Eunice Kennedy Shriver
National Institute of Child Health and Human Development
National Institutes of Health

Trans-NIH Fragile X Research Coordinating Committee
Research Plan for Fragile X and Associated Disorders

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Bethesda, MD

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Welcome
Yvonne Maddox, Deputy Director, Eunice Kennedy Shriver *National Institute of Child Health and Human Development (NICHD)*

Dr. Maddox greeted the participants and thanked them for their contributions to this endeavor. She acknowledged the unique opportunity presented by NICHD's being part of the leadership team. Many grant applications have come into NICHD related to fragile X syndrome (FXS) and *FMR1* gene expression, a reflection of their increasing importance to the research community as well as the people affected by fragile X disorders and their families. She spoke about the role of the trans-NIH Fragile X Research Coordinating Committee in organizing this meeting, the importance of having Congressional interest as an impetus for action, and the encouragement and support of fragile X advocacy groups, here and abroad.

The research agenda should serve as a means to fill gaps in knowledge about the spectrum of fragile X disorders. It is up to NIH to find ways to better coordinate activities, find ways to encourage Institutes and Centers (ICs) to collaborate amongst themselves as well as with other federal agencies, and explore setting up private-public partnerships with advocacy groups.

Introductions and Goals for the Meeting
Tiina K. Urv, NICHD

Dr. Urv acknowledged the various Institutes represented on the trans-NIH Fragile X Research Coordinating Committee (NICHD, the National Institute of Mental Health [NIMH], National Institute of Neurological Disorders and Stroke [NINDS], National Institute on Aging [NIA], National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], and National Institute of General Medical Sciences [NIGMS], and the NIH Library), which helped organize this meeting. The planning for this meeting started in March of last year. She thanked the scientists and partnering Institutes, as well as the Health Resources and Services Administration (HRSA), FRAXA, and the NIH Library staff.

The purpose of the meeting is to develop research objectives to incorporate into a report to Congress pursuant to S. Rept. 110-117:

> The Committee is encouraged by the efforts of all the Institutes supporting Fragile X research to coordinate Fragile X research and encourages them to intensify and expedite this effort. The Committee urges the NIH, through the NICHD and other participating Institutes, to convene a scientific session in 2008 to develop pathways to new opportunities for collaborative, directed research across Institutes, and to produce a blueprint of coordinated research strategies and public-private partnership opportunities for Fragile X. The Committee requests the NICHD to report to Congress by September 1, 2008, on its progress in achieving these goals.

Dr. Urv envisioned the outcome of this meeting as a report that will serve as a living document—a research framework to guide future research. The report must be based on sound scientific knowledge in each area of research: fragile X full mutation, fragile X–associated tremor/ataxia syndrome (FXTAS), and fragile X primary ovarian insufficiency (FXPOI). The idea is for NICHD to work collaboratively with and garner buy-in from other NIH ICs, the scientific community, additional federal agencies, and constituency groups. Another goal is to identify obstacles to collaboration, such as the failure to take full advantage of ongoing or completed research.

The three working groups comprise representatives of advocacy groups, the Centers for Disease Control and Prevention (CDC), HRSA, the research community, and the Fragile X Research Coordinating Committee. NICHD's goals are to provide the working groups with opportunities to work face to face and equip them with the resources they need to develop research goals and objectives.

Collaborative Efforts at NIH
Linda Brady, Director, NSC, NIMH

Dr. Brady provided background about fragile X syndrome and associated disorders. The most common cause of inherited mental retardation, it affects 1 in 4,000 males and 1 in 8,000 females. It is characterized by cognitive, behavioral, and emotional dysfunction. Fragile X mutations have also been identified in some individuals with autism spectrum diseases.

Fragile X syndrome stems from a triplet nucleotide (CGG) repeat expansion in the 5′ untranslated region of the *FMR1* gene. The full mutation (> 200 repeats) is characterized by transcriptional silencing of the gene, functional loss of FMRP protein, and dysregulation of glutaminergic synaptic plasticity. Premutation carriers (55–200 repeats) have a smaller number of repeats and are at risk for primary ovarian insufficiency (POI, formerly termed premature ovarian failure, POF) and FXTAS.

NIH is particularly interested in the neurobiology of FXS and autism, including their etiologies, pathophysiology, and treatment in terms of what might be done to reverse the effects of the genetic defect. Investigators are studying cellular and molecular targets as potential bases for intervention. Model systems and organisms, even a zebrafish model, have been fruitful. Imaging studies have provided information about how the *FMR1* mutation affects brain structure and function, including learning and memory, cognition, and executive function. Biomarkers are

being sought as a means to develop targeted therapies and gauge treatment response. Studies of genetics and epigenetics are critical to understanding the expression of the disorder.

Dr. Brady highlighted several conferences dedicated to advancing the field of FXS study. NIH has provided significant support for the Banbury Center conferences on fragile X as well as the Shared Neurobiology of Autism and Related Disorders Conference.

Dr. Brady then spoke about the NIH blueprint for neuroscience research. This is existing infrastructure; more than 16 ICs are supporting the development of tools and resources, such as high-quality monoclonal antibodies for studying involved molecules (http://neuroscienceblueprint.nih.gov). The blueprint theme for 2007 was neural development.

The blueprint theme in 2008 is neuroplasticity. Initiatives related to this theme are the development of probes, instruments, and other tools for study and manipulation of neural plasticity. Another initiative is creating a set of gene expression data in the developing rhesus macaque brain. The resulting gene expression map may provide a means for comparisons with other species (e.g., mouse, human).

Dr. Brady offered a compelling example of a collaboration to translate research into treatment. Dr. Randall Carpenter is exploring rationally based pharmacologic treatment with the potential to rescue cognitive and behavioral deficits based on studies in model organisms (mouse, fly). Under study is the development of metabotropic glutamate receptor subtype 5 (mGluR5) antagonists to treat fragile X syndrome and autism (U01 MH78270). The research is being supported by NIMH, NICHD, NINDS, the Fragile X Research Foundation, and Autism Speaks/CAN. This translational research should enable clinical studies to test the hypothesis that mGluR5 antagonists are an effective treatment for fragile X syndrome.

Dr. Brady described the mGluR5 theory of FXS (Figure 1). mGluR5 acts in a manner opposed to fragile X protein (FMRP). In FXS, FMRP is absent; unchecked mGluR5 translation causes an altered dendritic structure in the brain, suggesting that excess mGluR5 gene product plays a role in the fragile X phenotype. By interrupting the mGluR5 pathway, it has been possible to reverse some phenotypical defects in animal models.

Dr. Carpenter's group has obtained an mGluR5 compound (STX107) and is seeking to obtain an IND to test it in human subjects. Several back-up compounds are at the ready in case issues arise in terms of bioavailability or toxicities. The STX compound is ready for pre-IND studies. By the end of this year, the researchers should be ready to test safety, tolerability, and pharmacokinetics in healthy, normal subjects. In response to an audience member's question, Dr. Brady said that cardiotoxicity studies, as well as other toxicity studies, were negative.

Dr. Brady expressed a desire to see science serve as the driver behind the recommendations emanating from this meeting.

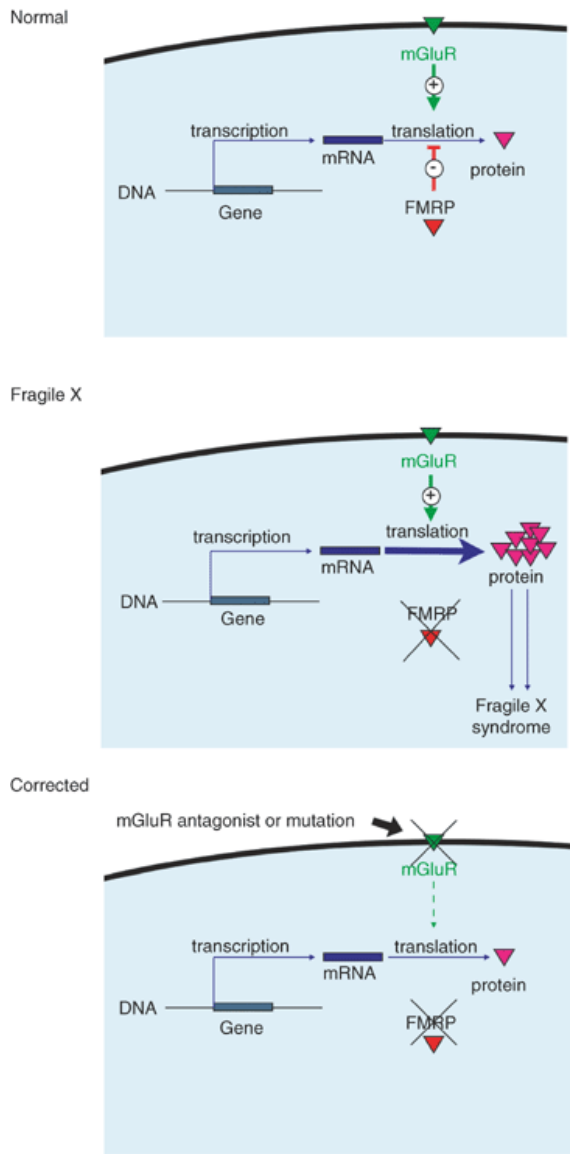

Figure 1. Model for the pathogenesis and correction of FXS
mGluR5 and FMRP regulate translation of mRNA at the synapse in a functionally opponent manner—mGluR5 activation initiates protein synthesis and FMRP suppresses it. In the absence of FMRP, as is the case in FXS, mGluR5-dependent protein synthesis proceeds unchecked, and consequent excessive translation leads to the diversity of clinical features that make up the syndrome (learning and memory deficits, seizures). Our results demonstrate that this progression can be corrected by genetic reduction of mGluR5 activity. (Dolen & Bear, J Physiol 2008)

Report to Congress—What Does That Mean?
George Gaines, Chief, Office of Program and Public Liaison, NICHD

Mr. Gaines explained the genesis of a report to Congress that will flow from this meeting and the work of the coordinating committee. When a bill comes out of committee for consideration by the full House or Senate, it must be accompanied by a bill report. Such a report provides a rationale for that particular legislation to help members take an informed position for voting. Most commonly, the language comes from advocacy groups and individuals lobbying on issues important to them. Both FRAXA and the National Fragile X Foundation (NFXF) are strong

proponents of research in fragile X and supported the language calling for a research plan and a report.

With regard to S. Rept. 110-117, the Senate committee requested a report by September 1, 2008, on progress toward the stated goals. The report will be drafted by NICHD staff with input from the assembled experts. It will then be circulated by the NIH Office of the Director to all relevant parts of NIH for comment. After NICHD revises the report, it will go through a clearance process in the Department of Health and Human Services (HHS) and be sent for parallel review by the Office of Management and Budget as well as the Executive Office of the President. From there, it will be forwarded to Congress for consideration by the appropriate appropriation and authorization committees, which are responsible for establishing and funding programs, respectively.

Mr. Gaines said in closing that he hopes that Congress is interested in providing adequate resources for this type of research. It is also possible that Congress could take other action, such as forming a Congressional caucus for discussing FXS. He expressed optimism in light of Congress's expression of interest in this area.

Successful Experience—Muscular Dystrophy Report
John Porter, Program Director, NINDS

Dr. Porter reported on a NINDS Congressional report in the area of muscular dystrophy (MD). He spoke about what his group learned during the planning process for developing an action plan for MD. They started with the Muscular Dystrophy Coordinating Committee (MDCC) and developed an action plan to drive MD activities. The key, according to Dr. Porter, is collaboration. He emphasized the need to look broadly at partnerships.

The MDCC is a Federal Advisory Committee Act (FACA) committee, a designation that entails certain requirements (e.g., notice of meetings in the *Federal Register*). Included are 15 members, 10 from NIH and other federal entities, 3 from major advocacy groups, and 2 with MD, 1 of whom participated in a trial of an experimental adenoviral-mediated gene therapy. Patient participation brings an interesting perspective to the MDCC's work. The diverse composition of the MDCC, with many independent players, meant that no one person or group could predominate. The report was a means to coordinate activities and stimulate people to think beyond NIH to increase planning breadth and specificity. The report preparation process also generated a plan for implementation, tracking, and assessment to ensure the report did not languish on a shelf.

The charge to the MDCC Strategic Working Group was to refine, focus, and inform the MDCC what the field needs in terms of data on quality of life and standard-of-care treatment, barriers to progress, and research needs that could be met by NIH. The next step was to develop research objectives to aid in the detection, diagnosis, treatment, and prevention of MD. The focus was to be on disease stages since some areas of research (particular disease stages and types of MD) are further along.

The final plan consisted of 100 pages, including 73 specific recommendations. Importantly, the plan had great credibility because the objectives were prioritized by respected experts used by many MD organizations as advisors. Each objective was assigned a risk value (how difficult it would be to achieve) and a time frame. This approach provided multiple views of the objectives.

The action plan can be used in several ways:

- To guide where research should focus.
- To inform funders; even small organizations can benefit from this model and utilize the plan, too.
- To coordinate activities.
- For accountability; at annual MDCC meetings, each player reports on what has been accomplished on the action plan.
- For grant coding by action plan component; Congress finds this concept very useful.
- To ensure transparency; this process and plan has improved relationships with patients, families, and advocacy groups. For example, instead of the heretofore somewhat contentious relationship, advocacy groups now advise Congress to fund NIH initiatives.
- To give a stage-specific focus by pushing toward preclinical research and clinical development; different diseases are at different research stages. The action plan affected RFA renewal plans for the Wellstone Center, which developed a biomarker program as an answer to the need expressed by investigators, and influenced how the center program was implemented.
- To serve as a basis for eliminating disparities through joint RFAs on understudied MDs.

Already, the action plan has yielded tangible progress; by bringing groups together with a plan, it is possible to leverage relationships to maximal advantage. Among the positive results flowing from the action plan are several large translational grants, international partnerships, and strong collaborations between NIH and advocacy groups.

The focus has been on translational programs to fill the gap between mechanistic studies and clinical trials. The goal of the translational research program is an IND for a drug, biologic, or device for *any* neurological disorder. Funding is generous for the U54 mechanism. The single component awards are up to $1 million per year for U01s. Milestones are in place with go/no-go cutoffs to ensure that research will meet criteria for continued development. Dr. Porter made it clear that supported research is not high-risk; only limited funding is at risk. Significantly, NINDS funding for MD research increased 51% in FY2007, a flat-budget year.

The action plan provides a means for the MDCC to track results. It is a way to increase cooperation in the field and avoid repeating mistakes. The key is that collectively, the groups are maximizing opportunities to apply scarce resources to this disease.

Dr. Porter said that too many drugs and devices fail during development. To avoid this situation, the group has identified a number of important launch-stage issues for novel MD therapies:

- Is the pathology clearly defined?
- Is the target druggable?
- What is the unmet medical need?

- Is there sufficient preclinical and clinical information (e.g., animal data, patient selection/stratification) to go to the clinic?
- How predictive are the preclinical models? Are animal efficacy data "real?"
- Can your preclinical program progressively increase confidence in mechanism (CIM)? In safety (CIS)?
- Is there an established path to regulatory approval (acceptable trial end points)?
- Are there backup compounds?
- What about intellectual property considerations?
- Is the compound commercially viable?

Dr. Porter concluded by saying that the action plan is changing mindsets of academic investigators who are now thinking in terms of milestones instead of mechanisms and of biotech and pharma who are now partnering with academia and transforming the risk assessment/economics for rare diseases because translational studies are being done. The plan is also changing partnerships with a new academic team model and international partnerships. Changes in funding strategies reflect the fact that no one group can do it all; instead, collaborations of academics, advocacy, corporate, and government are *essential* for rare disease study. The action plan is also encouraging experimentation and innovation on the part of funders.

One meeting participant inquired about funding transitions from R01 to U01. Another asked about communication with advocacy groups. Dr. Porter said that he cannot share confidential NIH information, but he knows exactly what the advocacy groups are doing. NIH can facilitate the formation of partnerships between advocacy groups and investigators.

Another person asked about the lack of representation by professional organizations on the MDCC. Dr. Porter acknowledged that more efforts are needed to include them and that they probably should have been involved from the beginning.

Fragile X Syndrome and Associated Disorders: How Far We Have Come
Randi Hagerman, Professor and Medical Director, University of California, Davis

Dr. Hagerman offered a 28-year perspective on FXS, starting in the 1980s with Gillian Turner's paper on FXS, which stimulated a great deal of testing for fragile X. Prior to that, few cases of fragile X were detected through metabolic studies, but with the new test, many positives were found. To provide educational materials to help patients and their families, NFXF was formed with the cooperation of parents and professionals in 1984. These developments were followed by a great deal of research to define the phenotype.

