

Clinical trials for neurodevelopmental disorders: At a therapeutic frontier

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A well-powered clinical trial that failed to replicate promising results in animal models of fragile X syndrome yields important lessons for clinical trial design (Berry-Kravis *et al.*, this issue).

Clinical trial design and interpretation are inherently challenging. Nonetheless, the search for targeted, effective treatments for neurodevelopmental disorders—fragile X syndrome, Rett syndrome, Down syndrome, and others—has been plagued by their heterogeneity and complexity. We often are quick to conclude that “negative” findings in a trial prove that a treatment is ineffective under all conditions or that the presumed underlying pathophysiological mechanisms are not valid. However, as reinforced by the well-designed, properly powered study in this issue by Berry-Kravis *et al.* (1), negative findings can provide an opportunity to reflect on clinical trial design and implementation, as well as the underlying mechanistic motivations, and provide lessons for treatment studies of neurodevelopmental disorders in general. Berry-Kravis *et al.* highlight key themes to consider in clinical trials: choice of treatment group, target engagement, selection of outcome measures, and the impact of the placebo effect.

The authors report results from two multi-site, phase 2b clinical trials of the metabotropic glutamate receptor (mGluR) antagonist mavoglurant for adolescents and adults with fragile X syndrome. Fragile X syndrome is among the most common inherited causes of intellectual disability and autism spectrum disorder, and children with fragile X syndrome also exhibit many of the common associated comorbidities—behavioral dysregulation, mood disorders, and attention deficit hyperactivity disorder (ADHD) (2). The discovery that mutations in the fragile X mental retardation (*FMR1*) gene cause disease, and nearly two decades of mechanistic work in animal model systems, identified potential targets for pharmacological treatments (3). The

choice of mavoglurant is based on the mGluR theory of fragile X syndrome (4), which implicates overactive mGluR signaling as a cause of exaggerated hippocampal long-term depression (LTD), slowed synaptic maturation, and ultimately cognitive impairment. Treatment with mGluR antagonists improves physiological and behavioral end points in preclinical studies of mouse and fly models (3, 5). In one clinical study, mavoglurant treatment did not show overall efficacy, but post hoc analysis indicated that the methylation status at the *FMR1* locus [perhaps indicative of different levels of residual FMRP (fragile X mental retardation protein) expression] might mark a subgroup of patients who did respond to this mGluR antagonist (6).

In the two parallel multicenter trials reported in this issue, the investigators tested a clear hypothesis based on these previous results in cohorts of adolescents and adults treated with three different doses of mavoglurant and stratified by *FMR1* methylation status. Noteworthy was the collaboration among investigators in 16 countries, an impressive effort that resulted in a sample size powered to test the selected primary outcome measures. Based on the prior study (6), outcome measures were derived from caregiver questionnaires of overall patient functioning, as well as from cognitive testing in the few individuals who could complete the protocol. None of the groups exhibited significant improvement in these outcomes over placebo, which led the authors to conclude that “under the conditions of our study, we could not confirm the mGluR theory of fragile X syndrome, nor the predictive value of the methylation state of *FMR1* promoter for mavoglurant efficacy.” What seemed like a reasonable a priori hypothesis based firmly on previous studies, including a clinical trial in patients, was not supported by the outcome data in this trial. The key question is, Why? Is this result a failure of the assumed mechanism of action, of inappropriate extrapolation from animal to human of target engagement, or of other clinical factors—outcome measures or simply the inherent liability of post hoc analyses of the previous human trial?

WHOM SHOULD WE BE TREATING?

The investigators used methylation status to establish two subgroups of patients, based on the hypothesis from the earlier post hoc analysis: The mGluR antagonist would have greater efficacy in patients with the fully methylated *FMR1* gene. The heterogeneity of neurodevelopmental disorders necessitates some level of informed stratification to identify subgroups that may respond better to a drug. More than two subgroups may be needed, but the patient characteristics that can guide further stratification have yet to be determined.

The age of the participants also requires further consideration. As with all neurodevelopmental disorders, the behavioral and cognitive characteristics of fragile X syndrome, such as social communication impairment, intellectual disability, and maladaptive behaviors, unfold early in infancy and continue to evolve over a lifetime. Pharmacological trials of adults may reflect, therefore, the fact that the fully developed nervous system has already suffered the neurobiological consequences of, in this case, *FMR1* gene loss (enhanced LTD and synaptic dysfunction). Optimism that adulthood is not too late to treat fragile X syndrome is reasonable, however, because preclinical studies in adult mouse models with fragile X syndrome showed symptom reversal. This may still be a valid conclusion. The outcome measures in mouse models may not directly translate to the parental report measures selected for these human clinical trials, although in this particular case, these measures were appropriately chosen to formally test the hypotheses generated from the previous clinical trial (6).

