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REVIEW ARTICLE

Challenges in Conducting Clinical Trials for Pharmacotherapies in Fragile X Syndrome: Lessons Learned

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Abstract Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and most common single gene cause of autism spectrum disorder (ASD). Even in the context of a single gene disorder like FXS, characteristic cognitive and behavioral heterogeneity creates challenges in conducting targeted pharmacotherapy trials. Neuroscientific advances have elucidated aspects of the underlying neurobiology in FXS and have guided targeted treatment development in the last decade. However, despite significant preclinical progress, recent clinical trials have failed to consistently demonstrate therapeutic efficacy based on behavioral outcome measures in patients with FXS. One potential explanation for these failures is that many behavioral measures are not capable of quantitively capturing clinically significant change in such short-term trials. Further, the use of parent and clinician report instruments as primary outcome measures creates additional challenges in clinical trials. Future trials may employ more quantitative measures of evaluating the pathophysiology of FXS to avoid placebo-response resulting from rater bias. Quantitative measures of language, eye gaze, molecular dysregulation, and brain function may be used to identify which individuals may best respond to a particular treatment and to capture potential treatment-associated change. Here, we present a thorough review and reconsideration of the challenges encountered in conducting clinical trials in FXS to allow for lessons learned to drive future success in this field.

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Key Points

Introducing pharmacodynamic approaches earlier in FXS trials may enhance future trial success through incorporation of quantitative biomarker measures designed to demonstrate target engagement using early pharmacokinetic-pharmacodynamic (pK:pD) relationships to enrich sample and outcome measure selection.

Defining the natural history of more objective, quantitative measures of pathophysiology in FXS will become increasingly important as FXS trials move from short-term to longer-term treatment periods.

Preclinical assays such as induced pluripotent stem cell (iPSC)-derived neuronal species may serve as tools in the translational treatment pipeline as potential findings are put into a developmental context impacting preclinical and clinical study.

1 Introduction

Fragile X syndrome (FXS) is the leading inherited cause of intellectual disability (ID) and most common monogenic cause of autism spectrum disorder (ASD). FXS has an estimated prevalence of 1 in 4000 males and 1 in 8000 females reflecting the locus of the Fragile X Mental Retardation (*FMR1*) gene on the X chromosome [1, 2]. The disorder is characterized by cognitive deficits and risk for behavioral and other features including anxiety, inattentiveness, hyperarousal, social and communication deficits,

and autistic-like behaviors [3]. Despite a common genetic etiology, the clinical presentations of FXS varies considerably within the affected population.

FXS is typically caused by an unstable cysteine–guanine–guanine (CGG) repeat expansion located in the 5′ untranslated region of the fragile X mental retardation gene 1 (*FMR1*) at Xq27.3. This large polymorphic trinucleotide expansion results in DNA hypermethylation and subsequent inhibition of *FMR1* transcription when larger than 200 CGG repeat [4]. Rarely, FXS can also be caused by deletions/disruptions within the *FMR1* gene code itself, but both mechanisms result in deficient production of fragile X mental retardation protein (FMRP) [5]. FMRP is an RNA-binding and transport protein critical to brain development and function that plays a pivotal role in synaptic plasticity, neuronal migration, and neurogenesis [6].

Advances in experimental models have led to increased understanding of the neurobiological underpinnings of FXS and, in turn, have directed the development of targeted treatments. The last decade of FXS research is characterized by many clinical trials following widespread preclinical pharmacotherapy reports of phenotypic rescue within *fmr1* knock out (KO) animal models. Clinical trial progress in the last decade in FXS is marked by the conduct of at least 15 double-blind, placebo-controlled trials. Despite this apparent progress and many successful preclinical interventions, recent clinical trials have failed to consistently demonstrate therapeutic efficacy. This disparity requires careful examination to identify possible factors hindering target pharmacotherapy development and to identify strategies that may yield more successful trials for FXS. Potential factors contributing to trial failure include lack of potential sensitive and specific outcome measure in FXS, inability to effectively and quantitatively define subpopulations of persons with FXS who may best respond to a particular treatment, and lack of reliable pharmacodynamic markers describing the impact of targeted pharmacotherapy on the underlying pathophysiology of FXS [7].