The *FMR1* gene was discovered by Warren, Nelson, Oostra, and others in 1991, and the knockout mouse model was developed in 1995. Greenough, Weiler, et al. opened the world of neurobiology in fragile X when they found that the knockout mouse model was characterized by having many immature dendritic spines. A flurry of reports in the 1980s–1990s recognized the association of POF (now termed POI) with the premutation. In 2001, the first article was published describing FXTAS. Jacquemont et al. brought international recognition to FXTAS when they published a prevalence study in *JAMA* in 2004. Although occasional cases of FXTAS are reported in females, the prevalence is lower due to the protective effect of the second X

chromosome (Berry-Kravis et al., 2004). An expanded phenotype of FXTAS involving autoimmune problems is now emerging (Coffey et al., 2008, Am J Med Genet).

Dr. Hagerman spoke about a bedridden patient with multiple sclerosis (MS) who was diagnosed at age 43 and died at 52. Pathology studies demonstrated MS and FXTAS (Greco et al., 2008, Arch Neurology). It is possible that the presence of fragile X has an additive effect, making MS course more severe or prolonged.

The premutation prevalence has been reported to be 1 in 250 or 300 in some international screening studies. It may have additive effects with other problems. The FXTAS complex includes not only tremor and ataxia, but also hypertension, neuropathy, limb pain, impotence, psychological problems (observed in up to a third of carriers, especially mood instability and anxiety), migraine headaches, impotence (perhaps related to low testosterone levels), urinary problems (perhaps associated with autonomic nervous system problems), and hypothyroidism. Clearly, there is a hypothalamus–pituitary–adrenal axis problem prevalent among premutation carriers.

Dr. Hagerman also discussed the possible association between FXS and autism, noting that it has been a controversial subject since it was first reported in 1982. Rogers et al. (2001) reported a 30% prevalence of autism in FXS. The prevalence of FXS in autism is between 2% and 6%, according to Dr. Hagerman.

FRAXA funding has led to some enormous advances, including the mGluR5 theory in FXS. Other possible pharmacologic interventions include ampakine receptors, minocycline, GABA agonists (e.g., ganaxolone), and fenobam, which have all demonstrated some promise in animal models. Lithium was shown to be helpful in a study of 15 patients. D'Hulst (2007) and Kooy et al. (2005) showed that the $GABA_A$ receptor is dramatically down-regulated in FXS. Dr. Hagerman anticipated that several targeted agents will be ready for clinical studies in the near term:

- Lithium and minocycline are available now.
- Fenobam has gone through initial toxicity trials, and a larger study in adults in FXS likely will be initiated this year.
- Novartis is carrying out an mGluR5 antagonist trial in Europe with Jacquemont and des Portes.
- Seaside Therapeutics will be ready to test R-Baclofen and then STX107 (mGluR5 antagonists) in human studies in a few years.

NFXF and FRAXA are working together—across the country and internationally—to establish a clinical trials network for treatment studies, set up and maintain a database, and increase awareness and diagnosis of FXS and related disorders.

Dr. Hagerman explained how FXS might serve as a model for autism research. FXS research will lead the way for targeted treatment and possibly enhance testing in the autism field. mGluR5 antagonists should be tested in FXS and in autism because there are commonalities among pathways. FMRP is the *über*-protein: It controls the translation of many messages associated

with autism. Fragile X could become a model for the additive effects of environmental toxins leading to autism or fragile X–associated disorders (e.g., smoking and FXPOI).

Working Group Reports

Fragile X Syndrome Working Group

Don Bailey, RTI International

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Andrea Beckel-Mitchener, Ph.D.
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Dr. Bailey described how the Fragile X Syndrome Working Group worked collaboratively to develop broad areas of need (goals) and specific research objectives under each goal. (*Note:* There was a subsequent discussion among all participants about definitions of "goals," "objectives," and "broad areas of need.") Before presenting the group's draft document, he offered a few caveats:

- The group was in a brainstorming phase and did not completely address the factor analysis problem (that is, how to cluster the objectives in a meaningful way).
- The goals varied with regard to the level of specificity and detail covered.
- Fragile X is an expansive topic; therefore, there was much territory to cover insofar as the goals and objectives were concerned. It was also difficult to decide how much detail to provide. For example, the working group wanted to address the psychosocial aspects of being a carrier. Ultimately, it was decided to include some perspective on this point but not to list any separate goals; rather, this aspect is embedded in the other goals.
- It was clear that there would be some overlap with the other working groups. For example, the group did not set up a separate goal on family research about how well children adapt. That question is something that will likely be addressed by the other groups. Conversely, there may be other overlapping topics that other groups did not address; therefore, Dr. Bailey requested help in discerning which areas might need to be "beefed up."
- The working group did not categorize the objectives in terms of high, medium, or low risk, nor did they assign time frames.

Below are the draft research goals (i.e., broad areas of need) and objectives advanced by the working group and modified after discussions within the plenary session:

Goal 1: Advance understanding of the pathophysiology of fragile X syndrome.

Rationale. The most effective treatment for a disorder is likely to emerge from the most complete description of its mechanisms in the cellular and molecular context of the disorder. Drugs developed for other purposes are unlikely to be suitable treatments for a gene that may modulate the expression of tens to hundreds of other gene products. For this reason, understanding FXS at the genetic, molecular, cellular, nervous system and behavioral levels is of paramount importance in the development of treatments.
Research objectives

Research Objective 1.1: Solidify knowledge of the relationships among CGG repeat length, promoter region methylation (or other covalent modifications), transcriptional silencing, and protein translation in the "classic" FX syndrome. Other syndromes related to deletions in the gene are likely to be rare and clinically insignificant but have the potential be very informative. (Note that in the near term, the most clinically useful available drugs may reveal little about the origins of the pathology.)

Research Objective 1.2: Delineate the cellular roles of FMRP and of the macromolecules with which it interacts.

Research Objective 1.3: Delineate the nervous system functional and structural phenotype of FXS and of appropriate mouse models. Evaluate for most ideal reporters of FMR1 function.

Research Objective 1.4: Delineate the behavioral phenotype of FXS and its relationship to the behavioral phenotype of appropriate mouse models. Evaluate for most ideal reporters of FMR1 function.

Goal 2: Improve appropriate and timely diagnosis of individuals with fragile X syndrome and conduct population-based screening studies.

Rationale. Fragile X syndrome is still underdiagnosed and many affected children are identified too late to maximize participation in early intervention programs. Strategies are needed to increase physician awareness of fragile X (FX) and promote early identification. Population-based screening is needed to determine the true prevalence of fragile X syndrome within and across ethnic groups; assess the frequency of allele/carrier frequencies in the general population; create a more complete description of the full range of phenotypic expression (both primary consequences and secondary conditions); and further explicate the relationship between genotype and phenotype in both carriers and individuals with the full mutation. Screening studies would also be useful to understand public willingness to participate in screening for FX status (both carrier and affected) at different life stages (preconception, prenatal, newborn, during childhood), and how families and individuals adapt to genetic information obtained from screening.

Research Objective 2.1: Promote earlier and more complete identification of males and females with the full mutation fragile X.
Fragile X has no physical features that are obvious at birth. Consequently most individuals with fragile X are identified after a family member becomes concerned about developmental or behavioral problems and a knowledgeable physician acknowledges these problems and specifically requests fragile X testing. For many families this process can take 2–3 years in the case of males with the full mutation and much longer for females. Some children are identified much later and many may never be diagnosed, although the extent to which this occurs is unknown. As a result, many children miss the opportunity to benefit from early intervention programs, families experience costs and frustrations associated with their "diagnostic odyssey," and families make reproductive decisions unaware of genetic risk for fragile X. In the absence of newborn screening, models for promoting earlier and more complete identification of all cases of full mutation FX need to be developed and evaluated. Candidate approaches that could be studied independently or in combination with each other include physician awareness about FX, the incorporation in pediatric practice of routine developmental screening for all infants, developing and evaluating the usefulness of FX-specific checklists, and prompt referral for genetic testing of any infant showing developmental delays.

Research Objective 2.2: Develop and validate cost-effective and highly accurate laboratory tools for screening large numbers of individuals with fragile X.

Currently fragile X syndrome is diagnosed using a combination of PCR and Southern blot testing. These procedures are highly accurate for identifying individuals with the full mutation as well as carriers, but the testing requires a blood draw and the cost ($250-$400) is too expensive for large-scale research or screening. An inexpensive ($1-$5) laboratory test would accelerate research to define the true incidence of FMR1 gene variants, advance knowledge about the full range of genotype-phenotype correlations, and make possible broad-based public health screening. Ideally such a test would be highly accurate; differentiate carriers from those with the full mutation; work equally well for males and females; require only a small amount of blood (as in the case of blood spots on filter paper for newborn screening) or other non-invasive biologic sample (e.g., saliva). The possibilities for useful applications will increase dramatically if fragile X screening could be part of a platform that simultaneously tested for multiple conditions (as in the case of tandem mass spectrometry or oligonucleotide microarrays).

Research Objective 2.3: Determine the true incidence of FMR1 gene variations and the extent to which the rate of these variants is consistent across major ethnic minority groups in the U.S.
Strength of Evidence: The true incidence of fragile X syndrome is unknown and debated. It is assumed that fragile X occurs in almost every ethnic group and throughout the world, but some studies have suggested variability as a function of ethnic group or region of origin. However, sample sizes have been far too small to provide definitive answers. Without this information, it is difficult to estimate the public health burden of fragile X, whether rates of fragile X are changing over time, or the extent to which some groups are at more or less risk for fragile X than others. Large-scale research is needed to provide answers to these questions. This research will depend on the development of more cost-effective screening tests. Ideally these studies would be able to describe variations in allele frequencies as opposed to categorical reporting (e.g., full mutation, premutation, gray zone, normal). Such studies could be done using anonymized samples such as newborn screening blood spots. Data on gender and ethnicity would greatly facilitate this work, although race, ethnicity, and region of origin are increasingly complex constructs; research on cross-ethnic or cross-country variation will need to be conducted carefully to take such complexity into consideration when interpreting results. Dissemination of findings on cross-ethnic variations should be done with sensitivity to how these groups might interpret the results; ideally members of groups studied should be included as stakeholders in designing an appropriate dissemination plan, should definitive cross-ethnic differences be determined.

Research Objective 2.4: Evaluate the full range of costs and benefits of various approaches to population screening for fragile X.
Strength of Evidence: Screening for fragile X syndrome could be offered at different life stages – preconception, prenatal, newborn, during childhood. Nowhere is such screening routinely offered. For example, currently newborns in the U.S. are routinely screened for 20–55 conditions, depending on state of residence. Conditions screened are primarily those for which an accurate and cost-effective screening test exists and for which an effective treatment exists that reduces morbidity and mortality associated with the disease. Some conditions (sometimes referred to as secondary targets) are necessarily identified when screening for the core conditions and are usually reported to parents, even though effective treatments are not available. Fragile X is not currently included in newborn screening because an inexpensive, accurate screening test has not been available and no medical treatments exist that must be provided in the first months of life to be effective. However, recent developments are encouraging with regard to screening alternatives and new medications may be more effective if started early in life. The desirability of newborn screening for fragile X is debated, especially if screening also detects carriers. Other issues arise if screening occurs at other times (e.g., prenatal screening). These factors make fragile X a good prototype for study issues that will emerge in a new era of technological capabilities for genetic screening. Research is needed to understand public willingness to participate in screening for FX status (both carrier and affected) at different life stages, how families and individuals adapt to genetic information obtained from screening, and the full range of costs and benefits of various forms and timing of screening.

Goal 3: Validate functional measures of the consequences of fragile X syndrome.

Rationale. Many studies have documented a wide range of effects of FXS at both the biological and behavioral level. However, specific conditions associated with FXS (e.g., autism, anxiety, arousal) have been measured in many different ways with variable findings. There is no consensus on a core battery of behavioral and psychosocial assessments. A variety of physiological and neuroimaging techniques show promise and should be investigated further to determine feasibility and validity. An intensive biomarker discovery program is needed, incorporating diverse technologies, methodological approaches, and models to provide targets for treatment,

accelerate the discovery of targeted pharmaceuticals, and measure their efficacy. Consensus is needed on a range of functional, objective measures to better assess phenotype and provide gold-standard indicators of treatment effectiveness.

Research Objective 3.1: Develop a biomarker discovery program, incorporating diverse technologies, methodological approaches, and models to provide targets for treatment, accelerate the discovery of targeted pharmaceuticals, and measure their efficacy.
Strength of Evidence: In recent years, a number of potential cell and molecular biomarkers that go beyond the genetic causes of fragile X syndrome have been developed. These range from hormonal responses to stress to differences in the levels of some neurotransmitters and/or their receptors to differences in enzymatic and molecular phenomena that alter cellular metabolism and function. Examples include genotype-associated patterns of response to the neurotransmitter glutamate and alterations in enzyme pathways involved in cellular signaling. Considerable research remains to be conducted on these biomarkers. Indeed, many of the biomarkers available for human studies are crude (e.g., FMRP levels), although the science is more advanced in the development of biomarkers useful in other animal models.
3.1a In the short term, there is a need to determine empirically the relationships among these markers to determine if they organize themselves into clusters or constellations of biomarker. In the case of human studies, this work is likely to involve physiological measures of CNS development and might include automated analysis of activity, movement or facial expression, skin conductance, HR, ERP, PPI, anatomic MRI, DTI, fMRI, fNIRS. In the case of nonhuman animal studies, this work will involve a wider range of variables, including neurotransmitters, enzymes, and other molecular measures. In studies of humans and other animals, it will be important to document how these measures differ from normal and how they relate to age-related change in the individual with fragile X syndrome and to variability among individuals with fragile X syndrome.
3.1b A medium-term but clearly attainable goal would be to develop a more complete list of biomarkers and characterize their responses to therapeutic drugs so that the field is positioned to identify the variables that might predict the efficacy and requisite dosage of new and traditional therapeutic drugs. This work can proceed in both humans and nonhuman animal studies, albeit with somewhat different sets of biomarkers.
3.1c A long-term goal would be to use these biomarkers to assess potential efficacy of new drugs during the drug development process. And finally, these biomarkers could be used to understand the biological systems that are affected in fragile X syndrome and that mediate and moderate the expression of the phenotype. Again, this work can proceed in both humans and nonhuman animal studies, albeit with somewhat different sets of biomarkers.

Research Objective 3.2: Develop a standard battery of functional, objective measures to better assess the phenotype, to distinguish features that are unique to the syndrome and those shared with other conditions, to map the developmental trajectory of the phenotype, to clarify gene-environment interactions, and to provide gold-standard indicators of treatment effectiveness.
Strength of Evidence: There is little doubt that fragile X syndrome is associated with effects on numerous dimensions of the phenotype, from the physical to the cognitive (e.g., executive function) and the social-affective (e.g., anxiety-related behaviors). The expression is variable across individuals, however, with some individuals, especially females, displaying milder and more circumscribed effects and others displaying more severe and pervasive effects leading to secondary diagnoses (e.g. autism). The measures used to characterize the nature and severity of phenotypic effects, however, have been variable across studies and have involved small samples of participants. In addition, many of the measures used have poorly understood psychometric properties, have not been hypothesis driven, and have lacked precision (i.e., they often are influenced by variables besides those of interest). The consequences of these measurement limitations are that the natural history of the disorder has not been fully characterized, the neurological underpinnings of many of the behavioral dimensions of the phenotype are unknown, and few phenotypic "targets" that could be used to measure treatment efficacy exist. Data are needed on both core (i.e., syndromes-specific) features of fragile X syndrome as well as on co-occurring conditions (e.g., autism, ADHD). Focusing on high-profile co-occurring conditions, such as autism, could advance knowledge about both conditions and perhaps advance understanding about core mechanisms of both biological and environmental influences.
3.2a In the short-term, there is a need in human studies to reach consensus about the utility of available measures for characterizing the phenotype and to develop new measures that have applicability to a broad range of ages and degrees of affectedness. There also is a need in human studies to apply the measures to a broader range of disorders (and age- and IQ-matched samples) to ensure that fragile X syndrome-specific profiles are identified and to map age-related changes in the measures across the lifespan.

3.2b A medium-term goal is to more completely elucidate the relationships among measures at different levels of analysis, with the mapping of behavioral features onto underlying brain pathologies and onto biomarkers of the condition being especially important. In addressing this goal in human studies, we must refine and optimize the sensitivity and specificity of physiological measures of CNS development and function that are correlated with robust indicators of genetic "affectedness" in fragile X syndrome at both the behavioral and molecular levels. In the case of nonhuman animal studies, the range of "systems" will be more varied and include measures obtained by more invasive means.
3.2c Another medium-term goal for human studies is to develop and refine behavioral genetics approaches so that we can identify specific environmental variables that moderate genetic risk for cognitive and behavioral adversity in fragile X (e.g., family cohesiveness, parental behaviors, classroom characteristics). This information can set the stage for development of fragile X specific environmental interventions that can optimize response to biological treatments.
3.2d A long-term goal would be to use these measures to evaluate the outcomes of various therapies, both pharmacological and educational, as a way of assessing treatment efficacy but also to refine our understanding of the measures and the fragile X phenotype. Developing a set of gold-standard measures will require testing with larger and more diverse samples than has been the case to date. Ideally, this set of measures should be simple, transportable, with hierarchically organized structure so that lower level components do not require expensive instrumentation or high level technical expertise. Explicit training manuals and materials should be produced with criteria for achieving "certification." This long-term goal will likely cross both animal and human studies.

