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Cognitive and behavioral improvement in adults with fragile X syndrome treated with metformin-two cases

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Abstract

Background: The majority of individuals with fragile X syndrome (FXS) have intellectual disability, behavioral problems, autism, and language deficits. IQ typically declines with age in boys with the full mutation. The results of preclinical studies demonstrated that metformin, a biguanide used to treat type 2 diabetes, rescues multiple phenotypes of FXS in both Drosophila and mouse models. Preliminary studies of patients with FXS demonstrated improvements in behavior.

Methods: Here, we present two cases of individuals who have been treated with metformin clinically for one year.

Results: Both patients demonstrated significant cognitive and behavioral improvements. They also improved eating habits and normalization of their weight percentiles.

Conclusion: Metformin may be a candidate drug for treatment of several types of symptoms in individuals with FXS.

KEYWORDS

cognition improvement, fragile X syndrome, metformin, targeted treatment

1 | INTRODUCTION

Metformin (N,N-dimethylbiguanide), the antidiabetic agent with a favorable risk/benefit profile, is one of the most widely prescribed drugs in the world. Metformin is in clinical use for more than 60 years and its pharmacokinetic and pharmacodynamic properties are well established. Metformin has been used in Europe for treatment of diabetes mellitus type 2 since 1957 (Foretz, Guigas, Bertrand, Pollak, & Viollet, 2014).

There are studies documenting that metformin could be a helpful medication to decrease weight in insulin sensitive and insulin resistant overweight and obese adult patients (Seifarth, Schehler, & Schneider, 2013). In addition, Metformin is effective in the treatment of obesity with and without diabetes mellitus type 2, in a cohort of children (Jones, Arslanian, Peterokova, Park, & Tomlinson, 2002; Klein, Cottingham, Sorter, Barton, & Morrison, 2006; Park, Kinra, Ward, White, & Viner, 2009). It can be used for the treatment of polycystic ovary disease, diabetic nephropathy, gestational diabetes and cardiovascular complications associated with diabetes mellitus type 2 (Foretz et al., 2014; Viollet et al., 2012). This medication has benefits associated with decreased cancer risk and improved cancer prognosis (Romero et al., 2017; Viollet et al., 2012).

There is a clear difference between the mechanism of action during acute and chronic use of metformin. Preclinical studies indicated that the mechanism involves AMP-activated protein kinase (AMPK)-dependent, as well as AMPK-independent pathways, inhibition of mitochondrial respiration and activity of the mitochondrial enzyme glycerophosphate dehydrogenase (Rena, Hardie, & Pearson, 2017).

The symptoms in patients with fragile X syndrome (FXS; phenotype MIM number: 300624) are related to the absence or deficiency of FMRP, a protein which plays a key role in protein translation which is important for neuronal synaptic connections. There is an upregulation of the metabotropic glutamate 5 (mGluR5) pathway, the insulin receptor and the mTOR pathway in the absence of FMRP (Gantois, Popic, Khoutorsky, & Sonenberg, 2019; Hagerman et al., 2017). This dysregulation leads to global developmental delays in males and continued IQ decline in childhood and adolescence (Wright-Talamante et al., 1996). Individuals with FXS typically manifest social and behavioral problems such as attention-deficit/hyperactivity disorder (ADHD), anxiety, autism spectrum disorder (ASD) and aggression (Rajaratnam et al., 2017) Many children and adults with FXS have eating problems, including overstuffing their mouth, overeating and lack of satiation after meals causing obesity in 31% (McLennan, Polussa, Tassone, & Hagerman, 2011).

The use of metformin in the animal models, such as Drosophila FXS and FXS knock out (KO) mouse demonstrated that metformin could improve FXS phenotypes, the circadian rhythm deficits and memory deficits (Gantois et al., 2017; Monyak et al., 2017). Pathogenically overactive signaling pathways in FXS in the central nervous system could also be normalized by metformin (Gantois et al., 2017,2019).

Recently, we published data which documented that metformin was helpful in seven individuals aged 4–60 years with FXS, characterized by a full mutation (>200 CGG repeats) of *FMR1* (gene/locus MIM number: 309550; the GenBank reference sequence and version number: NC_000023.11) (Dy et al., 2018). These patients demonstrated improvements in their eating habits, weight loss, and behavior as measured by the Aberrant Behavior Checklist. In addition, the families reported that the patient's language level improved and in several cases these patients were able to carry out conversations.