In this paper, we will review aspects of the FXS phenotype, heterogeneity in the presentation of FXS, examples of recent failed targeted drug trials in FXS, trial outcome measures, and quantitative assay development in FXS, all with an eye to appreciating the challenges and opportunities presented by each topic in order to facilitate successful drug development for patients with FXS.

2 Fragile X Syndrome Phenotype

Nearly all individuals with FXS demonstrate problems with attention, inhibitory behavioral control, and disorganized behavior [8]. These deficits are often milder in females as a result of inactivation of one of the X chromosomes [9]. While only approximately 25% of females with FXS will have an intelligence quotient (IQ) below 70, an additional 50% are reported to have a borderline IQ (70–79), learning disabilities, and executive function deficits [8, 10–12]. Males with FXS have an average IQ between 40 and 50 and universally have mild-to-severe cognitive deficits with distinctive weaknesses in visual spatial processing, visual motor coordination, and arithmetic [8, 13, 14]. Males with FXS are likely to experience significant delays in several language domains, including vocabulary, morphosyntax, and functional use of conversational language [15].

In addition to cognitive deficits, individuals typically present a wide range of functionally limiting behavioral concerns that may negatively affect quality of life [16, 17] . Behavioral difficulties in FXS are often grouped into the following symptom clusters: (1) irritability, which includes severe tantrums, aggression, and self-injury; (2) attention deficit/hyperactivity disorder (ADHD)-like symptoms, which include hyperactivity, distractibility, and impulsivity; (3) repetitive and stereotypic behaviors, which include perseverative speech, body rocking, and hand flapping; (4) anxiety-related symptoms that may be socially related and are often demonstrated by social avoidance or social withdrawal; and (5) autistic features, including impairments in social interaction and communication, lack of social awareness, and restricted interests [16, 17]. Likely depending on specific diagnostic methods and use of a broad or strict phenotype definition of autism, in the literature anywhere between 18 and 67% of males with FXS may meet criteria for ASD [18–21]. A reported 50–90% of males with FXS may display some ASD features, despite many with FXS not meeting full ASD diagnostic criteria [22–26]. An additional common feature of FXS in males (and some females) is hyperarousal to sensory stimuli. This heightened sympathetic response to sensory stimuli as measured by electrodermal response has been shown to be inversely associated with level of FMRP expression [27, 28].

2.1 Fragile X Syndrome Phenotype Heterogeneity: Driving Clinical Trial Challenges

One source of observable heterogeneity in behavioral and cognitive deficits in persons with FXS may be attributed to the variation in levels of FMRP [17]. These levels may be driven by possible CGG expansion size mosaicism, *FMR1* gene methylation levels, and in females, X chromosome inactivation patterns. All females with FXS are considered mosaic, with two X chromosomes leading to potential differential pattern of FMRP expression across tissue and organ types [9]. Mosaicism in males results from variations in the methylation pattern of the CGG expansion, which

can be driven in males by the presence of premutation and full mutation *FMR1* alleles in the same person [16, 29]. The correlation between severity of developmental disability and both *FMR1* activity and FMRP levels has been demonstrated in both males and females with FXS [8, 30, 31]. More quantitative and reproducible methods for measuring FMRP levels are needed to thoroughly explain the relationship between phenotypic presentation and FMRP activity. This is challenging given the uncertain relation between blood FMRP and brain protein expression. Additionally, individual genetic, treatment availability, and environmental factors may contribute to symptom manifestation, severity, and treatment responsivity [32].

As a rare disorder, phenotypic heterogeneity is particularly challenging for clinical trials. Methods such as stratifying trial participants by presence or absence of comorbid ASD, requiring a specific degree of developmental disability for study inclusion, utilizing biological heterogeneity, or requiring specific scores on behavioral inventories to enrich a study sample for specific features can be logistically challenging. Phase II clinical trials of medications in FXS have been charged with generating the decision-making data regarding potential subgroups who may best respond to a particular treatment, yet these trials may be under-powered to do so. This has resulted in decisions regarding subgroups of interest or specific behavioral measurements that have failed upon more rigorous and large-scale Phase III studies [33, 34]. One method to address this Phase II to Phase III gap may be to focus Phase II subgroup definitions on quantitative measures of FXS pathophysiology that are well linked to the pathobiology of the disorder. Such an approach may strengthen the ability to replicate initial positive trial results. Such work may focus on subgroups defined by EEG findings, eye tracking/pupillometry testing, or potentially findings from molecular blood assays among other potential biologically relevant quantitative measurements [33–35].