HOW DO WE DEFINE OUTCOMES?

The authors aptly state, “The results of the studies presented here highlight the difficulty of translating information on molecular mechanisms identified in animal models to humans.” Three prior studies of mGluR inhibition in adult mouse models of fragile X syndrome demonstrated “reversal of both neurobiological and behavioral phenotype.” Studies in flies also showed parallel morphological, physiological, and behavioral phenotypes, providing additional confidence that these observations were evolutionarily conserved (7). In mice, treatment normalized dendritic spine length in the hippocampus (8), improved prepulse inhibition (an index of sensory sensitivity) (8), and increased social interest as quantified by increased time spent with a “stranger” mouse (9).

At face value, the outcome measures used in these trials do not clearly parallel the most

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consistent behavioral changes seen in the mouse models after treatment. A caregiver report, while clinically convenient and a valid measure of overall adaptive functioning, may not properly reflect changes in dendritic spine length or startle response seen in mice, let alone changes in LTD or spatial learning. It has been argued that the underlying biochemical and physiological mechanisms serve as the target of treatment, so that parallel behavioral changes in humans and animal models are not required. Without a clear measure of target engagement or other biomarkers of treatment response, however, one must necessarily rely on behavioral outcomes in humans. Such outcomes likely reflect the combined effects of treatment on many synapses that comprise the circuits underlying behavior.

The original mGluR hypothesis is based primarily on data from the hippocampus and visual cortex; many of the human behaviors affected in fragile X syndrome, such as social cognition and anxiety, involve additional brain regions and circuits. Thus, one must consider cellular and regional variability. Indeed, the downstream effects of FMRP reduction differ among cell types and different brain regions in preclinical models. The amygdala, for example, is critical for processing emotional and social information and contributes to the anxiety and social impairment often seen in fragile X syndrome. But without a parallel behavioral or circuit readout in preclinical studies, one is forced to assume that synaptic function changes seen after inhibiting mGluR apply to all circuits, including the amygdala. Although the same mutation in humans and animals will not necessarily exhibit the same behavioral outcomes, it would be valuable to develop behavioral measures in animal models that map onto specific neural circuits and that could translate directly to patients. Such direct translation would facilitate the choice of behavioral targets in clinical trials.

Another effort toward greater precision in outcome measures lies in the growing interest in the identification of hypothesis-driven biomarkers as outcome measures in clinical trials for neurodevelopmental disorders. Particularly useful will be markers sensitive to change in short time periods that would correlate with or predict clinical behavioral change. On the basis of evidence from mouse models, one might design a study that directly assays LTD through electrophysiological measures of

prepulse inhibition, face processing, and relational memory and then relates these quantitative measures to downstream clinical measures of adaptive function, cognition, or social communication skills. Future objective measures of outcomes for clinical trials will require innovative methods such as event-related electrophysiological and eye-tracking correlates of attention and cognition. We are hopeful that the recently initiated NIH multisite initiative called the Autism Biomarkers Consortium for Clinical Trials (www.asdbiomarkers.org) will identify a useful set of such tools.

POWER OF PLACEBO

Quite striking in this study was the change in caregiver measures evidenced in the placebo group, perhaps reflecting the eagerness of caregivers of individuals with developmental disabilities for treatments that will improve symptoms and reduce the burden of disease. This enhanced placebo effect could have undermined the quan-

tification of change in the treatment group. A recent meta-analysis of placebo response in medication trials for autism spectrum disorder showed that in 25 data sets (1315 participants), there was a “moderate effect size” for overall placebo response [Hedges’ $g = 0.45$, 95% confidence interval (0.34–0.56); $P < 0.001$] (10). Might we expect a greater placebo effect in caregiver reports of patients with neurodevelopmental disorders than in other populations? If so, this effect would be attenuated with more objective measures, reinforcing the need for more quantifiable outcomes that are more proximal to the mechanism of action of the drug being studied and that relate to disease-impaired circuit function in addition to synaptic physiology.

CONCLUSIONS

Translational neuroscience has rightly placed significant confidence and resources in developing treatments based on understanding the causal genetic mechanisms underlying disease.



