

# Metformin for Treatment of Fragile X Syndrome and Other Neurological Disorders

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## Abstract

Fragile X syndrome (FXS) is the most frequent inherited form of intellectual disability and autism spectrum disorder. Loss of the fragile X mental retardation protein, FMRP, engenders molecular, behavioral, and cognitive deficits in FXS patients. Experiments using different animal models advanced our knowledge of the pathophysiology of FXS and led to the discovery of many targets for drug treatments. In this review, we discuss the potential of metformin, an antidiabetic drug approved by the US Food and Drug Administration, to correct core symptoms of FXS and other neurological disorders in humans. We summarize its mechanisms of action in different animal and cellular models and human diseases.

**FMRP:** fragile X mental retardation protein

## INTRODUCTION

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability, affecting 1:5,000 males and 1:6,000 females worldwide (1). FXS is caused by a CGG repeat expansion in the promoter region of fragile X mental retardation gene, *FMRI*. More than 200 repeats of CGG lead to hypermethylation and silencing of the gene, causing the loss of fragile X mental retardation protein (FMRP). In <1% of FXS individuals, absence of FMRP is caused by mutations in the coding region of *FMRI* (1). Males with FXS display intellectual disability (IQ ranging between 20 and 70) and a broad spectrum of symptoms including physical abnormalities (macroorchidism, prominent ears, elongated face and hyperextensible finger joints), and behavioral (anxiety, social avoidance and hand clapping), cognitive (executive functioning, visual-spatial processing and developmental delay), and neurological (epilepsy and disrupted sleep patterns) deficits (**Table 1**). Because the disease is X-linked, females with a full mutation are less affected than males, with an average IQ of 75–80 (1). Several animal models of FXS have been developed, including *Drosophila*, mouse, and rat (**Table 1**; see sidebar titled Animal Models of Fragile X Syndrome for details). In recent years, numerous preclinical and clinical studies have been carried out to develop and test novel therapeutics for FXS (2). In this review, we describe the latest advances in understanding the molecular basis of the disease pathogenesis and highlight the emerging therapeutic potential of the antidiabetic drug metformin.

## FRAGILE X SYNDROME AND TRANSLATIONAL CONTROL

Many neuropsychiatric symptoms of FXS are thought to be a consequence of dysregulated protein synthesis at the synapse (3). FMRP is an RNA-binding protein, targeting a subpopulation of neuronal mRNAs (1, 4). The cardinal function of FMRP is repression of translation, mainly of genes encoding synaptic plasticity-related proteins (1, 4). The absence of FMRP leads to

**Table 1** Overlapping phenotypes and the effects of metformin in humans with fragile X syndrome (FXS) and in FXS animal models<sup>a</sup>

Impaired phenotypes in FXS	<i>Drosophila</i> (references)	Mouse (references)	Rat (references)	Human (references)
Social interaction	Yes (101)	Yes (12, 15, 35, 91)	Yes (99, 100)	Yes (1, 2, 34)
Repetitive behavior	Yes (101)	Yes (15, 35, 91, 95)	No (100)	Yes (1, 2, 34)
Speech/ultrasonic vocalization	Not applicable	Yes (95)	No (100)	Yes (1, 2, 34)
Memory	Yes (33, 101)	Yes (12, 35, 90, 91, 95)	Yes (98, 99)	Yes (1, 2)
Hyperactivity	Yes (101)	Yes (15, 35, 90, 95)	No (100)	Yes (1, 2, 34)
Seizures	Not reported	Yes (15, 35, 90, 95)	Not reported	Yes (1, 2)
Sleep disorder	Yes (101)	Yes (91, 95)	Not reported	Yes (1, 2)
Circadian rhythm	Yes (33, 101)	Yes (91, 95)	Not reported	Yes (1, 2)
Long-term depression	Not applicable	Yes (12, 15, 35, 91, 93, 94)	Yes (98, 99)	Not applicable
Dendritic/synaptic morphology	Yes (101)	Yes (15, 35, 91, 92, 95)	Yes (98)	Yes (1, 2)
General protein synthesis	Not reported	Yes (12, 15, 35)	Yes (98)	Yes (3, 4)
Hyperactivation of mTOR	Not reported	Yes (12, 35)	Not reported	Yes (3, 4, 13)
Hyperactivation of ERK	Not reported	Yes (14, 35)	Not reported	Yes (3, 4, 13)
Macroorchidism	Yes (101)	Yes (12, 35)	Yes (99)	Yes (1, 2)

<sup>a</sup>Red text indicates phenotypes corrected by metformin.

