Review

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Fragile X syndrome as a rare disease in China – Therapeutic challenges and opportunities

Xiaowei Jin*, Li Chen

Hua Medicine, Shanghai, China

Summary **Recognized as the most common inherited from of intellectual disability (ID) and the most common known monogenic cause of autism spectrum disorders (ASD), Fragile X syndrome (FXS) is identified as an unmet medical need for the development of personalized medicine and targeted therapeutics for neurodevelopment disorders as a result of improved understanding of the genetic and cellular mechanisms. Consequently promising pharmacological targets have emerged from basic and translational research, are now being pursued by global pharmaceutical and biotech companies in early proof-of-concept clinical trials. With the world's largest rare disease population, China potentially has a large number of FXS patients, many of whom are under-diagnosed or even misdiagnosed, barely with any treatment. In spite of improved awareness of FXS in recent years, big gaps still exist between China and developed countries in multiple aspects. With increased public awareness, strong government support and investment, coupled with an increasingly large number of Western-trained experienced researchers engaging in new drug discovery and development, China has the potential to become an important player in the discovery of effective diagnostics and treatments for a rare disease like FXS.**

Keywords: Fragile X syndrome, *FMR1*, Drug development, mGluR5, Translational science

1. Introduction

Neurodevelopment disorders such as autism or ASDs are life-long conditions which historically have been viewed as intractable to pharmacological interventions. Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability and autism that affects all major ethnic groups and races (*1*). FXS and the related autism spectrum disorders (ASDs) now represent an urgent unmet need for effective treatment due to the rapidly growing patient population and the consequent huge burden on affected individuals, their families and care givers, and society as a whole (*2*).

Following the discovery of the genetic mutation of *FMR1* gene for FXS in 1991 (*3*), major advances have been made in the understanding of the underlying neurobiology and pathophysiology of FXS. The generation and analysis of FXS animal models, in particular the development of *FMR1* KO mice, have paved the way for testing targeted therapeutic agents based on the underlying mechanisms, many of which have in recent years advanced into further assessment in human patients (*4*). Clinical investigation of these novel therapies has subsequently enhanced our understanding of the challenges involved in the development of therapeutic treatment for this monogenic yet complex disorder (*5*). Despite tremendous progress made in bench-to-bedside translation, discovering novel treatments for FXS remains a daunting task.

Compared with the more established infrastructure for translational research in the US and many Western countries, there is a relatively weak basis for both basic science and clinical research in China, which is mainly reflected in the following areas: scattered distribution of resources, lack of expertise and severe shortage of resource for clinical investigations. Furthermore, there is rather limited awareness of FXS among the general population, medical professionals as well as healthcare policy makers. Although the first reported case of autism in mainland China was made over 30 years ago

**Address correspondence to:*
Dr. Xiaowei Jin, Hua Medicine, 275 Ai Di Sheng Road, Zhangjiang Hi-Tech Park, Pudong, Shanghai, China.
E-mail: xwjin@huamedicine.com

by the pediatric psychiatrist Dr. Tao Kuo-Tai in 1982 (*6*), autism is a relatively new area for researchers in mainland China. At present, ASDs including FXS have not been included in medical school training. As indicated in a recent survey by Duan *et al*., no more than 30% of the medical students have ever heard of FXS (*7*). Furthermore, there are very few qualified hospitals, healthcare centers and research institutes in China where FXS genetic testing can be reliably conducted. Consequently, there have been no clinical studies ever conducted on human FXS or ASDs patients in mainland China. Thus there is a need to establish a network of scientists and clinicians for sharing resources and forging collaborative relationships to support the direction and outgrowth of translational research with emphasis on human patients.

There is also an urgent need for establishment of standardized clinical practice. Currently in mainland China the branch of developmental psychiatry has not been established as an independent department. So far there has been no focused research team on autism or ASDs, therefore there is no unified department for the clinical diagnosis of autism and ASDs, including FXS, unlike the Western clinical settings, where there is generally a multidisciplinary team for the diagnosis and subsequent management of ASDs. In China, diagnosis of ASDs heavily depends on the judgment of the clinicians to whom the patient was referred. In addition, there are only a limited number of psychiatrists or pediatricians who are specialized in ASDs. In recent years, there has been increased awareness about autism and ASDs as a result of media publicity and the dissemination of related information *via* the internet. Usually when parents become concerned that their child may have autism, they normally try first to locate information online prior to seeking diagnosis. In sharp contrast to autism or ASDs, currently there is rather limited awareness of FXS in China among the general public as well as medical professionals, largely due to the shortage of an effective diagnosis of FXS in the clinic.

