Open-Label Treatment Trial of Lithium to Target the Underlying Defect in Fragile X Syndrome

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ABSTRACT: ***Objective:*** **In fragile X syndrome (FXS), it is hypothesized that absence of the fragile X mental retardation protein (FMRP) disrupts regulation of group 1 metabotropic glutamate receptor (mGluR and mGluR5)-dependent translation in dendrites. Lithium reduces mGluR-activated translation and reverses phenotypes in the *dfxr* mutant fly and *fmr1* knockout mouse. This pilot add-on trial was conducted to evaluate safety and efficacy of lithium in humans with FXS.** ***Methods:*** **Fifteen individuals with FXS, ages 6–23, received lithium titrated to levels of 0.8–1.2 mEq/L. The primary outcome measure, the Aberrant Behavior Checklist – Community Edition (ABC-C) Irritability Subscale, secondary outcome measures (other ABC-C subscales, clinical global improvement scale (CGI), visual analog scale for behavior (VAS), Vineland Adaptive Behavior Scale (VABS)), exploratory cognitive and psychophysiological measures and an extracellular signal-regulated kinase (ERK) activation assay were administered at baseline and 2 months of treatment. Side effects were quantified with a standardized checklist and lithium level, complete blood count (CBC), thyroid stimulating hormone (TSH), and chemistry screen were done at baseline, 2 weeks, 4 weeks and 2 months.** ***Results:*** **The only significant treatment-related side effects were polyuria/polydipsia (n = 7) and elevated TSH (n = 4). Although the ABC-C Irritability Subscale showed only a trend toward improvement, there was significant improvement in the Total ABC-C score (p = 0.005), VAS (p = 0.003), CGI (p = 0.002), VABS Maladaptive Behavior Subscale (p = 0.007), and RBANS List Learning (p = 0.03) and an enhanced ERK activation rate (p = 0.007). Several exploratory tasks proved too difficult for lower-functioning FXS subjects.** ***Conclusions:*** **Results from this study are consistent with results in mouse and fly models of FXS, and suggest that lithium is well-tolerated and provides functional benefits in FXS, possibly by modifying the underlying neural defect. A placebo-controlled trial of lithium in FXS is warranted.**

*(J Dev Behav Pediatr 29:*293–302, 2008) **Index terms:** fragile X syndrome, lithium, FMR1, dendritic translation.

Fragile X syndrome (FXS) is the most common genetically identifiable inherited form of mental retardation, autism, and learning disability, with an estimated prevalence of about 1/4000 males and females.[1] Clinically FXS is characterized by variable physical signs such as long face, large ears and macro-orchidism, seizures in about 15% of individuals, most commonly in childhood,[2] and cognitive disabilities ranging from mild to severe, often associated with debilitating behavioral problems such as hyperactivity, anxiety, sensory hyperarousal, mood lability, aggression, and autistic behaviors (for reviews see [3,4]).

FXS results from an unstable trinucleotide repeat expansion mutation of >200 CGG repeats in the promoter of the *FMR1* (Fragile X Mental Retardation–1) gene,[5] located on the long arm of the X chromosome. Due to the presence of a presumably normal *FMR1* allele on the second X chromosome, females heterozygous for a full mutation are more mildly affected than males. The mutation leads to transcriptional silencing of *FMR1* and thus, the gene product (FMRP, Fragile X Mental Retardation Protein) is reduced or absent in FXS.[6] A body of literature suggests that FMRP is an RNA binding protein which modulates dendritic maturation and synaptic plasticity through a mechanism involving inhibition of group 1 metabotropic glutamate receptor (mGluR1 and mGluR5)-mediated mRNA translation in dendrites.[7–11] In the absence of FMRP, there is enhanced mGluR-activated hippocampal[12] and cerebellar[13] long-term depression (LTD), due to loss of normal inhibitory control of dendritic protein synthesis. Numerous expected consequences of excessive activation of mGluR-mediated dendritic protein synthesis are found in the *fmr1* knockout (KO) mouse, including reduction of synaptic AMPA receptors,[14,15] immature-appearing elongated dendritic processes,[7,8,16] and abnormal epileptiform discharges.[17] Further, many phenotypic features of FXS including seizures, electrical excitability on EEGs, hypersensitivity to tactile stimuli, cognitive difficulty, strabismus, enhanced anxiety, coordination problems and even loose stools are effects that have been proposed to occur in a setting of enhancement of

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mGluR-mediated processes that would normally be inhibited by FMRP.[15,18]

New therapeutic options have been proposed to target this underlying disease mechanism in FXS. Indeed, MPEP (2-Methyl-6-(Phenylethynyl)-Pyridine) and other mGluR negative modulators have been shown to reverse multiple phenotypes including audiogenic seizures, epileptiform discharges and open field hyperactivity in the *fmr1* KO mouse[17,19] and impairments in courtship memory in *dfxr* mutant *Drosophila*[20] models, both of which have absent FMRP. Several mGluR5 negative modulators are currently being developed for treatment of FXS but are just beginning to enter clinical trials and are not available for general use in humans.