3 Fragile X Syndrome Translational Treatment Development: Learning from the Past

Novel neurobiological treatments have predominantly focused on a proposed excitatory/inhibitory neurotransmitter activity imbalance in FXS, with significant concentration on metabotropic glutamate receptor 5 (mGluR5) signaling [7]. Recent clinical trials in the FXS field have often been based on reports of phenotypic rescue in animal models followed by lack of consistent positive response in placebo-controlled human trials. Among targeted treatment development focused on excitatory/inhibitory imbalance in FXS, study of mavoglurant and arbaclofen exemplify both challenges associated with preclinical to clinical conversion of treatment response and the challenges noted above using Phase II findings to drive large Phase III programs in the field.

The most wide-spread preclinical success in FXS-related research has focused on attenuation of mGluR5 activity. The mGluR5 theory of FXS, outlined by Bear and colleagues, grew out of a number of key findings [36]. It has been demonstrated in animal models that deficient FMRP results in the up-regulation of mGluR5-mediated signaling leading to an exaggeration of mGluR5-mediated activation [37, 38]. *Fmr1* KO mouse studies employing treatment with selective mGluR5 antagonists [38, 39] or genetic knockdown of mGluR5 expression [40] have shown significant phenotypic rescue including resolution of audiogenic seizures, hyperactivity, dendritic spine abnormalities, and dysregulated basal protein synthesis among other features. Specifically, the selective mGluR5 antagonist mavoglurant showed similar promising preclinical results in models of FXS with rescue of dendritic spine abnormalities [41] and phenotypic improvements in the *fmr1* KO mouse [42].

In studies of patients with FXS, a small initial double-blind, placebo-controlled crossover of mavoglurant showed no group-wide improvement on the Aberrant Behavior Checklist (ABC) [43] or clinical global impression (CGI) scales [44] in 30 young adults with FXS [45]. Post-hoc analyses revealed that a subgroup of seven individuals with full methylation of the *FMR1* promoter showed treatment-related improvement on the ABC, as well as the CGI Improvement subscale (CGI-I). These individuals had more severe behavioral impairments, which may have contributed to the more positive treatment effect seen in this subgroup [46]. This seven-subject subgroup additionally exhibited an unexplained very limited placebo-response rate, which contributed to a conflated treatment effect size estimate. This finding prompted further efficacy studies utilizing molecular profiling to identify differential treatment response in individuals with complete versus partial methylation of the *FMR1* promotor [33]. Unfortunately, this series of two well-powered double-blind, placebo-controlled trials of mavoglurant, taking methylation status into consideration, did not demonstrate efficacy for the drug in any outcome measure. Further, there was no significant correlation between outcome and methylation status [33].

Another approach to excitatory/inhibitory imbalance in FXS, has focused on gamma aminobutyric acid (GABA) neurotransmission. Decreased GABAergic inhibition has been observed in multiple brain areas in *fmr1* KO mice, including the hippocampus, striatum, amygdala, and somatosensory cortex [19, 47]. GABA type B (GABA(B)) receptors are metabotropic G protein-coupled receptors

found both pre- and post-synaptically throughout the brain. They elicit both presynaptic and slow postsynaptic inhibition through the modulation of Ca^{2+} (presynaptic) and K^{+} (postsynaptic) channels, as well as modulating oscillatory activity in thalamocortical networks [48]. The GABA(B) selective agonist arbaclofen (a single enantiomer of baclofen), corrected dendritic spine abnormalities and aberrant protein synthesis [49] and reversed dysregulated feed-forward inhibition [50] in *fmr1* KO mice. An initial Phase II double-blind, placebo-controlled crossover trial of arbaclofen in 63 FXS patients aged 6–39 years focused on irritability as rated on the ABC Irritability subscale (ABC-I) as a primary outcome measure, taking guidance from the FDA approvals for use of risperidone and aripiprazole targeting irritability in youth with autism [46]. Arbaclofen was not associated with positive treatment response on the ABC-I primary outcome. Post-hoc analysis using a newly validated FXS-specific ABC Social Avoidance Scale (ABC-SA) noted a significant treatment effect in the full study population. A post hoc analysis of a subgroup of 27 subjects with more severe baseline social impairment showed treatment-associated improvement on several outcome measures including the Vineland Adaptive Behavior Scale (VABS) social domain, ABC-SA, and the CGI. This Phase II work led to the development of a large-scale Phase III arbaclofen in FXS program, which was focused on the ABC-SA as the primary outcome measure of importance. Unfortunately, arbaclofen failed to show significant treatment-associated improvement on the ABC-SA in a Phase III study [51]. Consistent with the failing of the mavoglurant program in FXS, a pivot in the outcome measure of interest made in arbaclofen trials was made from the Phase II to Phase III trial. Post-hoc analyses of smaller Phase II studies were important in the selection of outcome measures and other aspects of study design of Phase III programs. Also, in both programs, limited quantitative biologically relevant data were generated in Phase II to demonstrate drug target engagement prior to moving to the Phase III study.