Research Objective 3.3: Develop a standard battery of functional, objective measures for describing behavioral and psychosocial effects in carriers of the premutation and to serve as indicators of treatment need and treatment efficacy.
Strength of evidence: Despite earlier assumptions of lack of effects in carriers, more recent evidence has found that the premutation is associated with serious health effects (i.e., FXPOI and FXTAS). There is also evidence, however, of effects in the behavioral, neurocognitive, and psychosocial domains; however, these effects are more subtle and variable across studies, owing perhaps to a lack of robust measures for detecting effects and a reliance largely on small, non-representative samples of participants. Recent findings using in-depth clinical interviews suggest the effects in the psychosocial realm may be serious enough to warrant the provision of mental health services to a relatively large proportion of adult women. As in the case of affected individuals with the full mutation, studies of premutation carriers should focus on both core (i.e., condition-specific) features and co-occurring conditions and diagnoses (e.g., anxiety disorders, depression).

Goal 4: Initiate a broad-based program of research on the efficacy of treatments for fragile X syndrome.

Rationale. Although literally hundreds of studies have focused on the nature and consequences of fragile X syndrome, there is a dearth of studies on the efficacy of treatments. A few studies have examined the efficacy of traditional medications (e.g. stimulants) in humans, and recent early stage trials have begun using more targeted treatments. Promising studies in the past two years have shown significant treatment effects of targeted medications in selected non-human models. Virtually no research has been conducted on the efficacy of educational, psychological, or therapeutic interventions in fragile X. Major work is needed to study the efficacy of a wide range of treatment options, both known and emerging, in both animal and human models. This research needs to be hypothesis-driven, combining disease-specific knowledge about fragile X with more general knowledge about effective interventions derived from studies of other conditions. Maximal benefits will be shown when targeted pharmaceutical treatments are combined with optimal environmental conditions to maximize learning and promote adaptation.

Research Objective 4.1: Pursue treatment targets which have shown promise in preclinical studies, including those for which drugs are currently in development (mGluR5 antagonists, PAK inhibitors, etc.), and those for which approved drugs already exist (GABA agonists, minocycline, etc), and carry out controlled trials of these drugs in adults and children with FXS as appropriate.
Strength of evidence: Trials of mGluR5 antagonists including MPEP and fenobam have been carried out in animal models of FXS including the knockout mouse model, the Drosophila model and even the zebra fish model demonstrating evidence of efficacy in improving learning, seizures and behavior. Genetic studies including the crossing of mGluR5 deficient mouse with the fragile X KO mouse have demonstrated recovery of the synaptic deficits, growth abnormalities, cognitive deficits and behavior problems. This is strong support for the mGluR5

theory of mental retardation and other behavioral features including anxiety and hyperarousal in fragile X syndrome. Human studies in normals have demonstrated efficacy of fenobam as an anxiolytic in the 1980s with minimal side effects. Fenobam has been more recently identified as an mGluR5 antagonist. Toxicity studies have now been carried out and controlled trials can be initiated regarding fenobam in adults and subsequently children with FXS. Other mGluR5 antagonists have been developed and are also close to human studies. These studies are short to medium term with low risk but great benefit to the field.

Research Objective 4.2: Identify new treatment targets through translational research in basic disease mechanisms of Fragile X. Conduct further preclinical studies to validate treatment targets which show promise.

Research Objective 4.3: Screen drug libraries in cellular assays and animal models to identify potential treatments for the symptoms or the underlying mechanisms of FXS.
Strength of evidence: The screening of compounds in flies and other animal models has already been initiated leading to new avenues of treatment. Molecular studies in the KO mouse model have demonstrated that the GABA system besides the mGluR5 system is dysregulated in fragile X. This finding has also been documented in the Drosophila model of FXS where GABA and GABA agonists prolong life in the Drosophila model of FXS. The GABA A receptor is down regulated in the mouse suggesting that a GABA A agonist may be beneficial in the treatment of FXS. Animal studies are needed to demonstrate efficacy of these and other new treatments. Minocycline has been studied in the KO mouse model and it has helped to mature the synaptic connections in fragile X mice. These animal studies can lead into human trials. Short- and long-term studies are needed, low to high risk.

Research Objective 4.4: Evaluate and adapt current educational/behavioral interventions for Fragile X patients and develop new interventions where indicated.
Strength of evidence: Animal models have demonstrated that an enriched environment can normalize the synaptic structure in the KO mouse. There have been no studies to assess the efficacy of educational or behavioral interventions in FXS. The ABA interventions that have been demonstrated to be efficacious in autism have not been studied in those with FXS either with or without autism. Controlled studies must be carried out to assess behavioral intervention for autism in FXS and to assess educational interventions including the use of assistive technology devices in FXS. These issues become particularly important in the age of targeted treatments for FXS that can make the synaptic connections more receptive to interventions in the environment. This research is an exciting endeavor for both adults with FXS who may now be able to learn a lifetime of education and also for children whose development can be more normalized. Cutting edge educational interventions, such as the use of virtual reality paradigms should also be studied. Short and long term research both low risk and high risk.

Research Objective 4.5: Understand the best timing of treatments and interventions across the lifespan for FXS patients.
Strength of evidence: Human and animal studies to date have not yet determined how effective specific treatments and interventions for FXS may be across the lifespan – the newborn period, childhood, adolescence, and adulthood. Developmental critical periods may limit the usefulness of certain specific treatments to childhood. Further research on the developmental time course of FXS is needed. As more specific treatments are explored, it becomes increasingly important to understand when these treatments might be useful, so as to design appropriate animal and human trials.

Newborn screening studies are now being initiated to identify babies with FXS. In the near future many more babies will be identified and research is needed to evaluate the most effective intervention paradigms for these babies. New information regarding how infants process information can help to shape new intervention techniques and in the future new medical interventions will be developed that may add to new intervention programs. The development of new intervention programs may be beneficial to a larger population of delayed children than just FXS. Short to long term and high risk.

Research Objective 4.6: Increase understanding of symptoms and underlying pathophysiology in premutation carriers, both male and female, and develop effective interventions for individuals with the premutation who may experience developmental problems and aging problems.
Strength of evidence: A subgroup of individuals with the premutation, particularly males, may experience developmental problems that interfere with cognitive and social development. There is a need to identify which

subgroup is most vulnerable to developmental problems either from lowered FMRP levels, elevated mRNA levels or other additive factors to these molecular changes. The pathophysiology of the developmental problems must be researched along with interventions that would be efficacious in childhood. Short and long term and low to high risk.

Research Objective 4.7: Identify common cellular pathways underlying FXS and related disorders, like autism. Identify biomarkers which predict response to treatment in FXS and may also predict response in subpopulations of autistic patients.
Strength of evidence: Multiple lines of study suggest that overlapping pathways are affected in several developmental disorders. If this is the case, treatments being developed for Fragile X might effectively treat many other patient populations as well.

Goal 5: Advance understanding of the practical consequences of fragile X syndrome for individuals and families.

Rationale: Families of individuals with fragile X syndrome face two broad challenges. First, they must help their child (a) learn functional skills so that he or she can live and work independently; and (b) develop social skills, and regulate or control challenging behaviors so that he or she can function in social environments and develop meaningful and enduring relationships with others. Second, because FXS is inherited, families must take reproductive risk into account when making decisions about future children. They must inform siblings and their own parents and often must encourage them to obtain genetic testing. Families may deal with guilt for having transmitted a disorder to their child. They must decide when and how to tell carrier children about their reproductive risk status. Carriers may also be affected by FXS, may feel stigmatized and their reproductive risk status may affect their ability to find or keep a marital partner. Some mothers have the full mutation themselves and may face challenges in their capacity to care for their children. Research needs to develop a deeper understanding of the family consequences of fragile X, the school, work, and living environments of individuals with fragile X, and the nature of supports needed to promote positive adaptive living.

[Objectives to be determined]

Goal 6: Create a research infrastructure and resources to maximize research efficiencies, promote large-scale investigations, and accelerate the appropriate integration of human genome discoveries into health care and disease prevention.

Rationale: Most research to date has relied on individual investigator-initiated efforts with little in the way of broad infrastructure support. While this might have been sufficient in the early stages of research, the field has made sufficient advances so that specific core supports would now enhance the quality, timeliness and efficiency of research. Three examples of areas of needed infrastructure are (1) a broader array of appropriate non-human models of FXS easily accessible by researchers from a variety of institutions; (2) a human brain and tissue bank; and (3) a patient registry or research consortium to maximize access to a large number of individuals with FXS for both descriptive and intervention studies.

Research Objective 6.1: The availability of key research resources should be supported and expanded.
Strength of evidence: At this point in time, the field is sufficiently mature to offer a number of key resources that cross multiple scientific levels of investigation. For example, the original Fmr1 KO mouse is available on several genetic backgrounds as are cell lines for various FXS related conditions. The Drosophila model system is also available for study and increasingly being used by various investigators. At the tissue or brain bank level, there are relatively fewer resources available and these are not widely publicized. There are currently no human or animal imaging data libraries available.
6.1a Short-term goals include: 1) continued support for currently available resources is essential (e.g., KOs, brain and other tissue cell lines by NIGMS, FXS stem cells from the NFXF) – these should be widely available along with augmented brain and tissue banking; 2) new KO and transgenic mice should also be supported and be made more available (e.g., I304N KI, YAC Tg298, various CGG repeat expansion mice, and KOs for Fxr1/2); 3) development of improved reagents, including monoclonal antibodies, should be strongly encouraged and supported.

6.1b Medium-term goals include: 1) new model systems, in addition to Drosophila, should be developed and made available with emphasis on viable conditional KOs; 2) imaging data banks should be supported with data made available to the scientific community (see database objective below).

Research Objective 6.2: Support and develop research consortia and shared databases.
Strength of evidence: Enhancing inter-institution collaboration for multi-site studies, multi-disciplinary research consortia and shared databases provides clear-cut advantages for advancing the field as has been shown in other diseases such as cancer, autism and diabetes. These advantages include the ability to recruit larger numbers of more representative subjects for research studies and clinical trials, standardization of assessment and/or treatment methods, the production and availability of significantly larger samples of biological samples or digital data, cross-fertilization of ideas across investigators and institutions and, eventually, new and improved state-of-the-art practice parameters to improve outcome in affected individuals with disease-specific interventions.
6.2a Short-term goals include: (1) the development of a Website that describes ongoing fragile X related human research projects and clinical trials, similar to that implemented at NCI (http://www.cancer.gov/clinicaltrials); (2) NIH and CDC to support investigator research meetings explicitly focused on data sharing, registries and the development of new inter- and cross-disciplinary research consortia; (3) Efforts should be supported to expand ways to include subjects with FXS in NIH funded clinical trials. Efforts should also be made to include FXS subjects in autism spectrum disorder clinical trials. (4) Private and public funding agency personnel should meet with lead investigators to discuss mechanisms to enhance collaborative research including private-public funding partnerships, funding agency contingencies or mandates requiring data sharing, and funding preference for collaborative and multi-/interdisciplinary research proposals.
6.2b Medium-term goals include (1) the development of new and enhanced databases of tissue samples and digital (e.g., imaging, ERP) datasets that are accessible to new and established FXS investigators; (2) the development of a multi-site clinical research consortia that can rapidly test new fragile X specific treatments based on rapidly expanding knowledge of disease pathophysiology (mGluR, GABA, CR, cholinergic agents, etc.). These clinics would be prepared to use standardized methods for assessment and treatment, thus maximizing subject numbers and representation from the general population.

Discussion of Draft Goals and Objectives

Dr. Bailey observed that there is much more to understand about the *FMR1* gene and its protein. The knowledge obtained under Goal 1 could lead to targeted therapies. Also, work is necessary to elucidate the downstream effects of FMRP. Animal models are needed to delineate the nervous system functions and structures that are affected in FXS. It will also be necessary to delineate the behavioral phenotype of FXS and compare the mouse phenotype to the human one.

The objectives within Goal 2 represent a natural progression. Rapid referral for fragile X testing is needed for all children with developmental delays. Full identification is occurring too late; therefore, we need earlier and more complete identification of fragile X individuals. There is a need for population-based screening, because we do not know the true prevalence of FXS and the premutation. Most prevalence studies have involved small sample sizes or were conducted in clinics for people with other identified disabilities. An accurate diagnostic test is available, but it is too expensive for population-based studies. A much larger sample size is necessary. One possibility would be to perform anonymous blood spot testing of newborns. Also, an evaluation is needed to assess the full range of costs and benefits of various screening approaches (e.g., newborn, preconception).

Goal 3 calls for validating and using functional measures of the consequences of FXS for two main purposes: to drive the development and evaluation of new therapies and to develop a biomarker program. Much more work is needed in this area. Dr. Bailey identified the need to better define the phenotype to distinguish features that are unique to the syndrome and those

shared with other conditions. IQ measurement, for example, does not capture everything that is going on in FXS (e.g., impaired executive function). We need to understand FXS in its expressed form and its variants: Why do we see such a range of expression in the full mutation? When does self-injury begin and in whom? What is a prompt for self-injurious behavior in children? Another objective under this goal is to develop a standard battery of functional objective measures for describing the premutation and the carrier state.

Goal 4 entails a broad-based program of research on the efficacy of treatment for FXS. Dr. Bailey said that this is an exciting time. It is difficult to accrue sufficient numbers of children to test interventions in FXS. What psychosocial interventions are needed for mental disabilities in general and for FXS specifically? Dr. Bailey also spoke about the concept of critical periods and how it might apply in developing a life-perspective approach for developing interventions, including those applicable to infants.

Goal 6, according to Dr. Bailey, is intended to foster the creation of a broad-based research infrastructure for FXS, including train and tissue banks, stem cells, knockout and transgenic mice, and monoclonal antibodies. Also needed are research consortia and a patient registry. Setting up a data coordinating center is a goal of NFXF; it will allow studies of the effectiveness of interventions and help identify potential study subjects. A consortium would make such studies feasible.

A meeting participant observed that the level of detail to include in the goals and objectives is an issue for each of the working groups and speculated that the objectives related to pathophysiology (Goal 1), for example, probably need to be quite detailed.

FXTAS Working Group
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FXTAS is the most severe form of clinical involvement associated with premutation *FMR1* alleles; its core features are intention tremor and/or ataxia, with lower extremity neuropathy, autonomic dysfunction, and gradual cognitive decline beginning with memory and executive function deficits. Psychiatric features, including anxiety, dysinhibition, depression, and apathy, are also common problems. Preliminary data on life expectancy are variable, with a range from 5 to 25 years. The age onset of FXTAS correlates with the CGG expansion within the premutation range: the higher the repeat number, the earlier the tremor or ataxia. Penetrance of clinical features of FXTAS generally has onset after 50 years; occurs in both male and female carriers, although much less common in females; and is incomplete. MRI features of FXTAS include global brain atrophy, white matter disease in the subcortical, middle cerebellar peduncles and periventricular regions, and dilated ventricles. Both males and females with FXTAS display the

characteristic neuropathological feature of exclusively intranuclear, ubiquitin-positive inclusions in neurons and astrocytes. The density of the inclusions throughout the central nervous system (CNS) correlates with the size of the CGG repeat expansion.

Associated clinical features include peripheral neuropathy, dysautonomia, and—particularly in women with FXTAS—hypothyroidism, and muscle pain/fibromyalgia. Numerous lines of evidence from cell-, animal-, and human-based investigations point to an RNA "toxic" gain-of-function as the pathogenic basis of FXTAS: First, the disorder is largely confined to carriers of active premutation alleles of the *FMR1* gene. Second, *FMR1* gene expression is abnormal in that FMR1 mRNA levels are elevated by as much as eightfold, the mRNA itself is altered due to the presence of the expanded CGG repeat, and the start site for transcription is altered by the presence of the expanded repeat. Third, both mouse and Drosophila melanogaster models that harbor premutation CGG-repeat expansions (~90 to 100 CGG repeats), even without the coding portion of the *FMR1* gene, manifest features of the neuropathology of FXTAS.

FXTAS and FXS are entirely distinct disorders. It must be emphasized that the neurodegenerative disorder, FXTAS, and the neurodevelopmental disorder, FXS, are completely different syndromes in terms of whom they affect (premutation carriers v. carriers of full-mutation alleles), age ranges of onset (late adult onset v. childhood onset), and mechanism of pathogenesis (RNA toxicity due to increased gene expression v. absence of protein due to gene silencing). This distinction is often unrecognized among members of the health profession.

