

Fragile X Research: A Status Report

Editor's note: The National Fragile X Foundation's Fragile X Clinical & Research Consortium (FXCRC) is working to provide for shared clinical research trials among clinics across the U.S. (and one in Canada). Combining that development with promising news flowing in a regular stream from the research community, the editors thought an overview of the field was in order. We thus asked Elizabeth Berry-Kravis from the NFXF's Scientific and Clinical Advisory Committee to write the story that follows. Please note that much of the research she describes is being supported by organizations noted in the footnotes at the article's conclusion.

New ideas about treatment for individuals with fragile X syndrome (FXS) have recently come from scientific advances in two key areas:

- Understanding the functions of the Fragile X Mental Retardation Protein (FMRP), which is absent in those with FXS, and
- Abnormalities in brain mechanisms in the fragile X mouse and fly models.



Elizabeth Berry-Kravis

These new treatment ideas are directed at correcting mechanisms of underlying brain dysfunction caused by the lack of FMRP, which will allow us to treat fragile X syndrome itself. These are called “targeted” treatments because they are targeted to the FXS disorder as a whole, in contrast to supportive treatments that are already

available and consist of medications and therapies designed to treat a specific symptom (like hyperactivity, anxiety, or aggression) but that do not correct the underlying disorder.

Supportive treatments do not change the intellectual ability of those with FXS, although they may allow reduction of behavioral problems that interfere with their fullest potential function. We think that the newer targeted treatments may be able to improve cognitive functioning, but we do not know how early in life they would need to be started or how long they would have to be used to change intellectual potential.

THE ROLE OF mGluR

Ideas for targeted treatments have come mostly from scientific information about regulation of metabotropic glutamate receptor (mGluR) function by FMRP. Glutamate is the main chemical that mediates activation of one brain cell by another. It is everywhere in the brain. There are several kinds of glutamate receptors, some of which are involved in direct activation of the brain cells at a connection point (AMPA

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and NMDA receptors), and some of which regulate the responsiveness of the brain cells to activation (mGluR receptors) at that connection.

mGluR receptors exist in several groups that do different things, and the group important in FXS is 1 mGluR receptors—mGluR1 and mGluR5. When glutamate in the brain binds to mGluR1 or mGluR5 receptors, it activates protein synthesis at the brain cell connection that can cause the connection to

become stronger (with more AMPA receptors) or weaker (with less AMPA receptors), depending on the brain location of the connection. This strengthening and weakening process of brain connections is crucial for normal learning and memory to occur. FMRP regulates the protein synthesis activated by these mGluR receptors. In several studies performed in different laboratories, investigators have discovered that when FMRP is absent, as it is in FXS, the mGluR-mediated protein synthesis is unregulated and runs out of control, leading to excessive weakening of brain connections in the cortex and hippocampus (the thinking and memory centers of the brain).¹ New treatments proposed for FXS have thus been directed at:

- 1) reducing the excessive mGluR-mediated activation of protein synthesis,
- 2) reducing the activity of one or multiple proteins that are present in excess due to loss of regulation by FMRP,
- 3) directly strengthening the connection by replacing reduced AMPA activity, or,
- 4) correcting other neurotransmitter abnormalities in the brain.

In the rest of this article, these four different treatment categories will be reviewed individually. Information will be provided on how medications from these categories are thought to work in FXS, what scientific evidence exists to show that they do work for FXS, whether they are currently available, and if not, the stage and expected time frame of development.

CLINICAL TRIAL DEFINITIONS

Before discussing new treatments in the different categories, it is important to clarify some aspects of how new medications are developed through clinical trials. In this article a clinical trial refers to a testing process in which a group of people are given a medication on a trial basis with careful monitoring by researchers of the medication's effects (both positive and negative) in order to determine how well the medication works for the study group and whether there are side effects to be concerned about. This is one area where the NFXF's consortium plans to play a major role by organizing groups of clinics and the families who visit those clinics into large enough trials so that the results will be meaningful.

"Open label" trials are those in which study participants all take a new medication, possibly in addition to other medications they are taking. These trials are good for exploring whether a medication may be helpful, but they are subject to placebo effects (when people think they are better because they know they are getting a treatment that is supposed to help) because the participants and doctors know the participant is being treated.

"Placebo-controlled" trials are when some participants take the new medication and others take an identical-looking pill containing no medication ("placebo"). These trials are usually "double blind," meaning neither the researchers nor participants know who is taking the actual medication. After a period of treatment time, the effects of the treatment in the group on medication are compared with those on the placebo. This is the most accurate way to get information on the effects of a medication, and data from this type of trial is the requirement for getting a medication approved by the FDA and eligible to be prescribed by doctors for a specific problem.