Lithium is an alternative agent currently available for use in humans that is known to attenuate activation of the phospholipase C (PL-C) signaling pathway,[21–24] utilized by mGluR and other receptors to activate dendritic translation. Thus, lithium might theoretically correct excessive dendritic translation in FXS by acting as an inhibitory agent on signaling pathways that regulate translation (Figure 1). Lithium has been shown to improve defects in naïve courtship behavior, immediate recall and short-term memory in *dfxr* mutant flies[20] and to reduce audiogenic seizures in the KO mouse model.[25] These preclinical findings suggested that lithium might provide therapeutic benefits for behavior and/or cognition in humans with FXS. Although lithium has been used in the past to treat mood instability and aggression in FXS,[26] only anecdotal information on effectiveness is available. Therefore, this pilot study was initiated to test the concept of inhibition of a pathway involved in mGluR-mediated translational signaling as a treatment strategy for FXS by systematically exploring the effects of short-term (2 month) treatment with lithium on a broad range of phenotypes including behavior, cognition, and biophysical measures in a small cohort of subjects with FXS.

In addition, ERK (extracellular-signal regulated kinase) is a nodal point on which numerous signaling pathways (including the PL-C cascade) converge (Figure 1). As such, measurement of ERK activation in lymphocytes represents an attractive candidate assay to detect changes in cell signaling during treatment with agents such as lithium. Thus ERK activation was explored as a potential biomarker for effects of lithium on translational signaling in this cohort of subjects with FXS.

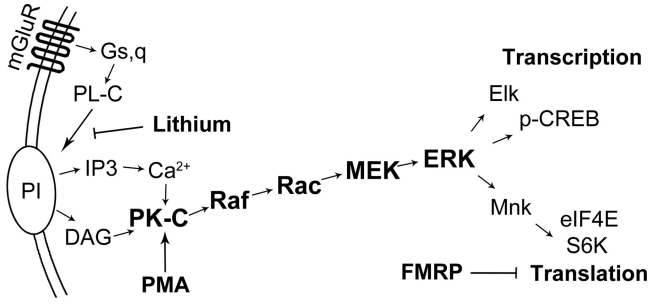

Figure 1. Mechanism of lithium action on translational activation and ERK. Note that FMRP normally inhibits translation so in the absence of FMRP, blockade of the pathway for mGluR-mediated activation of translation by lithium may partially correct defective translation and affect regulation of ERK. Abbreviations in figure are as follows: metabotropic glutamate receptor (mGluR), phospholipase C (PL-C), inositol phospholipids (PI), inositol 1,4,5-trisphosphate (IP3), diacylglycerol (DAG), protein kinase C (PK-C), MAPK/ERK kinase (MEK), extracellular signal-regulated kinases (ERK), cAMP response element-binding proteins (CREB). MAPK-interacting kinases (Mnk), eukaryotic initiation factor 4E (eIF4E), p70 S6 kinase (S6K), phorbol 12-myristate 13-acetate (PMA), fragile X mental retardation protein (FMRP).

SUBJECTS AND METHODS

Subjects

Subjects were recruited from the Rush University FXS Clinic or were self-referred after learning about the study from the FRAXA Research Foundation website or at the 10th International Fragile X Conference. Inclusion criteria included (1) diagnosis of fragile X syndrome with an FMR1 full mutation documented by DNA analysis, (2) age between 6 and 30 years, (3) at least mild behavioral dysfunction with an Aberrant Behavior Checklist - Community Edition (ABC-C)[27] Irritability score of 9 or more and a CGI-Severity score of at least 4, (4) normal hearing and corrected vision, (5) an available parent for all clinic visits and assessments with at least one parent having a 6th grade or higher reading level, and (6) stable doses of other psychotropic medications for at least 8 weeks prior to entry into the study. Subjects were excluded from participation if they had (1) a previous negative response to lithium as defined by a trial period of 4 weeks with serum level >0.7 mEq/L, (2) allergy to lithium, (3) kidney disease, (4) prior diagnosis of thyroid disease, (5) persistent psychotic symptoms, or (6) behavioral symptom severity judged likely to endanger personal safety or safety of others or which would preclude co-operation for necessary tests. Informed written consent was obtained from either the subject or the parent prior to participation. Assent from the subject was obtained in every case in which the subject was not his own legal guardian and had sufficient cognitive ability to agree to participate. The study was approved by the Institutional Review Board at Rush University Medical Center.

Baseline Evaluation

All subjects had an evaluation at the baseline visit that included the Stanford-Binet V[28] to measure IQ, a medical evaluation including detailed history, medication review and review of FXS test results, a physical exam and screening blood tests, including a CBC, thyroid stimulating hormone (TSH), comprehensive metabolic profile (including electrolytes, calcium, BUN, creatinine, protein, albumin, and liver function tests) and lithium level, to assess medical health and ensure the patient was not getting lithium through an alternative source. A Clinical Global Impression - Severity (CGI-S) scale for severity of behavioral dysfunction was completed by the PI (EBK) and a set of outcome measures (described below) was administered.

