

Case Report

Prepubertal Metformin Treatment in Fragile X Syndrome Alleviated Macroorchidism: A Case Study

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Abstract

The majority of males with Fragile X syndrome (FXS) have macroorchidism in addition to intellectual and language deficit, behavioral problems and autism. Macroorchidism is a characteristic feature of FXS and it is present in more than 90% of postpubertal males. The results of preclinical studies demonstrated that antidiabetic drug metformin rescues multiple phenotypes of FXS in both the *Drosophila* and mouse models including the presence of macroorchidism. Preliminary studies of patients with FXS demonstrated language and behavioral improvements. Here, we present one case of a male with FXS who has been treated with metformin clinically from 12 to 14 years of age. He was Tanner stage 3 at age 14 and his testicular volume was in the normal range which is rare in FXS. This is the first report of the alleviation of macroorchidism in a male with FXS treated with metformin and it parallels the changes seen with metformin in the mouse model of FXS.

Keywords: Fragile X syndrome, metformin, macroorchidism, targeted treatment

Introduction

Fragile X Syndrome (FXS) is the most common genetic form of intellectual disability and single gene cause of autism spectrum disorder (ASD) with a prevalence of approximately, 1 in 4000 males and 1 in 8000 females [1]. FXS is a neurodevelopmental disorder caused by full mutation (200 or more CGG repeats) of the fragile X mental retardation (*FMR1*) gene, located on chromosome Xq27.3. This mutation leads to methylation of the gene and a subsequent lack of the protein encoded by this gene, the Fragile X mental retardation protein (FMRP) [2-4].

Patients with FXS have variable clinical presentations. The classical physical phenotype in males with FXS includes large prominent ears, long narrow face, hyperflexible joints, double-jointed thumbs and macroorchidism (large testicles). All boys with FXS have one or more of these physical features. They can also have behavioral problems such as social anxiety, shyness, attention-deficit hyperactivity disorder (ADHD), irritability, aggression (including self-aggression), impulsiveness, language deficit, and seizures. All of the listed symptoms are a result of the *FMR1* protein (FMRP) deficit [5].

The management of FXS includes pharmacological treatments and non-pharmacological interventions including speech therapy, physical therapy, occupational therapy, and behavior therapy. The medications commonly

used include stimulants, selective serotonin reuptake inhibitors (SSRIs), atypical antipsychotics and medications for sleep disturbances. One of the most important aspects of the FXS field is the identification of targeted treatments. These targeted treatments have the potential to reverse the neurobiological aspects of FXS. One of these targeted treatments, metformin, has shown promise of being beneficial for patients with FXS [6,7]. Metformin is a well-studied, safe and effective antidiabetic medication that has been in clinical use for more than 60 years [8]. Metformin's mechanism of action includes several pathways including down regulation of the mTOR pathway that is upregulated in FXS [6], but metformin may also decrease the MMP9 level which is pathologically elevated in FXS [9-11]. Metformin is particularly important also for the weight problems in FXS because obesity and over eating or stuffing the mouth is common in FXS [12]. A subgroup of patients with FXS (<10%) have the Prader-Willi phenotype of FXS which is characterized by severe obesity, hyperphagia, small phallus, delayed puberty and lack of satiation after meals [13].

Here, we present a pubertal boy with FXS with significant benefits from the targeted treatment of metformin. This patient started metformin at 12 years of age and after two years subsequently has significant improvement in social skills, language and behaviour, significant weight loss and BMI reduction, and he did not develop macroorchidism in puberty.

Materials and Method

Participants

We are presenting one individual diagnosed with FXS that has been on metformin for the last 2 years. The parents have signed an informed consent for developmental and molecular testing approved by the institutional IRB and the family has consented to have his clinical response to metformin published.

We saw this child in the Fragile X Treatment and Research Center at the MIND Institute at University of California Davis Medical Center for management of FXS. Metformin was prescribed as a treatment for obesity and as a targeted treatment of FXS.

Case Report

Our patient is a 14-year old male with a history of the Prader Willi phenotype of FXS. He was diagnosed with FXS at age 5 due to developmental delays and was noted to have to have a methylation mosaicism with ~68% of cells having hypermethylated alleles >200 CGG repeats in size and the remaining with unmethylated alleles spanning the entire normal and premutation range (~30-200 CGG repeats). *FMR1* mRNA expression level was 0.45 (StErr 0.04). During this time, he was also diagnosed with intellectual disability, ASD and ADHD. His mother is a premutation carrier and has had significant problems with depression. His Prader Willi phenotype includes hyperphagia and obesity without satiety after meals.

The patient has a long history of daily irritability and aggression. When he was 8 years old, his aggressive behavior worsened and he was extremely difficult to handle. He often had melt downs with aggression. His mother was worried about his safety, her safety and the safety of other children. His anger and behavior problems caused significant difficulties in daily functioning. Along with these behavioral problems he also had difficulties with sleep. He would have day time naps and then sleep minimally at night, waking up at 3-4 AM every night.

During the time period of 5-10 years of age he had significant weight and BMI increases due to multiple factors which include his Prader Willi phenotype and lack of exercise. Parents reported that they tried to maintain a healthy diet and would lock the kitchen cabinets to prevent excessive eating but that it was not enough to control the weight gain. At 5 years of age he weighed 22.9kg with a BMI of 18.2 and by 10 years of age he weighed 73.1kg and a BMI of 34.3. On physical exam during these years it was noted he was Tanner Stage 1 with testes 4-5 ml bilaterally.