2. Epidemiology, diagnosis and current management of FXS

Fragile X, or Martin-Bell, syndrome was reported in 1943 (*8*) in the first pedigree with mental retardation linked to the X-chromosome. FXS is the most common single-gene cause of autism and inherited cause of intellectual disabilities especially among boys. It is a genetic disorder that manifests itself through a complex spectrum of intellectual disabilities ranging from mild to severe, as well as physical characteristics such as an elongated face, large or protruding ears, and large testes (macrooorchidism), and behavioral characteristics, such as stereotypic movement (*e.g*. hand-flapping), and social anxiety (*5*).

2.1. *Prevalence of FXS*

FXS is a genetic disorder that occurs as a result of a mutation of the *fragile X mental retardation 1* (*FMR1*) gene on the X chromosome, most commonly an increase in the number of CGG trinucleotide repeats in the 5'untranslationed region of *FMR1*. Although this accounts for over 98% of cases, FXS also occurs as a result of point mutations affecting *FMR1*. The general prevalence of males with a full mutation is estimated as ~1 in 4,000. The female prevalence is presumed to be approximately one-half of the male rate, or ~ 1 in 5,000-8,000. All major ethnic groups and races appear to be susceptible to expansion of the *FMR1* CGG region (*2*).

In China, the first documented report on FXS was published in 1986 by Zhou *et al*. (*9*), whereas 6 FXS families were identified with a total of 15 male FXS patients and 13 female carriers. There has been very limited number of FXS-related studies published to date by Chinese researchers. A literature search has indicated a steady increase in the number of published studies in autism or ASDs since the first case report in mainland China in 1982, accumulating in the thousands as listed in the CNKI database. Of particular note, there is a huge surge in the amount of research work in autism and ASDs since 2005 after the Chinese government issued the Five-year 11-5 (2006-2010) Outline of Development Plans for the Chinese Disabled Persons' Federation, wherein autism was for the first time specifically listed as an important area for government funding and support (*10*). However a serious gap still exists between China and the Western countries in this field, especially in translational science. So far there have been no clinical studies conducted in autism or ASDs. This is in stark contrast to Western countries, where there are over 500 total registered clinical studies focused on ASDs and more than 50 clinical studies specifically on FXS, according to the data provided by ClinicalTrials.gov (*11*).

China has the world's largest patient population of ASD, and presumably FXS as well. Due to the lack of full awareness and limited access to diagnostic tools and standards, epidemiology data on FXS remains scarce, and thus well-organized epidemiological studies are yet to be conducted to get an accurate account (*12*).

2.2. *Diagnosis of FXS*

Early diagnosis of FXS or carrier status is important for providing early therapeutic intervention including speech therapy, occupational therapy, psychotherapy and special education that can considerably improve the quality of the patient's life. It also allows for genetic counseling in regard to the potential implications for the parents and their extended families.

As a monogenetic disease, FXS is mainly caused by a CGG-repeat expansion that triggers hypermethylation

of this region and silences the *FMR1* gene which leads to FMR protein (FMRP) production deficiency. In general, the severity of the FXS physical phenotype and intellectual impairment is correlated with the magnitude of the FMRP deficit. The diagnosis of FXS is now performed through the detection of genetic mutations in the *FMR1* gene. Available tests used for diagnosis include both chromosome DNA analysis and various protein tests, although protein-based analysis has not been recommended at this time due to its limitations such as sensitivity, technical challenge and suitability for prenatal testing. At present, the DNA-based test has been predominantly utilized and can be administered with two different lab procedures, *i.e.*, the polymerase chain reaction (PCR) method and Southern blot analysis. The Southern blot analysis test determines if the gene has a full mutation, its approximate size as well as methylation status, while the PCR analysis can determine the actual number of "CGG repeats". Historically PCR has been not the test of choice to diagnose a full mutation, in particular when the CGG repeat size is over 250, due to limitations in terms of accuracy, although PCR is quite accurate in determining premutation and normal gene repeat numbers. In case of a full mutation, laboratories have to use more than one method because no single method can characterize all aspects of the *FMR1* full mutation. On the other hand, PCR is less expensive and quicker than Southern blot analysis. A major effort has been made to advance the PCR-based technology, resulting in improved ability to identify full mutations with a large CGG repeat size. Currently, as the best practice to determine full mutation, laboratories have to use both PCR and Southern blot analysis. Consequently, in their most updated policy statement, the American College of Medical Genetics and Genomics (ACMG) recommends that Southern blot analysis always be performed along with traditional PCR (*13*).

