




ORIGINAL ARTICLE

Metformin treatment in young children with fragile X syndrome

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Abstract

Background: Metformin is a drug commonly used in individuals with type 2 diabetes, obesity, and impaired glucose tolerance. It has a strong safety profile in both children and adults. Studies utilizing the *Drosophila* model and knock out mouse model of fragile X syndrome (FXS) have found metformin to rescue memory, social novelty deficits, and neuroanatomical abnormalities. These studies provided preliminary evidence that metformin could be used as a targeted treatment for the cognitive and behavioral problems associated with FXS. Previously, a case series of children and adults with FXS treated with metformin demonstrated improvements in irritability, social responsiveness, language, and hyperactivity.

Methods: Here, we present nine children with FXS between 2 and 7 years of age who were treated clinically with metformin and monitored for behavioral and metabolic changes.

Results: Parent reports and developmental testing before and after metformin are presented. There were improvements in language development and behavior (such as lethargy and stereotypy) in most of the patients.

Conclusion: These results support the need for a controlled trial of metformin in children with FXS under 7 years old whose brains are in a critical developmental window and thus may experience a greater degree of clinical benefit from metformin.

KEYWORDS

FMR1, fragile X syndrome, metformin, targeted treatments, translational medicine

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1 | INTRODUCTION

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability (ID) and is typically diagnosed between 2 and 3 years of age at the time language delays and behavioral manifestations emerge (Bailey, Cody Hazlett, Roberts, & Wheeler, 2011). It is caused by a trinucleotide repeat expansion of CGG in the promoter region of Fragile X Mental Retardation-1 (*FMRI*, OMIM: 309550) gene, leading to methylation, transcriptional silencing, and the absence or deficiency of fragile X mental retardation protein (FMRP).

Metformin was originally FDA-approved for its effects in lowering blood glucose levels in patients with type 2 diabetes (T2D). It is safe and effective at doses up to 1,000 mg twice a day in children 10–16 years old with T2D (Jones, Arslanian, Peterokova, Park, & Tomlinson, 2002). Because its mechanism of action does not directly stimulate insulin, metformin is unlikely to lead to hypoglycemic episodes (Bodmer, Meier, Krahenbuhl, Jick, & Meier, 2008; Bolen, Feldman, Vassy, & Feldman, & Vassy, 2007). Metformin has since expanded to the treatment of obesity and overeating in children and adults with and without T2D (Anagnostou et al., 2016; Klein, Cottingham, Sorter, Barton, & Morrison, 2006; Muzar, Lozano, Kolevzon, & Hagerman, 2016; Park, Kinra, Ward, White, & Viner, 2009). For patients who are nonobese, metformin has been effective in glycemic control (Ong, Molyneaux, Constantino, Twigg, & Yue, 2006). In FXS, overeating is commonly associated with psychiatric comorbidities including anxiety and obsessive-compulsive behaviors. Also, the Prader-Willi-phenotype (PWP) of FXS is found in less than 10% of children with FXS. The FXS-PWP is associated with hyperphagia, morbid obesity, delayed puberty, and lowered cytoplasmic FMRP interacting protein (*CYFIP1*, OMIM: 606322) expression (Muzar et al., 2016; Nowicki et al., 2007). The common use of atypical antipsychotics in children with FXS further increases their risk for obesity. Treatment with metformin showed clinical improvement in eating behaviors and weight loss in those with and without FXS-PWP and is now recommended for the treatment of obesity in FXS (Dy et al., 2018).

Recent research in animal models of FXS has demonstrated that metformin, a T2D medication, can rescue both behavioral and cognitive features of FXS. In the *Drosophila* FXS model, metformin rescued abnormalities of circadian rhythm and memory problems (Monyak et al., 2017). In the *FMRI* knock out (KO) mouse, metformin rescued many deficits including: social deficits, seizures, macroorchidism, and was also seen to improve extracellular signal regulated kinase (ERK) signaling, eukaryotic translation initiation factor 4e (*EIF4E*, OMIM: 133440) phosphorylation, and matrix metalloproteinase 9 (*MMP9*, OMIM: 120361) overexpression (Esfahanian et al., 2012; Gantois et al., 2017; Gantois,

Popic, Khoutorsky, & Sonenberg, 2018). Upregulation of *MMP9* has been shown to interfere with synaptic maturation and plasticity in both the *FMRI* KO mouse (Bilousova et al., 2009; Rotschafer, Trujillo, Dansie, Ethell, & Razak, 2012) and in patients with FXS (AlOlabi et al., 2017; Dziembowska & Wlodarczyk, 2012). Metformin has been shown to lower *MMP9* levels via reduction of *EIF4E* phosphorylation which promotes translation of a subset of mRNAs including *MMP9* (Gantois et al., 2017, 2018; Hoeffler et al., 2012; Li et al., 2017; Muzar et al., 2016). These developments have led to the off-label clinical use of metformin in patients with FXS. The first seven cases of patients ages 4–60 years old treated clinically demonstrated improvements on the Aberrant Behavior Checklist-Community (ABC-C), language, and conversational skills per parent report (Dy et al., 2018). The growing interest of metformin as a feasible alternative for this population led to the initiation of a controlled trial of metformin for individuals with FXS between the ages of 6–25 years old (NCT03479476). In addition, a smaller open label trial of metformin is also underway for those 10–40 years old with FXS (NCT03722290).