Trials are generally conducted in three phases for the development of a new medication.

Phase I trials simply involve giving the medication to a few volunteers, making sure it is safe, and testing how long the medicine stays in the body.



Phase II trials involve small groups of people—usually 20-50. These trials may be open-label or placebo-controlled, and are designed predominantly to evaluate the safety of the medication and make sure there are no major side effects over a short-term treatment.

Phase III trials involve many people, may involve short or longer-term treatment, and are virtually always placebo-controlled, although there may be options in the study design to ensure that everyone gets a chance to be on the trial medication by the end of the study. (This is so all study participants can benefit if the medication proves to be effective.) Phase III trials are predominantly focused on evaluating efficacy of the trial medication, e.g. how well it works for the targeted symptom or symptoms in the targeted population. New medications are not approved for use until they have been through all three phases of trials in sequence. This is important to protect people from drugs that may have unexpected dangerous side effects or may not actually work at all for the symptom they are meant to treat.

TREATMENT CATEGORY 1 (*reducing the excessive mGluR-mediated activation of protein synthesis*) This is the largest category, encompassing seven different treatment approaches.

Lithium

Lithium is a medication that has been used for decades to treat mood disorders (bipolar, or manic-depressive disease). Lithium blocks the signaling pathway from the mGluR receptor to protein synthesis and thus should reduce the unregulated protein synthesis in brain connections lacking FMRP. Lithium also has many other effects on the brain that might not have anything to do with defects in FXS, so it is not specific just to FXS pathways.

Lithium has been shown to reverse memory problems in the fragile X fruit fly. It has also been shown to reduce audiogenic (sound-induced) seizures and hyperactivity in the fragile X mouse. An open-label two-month pilot trial of

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lithium in 15 children and young adults with FXS1 showed it was helpful for problem behaviors, improved one measure of verbal memory, and improved a blood marker that relates to the protein synthesis dysfunction and that is abnormal in FXS. Many individuals in this study retained improvements even over a year of treatment. Only a few experienced side effects, and those were mostly increased drinking and urination, which are known side effects of lithium. Thus there is evidence that lithium can be helpful in FXS, although a placebo-controlled trial is needed to best understand its effects.

Lithium can be prescribed by any doctor off-label (meaning it was not specifically approved to treat FXS). It has been approved for use in children and adults, six years old and up. Although lithium is sometimes used clinically in younger children with mood disorders, there is very little information on its effects in children under age six. If it is prescribed for individuals with FXS, it is very important to monitor side effects, blood levels, kidney function (involving a blood test), and thyroid hormone (also a blood test).

mGluR5 Negative Modulators

These medications are also referred to as “mGluR5 blockers,” and they include medications such as fenobam and MPEP. MPEP is an old drug that for various reasons can be used only in animals. Nonetheless, it works in the brain the same as many of the newer mGluR5 blockers and has been very helpful in understanding the effects of mGluR5 blockers in animal models of FXS. These drugs decrease activity of the mGluR5 receptors, thus decreasing the unregulated protein synthesis in brain connections without FMRP.

MPEP has been shown to correct memory problems and structural brain abnormalities in the fragile X fly. It also corrects numerous abnormalities in the fragile X mouse, as analyzed in many different labs. The abnormalities include audiogenic seizures, hyperactivity, immature connections, protein synthesis abnormalities, visual plasticity defects, and multiple other behavioral defects.

Other mGluR5 blockers, including fenobam, have been shown to have the same effects as MPEP. MPEP also corrects the loss of surface AMPA receptors in neural cells without FMRP growing in culture. Further, if the fragile X mouse is bred with a genetically altered mouse that has one-half the mGluR5 receptors, the offspring show correction of all the fragile X-related brain abnormalities. All together, this is powerful evidence that the fragile X-related problems in the

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mouse can be corrected by reducing mGluR5 activity. mGluR5 blockers are likely to work on these defects in a more specific way than lithium.

No mGluR5 blockers are currently available for prescription—they are in various stages of development. One of them is fenobam, a medication that was tried in phase II trials for anxiety in

the 1970s but dropped because of concerns about erratic metabolism, borderline effectiveness, and various side effects. In the past few years, it was discovered that fenobam is an mGluR5 blocker (researchers did not know how it worked in the 1970s), and since it has already been used by people in studies, it may be faster to develop for FXS than other drugs that have never been tried in humans. Fenobam has been granted orphan drug status for FXS from the FDA (meaning FXS is a rare disease so the drug can be developed without having to clear quite as many hurdles).