In this report, we present two adult individuals with FXS who have been treated with metformin for 1 year and demonstrated improvements in their IQ scores and behavior.

2 | MATERIALS AND METHODS

2.1 | Ethical Compliance

Both patients signed an informed consent for developmental and molecular testing approved by the institutional IRB and all families have consented to have their case histories and clinical response to metformin published.

The cases described in this article are seen frequently for management of FXS at the Fragile X Treatment and Research Center at the University of California Davis MIND Institute. We described each patient with their medical histories, physical features, behavioral symptoms focusing on qualitative reports from the individuals' primary care providers, and IQ scores as well as their laboratory findings pre- and posttreatment. Both of the cases were prescribed metformin to treat their FXS. According to the results of our previous study, the medication was prescribed as part of their clinical care and was targeting irritability, social responsiveness, hyperactivity, social avoidance, as well as language and conversational skills (Dy et al., 2018; Gantois et al., 2019). The regular metformin adult dose is 1,000 mg twice a day. If no diarrhea occurs, Metformin can be titrated up to this dose. They were both observed for intellectual, behavioral and metabolic changes.

The level of cognitive functioning was determined through the administration of the Stanford–Binet Intelligence Scale, Fifth Edition (SB-5), a cognitive functioning test that has robust psychometric properties, minimal practice effects, and correlates highly with other measures of intelligence. This was performed before the introduction of metformin and after 1 year of usage of metformin. The Stanford Binet assessment was done by the same examiner for both participants at all time points. The behaviour observations correspond to his

age between 22 to 25 years. During this time period he was continuously treated with metformin.

All information for both individuals was obtained by parents' reports, as well as review of Clinic visit notes and review of previous reports.

3 | RESULTS

3.1 | Case 1

This case is a 25-year-old male diagnosed with FXS, autism and ADHD with a history of hyperphagia and lack of satiety after meals.

He met the criteria for autism (ADOS, Module 3 score of 18, above the cut-off score for ASDs) and his Full-Scale IQ was 57 with a verbal IQ of 64 and a nonverbal IQ of 57 on the Stanford-Binet scale when he was 13. He was diagnosed with FXS when he was 14 years old. He has full mutation of *FMR1* with 185, 440 and 1,100 CGG repeats, and without *FMR1* mRNA. His mother and his maternal grandfather are premutation carriers. He received an additional diagnosis of generalized anxiety disorder. He exhibited periods of aggressive outbursts, poor eye contact, tactile defensiveness and perseveration in speech. He has been prescribed several medications to treat his symptoms in the past including methylphenidate, citalopram, minocycline and sertraline. At the age of 22, he was on metformin with an increase from 500 mg at dinner to 500 mg bid. At present he is on simvastatin 10 mg/day and metformin 500 mg bid.

Currently, on physical examination, he has typical features of FXS. He has hand calluses and minimal arch on his feet. He had a normal neurological exam and DTRs were 2+ in all four extremities. Summary of the diagnostic findings is presented in Table 1.

Baseline (at the age of 19 years, i.e. before he started metformin) and follow-up IQ scores after 1 year of usage of metformin (at the age of 23/24 years) are given in Table 2. At age 23, he and his caretaker were interviewed with the K-SADS-PL (a semi-structured clinical interview). The patient met DSM-5 criteria for social phobia and agoraphobia, and the clinician noted a decrease in autism spectrum-related symptoms from the previous year.

He continued on metformin and had an excellent response to metformin over the subsequent 17 months. He does not have loose stools which is the most frequent side effect of metformin. Also, he does not have any other side effect of metformin. He has experienced significant improvement in his communication and behavior. He now enjoys outdoor activities. He socializes more and invites friends over. He works in a group doing landscaping and seems to be happy with his life. He is calmer and he has not had any outbursts over the last year. He is less verbally and physically aggressive to other children and adults. He talks less excessively and he has less repetitive speech. He pays more attention to the instructions. He is less distractible. His self-esteem has also improved considerably. He has new skills since he started on metformin including taking the bus independently and cooking meals. He wants to earn money for his work and he wants to spend it on his own things. The behaviour observations correspond to his age of 22–25 years. During this time period he was continuously on metformin. However, there were no changes in the patient's living conditions, additional therapies, or occupational status from time point 1 (age of 22 years) to time point 2 (age of 25 years).

