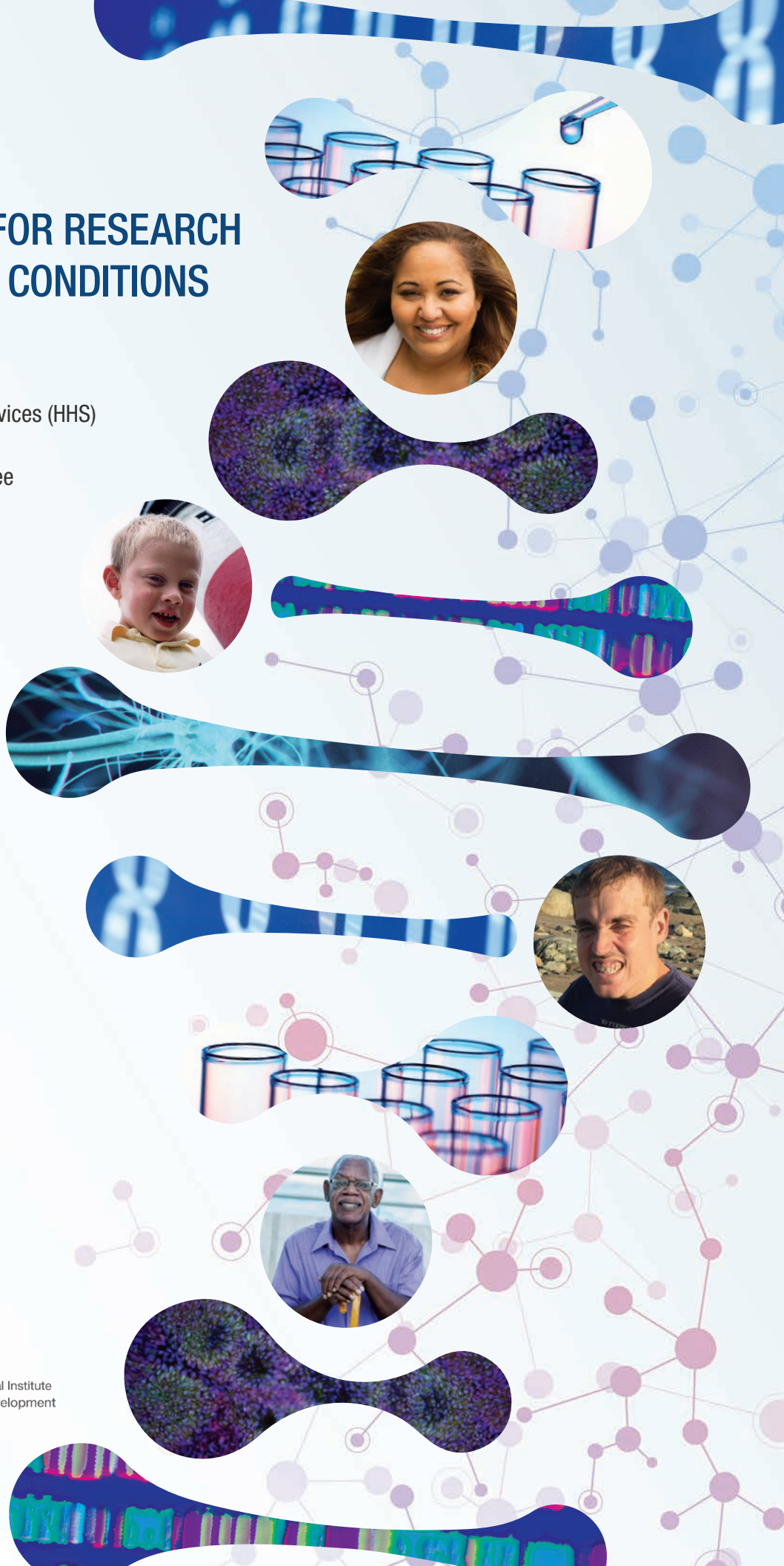
NIH STRATEGIC PLAN FOR RESEARCH ON *FMR1*-ASSOCIATED CONDITIONS

October 2019

U.S. Department of Health and Human Services (HHS)
National Institutes of Health (NIH)
Trans-NIH Fragile X Coordinating Committee



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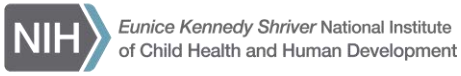

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Introduction

FMR1 is a gene on the X chromosome in humans. It contains the genetic code for a protein, FMRP, that plays an important role in the development and function of the brain and nervous system. This gene was discovered in 1991 and first linked to **Fragile X Syndrome (FXS)**, the most common inherited cause of intellectual and developmental disability (IDD). Since then, mutations in the *FMR1* gene have also been linked to two very different conditions: **Fragile X-associated Tremor/Ataxia Syndrome (FXTAS)**, which leads to disabling neurological symptoms in middle-age and elderly adults; and **Fragile X-associated Primary Ovarian Insufficiency (FXPOI)**, which can lead to infertility and/or early menopause in women. As research continues, researchers are discovering more connections between *FMR1* mutations and a range of symptoms and conditions. This document uses the term ***FMR1*-associated conditions** to refer to these conditions.

NIH support for research on *FMR1*-associated conditions crosses the translational spectrum, ranging from basic genetic, molecular, and cellular research to research involving animal models of disease to clinical trials in individuals with *FMR1*-associated conditions. NIH efforts also span multiple institutes and offices, reflecting the wide-ranging importance of understanding the gene and FMRP to develop treatments, preventive interventions, and cures for health issues experienced by those with *FMR1*-associated conditions.

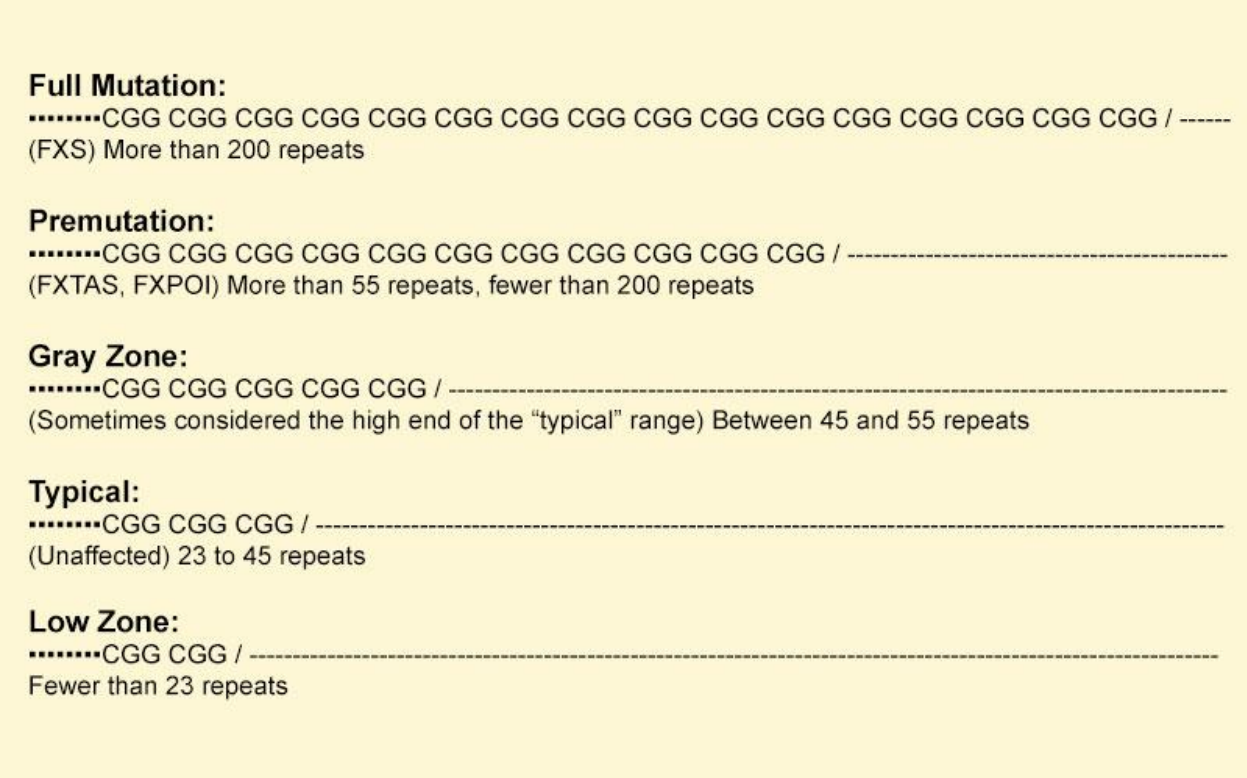
This strategic plan outlines priorities for NIH research on *FMR1*-associated conditions that will both enhance existing efforts and support promising new research to improve the health of individuals affected by *FMR1*-associated conditions.