## ANIMAL MODELS OF FRAGILE X SYNDROME

The best-studied mouse model, generated in 1994, has been instrumental in investigating fragile X syndrome (FXS) pathophysiology and preclinical testing of therapeutic strategies (**Table 1**) (90). *Fmr1* knockout (KO) mice display a range of phenotypes reminiscent of the human FXS, including a mild cognitive deficit, hyperactivity, increased sensitivity to auditory stimuli leading to epileptic seizures, decreased acoustic startle reflex, and macroorchidism (91). Similar to FXS individuals, *Fmr1* KO mice show increased dendritic spine density and immature spines (92). The mouse model shows a robust increase in metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) in the hippocampus and cerebellum (93, 94). In addition, *Fmr1* KO mice exhibit autistic-like behavior such as repetitive/stereotypic behavior and social interaction deficits (95). In 2006, a new *Fmr1* KO2 mouse was generated, which does not express any *Fmr1* mRNA, and impairments similar to the previously described KO mouse model were reported (96, 97). A rat model of FXS exhibits reduced long-term potentiation (LTP) and increased mGluR-LTD, impairments in learning and memory, altered dendritic spine morphology, and macroorchidism (**Table 1**) (98, 99). The *Fmr1* KO rat also exhibits deficits in social interaction (99, 100). *Drosophila* has been extensively used to investigate the basic mechanisms underlying FMRP (fragile X mental retardation protein) function and to test pharmacological treatments (101). The *Drosophila* FXS model exhibits learning and memory deficits, autistic-like behaviors such as abnormal grooming and social deficits, increased locomotion, and altered sleep and circadian rhythm, as well as impaired neural physiology and structure (**Table 1**).

augmented translation of its direct target mRNAs (4) and to the activation of two critical pathways regulating protein synthesis: the mTOR (mechanistic target of rapamycin) pathway and the MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) pathway (5).

### The mTOR Pathway

mTOR is a serine/threonine protein kinase, which is highly evolutionarily conserved from yeast to humans. The mTOR pathway regulates fundamental cellular processes, such as cell growth, proliferation, and metabolism, via controlling protein synthesis, lipid biogenesis, and autophagy (5, 6). In the nervous system, mTOR controls brain development, neuronal circuit formation, synaptic plasticity, and learning and memory (6).

mTOR exists in two distinct structural and functional complexes: mTOR complex 1 (mTORC1) and mTORC2. mTORC1 is rapamycin sensitive and phosphorylates two major downstream effectors: p70S6 kinase 1 (p70S6K1) and eukaryotic translation initiation factor 4E (eIF4E)-binding proteins (4E-BPs) (**Figure 1**) (5). 4E-BPs are translational repressors that bind eIF4E, the major mRNA 5'-cap binding protein, preventing the interaction of eIF4E with eIF4G (a large scaffold protein). eIF4E and eIF4G, together with eIF4A (an mRNA helicase), form a three-subunit complex, eIF4F. The formation of eIF4F is critical for the recruitment of the ribosome to the 5'-end mRNA cap structure (m<sup>7</sup>GpppN, where N is any nucleotide) and therefore is central to translation initiation. Phosphorylation of 4E-BP by mTORC1 causes dissociation of 4E-BP from eIF4E, allowing eIF4F complex formation and initiation of translation (5).

Dysregulation of mTORC1 has been implicated in numerous pathological conditions including cancer, obesity, and type 2 diabetes (T2D) (7). It has also been associated with neurological disorders, such as tuberous sclerosis complex (TSC), phosphatase and tensin homolog deleted on chromosome 10 (PTEN) hamartoma tumor syndrome, neurofibromatosis, autism spectrum disorder (ASD), FXS, epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease, major depressive disorder, and schizophrenia (6).

