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The Urgent Need for Molecular Imaging to Confirm Target Engagement for Clinical Trials of Fragile X Syndrome and Other Subtypes of Autism Spectrum Disorder

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Abstract

Promising therapeutic agents for the symptoms in animal models of fragile X syndrome (FXS) have not resulted in similar advances in clinical trials of humans with FXS due to the dearth of tools to quantify their key cognitive and behavioral outcome measures with optimal validity and reliability. Therefore, experts strongly recommended an effort to develop and implement use of biomarkers in unfolding clinical trials in FXS. Molecular imaging provides a spectrum of agents to serve as biomarkers to confirm that humans with FXS exhibit the molecular abnormalities of animal models of FXS. Thus, molecular imaging provides the mechanism to establish target engagement in humans for clinical trials of novel agents for FXS.

Keywords: Neurotransmission, Positron Emission Tomography, Receptors, Single-Photon Emission Computed Tomography, Transporters

1. Background

Fragile X syndrome (FXS) leads the way for targeted treatments in autism spectrum disorder (ASD) as its most common known single gene cause (1-3). FXS is typically caused by an expansion (> 200, full mutation) of a CGG repeat in the promoter region of the *fragile X mental retardation 1* (*FMR1*) gene, leading to complete or partial methylation of the promoter and insufficient fragile X mental retardation protein (FMRP) (4). The *FMR1* gene-encoded RNA-binding protein regulates the brain development by translation of several hundred mRNA ligands; many of them overlap with known ASD genes (5). FXS emerges in early childhood with deficits in motor, language, and communications skills as FMRP modulates the development of the brain synapses (6). Intellectual disability (ID) in males and behavioral features such as attentional network deficits, perseveration, hypersensory (4), and social interaction disorders such as social anxiety and ASD (7) are common as well. Individuals with FXS often present for treatment for a wide range of the aforementioned problem behaviors requiring a complex combination of pharmacological, behavioral, and educational interventions (4). At present, no target-symptoms or disease-modifying treatments for FXS have received regulatory approval.

2. Preclinical Advances

Preclinical advances in FXS and other types of ASD have provided the bases for promising targeted therapies for humans with FXS and other types of ASD. Although the *FMR1* mouse model has propelled much interest in the FXS field, number of failed clinical trials unfolded in an attempt to establish the efficacy of these compounds. Rigorous preclinical trials must be conducted to establish the efficacy of potentially beneficial agents before accomplishing human clinical trials (2). The vast majority of twenty-two controlled clinical trials in FXS, the most among all neurodevelopmental disorders, targeted the core inhibitory/excitatory imbalance characteristic of FXS (1). For these failed studies, tools to measure their key outcomes were deemed inadequate, with limited validity and reliability, including cognitive and behavioral (1).

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3. Biomarkers

Biomarkers associated with and sensitive to behavioral changes would be particularly useful, including those to measure the drug engagement of its targeted ligand. Recently, a group of experts in the field of FXS strongly recommended an effort to develop and implement use of biomarkers, such as electroencephalography (EEG), event-related potential (ERP), and eye tracking (8), in clinical trials in FXS (2).

4. Molecular Imaging

Molecular imaging, particularly positron emission tomography (PET) and single-photon emission computed tomography (SPECT), provides the tools to identify transporters, neurotransmitters, and receptors in the brain of humans. As such, molecular imaging can confirm that the targets identified in the *FMR1* animal models of FXS hold for humans with FXS. These tools can confirm the target engagement in other types of ASD (9), including Rett syndrome (10), and probably the targeted interventions in FXS. Measurement of the density and the distribution of targets in the brain pre- and post-clinical trials in FXS will provide the means to determine whether the desired target engagement was accomplished by the putative therapy. Since molecular imaging uniquely identies the density and the distribution of molecular targets in the brain, it is the crucial tool to advance effective clinical trials for FXS. Thus, molecular imaging is necessary despite the risks of those techniques. For example, SPECT demonstrated reductions of vesicular acetylcholine transporters in participants with Rett syndrome (Figure 1) (10).

Informed consent was obtained from each patient included in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki Subjects (11) as reflected in a prior approval by the Institution's Human Research Committee.

5. Risks of Molecular Imaging

Risks of molecular imaging are justified due to the enormous critical data that will result from these studies. The safety and efficacy of molecular imaging biomarkers have been established on healthy adults. Now investigations of molecular imaging biomarkers on adults of FXS and other types of ASD are urgently needed to conduct definitive clinical trials.