It is worth noting that since the current DNA analyses only tests for expansion of the CGG repeat, individuals with FXS due to missense mutations or deletions involving *FMR1* have to undergo sequencing of the *FMR1* gene in order to be properly diagnosed. Recent technical advances in prenatal testing have enabled reliable diagnosis of *FMR1* mutation while the fetus is in utero.

From a regulatory perspective, even in the US or Europe, there is currently no genetic test for FXS which has been officially approved by the regulatory agencies. Thus, all FXS genetic testing is now offered as a laboratory-developed test for clinical research as well as for diagnosis (*13*).

Prenatal testing for FXS is also available in Western countries using chorionic villi and/or culture, amniotic fluid cells, and/or culture or blood samples, although the use of a methylation-sensitive method is not suitable for early prenatal diagnosis because the methylation of a full mutation is not always present in DNA from chorionic villi, whereas it is established after the 14th week of pregnancy. In addition, in contrast to lymphocytes and amniocytes, the *FMR1* gene is not methylated on the inactive X chromosome in the chorionic villi of female fetuses (*13*).

Due to technical difficulties associated with accurate assessment of the large CGG expansion, only a handful of genetic testing laboratories in China, mostly academic institutes, are capable of performing comprehensive FXS testing, including prenatal diagnosis. Recently some commercial FXS testing trial packs or kits such as those from Asuragen, Abbott and Perkin Elmer, have been introduced in China (*7*). All these commercial kits adopt PCR-based methodologies, however, due to a lack of appropriate instruments, and more importantly, severe shortage of well-trained technicians in addition to the associated large cost which is not covered by health insurance, application of these kits is limited to a few research institutions. On top of all these, there is also an uneven distribution of knowledge of FXS across different regions in China. All these factors have hindered the accurate and timely diagnosis of FXS in China.

2.3. *Management of FXS*

There are currently no FDA approved drugs addressing the core symptoms of FXS, leaving patients and their caregivers with limited treatment options. Nevertheless several drugs that alleviate various aspects of behavioral symptoms are available. Current trends in managing and treating FXS include early intervention with both speech and language therapy, and occupational therapy with sensory integration techniques are also given to children with FXS as early as possible. Medical treatment of FXS is largely symptom-based. For example, it is a common practice to treat FXS patients with stimulants and selective serotonin reuptake inhibitors (SSRIs) for anxiety and obsessive-compulsive behaviors; and an atypical antipsychotic agent for self-injury, aggressive behaviors and autism. It is recommended to combine the pharmacotherapies with speech and sensory integration occupational therapy, together with individualized educational plans, and behavioral interventions in order to achieve the most optimal outcome (*14*).

New targeted medicine for FXS including mGluR5 antagonists, GABA A and B agonists, minocycline, and lithium *etc*., are now undergoing human clinical studies. Early reports are promising for some of these novel pharmacotherapies, which have disease modifying potential. It is noteworthy to mention that many of these targeted investigational drugs are often mixed with maintenance drug treatments outlined above for an optimal efficacy for FXS patients (*15*).

In China, due to its limited awareness, there have

been no guidelines or policies regarding the diagnosis and management of FXS. Currently Chinese doctors generally prescribe medications according to the clinical presentation of the patients, and the diagnosis of FXS normally has no impact on this pragmatic approach (*7*).

3. Therapeutic development for FXS

3.1. *Scientific rationale for development of personalized pharmacotherapies for FXS*

Our knowledge of FXS and related ASDs has advanced substantially over the past two decades. FXS is caused by expansion of an unstable CGG repeat region in the 5' untranslated region of the *FMR1* gene. The full mutation (>200 CGG repeats) is often accompanied by extensive methylation of the *FMR1* promoter, leading to transcriptional silencing, resulting in reduced expression or entire absence of FMRP protein which plays essential roles in neural development. Lack of FMPR expression appears to be at the core of the intellectual disability and other features characteristic of FXS. FMRP is a repressor of mRNA translation that is particularly important for the regulation of activity-dependent protein synthesis in neurons. The absence of FMRP leads to significant alteration in cognitive functions, dendritic spine morphology and intracellular signaling (*15*).