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**mTOR:** mechanistic target of rapamycin

**MAPK:** mitogen-activated protein kinase

**ERK:** extracellular signal-regulated kinase

**4E-BP:** eukaryotic translation initiation factor 4E (eIF4E)-binding protein

**T2D:** type 2 diabetes

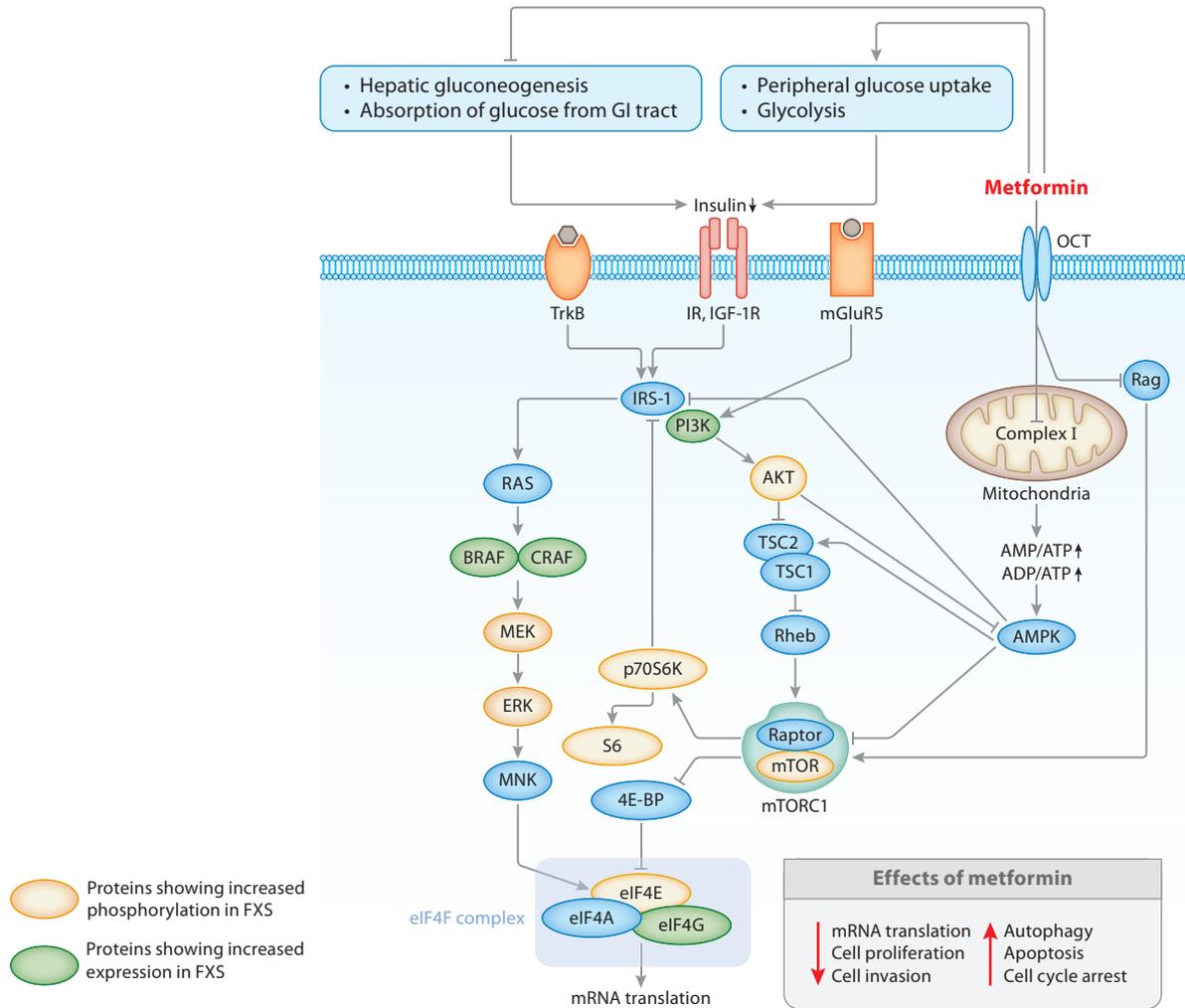
**TSC:** tuberous sclerosis complex

**ASD:** autism spectrum disorder

**AD:** Alzheimer's disease

**PD:** Parkinson's disease

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**Figure 1**

The mechanism of action of metformin on mTOR and MAPK signaling pathways in FXS. mTOR and MAPK pathways show increased activity in FXS. Metformin inhibits mTORC1 and ERK pathways and their downstream effectors at multiple levels. Metformin can act in an AMPK-dependent manner through inhibition of mitochondrial complex I, leading to the activation of AMPK, which in turn inhibits IRS-1, activates TSC complex, or inhibits Raptor. In an AMPK-independent manner, metformin can inhibit mTORC1 through inhibition of Rag GTPases. Additionally, metformin lowers systemic glucose and insulin levels, leading to the inhibition of the mTOR and ERK pathways. Abbreviations: AMPK, AMP-activated protein kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CRAF, v-raf1 murine leukemia viral oncogene homolog 1; ERK, extracellular signal-regulated kinase; FXS, fragile X syndrome; GI, gastrointestinal; IGF-1R, insulin-like growth factor 1 receptor; IR, insulin receptor; IRS-1, insulin receptor substrate 1; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mGluR5, metabotropic glutamate receptor 5; MNK, MAP kinase-interacting protein kinase; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; RAS, rat sarcoma; TSC, tuberous sclerosis complex.

## The MAPK/ERK Pathway

The MAPK/ERK pathway plays a central role in multiple cellular processes in the brain, such as proliferation, differentiation, and senescence, and it is critical for synaptic plasticity as well as learning and memory (8). In this pathway, Ras GTPase activates Raf proteins, which in turn activate the MEK-ERK (mitogen-activated protein kinase kinase/extracellular signal-regulated kinase) signaling cascade (**Figure 1**). ERK1 and -2 exert their function via multiple downstream effectors, in both the cytosol and nucleus. ERK downstream targets include MNKs (MAP kinase-interacting protein kinases), which phosphorylate eIF4E (9), promoting the translation of a subset of mRNAs. Dysregulation of the ERK pathway occurs in several cancer models and also in disorders of the nervous system such as neurofibromatosis type 1, Noonan syndrome, and FXS (4, 10).