3.2 | Case 2

This case is a mosaic 30-year-old male initially diagnosed with FXS when he was 21 months old. His mother and maternal grandmother are premutation carriers. Furthermore, he was diagnosed with anxiety disorder and ASD with a history of panic attacks, moodiness, becoming nervous interacting with unfamiliar people and shyness. His symptoms included hand flapping, poor eye contact, perseveration in speech, tactile defensiveness and periods of outbursts. He had early intervention from 20 months of age. He has been prescribed several medications to treat his symptoms in the past including mavoglurant and sertraline. He had been on metformin 1,000 mg ER once a day for 1 year. His current medications include calcium, multivitamins including Vitamin D and B, probiotics, magnesium citrate and Coenzyme Q10.

He was delivered at term and by normal delivery. He was hypotonic but grew very tall in early age. He was mildly delayed in milestones. His early developmental milestones included sitting at 6 months, crawling at 7 months, walking not until 31 months, and he said single words at 2 years of age, and he put together phrases at about 4 years of age. An autism assessment at age 22 showed an ADOS (Module 4) score of 11, which is above the cut-off for ASDs.

On physical examination, he spoke in short sentences, but sometimes mumbled and his eye contact was limited during examination. Typical features of FXS were present. He had flat, out-turned and pronated feet. He had a normal neurological exam with reflexes 2+ symmetrical in all four extremities but he could not tandem walk. Clinical presentation of this patient and his lab results are shown in Table 1.

He started on metformin when he was 29 years old, and baseline (at the age of 26 years; i.e. before he started metformin) and follow-up IQ scores after 1 year of metformin usage (at the age of 30 years) are given in Table 2. Usually, there is a decline in cognitive skills in FXS with age, which was not the case here.

Parents reported that metformin has had a significant effect on his language. He has been more verbal since starting metformin; he shares his thoughts more often, and he asks

TABLE 1 Summary of cases

Case Age Gender	Diagnosis	DNA testing results	Primary Concern	Physical Features	Laboratory Findings		Metformin Dose	Other Continuous Medications
					Baseline up	Follow		
Case 1. 25 yo Male	FXS Autism ADHD Generalized Anxiety Disorder	*CGG repeats*: 185, 440, 1,100 Full methylated No *FMR1* mRNA	Anxiety Aggression Hyperphagia[a]	Height: 172 cm, Weight: 79 kg, kg/m^2 BP: 126/81, HC: 58.5 cm HR: 83 Long and narrow face, prominent cupping ears, hyperextensible fingers, double jointed thumbs, high palate, macroorchidism, Tanner stage V, normal phallus, striae on abdomen, normal tone and strength	FBS: 89 mg/dl Normal CBC Normal CMP BMI: 30.22	HgbA1c: 5.6 FBS: 98 mg/dl Normal CBC BMI 26.56	Starting dose 500 mg increased to 500 mg bid	Simvastatin 10 mg
Case 2. 30 yo Male	FXS Generalized Anxiety Disorder ASD	*CGG repeats:* ~300, 800, (30–200); Methyl and size mosaic: >95% methylated *FMR1* mRNA = 0.20 (0.01)	Panic attacks Moodiness Shyness Anxiety Difficulty in expressive language	Height: 179.1 cm, Weight: 68 kg, BP: 106/70, HR: 83, HC: 59 cm, Long and narrow face, prominent ears and jaw, high and narrow palate, hyperextensible finger joints, double jointed thumbs, macroorchidism, unable to tandem walk	HgbA1c: 4.9 FBS: 84 mg/dl Normal CBC Normal CMP BMI: 20.4	HgbA1c: 5.2 FBS: 87 mg/dl Normal CBC Normal CMP BMI 21	Starting dose 500 mg increased to 1,000 mg ER qd	Calcium Multivitamins Magnesium Citrate Coenzyme Q10

Abbreviations: ADHD, Attention-Deficit Hyperactivity Disorder; ASD, Autism Spectrum Disorder; BMI, Body Mass Index; BP, blood pressure; CBC, Complete Blood Count; CMP, Comprehensive Metabolic Panel; FBS, fasting blood sugar; FXS, Fragile X Syndrome; HC, Headc circumference; HR, heart rate.

[a]He used to eat more before he started on metformin, he has lost approximately 10 kg over the last year and he is no longer focused on food as he had for many years.

