Review

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The neurobiology of the Prader-Willi phenotype of fragile X syndrome

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Summary **Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and autism, caused by a CGG expansion to greater than 200 repeats in the promoter region of *FMR1* on the bottom of the X chromosome. A subgroup of individuals with FXS experience hyperphagia, lack of satiation after meals and severe obesity, this subgroup is referred to have the Prader-Willi phenotype of FXS. Prader-Willi syndrome is one of the most common genetic severe obesity disorders known and it is caused by the lack of the paternal 15q11-13 region. Affected individuals suffer from hyperphagia, lack of satiation, intellectual disability, and behavioral problems. Children with fragile X syndrome Prader-Willi phenotye and those with Prader Willi syndrome have clinical and molecular similarities reviewed here which will impact new treatment options for both disorders.**

Keywords: Fragile X syndrome (FXS), Prader-Willi phenotype, *FMR1* gene, Hyperphagia, Autism, IGF-1, Growth hormone

1. Introduction

Prader-Willi syndrome (PWS) is the most common cause of obesity and intellectual disability, occurring in about 1 in 15,000 in the general population (*1-3*). PWS is characterized by hyperphagia and lack of satiation after meals. Typical physical features include: a round face, narrow palpebral fissures, short stature, small genitalia, and short fingers and toes. Behavioral and cognitive features include mild intellectual disability (ID), poor attention, obsessive behavior particularly around food so that food hoarding and food stealing are common, excessive skin picking, and remarkably good ability with puzzles. PWS is caused by an absence of expression of imprinted genes in the paternally derived PWS/Angelman syndrome (AS) region (15q11.2-q13) of chromosome 15 by one of several genetic mechanisms (paternal deletion, maternal uniparental disomy and rarely an imprinting defect) (*2*).

Fragile X syndrome (FXS) is the most common inherited cause of ID and Autism Spectrum Disorder (ASD) and it is caused by a CGG trinucleotide expansion of over 200 repeats (full mutation) in the 5' region of the fragile X mental retardation 1 gene (*FMR1*) at Xq27.3. This full mutation leads to hypermethylation of *FMR1* and a subsequent lack of transcription and translation, which in turn results in a deficiency of the FMR1 protein (FMRP). The FMRP is an RNA binding protein that mainly negatively regulates the translation hundreds of genes, many of which are critical for synaptic plasticity (*4*). The prevalence of FXS is 1 in 5,000 males and 1 in 8,000 females (*5*). The features of FXS include hyperactivity, attentional problems, poor eye contact, hand-flapping, anxiety, hyperarousal and

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a lack of habituation to sensory stimuli. The physical features of FXS include a long face, prominent ears, hyperextensible finger joints and flat feet. Males with FXS have macroorchidism during and after puberty with a normal phallus. Although the clinical phenotype is very different between PWS and FXS there is a subgroup of individuals with FXS that develop hyperphagia, obesity, and hypogonadism or delayed puberty. Because this phenotype looks like those with Prader-Willi syndrome, it is described as Fragile X syndrome - Prader-Willi phenotype (FXS-PWP) (Figure 1A and 1B). This phenotype in FXS was first reported by Fryns *et al*. (1987) and has subsequently been described by others (*6-10*). This phenotype is not related to a deletion of 15q11-q13 nor due to maternal uniparental disorders, and it occurs in less than 10% of individuals with FXS (*11*). Although short stature and small fingers and toes can sometimes occur in the FXS-PWP this is not seen in the majority of cases. Nowicki *et al*. (2007) found lower cytoplasmic interacting FMR1 protein 1 (CYIP1) levels in the blood of patients with the FXS-PWP compared to those with FXS without the PWP (*6*). They also found a higher rate of ASD in those with the FXS-PWP compared to FXS alone (*6*).

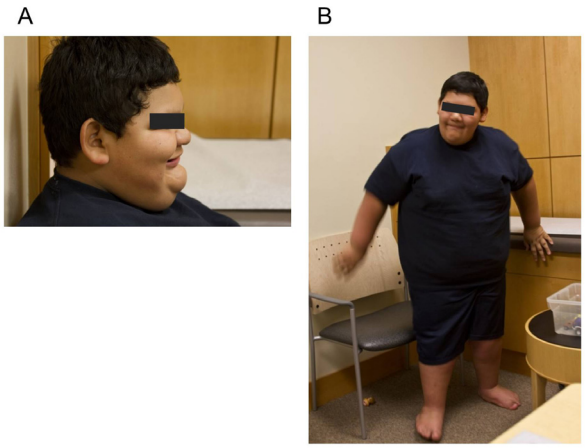

Figure 1. 11 year old with FXS and PWP, showing severe obesity, small hands and mild facial dysmorphic features