FXTAS represents a paradigm to study general process underlying neurodegeneration. In developing therapeutic approaches for any CNS disorder, several questions need to be addressed. For example, how is the clinical phenotype characterized or defined; how does one establish appropriate animal and cellular models; how does one gauge the efficacy of therapeutic agents? For the late-onset neurodegenerative disorders (e.g., late-onset Parkinson's and Alzheimer's disease), these questions are particularly difficult to address, since their generally sporadic nature largely precludes the identification of clear pathogenesis-phenotype relationships that are essential to fully address such questions. FXTAS, with a known pathogenic trigger, thus represents an important tool for research in the broad area of neurodegeneration.

This working group proposed eight draft objectives. Seven are related to specific FXTAS research questions, and the eighth is for a general infrastructure for FXTAS research. Dr. Hagerman noted that many infrastructure needs in terms of consortia and animal models will probably be the same for all three working groups. The overarching aim for the proposed objectives is to develop targeted therapeutic interventions. He mentioned mRNAs and the downstream effects of FMRP and noted that research in these areas could inform the larger field of neurodegeneration and neurodevelopment. All three working groups will likely be able to contribute in this regard.

Below are the draft research goals (i.e., broad areas of need) and objectives advanced by the working group:

Goal 1: Defining pathogenic mechanisms of FXTAS.

Rationale: FXTAS is a neurodegenerative disorder that results from the abnormal expression of moderately expanded (premutation) forms of the *FMR1* gene. Detailed understanding of the consequences of this abnormal

expression is likely to yield targeted (disease-specific) therapeutic approaches for FXTAS. Furthermore, since downstream events in FXTAS neurodegeneration are likely to at least partially overlap those of other neurodegenerative disorders (e.g., Parkinson and Alzheimer diseases), defining the pathogenic mechanisms of FXTAS should yield therapeutic approaches that may be more generally applied to more common neurodegenerative disorders.

Research Objective 1.1: Define the specific molecular mechanisms whereby expanded CGG-repeat *FMR1* mRNA triggers downstream events leading to pathology.
INTERMEDIATE TERM, LOW RISK

Research Objective 1.2: Define the mechanisms leading to overexpression of the expanded CGG-repeat mRNA.
INTERMEDIATE TERM, LOW RISK

Research Objective 1.3: Identify the molecular basis for incomplete penetrance of FXTAS, specifically, the extent to which penetrance reflects second-gene effects and/or environmental factors.
LONG TERM, HIGH RISK

Research Objective 1.4: Elucidate the molecular basis for the apparent difference in disease penetrance and phenotypic character between males and females who carry CGG-repeats of comparable size.
LONG TERM, HIGH RISK

Research Objective 1.5: Determine the mechanism of formation of the intranuclear inclusions of FXTAS and the functional relationship between the presence of inclusions and pathogenesis of FXTAS; that is, are the inclusions *per se* deleterious, neutral, or neuroprotective in the cells expressing the expanded CGG-repeat mRNA?
INTERMEDIATE TERM, MEDIUM RISK

Research Objective 1.6: Understand the pathogenic basis for the significant white matter present in FXTAS.
LONG TERM, HIGH RISK

Research Objective 1.7: Define the period in life that corresponds to the onset of identifiable cellular dysregulation, and whether these earlier events – those preceding the onset of clinical features – can be utilized as molecular/cellular indicators of incipient disease.
LONG TERM, HIGH RISK

Research Objective 1.8: Define the cell-death mechanism(s), and whether these mechanisms are intrinsically apoptotic or non-apoptotic.
INTERMEDIATE TERM, INTERMEDIATE RISK

Research Objective 1.9: Identify common/parallel pathways of neurodegeneration between FXTAS and other disorders (e.g., Huntington, Parkinson, Alzheimer, etc.).
LONG TERM, HIGH RISK

Infrastructure Needs

Research Objective 1.10: Establish invertebrate, other vertebrate, and alternative model systems to study pathogenetic mechanisms of FXTAS pathogenesis.
LONG TERM, INTERMEDIATE RISK

Research Objective 1.11: Establish additional mouse models, including mice with inducible and conditional expression of the CGG repeat in reporter contexts, and with cell-type-specific promoters for each, where the behavioral and cognitive phenotypes are optimal for the human clinical phenotypes.
LONG TERM, HIGH RISK

Research Objective 1.12: Establish a primate model of FXTAS, which will display the behavioral and cognitive phenotypes that are more similar to the human clinical phenotypes. The primate model will also be important for drug development.
LONG TERM, HIGH RISK

Goal 2: Defining clinical phenotypes.

Rationale: FXTAS is a neurodegenerative disorder primarily characterized by neurological symptoms of tremor and ataxia. However, more recent studies suggest a much broader range of neurological, as well as psychiatric and cognitive problems including nerve conduction abnormalities, autoimmune disease, anxiety and depression, and dementia. Research is needed to identify the full range of the clinical phenotype of FXTAS in order to discover pathogenic mechanisms and guide treatment. The risk and protective factors, and prevalence of the disease within clinical groups are largely unknown, making it very difficult to determine which individuals are at greatest risk for developing FXTAS. Prospective longitudinal studies, as well as studies of the natural course of FXTAS are needed to better understand the rate of progression of the condition, whether an early prodromal state can be identified, and how to identify those at risk who may benefit from neuroprotective treatment. Understanding the natural history of FXTAS is crucial to be able to identify the impact of future treatment on the time course of disease. Validation of tools to quantify clinical involvement (for motor, cognitive and psychiatric domains) is crucial for characterization of disease features and relating these to molecular and other parameters, tracking the time course of disease progression, and for monitoring potential effect of treatments.

Research Objective 2.1: Define the broader range of neurological, neurocognitive, behavioral, and emotional dysfunction associated with FXTAS.
SHORT TERM, LOW RISK

Research Objective 2.2: Develop and validate instruments that quantify clinical signs and progression of disease and instruments that are sensitive to early signs of FXTAS progression; identify the most sensitive and predictive parameters to include in these instruments through clinimetric testing.
SHORT TERM, LOW RISK

Research Objective 2.3: Identify molecular, clinical, and environmental risk and protective factors associated with the penetrance of FXTAS.
INTERMEDIATE TERM, MODERATE RISK

Research Objective 2.4: Prospectively identify the natural history of the premutation carrier and FXTAS progression, including factors associated with rapid and slow progression of disease.
INTERMEDIATE TERM, MODERATE RISK

Research Objective 2.5: Define the relationship between the FXTAS clinical phenotype, FMR1 molecular genetic abnormalities, imaging and measures of cellular pathology; identify other factors (molecular, clinical, environmental) associated with various FXTAS clinical signs.
INTERMEDIATE TERM, MODERATE RISK

Research Objective 2.6: Identify potential familial predisposition to FXTAS, association with POI, and FXS, and find molecular factors involved in family clustering.
LONG TERM, HIGH RISK

Infrastructure Needs

Research Objective 2.7: Develop mechanisms that allow multicenter studies combining data and banked tissues across larger samples.

Goal 3: Epidemiology of FXTAS and premutation alleles.

Rationale: FXTAS is a neurodegenerative disorder that results from the abnormal expression of expanded (premutation) forms of the *FMR1* gene. The incidence of FXTAS is currently unknown, and estimates are drawn from frequency measures for large normal and premutation repeat length alleles in the general and in more restricted populations. However, the incomplete penetrance of FXTAS, coupled with the potential for poorly defined clinical phenotypes suggests the need for more detailed surveys to establish the prevalence of FXTAS and related neurological disorders that result from *FMR1* premutation alleles.

Research Objective 3.1: Define the prevalence of the *FMR1* allele lengths in the general population ascertained in an unbiased fashion. Categorize these data for age; data across numerous age cohorts are desirable.
INTERMEDIATE TERM, LOW RISK

Research Objective 3.2: Determine the prevalence of elongated *FMR1* alleles in adult patients with genetically undefined movement disorders (e.g., ataxia, tremor, parkinsonism), memory impairment or dementia (e.g., Lewy body dementias, Alzheimer disease), dysautonomia, and/or peripheral neuropathy. As a component of this goal, additional consideration should be given as to how to establish the appropriate high-risk groups for screening. In particular, movement disorders or dementia clinics may not be the most appropriate venue for screening, since only a small minority of individuals with FXTAS may visit such clinics.
INTERMEDIATE TERM, LOW RISK

Research Objective 3.3: Identify patients ascertained through screening who are willing to participate in clinical studies defined in other Research Objectives. This goal would require appropriate design of studies in Goal 2 allowing follow up and referral of patients identified.
LONG TERM, LOW RISK

Goal 4: Early diagnosis/identification of individuals most at risk of developing FXTAS.

Rationale: Although the ascertainment of a CGG repeat in the premutation range confers carrier status and therefore the *a priori* possibility of developing FXTAS, with longer repeats associated with increased relative risk, neither the presence of a CGG repeat nor its length provides an accurate individual risk. There are carriers with a large premutation who never get disease and carriers with a small premutation who get relatively severe disease. In particular it is very difficult to identify which female carriers are in the 10% or so at risk for FXTAS, as this is dependent on both repeat length and activation ratios and even these parameters together are poor predictors. This, no doubt, relates to underlying interacting genetic and environmental risks that have yet to be defined. It is important to be able to identify both male and female carriers who are most at-risk for developing FXTAS to be able to deliver prognostic information that will help families with planning for the future, to be able to identify the individuals most appropriate for studies of preventative treatments targeted to the underlying mechanisms of cellular toxicity, and to understand the complex interactions that predispose to FXTAS.

Research Objective 4.1: Develop very sensitive clinical protocols and tools to determine when early symptoms of FXTAS are emerging, through standardized measurement of motor and psychological dysfunction.
SHORT TERM, LOW RISK

Research Objective 4.2: Develop molecular markers of cellular toxicity downstream of the CGG repeat that are assayable in a peripheral tissue of individual carriers to assist with prediction of impending disease before symptoms develop, finding those most at risk, and tracking cellular toxicity in carriers, with age, and after interventions.
INTERMEDIATE TERM, LOW RISK

Research Objective 4.3: Utilize these markers to assist with drug discovery for targeted treatments that block the mechanism of toxicity in the underlying disorder.
INTERMEDIATE TERM, MODERATE RISK

Research Objective 4.4: Develop radiological markers of CNS toxicity through more sensitive measures such as DTI or MR-SPECT, that will indicate individuals at risk for FXTAS before clinical symptoms or radiological signs of FXTAS become evident.
INTERMEDIATE TERM, LOW RISK

Research Objective 4.5: Use the cellular, radiological, and clinical markers to successfully track improvements in toxicity measures with preventative or symptomatic treatment in clinical trials.
LONG TERM, HIGH RISK

Goal 5: Define and develop therapeutic interventions both for supportive symptomatic treatment of FXTAS, and targeted treatments to reverse the underlying disease process.

Rationale: Currently there is no targeted treatment for FXTAS, and it is not known whether supportive treatments targeted to symptoms are truly helpful. Only one retrospective study exists to suggest that some individuals derive benefit from medication targeted to symptoms. Individuals with FXTAS are often treated with medications empirically and these may be unhelpful or even detrimental; however, there is no information available to guide practice. Further, although there are likely to be some benefits of currently available supportive treatments, these are likely to be limited, and development of new therapeutics that target the underlying disorder is crucial, aimed at both treatment to reduce symptoms and progression in symptomatic individuals and prevention of symptoms in at-risk presymptomatic individuals.

Research Objective 5.1: Evaluate currently available pharmaceutical treatments targeted at specific symptoms in FXTAS to determine if they are helpful in well-designed clinical trials. Examples would be memantine for neurodegeneration and cognitive decline, minocycline and other neuroprotective agents, and standard treatments targeted at reduction of tremor.
SHORT TERM, LOW RISK

Research Objective 5.2: Evaluate current supportive devices and physical therapies to determine if these improve outcome (e.g., increase time to becoming wheelchair-bound) or lengthen survival.
SHORT TERM, LOW RISK

Research Objective 5.3: Evaluate new drugs already in development that are targeted to symptoms (oxybate, mGluR5 blockers) in trials in animal models and human clinical trials.
INTERMEDIATE TERM, MEDIUM RISK

Research Objective 5.4: Complete screens of drug libraries in cellular toxicity assays and animal models to determine whether compounds exist that seem to reverse cellular toxicity.
INTERMEDIATE TERM, HIGH RISK

Research Objective 5.5: Evaluate agents that reverse cellular toxicity and reverse or prevent symptoms in animal models, in human clinical trial aimed at both treatment and prevention.
LONG TERM, HIGH RISK

Research Objective 5.6: Develop a consortium of clinics to work with FXTAS patients, with expertise in use of optimal outcome measures, through which clinical trials can be run at multiple sites in a standardized fashion for future multi-center trials.
LONG TERM, HIGH RISK

Goal 6: Quality-of-life issues associated with FXTAS.

Rationale: Although it is clear to clinicians working with families with FXTAS that these families struggle with many complex issues, there is little research to document effects of FXTAS on quality of life, both at the level of the individual and the level of the family. There is little information available on the impact of knowledge of premutation carrier status, and thus future risk of FXTAS, on quality of life.

Research Objective 6.1: Define attitudes of families with an FMR1 mutation and families in the general public regarding attitudes of men and women about receiving a premutation diagnosis, including the optimum time to receive the information about the premutation and FXTAS risk, the interest level of premutation carriers and family members regarding learning about FXTAS, how much information should be shared with parents when a child is diagnosed with FXS, whether the information is helpful or hurtful, and who in the family needs to know.
SHORT TERM, LOW RISK

Research Objective 6.2: Determine the availability and adequacy of genetic counseling for families, usefulness, complexity, and understandability of information provided through counseling after diagnosis as an FMR1 premutation carrier.
SHORT TERM, LOW RISK

Research Objective 6.3: Determine the impact of the FMR1 premutation carrier diagnosis on the quality of life of the carrier individual and of other family members, and design interventions to reduce negative effects on quality of life.
INTERMEDIATE TERM, MODERATE RISK

Research Objective 6.4: Determine the impact of the FXTAS diagnosis on the quality of life of the affected individual and of other family members, particularly those helping support both family members with FXS and FXTAS.
SHORT TERM, LOW RISK

Research Objective 6.5: Determine whether quality of life for those affected with FXTAS and family members is most affected by motor, psychological/psychiatric, or cognitive dysfunction in the individual with FXTAS.
SHORT TERM, LOW RISK

Research Objective 6.6: Determine the specific areas in which quality of life is most affected by FXTAS in the affected individuals and closely related family members and design and implement supportive interventions that will improve quality of life in these areas.
LONG TERM, INTERMEDIATE RISK

Research Objective 6.7: Determine the level of awareness among FXTAS-affected families regarding support systems at Fragile X Clinics and resource groups, and perception of need, adequacy and availability of expertise in neurological management, psychological/counseling support, and availability of information on possible treatment options.
SHORT TERM, LOW RISK

Goal 7: Broader implications for other neurodegenerative diseases.

Rationale: FXTAS is a late-onset neurodegenerative disease characterized by intention tremor and parkinsonism with progressive cognitive decline and moderate to severe global brain atrophy. Due to its unusual molecular etiology and characteristic clinical features, FXTAS serves as a model for other RNA gain-of-function disorders that affect the CNS including myotonic dystrophy (DM1, DM2), several spinocerebellar ataxias (SCA8,10,12) and Huntington's disease-like 2 (HDL2). More importantly, elucidation of the primary mechanisms and downstream consequences caused by FXTAS-associated $(CGG)_{55\text{-}200}$ expansions will likely have a significant impact on our understanding of degenerative events in other progressive CNS disorders, such as Parkinson's and Alzheimer's diseases (PD, AD) where specific pathogenic events leading to degeneration have not been adequately defined. The long term objective of this research is to develop novel therapeutic strategies that will be broadly applicable to late-onset neurodegenerative disorders.

Research Objective 7.1: Define the neuropathological similarities and differences associated with FXTAS compared to other microsatellite diseases caused by RNA-gain-of-function effects.
INTERMEDIATE TERM, MEDIUM RISK

Research Objective 7.2: Identify mechanistic features of the pathogenic pathways that are shared by FXTAS and more common neurodegenerative disorders, such as idiopathic PD and AD.
LONG TERM, MEDIUM RISK

Research Objective 7.3: Determine if PD-associated mechanisms (proteolytic stress/ubiquitin-proteasome system impairment, mitochondrial dysfunction/ROS generation) are relevant to FXTAS pathogenesis.
INTERMEDIATE TERM, MEDIUM RISK

Research Objective 7.4: Define the roles (causative, protective, innocent bystander) of FXTAS-associated intranuclear inclusions in disease onset and progression.
LONG TERM, HIGH RISK

Research Objective 7.5: Characterize the molecular cascade that results in formation of RNA-protein inclusions in FXTAS.
LONG TERM, HIGH RISK

Infrastructure Needs

Research Objective 7.6: Develop conditional/inducible animal models that recapitulate specific neurotoxic events common to FXTAS and other neurodegenerative diseases.
LONG TERM, HIGH RISK

Research Objective 7.7: Establish research initiatives and forums which promote collaborations between investigators studying FXTAS and other late-onset neurodegenerative disorders.
LONG TERM, LOW RISK

Goal 8: Establishing a general research infrastructure for FXTAS.