Fig. 1. Dissecting a failure to translate. Promising findings in mice suggesting that glutamate receptor antagonists could effectively treat fragile X syndrome failed to replicate in a well-designed clinical trial. Nevertheless, important lessons about designing clinical trials based on preclinical animal studies have emerged from this experience.

Such an approach has shown notable success in cancer treatment and considerable promise in autism spectrum disorders. The more parallel the findings in model systems and humans, and the more that preclinical work is conducted with the rigor of clinical trials, the easier this road to translating genetic findings into treatment will be. We stand at a therapeutic frontier, with much more to learn. As Berry-Kravis and colleagues show, the results of negative trials, while disappointing, can be crucial in refining our hypotheses and encouraging dialogue that will accelerate the process of bringing effective treatments to our patients.

REFERENCES AND NOTES

1. E. Berry-Kravis, V. Des Portes, R. Hagerman, S. Jacquemont, P. Charles, J. Visootsak, M. Brinkman, K. Rerat, B. Koumaras, L. Zhu, G. M. Barth, T. Jaecklin, G. Apostol, F. von Raison, Mavoglurant in fragile X syndrome: Results of two randomized, double-blind, placebo-controlled trials. *Sci. Transl. Med.* **8**, 321ra5 (2016).
2. R. J. Hagerman, The physical and behavioral phenotype, in *Fragile X Syndrome: Diagnosis, Treatment, and Research*, R. J. Hagerman, P. Hagerman, Eds. (Johns Hopkins Univ. Press, Baltimore, 2002), pp. 3–109.
3. M. R. Santoro, S. M. Bray, S. T. Warren, Molecular mechanisms of fragile X syndrome: A twenty-year perspective. *Annu. Rev. Pathol.* **7**, 219–245 (2012).
4. M. F. Bear, K. M. Huber, S. T. Warren, The mGluR theory of fragile X mental retardation. *Trends Neurosci.* **27**, 370–377 (2004).
5. S. Jacquemont, E. Berry-Kravis, R. Hagerman, F. von Raison, F. Gasparini, G. Apostol, M. Ufer, V. Des Portes, B. Gomez-Mancilla, The challenges of clinical trials in fragile X syndrome. *Psychopharmacology* **231**, 1237–1250 (2014).
6. S. Jacquemont, A. Curie, V. des Portes, M. G. Torrioli, E. Berry-Kravis, R. J. Hagerman, F. J. Ramos, K. Cornish, Y. He, C. Paulding, G. Neri, F. Chen, N. Hadjikhani, D. Martinet, J. Meyer, J. S. Beckmann, K. Delange, A. Brun, G. Bussy, F. Gasparini, T. Hilse, A. Floesser, J. Branson, G. Bilbe, D. Johns, B. Gomez-Mancilla, Epigenetic modification of the *FMR1* gene in fragile X syndrome is associated with differential response to the mGluR5 antagonist AFQ056. *Sci. Transl. Med.* **3**, 64ra1 (2011).
7. T. C. Dockendorff, H. S. Su, S. M. McBride, Z. Yang, C. H. Choi, K. K. Siwicki, A. Sehgal, T. A. Jongens, *Drosophila* lacking *dfmr1* activity show defects in circadian output and fail to maintain courtship interest. *Neuron* **34**, 973–984 (2002).
8. J. Levenga, S. Hayashi, F. M. de Vrij, S. K. Koekkoek, H. C. van der Linde, I. Nieuwenhuizen, C. Song, R. A. Buijsen, A. S. Pop, B. Gomez-Mancilla, D. L. Nelson, R. Willemsen, F. Gasparini, B. A. Oostra, AFQ056, a new mGluR5 antagonist for treatment of fragile X syndrome. *Neurobiol. Dis.* **42**, 311–317 (2011).
9. I. Gantois, A. S. Pop, C. E. de Esch, R. A. Buijsen, T. Pooters, B. Gomez-Mancilla, F. Gasparini, B. A. Oostra, R. D'Hooge, R. Willemsen, Chronic administration of AFQ056/Mavoglurant restores social behaviour in *Fmr1* knockout mice. *Behav. Brain Res.* **239**, 72–79 (2013).
10. A. Masi, A. Lampit, N. Glozier, I. B. Hickie, A. J. Guastella, Predictors of placebo response in pharmacological and dietary supplement treatment trials in pediatric autism spectrum disorder: A meta-analysis. *Transl. Psychiatry* **5**, e640 (2015).

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