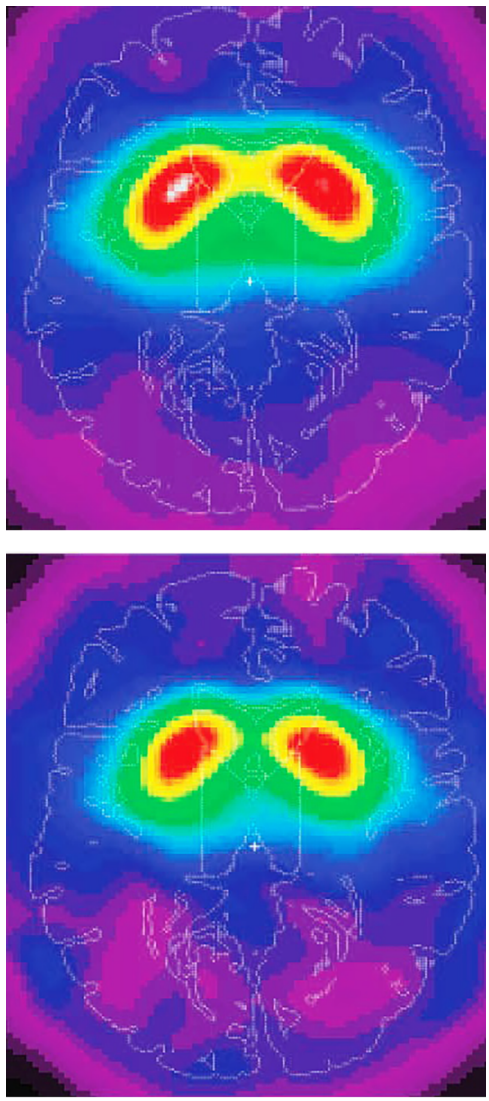

Figure 1. Vesicular acetylcholine transporters reduced in participants with Rett syndrome (lower panel) in contrast to healthy participants (upper panel). Images of mean uptake on single-photon emission computed tomography (SPECT) one day following the intravenous injection of approximately 333 MBq (9 mCi) (2)-5-[^{123}I]iodobenzovesamicol ([^{123}I]IBVM), a radiotracer for vesicular acetylcholine transporters to 8 healthy participants (upper panel) and 4 participants with Rett syndrome (lower panel). The left side of the brain is represented on the left of each panel. Panels demonstrate transverse sections at striatum. The lower row illustrates the reduced uptake in the participants with Rett syndrome in the striatum. Reproduced from (10), figure 6, page 478, with permission.

6. Radiation Exposure

Radiation exposure occurs during PET and SPECT. Participants in these studies are asked to follow the limits of radiation exposure for radiation workers, 5 rem per year. Radiotracers for PET and SPECT are initially studied on healthy adults to establish their safety and efficacy be-

fore being carried out in individuals with FXS and other subtypes of ASD. Thus, initially molecular imaging will be administered to participants with FXS who are 18 years of age or older, and primarily in males that carry the major burden in FXS. Participants are studied carefully to determine that they are physically well.

7. Stillness

Stillness is a necessary component of successful molecular imaging studies. Healthy participants typically can refrain from motion for a scan lasting 90 minutes. Individuals with FXS and other types of ASD may experience claustrophobia and anxiety when asked to enter a scanner. For this reason behavioral psychologists have developed mock scan techniques to desensitize individuals with FXS and other developmental disabilities to maintain a stationary position for the duration of scans (12-14). Higher functioning individuals with FXS can typically be trained with a mock scanner to complete the process with an actual scan. Lower functioning persons with FXS may not be able to complete scans despite mock scan training. Therefore, other options may be utilized. Sedation in the form of a benzodiazepine may relax a person with FXS and other types of ASD adequately to undergo a scan. However, general anesthesia may be required to maintain the necessary stillness for an adequate examination of individuals with ID. General anesthesia was employed for molecular imaging of participants with Rett syndrome, a type of ASD characterized by profound ID (10).

8. Ethical Standards

The work was carried out in accordance with the recommendations of the World Medical Association in the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (11) and the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (15). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (11) as reflected in a prior approval by the Institution's Human Research Committee.

9. Conclusions

In summary, adequately measuring target engagement in clinical trials for humans with FXS and other types of ASD is a crucial step to begin to establish the success of the putative therapies. Biomarkers are needed to demonstrate that the specific deficits identified in animal models of FXS can be translated into humans with FXS. Molecular imaging provides that necessary tool to demonstrate the success of promising interventions in humans with FXS and other types of ASD. The benefits from applying these tools in individuals with FXS and other types of ASD outweigh the risks of the utilization of the molecular imaging. Investigations with these biomarkers are urgently needed to provide the tools to meaningfully measure target engagement in clinical trials of adults with FXS and other types of ASD.

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Footnotes

Conflict of Interests: The authors have no conflicts of interest.

Ethical Approval: The study was approved by the Institutional Review Board of The Johns Hopkins University School of Medicine in Baltimore, Maryland.

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Patient Consent: All participants provided written informed consent to take part in this study.

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