Lithium Dosing and Titration

Oral lithium carbonate was started after procedures at the baseline visit were completed, initially at a dose of

300 mg TID for subjects weighing more than 50 kg and at a dose of 20 mg/kg/day rounded to the nearest 150 mg increment and divided into a TID dosing schedule to a maximum of 300 mg TID for subjects less than 50 kg (not all doses were necessarily equal). Lithium levels were obtained 2 weeks (±1 day), 4 (±3 days), weeks and 2 months (±6 days), after starting treatment and dose was titrated upwards at 2 weeks and 4 weeks as appropriate (in 150 or 300 mg increments) based on levels, to obtain a level as high within the 0.8–1.2 mEq/L range as tolerated. If significant side effects occurred at a specific dose, the dose was reduced to the highest previous dose on which side effects had not been present. The dose was reduced (also in 150 or 300 mg increments) for levels above 1.2 even if there were no side effects. The goal was to achieve a steady level within the range of 0.8 to 1.2 mEq/L for the final four weeks of the 2 month treatment period. No changes in psychotropic medications being taken at the baseline visit were allowed during the 2 months of lithium titration and treatment.

Safety and Adverse Event Monitoring

Blood tests for safety monitoring and to screen for lithium toxicity were obtained at 2 weeks (CBC and comprehensive metabolic panel (CMP)), 4 weeks and 2 months (CBC, CMP and TSH) with lithium levels. Information regarding adverse events was obtained using a complimentary dual approach with both clinician-elicited symptom reports and a structured data gathering tool, the Safety Monitoring Uniform Research Form (SMURF), an adverse events inventory used in a prior clinical trial assessing medication effects in developmentally disabled participants.[29] At 2 weeks, 4 weeks and 2 months, caretakers were questioned regarding health problems, intercurrent illnesses and concomitant medications. For all adverse events, information was documented regarding time of event, duration, severity and whether the event was considered related to lithium or not. These symptoms were grouped according to the standard COSTART (Coding Symbols for a Thesaurus of Adverse Reaction Terms) classification system for adverse event reporting. The caregiver was also formally questioned using the SMURF, which has a structured system of questions that evaluates symptoms pertinent to all body systems, including sedation, energy level, motor restlessness, nausea, vomiting, bowel and bladder problems, sleep changes and appetite changes. The SMURF covers all of the known side effects of lithium.

Outcome Measures

The primary outcome measure was the ABC–C Irritability Subscale.[27] The ABC-C is a caregiver-rated scale with 5 subscales and a score range of 0–174, assessing a variety of behaviors that was specifically developed to evaluate medication and other treatment effects in individuals with developmental disorders. This scale was chosen as the primary outcome measure as it has demonstrated good ability to detect medication responsiveness in a prior clinical trial in autistic spectrum disorders,[29] has good reproducibility when administered repeatedly to caregivers of individuals with FXS,[31] and has items across the 5 subscales that address many of the problematic behaviors observed in individuals with FXS, including outbursts, agitation, mood lability, hyperactivity, and perseverative behaviors. The Irritability Subscale (score range of 0–45) was chosen as primary in particular because lithium is typically targeted to mood, although it is important to recognize that behavior in FXS is complex and if partially targeting the underlying defect in FXS, lithium might well target phenotypic behaviors addressed in other subscales, hence the use of these subscales as secondary outcome measures (below).

Secondary outcome measures were the following: other subscales of the ABC-C including the Lethargy (score range 0–48), Hyperactivity (score range 0–48), Stereotypy (score range 0–21), and Inappropriate Speech (score range 0–12) Subscales, a Visual Analog Scale (VAS) for a parent-defined target behavior on which the parent marks where the symptom lies from worst ever to no problem at all on a 4-inch line and change is assessed by measuring the location of the mark in millimeters from the beginning of the line, the Vineland Adaptive Behavior Scale[30] which assesses adaptive functioning in a variety of areas, and the CGI-I which is a 7-point scale of clinical global impression of improvement that the PI (EBK) filled out after considering all the available information on the patient including the parent history, the examination in clinic, reports from the school and other sources.[31] These measures were chosen because, in addition to addressing typical problematic behaviors in FXS, the ABC Subscales and VAS showed good reproducibility in a prior clinical trial[31] for FXS subjects intraclass correlation coefficients (ICC 0.8–0.9). The Vineland was chosen because it has been used extensively to measure adaptive functioning in FXS populations and it was felt that improvements due to lithium might present initially as functional adaptive skills improvements prior to any detectable cognitive change on testing.