Previous studies have suggested that targeted treatments may work best for young children with FXS (Berry-Kravis et al., 2017; Greiss Hess et al., 2016; Leigh et al., 2013). Furthermore, recent research in the *FMRI* KO mouse shows that correction of key disruptions in neuronal development during the critical period in young animals results in restoration of synaptic development, produces a long-lasting rescue of somatosensory circuit function, and normalizes differentially expressed proteins (He et al., 2017). Such findings indicate a critical period of development during which targeted interventions may have significant and durable effects on developmental trajectory and outcomes in FXS. These data suggest that metformin would have a significant benefit in young children with FXS; here we describe nine boys between 2 and 7 years old with FXS who underwent off-label clinical treatment with metformin for a period of at least 3 months. It is important to emphasize that represented here are individual cases of young children with FXS treated clinically with metformin, not an open-label trial; therefore, there is no specified protocol homogenizing the sample.

2 | MATERIALS AND METHODS

Patients were seen clinically at the Fragile X Treatment and Research Center at the MIND Institute at the University of California, Davis Health for management of FXS. All patients had a full mutation FXS documented via *FMRI* (GenBank: NG_007529.2) DNA testing. Laboratory studies were attempted pre-treatment whenever possible and included fasting glucose, a chemistry panel, and HbA1c. When available, copies of previous FXS testing, including *FMRI* methylation

status, were also obtained from caregivers or patient records with permission. Post-treatment laboratory studies were collected in many cases at patients' primary care locations and were therefore not available to report here. All families signed an institutional review board-approved consent form for research involving the blood work and developmental testing and gave consent for publication of their clinical case histories.

Parents completed the ABC-C and reported on behavior and symptoms pre- and post-metformin treatment. The ABC-C was scored using the FXS algorithm (Sansone et al., 2012). The ABC-C is a 58-item global behavior checklist implemented for the measurement of treatment effects in individuals with ID; a factor analysis of the ABC-C specifically in FXS generated a 6-factor structure: irritability, lethargy, stereotypy, hyperactivity, inappropriate speech, and social avoidance (Berry-Kravis et al., 2017). Higher subscale scores are correlated with more severe aberrant behavior; therefore, a decrease in the scores across each domain is indicative of an improvement of that behavior. Length of time between pre- and post-treatment ABC-C administration ranged from 1 to 7 months due to the natural variability in duration of clinical follow-up and caregiver report.

Developmental testing was administered pre- and post-treatment for six of the nine subjects using the Mullen scales of early learning (MSEL) (Mullen, 1995). The MSEL includes four domains: fine motor, receptive language, expressive language, and visual reception, all of which have age equivalent scores generated. In place of the early learning composite (ELC) score, which is an age-normed standard score generated from the aforementioned domains, a global developmental age (GDA) score was used. The GDA averages the age equivalents across the four domains (Roberts et al., 2009) and thus provides a consistent metric that can be utilized as a measurement for improvement in this report. For one of the subjects, pre-treatment age equivalent scores from three of the four domains from the Bayley Scales of Infant and Toddler Development (Bayley, 2006) were used in place of pre-treatment MSEL data, which was not available. The moderate correlation between the two developmental tests is reported in the MSEL test manual (Farmer, Golden, & Thurm, 2016; Mullen, 1995). Due to the clinical nature of this study, there is variability in the pre- and post-testing time points for the MSEL among the cases which ranges from 7 to 26 months after pre-treatment testing.

Statistical analyses of pre- and post-treatment ABC-C and developmental testing scores are presented in median and interquartile ranges. They were done in Stata 14 utilizing a nonparametric test "signrank." Stata command tests the equality of matched pairs of observations by using the Wilcoxon matched-pairs signed-ranks test and generates *p*-values (Wilcoxon, 1945). The null hypothesis is that both distributions are the same. A pre-specified significance

level of $p < .05$ was assumed. Additionally, using MSEL age equivalent scores, mean linear slopes in all subscales and GDA were generated using the individual trajectories per patient. The slope was then compared to $m = 0.48$, the experimentally determined expected rate of developmental gains seen in children with FXS (Bailey Jr., Hatton, & Skinner, 1998).

3 | RESULTS

3.1 | Case 1

Patient 1 is a 4-year, 2-month-old boy diagnosed with FXS at 9 months of age due to missed motor milestones and delayed babbling. He was adopted at birth and lives with his adoptive family. His birth weight was 3.6 kg and he had respiratory distress requiring immediate treatment. He was diagnosed with neonatal pneumonia, requiring a 7-day Neonatal Intensive Care Unit stay. He sat alone at 8 months and took steps at 13 months. With the intention of improving speech, at 12 months he was started on sertraline but with limited results; his first words were spoken at 16 months, with very slow progress in language thereafter. He had tympanostomy tubes placed at age 2 without significant improvement in language. He has a history of awakening multiple times per night for a total of 2–4 hr. Behaviorally, he is generally a "happy and social child with mild anxiety."

On examination at age 2 years, typical physical features of FXS were observed, and baseline laboratory findings were normal (see Table 1). He was started on metformin at 25 mg of the liquid form that is 100 mg/ml at dinner, and his dose was gradually increased to 200 mg twice a day (bid) over 1 year (see Table 1). After initiation of metformin, his sleep disturbance resolved, only occasionally awakening once for roughly 30 min. Two weeks after initiation, he went from stacking 3–4 blocks to stacking a tower of 11 or more blocks; within a few more weeks, he began building more complex structures comprised of different size blocks. He showed marked improvement in self-help and motor activities, including toilet training, clearing the table and loading the dishwasher, brushing his own teeth, dressing independently, and learning how to make toast. His preschool teachers, who were unaware of metformin treatment, told his mother that "it's like something just clicked or he just woke up. He's a whole different kid."