## The Role of mTORC1 and ERK in Fragile X Syndrome

FMRP is thought to regulate the coupling between the metabotropic glutamate receptors (mGluRs) and the translational machinery via suppressing the ERK and mTORC1 signaling cascades (4). Accordingly, mTORC1 and ERK pathways are hyperactivated in brains of FXS patients and *Fmr1* knockout (KO) mice (4, 11). The activation of these pathways can explain the FXS pathological phenotypes, as inhibition of both mTORC1 and ERK signaling alleviates numerous deficits.

In *Fmr1* KO mice, genetic deletion of S6K1, a downstream effector of mTORC1, rescued behavioral (social and cognitive), morphological (dendritic spine dysgenesis and macroorchidism), and electrophysiological (mGluR–long-term depression) abnormalities (12). The genetic reduction of S6K1 also reversed the exaggerated general protein synthesis and elevated phosphorylation of translation components (S6 and eIF4B). Pharmacological inhibition of mTORC1 with temsirolimus (a rapamycin derivative) normalized the cognitive deficits in the object recognition task and the susceptibility to audiogenic seizures in *Fmr1* KO mice (1, 4, 13). The selective MEK1/2 inhibitor SL327 normalized elevated p90 ribosomal S6 kinase (RSK) activity and S6 phosphorylation in the neocortex of *Fmr1* KO mice, and abrogated audiogenic seizure activity (11, 14). The RSK inhibitor BI-D1870 prevented audiogenic seizures in *Fmr1* KO mice, pointing to the importance of the ERK pathway in FXS (14).

Lovastatin, a cholesterol-lowering drug, corrected increased ERK signaling, elevated general protein synthesis, and audiogenic seizures in the mouse model, and reduced hyperactivity of the ERK pathway in platelets of FXS patients (1). Consistent with increased ERK activity, eIF4E phosphorylation is elevated in the brains of FXS patients and *Fmr1* KO mice (4, 15). Phosphorylation of eIF4E promotes translation of a subset of mRNAs, including the mRNA encoding matrix metalloproteinase–9 (MMP-9), which has been implicated in FXS (13, 15, 16). Genetic and pharmacological reduction of eIF4E phosphorylation and MMP-9 rescued core pathologies in *Fmr1* KO mice (13, 15).

Since there is no cure for FXS, the search for new therapeutic targets is intensive and has already resulted in the identification of several candidates. Many of the drugs used in FXS studies converge on the mTOR and ERK pathways and have been shown to correct several of the core phenotypes (1, 2, 4, 13).