2. Neurobiology of FXS with PWP

Patients with the FXS-PWP have hyperphagia that arises in childhood (*6*), as is the case in PWS. Even though there is no clear molecular explanation underlying hyperphagia in FXS, it is hypothesized that it arises from dysregulation of gamma-aminobutyric acid (GABA) system in the hypothalamus. The lateral hypothalamus (LH) is a critical modulator of feeding (*12,13*). A previous study by Jennings *et al*. demonstrated that GABAergic (*Vgat*-expressing) neurons in the LH are responsible for producing appetitive and consummatory behaviors (*14*). FXS animal models have lowered GABA subunit receptors, synthesis of GABA, GABAergic input to many regions of the brain, and increased catabolism of GABA (*15,16*). Similarly, cerebral GABAA receptor expression is reduced in several brain regions of subjects with PWS (*17*).

There is a high rate of ASD (7 of 13, 54%) in the patients with FXS-PWP (*6*). This may be related to the reduction mRNA levels of the CYFIP1, which was found to be two to fourfold lower in the patients with FXS-PWP compared to individuals without FXS and patients with FXS without the PWP (*6*). CYFIP1 is localized to the critical region for PWS at 15q between breakpoint 1 and 2 (*18*). FMRP binds to CYFIP1 (*19*) in the execution of its role as a transporter and regulator of translation of mRNAs (*20-22*). CYFIP1 expression levels are vital for dendritic arborization and neuronal morphological complexity (*23*). Neurons from CYFIP1 haploinsufficient animals have smaller and less complex dendritic branching both *in vitro* and *in vivo* (*23*). Another reason could be due to dysregulation of oxytocin (OT) and arginine vasopressin (AVP) in the brain. The dysregulation of the OT system in animals and humans is linked with marked deficits in social behavior and anxiety (*24*). There is a scarcity of OT producing neurons in the paraventricular nucleus of the hypothalamus (PVN) in individuals with PWS (*25*) and in the *Fmr1* KO (knock-out) mice (*24*). OT administration increases trust and diminishes disruptive behavior in individuals with PWS (*26*); in those with FXS OT therapy improves social anxiety (*27*).

3. Treatment

In the treatment of autism, use of intensive early behavioral intervention, such as the Early Start Denver Model (ESDM) has been shown to improve developmental and social outcome in addition to normalization of the EEG abnormalities compared to those treated with community behavioral interventions (*28*). Such intervention is also recommended in young children with FXS both with and without autism or the PWP (*29,30*). A variety of medication use can be helpful for those with FXS both with and without the PWP including; stimulants which can help with attention and appetite; Selective Serotonin Reuptake Inhibitors (SSRIs), such as sertraline for anxiety (*31*); and atypical antipsychotics, such as aripiprazole to stabilize mood, improve autism, aggression and/or tantrums (*6*). However, aripiprazole can cause an increase in weight gain and particularly for those with a CYP2D6 polymorphism that can slow down the metabolism of aripiprazole (*32*).

There are a variety of new-targeted treatments that have been studied in those with FXS. To enhance the GABA deficits in FXS, the GABAB agonist, arbaclofen, showed initial benefit for those with ASD or low sociability (*33*). However, the subsequent phase 3 trial in children and adolescents and adults did

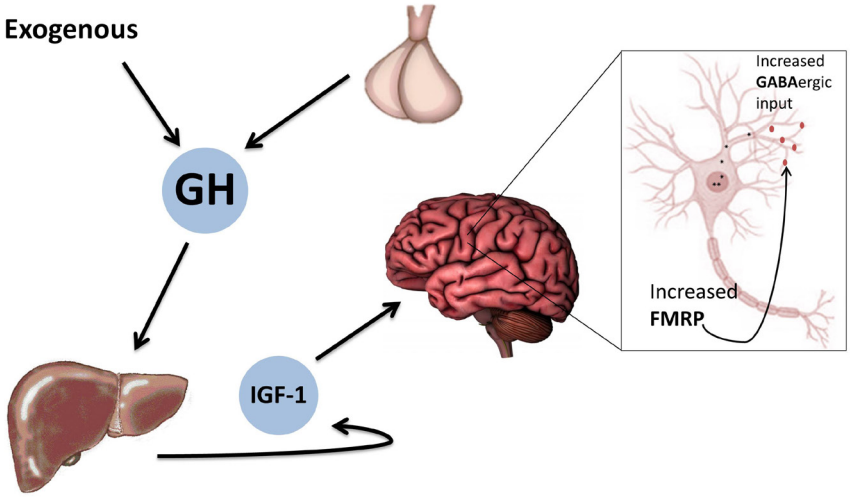

Figure 2. Suggested mechanism of action of GH. GH stimulates the liver to release IGF-1, which activates CREB, and this increases FMRP in neurons. The increase of FMRP leads to increased GABAergic input to some areas of the brain.