Although the hypothesis was that the primary and secondary measures would show treatment effects, exploratory measures were employed to help determine which of them might be most useful to detect cognitive changes resulting from treatment. Given that this was a pilot study, initiated with the idea that lithium might impact the underlying defect in FXS, and thus effect cognitive improvement, and that measures that will successfully address cognitive change in a clinical trial setting in FXS are not well defined, a panel of exploratory cognitive outcome measures covering a broad range of tasks was also administered. The tests in this panel were chosen because it was thought that they could be accomplished by a high percentage of individuals with FXS, based on a concurrent study of outcome measures in FXS.[32] This panel included the Peabody Picture Vocabulary Test–Third edition (PPVT-III),[33] a measure of single word receptive vocabulary, the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)[34] List Learning and Story Memory Subtests which assess immediate auditory recall, the NEPSY Tower Subtest,[35] a mea-

sure of cognitive flexibility and problem solving, a Computer-Based Card Task which evaluates visual memory, sequential memory, and working memory with pictures of cards, during which the subject must remember a progressively longer sequence of colors followed by combinations of colors and numbers,[32] Non-Verbal Associative Learning Task (NVALT),[32,36] a non-verbal measure of object discrimination learning and object discrimination reversal, and the Carolina Fragile X Project CPT (FXCPT),[32,37] a simplified computerized continuous performance test with separate measures of auditory and visual attention, inhibition, and impulsivity. The measures in the baseline exploratory cognitive battery are all fairly short and the entire battery took about one to two hours depending on the level of function and cooperation of the subject. Most tests in this battery were piloted in a cohort of 46 subjects with FXS in a concurrent study of reproducibility of outcome measures[32] and all measures had fair to good reproducibility except the NVALT (weighted kappa 0.5 for the Card Task, ICC or weighted kappa 0.7 or greater for the RBANS List and Story Memory, NEPSY Tower, FXCPT). The PPVT had excellent reproducibility (ICC 0.9) in the placebo group from a prior clinical trial in subjects with FXS.[31]

An exploratory panel of biophysical measures shown to be abnormal in subjects with FXS relative to controls, including heart rate variability, auditory processing, and eye tracking was obtained (method described in Heilman KJ, Harden E, Berry-Kravis E, et al. Atypical autonomic regulation in fragile X syndrome: Contrasts with normals and effects of lithium. *J Aut Dev Disord*. 2007, in preparation). An exploratory blood biomarker measuring ERK activation kinetics in leukocytes, shown to be prolonged in a cohort of individuals with FXS relative to controls, was also measured as described previously.[39] Time to half maximum ERK phosphorylation, after stimulation of blood leukocytes with the artificial PKC activator, phorbol ester, was defined as "ERK activation time." A shorter time (more rapidly reaching half maximum phosphorylation) means a more efficient second messenger signaling pathway. This method bypasses mGluR1 and 5 receptor activation to provide an internal standard for second messenger signaling efficiency.

All outcome measures were administered at the baseline visit and after 2 months of treatment. During these visits subjects came to the site for most of a full day, although ample breaks were provided to try to optimize performance.

Statistical Analyses

Data were analyzed as change at two months treatment from baseline for all safety and outcome measures. Mean group change and standard deviations were determined for all measures for which sufficient data existed from both assessments. Correlation coefficients for change in outcome measures with lithium dose or levels were evaluated. The Wilcoxon signed rank test was used to determine statistical significance of group change (p values were 2-sided, corrected for ties). Results with $p < 0.05$ were considered significant given this study is considered an exploratory pilot study; no correction was made for multiplicity of comparisons.

RESULTS

Sixteen subjects were enrolled into the study and started on lithium. One subject discontinued lithium and dropped out of the study after the first few weeks of treatment due to multiple life transitions. Fifteen subjects completed the lithium titration and treatment protocol for the full two month period. Demographic characteristics of the study population are shown in Table 1.

Safety and Adverse Events

All subjects but one were titrated to a therapeutic dose (at least 0.8 mEq/L) of lithium within the first 4 weeks. The one subject that did not reach a dose of 0.8 mEq/L was titrated to 1350 mg per day without side effects but maintained levels of around 0.5 mEq/L. Dose reduction was required for side effects in only one subject, who, after reaching a level just over 0.8 mEq/L, had substantial behavioral deterioration (agitation), and the dose was lowered to give a level of 0.69 mEq/L at two months. The mean lithium level for all other subjects at 1 month was 0.89 ± 0.22 mEq/L and at two months was 0.90 ± 0.26 mEq/L. There was no correlation between total dose per weight and lithium levels at two months, suggesting that lithium metabolism was quite variable in this cohort.

There were no clinically significant abnormal laboratory values and no clinically significant changes in blood

Table 1. Demographic Data for Subject Cohort in Open-Label Treatment Study of Lithium in FXS

Age	
Mean ± SD (range)	11 ± 5 (6–23)
IQ on Stanford-Binet V	
Mean ± SD (range)	50.5 ± 4.9 (47–61)
Race	
Caucasian	13
African American	1
Asian	1
Living setting	
Home with family	14
Community group home	1
Number of concomitant psychoactive medications	
None	1
One	7
Two or more	7
Type of concomitant psychoactive medications	
SSRIs[a]	7
Stimulants	6
Antipsychotic	5
Alpha-agonist	3
Anticonvulsants	3

[a]Selective serotonin reuptake inhibitors.

chemistries including liver functions, creatinine, glucose or blood counts during the two month treatment period. Four subjects had a slightly elevated TSH at one month, one of which normalized spontaneously by two months (Table 2). Only one of these individuals had an abnormal T4 (thyroxin) or T3 (triiodothyronine) value. TSH did not correlate with lithium level or daily dose per weight at two months.