## mTOR AND ERK SIGNALING IN AUTISM SPECTRUM DISORDER

Approximately 60% of males and 20% of females with FXS are diagnosed with ASD (1, 17). Behavioral symptoms of ASD in individuals with FXS include impairments in social interaction and communication, as well as restricted and repetitive behaviors, such as poor eye contact, perseverative speech, and hand flapping.

Dysregulation of the molecular machinery controlling synaptic protein synthesis is linked to ASD (18). Several monogenic disorders harboring mutations in proteins upstream of mTOR exhibit high incidence of autism, increased levels of synaptic proteins, and augmented connectivity (3, 18–20). Loss-of-function mutations in *TSC1/2*, upstream of mTOR, are associated with autism in humans (18, 21). Mutations in *TSC1/2* enhance mTOR activity, and 85% of affected patients display cognitive impairments tightly linked to autistic features, epilepsy, and abnormal or absent speech (20). *Tsc2*<sup>+/-</sup> mice display deficits in social interaction and memory (22, 23) as well as enlargement of neuronal somata and dendritic spines (24). Administration of rapamycin reverses the observed deficits (22, 25). Deletion of the translational repressor 4E-BP2, downstream of mTOR, engenders autistic-like behaviors and an increased ratio of excitatory to inhibitory synaptic inputs (26). It is noteworthy, however, that in the *Shank3* KO mouse model of ASD, mTOR signaling is decreased rather than increased (27). SHANK3 is a postsynaptic protein, and mutations or deletions in its gene have been linked to ASD (18). The *Shank3* KO mouse model shows social and repetitive behavioral deficits as well as impaired synaptic plasticity (28).

The important role of ERK and mTORC1 pathways in ASD etiology is further supported by studies in ASD patients. Genome-wide association studies and genomic copy-number variation analyses demonstrated enrichment in gene sets involved in MAPK signaling in ASD individuals (29). A 593-kb deletion in chromosome 16p11.2, one of the most common copy-number variations associated with autism, encompasses *ERK1* (30). Interestingly, a mouse model of the human 16p11.2 microdeletion displays elevated ERK activity in the developing cortex and hippocampus and recapitulates some ASD pathology, including hyperactivity, repetitive behavior, lack of habituation, and synaptic defects (31, 32).

## THERAPEUTIC APPROACHES IN FRAGILE X SYNDROME

Preclinical studies in animal models identified several drug candidates for treatment of FXS. The drug candidates include mGluR antagonists, GABA ( $\gamma$ -aminobutyric acid) agonists, minocycline and its derivatives, and targets of other pathways such as endocannabinoid. We refer the reader to recent reviews covering their mechanisms of action and latest clinical results (1, 2, 4, 13). Metformin, an antidiabetic drug, was recently shown to have beneficial effects in preclinical studies of FXS and in human case reports (33–35). In this review, we focus on the effects of metformin on FXS and other brain disorders.

### METFORMIN

Metformin is a dimethylbiguanide, derivative of guanidine, which was isolated in the 1920s from the extract of French lilac (*Galega officinalis*) flowers. Two other biguanides, phenformin and buformin, had been used as oral antihyperglycemic drugs, but owing to lactic acidosis and other toxic effects, their use has been discontinued in most countries (36). Metformin is mostly known for its ability to normalize blood glucose levels, which it achieves via a combination of mechanisms: inhibiting hepatic gluconeogenesis, decreasing intestinal glucose absorption, increasing glucose uptake in peripheral tissues, and improving peripheral insulin sensitivity (37). Nowadays, pleiotropic effects of metformin are well established and include regulation of lipid metabolism, decrease of food intake and body weight, cardioprotection, and antineoplastic activity (38). Metformin is used in the clinic as the first-line drug to treat T2D, and is one of the most commonly prescribed medications worldwide (39).

## Molecular Mechanisms Underlying the Beneficial Effects of Metformin

While metformin has been prescribed for decades, its molecular mechanisms of action remain largely elusive. Its absorption, distribution, and elimination from the body depend on the activity of several solute carrier (SLC) transporters: OCT1 (organic cation transporter 1), -2, and -3 (also known as SLC22A1, -2, and -3), OCTN1 (SLC22A4), MATE1 and -2 (multidrug and toxin compound extrusion; SLC47A1 and -2), and PMAT (plasma membrane monoamine transporter; SLC29A4) (40). Additionally, metformin is a cargo of THTR-2 (thiamine transporter 2; SLC19A3), which plays a role in intestinal absorption and tissue distribution of metformin (41). Metformin transporters are highly expressed on the cell membrane of hepatocytes, enterocytes, and renal epithelial cells (40). Although they are less abundant in the central nervous system (CNS), OCTs were found in the endothelial cells of the blood–brain barrier of human and rodent brains (42, 43). Metformin rapidly crosses the blood–brain barrier and is distributed to various brain regions (44).