About the *FMR1* Gene

Although *FMR1*-associated conditions have different clinical symptoms, they all result from the same type of mutation on the *FMR1* gene: expansions of a repeated sequence of 3 nucleotides, cytosine-guanine-guanine (CGG). These repeats are in the 5’ untranslated region (sometimes denoted 5’ UTR) of the gene, which is not typically transcribed into messenger RNA (mRNA), the molecule that is translated into a protein. Instead, this region of the gene usually plays a regulatory role by helping to determine when the gene is turned on and off. Changes to this region, therefore, affect the structure of the FMRP protein and how much FMRP protein is made.

The number of CGG repeats in the *FMR1* gene varies from person to person; these numbers are used to categorize *FMR1* mutations, as listed in Figure 1.

Figure 1: Categories of *FMR1* Mutations



- In individuals with the **full mutation** (more than 200 repeats), the CGG repeat region is silenced or turned off when methyl groups attach to the DNA (called methylation). A silenced *FMR1* gene does not produce any FMRP.
    - Because males have only one X chromosome, they also have only one copy of the *FMR1* gene. Males with the full mutation usually make no FMRP, resulting in symptoms of FXS.
    - Females have two X chromosomes, meaning that if they have a full mutation on one X chromosome, they often have a functioning gene on the other X chromosome. Because patterns of X-chromosome inactivation are random, symptoms of FXS in females can vary widely. Some have the full range and severity of features commonly seen in males with FXS, but others have mild symptoms or no symptoms at all.
- People with between 55 and 200 CGG repeats have an *FMR1* **premutation**. Individuals with the premutation are at increased risk of developing FXTAS and/or FXPOI. Some research suggests that the premutation may be associated with increased risk for certain neuropsychiatric symptoms, including anxiety, depression, chronic pain, chronic fatigue, and sleep disturbances. Although the mechanisms by which the premutation leads to these conditions are not fully

understood, some evidence suggests that abnormal transcription and translation of the CGG repeats themselves may generate toxic mRNAs and proteins, which may contribute to these conditions.

- Individuals with between 45 and 55 repeats are considered to be in the "**gray zone**." Until recently, the typical range included up to 55 repeats. However, newer research suggests that having between 45 and 55 repeats may be associated with specific health effects.
- **Typical** *FMR1* genes have between 24 and 45 repeats. People with typical *FMR1* genes are usually not affected by symptoms of *FMR1*-associated conditions.
- Some researchers recently found intriguing evidence that having a less-than-typical number of CGG repeats, 23 or fewer—called the "**low zone**"—may also be associated with increased risk of certain conditions and symptoms.

The stability of the number of repeats varies and seems to be influenced by multiple factors, including which parent the inherited *FMR1* gene comes from and the age of the parent(s). Expansion from one generation to the next, meaning a parent with a premutation could have a child with a full mutation, is well documented. Such situations are part of the reason people who have an *FMR1* premutation are sometimes called "carriers." But there are also cases of contraction, in which the number of repeats decreases in the next generation. Understanding this process remains an active area of study.

FMR1-Associated Conditions

Fragile X Syndrome (FXS)

In individuals with FXS, the *FMR1* full mutation is present at birth, and symptoms of FXS emerge in early childhood. More males are affected with the symptoms of FXS than females, and symptoms are often milder and more variable in females than in males, due to the random nature of X inactivation in females. The Centers for Disease Control and Prevention (CDC) estimates[1] that 1.4 per 10,000 males and 0.9 per 10,000 females have FXS.

Common FXS features include the following:

- **Intellectual and language impairments.** Most males with FXS have intellectual disabilities, as well as speech and language delays from a young age. Developmental delays typically become evident in early childhood and range from mild to severe. Females with FXS show a full range of intellectual function: some may have severe impairments, while others function at typical levels or

[1] https://www.cdc.gov/ncbddd/fxs/data.html

above. Many individuals with FXS have strong visual memory skills relative to their overall cognitive abilities.

- **Autism spectrum disorder (ASD) or features of ASD.** According to the CDC, between one-third and one-half of males[1] with FXS are diagnosed with ASD, making FXS the most common inherited cause of ASD. ASD is characterized by difficulties in social interactions (such as avoiding eye contact or not recognizing social cues) and restricted or repetitive behaviors (such as hand-flapping or insistence on certain routines). Features of ASD also occur in females with FXS, but, as with FXS-associated intellectual impairment, they are less common and more variable than in males.
- **Other behavioral or emotional characteristics.** Attention problems, hyperactivity, impulsivity, anxiety, or aggressive behaviors are common in individuals with FXS. At the same time, many individuals with FXS have high levels of sociability.
- **Sensory hypersensitivity.** Many individuals with FXS are extremely sensitive to loud noises, bright lights, or certain textures.
- **Physical features.** Common physical features of FXS include a long and narrow face, large ears, a prominent forehead, flexible joints, low muscle tone, and flat feet. These physical features are often not noticeable in early childhood and may not become evident until adolescence or adulthood.
- **Seizures/epilepsy.** The CDC estimates[1] that seizures occur in between 15 percent and 20 percent of males with FXS. These seizures may become chronic, meeting the criteria for epilepsy. As with the other features of FXS, they can also occur in females, but they are less common and more variable than in males.

There is currently no cure for FXS, but there are treatments, usually aimed at managing each person's specific symptoms. Individuals with FXS who receive appropriate educational interventions, therapy services, and/or medications have the best chance of maximizing their capabilities and skills.

Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

FXTAS occurs in *FMR1* premutation carriers and is characterized by problems with movement and cognitive functioning. FXTAS was first discovered when researchers noticed a cluster of specific neurological symptoms in relatives of patients with FXS; these relatives were then found to have *FMR1* premutations. Symptoms of FXTAS typically do not begin until later in adulthood, most often after age 50 years. These symptoms may include:

- **Intention tremor:** trembling or shaking of part of the body when trying to perform a voluntary movement, such as reaching for an object

- **Ataxia:** problems with balance and coordination
- **Parkinsonism:** a collection of movement problems often found in Parkinson's Disease, including resting tremor, stiff/rigid movements, and unusually slow movements (bradykinesia)
- **Decline in cognitive abilities,** including memory loss and loss of executive function skills (related to planning and problem solving)
- **Depression, anxiety, and/or irritability:** may be manifestations of FXTAS itself, but may also represent an individual's response to the occurrence or worsening of other FXTAS symptoms
- **Autoimmune disorders,** such as hypothyroidism or fibromyalgia, in some women with FXTAS, often before the onset of neurological symptoms

Estimates suggest that up to 40 percent[2] of male *FMR1* premutation carriers older than 50 years of age have FXTAS, with symptoms more common at older ages. However, FXTAS is often misdiagnosed because many symptoms of FXTAS are similar to other neurological conditions more commonly seen in older adults, including Alzheimer's disease and Parkinson's disease. Nonetheless, the overall proportion of premutation carriers who go on to develop FXTAS may be lower than estimated because many male premutation carriers are not aware of their carrier status, especially if they do not have a family member diagnosed with an *FMR1*-associated condition.

As with FXS, FXTAS occurs in both males and females, but is less common and more variable in severity in females compared to males.

There is also currently no cure for FXTAS. Like in FXS, treatments for FXTAS address a person's specific symptoms, and often include combinations of therapy services, counseling, and medications.

Fragile X-Associated Primary Ovarian Insufficiency (FXPOI)

FXPOI, like FXTAS, is a condition that occurs in *FMR1* premutation carriers and does not become evident until adulthood. The symptoms of FXPOI are similar to those of primary ovarian insufficiency (POI) from other causes. They can include:

- **Irregular menstrual cycles**
- **Subfertility** (difficulty becoming pregnant) or **infertility** (inability to become pregnant)
- **Early menopause,** in which the symptoms of menopause (absent menstrual cycles, hot flashes, insomnia, vaginal dryness) occur before age 40 years

[2] https://ghr.nlm.nih.gov/condition/fragile-x-associated-tremor-ataxia-syndrome

- **Osteoporosis** (thinning bones and an increased risk of fractures), a direct result of the hormone changes associated with early menopause

Approximately one-quarter[3] of adult female *FMR1* premutation carriers are estimated to experience FXPOI. Like FXTAS, however, the FXPOI diagnosis may be missed because there are few characteristics that distinguish FXPOI from POI due to other causes, and because many women with FXPOI may not be aware of their carrier status, especially if they do not have a family member diagnosed with an *FMR1*-associated condition.