There was no significant change in heart rate or blood pressure for any study subject. At two months of treatment, the mean weight increase was 1.3 kg and there was a proportionate mean height increase of 1 cm. No individuals crossed percentiles for weight relative to height during the treatment period. Adverse events observed during the treatment period are shown in Table 2. There were no serious adverse events. Of note, polydipsia was reported by 7 subjects, polyuria by 4 subjects, and aggravation of aggressive behavior by 1 subject, while no subjects developed tremor. All adverse events reported were transient, mild or moderate in intensity, and none were of sufficient severity to result in discontinuation of lithium during the treatment period. The one individual who had seizures on lithium had no change in seizure frequency or severity from prior to starting lithium therefore the seizures were considered unrelated to lithium treatment. There was no relationship across the cohort between higher lithium levels and occurrence of adverse events.

Efficacy

There was significant improvement in behavior across the cohort as measured by the Total ABC-C score during the period of lithium treatment (Table 3). Specifically the Hyperactivity and Inappropriate Speech subscores showed significant improvement and the Irritability, Lethargy, and Stereotypy subscores showed a trend towards significant improvement. The CGI also showed significant improvement, with only one subject failing to improve and one subject showing worsening of functioning (Table 3). Mean improvement in the CGI rating of 1.3 corresponded to a mild-to-moderate overall improvement. Likewise, the VAS showed significant improvement across the group in parent-defined target behaviors, including ag-

Table 2. Adverse Events During Lithium Treatment Period

Symptom	Time Period[a]		Total Events[b]	Total Subjects[c]	Relationship to Lithium Treatment				
	0–4	4+			Unrelated	Unlikely	Possible	Probable	Definite
Aggression/irritability	2	2	4	2			√		
Appetite decrease	4		4	4			√		
Appetite increase	2		2	2				√	
Bed wetting	5	1	6	5				√	
Constipation	1	1	2	1			√		
Dental problems	1		1	1	√				
Diarrhea	3	1	4	4			√		
Drooling	1		1	2		√			
Ear infection	1		1	1	√				
Headache	2	2	4	2			√		
Polydipsia	7	1	8	7					√
Polyuria	5	1	6	4					√
Nose bleeds		1	1	1		√			
Rash	1		1	1		√			
Reclusive	1		1	1		√			
Ringing in ears	1		1	1			√		
Sedation	1		1	1			√		
Seizures		1	1	1	√				
Sleep problems	4	1	5	3			√		
URI/congestion	4	7	11	8	√				
Tiredness	2		2	2			√		
Vomiting	4		4	4			√		
Labs									
High TSH	4	3	7	4					√
Low T4		1	1	1					√

[a]Time period is in weeks such that 0–4 represents the first 4 weeks of treatment and 4+ represents the time between 4 weeks and 2 months.

[b]Total events is the total number of events that occurred during the treatment period.

[c]Total subjects is the total number of subjects experiencing the event (some subjects had an event more than once).

Table 3. Baseline Scores and Change in Behavior and Adaptive Measures after Treatment with Lithium for 2 Months

Measure	Baseline Group Mean ± SD	Group Mean Change ± SD	Number Improved	p[a]
VAS	18.3 ± 13.0	22.5 ± 21.8	12	0.003
CGI	4.7 ± 0.9	1.3 ± 1.1	13	0.004
ABC-C[b]				
Irritability	14.8 ± 8.9	−4.7 ± 9.5	9	0.105
Lethargy	11.4 ± 6.8	−3.9 ± 6.7	9	0.052
Stereotypy	7.0 ± 4.4	−1.8 ± 3.5	10	0.068
Hyperactivity	21.3 ± 9.8	−6.3 ± 7.2	12	0.002
Inappropriate speech	6.1 ± 2.7	−1.7 ± 2.8	11	0.035
Total	60.6 ± 19.9	−18.5 ± 23.6	13	0.005
Vineland communication				
Receptive	22.6 ± 2.6	0.9 ± 1.7	9	0.073
Expressive	35.9 ± 12.3	1.8 ± 5.0	10	0.077
Written	8.2 ± 7.5	0.3 ± 2.4	6	0.478
Vineland daily living skills				
Personal	55.1 ± 12.1	1.7 ± 1.7	10	0.005
Domestic	13.3 ± 6.8	0.0 ± 4.3	6	0.663
Community	14.1 ± 10.6	0.9 ± 3.0	7	0.221
Vineland socialization				
Interpersonal relationships	32.9 ± 7.5	0.6 ± 6.2	7	0.887
Play and leisure	21.3 ± 7.7	0.1 ± 4.5	8	0.733
Coping	11.7 ± 8.4	1.9 ± 4.7	8	0.190
Vineland motor skills				
Gross	32.1 ± 5.4	−0.3 ± 2.8	5	0.816
Fine	25.2 ± 4.2	0.3 ± 1.6	8	0.398
Vineland maladaptive behavior[b]				
Part 1	18.2 ± 4.8	−2.6 ± 4.3	10	0.040
Part 2	4.7 ± 2.8	−1.8 ± 1.9	11	0.004
Total	22.9 ± 6.4	−4.4 ± 4.9	12	0.007

[a]Significance.