3.1 Outcome Measure Challenges in Fragile X Clinical Trials

Several challenges to the field of FXS clinical trial research are presented by the mavoglurant and arbaclofen Phase III study failures, which provide lessons to aid future trials. Defining subgroups using traditional "pen and paper" parent report instruments may continue to pose challenges when used as the primary outcomes for clinical trials in this disorder. Given the large number of parent and clinician report measures used across treatment trials, combined with the many measure subscales for the rating instruments, there is significant potential for Type 1 errors and sample-dependent effects driving results of post hoc analyses.

Further, both the arbaclofen and mavoglurant development programs highlight the risk and challenges of animal model to human response extrapolation in this field. While findings of dendritic change or global correction of aberrant protein synthesis with use of many targeted treatment agents in the *fmr1* KO mouse have been striking, the behavioral and other outcome assays used in trials of these same agents have not been directly linked to biological changes seen in animal findings. It is unclear if the outcome measures used in drug developmental programs had potential to capture clinical improvement in the population associated with each treatment, particularly during short-term treatment periods. If one or both drugs in humans with FXS was having similar biological effects as in the *fmr1* KO mouse, the manifestations of dendritic normalization and correction of excessive protein synthesis could have potentially been missed in both trial programs as designed. Essentially, the previous efforts to move from preclinical to clinical findings simultaneously shifted from mouse to human, and from primarily biological to behavioral outcomes with unclear effects on clinical trial outcome.

The FXS clinical trial field began with initial reliance on the ABC given the use of this measure in the US FDA approvals for use of risperidone and aripiprazole targeting irritability in youth with ASD [52–55] and given the extensive validation data using the ABC in persons with developmental disability [56], including FXS [57]. Despite clear justifications for this approach, many years into the current era of FXS trial development, there are reasons to consider that this type of outcome measure approach could be suboptimal. While behavior is very important in FXS, it is unclear if short-term treatment with a potential efficacious targeted treatment will result in consistent and relatively rapid behavioral improvement across a heterogeneous cohort of persons with FXS. Trying to more broadly capture behavior in FXS using the ABC has resulted in use of the ABC total score, rather than particular subscale scores [45]. However, the ABC total score has not been validated as an outcome measure and its use may have resulted in dilution of the ability to detect meaningful change with treatment in FXS trials. Efforts to factor the ABC specifically in FXS have been important, but also did not result in trial success when the arbaclofen program employed the FXS-specific ABC-SA.

Other parent and clinician report methods have been employed in addition to the ABC in FXS-specific clinical trials. We will briefly review several illustrative, but non-inclusive, examples of this approach. Given the prominence of anxiety in FXS, the anxiety, depression, and mood scale (ADAMS), which has been validated in persons with developmental disability [58] has been employed in this

field [59, 60]. However, while use of such outcomes is logically reasonable, the ADAMS has not yet been shown sensitive to change in large-scale shorter-term controlled FXS trials. Given the prominence of attention deficit hyperactivity disorder (ADHD) symptomatology in FXS, the ADHD Rating Scale 4th edition (ADHD-RS-IV), a prominent measure in trials leading to ADHD drug approval, has been employed in FXS trials. However, the ADHD-RS-IV lacks normative data in the FXS and developmental disability fields, and the measure did not detect change with treatment in a recent Phase II study of metadoxine [61]. Adaptive behavior has been extensively assessed in FXS trials mainly through use of the well-validated VABS, 2nd Edition (VABS-II) [62–64]. The VABS-II has also been extensively utilized in FXS descriptive studies [65, 66]. While the VABS-II has been used as a secondary outcome in several FXS trials, the ability of the measure to detect meaningful change during short-term trials remains to be established. While the list of available behavioral parent and clinician report outcome measures for trials has grown significantly in the FXS field, this work to date has not demonstrated its utility for detecting meaningful, robust change with treatment. Given these issues, it is possible that moving towards quantitative and translational evaluations of FXS pathophysiology may provide a more promising direction for selecting outcome measures especially for Phase II studies.