Patient 1's ABC-C composite score improved on metformin (see Table 2). With respect to language acquisition, he had 10–20 verbal words and 20 signs at the time of initiation. Six months later, he demonstrated use of 60–80 words, including several 2-word phrases and 100 signs, and his mother reports that his cognitive skills have significantly improved as well. He tolerated all medication increases with only minor self-limited diarrhea for 1–2 days. Fasting blood glucose has

TABLE 1 Summary of all nine cases

Case/Age at initiation/sex	Diagnoses	Family history	Physical features	CGG repeats	Methylation status	<i>FMR1</i> mRNA expression level	Pre-Tx laboratory findings	Metformin dose	Other medications
Case 1. 2-year-old, male	FXS	Adopted; family history unknown	Broad forehead, epicanthal folds, hyperextensible joints to 90 degrees, double jointed thumbs, slight hypospadias, 4 ml testicular volume	320, 490, 790, 1,280	Methylation mosaicism with a full mutation of the cells	0.18 (± 0.001)	HbA1c: 5.0, FBS: 84	25 mg QD increased gradually over 1 year to 200 mg BID (31.01 mg kg ⁻¹ day ⁻¹)	Sertraline 2.5 mg, folic acid 5 mg, <i>N</i> -acetyl cysteine, melatonin, probiotic, vitamins C, D & E
Case 2. 4-year-old, male	FXS, ASD, OSA and CSA	Mother (carrier, 99 CGG repeats), maternal grandmother (carrier, no FXTAS)	Less than 6 ml testicular volume, small umbilical hernia, finger joints hyperextensible with MP extension to 90 degrees, single palmar crease, bilaterally, flat feet, bilaterally	>200	Full	None	HbA1c: 4.9, FBS: 92	50 mg at dinner increased after 1 week to 50 mg BID. Increased after 6 months to 100 mg BID (12.91 mg kg ⁻¹ day ⁻¹)	Clonidine (0.05 mg qHS, 0.025 mg in the morning), melatonin 1.5 mg qHS, omega-3, folic acid
Case 3. 6-year-old, male	FXS, epilepsy	Maternal grandfather (carrier, FXTAS)	Heterochromia on the right eye, fingers hyperextensible, DTRs 1+ bilaterally on UE	470	Full	None	HbA1c: 5.3, FBS: 86	50 mg at dinner increased to 50 mg BID (5.62 mg kg ⁻¹ day ⁻¹)	Sertraline 4 mg QD, minocycline 25 mg QD, guanfacine 0.5 mg BID, oxcarbazepine 300 mg BID, melatonin 0.25 mg qHS
Case 4. 4-year-old, male	FXS, ASD	Mother (carrier, 77 CGG repeats), maternal grandfather (carrier, 65 repeats), maternal great-grandmother (carrier), 2 aunts (carriers), 2 cousins (carriers, 77 and 75 CGG repeats, respectively).	Forehead is broad, ears are prominent with cupping bilaterally. Finger joints are hyperextensible with MP extension to 90 degrees. Thumbs are double jointed, feet are completely flat with mild pronation. 4 ml testicular volume	550	Full	None	Not Available	50 mg BID increased after 3 months to 100 mg BID (10.75 mg kg ⁻¹ day ⁻¹)	Sertraline 5 mg QD, guanfacine 50 mg BID
Case 5. 7-year-old, male	FXS, ASD, ADHD, seizures, cleft lip S/P repair	Mother (carrier)	Cleft lip repair on the left, 3 ml testicular volume	350, 510, 860	Methylation mosaicism with a full mutation methylated in >95% of the cells	0.12 (± 0.004)	HbA1c: 5.4, NF glucose: 102	500 mg QD at dinner increased after 6 months to 500 mg BID (44.26 mg kg ⁻¹ day ⁻¹)	Clonidine 0.1 mg TID, clonidine 0.2 mg qHS, adderall 2.5 mg TID, oxcarbazepine 420 mg BID, CBD tincture 0.3 ml, melatonin 10 mg qHS
Case 6. 4-year-old, male	FXS, ASD	Mother (carrier)	Mildly prominent ears, prominent forehead, decreased tone, transverse palmar crease on right palm, bridged palmar crease on left palm, hyperextensible finger joints	380, 650	Methylation mosaicism with a full mutation methylated in >95% of the cells	0.18 (± 0.03)	HbA1c: 4.7, FBS: 87	50 mg QD at dinner increased after 1 week to 50 mg BID (6.85 mg kg ⁻¹ day ⁻¹)	Sertraline 2.5 mg QD, lithium orotate 2.5 mg QD, methylfolate 4 mg QD, vavarin 1 capsule QD, multivitamin with folate QD, fish oil QD, melatonin QD
Case 7. 2-year-old, male	FXS	Mother (carrier), maternal grandmother (carrier), 2 maternal great-aunts (carriers), 6 maternal cousins (carriers)	Mildly hyperextensible joints in his fingers and hands, flat feet, low tone in his ankles/core.	480	Full	None	HbA1c: 4.8, FBS: 78	50 mg BID increased after 5 months to 150 mg BID (20.27 mg kg ⁻¹ day ⁻¹)	Sertraline 2.5 mg QD, melatonin 1 mg qHS, multivitamin with folate QD, fish oil QD
Case 8. 3-year-old, male	FXS, ASD	Mother (carrier)	Long face with high forehead, high palate, large prominent ears, 3 ml testicular volume, flat feet bilaterally with mild pronation	430, 560, 800	Methylation mosaicism with a full mutation methylated in ~94% of the cells	None	HbA1c: 5.1, FBS: 77	50 mg at dinner increased after 1–2 weeks to 50 mg BID (6.38 mg kg ⁻¹ day ⁻¹)	Sertraline 1.25 mg QD
Case 9. 4-year-old, male	FXS	Mother (carrier)	Prominent ears, bilateral tympanostomy tubes present, mild epicanthal folds bilaterally, hyperextensible fingers, flat feet	360, 520, 660, 860	Full	None	FBS: 80	50 mg QD gradually increased to 150 mg BID (16.67 mg kg ⁻¹ day ⁻¹)	Sertraline 5.0 mg QD

