

FRAXA UPDATE

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FRAXA RESEARCH
FOUNDATION

"NEVER

DOUBT

that a small

group of

thoughtful,

committed

citizens can

change the

world.

INDEED,

it's the only

thing that

ever has."

— Margaret Mead

FRAXA Funds New Research

by Katie Clapp, president & co-founder

FRAXA Research Foundation FRAXA kicked off 2004 by funding 10 grants and fellowships for a total of \$555,000! Over the past several years, FRAXA has funded over one million dollars in research grants each year, accelerating the pace of progress, scientific publications, and discoveries towards our goal: specific treatments and ultimately a cure for Fragile X syndrome.

The new projects pursue two lines of inquiry:

1. Solving the mystery of FMRP, the protein lacking in brain cells of people with Fragile X. Scientists are identifying the molecular targets of this protein in order to guide the design of effective new treatments and to identify existing treatments to help people with Fragile X.
2. Pursuing the mGluR theory of Fragile X and the drug therapies (Ampakines and mGluR antagonists) that it suggests.

FRAXA's next deadline for new research proposals is May 1, 2004. After peer review by our Scientific Advisory Board and other scientists expert in Fragile X research, the additional awards will be announced in July.

As you will see from the descriptions starting on page 3, there has been great progress in Fragile X research. In the words of Dr. Stephen T. Warren, a FRAXA Scientific Advisor and co-discoverer of the Fragile X gene in 1992, "although a great deal of work remains to be done before clinical trials [based on the mGluR theory] can be initiated, for the first time in 22 years I can see a rational therapeutic approach."

"... for the first time in 22 years I can see a rational therapeutic approach."

— Dr. Stephen T. Warren

10th Anniversary FRAXA Galas Set for this Spring

May 6th in Omaha

The Nebraska Fragile X Families Association presents the Seventh Annual Mary Higgins Clark Fragile X Gala, with Special Guests **Senator Chuck Hagel, TIME Magazine president Eileen Naughton, and author Mary Higgins Clark.**

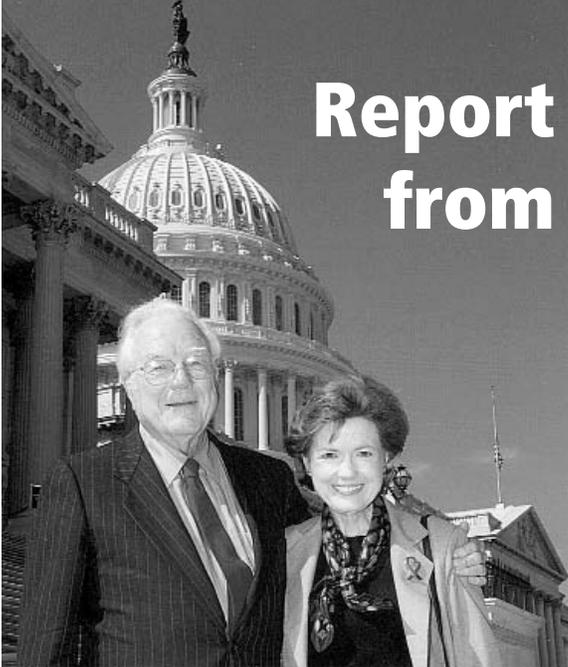
Also in this issue:

- Report from Washington
- New Research Reports
- Research Studies Looking for Subjects

May 19th in Boston

Harry Manion agreed to chair FRAXA's 10th Anniversary Gala at the Copley Plaza Hotel in Boston and has assembled a spectacular evening with celebrity guests including **Governor Romney, Boston's Mayor Menino, author Mary Higgins Clark, Roger Mudd, Boston sportscasters Bob Lobel and Bob Neumeier, and sport stars Danny Ainge, Howie Long, Sean McDonough, Willie McGinest, Rick Middleton, and Terry O'Reilly.** **Ruth Pointer** of the fabulous Pointer Sisters will perform. This is not an event to miss! Contact Katie Clapp at 978-462-1866, FRAXA.org or kclapp@fraxa.org.

FRAXA is a nonprofit, tax-exempt charity run by parents of children with Fragile X syndrome. Fragile X syndrome is the most common inherited cause of mental retardation and developmental disabilities, affecting approximately 1 in 4000 males and 1 in 6000 females. FRAXA's goal is to accelerate research aimed at the treatment and cure of Fragile X, by direct funding of promising research projects and by raising awareness of this disease.