not demonstrate efficacy (*34*). The GABAA agonist ganaxolone is being studied in children 6 to 17 years old at the MIND Institute at UC Davis Medical Center and in Belgium utilizing the same protocol (*https://www.clinicaltrials.gov* [NCT]: ID number NCT01725152). Results from this study are expected before of the end of this year. The mGluR5 antagonists developed by Roche and Novartis have not demonstrated efficacy in adult and childhood studies, but further studies are planned for an mGluR5 antagonist. Minocycline has been studied in children with FXS and has demonstrated some efficacy so it is often utilized clinically (*35,36*). Other trials included at multiple centers included metadoxine for improving attention and focus and the IGF-1 analogue developed by Neuren for the treatment of behavioral problems. However, those with the FXS-PWP of are not typically included in such clinical trials because their level of obesity is beyond what is acceptable for inclusion in these trials. Therefore, there are no studies of the treatment in those with FXS-PWP.

In contrast, human growth hormone (GH) has been the panacea for treatment of PWS over the last decade (*37,38*). Most individuals with PWS, but not all, are deficient in GH and studies that were initially focused on improving growth have not only demonstrated this effect, but also improvements in metabolism, body composition, behavior and cognition (*38-44*). However, GH therapy on occasion can be associated with significant side effects, such as the stimulation of adenoid tissue leading to obstructive sleep apnea so it should be used carefully in the treatment of PWS. GH can also promote the growth of some malignant tumors so such a history is a contraindication for GH therapy. Since GH therapy has been so beneficial to those with PWS and because there are remarkable similarities between PWS and the FXS-PWP, it is possible that those with the FXS-PWP will benefit from GH therapy. The observed benefits of the IGF-1 analogue in the KO mouse FXS model support the likelihood that GH therapy should be beneficial in the FXS-PWP. GH stimulates the release of IGF-1 by the liver and this may be the mechanism for the benefit of GH therapy. IGF-1 enhances GABA activity that is deficient in FXS both with and without the PWP (Figure 2)(*45*). In addition, the GABAB receptor-mediated transactivation of IGF-1 receptors leads to cAMP response element-binding protein (CREB) activation which in turn binds to *FMR1* and increases FMRP levels (*46*). Therefore, GH in FXS-PWP could stimulate the release of IGF-1 to enhance the GABA system, and increase the residual expression of *FMR1* particularly in those who are mosaic or partially unmethylated. Further studies are necessary to determine the molecular benefits of IGF-1 analogues and GH in FXS.

In general, metabolic anomalies are suspected in FXS because about 30% are obese (*47*). Metabolic anomalies including increased glucose uptake and excess protein synthesis in the brain have been reported in *Fmr1* KO mice, while in the fly (*dfmr1*), it has been shown that FMRP is required during brain development and may function in neuroblast reactivation by regulating an output of the insulin signaling pathway (*48-52*). Metabolic profiling in the *Fmr1* KO mice also revealed profound consequences in brain metabolism, which in turn lead to alterations in the metabolic response, along with anomalies in other physiological processes and behaviors (*53*). Using Drosophila as a model of FXS it has been shown that the *dfmr1* has elevated levels of drosophila insulin-like peptide 2 (Dilp2) in the insulin-producing cells which result in elevated insulin-signaling *via* the PI3K/Akt/mTOR pathway (*54*). It is also known that increased insulin-signaling leads to defects in the circadian output pathway and in short and long-term memory