Abbreviations: ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; BID, two times a day; CBD, cannabidiol; CSA, central sleep apnea; DTRs, deep tendon reflexes; FBS, fasting blood sugar; FMRI (GenBank: NG_007529.2), fragile X mental retardation 1; FXS, fragile X syndrome; FXTAS, fragile X with tremors and ataxia syndrome; MP, metacarpophalangeal; NF, non-fasting; OSA, obstructive sleep apnea; Pre-Tx, pre-treatment with metformin; QD, once daily; qHS, at hour of sleep; S/P, status post; TID, three times a day; UE, upper extremity.

TABLE 2 Aberrant Behavior Checklist-Community (ABC-C)—prior to metformin treatment and after 1–8 months of treatment

	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6		Case 7		Case 8		Case 9	
	Baseline	After 4 mos.	Baseline	After 4 mos.	Baseline	After 3 mos.	Baseline	After 3 mos.	Baseline	After 7 mos.	Baseline	After 1 mos.	Baseline	After 8 mos.	Baseline	After 7 mos.	Baseline	After 6 mos.
ABC-C composite score	15	7	109	39	29	29	32	28	113	59	22	20	33	48	67	59	62	51
I. Irritability	8	3	32	12	12	4	4	6	40	19	5	3	14	17	21	25	29	22
II. Lethargy	1	0	20	4	1	4	3	3	23	12	0	0	0	1	11	5	0	0
III. Stereotypy	2	0	11	5	0	4	4	4	10	5	2	0	2	1	9	6	9	8
IV. Hyperactivity	4	4	29	13	13	9	16	12	29	14	12	11	17	27	22	16	17	14
V. Inappropriate speech	0	0	6	2	2	0	0	0	9	8	3	6	0	2	4	6	7	7
VI. Social avoidance	0	0	11	3	1	4	3	3	2	1	0	0	0	0	0	1	0	0

remained stable at all doses. He is tolerating his current dose without problems.

3.2 | Case 2

Patient 2 is a 6-year, 1-month-old boy diagnosed with FXS at the age of 2 years 7 months after parental concern regarding developmental delays. He was born at term and weighed 3.2 kg. He sat alone at 9 months, walked at 19 months, and fed himself and said his first word at 3 years. Past medical history includes reflux since infancy as well as more than 10 episodes of acute otitis media (OM), prompting placement of his first set of pressure equalization (PE) tubes at 3 years, 11 months. After a possible seizure at 7 months, patient 2’s EEG and MRI were normal; no further seizure-like episodes have occurred since. Once his parents noted loud snoring and brief pauses in breathing, a diagnosis of obstructive and central sleep apnea was made and an adenoidectomy was performed.

Behaviorally, patient 2 chews on things, babbles to himself, and hand flaps when he is excited or anxious. His eye contact is poor with his peers but better with adults; he is hyperactive and engages in perseverative behavior, such as repetitively pouring liquids.

After diagnosis, patient 2 was evaluated for an individualized education program and started school where he is receiving speech, occupational, and physical therapy. At 4 years, he initiated metformin at a dose of 50 mg bid which was gradually increased to 100 mg bid (see Table 1). His pre- and post-metformin ABC-C scores demonstrate an overall decrease across all measured behaviors (see Table 2). Since starting metformin, he has also made progress in expressive language, specifically in expanding his vocabulary. Although baseline MSEL testing was not available for comparison, a post-treatment MSEL at 58 months yielded age equivalent scores (in months) of 25 for visual reception, 27 for fine motor, 18 for receptive language, and 15 for expressive language, with an ELC of 49. His parents reported no significant side effects aside from loose stools, which resolved spontaneously within two days of metformin initiation.

3.3 | Case 3

Patient 3 is an 8-year, 6-month-old boy with a normal birth history. However, significant developmental delays and hypotonia led to a FXS diagnosis at 18 months. Due to severe anxiety, at 3.5 years he was treated with sertraline, which was beneficial. Attention deficit hyperactivity disorder symptoms emerged at 3 years, and he began guanfacine a year later. The stimulant methylphenidate was introduced at 5 years with beneficial effects on hyperactivity and short attention span. Past medical history includes intermittent exotropia

and mild astigmatism, two episodes of OM, and occasional constipation. He currently receives physical, occupational, and speech-language therapy.

At the age of 6 years, 7 months, patient 3 initiated metformin at a starting dose of 50 mg at dinner and increased to 50 mg bid without any side effects (see Table 1). He experienced initial improvement in behavior, but after one month he suffered a generalized seizure in the early morning with tonic-clonic movements and was taken to the ER. An MRI revealed grey matter heterotopias and a possible cortical abnormality of the posterior cingulate gyrus; furthermore, an EEG showed spike wave discharges that were diffusely dispersed throughout the reading. ER laboratory reports showed a normal glucose. At this time, he was started on oxcarbazepine for treatment of his seizures. Due to the occurrence of a second generalized seizure 1 week later, metformin was discontinued.