[b]Negative value indicates a positive change.

gression, abnormal vocalizations, self-abuse, work refusal, outbursts, overemotionality, anxiety, meltdowns, mood swings, tantrums, perseveration, and crying, and substantial improvement in these behaviors in two-thirds of the individual subjects (Table 3).

Adaptive functioning, as assessed by the VABS, improved significantly in two specific areas (Table 3). Consistent with the results on the ABC-C and the VAS, Maladaptive Behavior on the VABS improved significantly as did Personal Daily Living Skills, suggesting that behavioral improvement was associated with functional improvements in day-to-day life skills.

The exploratory cognitive battery proved difficult to complete for many subjects in the cohort enrolled in this study, largely due to their challenging behavioral profile at study entry (see Table 4 for completion rates and score changes). All subjects did complete the PPVT at baseline and two months and although subjects demonstrated improved performance, this was insufficient to show significance (Table 4). Only one cognitive measure, the RBANS List Learning showed significant improvement over baseline performance after lithium treatment in the ten subjects able to complete the task at both assessments (Table 4). Five subjects were able to attempt more tests after lithium treatment than before (4 subjects did one more test and one subject did two more tests).

Some of the biophysical measures proved difficult to obtain consistently from this cohort of study subjects. Autonomic regulation could be assessed in most FXS participants (n = 14). When the FXS participants were contrasted with typically developing individuals, subjects with FXS expressed atypical autonomic regulation with lower amplitude respiratory sinus arrhythmia (i.e., an index of the cardiac vagal tone), less heart rate variability, faster breathing rates and faster heart rate (Heilman KJ, Harden E, Berry-Kravis E, et al. Atypical autonomic regulation in fragile X syndrome: Contrasts with normals and effects of lithium. *J Aut Dev Disord*. 2007, in preparation). Nine FXS participants completed measures of autonomic regulation at baseline and following two months of lithium

Table 4. Baseline Scores and Change in Cognitive Measures after Treatment with Lithium for 2 Months

Measure	Baseline Group Mean ± SD	Subjects Attempting Test at Baseline (N)	Subjects Attempting Test at 2 Months (N)	Group Mean Change ± SD	Subjects Attempting Test at Both Visits (N)	Number Improved	p[a]
Card task-color	3.0 ± 1.6	10	8	0.9 ± 2.2	8	4	0.315
Card task:-number	1.9 ± 1.9	7	7	−0.8 ± 1.3	4	1	—[b]
CPT: visual-omissions	0.7 ± 0.8	11	11	0.4 ± 1.2[c]	10	2	0.709
CPT: visual-commissions	16.3 ± 23.8	11	11	0.6 ± 13.7[c]	10	6	0.389
CPT: auditory-omission	2.2 ± 4.4	9	9	−1.5 ± 3.4[c]	8	3	0.789
CPT: auditory-commissions	13.8 ± 21.7	9	9	5.8 ± 11.0[c]	8	3	0.389
NVALT learning[d]	34.0 ± 16.4	12/6[e]	14/9[e]	0.2 ± 7.0	12/7[d]	6	—[b]
NVALT reversal[f]	35.7 ± 14.7	6/3[e]	9/5[e]	2.7 ± 14.7	6/4[d]	2	—[b]
NEPSY tower	2.1 ± 1.1	9	9	−0.2 ± 1.1	9	3	0.621
RBANS: list learning	10.0 ± 7.4	10	11	4.1 ± 5.0	10	8	0.028
RBANS: story memory	5.6 ± 4.5	9	9	3.6 ± 1.1	9	4	0.512
PPVT	56.9 ± 16.6	15	15	1.7 ± 13.0	15	9	0.512

[a]Significance.

[b]Not enough data points to calculate p value.

[c]Negative value indicates a positive change.

[d]Scores on the NVALT are presented as the number of trials the subject required to make the association between stimulus and reward.

[e]Subjects who took the test/subjects who passed the test (made the association between stimulus and reward in less than 50 trials).

[f]Only subjects who pass the NVALT learning take the NVALT reversal.

treatment. Autonomic regulation was not normalized by the lithium treatment. Evaluation of central auditory processing and eye tracking was difficult for FXS participants with only 4 participants completing the auditory processing tasks and only two participants completing the eye tracking task in both the baseline and two month testing sessions. However, data from those who could complete the initial baseline measures suggests both difficulties in auditory processing and substantial gaze avoidance in subjects with FXS relative to controls (Heilman KJ, Harden E, Berry-Kravis E, et al. Atypical autonomic regulation in fragile X syndrome: Contrasts with normals and effects of lithium. *J Aut Dev Disord*. 2007, in preparation).