Awareness of FXPOI as a cause of early menopause is growing. The American Society for Reproductive Medicine and the American College of Obstetrics and Gynecology now both recommend that all women with symptoms or laboratory evidence of POI undergo *FMR1* testing.

Women with subfertility or infertility due to FXPOI may choose to pursue assisted reproductive technologies (ART) to increase their chances of becoming pregnant. However, some ART treatments appear to be less effective in women with FXPOI than in women with infertility from other causes. Furthermore, all women diagnosed with FXPOI (in fact, all individuals diagnosed with the *FMR1* premutation) should meet with a genetic counselor before seeking to become pregnant because premutation carriers may pass their premutation, or even a full mutation, on to their children.

Development of the NIH Strategic Plan for Research on *FMR1*-Associated Conditions

As scientific discovery has accelerated over time, NIH has expanded research on *FMR1*-associated conditions, involving a greater number of researchers and NIH institutes and centers. In 1991, NIH-supported researchers discovered the *FMR1* gene and its relationship to FXS. In the early 2000s, NIH-supported researchers studying FXS made connections between symptoms experienced by family members of individuals with FXS and the *FMR1* premutation. In 2007, NIH formed the Trans-NIH Fragile X Working Group. In 2009, the Trans-NIH Fragile X Research Plan established recommendations for specific, high-priority research objectives in FXS, FXTAS, and FXPOI. Developed with the input of research experts, patient advocates, and other stakeholders, the 2009 NIH plan incorporated 19 broad research goals, encompassing three *FMR1*-associated conditions (FXS, FXTAS, and FXPOI) and addressing research gaps in basic, translational, and clinical studies. In 2012 and 2013, NIH obtained additional input from scientists and the public and incorporated this feedback into a funding opportunity announcement for the Centers for Collaborative Research in

[3] https://ghr.nlm.nih.gov/condition/fragile-x-associated-primary-ovarian-insufficiency

Fragile X (RFA-HD-14-033). Three Centers were awarded through this funding opportunity.

The intervening years have yielded numerous scientific and clinical advances relevant to *FMR1*-associated conditions. A better understanding of the wide range and complexity of FMRP's functions in regulation of transcription and translation has yielded new insights into previously studied pathways—such as the metabotropic glutamate receptor 5 (mGluR5) pathway—and opened many promising new avenues for study and potential targets for interventions. Recognition of RAN (*r*epeat-*a*ssociated *n*on-AUG) translation as a key pathological mechanism in nucleotide repeat expansion disorders has provided new insights into possible disease mechanisms, especially for premutation-associated conditions. Dissemination and implementation of recommendations regarding *FMR1* testing among individuals with certain clinical features have increased rates of diagnosis and provided a more complete picture of the range of *FMR1*-associated phenotypes. Growing recognition of clinical phenotypes associated with a wide range of repeat lengths, not just the full mutation and the premutation, are opening avenues of investigation that promise to provide even more insight into the *FMR1* gene and its function.

Advances in related areas of research are also providing critical tools for advancing *FMR1*-related research. NIH funding opportunities focused on outcome measures for individuals with IDDs have led to the development and validation of more sensitive and specific tools for measuring outcomes of interest in clinical trials with these populations. Researchers have made great strides in identifying and validating biomarkers that provide insights into basic mechanisms of disease, and that can be translated between animal models of IDD-associated conditions to humans and back to animal models for further study. With better outcome measures and biomarkers, studies of therapeutic interventions (whether in animal models or humans) are more likely to identify changes resulting from those interventions and may help identify which individuals most likely to benefit from certain interventions. Development of model systems based on cells from individuals with *FMR1*-associated conditions, such as human induced-pluripotent stem cells (hiPSCs) and human brain organoids, may accelerate the translation of research findings from the laboratory to the clinical studies. The development of genetic and epigenetic tools, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene-editing and DNA methylation editing, raise the prospect of perhaps someday having gene-level therapies for *FMR1*-associated conditions.

At the same time, critical gaps in knowledge remain. Many aspects of the natural history of *FMR1*-associated conditions remain unknown. Awareness of some premutation-associated conditions remains low. Some promising targets for interventions in animal models have not yet been successfully translated to interventions in humans. Many

segments of the population remain underrepresented, and thus understudied, in *FMR1*-related research.

Reflecting both these scientific advances and remaining gaps in knowledge, in 2017 NIH embarked on a process to develop a new NIH Strategic Plan for Research on *FMR1*-Associated Conditions.

NIH obtained public input for the new plan through a Request for Information (NOT-HD-17-033) issued in January 2018.[4] Working groups, convened in the spring of 2018, were asked to review scientific progress and accomplishments since the last research plan, and to describe remaining gaps and challenges, priorities and new directions, and significant advances from other fields that might help advance future research in *FMR1*-associated conditions. These working groups, led by researchers with expertise across basic, translational, and clinical research, included scientific experts and representatives from patient advocacy organizations, NIH institutes, and other federal agencies. An in-person meeting of these experts was held to facilitate discussion of research needs and priorities for *FMR1*-associated conditions.[5] Staff at NICHD drafted the strategic plan, then refined the document based on feedback from the Trans-NIH Fragile X Coordinating Committee[6] and other participants.

This document is the culmination of those efforts and reflects input from all these sources, including NIH staff, researchers, and the public.

Long-Term Vision: Effective Prevention and Treatment Interventions for *FMR1*-Associated Conditions

NIH's overarching vision for research on *FMR1*-associated conditions is to accelerate the development of effective interventions to prevent or treat these conditions. This includes interventions that minimize the impact of symptoms and optimize quality of life for affected individuals, their families, and their communities.

Achievement of this long-term vision will require continued research and new discoveries at every level of biomedical research, from basic genetic and molecular biology to innovative studies using animal models to clinical trials and implementation research. Research experts for different *FMR1*-associated conditions have identified different goals and advances needed to move their fields forward, reflecting the varying levels of prior knowledge and scientific progress among the different conditions. For example, many experts noted the relative paucity of knowledge about the mechanisms

[4] A summary of the comments received in response to the RFI is included in this report as Appendix I.
[5] A list of participants at this meeting is included in this report as Appendix II.
[6] Current members of the Trans-NIH Fragile X Coordinating Committee include representatives from NICHD, the National Institute of Mental Health, the National Institute of Neurological Disorders and Stroke, the National Center for Advancing Translational Sciences, and the National Institute of General Medical Sciences.

and natural history of FXTAS and FXPOI compared to FXS, which has been studied much more extensively. There was broad consensus on the need for more basic research on mechanisms and phenotypes associated with the *FMR1* premutation because further advances in translational and clinical research are likely to be limited until such basic knowledge becomes available.

Because specific research needs and goals vary among the *FMR1*-associated conditions, this strategic plan is organized by condition. The order of the goals in this plan do not reflect any inherent priority or ranking; rather, they are intended to represent a range of goals across basic, translational, and clinical research domains.

Fragile X Syndrome (FXS)

Specific goal areas for FXS reflect both the rapid pace of recent advances in tools for advancing this research in cellular, animal, and human models of the disease, as well as the many gaps that remain in understanding the variability and developmental trajectories of the condition and how best to translate research findings between animals and humans.

Goal 1.1: Identify novel mechanisms and targets for intervention

Novel mechanisms, pathways, and potential targets for intervention should be identified at multiple stages of development and multiple levels of action—including genetic, molecular, cellular, and circuit-level effects—setting the stage for development of novel therapeutic interventions.

Advances in research on the basic mechanisms underlying FXS show that many brain and cognitive development pathways are disrupted by lack of FMRP. Many past and ongoing research efforts in this area focus on one or more of these pathways as potential targets for intervention. While much of this research has been groundbreaking in elucidating the basic mechanisms underlying FXS, efforts to translate these discoveries to interventions for individuals with FXS have had mixed results.

Insights into the breadth of *FMR1* activity have also highlighted that interventions directed toward a single molecular pathway are unlikely to fully reverse or cure FXS. Broadening the search for interventions beyond traditional cellular and small-molecule approaches may open new paths of inquiry and foster the development of novel therapeutics, including genetic and genomic interventions (such as gene editing and gene reactivation) and circuit- or network-level treatments (such as brain stimulation therapies to treat aberrant brain activity rhythms).