Preclinical Research Infrastructure

Research Objective 8.1: Establish standardized end points for preclinical trials in mouse models, and ensure that facilities are available that enable testing of drugs and other therapeutic approaches.
LONG TERM, LOW RISK

Research Objective 8.2: Create a mechanism to maintain mouse models of FXTAS at approved vendors in a live state, available for easy and rapid importation into academic colonies.
MEDIUM TERM, LOW RISK

Research Objective 8.3: Develop optimized models for mechanistic studies of FXTAS, including models appropriate for therapeutic development screens.
MEDIUM TERM, HIGH RISK

Research Objective 8.4: Encourage the development of cell-based assays that target aspects of pathogenesis and pathophysiology in FXTAS, to enable high throughput drug screening.
MEDIUM TERM, HIGH RISK

Clinical Research and Trial Infrastructure

Research Objective 8.5: Establish FXTAS patient sample repositories, including DNA, cells and tissues.
INTERMEDIATE TERM, MEDIUM RISK

Research Objective 8.6: Identify, develop, and encourage the use of standardized instruments to measure quality of life, cognitive, and central nervous system function using existing databases, and potentially develop new common element databases to extend research capabilities.
MEDIUM TERM, INTERMEDIATE RISK

Research Objective 8.7: Monitor, coordinate, and communicate the rehabilitation and educational assessment activities of the various Federal agencies, voluntary, and patient advocacy groups.
LONG TERM, LOW RISK

Communication and Education

Research Objective 8.8: Design and implement a web site that provides information and links to all existing resources in both the USA and internationally.
SHORT TERM, LOW RISK

Research Objective 8.9: Provide a publicly accessible listing of available training grants and resources so that opportunities for physicians and scientists are transparent.
SHORT TERM, LOW RISK

Research Objective 8.10: Stimulate international collaborations and infrastructure sharing to ensure that opportunities are exploited and resources are used to maximum advantage.

Discussion of Draft Goals and Objectives

Regarding Goal 1, Dr. Hagerman raised questions about the incomplete penetrance of FXTAS. Other gene and/or environmental effects are probably at play. Hormonal effects should be explored in light of the sex differences seen in the phenotypic effects of similar trinucleotide repeats.

Dr. Hagerman also expressed interest in looking for the onset of dysregulation—prodromes to phenotypic expression. Can the onset of FXTAS be predicted on the basis of earlier appearance of cellular dysregulation? Such studies might lead to the discovery of biomarkers.

It will also be important to establish other animal models to elucidate the consequences of turning the offending gene off and perhaps discover commonalities with other neurodegenerative diseases.

For Goal 2, Dr. Hagerman explained that tools are needed to define the syndrome and measure clinical response. Many affected individuals might be patients in other clinics, being evaluated or treated for Alzheimer's or Parkinson's disease, for example. Other avenues of research include molecular, clinical, and environmental risk/protective factors. Familial clustering will have ramifications for all three working groups.

Goal 3 relates to the epidemiology of FXTAS, although the findings will extend across all three syndrome domains. Dr. Hagerman mentioned some recent articles by Deborah Hall, M.D., and noted that initially most FXTAS individuals were being seen in clinics for Parkinson's disease, dementia, or movement disorders. He mentioned the need to screen high-risk groups for FXTAS.

Goal 4 addresses early diagnosis, perhaps based on factors in young adulthood that might affect the predilection to develop FXTAS. Research under this goal would involve defining mechanisms and markers that might assist in developing drug therapies.

Goal 5 pertains to the trials to evaluate the efficacy of supportive and targeted therapeutic interventions. Are symptomatic treatments helpful? Some currently available pharmaceuticals and supportive devices might ameliorate the debilitating aspects of FXTAS. A screening program could be used to evaluate them in FXTAS.

Goal 6, according to Dr. Hagerman, is about quality of life. Families with fragile X mutation or premutation (or both) are dealing with many complex issues. There is little research to document the effects of FXTAS on quality of life at the levels of the individual and the family. The objectives under this goal are aimed at defining the impact of FXTAS status or diagnosis on quality of life. Again, what aspects of the phenotype are more debilitating psychologically to the individual and his family? There needs to be more awareness in the various support structures, as well.

Goal 7 is based upon the recognition that fragile X disorders are orphan diseases; however, they can be a paradigm for other neurodegenerative events in other progressive CNS disorders, such as Parkinson's or Alzheimer's disease. The working group is interested in study of the overlap

between FXTAS and other microsatellite diseases. It is hoped that targeted agents might be broadly useable in other neurodegenerative diseases.

Goal 8 outlines the need for FXTAS research infrastructure: animal models, research initiatives, and forums to promote collaborations. Particular needs include (1) standardized end points in animal models and for clinical trials, (2) resources to keep models at the ready, (3) optimization of models, and (4) development of new cell- and animal-based assays. Also required is clinical trial infrastructure, e.g., patient sample repositories. Communication and education are very important. A Web site could be used to link all existing resources. It should be broad-based and information-laden for families and caregivers. Research training grants are also needed as well as means for fostering international collaborations.

William Greenough, Ph.D., called attention to the risk aspect of having too much detail in the research objectives. The current model for many R01 proposals is to find a program that matches an investigator's interest in terms of present program goals. Aspects of the syndrome were not originally recognized, e.g., loss of dopaminic neurons; therefore, it is critical to avoid exclusionary lists. He encouraged cutting down on the number of details and focusing on broad objectives that are flexible to accommodate new developments. One purpose of this meeting should be to agree on the level of detail. It will come down to a balance and the purpose of the report. It was generally agreed that the research plan should include a qualifying statement (perhaps in the preface) to say these goals and objectives are not meant to be limiting.

Dr. Randi Hagerman commented on the problem of emerging terminology: There is a spectrum of phenotypic expression associated with the premutation (e.g., neuropathy but no ataxia; anxiety). She recommended thinking outside of these three "boxes" (FX full mutation, FXPOI, FXTAS). Ms. Katie Clapp agreed that these boxes are not fitting well and noted that she is the parent of a girl with the full mutation who is an honors student. Dr. Paul Hagerman cited the example of cystic fibrosis, which is a spectrum disorder. FXTAS is named for the motor component, but there are other issues faced by those with the premutation.

Dr. Bailey observed many common themes developed by the FXS and FXTAS working groups. He speculated that some of the research objectives may collapse as we look across groups.

FXPOI Working Group
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Only 1% of women experience menopause before the age of 40 years (Coulam et al., 1986), termed premature ovarian failure (POF). The disorder can be better described as primary ovarian insufficiency (POI) to reflect the continuum of ovarian dysfunction it encompasses (Welt, 2007). Women with POI not only have loss of normal fertility, but also are at increased risk for osteoporosis (Kritz-Silverstein, Barrett-Connor, 1993; Richelson et al., 1984), cardiac disease (de Kleijn et al., 2002; van der Schouw et al., 1996), and overall mortality (Jacobsen et al., 2003; Jansen et al., 2002). Despite the clinical importance, the causes of POI have been elusive, with the exception of X chromosome deletions and translocations and rare X chromosome and autosome gene mutations. In the absence of an etiology and appropriate diagnostic testing, POI cannot be predicted in a majority of cases, nor can the longitudinal course of ovarian function be defined. Genetic testing for fragile X premutations will provide one of the only opportunities to identify women at risk for POI and to study the risk factors and longitudinal course of the disease.

More important to women who carry the premutation, genetic testing for carrier status identifies women at risk for progeny with mental retardation due to FXS. Carriers of the premutation have the risk of transmitting a further expanded full-mutation allele to their progeny. Among female carriers, a repeat length of ~100 or greater has almost a 100% chance of expanding to a full mutation in one generation, whereas repeat lengths of 70–79 have a 31% chance. Interestingly, when passed through the father, the premutation repeat length is more stable and rarely, if at all, expands to a full mutation.

The prevalence of the premutation in women is 1/259 in an unselected population of more than 10,000 French Canadian women (Rousseau et al., 1995) and as high as 1/100 in Israel (Pesso et al., 2000). In a series of women ascertained from the general population in metropolitan Atlanta, the prevalence was 1/151 (Sherman, unpublished data). Therefore, the premutation is quite common. The prevalence is much higher in women with POI at 1/33 in women with sporadic POF and 1/8 in women with familial POF (for review, see Sherman et al., 2007). Therefore, detecting this common triplet repeat disorder is one of the most fruitful means of identifying women at risk for POI. Conversely, since 20% of couples presenting to infertility practices show evidence of POI with increased follicle-stimulating hormone (FSH) levels, idiopathic infertility, or a poor response to gonadotropins (Speroff L et al., 1999), it is critical to screen for the premutation in infertility patients, because they may be at risk for progeny with FXS.

There is no recommended standard of care for a woman known to carry the premutation with respect to her prognosis for a shortened reproductive life span. Determining the risk factors and time course of the reproductive abnormalities associated with premutations using *in vivo* and *in vitro* models could have an enormous impact on counseling about prognosis, fertility potential, fertility risks such as mental retardation, and fertility treatment and could help define patient recommendations for testing. From a broader health perspective, the time course could also impact counseling regarding the risks of osteoporosis and cardiovascular disease. Taken together, the translation of the premutation carrier's observed ovarian insufficiency to the bench and back to predictive models for the patient will provide information that is critically important for the clinical care of these women and the broader group of infertility patients with high FSH levels.

Dr. Sherman underscored the importance of taking into account the stage appropriateness of various research approaches. FXPOI research is lagging compared with the other two FX-associated disorders because the mechanism has not yet been elucidated.

Below are the draft research goals (i.e., broad areas of need) and objectives advanced by the working group:

Goal 1: Disease-specific mechanism and therapeutic targets.

Rationale: In contrast to FXPOI, in which there has been little mechanistic research, there have been a number of studies examining the mechanism of neurological dysfunction in men with the premutation who have symptoms of FXTAS. Although FXTAS appears to behave as an X-linked recessive due to its rare manifestation in women, findings from FXTAS studies may be relevant to FXPOI.

In the presence of the full fragile X mutation, *FMR1* is methylated, thereby blocking FMRP transcription. In contrast, premutation carriers produce transcripts with large CGG repeat tracks (rCGGs). Furthermore, *FMR1* transcript levels increase with increasing repeat size, but FMRP levels are slightly decreased, as assessed in lymphocytes and neurons (Adams et al., 2007; Allen et al., 2004; Kenneson et al., 2001; Tassone et al., 2000b; Tassone et al., 2000a; Tassone et al., 2007a). Since FXTAS and FXPOI have only been demonstrated among premutation carriers, not full mutation carriers who have absence of *FMR1* mRNA and FMRP, the premutation disorders cannot be the result of decreased protein. Rather, it has been suggested that the large track of rCGGs acts in a toxic manner (RNA gain-of-function). Drosophila and mouse models of the premutation support this postulate. These studies have shown that *FMR1* mRNA with expanded repeats (90-98 repeats) is overexpressed in the neurons of Drosophila and mice (Jin et al., 2003; Willemsen et al., 2003). These studies demonstrate 2-3 fold elevated FMR1 mRNA levels, neurodegeneration and intranuclear inclusions that increase with age.

The RNA gain-of-function effects are thought to be related to the increased mRNA levels or the unusual structural features of the large repeat tracks in the transcripts. Such structures could bind other important proteins in the cell, rendering them inactive or targeting them for degradation (Jin et al., 2003). Alternatively, proteins or transcription factors that bind to the CGG repeats may be sequestered, preventing them from performing their normal functions (Ranum and Day, 2002). It is also possible that the repetitive RNA hairpins are processed by the RNA interference pathway, leading to the silencing of genes that contain short complementary repeats in their transcripts (Handa et al., 2003; Jin et al., 2004). Finally, although intranuclear inclusions may not be pathogenic, they do stain with anti-ubiquitin antibodies, suggesting a link to proteasome protein degradation (Greco et al., 2002).

As stated previously, the studies and understanding of the mechanism for FXPOI lag far behind the FXTAS field: there is no well-described model system to aid in the identification of mechanism, and the parallels to FXTAS are obvious only on "paper," not by experimental observation. Thus, the effect of the high CGG repeat on reproductive biology is unknown and must be understood to effectively identify potential treatments.

Research Objective 1.1: **Develop model systems for FXPOI in order to identify mechanism and examine potential treatment time points; create additional models if needed. (short term, intermediate risk)**
At least two premutation mouse models exist to date; however, the characterization of their reproductive phenotype has not been completed as yet. The need for a system in which the target tissue affected by the CGG repeat can be identified is of great importance. This type of model has proven invaluable for studies of both FXS and FXTAS.

Short/Mid/Long range goal

1.1a Characterize reproductive phenotype among existing premutation animal and cell models.
1.1b Create additional models, including those with inducible and conditional expression of the CGG repeat in reporter contexts and with cell-type-specific promoters for each to examine treatment time points.

Research Objective 1.2: **Identify the reproductive target tissues and biological systems affected by the high CGG repeat. (intermediate term, low risk)**

Research Objective 1.3: **Determine the specific molecular mechanism that causes the CGG repeat to be toxic to the reproductive system. (intermediate term, low risk)**

Research Objective 1.4: **Investigate the non-linear association of repeat size and reproductive phenotype as a method to identify mechanism (intermediate term, low risk)**
Studies have demonstrated that ovarian dysfunction depends on repeat size, but the relationship is not linear (Allen et al., 2007; Ennis et al., 2006; Sullivan et al., 2005). Women with mid-range repeats (80–100 repeats) experience POF earlier and at higher frequencies (32%) than other carrier groups and their mean age at menopause is youngest at 44.9 years (Allen et al., 2007). In contrast, the mean age of menopause is 52 years in controls, 49 years in premutation carriers with a low repeat number (59–79 repeats) and 48 years when the repeat size reached > 100. The non-linear relationship between ovarian function and repeat number is not paralleled by the relationship between repeat number and *FMR1* mRNA levels in lymphocytes: this relationship is linear, with a 2-fold mean increase in the lymphocyte mRNA levels in carriers compared to controls (Allen et al., 2004; Tassone et al., 2007b). It is possible that the mid-range repeat lengths result in the greatest amount of mRNA and therefore the greatest toxicity. It is also possible that repeat size confers a specific mRNA conformation that interacts with different proteins or mRNAs. The toxicity of mRNA quantity vs. repeat size needs examination in a human model. It is important to note that the repeat size class definitions of premutation, intermediate, and non-carrier groups only relate the risk for instability and expansion to the full mutation. They do not necessarily correspond to the risk for premutation-associated disorders such as FXPOI. Studies to identify at risk alleles have not been completed.

Research Objective 1.5: **Based on the identified mechanism, examine treatment strategies that extend the reproductive life of a carrier with the premutation. (long term, high risk)**

Research Objective 1.6: **Identify common pathways of ovarian dysfunction between FXPOI and other forms of ovarian insufficiency. (long term, low risk)**

Goal 2: Disease progression and preventive medicine.

Research Objective 2.1: **Establish longitudinal studies to characterize onset with respect to menstrual cycle alterations, hormonal profile, fertility and menopause transition. (intermediate term, low risk)**
Women who carry the fragile X premutation are at risk for FXPOI. However, the risk for an individual woman has not been defined and the manifestations may be variable. Indeed, the course of ovarian function appears to differ even in women with the same premutation length. Studies are needed to identify the manifestations from POI in women who carry the fragile X premutation, ranging from the first evidence of infertility through the cessation of menstrual cycles due to loss of all ovarian follicles. Therefore, longitudinal studies are needed to characterize the reproductive function in women who carry the premutation. Of note, parallel longitudinal studies will be performed in mouse models.

Women with the fragile X premutation and regular menstrual cycles have been demonstrated to have elevated FSH and decreased inhibin B levels in one small study. Furthermore, they have higher FSH and lower AMH levels compared to non-carriers on average, suggesting early ovarian aging in these women. Finally, women who carry the premutation have a 5–7 year earlier menopause on average than non-carriers, with age at menopause varying by premutation length. Thus, preliminary and pilot data suggest that women who carry the premutation exhibit the continuum of POI, from elevated FSH levels and regular menses to early menopause. However, the onset, longitudinal course of these findings in the group and the variability in the course need definition.

2.1a The short-term goal (low risk) is to establish longitudinal studies to examine the course of ovarian function in fragile X premutation carriers. These studies will require a collaborative network, definition of the parameters for measurement such as in the history, physical exam and serum biomarkers (see Research Objective 3, below). For examples of parameters necessary to acquire in the history see Research Objective 2, below.
2.1b The intermediate goal (low risk) is to define the course of reproductive function using data obtained from a longitudinal study of fragile X premutation carriers.

In tandem with these goals, longitudinal studies of reproductive function will be performed in mouse models for comparison.