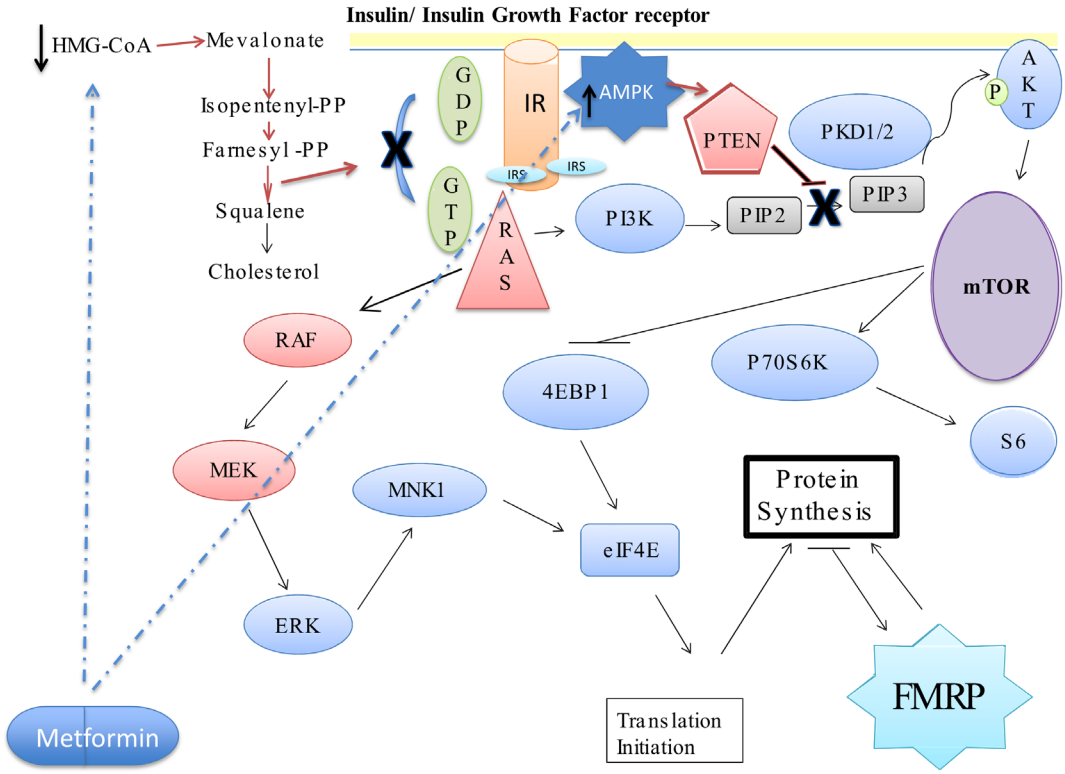

Figure 3. Mechanism of action of metformin. Metformin decreases protein synthesis and insulin signaling (IS) *via* the AMPK/Akt/mTOR pathway, it also inhibits the lipid and sterol biosynthetic pathways

deficits. Interestingly, pharmacological restoration using metformin, rescued memory deficits in the *dfmr1* (54). Metformin is known to decrease body mass index (BMI) and to prevent cognitive deficits in individuals with diabetes (*55-59*). Metformin decreases protein synthesis and insulin-signaling *via* the AMPK/Akt/mTOR pathway, it also inhibits the lipid and sterol biosynthetic pathways (*60-63*) (Figure 3). Therefore, metformin in FXS may decrease insulin-signaling and restore the circadian output pathway and in turn have positive effects on memory and sleep. A pilot, open-label study of response to metformin in 21 children with PWS and six with early morbid obesity (EMO) showed significant improvements in food-related distress, anxiety, and ability to be redirected away from food. Within the PWS group, responders to metformin had higher 2-hour glucose levels on oral glucose tolerance test and higher fasting insulin levels. Additionally, parents of 5/13 individuals with PWS and 5/6 with EMO reported recognition of satiety (*64*). Further studies are necessary to determine the safety and efficacy of metformin in FXS, PWS and FXS-PWP.

4. Future directions for research

Understanding the similarities and differences between PWS and the PWP of FXS will lead to new treatments perhaps for both disorders. Since *CYFIP1* is down-regulated in the PWP and because the clinical phenotypes are so similar across both disorders, it is likely that epigenetic changes or methylation differences may be down-regulating other genes in the 15 q 11-13 region in those with the PWP. Further studies are warranted to determine whether the reduced expression of multiple genes in PWS also occur in FXS-PWP. These genes include, *MKRN3* (*65*), *MAGEL2* (*66*), *MAGED1* (*67*), *NECDIN* (*68,69*) and *SNURF-SNRPRN* (*70*). To understand more about hyperphagia phenotype in FXS-PWP, studies regarding the GABA network in the brain are required. In addition, as chronic hyperghrelinaemia promotes hyperphagia in PWS (*71*), it would be interesting to see ghrelin levels in patients with the FXS-PWP.

A variety of new treatments are currently being studied in PWS and reviewed in Miller *et al*. (2015). Although GH treatment has many beneficial effects, it does not significantly help the hyperphagia. Some of the new medications that are being studied in PWS include Diazoxide, a potent K^+-ATP channel agonist that hyperpolarizes hypothalamic neurons whose activity is impaired by a defective leptin signaling pathway in PWS (NCT02-34071); AZP-573, an unacylated ghrelin analog; Exanatide/Liraglutide, glucagon-like peptide 1 (GLP-1) receptor agonist which can suppress appetite and reduce weight in PWS and obese patients (NCT014448981/ NCT01542242). Clearly the study of those with FXS-

PWP will determine whether they have the potential to benefit from some of these new trials that have occurred in PWS. The future of treatment of both disorders looks bright with the advent of targeted treatments based on the neurobiological studies of both disorders.

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