One of the primary targets of metformin action is thought to be the mitochondrial respiratory chain complex I (45). Metformin transiently inhibits complex I, resulting in decreased ATP production and, as a consequence, increased AMP and ADP levels. The increase in AMP/ATP ratio causes inhibition of glucagon-induced cAMP synthesis and activation of AMP-activated protein kinase (AMPK) complex, a pivotal cellular energy sensor (39, 46) (**Figure 1**). Activation of AMPK was thought to be required for the inhibition of gluconeogenesis in hepatocytes (47), but it was later demonstrated that metformin inhibits hepatic gluconeogenesis in transgenic mice lacking AMPK or its upstream activator LKB1 (liver kinase B1) (48). This finding suggests AMPK-independent effects of metformin on gluconeogenesis. Moreover, metformin has an effect in erythrocytes, which lack mitochondria (49), again implying the existence of alternative pathways.

### Metformin and the mTOR Pathway

Metformin impairs mTORC1 pathway activity in an AMPK-dependent manner via several mechanisms. First, AMPK phosphorylates and directly inhibits Raptor, a key component of mTORC1 (39). Second, AMPK activates TSC2, and it suppresses mTORC1 through Rheb (50). Third, AMPK inhibits IRS-1 (insulin receptor substrate 1), a component of insulin/IGF-1 (insulin-like growth factor 1) signaling that activates Akt (39) (**Figure 1**). Akt, in turn, can inhibit AMPK (51) as part of a negative feedback mechanism. Metformin can also inhibit mTORC1 independently of AMPK, through inactivation of the Rag family of GTPases (52), or in a p53-dependent manner, through an increase of REDD1 (regulated in development and DNA damage responses 1) (53). REDD1 is a negative regulator of mTORC1, controls cell survival, and is activated in response to DNA damage, nutrient depletion, glucocorticoid, and insulin (53).

### Metformin and the MAPK Pathway

Metformin impairs the MAPK signaling pathway, but the underlying mechanisms are not well understood. Metformin ameliorates the disrupted Raf/MEK/ERK signaling in cells expressing multiple rare variants of KSR2 (the kinase suppressor of Ras protein 2) (54). Since KSRs are stabilizing components of the ERK pathway, it is conceivable that metformin could impinge on the stability of the MAPK complex through KSR and thereby affect the ERK pathway.

The inhibitory effect of metformin on the MAPK pathway was demonstrated in different *in vitro* models, including ovarian, bladder, and pancreatic cancer, as well as in models of nerve injury and inflammatory pain (55–58). In the CNS, treatment of *Fmr1* KO mice with metformin

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#### AMPK:

AMP-activated protein kinase

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decreased ERK signaling (35). Conversely, in leukemia cells, metformin activated ERK (59). Because the effects of metformin on ERK activity can vary depending on the examined tissue and model, and because there are numerous feedback loops between mTORC1 and MAPK pathways, a better understanding of the effect of metformin on these pathways in different brain areas and developmental stages is imperative for future therapeutic use.

## **METFORMIN TREATMENTS IN NEUROLOGICAL DISORDERS**

The current understanding of metformin's mechanisms of action raises the intriguing possibility of repurposing this safe and effective drug for the treatment of neurodevelopmental, neurodegenerative, and psychiatric disorders. Metformin treatment in a mouse model of FXS alleviated impaired core phenotypes (see below) (35). Metformin was also reported to confer neuroprotection against apoptotic cell death in primary cortical neurons (60), to promote neurogenesis and augment spatial memory (61, 62), and to extend life span in mice (63).

