Individual Subjects

The following criteria will be used to identify possible adverse treatment events, which will indicate the need to halt active participation of the subject in the oxytocin study:

- Withdrawal of Consent
- PI or any regulatory authority (Safety Monitoring Board, IRB) believe withdrawal is necessary for the subject's health, well-being, or best interests.
- PI or any regulatory authority believe that withdrawal is necessary for the subject's health, well-being, or best interests
- Laboratory: any abnormality on any test with adverse event (AE, Common Terminology Criteria for Adverse Events, National Cancer Institute, scales) ≥ 3 or greater at any time in the study
- Any AE of any sort (clinical, laboratory) ≥ 4 will result in halting for the individual and also for the study as a whole.

To avoid bias, all analyses will include all subjects, including those withdrawn from the study, regardless of adherence to study protocol. Especially in an exploratory trial of a novel, unproven treatment, the safety of subjects is our primary *overriding* concern. If there is doubt concerning a subject's safety, the default mode will be withdrawal from treatment or active study participation, followed by close observation (safety follow up visits) and recommendation of standard treatment.

Return of a subject to active study participation will not be permitted, except if a transient clinical or laboratory abnormality unrelated to study treatment has occurred and subsequent permission of the Safety Monitoring Board to return the patient to active study has been provided.

If a subject is withdrawn from active study during screening or observation phases of the study, they will be returned to the referring physician and standard care will be recommended; in addition we will request the subject's participation in an end-of-study visit.

If a subject is withdrawn from active study during oxytocin treatment, they will also be returned to the referring physician and standard care will be recommended; however, in this case, we will in addition request the subject's participation in clinical and laboratory safety assessments and follow up per protocol schedule.

Stopping Conditions for Study as a Whole

The study will be halted* if two patients experience stopping conditions. (Exceptions for this criterion: Patients who withdraw voluntarily for reasons not directly related to or intrinsic to the study, e.g. incidental considerations such as concerns about travel time to study visits, unexpected pregnancy in the family, etc. Clearly, patients who experience adverse effects during oxytocin treatment would count towards this criterion for whole study stopping).

In addition, the study will be halted if one patient experiences a serious related adverse effect (grade ≥ 4 AE).

All safety data will be reported to the Safety Monitoring Board and IRB every six months, or, in the case of any major safety concern or question, immediately. If any study stopping condition occurs, this will be reported immediately and the study halted, pending review by the Safety Monitoring Board and IRB, and until the decision by regulatory authorities to resume, suspend or close the study has been made.

*Operationally, "halting" will ordinarily mean that no further screening of new subjects and no treatment initiation will occur until the safety issue has been investigated and resolved (i.e., a final decision has been

made to resume, suspend or close the study has been made). Enrolled study participants who have no new symptoms or adverse effects will ordinarily be allowed to continue study observation or treatment without interruption while the safety issue is being investigated, unless it is the contemporaneous judgment of the PI or subsequent judgment of the Safety Monitoring Board or IRB that it is unsafe to do so, in which case all observation or treatment interventions will be suspended forthwith.

Tables

Table 1. Safety and Screening Measures

Measures	Screening	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 24	Post-study
Physical exam	X	X	X	X	X	X	X	X
Neurological exam	X			X			X	
Electrocardiography	X			X			X	X **
Pregnancy test	X			X			X	X
Height	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Side effect monitoring*	X	X	X	X	X	X	X	X

*In addition to the above in-person visits, side effect monitoring will also occur at Week 2 and Week 14 (after the first two weeks in each phase) or at any time throughout the study should side effects emerge.

** To be completed only if EKG at Week 24 was abnormal.

Table 2. Laboratory Safety Measures

Measures	Screening	Wk 12	Wk 24
Electrolytes (Na, K, Cl, CO2)	X	X	X
Renal function (BUN/Cr)	X	X	X
Glucose	X	X	X
Liver function (AST/ALT)	X	X	X
Prolactin	X	X	X

INCLUSION OF WOMEN AND MINORITIES

The Seaver Autism Center has had great success in recruiting subjects for ongoing studies and receives over 600 new referrals each year for research and/or clinical services. Of the individuals referred, approximately 55% are white, 23% are African American, 13% are Hispanic, 5% are Asian, and 3% are other ethnicities. PMS is a rare condition and we anticipate that referrals will come primarily through the national Phelan-McDermid Syndrome Foundation (PMSF) which has an adequate number of families to support our recruitment needs. Estimates of demographic statistics are available from the PMSF database: 43% are males and 57% are females 89.4% are Caucasian, 2.2% are African American, 2.2% are Asian, and 6.2% are Hispanic. Among the 45 cases of PMS evaluated to date at our Center, the gender ratio is 58% males and 41% females; 96% are White and 4% are Asian; 98% are non-Hispanic and 2% are Hispanic.

Inclusion of Women: There will be no exclusion based on gender. Based on patients with PMS evaluated to date at our Center, the gender ratio is 58% males and 41% females. Autism in general is disproportionately present in males compared to females at a ratio of 4:1. Active recruitment efforts in the past have ensured the inclusion of appropriate numbers of participants from each gender. In three recently completed double-blind, placebo controlled trials in children and adolescents with autism, approximately 20% of the final sample were female patients. Every effort will be made to include a representative number of females.

Inclusion of Minorities: There will be no exclusion based on minority status. Our center has demonstrated the ability to recruit adequate minority participation in child and adult autism research. In a recent child autism clinical trial in our center, the final sample consisted of approximately 32% minority participants including Hispanic, Black, and Asian individuals. Among the 45 cases of *SHANK3* deficiency evaluated to date at our Center, 96% are White and 4% is Asian; 98% are non-Hispanic and 2% is Hispanic. There are limitations given the rare nature of *SHANK3* deficiency, but every effort will be made to include a representative sample of minorities.

INCLUSION OF CHILDREN

PMS is a genetic syndrome with manifestations that begin at birth. It is therefore crucial to study potential treatments in children and the target study sample of the proposed study is 40 children ages 5-17. Our research group has significant expertise working with children and adolescents with PMS, idiopathic ASD, and other neurodevelopmental disabilities. All co-investigators have specific training working with children and the treating physicians include a child and adolescent psychiatrist (Kolevzon) and a pediatric neurologist (Frank).

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