Much of our understanding of the neurobiology of FXS has been gained from studies of the *FMR1* knockout (KO) mouse, which shares many anatomic and behavioral phenotypes with human FXS (*16*). These KO mice recapitulate many symptoms observed in human patients, with defects in neuronal development, dendritic spine morphology, synaptic plasticity, pain processing, and behavior (*16*). By definition, the *FMR1* KO mouse model does not have perfect construct validity because genetically it does not exactly mimic the initial pathological lesion underlying human FXS, which is due to the CGG trinucleotide expansion. As such they are not a perfect model to test all therapeutics targeted in human FXS. For instance, some potential therapeutic targets cannot be tested using these models, including those related to DNA methylation. Nevertheless this KO mouse recapitulates the human protein abnormality, *i.e.*, loss of FMRP protein expression, thereby giving it high face validity. Indeed this animal model has tremendously advanced our understanding of the underlying mechanisms of FXS and provided valuable insights for testing novel therapeutic approaches in human patients with FXS and potentially ASDs.

Based on accumulating evidence from early preliminary clinical studies, and/or studies in FXS animal models, many promising therapeutic targets have been identified or proposed. These targets can be further grouped into the following three major groups: (*i*) transmembrane receptors such as metabotropic glutamate mGlu1/5, dopamine D1/5, and GABA receptors; (*ii*) intracellular central signal transduction molecules including ERK1/2 or PI3K; and (*iii*) further downstream signaling molecules or proteins such as MMP-9, mTOR, GSK3β, or PAK (*15,16*). The ultimate validation of these novel therapeutic strategies will strongly depend on the availability of target-specific drugs that are safe for use and effective for long-term in patients. Currently several of these potential targets have been validated in early proof-of-concept clinical studies in FXS patients, although further studies are needed to investigate which of these therapeutic strategies might be the most beneficial for each distinct sub-population of FXS patients with a different clinical phenotype, though reliable biomarkers will be needed for this personalized or stratified medicine approach.

Based on numerous elegant studies using the *FMR1* KO mouse, mGluR5 was identified as one of the key players upstream of the FMRP-mediated pathways and activation of mGluR5 leads to protein translation. A landmark study by Huber *et al.* (*17*) reported that mGluR1/5-medited long-term depression (LTD) is elevated in *FMR1* KO mice. This work laid the foundation for the mGluR theory of FXS, which hypothesized that dysregulated mGluR1/5-mediated protein synthesis resulted in abnormal plasticity thus contributing to the pathology of FXS. Further genetic validation of the mGluR theory was provided by Dolen *et al.* (*18*) by genetic rescue of FXS phenotypes in *FMR1* KO mice that were crossed into an mGluR5 heterozygous background. It is now widely accepted that the loss of FMRP allows for excessive mGluR5 signaling, which in turn results in excessive protein translation and synthesis. Continuous treatment of FMRP mutant mice with mGluR5 inhibitors commencing early in life eliminate seizure and other phenotype abnormalities (*19,20*). These findings have provided a foundation and led to development of potent and selective mGluR5 inhibitors, including AFQ056, RG7090 and STX209, which have advanced into human clinical studies (*21-23*).

Although there is compelling evidence for the involvement of the mGluR pathways in the development of FXS, it is unknown to what extent dysregulation of these pathways contributes to the overall disease severity in FXS patients. FMRP is widely expressed throughout the brain and has recently been proposed to regulate approximately half of the known genes associated with ASDs (*24,25*). In light of this, it is not surprising that some observed phenotypes in the *FMR1* KO mouse model cannot be explained simply by dysregulation of mGluRs. One implication is that in order to test the clinical effectiveness of mGluR5 antagonists in human FXS patients, we need to choose mGluR5-specific abnormal phenotypes as

outcome measures. Besides serving as an excellent translation model for subsequent testing of a mGluR5 based therapeutic approach in human FXS patients, the *FMR1* KO mouse may in fact represent an ideal model for teasing out those abnormal phenotypes mostly likely mediated by excessive activity of mGluR5, and identifying potential biomarkers that can help us in patient stratification and subsequent selection of the right subgroup of FXS patients who are most likely responsive to mGluR5 antagonist treatment. Furthermore, the *FMR1* KO mouse model may also provide insight into the selection of specific outcome measures that are most relevant and robust for human clinical studies.

3.2. *Challenges in discovery of novel treatment for FXS: a drug development perspective*

CNS drug discovery has long been regarded as one of the most challenging areas in the pharmaceutical industry with disproportionally lower chances of success compared with other therapeutic areas. As a result, several major global pharmaceutical companies like GSK and AstraZeneca have in recent years decided to dramatically reduce their R&D investment in the CNS area, in particular, in the area of neurological diseases including mental disorders such as ASDs and FXS.