In patients with chronic psychiatric illness, such as schizophrenia, metformin was reported to be safe, well-tolerated, and effective in both the prevention of antipsychotic-induced weight gain and improvement of metabolic impairments (64). Similar salubrious metabolic effects of metformin were observed in ASD children and adolescent patients treated with antipsychotics (65, 66). Another study showed that metformin failed to improve memory in overweight youth with ASD who were treated with atypical antipsychotic medication, but due to the small sample size and challenges the study encountered with regard to evaluation of cognitive functioning and irritable behavior in the tested ASD persons, additional studies are necessary to confirm the conclusions (67). Recently, metformin was reported to rebalance early neuronal network alterations and to reverse behavioral aberrations in a mouse model for Huntington's disease (68). Metformin was also shown to alleviate gastrointestinal tract symptoms (69). Gastrointestinal tract deficits were reported in numerous neurological disorders including ASD, amyotrophic lateral sclerosis, PD, AD, and transmissible spongiform encephalopathies (70).

### **Metformin and Alzheimer's Disease**

AD is a neurodegenerative disease associated with increased amyloid beta ( $A\beta$ ) depositions and hyperphosphorylation of tau (71). A link between T2D and AD was suggested because of the chronic insulin resistance in the brain of AD patients. Several basic and clinical investigations on the impact of metformin on the metabolism of  $\beta$ -amyloid precursor protein (APP) and cognitive functions generated conflicting results. A prospective cohort study reported that T2D is associated with a twofold higher risk of dementia and that metformin may be neuroprotective in T2D patients, thus decreasing the risk of AD (72). Conversely, increased risk of cognitive impairments in T2D patients treated with metformin was also reported (73).

In obese (*db/db*) mice, metformin attenuated AD-like biochemical abnormalities; it reduced total and phosphorylated tau levels but did not rescue spatial learning and memory deficits (74). In a study of CNS neurons in APP/PS1 mice (a double transgenic mouse model expressing a chimeric mouse/human amyloid precursor protein and a mutant human presenilin 1), metformin prevented spatial memory impairments, amyloid plaque deposition, and neuronal loss in the hippocampus by triggering neurogenesis and reducing inflammation through the suppression of mTOR activity (75). At variance with the latter studies, metformin increased the production of  $A\beta$  peptides via transcriptional upregulation of BACE1 ( $\beta$ -secretase 1;  $\beta$ -site APP-cleaving enzyme 1) activity in vitro and in vivo, suggesting that metformin might have negative effects in elderly diabetic patients (76).

## Metformin and Parkinson's Disease

PD is a neurodegenerative disorder characterized by the accumulation of intracellular aggregates of  $\alpha$ -synuclein ( $\alpha$ Syn) in Lewy bodies, the loss of nigrostriatal dopaminergic neurons, and motor symptoms such as tremor and bradykinesia (77). In a Taiwanese population cohort, metformin reduced the risk of PD co-occurring with T2D in patients on sulfonylurea therapy (78). In PD mouse models, metformin reversed some PD phenotypes through AMPK-dependent and -independent mechanisms (77, 79–81).

In a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mouse model, metformin attenuated neuronal loss, improved antioxidant activity, and improved locomotor and muscular activities (79). Similar results were observed in another study, where metformin ameliorated MPTP-induced degeneration of dopaminergic neurons, increased striatal dopamine content, and improved motor impairments in mice via enhancement of AMPK-mediated autophagy and inhibition of microglia-overactivation-induced neuroinflammation (77).

The dopamine precursor L-3,4-dihydroxyphenyl-L-alanine (L-DOPA) is commonly used to treat severe motor deficits in PD patients, but prolonged use of L-DOPA can induce dyskinesia (involuntary movements) (80). In a mouse model of PD, metformin inhibited the development of L-DOPA-induced dyskinesia and impaired glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) activity in an AMPK-independent manner, without affecting elevated mTOR or ERK signaling (80). This raises the possibility that metformin can serve as a therapeutic in suppressing L-DOPA-induced motor complications in PD. Similarly, metformin exhibited neuroprotective effects and prevented nigrostriatal dopamine degeneration in an *AMPK* KO PD mouse model (81).

## Metformin and Epilepsy

Epilepsy is a common neurological disorder characterized by unpredictable seizures. Metformin suppressed the progression of seizures, ameliorated learning and memory impairments, and decreased brain oxidative damage induced by pentylenetetrazol (PTZ) (82). Metformin treatment decreased seizure susceptibility, facilitated seizure termination, and reduced seizure number and length (83). Metformin also reduced the progression of pilocarpine-induced seizures and blocked seizure-induced overexpression of brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine receptor kinase B (TrkB), and it decreased mTOR activation through an AMPK-dependent mechanism (84). Epilepsy is manifested in 25% of males with FXS (85). On the basis of the beneficial effect of metformin in alleviating audiogenic seizures in a mouse model of FXS (35), and the above-mentioned findings, metformin emerges as a promising drug in FXS due to its anticonvulsant and antiepileptic properties.