The advent of new techniques for gene editing, gene reactivation, and cell type-specific molecular phenotyping presents exciting new opportunities for FXS research. Further development of laboratory and computational methods is needed for scientists to take advantage of these tools. For example, FMRP may play different roles in specific cell types at varying stages of development. Tools that better delineate the function and activity of FMRP in specific cell types and in specific brain regions and at specific developmental time points, using cell-based and animal-based models, could provide a better understanding of disease mechanisms and potential therapeutic avenues.

Many FXS symptoms, including problems with memory, attention, anxiety, communication, emotional regulation, and social processing, arise from interactions among large populations of neurons. These large-scale interactions occur at the brain circuit/network level, rather than at the level of a single molecule or cell. Describing abnormal circuit mechanisms and functions associated with stimuli or behaviors would help scientists develop hypotheses of brain dysfunction and guide the development of therapies that could act at this level. Manipulating circuits could correct behavioral phenotypes, using techniques such as optogenetics or chemogenetics (in animals) or brain stimulation (in humans). Identifying core mechanisms of dysfunction at brain circuit and network levels may also reveal novel targets for interventions across the different causes of ASD and IDD. This type of knowledge would enable researchers to distinguish more clearly among conditions and better tailor interventions to individual needs.

The role of FMRP across different stages of development is not fully understood. Although it seems to be most essential during childhood, FMRP is also present throughout life and may serve specific functions as individuals grow older. In addition, FMRP levels are not highly correlated with the severity of the FXS phenotype. This finding may be partially due to difficulty in measuring FMRP levels accurately, but it seems more likely that the role of FMRP changes with age and time. Understanding exactly what FMRP does during various developmental windows could help scientists gain insight into when and how treatment is likely to be most effective. Improved animal and cell models could provide opportunities to determine the developmental time course for FXS, identify the level of FMRP required for functional correction, and test gene therapy and reactivation techniques.

Goal 1.2: Develop and refine etiologically and physiologically relevant models

Cellular and animal models should more closely mimic the genetics, physiology, phenotypes, and development of FXS in humans.

Thanks to broad programs of preclinical research in FXS, neuroscientists are gaining a better understanding of the mechanisms by which the condition affects brain function.

Work in *Fmr1*-knockout mouse models has influenced researchers well beyond the field of FXS and has led to discoveries of genetic factors related to ASD. However, as noted previously, many treatments that have rescued key aspects of the FXS phenotype in animal models have thus far not proven efficacious in humans. Scientists agree that mouse models have facilitated groundbreaking discoveries in FXS and identified a variety of potential targets for intervention, and that they continue to play a critical role in FXS research. At the same time, gaps remain in the knowledge needed to translate findings from mouse models into successful treatments for humans. Development of additional animal models that more closely mimic human genetics and physiology of FXS could help bridge this gap.

Currently, there are no animal models that recapitulate the excess CGG repeats, hypermethylation, and subsequent silencing of the *FMR1* gene seen in humans with FXS. Models that replicate the human genetic defect could enable testing of interventions that could ultimately reactivate the *FMR1* gene or modify the number of CGG repeats in humans. The wide range of cognitive, physical, and behavioral phenotypic features found in humans with FXS suggests that multiple types of animal models may be needed to better understand disease mechanisms and uncover more appropriate targets for intervention. Such models include those currently in wide use, such as knockout mice and Drosophila models, but may also include rats, pigs, or non-human primates, such as marmosets. Non-human primate models may offer particular advantages because they are often housed in social groups and display complex cognitive and social behaviors similar to humans. Identifying the mechanisms that underlie environmental and genetic influences on both animal and human phenotypes could help explain the variability seen among affected individuals and could be transformative in advancing knowledge about FXS. Discoveries in these areas could help identify the most promising subpopulations, age ranges, and mechanistic targets for early-phase therapeutic trials.

Goal 1.3: Develop and validate biomarkers and outcome measures

Physiologically relevant, clinically significant, easily measurable biomarkers and outcome measures should be developed and validated for use in clinical trials.

To rigorously test proposed interventions, researchers must be able to assess their impact with outcome measures that correspond to what patients find important in real-world settings. While considerable progress has been made in the development of appropriate cognitive and language measures, there is still a need for measures that capture academic, behavioral, and quality of life outcomes across the age and developmental spectrum. For example, validating outcome measures to characterize anxiety could indicate how well interventions can address this challenging feature of FXS. It will also be important to design specific measures to address crucial functional outcomes during key developmental windows.

Cross-cutting Issue: Infrastructure, Research Training, and Career Development

Although a strong cadre of research scientists is dedicated to increasing knowledge of *FMR1*-associated conditions, there is still a clear need for more scientists to build on current efforts. Because of the broad phenotypic implications of *FMR1* mutations and premutations, the next generation of scientists must be properly prepared with knowledge of a wide range of specialty conditions. Expertise is urgently needed in genetics, pediatrics, neurosciences, gynecology, fertility, stem cells, mental health, and other areas. Identifying and training promising researchers now will maximize their opportunities to capture and expand on the knowledge of senior researchers and clinicians in the field.

Moreover, the infrastructure that currently supports scientists in *FMR1*-associated condition fields may be insufficient to meet the needs for new animal models, tissue and cell culture studies, and larger scale human phenotyping and clinical studies at facilities that are accessible to families and patients. Supporting new collaborative efforts in these areas may require consortia, tissue and data banks, and enhanced training efforts.

Biomarker[7] development is also urgently needed to facilitate clinical trials in FXS. Biomarkers have the potential to help researchers define the scope of a clinical trial population, identify key time windows for intervention testing, and track disease progression. They have the potential to provide precise, objective measures that avoid many of the biases and pitfalls of caregiver or clinician reports. Because the FXS phenotype varies widely in severity and across functional domains in affected individuals, biomarkers to stratify subsets of patients or monitor specific disease features may be a crucial component of clinical trial design for many interventions. Studies involving human induced pluripotent stem cells (iPSCs) and molecularly accurate *in vitro* cell models, especially in conjunction with computational modeling, could help develop such biomarkers. The ideal set of biomarkers for use in FXS would:

- Correlate with behavioral and other consensus outcome measures
- Have the potential to identify subsets of individuals most likely to benefit from a particular intervention
- Be as noninvasive as possible, to facilitate use in young children and lower functioning individuals

[7] A *biomarker* is a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention, including therapeutic interventions. (BEST [Biomarkers, EndpointS, and other Tools] glossary)

- Have a strong link to clinical outcome measures identified as most meaningful to affected individuals and their families, including quality of life
- Be conserved across species, facilitating bidirectional translation of discoveries between humans and animal models

Goal 1.4: Characterize human phenotypes and risk factors across the lifespan

More precise, detailed, and comprehensive descriptions of the natural history of FXS should be developed to include characterization of the key genetic, epigenetic, environmental, and behavioral risk factors that influence the severity and progression of FXS at different stages across the lifespan.

More research is necessary to understand the trajectory and natural history of FXS across the lifespan. Currently, individuals with FXS are typically not diagnosed at the first sign of developmental delay. A standardized, validated battery of developmental tests for infants with FXS is necessary to obtain information on how the condition develops in the earliest stages of life. Pilot newborn and early infancy screening projects may enable researchers to identify FXS in infants and begin collecting information at earlier stages of development. Another approach could be to study children diagnosed with FXS at a very young age due to a family history of FXS or other *FMR1*-associated conditions.

The FXS phenotype is highly variable. Full genome sequencing of individuals with FXS may help to establish genotype-phenotype correlations. Comparing severely affected individuals with FXS to high-functioning individuals with FXS may be helpful in identifying risk factors, including modifier gene effects, epigenetic factors, and environmental influences. Another key challenge comes from accounting for the influence of educational, family, social, and community factors on cognitive and behavioral symptoms of FXS. Phenotyping studies of FXS should incorporate a wider range of physiological and behavioral measures. Whenever possible, multiple measures—such as imaging, electroencephalography (EEG)/event-related potential (ERP), quality of life instruments, and others—should be used simultaneously to facilitate validation and allow scientists to compare findings across studies.