TABLE 2 The results of Stanford–Binet IQ testing

Case	Time of testing	Nonverbal IQ	Verbal IQ	Full scale IQ	Fluid reasoning	Knowledge	Quantitative reasoning	Verbal-spatial processing	Working memory
Case 1	Baseline	50	61	53	65	72	53	59	51
	Follow-up	52	66	57	65	66	56	71	60
Case 2	Baseline	47	58	50	68	66	50	48	57
	Follow-up	51	68	58	68	72	61	56	63

Note: Baseline-IQ testing before introduction of metformin; Follow-up—IQ testing after one-year usage of metformin.

more questions. The expressive language improvements occurred right away after metformin was titrated to the full dose, but he still has mild articulation difficulties. Since the start of metformin treatment, he has started to do familiar tasks/chores more often on his own initiative. He does not have loose stools, and no other side effects were reported. He works 4 days a week at a grocery store, but there were no changes in the patient's living conditions and additional therapies from time point 1 to time point 2. He continues to be physically active, going to the gym with his parents. He enjoys walking. The time period of behavioral observation corresponds to metformin usage: the baseline for the behavioral evaluation was when he was 29 years old, the follow-up when he was 30 years old.

4 | DISCUSSION

This is the first paper which describes individuals with FXS who have shown an improvement in IQ scores after treatment with metformin.

On baseline, scores of both patients on overall intellectual ability, nonverbal ability, and verbal ability, were lower relative to the normative sample. After one year of metformin, test results reveal an increase in Full Scale IQ (from 53 to 57 and from 47 to 51), Nonverbal IQ (from 50 to 52 and from 58 to 68) and Verbal IQ (from 61 to 66 and from 50 to 58) for both patients (See Table 2). Moreover, improvements in factor index scales were seen in measures of visuospatial processing and knowledge of spatial relations, working memory, as well as numeracy skills and quantitative reasoning (see Table 2). These changes were driven by better performances in both in nonverbal and verbal tasks (see Table 2), with the latter suggesting subtle, qualitative improvements in their capacity to use language more effectively to comprehend instructions, recall information, and provide appropriate responses. While an IQ increase of 1–5 points is not statistically significant and could be within normal limits, since a 10-point increase (1 standard deviation) is needed to be considered significant, the fact that both adults remained stable in most areas measured on the Stanford–Binet and did not show a decline in their cognitive functioning, is noteworthy. These results suggest that regression or deterioration in abilities in most domains on the cognitive measure did not occur. In fact, gains were also seen on item level analysis in terms of their number of correct responses some tasks. More importantly, these contrast with known IQ trajectories in FXS among young adult males, including those with comorbid ASD, where an early plateau in mental age eventually leads to the widening gap between expected capacities and cognitive performance over time.

Previous studies documented IQ score declines with aging in males and females with FXS (Schneider, Ligsay, & Hagerman, 2013). Lachiewicz, Gullion, Spiridigliozzi, and Aylsworth (1987) first published data about IQ declines in individuals with FXS during childhood. Borghgraef et al. (2002) described IQ changes in ten individuals with FXS aged from 33 to 65 years. They concluded that the declines were most notably in the verbal communication skills and that the use of language declined in these individuals over time (Borghgraef et al., 2002). IQ decline was also found in 39 males with FXS aged from 4 to 26 years (Wiegers, Curfs, Vermeer, & Fryns, 1993). Hagerman et al. (1989) published that individuals with FXS with the highest IQs had the greatest cognitive decline. Also, similar results were published in 1996 by Wright-Talamante et al. (1996).

Here we present two adult individuals with FXS who experienced benefits for language and cognition from metformin. These two patients have been treated clinically with metformin, as a targeted treatment in FXS. In addition to the general improvement in cognitive skills, both individuals have notable improvements in communication, social activities and behavior. They also demonstrated improved eating habits. Metformin has seemed to slightly decrease anxiety in these individuals with FXS.

The use of metformin in the Drosophila FXS model rescued the circadian rhythm deficits and memory deficits (Monyak et al., 2017). Preclinical studies performed in FXS KO mouse demonstrated that metformin could improve FXS phenotypes, including grooming, social novelty, electrophysiology in eCA1 of the hippocampus and dendritic spine morphology. Pathogenically overactive signaling pathways in FXS in the central nervous system, mTOR and PI3K, could be normalized by metformin (Gantois et al., 2017). This medication inhibits MMP9 production which is elevated in FXS (Dziembowska et al., 2013; Hoeffer et al., 2012; Muzar, Lozano, Kolevzon, & Hagerman, 2016).