The first phase II trial of fenobam in males and females with FXS age 18 and over has recently been completed.² It was a one-dose safety trial during which blood levels in the body and positive and negative effects of the dose were monitored. The results of that trial are being analyzed and will need to be reviewed by the FDA before further trials can take place.

STX107 is another drug being developed for FXS.³ It is in preclinical development but may be ready for trials in the next year or two. STX107 is also expected to receive orphan drug status when it is ready for clinical trials.

Other mGluR5 blockers are also being developed for a variety of conditions, and these drugs may also show potential for use in FXS.⁴ mGluR5 blockers will be available only through clinical trials for the next several years, and it is not yet known how soon individuals with FXS under 18 years old will be allowed in these trials.

GABA Agonists

GABA agonists are drugs that increase activity at brain GABA receptors. Since GABA systems often inhibit glutamate systems in the brain, researchers think these drugs would reduce excessive mGluR receptor activity by decreasing the amount of glutamate to activate the receptor. There is also some evidence

in the fragile X mouse that GABA receptors are deficient.

GABA agonists have been shown to reverse some of the problems in the fragile X fly, and work is ongoing to determine how well they work in the fragile X mouse. GABA agonists include baclofen, which is currently available as a prescription drug for spasticity (stiffness) in diseases like cerebral palsy and multiple sclerosis. Baclofen has been shown in one very old open-label study to help with aggressive and self-injurious behavior in patients with cognitive impairment and severe behavioral disturbances. This drug has resulted in anecdotal behavioral improvements in some patients with FXS, and improved forms of the drug may be available for clinical trials sometime in the next year.

Ganaxolone is a different kind of GABA agonist, and it is in phase II/III clinical trials for conditions involving seizures. Trials with this drug in FXS have been proposed.

PAK Inhibitors

PAK, or p21-activated kinase, is an enzyme that activates the pathway between mGluR activation and protein synthesis. Inhibition of PAK (similar to lithium) would be expected to decrease the unregulated protein synthesis due to loss of FMRP.

In one study, breeding a PAK-mutant mouse with the fragile X mouse resulted in correction of the abnormal brain connection and behaviors in the fragile X mouse.¹ PAK inhibitors that are specific for the form of PAK in the FXS pathway are in development because they may also treat a condition called neurofibromatosis. These drugs are still very early in preclinical studies and have not started clinical trials yet.

There are compounds that indirectly inhibit various forms of PAK, which are used as chemotherapy for treatment of cancer. But they are very toxic and would not be used to treat FXS.

GSK3 β Inhibitors

GSK3 β , or glycogen-stimulated kinase, is an enzyme that activates the pathway between mGluR activation and protein synthesis. Inhibition of GSK3 β (similar to lithium) would be expected to decrease the unregulated protein synthesis due to loss of FMRP. In fact, lithium likely achieves part of its effect by inhibiting GSK3 β activity. Drugs that act on GSK3 β exist—one of them has been used in the fragile X mouse and is very effective at reducing audiogenic seizures. These drugs are still very early in biochemical and preclinical studies and are not ready for clinical trials.

mGluR2/3 Agonists

These activators of mGluR2 and 3 receptors result in decreased

glutamate release into the neural cell connection. Theoretically, they would result in decreased glutamate activation of the mGluR1 and 5 receptors, thus decreasing the overactive signaling of protein synthesis. It has thus been theorized that these molecules would be helpful in FXS, but no information on how they work in the fragile X mouse is available. An mGluR2/3 agonist called LY2140023 is currently in phase II clinical trials for schizophrenia and has shown some effectiveness.⁵ No fragile X trials are currently planned.

Acetylcholine Muscarinic M1 Blockers

Last year it was shown that acetylcholine M1 receptors in the brain activate protein synthesis in a similar way to mGluR1/5 receptors, and this protein synthesis is similarly deregulated in the fragile X mouse due to lack of FMRP. These drugs would theoretically block acetylcholine M1 receptors and thus reduce the excessive protein synthesis in neural cells expressing M1 receptors. Drugs that block these receptors are being studied for effects in the fragile X mouse. These drugs include currently available prescription and non-prescription drugs such as scopolamine (weak drug, used for motion sickness, over-the-counter) and atropine (used to increase the heart rate in people who are being resuscitated and would not be a good candidate for a trial). Clinical trials have not been planned for FXS pending more results in the mouse and because it is not clear what would be the best drugs for trials.