The effects of environmental factors—including toxic exposures, diet, and social determinants of health—on the natural history of FXS also need to be better understood. The receipt of specialized medical care, special education services, and support for the transition from adolescence to adulthood should be better characterized because these factors are also likely to influence the FXS phenotype and response to interventions. Longitudinal studies of individuals with FXS will be important to help determine how individuals with FXS age, how their needs (and the needs of family caregivers) evolve over time, and how these patterns differ from individuals diagnosed

with ASD but not FXS. Such studies are particularly important for females with the full FXS mutation, for whom greater understanding of the variability in phenotype and natural history is urgently needed.

Finally, it is important to keep in mind that FXS impacts not only the individual with the mutation, but also family members, caregivers, educators, and others who play important roles in the lives of individuals with FXS. Better understanding of the impact of FXS on factors such as family cohesion and the mental health of family members is thus also an important area for future study.

Goal 1.5: Assess the safety and effectiveness of prevention and treatment interventions

The safety and effectiveness of prevention and treatment interventions—including pharmaceutical, behavioral, educational, and multimodal interventions—should be assessed across multiple domains in individuals with FXS, using rigorous clinical trial methods that include participants with a range of backgrounds, demographic characteristics, and comorbid conditions.

Randomized clinical trials provide the type of rigorous evidence that is crucial for achieving the long-term vision of this strategic plan. New therapeutic targets are likely to emerge from advances in basic research on FXS. Repair of the primary gene and/or gene product has the potential to address multiple disease mechanisms simultaneously, in contrast to downstream pathway-specific treatments. However, considerable uncertainty remains about the potential benefit and feasibility of genetic- or genomic-level interventions in FXS. Downstream treatments are much more likely to be feasible in the near future than gene repair or gene product repair. In particular, repurposing drugs that are approved by the U.S. Food and Drug Administration for other conditions may make additional treatments available for persons with FXS in a shorter time frame than is required for the development and testing of new agents. Such medications include mental health treatments like selective serotonin reuptake inhibitors, metabolic treatments like metformin, and others.

Educational and behavioral interventions—either alone or in combination with drug treatments—are also key areas for additional research. A variety of educational and behavioral interventions are commonly used in schools, families, and the community to improve cognitive and behavioral functioning for children with developmental delay, ASD, and other conditions similar to FXS. Most of these interventions have not been evaluated specifically in children with FXS, and many have never been subjected to rigorous scientific testing in any population. Therefore, rigorous trials are urgently needed to assess the potential benefits of such interventions for individuals with FXS.

The effectiveness of specific interventions may vary with age, developmental stage, and setting; well-designed studies are needed to identify the features critical for success. Some scientists have suggested that, while certain interventions did not seem to be effective in clinical trials, the same interventions might have been effective if administered earlier in life. In addition, participants in many previous studies, typically drawn from children seen in specialized clinics, are not necessarily representative of all individuals with FXS. Therefore, the results from these studies may not accurately reflect the potential impact of interventions on all people with FXS. These factors must be considered in the design and conduct of future trials.

Beyond developing interventions, educating parents, caregivers, and educators on the benefits of and how to implement interventions with fidelity is critical to improving the lives of FXS patients. Such educational and outreach efforts will help clinicians, parents, and educators implement the evidence-based interventions that are most likely to improve outcomes for their children.

Goal 1.6: Extend outreach, particularly to groups underrepresented in FXS research

Outreach efforts are needed to promote awareness and participation in FXS research, especially among groups currently underrepresented in FXS studies.

The FXS community includes families, clinicians, researchers, and advocacy organizations that are highly committed to supporting research and improving clinical care for people with FXS and their families. Researchers have expressed concern, however, that individuals typically seen in specialty clinics and enrolled in research studies may not be representative of the broader population with FXS. Participants in FXS research studies often have the most severe manifestations of the condition. They may also come from families with greater means to travel to specialty clinics or research study sites. Emerging health technologies to conduct in-home visits or testing may provide more access for individuals who have not previously been able or willing to participate in clinical trials. Early screening for pre-symptomatic individuals also occurs more often among families and communities with higher levels of educational attainment or income. Larger studies with participants from a more diverse range of backgrounds and symptom severity will be critical for understanding FXS from both a clinical and basic science point of view. Engagement of affected families from all backgrounds will also be crucial for disseminating promising research findings and promoting their uptake into routine clinical care.

Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

Many of the goals for FXTAS research reflect the need for more basic mechanistic knowledge regarding the condition, and for improved diagnostic and research tools to better characterize the population of affected individuals and lay the foundation for future studies.

Goal 2.1: Describe pathogenic mechanisms underlying FXTAS

Better understanding of the risk factors and pathogenic mechanisms that lead to the development of FXTAS is needed to identify promising targets for intervention.

Researchers have suggested a variety of mechanisms for FXTAS, including RNA sequestration, RAN translation, and DNA damage. However, the relative contribution of these mechanisms to the cellular and clinical manifestations of FXTAS is not fully understood. The relationship between FMRP levels and clinical phenotypes among people with FXTAS is also unclear, although data from animal studies suggest a continuing need for FMRP throughout life. Most people with the full *FMR1* mutation never develop FXTAS symptoms, so it appears unlikely that reductions in FMRP levels are a major cause of FXTAS symptoms; however, the relationship, if any, between FMRP and the development or progression of FXTAS is not yet understood.

Cross-Cutting Issue: Promoting Collaborations Between Basic Scientists and Clinicians

Clinicians in the community are crucial to the success of efforts to better understand, diagnose, prevent and treat *FMR1*-associated conditions. Improving collaborations with community-based providers may enable researchers to better understand phenotypes, document variations in how the disorder presents itself, identify potential biomarkers and outcome measures, and develop new interventions.

For FXTAS and FXPOI, community partnerships will be especially critical to raising awareness and encouraging participation in clinical research. For all *FMR1*-associated conditions, collaborations with the broad community of clinical providers are a critical step in ensuring that promising research findings are disseminated and implemented so that *all* affected individuals may benefit, not just those with the means and wherewithal to access care at academic medical centers.

Multiple cell types appear to be involved in the pathology of FXTAS—neurons, glia, microglia, and other cell types have been implicated—yet there is only limited evidence to identify how these cells are affected and how their functions are related to the FXTAS

phenotype. Mitochondrial dysfunction within various cell types is also thought to be related to FXTAS, but its role is not well understood. Also unclear is how methylation and mosaicism may affect FXTAS and other premutation-associated conditions. Basic research that sheds light on these processes is needed to build this body of knowledge and lay the groundwork for future therapies.

Recent insights from imaging studies in Alzheimer's disease and related neurological conditions have explored deposition and ubiquitination (degradation) of specific brain proteins and highlighted their role in the pathophysiology and progression of disease. Because FXTAS shares some symptoms with these conditions, exploring whether similar mechanisms are operating in FXTAS could be illuminating.

These potential pathways are not mutually exclusive, and any or all may play a role in the development, severity, and/or progression of FXTAS. Synergistic effects among various disease mechanisms warrant investigation as well. In addition, genetic and environmental risk factors may interact to affect the development, severity, and progression of FXTAS, but little is known about what these factors are or how they interact. Understanding the contributions of different risk factors and disease mechanisms to FXTAS may aid efforts to identify promising therapeutic strategies, such as gene reactivation or other gene-editing approaches, and approaches that capitalize on targeting RNA mechanisms or RAN translation.

Goal 2.2: Develop precise, easily measurable diagnostic criteria

Precise, easily measurable consensus diagnostic criteria for FXTAS should be developed to accurately distinguish FXTAS from other neurological conditions with similar symptoms.

Motor problems, especially tremor and gait ataxia, are key early symptoms of FXTAS, and many FXTAS symptoms are shared with other neurological diseases, like Parkinson's disease and amyotrophic lateral sclerosis. However, FXTAS differs from other disorders in how the symptoms manifest; for example, tremor is often milder in FXTAS compared with Parkinson's disease, but affected individuals have more bradykinesia (slowness of movement) and rigidity. Some clinicians have observed subtle differences in sway in FXTAS individuals compared to individuals with other neurological disorders. The onset of FXTAS is also difficult to detect. By the time they are seen in a clinic, men and women with FXTAS have typically exhibited symptoms for some time, and many have been initially diagnosed with other conditions. While neuroimaging is often abnormal in FXTAS, imaging findings are often nonspecific. The frequent co-occurrence of psychiatric and cognitive features also poses challenges for clinicians and researchers seeking to document the FXTAS phenotype.