Researchers embarked on the search for novel targeted FXS therapies based on a solid understanding of the underlying mechanism as well as a well-characterized highly disease-relevant mouse model. Over the past two decades, tremendous progress has been made which has provided valuable insights for more future translational studies. Some major lessons the field has learned thus far over the past 20 years:

Lesson 1: Preclinical challenge – target selection is a critical first step toward a safe and effective treatment for neurodevelopmental disorders including FXS

In general, one of the crucial considerations in the target selection for a drug discovery program is whether such targets are tractable for drug development. A druggable target often refers to a protein which can be modulated with drug-like molecules, which often times for CNS indications, are small molecule drugs that can easily cross the blood-brain barrier. In this respect, transmembrane receptors or enzymes have historically been one of the most popular classes of investigational drug targets, especially for CNS disorders. An in-depth understanding of the underlying disease biology provides the foundation for the selection of a druggable target, which consists of identifying molecular processes that can be enhanced or inhibited in order to restore homeostasis disrupted under the disease condition. In many cases such as FXS and ASDs, instead of working directly on the genetic defect of the *FMR1* gene or the FMRP protein deficit, drugs work by acting on proximal or distal processes through compensation for the functional defect caused by the genetic mutation. Therefore, based on their key roles in the underlying pathophysiology elucidated preclinically, mGlu5 and GABA-A receptor, are ideally suited as drug targets for pharmacological treatment of FXS.

Even after the selection of a good druggable target, the ultimate creation of a drug for a CNS disorder normally requires a highly rigorous medicinal chemistry effort. A unique challenge for indications like FXS and ASDs, the prospect of life-long treatment and likely optimal starting at a young age, also puts stringent requirements on the drug safety with no side-effects or long-term toxicity. These important factors would exclude potential drug targets such as GSK3β and mTOR as these proteins have pleiotropic functions and are essential for body growth, even though there appears to be solid scientific basis for involvement of these proteins in the disease pathology of FXS (*15,16*). Furthermore, the possibility for transgenera-tional toxicity should also be evaluated in appropriate species. Another important factor to consider is that many FXS patients are already receiving other pharmacological treatments for symptomatic management, thus a novel treatment should be compatible with an ongoing treatment regimen with minimal drug-drug interaction. Overall, pediatric drug development poses additional challenges in terms of safety and tolerability. Drug metabolism is another important yet complicated factor since drug metabolism is more variable and less well characterized for pediatric patients. As such, the translation from *in vitro* testing to *in vivo* pharmacodynamic and pharmacokinetic predictions is much less established in young children than in adults.

Lesson 2: Preclinical challenge – FXS animal models need to be used wisely in order to realize its full translational power

One of the essential steps in the drug discovery and translational research process is the evaluation of pharmacological effects of prospective therapeutics using appropriate and/or validated animal models that can be reflected on the endpoint of its clinical treatment. For CNS disorders like FXS and ASDs, this step often relies on a broad repertoire of behavior tests related to the core and co-morbid symptoms. Relevant information gathered in animal studies should be translated into clinical relevance and vice versa, this 2-way communication between clinical and preclinical discovery scientists during the drug development process are likely to help in the development of more relevant, predictive animal models as well as biomarkers providing conceptual basis for development of effective treatment. The identification of the disease-causing mutation in the *FMR1* gene more than 20 year ago enabled the generation of genetically engineered mice carrying a similar defect, *i.e.*, loss of FMRP protein. As discussed above, this FXS mouse model

in many aspects recapitulates disease symptoms and pathophysiology present in human FXS patients, including behavioral, cognitive, neurochemical and physiological abnormalities.

Drug discovery and development belongs to translational science, therefore should be conducted as patient-oriented research. Convincing evidence has shown that FXS patients demonstrate abnormalities in sensory processing and communication. Clinical, behavioral, and electrophysiological studies consistently show auditory hypersensitivity in humans with FXS. One important characteristic of FXS is the co-occurrence of seizures in 10-40% of individuals with full mutations, which normally begin at a very young age, mostly between ages 4 and 10 years. Although seizures in FXS patients are infrequent and easily managed, there appears to be a strong correlation between seizure and overall disease severity, in particular comorbidity including autism and anxiety that impacts FXS patients' function and quality of life (*26,27*).