Patient 3 exhibited an overall improvement across all measurements on the ABC-C before metformin discontinuation (see Table 2). Likewise, developmental testing shows an overall improvement pre- and post-metformin (see Table 3). Within the first month of his treatment with metformin, patient 3's parents reported gains in his language and cognition; specifically, his mother noted more complex language, thoughts, and statements from him. One year later, he continues on oxcarbazepine without seizures.

3.4 | Case 4

Patient 4 is a 6-year-old boy born via emergency C-section due to premature labor at 33 weeks gestation; his mother had partial placental abruption. Developmentally he sat at 6.5 months, crawled at 12 months, walked at 12.5 months, and began speaking at 3 years. Patient 4 exhibits some hand flapping when excited, tactile defensiveness, and anxiety in new situations. Past medical history includes recurrent OM treated with PE tube placement, gastroesophageal reflux disease (GERD), milk intolerance, chronic rhinitis, partial adenoidectomy, and two episodes each of bronchiolitis and croup before 12 months.

Patient 4 began metformin at 4 years, 1 month (see Table 1). His pre-treatment MSEL was performed at a chronological age of 39 months and yielded age equivalent scores (in months) of 23 for visual reception, 24 for fine motor, 28 for receptive language, and 14 for expressive language, with an ELC of 54. Follow-up testing post-treatment was not obtained. Since initiating metformin, his vocabulary has increased from 2 words to about 40 words. His mother notes improvement in gross motor function, particularly proprioception and ability to imitate actions on command. She reports that metformin treatment has “unlocked” him, noting “he now has a general increased connectedness to [family] and others around him that he didn't have before.” His creative and independent play

TABLE 3 Mullen Scales of Early Learning (MSEL)—prior to metformin treatment and after 7–26 months of treatment

	Case 1		Case 3		Case 6		Case 7		Case 8		Case 9	
	Baseline ^a 22 mos.	Post 30 mos.	Baseline 63 mos.	Post 89 mos.	Baseline 34 mos.	Post 47 mos.	Baseline 23 mos.	Post 31 mos.	Baseline 46 mos.	Post 53 mos.	Baseline 30 mos.	Post 50 mos.
Visual reception	NA	27	30	40	38	40	15	25	50	52	18	29
Fine motor	13	21	20	23	29	30	18	21	26	27	21	27
Receptive language	19	27	36	36	32	36	10	17	31	36	24	33
Expressive language	16	17	42	53	21	29	7	14	18	27	22	48
Early learning Composite	75	70	52	55	61	61	54	54	63	62	61	61
Global development age score ^b	UTG	23	32	38	30	33.75	12.5	19.25	31.25	35.5	21.25	34.25

Abbreviations: NA, not administered; UTG, unable to generate.

^aBaseline testing scores taken from Bayley Scales of Infant and Toddler Development, 3rd edition.

^bGlobal developmental age was calculated based on the average age equivalents of the Mullen subscales.

have soared, and he is now initiating play with his brother and dogs. Mother attributes these gains to metformin because of minimal to no therapy since initiation, aside from continuing to attend his ongoing applied behavior analysis (ABA) sessions.

Patient 4's only reported side effect is increased verbalization of frustration when he does not get his way. Indeed, on the ABC-C, he exhibited a slight increase in irritability but an overall decrease across all other behaviors except stereotypy, which remained constant (see Table 2).

3.5 | Case 5

Patient 5 is an 9-year, 3-month-old boy who was diagnosed at 2 years with FXS. Mother had a normal pregnancy until 41 weeks when an emergency C-section was performed for fetal distress. At 3 months, patient 5 underwent surgery for the repair of cleft lip and recovered well. He crawled at 10 months, walked at 18 months, and spoke a few words at 2 years; however, emergence of more words was delayed until approximately 5 years, with phrasing at 6 years. Due to recurrent OM, PE tubes were placed at 2 years and again at 4 years. He has also been diagnosed with autism spectrum disorder (ASD).

At 5 years old he was found seizing in his bedroom with a grand mal seizure. For several days afterwards, he was less verbal and off balance but he gradually recovered. An MRI at the time showed mildly dilated ventricles with a somewhat thin corpus callosum, and an EEG demonstrated right-sided slowing and spike wave discharges. He began levetiracetam but discontinued due to increased aggression and other behavioral problems and instead started oxcarbazepine. On oxcarbazepine, patient 5 continued to have staring spells that were often associated with projectile emesis and falling to the ground; these partial motor seizures occurred once or twice a week.

Behaviorally, patient 5 was very aggressive, hitting his parents and his grandparents. He had echolalia and sensitivity to noises and touch, and he engaged in both hand flapping and hand biting. Overall, he was very hyperactive, perseverative, and anxious.

At 7 years, patient 5 started metformin at a dose of 500 mg at dinner and increased to 500 mg bid a few months later (see Table 1). His pre-treatment MSEL was performed at a chronological age of 93 months and yielded age equivalent scores (in months) of 27 for visual reception, 20 for fine motor, 23 for receptive language, and 18 for expressive language, with an ELC of 49. Follow-up testing post-treatment was not yet obtained. Since initiation of metformin, he has not experienced any seizures or seizure-like symptoms. He is now able to eat at normal frequencies rather than continuously throughout the day. Behaviorally, his parents report improvement in irritability, hyperactivity, anxiety, tantrums, and aggression. His communication has also improved to the

extent that he is now able to engage in a two-way conversation with multiple exchanges. Previously, his mother had constant complaints from school teachers about his behavior, but after metformin initiation she has received consistently positive feedback. The only side effect he has experienced is intermittent loose stools.