3.2 Developing Quantitative Assays in Fragile X Syndrome for Use in Clinical Trials

Quantitative measures of pathophysiology and phenomenology in FXS hold promise to both identify those with FXS who may best respond to a particular treatment and track potential change with treatment. These lines of research may also provide tools to enhance the direct translation from promising preclinical findings to successful human study. Quantitative measures of language, eye gaze, molecular dysregulation, and brain activity among other quantitative measures in development hold promise as means to avoid placebo-response resulting from rater bias, thus potentially enhancing sensitivity to detect drug effects, even in short-term trials.

In FXS, communication deficits are common and may even drive other interfering behaviors such as irritability due to resultant frustration generated by communication delay. Recent efforts have worked to quantitatively measure expressive language in FXS using recording of real time conversation and narration [67]. This methodology, which includes recording conversations from persons with FXS, transcribing the recordings, and analyzing the data in Systematic Analysis of Language Transcripts (SALT) software, has shown good test–retest reliability (ICCs >0.7) in 36 persons with FXS (mean age 18 ± 1.7 years) tested twice 12–37 days apart [67]. Additionally, mean length of utterance (MLU) and different word roots used correlated with VABS language scores. This expressive language sampling methodology holds significant promise as a quantitative, reproducible measure of language in FXS, thus removing parent and clinician report placebo-response risk. We and others are currently utilizing this sampling methodology in neurodevelopmental disorder study (clinicaltrials.gov NCT 01813318, 01911455).

Gaze aversion is a classic phenotypic feature of FXS. Hands-free infrared eye tracking in FXS has described reduced scanning of eye regions when viewing faces [68, 69] consistent with the clinical presentation of the disorder. Enhanced pupillary response to fearful, calm, and happy faces has also been noted during this testing [68, 69]. This finding is likely consistent with enhanced autonomic responsivity and social anxiety that are characteristic of FXS [70–72]. This eye tracking and pupillometry protocol has demonstrated good test–retest reliability [69] and has been utilized in a number of FXS clinical trials, though results of eye tracking in FXS trials remain largely unpublished. The field remains hopeful that eye tracking will be a means to quantitatively capture relevant phenotypic features of FXS-gaze avoidance and dysregulated autonomic response in the context of clinical trials. Publication of the use of these measures from recent and ongoing trials will enhance the ability of the field to move forward this methodology in future work, including the need to establish across-site reliability.

Given the central role of FMRP in regulating the expression of many other proteins, it is not surprising that deficient FMRP results in a number of downstream cellular signaling abnormalities in FXS. Two primary molecular abnormalities in FXS have been assayed in blood and have published clinical trial setting results. Extracellular signal related kinase (ERK) is a nodal point for several intracellular signal cascades and has been shown to be dysregulated in human with FXS and in the *fmr1* KO mouse [73–76]. Specifically in human lymphocytes, ERK activation has been demonstrated to be delayed in FXS compared to control subjects following activation with the protein kinase C activator phorbol ester [76]. This aberrant lymphocytic activation has been studied in two open-label clinical trial settings. In a small six-adult subject open-label pilot study of riluzole, a putative glutamate and GABA modulator FDA-approved for the treatment of amyotrophic lateral sclerosis (ALS), ERK activation kinetics uniformly normalized with 6 weeks of riluzole treatment (100 mg/day) [77]. Despite uniform normalization of ERK kinetics, no significant behavioral improvements were noted. Similar to the molecular impact from riluzole, the

FDA-approved mood stabilizer for the treatment of bipolar disorder, lithium, was associated with normalization of lymphocytic ERK activation kinetics in sixteen 6–23-year-olds with FXS in an open-label short-term trial [78]. In this latter report, lithium use was also associated with reductions in maladaptive behavior. This ERK assay has been employed in additional placebo-controlled trials of novel molecule FXS targeted treatments and in an ongoing acamprosate-controlled trial (NCT 01911455), although all of these results remain unpublished.