Interestingly, the most promising behavioral assays established thus far using FXS mouse models are those based on neuronal hyperexcitability, which have strong correlates in FXS patients, such as epilepsy and hyperactivity. In fact, one of the most robust assays tests the susceptibility to audiogenic seizures (AGS). Higher susceptibility to AGS has been reproducibly observed in *FMR1* KO mice of various genetic backgrounds and has become very useful in evaluating potential therapeutic strategies (*28,29*). Recently, a kindling paradigm also demonstrated higher seizure susceptibility in *FMR1* KO mice compared to wild-type controls, and thus potentially can serve as an additional tool to assess hyperexcitability using FXS mouse models (*30*). Numerous preclinical studies have suggested that auditory hyper-excitability can serve as a robust and reliable endpoint, and potentially as a translatable biomarker from *FMR1* KO mice to FXS patients.

Hence auditory hypersensitivity indeed provides a unique opportunity to integrate molecular, cellular, circuit level studies with behavioral outcomes in the search for therapeutics for FXS and ASDs. Theoretically this type of auditory deficits observed in FXS patients as well as FXS animal models should serve as relatively more tractable and more objective outcome measures than more complex and subjective social behaviors that are typically studied in FXS and ASD patients currently.

It is noteworthy that mGluR5 appears to play an essential role in mediating the hyperexcitability seen in FXS patients, as demonstrated in the *FMR1* KO mouse model (*18,19,29*). It is conceivable that this particular FXS model may represent a sub-population of FXS patients who exhibited significant dysregulation of the mGluR5-mediated pathways leading to the manifestation of FXS symptoms including susceptibility to seizure and epilepsy. Besides the convincing support from the seminal work by Dolen (*18*) showing that genetic knockdown of mGluR5 resulted in almost complete rescue of the AGS susceptible phenotype in *FMR1* KO mice (*18*), several mGluR5 negative allosteric modulators (NAMs), including MPEP, CTEP, RG7090 (Roche's clinical-stage drug that completed Phase II trial and achieved proof of concept status in treatment resistant depression patients) (*19,29,31*) have all been shown to be effective in ameliorating many defects including the susceptibility to AGS in FXS mice. Using the same study protocol, we tested HME01, one of the lead mGluR5 NAMs developed at Hua Medicine (*32*). Our data further confirmed that AGS is a robust objective endpoint for testing the efficacy of mGluR5 NAMs in FXS (Figure 1). One challenge of this AGS testing is that it has to be conducted on mice as young as 21-days old. This in fact reflects a reality with respect to the therapeutic window of this approach. In order to demonstrate the clinical effectiveness of an mGluR5 NAM with a more objective outcome measure

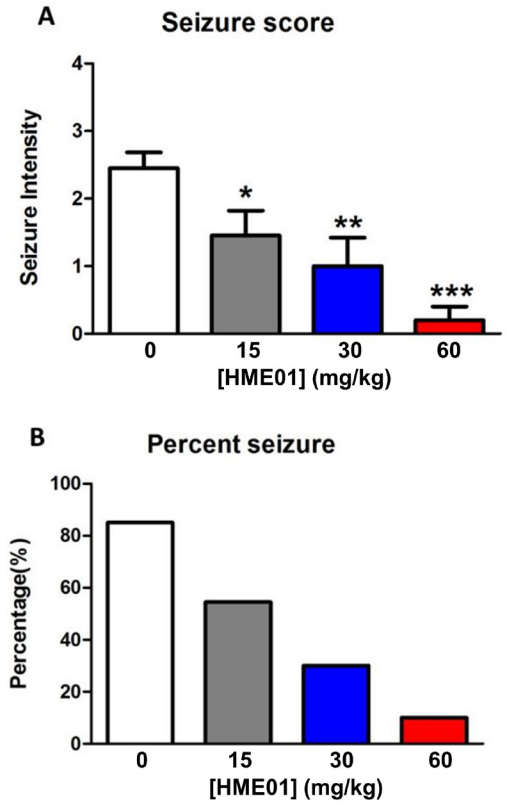

Figure 1. Audiogenic seizure (AGS) susceptibility was reduced in *FMR1* KO mice by the mGluR5 NAM HME01. (A) HME01 reduced severity of seizure as measured by intensity score in *FMR1* KO mice. Seizure intensity score 0 = no response; 1 = wild running and jumping; 2 = clonic seizures; 3 = tonic seizures; 4 = respiratory arrest. **(B)** HME01 decreased incidence of seizure as measured by percentage of *FMR1* KO mice exhibiting each phase of the sequential seizure response. Single injections of 0, 15, 30, or 60 mg/kg HME01 were administered 15 minutes before the test. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

based on the hyperexcitability phenotype, it will be desirable for the therapeutic engagement to start earlier, no later than an age of 5 to 14 when seizure co-occurs at the highest frequency among FXS patients. Of course this argument requires further validation in basic and clinical research before it can be translated into clinical practice.