3.6 | Case 6

Patient 6 is a 5-year, 4-month-old boy born via C-section due to breech presentation at 35 weeks and weighing 2.6 kg. He had mild jaundice and was treated with phototherapy for 12 hr before being discharged 4 days later. Past medical history includes reflux treated with omeprazole in the first year. He sat at 9 months, never crawled, and walked at 15 months. He said his first words at 24 months and when seen pre-treatment at 34 months he could combine 2 words (see Table 3). At 47 months, he was diagnosed with ASD.

Patient 6's behavior at 34 months included excessive chewing, shyness, perseveration, hyperactivity, and a short attention span. He had mild sleeping problems and occasional tantrums but was not aggressive. Ongoing interventions included ABA, speech and language, in home, and physical therapy.

Patient 6 was started on metformin at an initial dose of 50 mg at dinner and then increased to bid after 1 week (see Table 1). Although his initial response to metformin was positive (see Table 2), after 4 months increased hyperactivity and anxiety, decreased attention, and language regression were observed. Metformin was discontinued and the aforementioned problems subsequently resolved.

3.7 | Case 7

Patient 7 is a 3-year, 11-month-old boy diagnosed with FXS at 16 months due to delayed communication milestones and a positive family history (see Table 1). He was born at term via normal spontaneous delivery without complications and weighed 4.6 kg. He sat independently at 6 months, took his first steps at 14.5 months, and said his first word at 15 months. At 15 months a diagnosis of Global Developmental Delay was made. At 20 months patient 7 was started on sertraline, and parents observed some improvement in self-regulation and a reduction in separation anxiety (see Table 1).

Behaviorally, patient 7 is a social child who arm-flaps when excited. He experiences mild to moderate separation and performance anxiety. He has significant verbal and non-verbal communication delays and difficulty with imitation. Prior to metformin initiation, he could express himself using 3–5 verbal words and 15 signs, and he stacked approximately 3–4 blocks.

At 26 months patient 7 started taking 50 mg of metformin bid, and over the following 5 months his dose was

gradually increased up to 150 mg bid. Over time he has shown dose-dependent improvement across multiple areas of development, including language acquisition (see Table 3). Six months after initiation, he demonstrated use of 30 verbal words and 25–30 signs, including some combinations of 2–3 words and signs together. His therapists have noted improvements in his sustained attention, listening, focus, ability to imitate, and the speed with which he learns new words and signs. He is now stacking interlocking blocks greater than 15, and his family reports increased engagement in pretend play. He more regularly performs self-help activities and daily tasks, such as dressing himself, picking up toys, throwing away trash, putting his dirty clothes in the hamper, and independently washing his hands. Behaviorally, his family has noted a significant increase in hyperactivity and impulsivity over the last four months, the former of which is reflected in his ABC-C composite scores (see Table 2).

Overall, patient 7 tolerated all metformin dose increases fairly well. Besides the increase in hyperactivity and impulsivity, his only side effect was an increase in frequency of nightly awakenings upon initiation and with each subsequent increase in dose, but sleep returned to normal within 2 weeks each time.

3.8 | Case 8

Patient 8 is a 5-year, 7-month-old boy who was diagnosed with FXS at 22 months. His mother had a normal pregnancy, but delivery via C-section was delayed 1 week due to lack of progression. At birth he weighed 3.8 kg and was tongue-tied; his tongue was clipped the next day without sequelae. He sat independently at 6 months, crawled at 8 months, and walked independently at 9 months. Significant language delay and behaviors such as hand flapping, poor eye contact, and tactile defensiveness prompted evaluation and ASD was diagnosed at 18 months. He subsequently enrolled in an early intervention preschool with expertise in ASD, speech and language therapy, and occupational therapy. He had reflux as an infant with significant vomiting and poor weight gain but since stabilizing on a paleo diet, he has had less reflux but still suffers occasional diet-related constipation. He was started on sertraline at 25 months with a positive response (see Table 1).

On examination at 3 years, he was hyperactive and impulsive, demonstrating poor eye contact. He could only verbalize single words such as “bye-bye” or 2-word combinations such as “big dog” or “black dog” (see Table 3). His mother reported that he would play chase games with other children but would not socially interact with them.

At 46 months he started on metformin at 50 mg at dinner and increased to 50 mg bid after a few weeks; he tolerated the dose increase well. Initially, he had some soft

stools but no diarrhea. He is no longer aggressive, although he continues to have poor eye contact, hand flapping, and biting. Since metformin initiation, he has been eating more and maintains a healthy weight. He exhibited an overall decrease across all measurements on the ABC-C (see Table 2) and no cognitive decline on follow-up developmental testing (see Table 3).

3.9 | Case 9

Patient 9 is a 5-year, 3-month-old boy born at term via C-section after a pregnancy complicated by gestational diabetes. His developmental milestones included sitting at 8 months, crawling at 13 months, and walking at 16 months. He was diagnosed with FXS at 26 months of age and started on sertraline with a positive response in language development (see Table 1). Behaviorally, patient 9 hand flaps when excited and occasionally bites his fingers. He is social, making good eye contact, and often complimenting others and asking questions. He can be inattentive and hyperactive at times. He exhibits tantrums, irritability, and aggression (sometimes hitting and pushing people or objects). Past medical history includes seasonal eczema and three episodes of OM with PE tubes placed at 2 years, which led to a dramatic improvement in his language. However, by 3 years his mother noticed an increase in dysfluency and stuttering.