Improved diagnostic criteria must be a high priority for FXTAS research. Better diagnostic criteria will help ensure that individuals participating in clinical studies have the *FMR1* premutation and manifest the symptoms and brain abnormalities characteristic of FXTAS. Research advances in describing the FXTAS phenotype, documenting neuroimaging findings, and improving symptom measurement can facilitate the development of more explicit diagnostic criteria, which will, in turn, require validation and refinement. Ultimately, better diagnostic criteria will increase identification of individuals with FXTAS who are currently undiagnosed or misdiagnosed, facilitate diagnosis at earlier stages of disease, and increase the pool of potential participants for clinical FXTAS research.

There is also a significant need for biological assays that can be used for diagnostic purposes, but such assays require better understanding of the mechanisms behind the disease. Researchers report that promising neuroimaging and behavioral biomarkers may be able to predict FXTAS even before clinical symptoms appear, but these need to be further refined, tested, and validated. Additionally, including multiple elements—e.g., imaging, behavior, genetics, postmortem pathology—in the diagnostic criteria could be helpful for recognizing the wide variability in how individuals present with FXTAS, and perhaps help pinpoint individuals most likely to benefit from specific interventions.

Goal 2.3: Develop and validate biomarkers and outcome measures

Physiologically relevant, clinically significant, feasible biomarkers and outcome measures that can accurately assess disease progression and response to intervention in FXTAS should be developed and validated for use in clinical trials.

Many measures of motor function used in FXTAS studies were originally developed for other conditions. Motor function measures that are more specific to FXTAS, or that are tested and validated specifically in FXTAS populations, would be helpful both for clinicians and for researchers developing clinical trials to assess intervention effectiveness. The same is true for FXTAS behavioral and cognitive function measures in that most were originally designed and tested in individuals with other disorders.

To assess the effectiveness of interventions for FXTAS, better tools are needed to more precisely measure the onset, symptoms, and progression of the condition. Quality-of-life instruments used in individuals with neurological or motor function diseases have been applied in FXTAS, but additional work is needed to ensure that these instruments are truly valid and reliable in the FXTAS population. Without such measures, clinical trials in FXTAS may be difficult to interpret or may even be misleading.

Goal 2.4: Characterize human phenotypes and risk factors across the lifespan

More precise, detailed, and comprehensive descriptions of the phenotype and natural history of FXTAS should be developed to include characterization of the key genetic, epigenetic, environmental, and behavioral risk factors that influence the severity and progression of the FXTAS phenotype, including the role of the size and structure of the FMR1 premutation in FXTAS onset and progression.

FXTAS symptoms often appear when individuals are in their 50s, and the mean age of diagnosis for individuals with FXTAS is around 64 years. Data from brain imaging studies and patient histories suggest that FXTAS-induced changes in the brain and central nervous system occur long before symptoms become apparent. Establishing pre-symptomatic features of FXTAS could be especially helpful for understanding the progression of the disease and for diagnosis.

Because progression of the FXTAS phenotype is highly variable, some researchers have proposed different subtypes of FXTAS based on the degree of tremor or cognitive decline. Some manifestations—particularly psychiatric disorders, such as anxiety disorders and depression, as well as agitation—occur frequently but are not specific to FXTAS. Most of the available phenotypic information on FXTAS comes from convenience samples of participants drawn from specialty clinics. These individuals are predominantly male, reflecting the higher occurrence of FXTAS in men relative to women. As a result, FXTAS in women is not as well understood, and sex differences in FXTAS require further study.

Not all premutation carriers develop FXTAS, and it is unclear which risk or protective factors account for variations in penetrance or rate of progression. Natural history studies of FXTAS are necessary to describe its penetrance by symptom, sex, repeat size, and age, and to identify the role of genetic, epigenetic, environmental, and social exposures in its emergence and progression. It is especially important for such studies to actively recruit diverse populations because participants in previous studies have been disproportionately white, urban, and from higher income families. Moreover, many FXTAS studies have been short, focusing on short-term symptoms and outcomes. Longitudinal research in a cohort of participants would be especially helpful to illuminate factors associated with risk for FXTAS over the lifespan, and to help characterize disease trajectories in premutation carriers.

Understanding the natural history of FXTAS is also critical to the development of effective therapies. In animal models, FXTAS is a progressive neurological disease that leads to death, but FXTAS does not seem to progress in a similar way in humans. There is a crucial need for more research to characterize the relationship between underlying pathogenic mechanisms and disease progression. Genetic and epigenetic factors are

thought to play roles in disease progression, but the roles of these factors have not been effectively replicated in animal models. This suggests that mechanistic research on FXTAS may ultimately rely less on animal models and more on research conducted in banked human samples or human iPSCs.

Goal 2.5: Assess safety and effectiveness of prevention and treatment interventions

The safety and effectiveness of interventions approved to treat other neurological conditions should be assessed in individuals with FXTAS using rigorous clinical trial methods that include participants with a range of backgrounds, demographic characteristics, and comorbid conditions.

For many neurological conditions that share features with FXTAS, various interventions have proven helpful in managing symptoms. For example, scientists have recently developed new drugs to address the symptoms of Alzheimer's disease, Parkinson's disease, multiple sclerosis, and other disorders. Physical therapy and lifestyle changes also have demonstrated effectiveness in improving function and/or quality of life in several neurodegenerative disorders. FDA-approved medications exist for other conditions with overlapping symptoms. Testing these interventions in individuals with FXTAS may yield helpful insights into disease management and shorten the time required to develop new treatments. Once natural history studies have been conducted to establish a baseline for FXTAS progression, testing in individuals with early-stage FXTAS could determine whether these interventions can provide symptomatic relief or slow motor, cognitive, and psychological declines.

Goal 2.6: Extend outreach to the community

Outreach efforts should seek to inform providers, families affected by FMR1-associated conditions, and the public about FXTAS and encourage participation in FXTAS research studies.

Although scientists believe that a high percentage of premutation carriers may eventually develop FXTAS, as noted previously, many individuals with FXTAS are likely to go undiagnosed or be misdiagnosed. FXTAS is not well known among clinicians and the public, and many people with FXTAS present to specialty clinics only after having lived with FXTAS symptoms for some time.

Additional outreach, community engagement, and communication activities are needed to boost awareness of FXTAS, which, in turn, may allow for earlier diagnosis and treatment and encourage participation in research. These efforts could begin by targeting families affected by *FMR1*-associated conditions; community health providers, including specialists who are likely to encounter FXTAS in their practices; and state and

public health officials who are involved in providing health information to the public. Advocacy groups are critical partners in these efforts.

Fragile X-Associated Primary Ovarian Insufficiency (FXPOI)

The scientific evidence base for FXPOI is far more limited than the evidence base for FXS or even FXTAS. As a research topic, FXPOI presents many of the same scientific challenges as FXS and FXTAS research, but even less is known about the underlying mechanisms and how FXPOI can be distinguished from other causes of ovarian insufficiency. Consequently, understanding FXPOI mechanisms and improving diagnostic accuracy will be essential before large-scale human studies are feasible.

Goal 3.1: Identify the mechanisms leading to ovarian dysfunction

Better understanding of the risk factors and mechanisms by which the FMR1 premutation leads to ovarian dysfunction is needed to identify promising targets for intervention.

The mechanisms by which the *FMR1* premutation impacts ovarian function are largely unknown. Basic science research is needed to better understand the developmental origins of ovarian dysfunction and how premutation-associated products, or even FMRP itself, may interact with known ovarian processes and associated tissues or organ systems (e.g., oocytes, granulosa cells, stromal cells, and the hypothalamic-pituitary axis).

Natural history studies will be especially important in identifying the role of specific genetic and environmental factors in the development of FXPOI. Some potential factors to evaluate are *FMR1*- and FMRP-related factors (e.g., repeat structure, mRNA, FMRP pathways), epigenetic factors (e.g., methylation), modifying genes (e.g., interactive versus additive genes, variants associated with age at menopause or POI), and environmental factors (e.g., smoking, endocrine disruptors, body-mass index, occupational exposures, stress). Knowledge about these factors' involvement could allow scientists to create a predictive model of FXPOI.

Goal 3.2: Develop biomarkers to facilitate early diagnosis and risk stratification

Biomarkers should be developed that can distinguish FXPOI from other conditions at early stages of ovarian insufficiency, and that can predict which premutation carriers are at higher risk for FXPOI.