In terms of clinical translation, it appears to be rather challenging to utilize this endpoint as a viable outcome measure in the current setting of clinical studies, which in fact reflect the current gap between basic and clinical science in bringing novel neuroscience discovery into new treatment for FXS.

Lesson 3: Clinical Challenge – therapeutic development for a complex condition like FXS, predicting the outcome that will improve during a short trial is essentially guesswork without objective measures

The appropriate selection of patients is crucial for the success of any clinical research. Although a monogenic condition, clinical heterogeneity of FXS patients have demonstrated various severities with a wide range of co morbidities (such as mood and anxiety disorders, epilepsy and other behavioral problems) in addition to the core symptoms, which include physical impairment, cognitive, emotional and behavioral deficits. Results from recently completed clinical trials of novel FXS therapies have indeed highlighted several challenges including patient stratification with possible differential responses, the need for FXS-specific more objective outcome measures, and the lack of biomarkers for predicting a patient's response to a specific treatment.

With respect to patient stratification, drug development for childhood FXS poses unique challenges due to the compounded challenges associated with studying both a rare disease and pediatric population. Unique considerations include potential age-based differences in drug metabolism and toxicities, the long term consequences of a drug's effect on a growing child's physiology and development, *etc*. Based on convincing preclinical data, the optimal efficacy for pharmacological treatment of FXS and ASDs, most likely comes from early intervention in childhood, which theoretically offers the prospect of disease modification or correction of a developmental trajectory. On the other hand, traditional drug development and regulatory pathways require demonstration of safety and potential prospects of direct benefit in adult populations before pediatric studies can be conducted. While it is understandable that stringent safety requirements should be met before younger populations are exposed to experimental medicines, there is reason to believe that efficacy in adult patients might not be achievable in this case, or might not be fully predictive of the potential therapeutic efficacy in younger patients.

Currently there are no FXS-specific outcome measures that have been established and validated in the clinic. The widely used Aberrant Behavior Checklist – Community Edition (ABC-C) was developed to assess problem behaviors in children and adults with intellectual disability and has been effectively employed in trials for ASD treatments. Nonetheless it was unclear whether the ABC-C is specific and sensitive enough to detect disease modification by targeted novel therapies in all FXS patients. Although based on the extensive preclinical mechanistic studies, many novel targeted therapies including mGluR5 NAMs and GABA-A agonists, have the potential to normalize or partially normalize the core mechanism underlying FXS, which translate into a stabilization or improvement in symptoms. A key challenge to assess disease modifying therapies is identifying an appropriate outcome measure, considering the wide range of symptoms and the big variations in the severity of each individual symptom observed in FXS patients.

Successful development of effective FXS treatments in the future will most likely rely heavily on the availability of a robust biomarker. The availability of the well-characterized highly relevant FXS mouse model has provided an excellent opportunity to identify a reliable biomarker which potentially correlates with clinical improvement. For example, auditory hypersensitivity including seizure susceptibility may actually represent one of the most promising candidates as a biomarker for patient stratification, in particular for targeted therapies that have demonstrated robust efficacy in the FXS mouse models, such as mGluR5 NAMs and GABA-A agonists, as discussed above.

Even though China potentially has a large number of FXS patients who urgently need medical care, conducting clinical trials of novel therapies for FXS patients in China has to face additional challenges. Historically in mainland China, the diagnosis of autism or ASD is only given by pediatrician or pediatric neurologists, and there is essentially no diagnosis for adult autism patients. At present, autistic individuals including FXS patients who are seeking professional help are almost exclusively young children under intensive parental care. Adult autistic patients have historically been a neglected population; many of them are no longer under the proper medical care and therefore not registered under the right category. Furthermore many adult autistic patients are often misdiagnosed as other mental disorders such as mania or schizophrenia. Therefore one major obstacle in conducting FXS clinical trials in China is the identification and recruitment of adult FXS patients.

4. Conclusions

The monogenic cause of FXS leads to a relatively genetically homogeneous patient population, and offers a unique and favorable opportunity for drug development of effective therapies which has been

facilitated by the availability of a variety of transgenic animal models mimicking the FXS phenotype. Despite a common genetic etiology, individuals with FXS display significant heterogeneity in clinical phenotype and drug response.