He began metformin at 4 years, initially taking 50 mg at dinner and gradually increasing to 150 mg bid. Since initiation, he has exhibited improved language development: increased vocabulary and he now uses full sentences and carries out reciprocal conversations. His mother reports that he more frequently attempts and succeeds at problem solving without looking to others for help. Furthermore, he has made progress in his social development and is more regularly seeking interactions with his peers for longer periods of time. He has experienced some loose stools and occasionally complains of stomach aches, but he continues to have a good appetite and overall tolerates metformin well.

Patient 9's follow-up developmental testing reflects improvement across all areas but most notably expressive language (see Table 3). His ABC-C showed a decrease across all subscales except for inappropriate speech and social avoidance, which remained static (Table 2).

3.10 | Statistical results

Statistical analysis on the ABC-C pre- and post-treatment for all nine cases yielded a statistically significant ($p < .05$) improvement in two of the six factors: lethargy and stereotypy (Table 5). These findings differentiate this cohort from others in previous observational studies that saw an increase in these two factors over time (Hustyi, Hall, Jo, Lightbody, & Reiss, 2014). Analyses on the Mullen results

were performed for at least five of the nine cases; p -values ($p < .05$) suggest a statistically significant effect in all subscales and the global developmental age. Additionally, for all patients with both pre- and post-treatment developmental testing data available, mean linear slope trajectories were calculated (Table 4). Among the MSEL subscales, the rate of growth for receptive and expressive language—0.56 and 0.77, respectively, exceeded previously published literature estimating a rate of growth with 0.48 in this population (Bailey Jr. et al., 1998); in a larger sample size, this finding might indicate accelerated gains that could be attributable to metformin, but given the small sample size and noncontrolled nature of the treatment, no definitive conclusions can be drawn except that no regression was observed.

4 | DISCUSSION

In this study, we describe nine cases of young children with FXS between the ages of 2–7 years who were treated clinically with metformin with beneficial effects in language and some areas of aberrant behavior as measured by the ABC-C, developmental testing, and parent reports (Tables 2 and 3). As is common in this population, all children described in this report were receiving interventions in the community or at school, including speech and language therapy, physical and occupational therapy, and special education support. Those with ASD were also receiving ABA.

In comparison to typically developing children (TDC), those with FXS have a significantly lower rate of development; because of this, their IQ is found to decrease over time (Wright-Talamante et al., 1996). This observation can be attributed to the lack of FMRP, which is essential for synaptic plasticity and cognitive development. Those with FXS have significant deficits in abstract reasoning and higher symbolic language skills so the IQ decline increases in later childhood and adolescence; they neither lose skills nor regress but fall further behind TDC over time (Bailey Jr. et al., 1998; Hagerman et al., 1989;

Hodapp et al., 1990; Lachiewicz, Gullion, Spiridigliozzi, & Aylsworth, 1987; Roberts et al., 2009). Although babies with FXS are usually quiet in the first year of life, behavioral problems including irritability, hyperactivity, and tantrums emerge in the second and third years of life, and sleep disturbances are common (Roberts, McCary, Shinkareva, & Bailey, 2016). Language is expected to improve somewhat in the early years even in those with FXS who have not received pharmacological or nonpharmacological intervention, but approximately 10% of children with FXS remain nonverbal by age 7 (Komesidou, Brady, Fleming, Esplund, & Warren, 2017).

Treatment of children younger than 6 years of age has the potential of improving brain development in the absence of FMRP by reversing the upregulation of the mTORC1 and MEK-ERK pathways and lowering the elevated *MMP9* levels that are deleterious to synaptic development in FXS. Cognitive impairment is universally seen in males with the methylated full mutation due to the complete lack of FMRP. Counteracting the negative effects due to the lack of FMRP early in development has the potential to reverse the protein upregulation seen early on in FXS and prevent cognitive deficits before they are entrenched.

The parents of the children in this report were positive overall about metformin treatment; while there is a possibility that these impressions were due to a placebo response, per parent account there was demonstrated improvement in language acquisition rate and practical expressive ability. Parents noted gains not only in verbal and nonverbal communication but also in problem solving, motor abilities, and daily living skills. Behaviorally, tantrums and aggression were generally decreased, and improvement was observed in overall aberrant behavior as measured by the ABC-C pre- and post-treatment. The clinical treatment of the children in this report is not dissimilar to an open label treatment, and therefore bias can be introduced since the caregivers know that their child is being treated with a medication considered to be a potential targeted treatment for FXS. Informant based questionnaires, such as the ABC-C, can introduce such bias and recall effects. A potential possibility for the improvement in

TABLE 4 Statistical Analysis of Mullen Scales of Early Learning

Mullen subscale	Pre-metformin ^a	Post-metformin ^a	Slope ^{a,b}	p -value [*]
Visual reception ^c	30 (18–38)	40 (29–40)	0.52	.04
Fine motor	20 (18–26)	25 (21–27)	0.34	.02
Receptive language	27 (19–32)	34 (27–36)	0.56	.03
Expressive language	19 (16–22)	28 (17–48)	0.77	.02
Early learning composite score	61 (54–63)	61 (55–62)	NA	.5
Global developmental age ^{c,d}	30 (21.2–31.2)	34.2 (33.7–35.5)	0.52	.04

^aAll values measured in median and interquartile range.

^bRate of Growth.