The goal will be met if women carrying the fragile X premutation are enrolled in longitudinal studies of reproductive function in a number meeting a power estimate for the objective. Publications and meeting presentations will need to be tracked.

Determining the time course of the reproductive abnormalities associated with premutations could have an enormous impact on counseling about prognosis, fertility potential, fertility risks such as mental retardation and fertility treatment. From a broader health perspective, the time course could also impact counseling regarding the risks of osteoporosis and cardiovascular disease.

Research Objective 2.2: **Characterize menopausal symptoms, estrogen-deficiency-associated disorders, and neuropsychological profile using cross-sectional studies. (short term, low risk)**
Although preliminary studies have identified evidence for reproductive dysfunction, there has been little attention toward the resulting menopausal symptoms and estrogen deficiency disorders related to reproductive dysfunction. For example, studies of hot flashes, bone density, sleep disorders, cardiovascular risk markers and depression need examination. In addition, the neuropsychological profile of women with FXPOI in relation to biomarkers such as repeat number and prognosis for fertility need examination.

Preliminary studies have demonstrated that women who carry a mid range fragile X premutation are more likely to have short, irregular or missed menstrual cycles compared to non-carriers and were more likely to have visited a doctor for infertility. However, there are no studies examining the symptoms and neuropsychological profile related to reproductive dysfunction in women who carry the fragile X premutation. Furthermore, men and women with FXTAS symptoms reported higher levels of several types of psychological symptoms and higher levels of obsessive-compulsive symptoms (Hessl D, 2005). These findings have not been examined in relation to the reproductive phenotype.

2.2a Short/Mid/Long range goal
The short-term goal (low risk) is to examine women who carry the fragile X premutation with history, bone density, blood sampling, and neuropsychological testing to further define the menopausal symptoms, estrogen-deficiency-associated disorders, and neurological and psychological manifestations.

Progress toward the goal will be tracked by publications and published recommendations for patient treatment.

Defining these characteristics will help develop recommendations for testing for estrogen-deficiency–associated disorders, alert physicians to possible need for counseling or psychiatric referral and help patients consider possible treatment options for menopausal or neuropsychiatric symptoms.

Research Objective 2.3: **Identify biomarkers that predict length of reproductive life span and associated disorders and establish a prospective study to determine their efficacy. (intermediate term, medium risk)**
POI cannot be predicted in a majority of cases, nor can the longitudinal course of ovarian function be predicted. Identifying women who carry the fragile X premutation provides a unique opportunity to identify women at risk for POI and to study the biomarkers that might predict the longitudinal course of the disease. Identifying these biomarkers will also be critical to counsel individual women about their reproductive potential.

Studies of reproductive aging demonstrate a number of markers that predict fertility and the decline in follicle number across reproductive aging. These markers include age, FSH level (Scott RT 1989), and can predict the time to menopause in women who are studied in the menopausal transition. Inhibin A and inhibin B levels can predict the response to gonadotropin stimulation in women undergoing fertility treatment. Anti-Mullerian hormone (AMH) also appears to reflect the number and health of small antral follicles in the ovary, predict the ovarian response to gonadotropin stimulation and to mark the decline of ovarian function in reproductive aging. AMH may change ahead of other markers. Indeed, in a cross-sectional study, AMH appears to be a better marker than FSH in identifying this early decline in women who carry the fragile X premutation. Thus, biomarkers have been used to predict ovarian response and ovarian function in women.

In addition to these hormonal biomarkers, genetic markers and environmental factors may predict the reproductive life span. These factors may include smoking, CGG repeat number, interspersed AGG sequence and XIR. These biomarkers and environmental factors may also be important to predict the course of reproductive function.

2.3a The short-term goal (low risk) is to design a longitudinal study in which a history and blood sample are obtained at regular intervals.
2.3b The mid-term goal (medium risk) is to correlate the biomarkers identified with reproductive manifestations. These include infertility, menstrual irregularity, hot flashes, etc.
2.3c The long-term goal (high risk) is to create a predictive model of reproductive function in fragile X premutation carriers using history and biomarkers defined and design a prospective study to establish the validity of these defined markers.

This goal will be met by the complete yearly study of a sufficient number of women who carry the premutation, as defined by a power analysis. Tracking publications that define predictive biomarkers and create a predictive model will serve as criteria for meeting the goals. Setting up a prospective study of the newly defined predictive model will meet the overall goal of this research objective.

Determining the biomarkers that predict reproductive abnormalities associated with premutations could have an enormous impact on counseling about prognosis, fertility potential, fertility risks such as mental retardation and fertility treatment and could help define patient recommendations for testing. From a broader health perspective, the time course could also impact counseling regarding the risks of osteoporosis and cardiovascular disease. These studies will provide information that is critically important for the clinical care of women who carry the premutation and possibly the broader group of infertility patients.

Goal 3: Genetic and environmental factors that influence onset and severity.

Research Objective 3.1: **Characterize effects of "known" modifying factors: CGG repeat structure, XCI, environment factors associated with age at menopause. (short term, low risk)**
Additional studies that examine *FMR1*-related risk factors and other known risk factors for ovarian function in known premutation carriers are needed. Women with the premutation should be ascertained in a variety of ways, including from families with a relative affected with FXS, from patients diagnosed with clinical POI, and from large cohort studies that include stored DNA samples and reproductive outcome measures such as age at menopause. These data should be examined for effects of these and other, currently unknown, risk factors on both qualitative traits (e.g. POI) and quantitative traits (age at menopause, hormone levels, menstrual cycle characteristics, number of pregnancies, etc). Both cross-sectional and cohort studies would be useful for this aim. Large consortia would be helpful in the accomplishment of this aim.

Studies have demonstrated that ovarian dysfunction depends on repeat size, but the relationship is not linear (Allen et al., 2007; Ennis et al., 2006; Sullivan et al., 2005). Women with mid-range repeats (80-100 repeats) experience POF earlier and at higher frequencies (32%) than other carrier groups and their mean age at menopause is youngest at 44.9 years (Allen et al., 2007). In contrast, the mean age of menopause is 52 years in controls, 49 years in premutation carriers with a low repeat number (59–79 repeats) and 48 years when the repeat size reached >100. The non-linear relationship between ovarian function and repeat number is not paralleled by the relationship between repeat number and *FMR1* mRNA levels in lymphocytes: this relationship is linear, with a 2-fold mean increase in the lymphocyte mRNA levels in carriers compared to controls (Allen et al., 2004; Tassone et al., 2007b). It is possible that the mid-range repeat lengths result in the greatest amount of mRNA and therefore the greatest toxicity. It is also possible that repeat size confers a specific mRNA conformation that interacts with different proteins or mRNAs. The toxicity of mRNA quantity vs. repeat size needs examination in a human model.

Importantly, it is well documented that the *FMR1* repeat alleles also vary by AGG interspersion patterns. AGG interruptions have been shown to decrease the risk for expansion of a premutation to a full mutation (Eichler et al., 1994). For example, repeat alleles with pure repeats are more unstable than those with one or two AGG interruptions every 10 CGG repeats in the 5' end. In *in vitro* studies, AGG interruptions in a CGG repeat track prevented the formation of branched hairpin structures, which may alter the association between repeat size and presence of disease (Napierala et al., 2005). In one small preliminary study, at least one AGG interruption was demonstrated in controls with intermediate sized alleles and normal age at menopause, whereas women with POF and intermediate

sized alleles had no AGG interruption (Bodega et al., 2006). Interestingly, despite the potential protective effect of AGG interruptions on the stability of the repeat and on ovarian function, the AGG interruptions do not appear to decrease the mRNA transcript level in the lymphocytes (Di Pasquale et al., 2006; Tassone et al., 2007b). Thus, further examination is needed to determine whether there is a protective effect of interspersed AGG repeats.

Determining the relationship of risk factors to POI risk in a very large sample of women would yield data to provide insight into the biological mechanism of the CGG repeat. In addition, these data would provide predictive markers to increase the accuracy of counseling women who carry a premutation or high repeat concerning their risk of POI. It is possible that these data could also provide guidance for physicians treating such women.

Research Objective 3.2: **Conduct genome-wide association studies (GWAS) among premutation carriers including analysis of single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and epigenetic factors to identify interacting genes and variants that explain onset and severity of FXPOI. (long term, high risk)**
Form consortium to obtain the very large number of premutation carriers that would be required to perform a GWAS of FXPOI and of the related quantitative traits such as age at menopause, hormone profile, etc.

Based on family studies, results have indicated significant familial aggregation of age at menopause once the effect of CGG repeat size was removed. There was an estimated additive genetic variance of 0.55 to 0.96 depending on the parameterization of *FMR1* repeat size and definition of age at menopause (P-values between 0.0002 and 0.0027). This result suggests that there are other genetic variations that explain the onset and severity of FXPOI. Given the biology of ovarian function, it is highly likely that at least some genes have common risk alleles that have small to moderate effects on FXPOI.

Collecting large samples of premutation carriers with and without FXPOI or with known age at menopause and performing a GWAS; replicating the significant results in an independent sample.

A significant association would identify biological pathways that modify the penetrance of the premutation allele. In addition, identification of risk alleles would provide predictive information for POI among women who carry the premutation. Such information may improve counseling of risk and better guidance for physicians treating such women.

Goal 4: Treatment and management.

Research Objective 4.1: **Establish best genetic counseling guidelines for women of known premutation carrier status to inform about the risk for FXPOI and among women with ovarian insufficiency to inform about the risk for carrying the premutation and develop communication materials accordingly.**

In this situation there are two distinct groups of women. Firstly, there are women who have a family history of fragile X syndrome and are at risk of being premutation carriers or know they are premutation carriers and are therefore at risk of POI; these women will have some knowledge and experience of fragile X syndrome in their family. The second group is women who have ovarian insufficiency but have no family history of fragile X syndrome. These women could be offered carrier testing through their specialist and may be found to be a premutation carrier in this way. Women in this group are unique in that they do not have a known family history of fragile X and thus will have different counseling needs.

Below we refer to women in the first group as those with a family history of fragile X and women in the second group as those without a family history of fragile X.

1. Identified need or opportunity
    a. FAMILY HISTORY
        i. Currently guidelines state that women who are premutation carriers should be informed about their risk for POI (McConkie-Rosell, Finucane et al., 2005).

ii. However, there are limited guidelines for genetic counseling for premutation carriers regarding POI and research is needed to explore the counseling needs of these women.

b. NO FAMILY HISTORY

i. An interdisciplinary focus group on fragile X-associated POF recommended that obstetricians, gynecologists, reproductive endocrinologists, and general practitioners should offer *FMR1* testing to women with ovarian insufficiency (McConkie-Rosell, 2007).

ii. There are no guidelines regarding genetic counseling for women with ovarian insufficiency who are offered carrier testing through their specialist or for women who are identified as premutation carriers as a result of their ovarian insufficiency. As these women will not have a family history of fragile X they will not have the knowledge of fragile X nor the experience of fragile X in their families and thus will have different needs to those women who do have a family history of fragile X. Little is known about the genetic counseling needs of women in this category and therefore further research is required in this area.

c. Research is needed to explore the counseling and informational needs of women in each of these groups. Findings from this research will inform the development of best practice guidelines which will in turn improve the care of women with POI or at risk of POI as a result of their premutation carrier status.

2. Strength of evidence for approach

a. The interdisciplinary advisory group on FXPOF identified the following issues that need to be discussed with premutation carriers with POI: family planning in view of the potentially reduced reproductive timeline in women carriers; the risk of having a child with fragile X syndrome; reproductive options including: child-free living, adoption, use of egg donors, and embryo adoption; the reduced success of preimplantation genetic diagnosis related to a decrease in the number of harvested eggs; and prenatal diagnosis for those women who achieve pregnancy.

b. Women ascertained in the infertility setting who have ovarian insufficiency and do not have a family history of FXS may encounter additional challenges including: lack of knowledge about FXS; already experiencing fertility issues; discovering the cause for their reduced fertility is due to an inherited condition and that there may be health risks to other family members; altering their life plans related to child bearing. In addition, specialists who are offering the carrier testing may not be well informed about fragile X and related conditions.

c. Research with women who have ovarian insufficiency has found that the majority of these women were interested in carrier testing for fragile X (Pastore et al, 2006). A study offering carrier screening for fragile X to women from the general population found that women who were found to be premutation carriers were unprepared for their result (Anido et al. 2005). Women with ovarian insufficiency who do not have a family history of fragile X may be similar to those women from the general population identified as carriers and may be unprepared for their result.

3. Short-/mid-/long-range goal

a. Short term, low risk

4. Criteria for meeting goal

a. Conducting research to explore the needs of women who are premutation carriers and are at risk for POI or who have POI and women who have ovarian insufficiency and have no family history of FXS.

i. Research could start with a qualitative approach to explore the range of issues and needs of women.

ii. The results from the qualitative research could inform the development of a survey to determine the main issues for women in these groups.

iii. Research could also explore the views of specialist healthcare providers (gynecologists, reproductive endocrinologists etc.) towards offering carrier testing to women with ovarian

insufficiency. In addition, information materials could be developed to educate specialists who care for these women.

b. Research findings will inform the development of guidelines for best genetic counseling practice for women of known premutation carrier status to inform about the risk for FXPOI and among women with ovarian insufficiency to inform about the risk for carrying the premutation.

c. Research findings will also inform the development of information about POI for women and healthcare providers.

5. Impact

a. Developing genetic counseling guidelines will improve the care of women at risk for POI or with POI.

b. Informational materials will help educate women and healthcare providers about POI.

Research Objective 4.2: **Examine the ethical and social issues related to preventive treatments such as fertility preservation that would be best used among premutation carriers prior to their ability to consent.**

Research Objective 4.3: **Establish prevalence of the premutation carriers among women with ovarian insufficiency by ethnic groups. (short term, low risk)**

Research Objective 4.4: **Characterize success of currently applied treatments for infertility, menopause transition, osteoporosis and other estrogen-deficiency-related health problems used for premutation carriers based on existing clinical data. (intermediate term, low risk)**

Research Objective 4.5: **Determine the role of fertility preservation in females with premutation who are deemed to have high risk of POI.**

FXPOI is likely to be one of the very few conditions where risk of POI can be judged prospectively. It also is likely to be one of the most common genetic conditions associated with future infertility.

Research Objective 4.6: **Establish clinical trials to examine efficacy of treatment strategies based on identified mechanism of *FMR1* repeat. (long term, high risk)**

Goal 5: Infrastructure needs.

Rationale: Most research to date has relied on individual investigator-initiated efforts with little in the way of broad infrastructure support. While this might have been sufficient in the early stages of research, the field has made sufficient advances so that specific core supports would now enhance the quality, timeliness and efficiency of research. Three examples of areas of needed infrastructure are (1) a broader array of appropriate non-human models of FXS easily accessible by researchers from a variety of institutions; (2) a human brain and tissue bank; and (3) a patient registry or research consortium to maximize access to a large number of individuals with FXS for both descriptive and intervention studies.

Research Objective 5.1: **Establish clinical research consortium to share knowledge, data and biological samples. (short term, low risk)**

1. Identified need or opportunity
   a. Longitudinal data to determine natural history/phenotype of FX spectrum disorders (including FXPOI and other conditions affecting premutation carriers) in a large population.
   b. Bank of blood/tissue/DNA samples from carriers for research purposes outlined in other goals (i.e. biomarkers for FXPOI).
   c. Larger number of individuals for epidemiological studies (e.g. to determine incidence of conditions affecting premutation carriers). Having larger numbers will reduce sample bias.

2. Strength of evidence for approach

Similar clinical consortiums and surveillance studies have been set up for other rare conditions such as pediatric cancers. Consortiums such as the Pediatric Oncology Group and Children's Oncology Group have large numbers of members from hospitals nationally and internationally and have made significant advances in pediatric cancer research leading to the development of new treatments and improved survival (Children's Oncology Group: http://www.childrensoncologygroup.org/). The organizational models that have been developed by others can be used as a model for development of a consortium for fragile X research.

3. Short/Mid/Long range goal
   Short term, low risk – building on existing collaborations and including groups who are already motivated to conduct or be involved in fragile X research will increase the likelihood of success.

4. Criteria for meeting goal
   a. Convene group of interested parties (the consortium). Start with existing consortium and build on this by including clinics, advocacy groups, funding institutions, people with previous experience in developing a clinical consortium and longitudinal databases. Starting with those groups who are already involved and informed about fragile X syndrome will ensure success in the early stages.
   b. Decide type of information to be collected (data and biological samples) and create the database template for inputting data. Data collection tool enables data to be collected the same way across sites and allows pooling of the data.
   c. Establish data coordinating center which will house the pooled data. Establish sample repository site.
   d. Establish coordinating center to develop template protocols, informed consents, and educational documents.
   e. Educate FX community on the clinical consortium.

5. Impact
   a. Increase the knowledge level on FX spectrum disorders – phenotype, prevalence, effective treatments, biomarkers, age of onset, associated modifying factors etc.
   b. Identify individuals for clinical trials.