Researchers agreed that there is an urgent need to be able to identify women with FXPOI at much earlier stages of ovarian insufficiency. Currently, ovarian insufficiency

has often progressed to an advanced stage before women are referred for the genetic testing that reveals the presence of the *FMR1* premutation. Knowing their risk for FXPOI could be useful for women with the premutation in making family planning decisions. Although some biomarkers for FXPOI have been proposed, these biomarkers are all affected by age—for example, anti-Müllerian hormone, which is measured differently in infancy than later in life; and antral follicle count, which is typically too invasive for teens. Noninvasive and cost-effective biomarkers for FXPOI that are detectable and clinically relevant need to be identified and validated in representative community samples. Such biomarkers could aid in tracking the progression of the disease, screening individuals for FXPOI to assess public health impact, and identifying participants for inclusion in human studies.

Cross-Cutting Issue: Ethical, Legal, and Social Issues in Premutation Screening and Testing

Advances in technology have rapidly increased the use of genetic screening and testing. Carrier screening and genetic sequencing for *FMR1*-associated conditions raise many ethical and social issues, including (among others):

- Ethical issues related to prenatal diagnosis
- Diagnosis of conditions with limited or no treatment options
- Ethical issues related to identifying risks for other family members based on screening of one relative
- Ethical implications of correcting human disease via gene editing technologies that impact the germline and future generations

It will be critical for researchers on *FMR1*-associated conditions to be cognizant of these ethical issues, and to incorporate them into the design and conduct of future research.

Goal 3.3: Extend outreach to the community

Outreach efforts should seek to inform providers, families affected by FMR1-associated conditions, and the public about FXPOI and encourage participation in FXPOI research studies.

The prevalence of FXPOI in the community is unknown, in large part because of the difficulties in distinguishing between FXPOI and other conditions involving ovarian insufficiency. FXPOI is also not as well understood among gynecologists and fertility specialists as other conditions, and clinicians' primary concern may be less focused on etiology and more on infertility solutions. As a result, the population of individuals available for clinical studies is limited, and likely not representative of the FXPOI population as a whole.

Additional outreach, community engagement, and communication activities are needed to increase awareness of FXPOI and to encourage participation in research. These efforts could begin by targeting families affected by *FMR1*-associated conditions, gynecologists and fertility specialists who are likely to encounter FXPOI in their practices, and advocacy groups who are involved in providing women's health information to the public. These stakeholders will also be critical participants in any subsequent effort to develop and implement consensus guidelines for clinical diagnosis, referral, and management of FXPOI.

FMR1 Premutations

Although *FMR1* premutations have been clearly identified as conferring risk for FXTAS and FXPOI, the full range of *FMR1* premutation effects remains undefined. For example, some researchers have suggested that premutations may be associated with certain mental health conditions, including anxiety and depression. Scientists are still far from a complete understanding of how *FMR1* premutations affect human health and disease.

Goal 4.1: Understand the stability of the premutation

Better understanding of the stability of the FMR1 premutation over the lifespan is needed, as well as better understanding of the role of stability in health and disease.

Researchers have suggested that *FMR1* premutation size may not be the only factor important for understanding *FMR1*-associated conditions; its stability over time and through human developmental stages may also play a role. However, this theory remains largely unexplored. To investigate the concept, researchers will need to obtain genetic data from a sizeable number of premutation carriers on a repeated basis. If stability of the premutation turns out to play a significant role, design and implementation of interventions will also need to account for this information.

Goal 4.2: Develop technologies for carrier testing and premutation screening

Advanced technologies are needed that improve the accuracy, reliability, accessibility, and information value of FMR1 premutation carrier testing and screening.

Geneticists and experts in *FMR1*-associated conditions have suggested that more widespread screening for *FMR1* premutations and testing of oocytes from known *FMR1* premutation carriers may provide helpful information that could be applied to individuals affected by *FMR1*-associated conditions. However, such a program would require

efforts to improve the accuracy, consistency, and reliability of premutation screening. Additional calibration and validation efforts would be needed.

A broader testing and screening program would also require researchers across the range of *FMR1*-associated conditions to increase the practical value of screening knowledge for the person being screened. Currently, once individuals learn that they carry an *FMR1* premutation, few practical steps are available to help them manage or even understand their health risks. As the knowledge base for the premutation expands, scientists will need to provide clear, useful, scientifically sound information about new discoveries and the accompanying risks for *FMR1* premutation carriers.

A broad array of clinical tools is needed to fully uncover the implications of the *FMR1* premutation. Large databases and/or biobanks with detailed data on a large group of premutation carriers could inform phenotyping and mechanistic research on a range of associated disorders. Biobanks and natural history projects could shed light on understanding the progression of *FMR1*-associated conditions, as well as on factors that contribute to the variability in how these conditions lead to different phenotypes across individuals. Initiatives to identify gene-behavior connections and to better understand premutation biomarkers and phenotypes would have wide-ranging implications for clinical research.

Goal 4.3: Characterize premutation-associated conditions and risk factors

More comprehensive characterization of the conditions associated with FMR1 premutations is needed, including better understanding of the roles of repeat size, biological sex, other genetic and epigenetic factors, and environmental and behavioral risk factors in the onset and progression of symptoms.

Because *FMR1*-associated conditions can appear later in life and are associated with such a wide variety of issues, families face considerable uncertainty in the context of diagnosis. Families that include premutation carriers often want information on their risks of developing a particular condition (e.g., FXTAS and/or FXPOI), which symptoms to watch for, and how to help prevent the condition—but those answers are not currently available. Family support is essential, yet there is very limited research data to help guide choices about which types of support can be most helpful. Women with *FMR1* premutations who are caring for a child with FXS may also experience adverse health and mental health effects, but the extent to which such effects are attributable to the premutation (above and beyond the stresses of caring for a child with FXS) is unclear.

While existing animal models have been helpful, they have limited utility in uncovering molecular mechanisms, especially for premutation-associated conditions. Existing animal models have also proven insufficient to study disease onset, progression, and

psychological symptoms often found in premutation carriers. Whether knockout models can be applied to expansion-related premutation conditions needs to be clarified and, if necessary, new animal models should be developed to mimic premutation expansions. A wider array of species for animal models should also be investigated, including non-human primates and other mammalian models. Greater insight into reported inconsistencies in findings among animal models and human studies might be gained by comparing results across different animal models.

Because the role of repeat size in *FMR1*-associated conditions is ambiguous, further research on individuals in the so-called "gray zone" would be helpful in establishing its precise role in symptom onset and severity. Work is still needed to determine the best way to measure the size of the premutation and the characteristics of individuals in the gray zone. Some researchers have suggested looking at the repeat spectrum as a continuous variable, rather than calling out the gray zone. A spectrum approach could be applied to both clinical features and the size of the expansion.

Conclusion

Mutations in the *FMR1* gene, on the X chromosome in humans, are known to result in three specific medical conditions—FXS, FXTAS, and FXPOI—and could be associated with other health conditions and symptoms. NIH supports and conducts research on the causes, mechanisms, diagnosis, treatments, and management of *FMR1*-related conditions as well as concomitant symptoms observed in individuals with these conditions.

This strategic plan was developed by the Trans-NIH Fragile X Coordinating Committee with significant input from the scientific community outside of the NIH, alongside key organizations that include researchers involved in this area and advocates for individuals with *FMR1*-associated conditions and their families. The plan will provide guidelines for prioritizing and coordinating future NIH research related to *FMR1*-associated conditions. The plan will also help NIH communicate its priorities for research related to these conditions to the wider scientific community and hopefully encourage the submission of new research applications specific to these priorities.

Although NIH's long-term vision involves new prevention and treatment interventions to reduce the public health effects of *FMR1*-associated conditions, a path toward this vision will require a sustained effort to support basic, translational, and clinical research. The wide-ranging features of these conditions continue to challenge funding agencies, researchers, and advocates; different types of expertise must work together to effectively support affected individuals and their families.

Acknowledgements

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Appendix I: Summary of Responses to Request for Information (RFI)

To gather public feedback, NIH published a guide notice requesting information on future directions for research on Fragile X syndrome and *FMR1*-related conditions (NOT-HD-17-033). The notice was open for comments between January 29 and March 2, 2018. This RFI requested comments and suggestions from researchers in academia and industry, health care professionals, patient advocates, representatives of health advocacy organizations, members of scientific or professional organizations, and other interested members of the public. This section contains a summary of the ideas from 47 responses to this RFI.

Common Themes

These themes were highlighted in each group by multiple people. To avoid repetition, these items are not listed separately in each subgroup.