Based on the potential regulation of intracellular signaling pathways, the results of preclinical studies and our previous and current clinical studies, metformin appears to be a good targeted treatment for some of the disrupted intracellular functions in neurons of individuals with FXS. Thus, metformin may be a candidate drug for treatment of several types of symptoms in individuals with FXS. Preventing the cognitive decline in FXS can increase the quality of life and prognosis for semi-independent living skills (Gorelick, 2018). However, the limitation of this study could include possible placebo effect. Specifically, parent reports about the behavior changes in both individuals were not quantified with an adaptive behavior measurement, and other standardized scales.

The first randomized, double-blind controlled clinical trial of safety, tolerability and efficacy of metformin in the treatment of language deficits, behavior problems, and obesity/excessive appetite in individuals with FXS is currently taking place for individuals ages 6–25 years (NCT number: NCT03479476). The dose of metformin is titrated in a weight-dependent manner over the four weeks. The level of MMP9 as a biomarker is assessed. The first results will be expected in 2021 and the study will access the efficacy of metformin for behavior and cognition in patients with FXS.

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CONFLICTS OF INTEREST

RH has received funding from Roche, Novartis, Neuren, Marinus, and Alcobra for carrying out treatment studies in patients with fragile X syndrome. She has also consulted with Fulcrum, Ovid and Zynerba regarding treatment studies in individuals with fragile X syndrome. The other authors declare no conflicts of interest.

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REFERENCES

Borghgraef, M., Sacco, S., Gomot, M., De Vos, B., Jacobs, A., Buret, V., & Desportes, V. (2002). Neuro-cognitive and behavioural aspects in non-specific mental retardation. A proposal for phenotyping new XLMR genes. *Genetic Counseling (Geneva, Switzerland)*, *13*, 195–198.

Dy, A. B. C., Tassone, F., Eldeeb, M., Salcedo-Arellano, M. J., Tartaglia, N., & Hagerman, R. (2018). Metformin as targeted treatment in fragile X syndrome. *Clinical Genetics*, *93*, 216–222. https://doi.org/10.1111/cge.13039

Dziembowska, M., Pretto, D. I., Janusz, A., Kaczmarek, L., Leigh, M. J., Gabriel, N., … Tassone, F. (2013). High MMP-9 activity levels in fragile X syndrome are lowered by minocycline. *American Journal of Medical Genetics Part A*, *161A*, 1897–1903. https://doi.org/10.1002/ajmg.a.36023

Foretz, M., Guigas, B., Bertrand, L., Pollak, M., & Viollet, B. (2014). Metformin: From mechanisms of action to therapies. *Cell Metabolism*, *20*, 953–966. https://doi.org/10.1016/j.cmet.2014.09.018

Gantois, I., Khoutorsky, A., Popic, J., Aguilar-Valles, A., Freemantle, E., Cao, R., … Sonenberg, N. (2017). Metformin ameliorates core deficits in a mouse model of fragile X syndrome. *Nature Medicine*, *23*, 674–677. https://doi.org/10.1038/nm.4335

Gantois, I., Popic, J., Khoutorsky, A., & Sonenberg, N. (2019). Metformin for treatment of fragile X syndrome and other neurological disorders. *Annual Review of Medicine*, *70*, 167–181. https://doi.org/10.1146/annurev-med-081117-041238

Gorelick, P. B. (2018). Prevention of cognitive impairment: Scientific guidance and windows of opportunity. *Journal of Neurochemistry*, *144*, 609–616. https://doi.org/10.1111/jnc.14113

Hagerman, R. J., Berry-Kravis, E., Hazlett, H. C., Bailey, D. B., Moine, H., Kooy, R. F., … Hagerman, P. J. (2017). Fragile X syndrome. *Nature Reviews Disease Primers*, *3*, 17065. https://doi.org/10.1038/nrdp.2017.65

Hagerman, R. J., Schreiner, R. A., Kemper, M. B., Wittenberger, M. D., Zahn, B., & Habicht, K. (1989). Longitudinal IQ changes in fragile X males. *American Journal of Medical Genetics*, *33*, 513–518. https://doi.org/10.1002/ajmg.1320330422

Hoeffer, C. A., Sanchez, E., Hagerman, R. J., Mu, Y., Nguyen, D. V., Wong, H., … Tassone, F. (2012). Altered mTOR signaling and enhanced CYFIP2 expression levels in subjects with fragile X syndrome. *Genes, Brain and Behavior*, *11*, 332–341. https://doi.org/10.1111/j.1601-183X.2012.00768.x