Research Objective 5.2: **Expand infrastructure to integrate knowledge from fragile X spectrum disorders to increase cross-disciplinary studies.**

1. Identified need or opportunity
   Longitudinal studies on premutation carriers without a family history of FXS - Expand consortium to include specialty physicians (OBs, neurologists, genetics) to attain data on carriers identified through specialists (e.g. POI and FXTAS) who may not have a family history of FXS.

2. Strength of evidence for approach
   Other consortiums/surveillance systems (see 2a above).

3. Short/Mid/Long range goal
   Mid range, medium risk – The success of expanding the consortium is dependant on the effectiveness of the initial consortium in raising awareness of other specialists. In addition, epidemiological data on the frequency of fragile X spectrum disorders in different populations will influence the expansion of the consortium.

4. Criteria for meeting goal
   a. Consortium members identify specialists in their own community who potentially see premutation carriers (i.e. reproductive medicine providers, neurologists).
   b. Develop educational material to educate specialists on FX spectrum disorders and the consortium (specifically FXPOI for OBs/reproductive medicine specialists).
   c. Provide specialists with protocol, consents, etc.
   d. Specialist clinics input medical information into template. Information is pooled into the central database.

e. Tissue/blood/DNA samples sent to consortium bank.

5. Impact
   a. Discover genetic and/or phenotypic differences between carriers identified through family history of fragile X syndrome and those without.
   b. Increase knowledge of FX spectrum disorders.
   c. Increase knowledge and awareness of specialists on FX spectrum disorders.

Research Objective 5.3: **Develop education tools and methods to effectively disseminate knowledge to public and health professionals. (intermediate term, low risk)**

1. Identified need or opportunity
   Increase awareness of fragile X spectrum disorders and publicize findings of consortium among providers and public.

2. Strength of evidence for approach
   Disseminating research findings and educating the healthcare providers and the public will increase knowledge and raise awareness about fragile X and FX spectrum disorders. There is a significant body of research which supports health education as a successful approach to improve knowledge which in turn contributes to adopting health behaviors and utilization of resources to improve quality of life and health outcomes (Glanz et al., 2002).

3. Short/Mid/Long range goal
   Mid range, low risk – Disseminating research findings and educating healthcare providers and the public will be an ongoing process.

4. Criteria for meeting goal
   a. Publications in peer-reviewed journals.
   a. Presentations at professional society and advocacy group meetings.
   b. Interviews and articles with the news media.
   c. Information on websites of advocacy groups and funding institutions.

5. Impact
   a. Increase awareness of FX spectrum disorders in the general public.
   b. Increase knowledge of health care professionals surrounding FX spectrum disorders.
   c. Improved treatment/outcomes for families and individuals affected by FMR1 mutations.
   d. Improvement in diagnostic practices of physicians in identifying individuals with FX-associated disorders.

Discussion of Draft Goals and Objectives

According to Dr. Sherman, elucidating the mechanism and identifying possible therapeutic targets are top priorities in FXPOI research. Currently, no model system exists to address such questions as the following: Is FXPOI primarily a problem of the ovaries or another point in the pituitary-gonadal axis? Why is the reproductive system particularly affected by the CGG repeat?

Regarding Objective 2.2, it was suggested that the group delete the term “cross-sectional” so that other study designs could be employed.

Dr. Sherman explained that Goal 3 (genetic and environmental factors that affect gene penetrance) is important to find out why only 20% to 30% of women with the premutation have POI. It would be important to characterize the effects of modifying factors.

Regarding Goal 4, which deals with treatment and management, Dr. Sherman noted that two approaches for genetic counseling are needed: one for women who experience POI and are diagnosed as having the premutation subsequently and another for women are first found to have the premutation. She also mentioned issues regarding fertility preservation, which would be best used among premutation carriers prior to their ability to give consent. One meeting participant suggested adding an objective under this goal to address the question of what it means to a woman when she is given the diagnosis of POI. What can we offer in terms of support and coping mechanisms?

Dr. Corrine Kolka Welt inquired about the meaning of "premutation" versus high-normal CGG repeat numbers. Another person mentioned the importance of the repeat number and risk for instability, noting that this area is a high research priority. Dr. Randi Hagerman said that some people with the premutation are in the "gray zone" and present with some FXS-like aspects.

Dr. Allan Reiss commented on the direct and indirect effects of *FMR1* mutation/premutation on phenotype and underscored the importance of defining risk alleles. Regarding premutation status, there have been issues related to emotional effects and so on. POI may be a mediator so that some mental and psychological symptoms are related to the hormone status of affected females.

Dr. Allyn McConkie-Rosell spoke about crossover among the goals and objectives identified by the three working groups. Perhaps research could be framed in terms of time points. For example, during childhood, the paramount issues are mental retardation, behaviors, how to talk to girls about the risk for POI and fertility preservation, and minor consent issues. Adult-onset problems would include POI and, in later life, FXTAS.

Dr. Kutluk Oktay recommended emphasizing that this is a spectrum disorder; therefore, the criteria should be broad and not just focused on POI. We have to keep a flexible approach.

Discussion of Working Group Reports
Facilitator: Tiina Urv, NICHD

Dr. Randi Hagerman discussed the case of an adult male construction worker with 150–200 CGG repeats. He demonstrated emotional volatility, intermittent anxiety problems, slight paranoia, mild hypertension, and facial flushing. He presented with a mild tremor. His wife was aware that he was an FX carrier and was concerned about possible FXTAS; his brother had the full mutation. On exam, he had large testicles, seen in about 30% of premutation males.

Dr. Hagerman observed that in the knock-in mouse, at about 150 repeats, FMRP is about 60% of normal. Fragile X boys sometimes have facial flushing. At the upper end of the premutation range, there is a decrement of POI problems in women. Perhaps there is a protective effect against FXTAS or POI problems by having a slight FMRP deficit. How does the protein deficit relate to some premutation signs and symptoms? What is the effect of mRNA overexpression?

Some patients might be in this gray area. We know how to treat some emotional problems. We are learning about some neuroprotective measures to reduce premutation involvement, e.g., treating with antioxidants. We can learn much from patients seen in clinics.

Dr. Bailey emphasized that from a genetic/epigenetic perspective, larger studies are critical to get at such questions. Dr. Paul Hagerman observed that some symptoms/signs that are common in old age resemble premutation effects: essential tremor, high blood pressure, dementia. We need to define the gene and allele frequencies carefully, looking at frequency distributions across the spectrum. Another question is penetrance. He recommended carefully examining, for example, the group with 100 repeats in FXTAS to see what other symptoms they have. FXTAS has many faces.

Another participant said that the goals and objectives do not include adequate emphasis on the family system. The FXTAS group has addressed quality of life, but we need more information in the FXS goals/objectives. FX-associated disorders have a big effect on the family system. We know very little about aging within individuals with the full mutation. What services do they need? One person asked whether the lifespan of full-mutation individuals is the same as normal. They are dealing with a compensated system.

A discussion ensued about the level of detail to include in the goals/objectives. What will get the right attention in the right quarters? What are the risks of providing too much detail? Several people cautioned about taking a prescriptive approach because the aim is to have people bring in new ideas. Simpler may be better.

Dr. Randi Hagerman noted that members of Congress are thinking about aging. The research plan should emphasize how fragile X mutations are portals for understanding many other disorders, e.g., autism, anxiety, Parkinson's disease, Alzheimer's disease, infertility, and dementia. Fragile X mutations can help us understand these other disorders. It would be advantageous to move away from the perception of fragile X as a rare disorder, because it can give valuable insights into more common diseases. Fragile X research will have a broad impact on all brain-related research.

Several participants recommended collapsing themes that cut across the three working groups and then having the working groups focus on their specific areas. Each group is at a different place in terms of developing its goals and objectives. Dr. Urv reminded the participants that the goal is to make the report a living document that will give NIH some parameters about what direction to go in the science. There must be a balance in terms of the level of specificity in the stated objectives.

Summary of Working Group Progress
Working Group Chairs

Dr. Urv discussed the format for the research plan. The goal will be the larger unit, with objectives being secondary. She laid out the desired format:

- The goal: An identified need or opportunity.
- Strength of evidence for the approach: We need to avoid a prescriptive effect. What is the rationale for setting the goal in the first place?
- Short-/mid-/long-range term for the objectives: This is a way of categorizing the objectives. There will also be an introductory paragraph for each objective.
- Impact as measured by outcome measures: The trans-NIH planning group will come up with the milestones, as they will be the ones accountable for them. Outcome measures help Congress set funding priorities. Low-risk investments are most likely to pay off. A drug trial is high risk because it is expensive and might not work. Risk has a financial connotation. Risk when it comes to science represents the odds of success. If the odds of success are high, the risk is low. If all NIH projects succeed, maybe we are not taking enough risks. Dr. Lawrence Nelson mentioned risk-benefit assessment. A field of endeavor might be high risk but offer high benefit, too, as an offset. Risk is subjective.

Summation of Day
Randi Hagerman, University of California, Davis

Noting the many commonalities among the working groups' goals and objectives, Dr. Hagerman opined that NIH will be able to collapse these documents into a readable report. Almost every group, for example, cited the need for mechanistic studies and for infrastructure. Also, treatment needs have been widely identified.

Dr. Hagerman addressed the interplay and interaction among disorders. Some comments were heard about not looking at a disorder in isolation; rather, it is important to consider shared features. She suggested that the group should consider a term to describe the continuum of FX-associated disorders, e.g., *FMR1* spectrum disorder. Such an overarching name would relate better to what is seen in the clinic, as for autism. It will be critically important to relay to Congress how important fragile X is in terms of all its features and that it may be additive with other problems.

The meeting participants expressed divergent views about applying a new term. Some said that a continuum or dimensional view would be better than the present categorical approach. Others warned about the circularity of the autism spectrum nomenclature. Some recommended a move away from categorization and instead trying to impart a message without boundaries. Another

participant encouraged a *lingua franca* for the field. He advised "giving it a brand." Dr. Randi Hagerman said that it is disconcerting that many clinicians are calling FXTAS "Fragile X" and so forth. There are some learning barriers and it will be important to raise awareness among clinicians. Dr. Nelson suggested the name *FMR1* spectrum disorder. Dr. Reiss said the challenge would be clarifying the penetrance issue. Even those with full mutation are affected to different extents. He agreed with adopting a term such FMR1-associated disorder because the categories would not be mutually exclusive, which they are not. Other said that the term "fragile X" is already familiar to many. Also, it was noted that some people do not like the "MR" in FMR1 because it refers to mental retardation and that FMR1-associated disorder would likely become known as the acronym FAD. Dr. Joan Bailey-Wilson recommended not changing the abbreviations. In addition, "fragile X" might have to be used for billing purposes. Others recommended developing a unifying theme. The gene has been identified. The theme may well be RNA toxicity, which is likely affecting the whole body. One person recommended that the group consider changing the name of the gene since "mental retardation" has nothing to do with POI or FXTAS. One possibility was *RT1* for RNA toxicity gene. Others disagreed, saying that gene names are legacy. The trend now is to name genes for functions.

Overview of Thursday
Andrea Beckel-Mitchener, NIMH

The focus for the next day's meeting was overarching issues and goals. The working groups' goals and objectives documents will require some retooling. It was decided to handle this task after the meeting via the wiki site. The focus for now should be on cross-fertilizing the science.

THURSDAY, MAY 8, 2008

FX, FXTAS, POI—Overarching Issues: A Clinical Perspective
Elizabeth Berry-Kravis, Rush University Medical Center

Dr. Berry-Kravis discussed issues that span the spectrum of FXS, FXTAS, and FXPOI. She addressed how these *FMR1*-related disorders overlap within families and clinical situations.

She spoke about several families where these issues interact. One family presented in the clinic when triplet children were born, all with fragile X. One boy is severely impaired, one girl is high functioning but with behavioral problems, and one boy has moderate impairment. The mother had received fertility treatments. The father has some emotional problems and gait issues. The mother appears to be approaching menopause at any early age. This case raises the question of who should have an *FMR1* DNA test before using a fertility intervention. How often does FXS occur due to fertility interventions? We need research to guide OB/GYN practice regarding testing in the context of infertility treatment.

Another family was referred because the pediatrician saw a child with Down syndrome and fragile X. An older daughter had fragile X, mild cognitive issues, and attention deficit disorder. Because the older child had fragile X, the baby was also tested and found to have the full mutation (methylated). The questions raised by this case are: Does Down syndrome occur more frequently in premutation carriers due to POI? Is there an ovarian aging effect that might

increase frequency of Down syndrome births to POI women? Is FXS ever diagnosed if there is Down syndrome and no family FXS? Is FXS a cause of Down syndrome with severe behavior manifestations?

Dr. Berry-Kravis spoke of a woman who was really caught in the middle. She has a significantly involved FXS child with severe anxiety, hyperactivity, autistic behavior, and poor verbal skills. Her father has progressive ataxia and is confined to a wheelchair. He can no longer care for himself. The woman is sandwiched between these two generations of affected family members. Are there adequate resources available to families? How does the middle generation cope? How much anxiety is attributable to genetic causes and how much is environmental? What are her risks (e.g., endocrine, psychological) as a female carrier? Who is at risk for FXTAS? Is a person's risk of developing FXTAS greater if his or her father (or mother) has it? How much of this woman's stress is due to having the premutation and how much is due to the stress of caring for her two family members?

Another child presented at the clinic at age 4 with hyperactivity. The mother's father was an architect who had tremor/ataxia/Alzheimer's disease. He died. The girl had fragile X; her normal allele had 56 repeats, so she had a premutation and the full mutation; both chromosomes were affected. The husband was very anxious after learning that fragile X was in his family as well. This case raises questions about the frequency of these alleles. What is the real frequency? Are the husband's risks for FXTAS the same as in an FXS family? Can one person have FXS, FXTAS, and POI if he or she has the premutation and the full mutation? How often could FXTAS lead to FXS diagnosis or prevention?

Another case discussed by Dr. Berry-Kravis brought to the fore the issue of family clustering. A young woman in her 30s had mild cognitive problems. Her mother learned about fragile X on the Internet and recalled that throughout her family, people had tremor/ataxia/neurologic disorders as well as some cases of neuropathy and executive function disorders. Is there family clustering? Are some families more susceptible to FXTAS? Is POI more frequent in families with more FXTAS or vice versa, or are the risks separate? Dr. Randi Hagerman commented that her group did not observe increased incidence of POI in 18 women with FXTAS compared with the women without FXTAS. There might be some similar RNA toxicity, but it does not seem to be linked with FXTAS.

Finally, Dr. Berry-Kravis presented the case of a boy she has followed since age 2. He has mild fragile X deficits, good social skills, and a mild intellectual disability. There is a large scatter between his verbal and physical abilities. The mother has significant problems with anxiety. Her brother has been treated for attention deficient disorder, depression, and anxiety that has increased throughout his adult life. Questions raised by this case include the following: What is "mild FXS" (static) and what is "early FXTAS" (CNS toxic)? Are there really more problems in carriers, or is this hypervigilance and ascertainment bias, generating over-reporting of the expected frequency of these problems in the carrier population? Why do carriers have developmental problems? Are the deficits and problems the consequence of cellular mechanisms caused by decreased FMRP (as in FXS) or of increased mRNA (as in FXTAS), which could lead to RNA toxicity in the developing brain? Or are they the result of a combination of these factors?

More research is needed. These problems span the entire spectrum of the tangled web of fragile X and *FMR1*-associated disorders.

Dr. Randi Hagerman said that clinicians see such problems every day and that is what drives the research. She acknowledged the potential for bias problems in studies that focus on clinic populations. Would the prevalence rates be the same if population screening were done? Dr. Reiss commented on the sex chromosome aneuploidy study. When ascertainment bias was eliminated, the incidence of problems decreased.

Dr. Sherman pointed our some problems with studies looking at anxiety in premutation carriers because their anxiety levels increase when they learn that they are carriers. There are ethical problems. Looking at phenotypes would provide a sound epidemiologic basis. Dr. Reiss said that if we are going to start providing knowledge to clinicians and families, we need to screen them to see if they are at risk. Another person disagreed because of the detrimental effect of telling people they are premutation carriers. There are good epidemiologic studies that can be designed without these ethical issues.

Dr. Bailey recommended conducting a prospective study to see how people receive the information; the problem is that this is a particularly vulnerable population to receive this sort of news. The stress in affected families is incredible. If one looks at depression in a cross-sectional way, rates are low, but if you look at depression over the lifetime, rates are very high.

Dr. Randi Hagerman said that, among the premutation group, at the upper end of CGG repeats, we see a number of problems related to diminished levels of FMRP. Newborn screening could lead to earlier detection and treatment. If screening reveals a newborn with FXS, however, it will be necessary to deal with an entire family tree.