ERK activation times, defined as the time in minutes for ERK phosphorylation to reach the half maximal level, were successfully obtained at baseline and two months for 11 subjects, and normalized (decreased) for all 11 subjects after lithium treatment (Figure 2). The baseline mean activation rate of 4.872 minutes was reduced to 4.109 minutes after 2 months' lithium treatment ($p = 0.007$). The change of about 0.75 minutes represents about 75% of the 1 minute difference in activation time between FXS and control samples in a prior study.[39] Percent change in ERK activation rate did not correlate with lithium levels or daily lithium dose per weight at two months.

At the end of two months of treatment, subjects were offered ongoing treatment with follow up through an extension study for 1 year. At two months, twelve families independently reported an impression of improvement in language use and thirteen reported improvement in some aspect of functioning. Of these, 12 elected to continue into the one year extension (one did not continue due to the limiting side effect of polyuria with bedwetting). One family reported no change but no side effects and elected to continue. The one subject who deteriorated behaviorally during treatment and showed no improvement in any area did not continue. Neither lithium level nor total daily dose per weight at 2 months of treatment correlated with magnitude of improvement on any ABC-C subscore or the total ABC-C score, CGI or VAS ratings, VABS Maladaptive Behavior or Personal Daily Living Skills subscales, or the RBANS List Learning score.

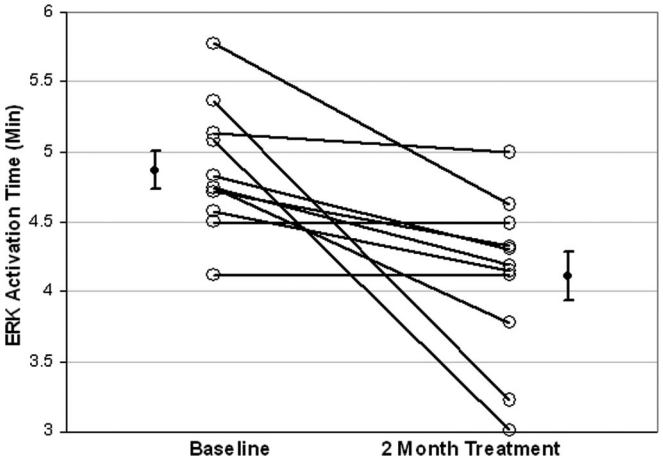

Figure 2. Change in ERK activation times from baseline to two months of lithium treatment in FXS study participants (N = 11). Reduction of activation time (faster activation) represents normalization as FXS subjects show longer activation times than normal controls.

DISCUSSION

The results of this pilot open-label trial suggest that lithium has positive effects on behavioral, adaptive skills

and a single cognitive measure for individuals with FXS. Despite the trend to improvement, significance was not reached for the primary endpoint, change in the ABC-C Irritability Subscale, although significance was achieved for the Total ABC-C score, two ABC-C subscales, and multiple other measures. Since all the ABC-C subscales assess areas of behavioral dysfunction seen in FXS, delineation of a specific subscale as primary is empiric and mostly dependent on known effects of lithium on behavior in non-FXS cohorts. If, as hypothesized for this study, lithium is targeting the underlying defect in FXS, different patterns of response and improvement in multiple additional areas of dysfunction might actually be expected and thus the Total ABC-C score may be most reflective of this sort of overall change in multiple areas.

Positive responses were distributed across the age range of the study cohort, suggesting that both children and young adults with FXS can benefit from lithium treatment. Beneficial effects of lithium in this small cohort did not seem to relate strongly to dose or lithium levels, although it is certainly possible that in a larger treatment cohort, such relationships would emerge. Because this was a trial of lithium as adjunctive or add-on therapy, most subjects were on other medications to manage their behavioral dysfunction and even though doses were held stable, it is certainly possible that interactions between lithium and these medications could have contributed to lithium response. However, the magnitude of improvement in scores for the entire cohort was similar to the magnitude of improvement for subgroups of subjects treated with stimulants, SSRIs (selective serotonin reuptake inhibitors), or antipsychotics for all measures showing significant improvement in the study, suggesting these interactions did not have a major effect on lithium response. All subjects were in a consistent educational or work program throughout the 2 months in the study. Although environmental factors could certainly contribute to lithium response, and minor environmental changes would be difficult to control, there were no substantial changes in programming, family environment or living setting during the treatment period for any subject.

The study also suggests that lithium at high therapeutic levels may not be associated with substantial toxicity in individuals with FXS, although conclusions about less common forms of toxicity are limited by the small study size. Side effects were not specific to age although younger boys with FXS (<12 years) may have had slightly more side effects. Lack of relationship between side effects and dose or lithium level suggests wide interpersonal variation in susceptibility to lithium toxicity in FXS. Lack of correlation between lithium dose and levels also suggests wide interindividual variation is likely for lithium absorption and processing and thus dosing needs to be individualized and based on levels and side effect review. The frequency of polyuria/polydipsia in this study was similar to that observed in a previous study of lithium treatment in a cohort of children with developmental disability, and this side effect was the predominant side effect in both studies. Aggressive behavior, appetite increase or decrease, enuresis, constipation, diarrhea, and headache were other complaints occurring infrequently during treatment in this cohort that may represent side effects of lithium in FXS. The observation of an elevated TSH in 20% of the cohort without attendant abnormal T3 or T4 is consistent with but less frequent than descriptions in previous literature on thyroid abnormalities induced by lithium. Thyroid dysfunction normalized or adapted in some subjects and this may have to do with the relatively young age of the subjects (more potential for compensatory changes and less likely to have underlying pre-existent thyroid disease), monitoring of TSH at an earlier time point than would be done in standard clinical practice, or unknown FXS-specific factors. Lack of tremor or weight gain as side effects is likely due to the young age of the participants and the short duration of the study, respectively.