Amyloid precursor protein (APP) is involved in several complex aspects of neuronal growth, maturation, and activity [79]. APP is metabolized down two distinct pathways including an amyloidogenic path leading to production of the neurotoxic molecules Aβ40 and Aβ42, implicated in the pathophysiology of Alzheimer disease and a non-amyloidogenic pathway resulting in the neurotrophic species amyloid precursor protein alpha (sAPPα) [80]. FMRP has been shown to directly regulate APP mRNA expression [81, 82]. In the *fmr1* kKO mouse, baseline APP levels are elevated [82] and APP derivative levels show elevation in humans with FXS [83, 84]. In an open-label pilot study setting, plasma APP total, sAPPα, Aβ40, and Aβ42 were assayed pre- and post-10 weeks of acamprosate treatment in nine persons (mean age 10.9 years) with FXS [83]. In this report, APP total reduced by 8.28 ng/mL ($p < 0.05$) and sAPPα reduced by 1.78 ng/mL ($p < 0.05$) following treatment, and levels of Aβ40 and Aβ42 did not change with treatment. Clinical improvement on the ABC social withdrawal subscale correlated with both molecular changes noted in this report. APP plasma assay is currently employed in two ongoing clinical trials in FXS and ASD (clinicaltrials.gov NCT 01813318, 01911455).

In recent years, electroencephalogram (EEG) testing in FXS has demonstrated cortical activation abnormalities that may hold promise for future use in trial settings. Auditory event-related potentials (ERPs) have been studied in both humans with FXS and in the *fmr1* KO mouse. In school-aged children with FXS, the N1 and N2 amplitudes were significantly increased following standard tones, and N1 habituation and N2 sensitization were reduced compared to control subjects [85]. Enhanced early sensory N1 ERP responses in response to auditory stimuli have been replicated in additional FXS studies consistent with a cortical hyper-excitability model of FXS [86, 87]. In 14 adolescents and adults with FXS (mean age 28.5 ± 11.7 years) compared to 15 typically developing control subjects, N1 habituation was shown to be reduced with reductions significantly related to clinical measures of sensory deficits and social communication [88]. Additionally, in this report, Ethridge et al. [88] noted enhanced gamma power and reduced gamma phase-locking during the early stimulus registration period that also correlated with behavioral deficits. These FXS EEG findings are consistent with the human FXS phenotype marked by sensory hypersensitivity and associated high levels of anxiety.

The ERP deficits in human FXS studies are translationally relevant as similar findings in the *fmr1* KO mouse have shown rescue with targeted molecular knockdown approaches. Genetic deletion of matrix metalloproteinase-9 (MMP-9) in the *fmr1* KO mouse has been associated with rescue of auditory ERP habituation deficits [89]. In a placebo-controlled human study of the MMP-9 inhibitor minocycline, a subset of 12 youths with FXS completed pre- and post-treatment auditory ERP studies using a passive oddball paradigm [90]. Following 3 months of minocycline treatment, N1 amplitudes reduced and habituation to auditory stimuli improved with minocycline treatment compared to placebo. EEG evaluation in FXS holds promise given initial reports demonstrating potential translation fidelity of findings from mouse to man, replication of auditory ERP results across labs, and potential relevance of the cortical hyperexcitability noted in EEG studies to aspects of the human FXS experience and behavioral problems.

Pursuit of quantitative measures of pathophysiology in FXS for use in clinical trials holds significant promise, although clearly more work remains to be done to establish and validate the relevance of different targets and measures. These promising areas of study all show potential links to the human phenotype. Molecular and EEG testing show considerable consistency across animal and human studies with EEG work most poised for bridging the translation assessment gap from preclinical work to clinical trials. For all of these measures, additional test–retest and inter-site evaluations are needed to build the understanding necessary for assay use in large-scale FXS clinical trials. Continuing to establish the clinical relevance of quantitative measures will also be required. For each measure, understanding the longitudinal trajectory of assay results and developmental influences will be essential to develop these tools for use in assessing and predicting response to treatment in clinical trials.