Explanation of Overarching Goals: *FMR1* Sliced Another Way
Facilitator: Robert Riddle, NINDS
Scribe: Tiina Urv, NICHD

Dr. Riddle said that there is much to be learned about the normal function of FMR1 protein. We need a common approach to the spectrum of *FMR1* disorders that could lead to faster results, and we need to improve the way we do research on fragile X.

Dr. Riddle initially proposed breaking into two groups—one working on basic FMR1 biology and the other on clinical aspects of *FMR1*-associated disorders—to look again at the documents to uncover overarching objectives and see how the goals fit into the bigger picture. After a subsequent discussion, the participants decided to stay together to elucidate cross-cutting themes.

1. What is the biology or pathophysiology underlying this part of the *FMR1* gene?
Toxic mechanism of elevated mRNA: Elevated message occurs in FXTAS and POI. Some people affected by fragile X also can have elevated message, especially in mosaic individuals who have a significant number of cells with the premutation. Those who are double heterozygotes—one allele with the full mutation and one allele with the premutation—are likely to have elevated message. What is the effect of having depressed levels of FMRP or having both

decreased protein and elevated message? Having decreased FMRP may blunt some effects on phenotype. In males with 180 repeats, for example, FXTAS is rarely seen. There is a subgroup of people with the full mutation who appear to have some RNA toxicity. Down-regulation of the *FMR1* gene occurs late in fetal development.

Instability of the CGG repeat: What about the CGG repeat causes an increase in message and decreased FMRP and how do those affect phenotype? Geneticists would think in terms of expression and function. Dr. Lawrence Nelson recommended starting with the final common pathway to see the effect on the cells that are the end of the pathways. Dr. Paul Hagerman discussed the importance of nucleic acid research because that will answer the question of how the CGG repeat and its stability affect what we are interested in with regard to the disease. Predictions can be made on the basis of RNA toxicity. For example, one can see a decrease in number of individuals who have POI at the high end of the mutation. If the mechanism is RNA toxicity, some with the full mutation should also get POI. He recommended trying to use basic mechanisms to make clinical predictions. Dr. Randi Hagerman introduced the overarching issue of translation. Some with premutation with an expanded CGG repeat are producing mRNA that is not translated well.

Resource development: Dr. Paul Hagerman said mouse models are needed to test specific issues related to RNA toxicity. This is an overarching resource need.

Dr. Berry-Kravis stated that there is some risk of premutation toxicity in the elderly. There could be some aging problem related to low FMRP levels that has not been studied in individuals with the full mutation. We need to look at the possibility of decline.

2. What is the incidence rate of allele variation?
If a person has X number of repeats, what are his or her chances of developing FXTAS, POI, or retardation? These phenomena develop on the trajectories of development as driven by the genotype. Are they also driven by experience? If we could do a longitudinal prospective study, these questions could be answered.

Newborn screening: Dr. Paul Hagerman discussed newborn screening (1) conducted with informed consent and with follow-up, or (2) done anonymously. The first approach would be biased because of the consent issue. Both types would provide valuable information on gene penetrance. For example, if we know the prevalence of the full mutation is 1 in 3000, and the phenotype is evident in 1 in 2000, what is the cause of the difference? It was recommended to investigate penetrance and gene frequency for all three disorders. Large-scale screening would cut across all three domains. Dr. Bailey said that anonymous screening would be cheaper and give important information but would not yield any data on phenotype. Some quick anonymous studies would be a good place to start and would provide some information on race/ethnicity distribution of the genotypes, too. Anonymous screening would show allele incidence. Until we do a good study in which ascertainment bias has been eliminated (study of the whole population), we will not know the incidence rate for genetic alleles for the mutation, and within the mutated rates what the incidence rates of clinical problems of interest are.

Screening could be done very cost-effectively by using stored biologic samples and using the blood-spot screening test. The Isle of Wight study was mentioned.

Screening of reproductive-age individuals: One person recommended screening reproductive-age people for POI. Dr. John March mentioned doing a case-control study using an overlapping cohort design. Be careful about selection bias so the sample is representative of the population.

3. Genotype, phenotype, environment

Use of existing data and samples: Dr. March pointed out a potential problem with trying to use existing data sets; they are unlikely to have collected the data elements that would be needed for correlating fragile X genotypes and phenotypes. The National Children's Study was mentioned as a possible source of data. The group recommended that an inventory of data sets and biologic samples be done across the Institutes to see if they include the necessary data elements and, if so, whether the data can be harmonized.

Clustering of disorders: Dr. Richard Anderson commented on depression in premutation carriers. We need more longitudinal studies of fragile X disorders and other conditions. He cautioned about being too quick to make links. Others mentioned clustering of fragile X disorders with other problems and the possibility that genetic modifiers might be subgroup specific.

Needed studies: All the working groups are concerned about allele variation, the full range of phenotypic expressions, and how they relate to gene expression. Most likely, prospective longitudinal studies and population-wide screening would be the way to gain such knowledge.

Environmental factors and toxicities: For example, smoking can lead to earlier POI. FXTAS can be exacerbated by excessive alcohol intake and surgeries. Oxidative stress problems can be observed in the brain, especially in full mutation, but also in the premutation. The result is that these populations may be more vulnerable to fragile X disorders.

4. Management

Treatment studies: There are few treatment studies that include all three fragile X–related disorders. We need to consider two categories of treatment studies: (1) explanatory trials that ask whether the treatment works, and (2) studies to determine who the treatment works for. The latter category introduces moderator variables. The intervention would be tested across different sorts of populations to see what works in whom. We are in the explanatory phase and are ready, in the case of FXS, to begin targeted treatment trials. Nevertheless, challenges remain with regard to trial design and outcome measures. What sample size is needed to detect a particular difference? Addressing the question of moderators requires very large sample sizes. "Noise" decreases power by increasing variation.

FXTAS-focused studies: There is a great deal of anecdotal information about effects of various interventions on FXTAS on symptoms and so forth. We have a short-term need to start testing some of these. One person argued that we need to develop an evidence base, especially in

FXTAS, that certain drugs work, and one advised conducting a study of the effect of occupational therapy.

POI-focused studies: For POI, we need studies of pharmacologic and nonpharmacologic mental health interventions. According to one meeting participant, NIMH is sponsoring some studies along these lines. Dr. Reiss would not advocate trying existing drugs for purely symptom-based, behavior-based studies. From the methodologic side, the field has matured beyond that. The real hope for effective treatment in the clinical setting is going to come from the basic science/translational spectrum. There might be some rationale that certain classes of drugs might have activity in this spectrum disorder. Another idea was to study the effect on women when they alter their reproductive plans after learning they are at risk of POI.

Innovative study designs: Also mentioned were adaptive treatment strategies, including sequential multiphase design and treatment strategies that allow trials to move through a series of treatments, and fractional ANOVA analysis. One participant recommended recruiting subjects with FMR1-associated conditions in conjunction with Alzheimer's disease studies to get some data on drugs already being studied (piggy-backing). Another person noted that for clinical, real-world indications, we underutilize single-case designs.

Research consortia: It has been 17 years since the gene was discovered, but not a single treatment is available yet. No psychosocial intervention studies have been conducted. It is time to ramp up the treatment agenda. We need to be smart about design, selection of interventions, and so forth. A research consortium could help investigators find the population they need in terms of target age, phenotype, and so forth to evaluate not just medical treatments but the full range of possible interventions. We also need to keep in mind the timing of interventions.

Prevention themes: Participants mentioned oxidative stress as well as seizures, which are known to exacerbate autism. Perhaps we need to screen for signs of seizure activity in the brain.

5. Fragile X and the effects on the family

Support: How do families cope with fragile X? What are the ramifications of intergenerational effects? How can we minimize stress? The first step is finding out what sort of supports would be most helpful.

Gene-environment interaction with respect to coping: Dr. Reiss asked about how to control for the overt stresses that are part of the phenotype as opposed to stress brought on by learning about risk of fragile X disorders or receiving a diagnosis. This is the area where biology and sociocultural effects interact. Fragile X is similar to many other disabilities but with added intergenerational aspects plus heritability.

Coordination of care: These families are under stress because their clinicians lack knowledge. Provide a network of providers (medical, counseling, therapists, respite care) for a range of needed services, including case referral. For individuals who are aging, challenges include developmental problems, respite care, home services, and counseling.

The problem that most clinicians have is not how to treat the disorder; rather, it is the problem of comorbidity. In the cognitive therapy literature these days, people are moving away from disorder-based manuals and taking elements within the manuals to develop modules that can be integrated to address each individual's symptoms. Emotional regulation is very important and cuts across all three disorders.

Family changes/treatment of multiple generations: Disorders need to be managed all along the lifespan and in the context of whole families and through generations. Clinicians need to work with the whole family in an integrated way.

Other cross-cutting themes that emerged during discussions were how to deal with stigma and how to provide living settings and work settings for adults with disabilities (FXS and FXTAS). The participants acknowledged the contributions of the families who have been involved in shaping research agendas for FXS.

6. Infrastructure

Measures: Dr. Kolka Welt pointed out the need to characterize the phenotypes and develop some standard metrics for assessing these disorders in large-scale screening. Harmonizing the metrics would be to great advantage as long as the process does not "hide" something that is important. One participant observed that there are many quantitative measures to evaluate extent of affectedness. Dichotomous yes/no responses might not be sufficient.

Researchers all have their own favorite measures. We need to delegate the final authority to a governance structure to decide which measures will be used based on their feasibility and standardization. A good example is the clinical trials network at NIAID that is involved in myriad small studies. Measures are often collected by clinicians, not researchers.

Dr. Bailey posed a hypothetical question: If we had a pot of money and a sample of children available, would we have the measures in place to do a study? Dr. Beckel-Mitchener said that some relevant measures have not been validated yet, but are close. Behavioral measures and psychological functioning measures are well established.

Animal models: Dr. Paul Hagerman said there is a need for additional and better mouse models—inducible, conditional models, in particular. Dr. Nelson agreed this is a significant need, but funding is a challenge. He mentioned that the cost of maintaining an aging mouse population is great. A consortium effort, broader than just one lab, would be needed to maintain live colonies. NIA makes available some aging mouse models. Another participant suggested identifying new models with a faster onset of disease.

Research consortium: The NFXF has embarked on the effort to create a consortium of those undertaking fragile X research. Research could be conducted at multiple sites with a common theme and in a coordinated way. The field is ripe for a major consortium that would provide shared infrastructure, e.g., a sample repository and a data coordinating center. Funding agencies would need to support planning meetings. NFXF has been largely filling this role to this point.

Drug screening: We need to screen drug libraries for drugs that can block RNA toxicities. The

Roadmap program has a library of 300,000 compounds that could be screened. (Dr. Linda Brady can provide more information.)

Other overarching infrastructure needs identified by the group included:

- Tissue and brain banking, including 500,000 blood spots.
- Larger scientific workforce dedicated to this field.
- Information dissemination to the public to keep support high.
- Database of those who have the alleles.
- Technology transfer/intellectual property agreements to capitalize on existing resources.
- Imaging databases.
- Web resources.

Fragile X Research Centers
David Nelson, Baylor College of Medicine

In the 2000 time frame, there were efforts by NFXF and FRAXA to establish fragile X research centers. Funds were dedicated to create three centers. NICHD put together an RFA in January 2002 based on a novel funding approach. The Mental Retardation and Developmental Disabilities Research Centers (now known as the *Eunice Kennedy Shriver* Intellectual and Developmental Disabilities Research Center—EKSIDDRC) were already working on intellectual disabilities.

Three centers were funded with modifications:

- Baylor–Emory center.
- UNC/Wisconsin/Kansas.
- Seattle/Davis.

Grantees were directed to develop at least one novel core, set up a new administrative core, and to use at least one existing core. Projects must show significant synergy.

The Baylor College of Medicine–Emory funding was initiated in August of 2003 as a supplement to the Developmental Disabilities Research Center. Dr. Nelson outlined the research projects.

Fragile X Clinical and Research Consortium
Robert Miller, National Fragile X Foundation, and Don Bailey, RTI International

Families affected by fragile X disorders were flying from coast to coast to seek care at certain clinics. This posed a tremendous burden to them, especially since many had to travel long distances with cognitively impaired children. Another problem was that various institutions were not aware of what others were doing. To address these problems, in February 2007, representatives from thirteen U.S. and one Canadian fragile X clinic met in St. Louis, Missouri, for the first formal meeting of the Fragile X Clinics Consortium. The meeting was organized by the NFXF, which is providing the administrative structure along with facilitation, coordination, and seed money. Among the consortium's goals are to

- Provide evaluation, treatment, or treatment recommendations while expanding the knowledge base of effective interventions, particularly for difficult cases.
- Offer comprehensive services in fragile X clinics to families and reduce the need for them to travel long distances for care.

For the consortium, which now consists of 14 clinics, Mr. Miller focuses on clinical aspects, and Dr. Bailey focuses on the research. An international subcommittee is looking into partnerships with institutions around the world. The consortium is working on establishing best practices, developing standard forms, and building a body of knowledge for others who want to open a center. NIH and CDC have been interested and supportive with their advice. Mr. Miller mentioned the upcoming international meeting in St. Louis.

Dr. Bailey said that he serves as a member of the board of NFXF and as an intermediary between the foundation and the clinics. This is very much a bottom-up initiative coming from the clinics and the foundation. The enthusiasm for participating in the absence of major funding has been impressive. Almost the entire scope of knowledge is represented among the clinics. It is very inclusive by design. About 1,000 families are involved—a tremendous resource for aggregating larger samples for clinical trials of medicines and psychosocial interventions.

A letter of intent has been forwarded to CDC for submitting an application for infrastructure support. The goal is to set up a data coordinating center. The first tasks will be data aggregation and description, surveillance in communities, and treatment studies.

Summation of Day
Randi Hagerman, University of California, Davis

This meeting provided an opportunity for communication across disciplines and interactions of top clinicians and researchers working on different facets of *FMR1*-associated disorders. Translational research is an important theme, because we need to get the latest information into the clinical realm. The Fragile X Clinical and Research Consortium may provide a means of disseminating information as participants share with others in their local areas. Translation must stay at the forefront.

Clinicians see the chaos experienced by families affected by *FMR1* disorders. What is most important to families is treatment. Therefore, we need to get clinical treatment trials under way, including studies with existing treatments for other neurological conditions, new targeted agents, and preventive treatments.

We are at the forefront with regard to our understanding of neurodevelopment and neurodegeneration. Fragile X is a portal for understanding many disorders that cut across the Institutes. We want to be sure that fragile X testing is part of other studies looking at aging effects, psychobiology, and so forth. We need to find out to what extent fragile X plays a role in Alzheimer's or Parkinson's disease, psychopathology, and autism. To do so, we should start communicating across research consortia outside of the fragile X field.

In terms of neurobiological pathways, we need to learn more about what is stimulating mitochondrial dysfunction, apoptosis, and so on. We are starting to understand the layering that might affect disease progression. Also, environmental toxicology and fragile X may be fruitful areas of research.

Dr. Paul Hagerman commented that fundamentally, what we are talking about is translational research. There is some history of tribalism in the fragile X field with the realization that there are different phenotypes. The trend should toward greater inclusivity. We need to continually emphasize the theme that, although fragile X is an orphan disorder, it can provide key information for the study of neurodevelopment and more common neurologic and neurodegenerative disorders, such as autism and Alzheimer's disease. We need to keep in mind the broader impact of what we are doing.

Dr. McConkie-Rosell said that fragile X is also a model for studying women's health because of the implications of POI, especially with regard to family management and genetic counseling across the lifetime trajectory.

Next Steps
Tiina Urv, NICHD

Dr. Urv asked that the working groups provide their final drafts by June 30, 2008. NICHD will conduct several levels of review in July and August to synthesize and refine the unified document and obtain necessary approvals.

Dr. Randi Hagerman again urged the group to consider renaming fragile X disorders as a spectrum disorder to capture those who do not fit cleanly into one of the three groups. Considering these disorders as part of a spectrum would stimulate further research into how they are additive to other neurological and psychopathological disorders and also would help alleviate stigma and anxiety.

Mr. Miller invited Dr. Urv to use the upcoming international NFXF meeting as a forum to handle any last-minute details.

Concluding Comments and Adjournment
Duane Alexander, Director, NICHD

Dr. Alexander thanked the participants and observed that the resultant report will guide NICHD research and help carry out the necessary studies on fragile X. He recounted briefly the history of how the *FMR1* gene was discovered and highlighted the pivotal role of NIH. Many Institutes now have fragile X within their mission. This group has provided essential support and camaraderie as it works together on fragile X syndrome and other *FMR1* disorders.

The products will be far better thanks to the advance work for this meeting, which was overseen by Dr. Urv. He emphasized that the outcome will be an excellent progress report for Congress and a working document for NICHD. It will be particularly important to get clinical trials and newborn screening under way.

We are poised to make meaningful changes in patients' lives. Dr. Alexander has great confidence, based on the quality of science, commitment, and dedication of NIH staff and others here, that this effort will be a successful one.

The meeting was adjourned at 2:00 p.m.