Jones, K. L., Arslanian, S., Peterokova, V. A., Park, J. S., & Tomlinson, M. J. (2002). Effect of metformin in pediatric patients with type 2 diabetes: A randomized controlled trial. *Diabetes Care*, *25*, 89–94. https://doi.org/10.2337/diacare.25.1.89

Klein, D. J., Cottingham, E. M., Sorter, M., Barton, B. A., & Morrison, J. A. (2006). A randomized, double-blind, placebo-controlled trial of metformin treatment of weight gain associated with initiation of atypical antipsychotic therapy in children and adolescents. *American Journal of Psychiatry*, *163*, 2072–2079. https://doi.org/10.1176/ajp.2006.163.12.2072

Lachiewicz, A. M., Gullion, C. M., Spiridigliozzi, G. A., & Aylsworth, A. S. (1987). Declining IQs of young males with the fragile X syndrome. *American Journal on Mental Retardation*, *92*, 272–278.

McLennan, Y., Polussa, J., Tassone, F., & Hagerman, R. (2011). Fragile x syndrome. *Current Genomics*, *12*, 216–224. https://doi.org/10.2174/138920211795677886

Monyak, R. E., Emerson, D., Schoenfeld, B. P., Zheng, X., Chambers, D. B., Rosenfelt, C., … Jongens, T. A. (2017). Insulin signaling misregulation underlies circadian and cognitive deficits in a drosophila fragile X model. *Molecular Psychiatry*, *22*, 1140–1148. https://doi.org/10.1038/mp.2016.51

Muzar, Z., Lozano, R., Kolevzon, A., & Hagerman, R. J. (2016). The neurobiology of the Prader-Willi phenotype of fragile X syndrome. *Intractable & Rare Diseases Research*, *5*, 255–261. https://doi.org/10.5582/irdr.2016.01082

Park, M. H., Kinra, S., Ward, K. J., White, B., & Viner, R. M. (2009). Metformin for obesity in children and adolescents: A systematic review. *Diabetes Care*, *32*, 1743–1745. https://doi.org/10.2337/dc09-0258

Rajaratnam, A., Shergill, J., Salcedo-Arellano, M., Saldarriaga, W., Duan, X., & Hagerman, R. (2017). Fragile X syndrome and fragile X-associated disorders. *F1000Research*, *6*, 2112–https://doi.org/10.12688/f1000research.11885.1

Rena, G., Hardie, D. G., & Pearson, E. R. (2017). The mechanisms of action of metformin. *Diabetologia*, *60*, 1577–1585. https://doi.org/10.1007/s00125-017-4342-z

Romero, R., Erez, O., Hüttemann, M., Maymon, E., Panaitescu, B., Conde-Agudelo, A., … & Grossman, L. I. (2017). Metformin, the aspirin of the 21st century: Its role in gestational diabetes mellitus, prevention of preeclampsia and cancer, and the promotion of longevity. *American Journal of Obstetrics and Gynecology*, *217*, 282–302. https://doi.org/10.1016/j.ajog.2017.06.003

Schneider, A., Ligsay, A., & Hagerman, R. J. (2013). Fragile X syndrome: An aging perspective. *Developmental Disabilities Research Reviews*, *18*, 68–74. https://doi.org/10.1002/ddrr.1129

Seifarth, C., Schehler, B., & Schneider, H. J. (2013). Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. *Experimental and Clinical Endocrinology & Diabetes*, *121*, 27–31. https://doi.org/10.1055/s-0032-1327734

Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M., & Andreelli, F. (2012). Cellular and molecular mechanisms of metformin: An overview. *Clinical Science*, *122*, 253–270. https://doi.org/10.1042/CS20110386

Wiegers, A. M., Curfs, L. M., Vermeer, E. L., & Fryns, J. P. (1993). Adaptive behavior in the fragile X syndrome: Profile and development. *American Journal of Medical Genetics*, *47*, 216–220. https://doi.org/10.1002/ajmg.1320470215

Wright-Talamante, C., Cheema, A., Riddle, J. E., Luckey, D. W., Taylor, A. K., & Hagerman, R. J. (1996). A controlled study of longitudinal IQ changes in females and males with fragile X syndrome. *American Journal of Medical Genetics*, *64*, 350–355. https://doi.org/10.1002/(SICI)1096-8628(19960809)64:2<350:AID-AJMG23>3.0.CO;2-D

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