He has been prescribed several medications to treat his symptoms and behavioural concerns. These included guanfacine hydrochloride, minocycline, aripiprazole, sertraline, divalproex sodium, lovastatin, lithium, methylphenidate hydrochloride and alprazolam. While some of these provided limited symptom relief, they were not long lasting. At the age of 12 years it was decided to start him on 500 mg of metformin with dinner. This was started as a target treatment for help with his language and cognition along with the benefit of appetite suppression and weight loss. To maximize the benefits of metformin he was titrated up to 500 mg twice a day (1000 mg/d). At the onset of metformin treatment his height was 156.7cm, his weight was 70.7 kg, and a BMI of 28.8. His lab values were as follows: HgbA1c 4.7, fasting blood glucose 88, alkaline phosphatase 253, and ANA was negative. All of these labs were normal. On physical exam, he was Tanner Stage 1 with small phallus hidden beneath a fat pad and testes were 8 ml bilaterally.

One year later, at 13 years of age, parents reported that since starting the metformin he had significant improvements in his behavior. He was calmer, had decreased agitation and aggression. They also report that he followed directions better; he could focus for longer periods of time and was more patient. The teachers in school reported that he was able to understand things in class and was more responsive to reward based behavior management. His only side effect was loose stools, a well-documented side effect, which quickly resolved shortly after starting the metformin. At this visit (age 13 years) his height was 157.7 cm, weight was 69.9 kg, and BMI of 28.1. In a year he grew 1cm, lost almost 1 kg, and decreased BMI by 0.7. Parents report that the weight loss and decrease in BMI was due to the metformin as they had not changed his diet or exercise program. They reported that he had decreased appetite and was sleeping better. On physical exam his genitalia showed prepubertal development with a small phallus and testicular volume of 10 ml bilaterally.

After two years on metformin he continues to show improvement in language and behavior along with weight loss. His height is 168.1 cm, weight is 68.1 kg, and BMI is 24.1. Since starting metformin, he has grown 11.4 cm, lost 2.6 kg, and reduced his BMI by 4.7. Parents report that he started puberty a few months prior to his current visit and on physical exam was noted to be Tanner Stage 3 with testicular volume of 25 ml bilaterally. The lack of macroorchidism was remarkable compared to other males with FXS who are Tanner stage 3. Typically, males with FXS develop an increase in testicular size at age 8yo and by 14 or 15 years the average testicular volume is 50 ml [14].

Discussion

This is the first report regarding the lack of macroorchidism in an adolescent patient with FXS treated with metformin as a target treatment of FXS. Macroorchidism is a characteristic feature of FXS and it is present in 92% of postpubertal males with a fully methylated full mutation and 83% of postpubertal males with <50% methylated full mutation of *FMR1* gene. The majority of adult males with FXS have a testicular volume of 40 to 60 ml. The frequency of macroorchidism is lower in prepubertal boys with FXS (39% and 30%) and typically the testicle size increase begins at 8 to 9 years of age [5,14]. However, the males with FXS are fertile and capable of reproduction [15,16].

Normal growth of testicles are as follows: 1-3 ml in childhood, 4-6 ml in early puberty, 8-10 ml in middle puberty, 12-15 ml in late puberty, and 20-25 ml in adulthood. Macroorchidism is noted to be larger than 4 ml prior to puberty. FXS is noted to have testicular volumes up to 4x the average size of 18ml. It is known that boys aged 2-7 years with FXS have significantly larger mean testicular volume but no significant macroorchidism. From 8-10 years of age all boys had significantly enlarged testicles with the majority >4.0 ml [17].

Currently, there has not been a clinical study aiming to reduce macroorchidism in patients with FXS. The preclinical study showed that a 10-day treatment of the *FMR1* KO mice with metformin (200 mg/kg/day) significantly reduced testicular weight [18]. Although, metformin is in clinical use for more than 60 years and it was approved in Europe since 1957 and in the US since 1994, its precise mechanism of action is not clear [8]. It is well known that it acts

through AMPK-dependent and AMPK-independent mechanisms, and its mechanism of action involves mTOR and MAPK signalling pathways. In FXS *Drosophila* and mouse models, metformin significantly improved core phenotypes at the levels of behavior, morphology, and synaptic plasticity and selectively normalized the MAPK signalling pathway [18,19]. In our recent clinical evaluation of metformin treatment, seven patients with FXS showed improvement in behaviour and language with metformin treatment [7]. According to our unpublished data, metformin can improve cognition in 2 adult males treated for one year but in adulthood the testes did not change from their large size [20] This data is in accordance with the results from preclinical studies of adult animal models of FXS [6,18,19].

Conclusion

Our patient's course of metformin for two years prior to puberty matches the mouse model with prevention of macroorchidism [18]. Metformin has been shown to be a targeted treatment for FXS with some limited evidence for improvement in language and cognition. The clinical course of our patient suggests that it may eliminate the development of macroorchidism if started before puberty. This effect is likely related to metformin's ability to lower the excess protein production that is driving testicular growth in FXS. Currently a controlled trial is taking place (www.clinicaltrials.gov; NCT number: NCT03479476) and the size of the testicles will be monitored during this trial in addition to cognitive, language and behavioral changes.

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Author Contributions

D. P. coordinated and wrote the work, P. K. helped in writing paper, F. T. contributed with the writing of the section on molecular and genetic data R. H. suggested the idea for the paper, wrote and edited the paper.

Conflict 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 and Zynerva regarding treatment studies in individuals with fragile X syndrome. The other authors declare no conflicts of interest.

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