## Metformin and Adult Neurogenesis

Metformin acts on two distinct molecular pathways to control neurogenesis: It enhances adult neural precursor cell (NPC) proliferation/self-renewal by elevating the p53 family member (tumor suppressor) and transcriptional factor TAp73, and it promotes adult NPC differentiation via activation of the atypical protein kinase C–CREB binding protein (aPKC–CBP) pathway (62).

In a hypoxia-ischemia injury model, metformin activated endogenous NPCs and promoted their migration and differentiation in the murine neonatal brain, restoring sensory-motor function after hypoxia-ischemia injury (86). Similarly, in a mouse model of transient middle cerebral artery occlusion, metformin, in an AMPK-dependent manner, promoted angiogenesis and neurogenesis, attenuated ischemia-induced brain injury, and improved functional recovery (87). These findings support testing metformin as an ischemic stroke therapy.

Recently, in human bone marrow–mesenchymal stem cells (hBM-MSCs), metformin was shown to promote neuronal differentiation and neurite outgrowth in an AMPK-dependent manner (88). Conversely, using a loss-of-function approach, it was reported that AMPK activity was not required for cortical neurogenesis, neuronal migration and polarization, and cell survival, but it was required for the regulation of axogenesis via mTOR pathway inhibition (89).

## Metformin and Fragile X Syndrome

Elevated insulin signaling was recently described in the brain of the *Drosophila* model of FXS (33), *dfmr1* mutants. These mutants showed impaired circadian behavior and learning and memory. Reducing insulin signaling by selective expression of *dfmr1* in insulin-producing cells or through genetic reduction of the insulin pathway rescued memory and circadian rhythm deficits in *dfmr1* mutants. Treatment with metformin (5 mM) for 4–6 days after eclosion restored short-term memory but failed to rescue circadian rhythmicity. Additionally, an acute treatment with metformin ameliorated olfactory learning and long-term memory deficits (33). Furthermore, a recent study showed that a 10-day treatment of *Fmr1* KO mice with metformin (200 mg/kg/day) corrected enhanced grooming and social behavior deficits, decreased audiogenic seizures, and partially reduced testicular weight, but it did not have an effect on hyperactivity (35). In addition, exaggerated long-term depression and impaired spine morphology were rescued, as well as excessive translation and increased ERK signaling but not mTOR signaling (**Table 1**) (35). A prolonged treatment with metformin was required, since one- and five-day treatments with the same dose failed to rescue the core FXS phenotypes. In a recent report, six adults (13–60 years old) and one child (4.5 years old) with FXS were treated with metformin for at least six months at doses of 500–2,000 mg/day for adults and 50 mg/day for the child (34). Most patients tested were obese and showed significant weight loss owing to treatment with metformin. Although there was high variability between patients, improvements in speech, irritability, social unresponsiveness, social avoidance, and hyperactivity were reported (**Table 1**) (34). These results demonstrate that metformin could be beneficial for behavior and cognition in FXS. These promising results formed the basis for a clinical trial using metformin for treatment of FXS (<https://clinicaltrials.gov/ct2/show/NCT03479476>). Additional clinical trials are in the planning stages.

### SUMMARY POINTS

1. To date, no therapy for FXS exists. Several drug candidates have been developed, but, despite promising preclinical results, no treatment has provided successful clinical outcomes, due to either major side effects or the lack of improvement of the disease.
2. Metformin treatment of FXS *Drosophila* and mouse models dramatically improved several core phenotypes at the levels of behavior, morphology, and synaptic plasticity and selectively normalized the ERK signaling pathway.
3. Metformin, an antidiabetic drug approved by the US Food and Drug Administration, acts through AMPK-dependent and AMPK-independent mechanisms, but additional research is necessary to clarify its precise mode of action.
4. A recent case study reported behavioral improvement in six FXS patients after several months of metformin treatment.
5. Metformin exhibits beneficial effects in AD, PD, and epilepsy, underscoring its potential to be repurposed to treat several devastating neurological disorders.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding or financial holdings that might be perceived as affecting the objectivity of this review.

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