Key Research Needs Across FMR1-Related Conditions (FXS, FXTAS, FXPOI)

- More research into gene editing/gene reactivation/gene therapy, including CRISPR technology (single most common response)
    - Better animal models, including non-human primate models
    - More longitudinal/lifespan research
    - A set of validated, consensus biomarkers that:
        - Are conserved across species
        - Can be used to stratify participants in clinical trials
        - Are specific to targeted treatments
        - Are valid over both the short and long term
    - A set of validated, consensus outcome measures, specific to each disorder, that are suitable for use in observational studies and clinical trials
    - Better understanding of the inconsistencies in reported findings among animal model and human studies
    - More effective support strategies for families affected by FXS and other *FMR1*-related conditions
    - Additional biobanks and shared databases
    - More cross-cutting research across *FMR1*-related conditions

- Stronger and more frequent collaborations between researchers working on *FMR1*-related conditions and scientists investigating other neurological disorders
- Stronger and more frequent collaborations, through consortiums and partnerships, that bring together basic research, clinical research, and families living with FXS
- More opportunities for families to have input into research

Condition-Specific Feedback

Similar comments have been combined to avoid repetition.

Fragile X Syndrome (FXS)

1. What are the most important questions for FXS research to address?
    - Understanding the degree to which brain and behavioral changes are reversible in individuals with FXS, and how this may be influenced by developmental trajectory
    - Identifying new molecular pathway/circuit mechanisms in FXS
    - Establishing the links among *FMR1* mutations, loss of FMRP, and cellular, network, and cognitive deficits
    - Exploring the potential for transcriptomics and proteomics for FXS research
    - Understanding the effects of animal strain and sex differences in preclinical models
    - Developing a better understanding of how gene/environment interactions (e.g., exposures to pesticides, stress) affect FXS
    - Within epidemiological studies, focusing on metabolic defects associated with FXS
    - Examining how FXS progresses across the life course, using longitudinal and brain imaging studies
    - Expanding research into older adults with FXS
    - Examining heterogeneity in illness expression in both mice and humans, and their biological substrates
    - Targeting novel disturbed cellular pathways to increase success in moving from animal to human models
    - Determining how to replace critical functioning of FMRP lost in the disease

- Determining if cognitive, adaptive, and/or linguistic development in FXS can be accelerated through certain educational, cognitive, or behavioral therapeutic approaches
- Establishing which currently used interventions are most effective in promoting development and reducing behavioral and clinical problems
- Conducting clinical trials to determine whether medications already approved for other indications (such as metformin or certain mental health medications) can be useful treatments for individuals with FXS
- Developing new targeted interventions
- Identifying the most critical developmental time frames for interventions, with a special focus on early life

2. What tools, resources, and partnerships are needed to address these questions?
    - Animal models that are more molecularly accurate (including non-human primates)
    - Established primate models where animals are housed in social groups
    - Cellular models (e.g., human Embryonic Stem Cell [hESC] or human iPSC models, organoids) that are more molecularly accurate
    - More computational models
    - Tools to remove FMRP from specific cell types from specific brain regions and at specific developmental time points
    - Support for clinical resources that support research (e.g., supplemental support for CDC-funded clinics)
    - More collaboration among funders, particularly public private partnerships
3. What are the most significant scientific and clinical advances in the field that can inform future progress toward these questions?
    - Successful translational electrophysiological research
    - Single-cell transcriptomics
    - Advances in machine learning
    - Large-scale studies of early phenotypes
    - Growing evidence for brain maturational profiles may provide rationale for early intervention
    - Better understanding of the impact of treatment earlier in childhood, compared with treatment received only when children are older
    - Treatment studies examining efficacy of non-medication interventions

- Studies of other neurodevelopmental disorders with lower FMRP levels could be expanded, and clarified with valid FMRP measurements, to inform understandings of commonalities with FXS
- Studies of gene reactivation in interventions for other disorders (e.g., spinal muscular atrophy, Angelman syndrome)
- Schizophrenia research has created a large-scale human patient iPSC lines involving multiple academic and industry institutions, and has made these cell lines available to other researchers
- Improved understanding of functioning in normal brains

Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

1. What are the most important questions for FXTAS research to address?
   - Specifying the pathogenic mechanism underlying FXTAS
   - Identifying the factors that determine when the premutation progresses to FXTAS
   - Describing how different mechanisms that have been proposed in FXTAS might fit together, focused on what happens first, and why
   - Identifying the role of FMRpolyG in FXTAS
   - Describing the role of methylation mechanisms in the development and progression of FXTAS
   - Understanding mosaicism
   - Understanding the onset and progression of FXTAS through biomarkers, with a specific focus on developmental aspects of FXTAS
   - Delineating key genetic risk factors
   - Developing targeted therapeutics based on pathogenic targets
2. What tools, resources, and partnerships are needed to address these questions?
   - Computational models
   - Refined and validated FXTAS rating scale and behavioral measures
3. What are the most significant scientific and clinical advances in the field that can inform future progress toward these questions?
   - Discovery of repeat-associated non-ATG (RAN) translation and its role in neurological disease
   - New imaging technology and methodologies

- Better understanding of how the number of AGG interruptions in the CGG repeat affects stability
- Insights from new methods in autism research, including optogenetics and machine learning
- Insights from Alzheimer's research, especially progress in imaging studies that explore deposition of amyloid and tau and their role in pathophysiology and progression of disease. This could be applied to consider a potential role in FXTAS for the deposition of other proteins.
- Insights from Parkinson's disease and other neurological disorders, including a pattern of increased and decreased intensity on Positron Emission Tomography (PET) scans. This could be applied to see if specific patterns are associated with FXTAS.

Fragile X-Associated Primary Ovarian Insufficiency (FXPOI)

1. What are the most important questions for FXPOI research to address?
   - Delineating the impacts of the mutation and premutation on oogenesis
   - Defining basal reproductive functions for ovarian FMRP
   - Identifying regulators of the FMRP, and assessing whether alterations to levels of those regulators could cause POI
   - Identifying the molecular mechanism(s) that leads to infertility in *FMR1* female carriers
   - Identifying the underlying cause of oocyte loss
   - Determining factors that affect expansion and contraction of the premutation
   - Evaluating inhibitors/activators of identified pathways that are altered by reduced FMRP on the ovary
   - Determining whether there is an additive ovarian impact of obesity for premutation carriers and individuals affected by FXS and/or FXPOI
2. What tools, resources, and partnerships are needed to address these questions?
   - Conditional alleles in mice, to help understand oocyte loss
   - Follicle stage-specific knock in and knock out models
   - Cultured human ovarian cell lines, of various repeat lengths
   - Funding specific to FXPOI research
   - Increased utilization of pluripotent stem cells in FXPOI-related research

3. What are the most significant scientific and clinical advances in the field that can inform future progress toward these questions?
    - Better understanding of epigenetic alterations, including those that could be inheritable
    - Non-coding RNA contributions to *FMR1* related conditions
    - Discoveries in other repeat disorders, including FXTAS, can help inform FXPOI research

FMR1 Premutation

1. What are the most important questions for FMR1 premutation research to address?
    - Determining if there is evidence that gene and environment interactions affect premutations
    - Establishing mechanisms of tri-nucleotide expansion and normal functioning FMRP
    - Identifying the best ways to deliver targeted epigenetic treatments to the human brain
    - Identifying, through epidemiological studies, the extent of co-occurring symptoms of premutations
    - Establishing better ways to measure in living persons and their tissue the pathological processes that occur in carriers
    - Improving methods to identify premutations across the life course
    - Better delineate the association between characteristics of premutations and premutation carriers and the development and progression of both *FMR1* related conditions and comorbid symptoms, including mental illness
    - Determining if caring for a child with FXS creates additional risk for medical and/or mental illnesses in premutation carriers
2. What tools, resources, and partnerships are needed to address these questions?
    - More sophisticated pre-clinical models for screening potential therapeutics
    - More sophisticated and affordable circuit analysis tools and cognitive/behavioral assays for measuring the effectiveness of proposed therapies in mouse models
    - Novel approaches for population screening

3. What are the most significant scientific and clinical advances in the field that can inform future progress toward these questions?
    - Moving beyond a categorical understanding of premutations, toward a spectrum approach
    - Improved understanding of linear and non-linear associations of premutation biomarkers and phenotypic characteristics
    - Initiatives to identify gene-brain-behavior connections

Appendix II: NIH Workshop on Updating the Fragile X Research Plan

March 8-9, 2018

Bethesda, Maryland

Attendees

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