Because current clinical testing of targeted novel therapies are mostly conducted as a monotherapy, patients will display optimal responses to different targeted treatments based on individualized complex interactions between genetic variability, neuronal pathways and synaptic function. It is also very likely that target-based monotherapy may not be sufficient as a successful 'cure' for FXS, rather, a combination therapy will be more efficacious for long-term treatment. Thus, it is likely that different patients will function best with certain pathway targets or certain combinations of treatments. In regard to this, a personalized approach seems ideally suited for this complex condition.

There is hope that ongoing development of new targets will gradually build on previous knowledge to result in progressive improvement in treatments and ultimate reverse of core deficits in FXS patients in the future.

Clearly, there is overlap in molecular and synaptic pathways between FXS and autism. Thus targeted treatments for FXS will likely be effective in a subgroup of ASD patients who display dysregulated synaptic defects in the same pathways that are abnormal in FXS. The great progress made thus far holds a strong promise for continued development of target-based therapeutic strategies for FXS.

In lieu of the disconnection between the efficacy observed in the preclinical animal studies and the apparent lack of efficacy in the human clinical studies through statistics, as highlighted by the recent failed trials by Novartis and Roche, it has been proposed that future efforts are needed to help unravel the exact reasons for the lack of translation between preclinical data and clinical outcome. A well-defined clinical end point should be developed based on preclinical and clinical research on *FMR1* gene mutation and clinical symptoms, which will bridge the gap between the scientific and medical community and create a novel path to management of FXS. Furthermore, more coordinated efforts are needed to expand and refine the preclinical toolbox in order to increase confidence in predicating therapeutic benefits in FXS patients. In the future there is also a need to optimize behavioral assays in *FMR1* KO mice, including better standardization for experimental paradigms, age, and genetic background to provide a valuable and reproducible tool for the evaluation of novel therapeutic strategies in FXS.

5. Looking forward to the future in China

Despite abundant cases of this rare disease, the related work in China such as research, regulations and social support have only recently been initiated. In China, the level of care for common diseases such as tumors and cardiovascular disease has significantly improved. Now, the prevention and treatment of rare diseases including FXS is also drawing more public and government attention. At the moment, a program for collaboration on rare disease research is being implemented at the national level. This program is committed to promoting the study of rare diseases in China and will encourage international collaboration in this regard (*33*).

The past few years have seen great advancement in our understanding of the genetic and molecular basis for FXS and ASD, a gradually increased number of researchers in China have gained greater interest in FXS and ASDs, especially in basic science research. Furthermore, a growing number of clinicians, researchers, and government health officials began to become aware of the importance of resource allocation, epidemiological study, and clinical study of rare diseases in China. Furthermore, there have been an increasingly large number of Western-trained experienced researchers who are devoted and engaged in novel drug development in China and for those most afflicted.

Although novel targeted treatments for FXS are still in the research and development stage, many affected Chinese families have expressed their interest in participating in the clinical trials, though safety is their most important concern.

The prevention of a rare disease like FXS is a comprehensive work and requires a coordinated effort at various levels, including healthcare, medical insurance and civil affairs. In order to tackle complicated diseases such as FXS and ASDs, it is hoped that a specialized clinical and research consortium in China can be organized to provide the most up-to-date knowledge and recommendations for Chinese medical professionals and to provide optimal assistance to affected individuals and their families.

Policy wise, current nationwide insurance programs in China cover basic general health care expenses for 95% of the Chinese population. For example, general screening for Downs syndrome has been conducted as part of routine prenatal tests. Unfortunately, no major plans are available today to cover the relatively high cost of genetic testing for FXS which is unaffordable for many affected families, particular those in the underdeveloped rural areas.

Building upon the remarkable progress made over the past few years in the understanding of drug development for FXS and other neurodevelopmental disorders, with strong government support under the 12.5 plan, Hua Medicine, is currently taking a personalized medicine approach actively engaged in bringing new concepts and novel treatment to the FXS and autism patients in China. It can be anticipated that the path leading to an effective novel treatment in China

may be filled with even more challenges. As a pioneer pathfinder, we need to work with all relevant parties at various levels, both public and regulatory agencies. By focusing on the need of the patients it will be possible to dramatically change the therapeutic landscape for neurodevelopmental disorders including FXS, creating new medicines that will improve the lives of patients and their families.

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