^cAnalysis with $n = 5$, all others were calculated with $n = 6$.

^dGlobal developmental age was calculated based on the average age equivalents of the Mullen subscales.

*A pre-specified significance level of $p < .05$ was assumed.

scores is that increases in adaptive behavior with time along with the natural course of development may be responsible in part for decreases (or the perception thereof) in aberrant behavior; likewise, caregivers could be habituating to the severity of their child's behavior over time. Despite this, a longitudinal study with 124 children and adolescents with FXS demonstrated a significant decrease in hyperactivity and irritability over time (Husty et al., 2014). Not all children demonstrated an improvement on metformin. One patient (Case 7) had an increase in hyperactivity several months after metformin initiation, though this behavioral observation improved after discontinuation of treatment, it may also be unrelated since increased hyperactivity is expected in the developmental course of FXS in the early years of life (Grefer, Flory, Cornish, Hatton, & Roberts, 2016).

Seizures are experienced by approximately 14% of male and 6% of female children with FXS and is often associated with a diagnosis of ASD. Seizures are more often partial, although generalized tonic-clonic seizures may occur. Common age of seizure onset is in young and mid-childhood (Berry-Kravis et al., 2010). After one month on metformin, patient 3 experienced a seizure and despite subsequent initiation of the anticonvulsant oxcarbazepine, within 1 week a second episode occurred. Patient 3's MRI after the first episode revealed grey matter heterotopias, which have been previously reported in FXS (Moro et al., 2006) and likely constitute the cause of the seizures. Metformin was discontinued at the discretion of the attending physician; however, the only recognized association of metformin with the presentation of seizures and other neurological symptoms has been reported in those with vitamin B12 deficiency (Lee, Chang, Wu, Weng, & Chen, 2005; Naha, Dasari, Vivek, & Prabhu, 2012) that can be occasionally seen after treatment for more than 4 months (Langan & Goodbred, 2017). Patient 3's complete blood count, comprehensive metabolic panel including blood glucose, and urinalysis results were unremarkable at baseline and at time of hospital discharge after the first episode of a seizure. Furthermore, no drug interaction is known to exist between metformin and oxcarbazepine.

Contrary to what was seen in patient 3, metformin has been reported as exhibiting beneficial effects in epilepsy such as decreasing seizure susceptibility, reducing seizure number and length, suppressing progression of seizures and ameliorating learning and memory impairments (Gantois et al., 2018; Mehrabi et al., 2018; Yang et al., 2017; Zhao et al., 2014). Metformin is also known to improve seizures in the mouse model of FXS (Gantois et al., 2017). Indeed, in one patient (Case 5) the partial motor seizures were improved with metformin. The most commonly reported side effect of treatment was self-limited loose stools after initiation or subsequent dose increases. Thus, it is necessary to start at a low dose and increase dosage gradually until a maximum tolerated dose is reached. While the nine patients presented in this

report demonstrate relative safety, further studies regarding safety are warranted. Close monitoring is recommended to detect any adverse behavioral changes that may accompany dose modification so that treatment can be discontinued if needed. In addition, further studies of young children treated with metformin should include careful monitoring for possible seizures and hyperarousal.

The results of this preliminary clinical treatment of children with FXS 2–7 years old indicate the potential of metformin as a targeted treatment for young patients with FXS as data suggests language and cognitive benefits with no regression.

5 | LIMITATIONS

Even though certain p-values may suggest statistical significance, it is important to address the limitations of our preliminary data. The material presented here are several clinical case reports, not an open-label study, hence no specified protocol is used. This cohort was treated clinically so there is variability in the time points for data collection; therefore, it is difficult to differentiate between the influence of time on the effects of metformin seen and the natural developmental progression of each patient. The small number of patients is a limitation in terms of statistical analyses; the presented statistical values could be the result of a large random error. Therefore, interpretation of the results as definitively providing evidence of an effect when $p < .05$, or a lack thereof when $p > .05$, is not possible. While there are limitations to the conclusions which can be drawn from the data, there were positive effects seen while the patients were treated with metformin. Likewise, our findings support a biologically plausible hypothesis based on previous work by Gantois and colleagues (Gantois et al., 2017) where metformin was shown to improve outcomes among the *FMRI* KO mouse model of FXS. Our preliminary data, although promising, suggests that only a controlled trial of metformin in young

TABLE 5 Statistical Analysis for Aberrant Behavior Checklist-Community

ABC-C domain	Pre-metformin ^a	Post-metformin ^a	p-value [*]
Irritability	14 (8–29)	12 (6–19)	.1
Lethargy	1 (0–11)	1 (0–4)	.04
Stereotypy	4 (2–9)	4 (0–5)	.01
Hyperactivity	17 (13–22)	13 (11–14)	.07
Speech	3 (0–6)	2 (2–6)	.6
Social avoidance	0 (0–2)	1 (0–1)	.2
Composite score	33 (29–67)	39 (20–51)	.06

Note: All calculations were done with an $n = 9$.

^aAll values measured in median and interquartile range.

^{*}A pre-specified significance level of $p < .05$. was assumed.

children (two and older) with FXS can ascertain the influence of the treatment in this population (Table 5).

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CONFLICTS OF INTEREST

RJH has received funding from Zynerva, Ovid and the Azrieli Foundation for carrying out treatment studies in patients with fragile X syndrome. She has also consulted with Fulcrum, and Zynerva regarding treatment studies in the same population. FT received funds from Asuragen, Inc, Roche and Zynerva. The other authors declare no conflicts of interest.


DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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