TREATMENTS IN CATEGORY 2 (*reducing the activity of one or multiple proteins that are present in excess due to loss of regulation by FMRP*)

Minocycline

Recently it was discovered that minocycline may work on a protein regulated by FMRP. Minocycline can be prescribed by any doctor, and is often used for acne treatment. It is currently in clinical trials to see if it helps autism. Minocycline can have some negative side effects such as permanent discoloration of the teeth in young children and some less frequent but more serious problems such as drug-induced lupus and pseudotumor cerebri (“false brain tumor” characterized by headache, nausea and other symptoms that mimic actual tumors). The drug should always be taken with careful monitoring by a physician. An open-label clinical trial of minocycline is currently ongoing.⁶ If this trial shows success, a placebo-controlled trial will be needed to fully understand the effects of this drug in FXS and to know if the benefits exceed the risks.

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TREATMENTS IN CATEGORY 3 (*directly strengthening the connection by replacing reduced AMPA activity*)

Ampakines

Ampakines activate AMPA receptors, which show lower activity in the weak connections in FXS. These drugs can also help bring AMPA receptors back to the surface of the connections where they are deficient because of the mGluR overactivity.

Ampakines activate BDNF (brain derived neurotrophic factor) which has been shown to improve connectivity in the fragile X mouse. A single ampakine, CX516, was studied in a phase II placebo-controlled trial in humans with FXS.⁷ This drug did not help the treated group as compared to the placebo group, but there were no substantial side effects. It appeared that in just the individuals co-treated with an antipsychotic, the CX516-treated group showed more improvement than with the placebo.

Since antipsychotics increase the activity of ampakines, and CX516 was subsequently shown to be a very weak ampakine, this suggests that ampakines may well be helpful in FXS, but stronger forms are needed. There is a stronger ampakine, CX717, in phase II clinical trials, but CX717 does not have much effect on BDNF, and it is felt that other stronger ampakines that robustly activate BDNF would be best for FXS. These are being studied in the fragile X mouse but are still in preclinical studies.

TREATMENTS IN CATEGORY 4 (*correcting other neurotransmitter abnormalities in the brain*)

Cholinergic Agonists

There are suggestions that acetylcholine activity is underactive in FXS. Parts of the brain that use acetylcholine do not activate as well in girls with FXS relative to controls, and acetylcholine activators were found to reverse effects in the fragile X fly in a drug screen. These drugs have not been used in the fragile X mouse. They are in clinical use to treat Alzheimer's disease, and they include agents such as Aricept (donepezil). An open-label trial of donepezil in FXS is now in progress.


Oxytocin

Oxytocin is a hormone involved in aspects of social behavior, which is problematic in FXS. It has not been used in the fragile X mouse or fly. There is a single-dose placebo-controlled study of oxytocin in FXS with a two-week extension currently in progress.

CONCLUSION

Clearly, there are many exciting possibilities for future medication treatment to target the underlying mechanism of FXS. Just as clearly, there is much work to be done to figure out what works and what does not, and what parts of FXS are best treated by which agents. More ideas for treatments will continue to emerge as we come to understand more about the neurobiology of FXS. It may be that certain combinations will work best due to the ability of medication combinations to target multiple places in the pathways that are rendered dysfunctional due to lack of FMRP. Some of those dysfunctional pathways are likely to overlap others that are active in autism, raising the possibility that new treatments for FXS will also be effective for autism.

Clinical trials will be needed to sort all this out and find the best treatments in the long run. Without good placebo-controlled trials, we will never know what really works, what the best doses are, at what ages each treatment works, and what works best in combination. Continued development of the NFXF's Fragile X Clinic and Research Consortium will eventually allow for clinical trials of the magnitude required to answer these questions.

Recent research findings have led to tremendous hope for treatment of the underlying disorder in FXS. It is an exciting time for FXS research, but careful, systematic and well-designed clinical trials will be crucial in upcoming years if we are to gain approval for new therapeutic agents, and to make such agents available to those with FXS at the youngest age possible. 

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Footnotes

- 1,7 Research/clinical trial funding from the FRAXA Research Foundation.
- 2 Clinical trial sponsored by Neuropharm LTD, a company based in the UK currently developing fenobam, in collaboration with FRAXA.
- 3 STX107 is being developed by Seaside Therapeutics of Cambridge, Massachusetts.
- 4 Companies such as Novartis Pharmaceuticals, Addex and Roche are developing these medications for conditions other than FXS.
- 5 LY2140023 is in development at Eli Lilly and Company of Indianapolis, Indiana.
- 6 Research/clinical trial funding from the FRAXA Research Foundation.