Many of the exploratory cognitive outcome measures were evaluated in a study of reproducibility of these measures in an FXS cohort with widely variable age. Reproducibility was fair to good for most of the measures[32] and this lithium trial provided an opportunity to test drug responsiveness for the measures. Completion rates for identical measures were higher in the reproducibility study[32] but the subjects in the lithium trial were more behaviorally challenging and are likely more representative of a typical FXS population for clinical trials. Some of the cognitive measures explored (e.g. RBANS Story Memory, FXCPT, NEPSY Tower) did not seem to be sensitive to lithium treatment and it remains unknown if they would be sensitive to other treatments with different mechanisms. Others (e.g. Card Task, biophysical measures) were simply too difficult to complete and appeared too complex for many of the study participants. A third problem, particularly for the RBANS Story Memory, had to do with perseverative responding, such that the story from the baseline visit (form A on the test) was what the subjects reported at 2 months after they had been read a different story (on form B). The RBANS List Learning is one of the simplest measures to use and requires only single word responses, rendering it easier to complete with a measurable score. This test did show significant improvement on lithium, consistent with parent reports of better quality of verbal communication. Thus, the RBANS List Learning appears to show drug responsiveness and would be a good measure to continue to utilize in future placebo controlled trials targeting cognition in FXS.

The measures of autonomic regulation were not responsive to lithium treatment and other neural-based measures, such as eye tracking, were too difficult to complete for the study group to provide a useful measure in future clinical trial designs for pharmacological interventions in FXS. On the other hand the ERK activation measure showed good promise as a biomarker for future clinical trials targeting pathways of translational activation involving ERK signaling in FXS.

There are a number of limitations to this study, most obviously the small size of the cohort treated and the open-label design, which allows for potential placebo

effect and rater bias. Placebo effects observed over one month in a prior placebo-controlled study of ampakine CX516 in FXS[31] for the ABC, VAS, CGI, and RBANS List Learning were all much smaller than the magnitude of the group mean changes on these same measures in the current lithium trial, suggesting the changes observed may be more than placebo effect. These placebo results are from a different study, however, and it is not clear they are comparable, and rater bias in parent- and investigator-rated scales may be a greater source of overestimation of lithium effects in this study than placebo effects. Use of a second rater and inter-rater reliability comparisons would have helped improve validity of these measures. Rater bias would, however, not play a role in the changes in the RBANS list learning and the ERK activation measure.

The concept motivating this study was to extend to a human population, findings demonstrating normalization of phenotypes in animal models of FXS with lithium. In that regard the trial was aimed at targeting the underlying defect in FXS as a mode of treating the behavioral and cognitive phenotype in humans. Theoretically lithium should act on a common pathway which would result in normalization of mGluR and other activation pathways leading to dendritic translation in FXS throughout all brain areas, and thus the treatment would be directed at "correcting" downstream aberrant translational regulation of all proteins normally under control of FMRP. However, lithium is certainly not selective for mGluR or even other translational regulatory pathways and is expected to have many additional actions in other cellular pathways in the CNS unrelated to FXS mechanisms. It has been demonstrated that PK-C over-activity produces prefrontal working memory deficits in rats and monkeys that are reversed by lithium treatment.[40] Although working memory is a particular area of cognitive weakness in individuals with FXS,[41] clinical benefits and problems related to lithium treatment would result from the balance of the diffuse activities of lithium and the relative weight of mGluR-related and other effects. Indeed benefits seen in this study may be non-specific and related to the mood stabilizing effects of lithium.

Nonetheless, the results of this trial are consistent with positive effects of lithium in animal models of FXS and thus are consistent with the concept that correction of pathways involved in excessive translational activation, including the hypothesized mGluR pathway, would be helpful in FXS. The results suggest a placebo-controlled trial of lithium in FXS should be done as a next step to confirm effectiveness, before recommending lithium treatment generally to individuals with FXS, and that adverse events would not be expected to be a limiting problem in such a controlled trial. This pilot study serves as an example to demonstrate translation of information from basic science and animal model research to clinical treatment in a cognitive disorder. Finally, this study can be used to inform clinical trial design. The results suggest that even behaviorally difficult subjects with FXS can be included in clinical trial design and complete many measures in a formal trial setting. Outcome measures successfully utilized in this study, including the ABC, VAS, CGI, VABS, RBANS List Learning, and ERK activation biomarker, could be considered for use as endpoints in a more extensive placebo-controlled trial of lithium in FXS and in future trials of mGluR5 blockers in FXS.

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