4 Discussion

Despite recent struggles to develop treatments for FXS, clear opportunities remain in the field. These struggles have defined a field that currently is well poised to learn from past failures. Several concerns developed during treatment failures continue to be addressed by ongoing treatment-focused research in FXS. Use of EEG assessment is becoming increasingly wide-spread in FXS studies. This

work holds significant promise to quantitatively assess drug-specific pharmacodynamic effects, while at the same time if appropriately powered, begins to identify potentially treatment-responsive subgroups of persons with FXS. With this development, the field will need to continue to build expert clinical trial infrastructure given the technical requirements of the growing list of quantitative metrics developed for use in the field. Decisions will be required specific to EEG use in trials regarding specific sensory and cognitive paradigms utilized and their optimal measurement. Additionally, across-site and test–retest reproducibility over trial durations appropriate for different protocols will need to be demonstrated in patients with FXS. The same needs and pathways exist for other areas of quantitative biomarker studies including molecular blood assay and eye tracking methodology.

With the emphasis on quantitative measures in clinical trials, and their potential value, especially in moving from preclinical to phase II trials, it will be important to not lose sight of final FDA approvable endpoints in the field. It will likely be necessary to establish further the relationship between quantitative measures and the clinical presentation of FXS both at one single time point and over the natural history of the disorder. For example, resolution of auditory habituation deficits during EEG testing in FXS would be predicted to have clinical correlates such as anxiety or sensory sensitivity reduction, but such relationships will need to be established to further enable the utility of such measures in FXS trials. The natural history of quantitative measures of pathophysiology in FXS will be more important as FXS trials may move more from short-term treatment periods to longer-term study as a means to capture potential drug-associated pro-learning effects that may mirror potential dendritic and synaptic improvements demonstrated in preclinical models.

Given recent developments in the field, we see a window of opportunity to modify the traditional Phase II to Phase III FXS drug-development approach. Bringing pharmacodynamic approaches into FXS human study earlier may provide enhanced future trial success. Such work may require Phase Ib study approaches first in FXS studies that incorporate a heavy load of quantitative biomarker measures that are not designed to demonstrate drug efficacy, but designed to demonstrate, early on, target engagement, potential early pharmacokinetic-pharmacodynamic (pK:pD) relationships, and potential use in patient stratification. Such early profiling with quantitative measures would help inform Phase II studies, even potentially providing a means to enrich sample selection and outcome measure selection much earlier in the drug development process. This early stage work may even be able to be conducted in an open-label setting given the purpose is not efficacy assessment.

Developmental issues in FXS treatment remain important yet largely unexplored. As a childhood-onset developmental disorder, we cannot assume that a particular drug treatment or even combination of drug treatments given at one stage of development may have a similar impact given much earlier, or for that matter, later in development. While the general mantra of "the earlier the better" in treatment development does permeate the field, such emphasis is not yet empirically supported. The need to assess treatments across developmental windows clearly adds expense to drug development and often moving from adult, to adolescent, to child drug development is required, as this approach mirrors the generation of appropriate safety data to justify human study at each stage. The youngest treatment trial in FXS to date was a controlled trial of low-dose sertraline, a selective serotonin reuptake inhibitor (SSRI), in individuals aged 2–6 years that revealed very modest improvement effects on visual perception, fine motor skills, and social participation [91]. Efforts to take trials into younger cohorts are currently underway as the upcoming Neuronext trial will initiate and combine mavoglurant and parent-implemented language intervention in children aged 3–6 years with FXS.

Developmental challenges will continue and develop more complexity even as new preclinical assays such as induced pluripotent stem cell (iPSC)-derived neuronal species enter the FXS field as potential tools in the translational treatment pipeline. iPSC-derived species are often very developmentally immature and therefore potential future findings from such assay must be put into a developmental context impacting preclinical and clinical studies.

5 Conclusion

Overall, refinement of FXS clinical trial methodology has been informed by many recent trials in the field, the majority of which have failed to move targeted treatments towards FXS-specific approval for use. We remain optimistic that lessons learned will significantly enhance our ability as a field to personalize the medicine of FXS to bring forth new potential treatments. Such work could even lead to important advances for a portion of persons with FXS defined by specific phenotypic characteristics that may be defined by quantitative measures of pathophysiology. While the days of thinking one treatment for all persons with FXS at all stages of development is on the precipice of discovery may be outdated, a more personalized and quantitative assessment-driven approach to FXS drug development holds significant promise for near-term discovery in the field.

Compliance with Ethical Standards

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