Report from Washington:

By Mary Beth and David Busby

Congress is hard at work, and the annual Reports of the House and Senate Appropriations Committees are our major focus. These Reports accompany the appropriations bills of the House and Senate and tell the administrative offices of the federal government (the National Institutes of Health (NIH) the Centers For Disease Control and Prevention (CDC) and the Health Resources and Services Administration (HRSA)) how to spend the appropriations. We are asking our FRAGILE X ADVOCATES (that's you!) to urge members of Congress to provide funds for the following ten requests:

1. the National Institute for Child Health and Human Development (NICHD) to issue a Request for Applications to enhance its new Fragile X Research Centers and to recruit new Fragile X researchers.
2. the National Institute of Mental Health (NIMH) for its studies of causes of, and pharmacological treatments for, Fragile X and related disorders such as autism.
3. the Office of the Director of the NIH for coordination of Fragile X research.
4. the program of the Director of the NIH to

enhance pediatric training and career development grants to include new Fragile X researchers under the Children's Health Act of 2000.

5. the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) to study the effects of Fragile X outside the brain.
6. the CDC's National Center on Birth Defects and Developmental Disabilities to develop a public health and epidemiological research initiative on Fragile X
7. the CDC to screen and provide help for families and individuals affected by Fragile X and other heritable disorders.
8. HRSA to expand the Title XXVI newborn screening, counseling, testing and special services program for newborns and children at risk for heritable disorders, including Fragile X.
9. the National Institute of Neurological Disorders and Stroke (NINDS) to study the effect of Fragile X on fundamental brain circuitry, especially on older carriers.

FRAXA's Law Firm Wins Award

DORSEY & WHITNEY LLP, which has served pro bono publico as FRAXA's counsel for the last 10 years, was honored to receive the coveted National Law Journal's 2003 Pro Bono Award to large firms for its long-time commitment to pro bono service. By agreement with the American Bar Association, the Dorsey firm donates at least 3% of its billable hours to charitable organizations. FRAXA is a major recipient of this program. The firm is headquartered in Minneapolis and has 21 offices worldwide. David Busby, a father of two Fragile X sons, is "of counsel" to the Washington, D.C. office and serves as, among other things, FRAXA's liaison with the Federal Government.

10. NINDS to expand research of Fragile X-associated tremor/ataxia syndrome and provide counseling for daughters of FXTAS patients about their carrier status of the Fragile X mutation.

Here is how you can help make these requests become reality:

Write, call or visit your Senators and Congressional representatives. Explain your personal interest in Fragile X. You will be surprised how much impact just a few letters or calls or visits can have!

HOW TO CONTACT CONGRESS

Write to Senators at:

Senate Office Building
Washington, D.C. 20510

Write to Representatives at:

Cannon House Office Building
Washington, D.C. 20515

Or, visit www.congress.org on the web.

HOW TO JOIN OUR FRAGILE X ADVOCATES

David Busby maintains an email list of ADVOCATES who are willing to contact members of Congress at critical moments in our advocacy efforts. You can join this list by calling David at (202) 442-3512 or emailing Busby.David@dorseylaw.com

Megan Massey Appointed to Federal Post

FRAXA Board member, Megan Massey, has been appointed by the Secretary of Education to the Federal Interagency Coordinating Council, which was formed as part of the Individuals with Disabilities Education Act (IDEA) in 1991. Megan's two sons, Jack and Jacob, both have Fragile X.



The FICC advises cabinet secretaries from the Departments of Education, Health and Human Services, Agriculture, Defense, and the Interior, as well as the commissioner of the Social Security Administration. The council's goal is to improve opportunities for children with disabilities. The FICC meets quarterly to identify gaps in programs and services, ensure the provision and support to children and their families, coordinate technical assistance activities across agencies, and identify barriers to this coordination of services.

These meetings take place in Washington, so Megan will be making the trip from Scottsbluff, Nebraska, on a regular basis. Congratulations to Megan for taking on this important work.

Kathy May is Public Citizen #1

FRAXA co-founder Kathy May of Fairfax, VA, was recently selected as the 2004 Public Citizen of the Year by the Virginia Chapter of the National Association of Social Workers. Kathy will now compete with winners in all 50 states for the overall national title.



Kathy May helped to start FRAXA in 1994; without her unflagging enthusiasm, there would not be a FRAXA. Kathy's son Sam, now 14, has Fragile X. Kathy has worked for the ARC of Northern Virginia for 10 years.

In her current position as Lead Advocate she focuses on influencing outcomes that directly affect the lives of individuals with developmental disabilities.

FRAXA Financial Report for 2003

Our audit for 2003 is done, and again FRAXA excels at efficiency.

2003

Income

| | |
|---|-------------|
| Donations, fundraising, investments, book sales | \$1,841,000 |
|---|-------------|

Expenses

| | |
|-------------------------|-------------|
| Research (FRAXA Grants) | \$1,103,000 |
|-------------------------|-------------|

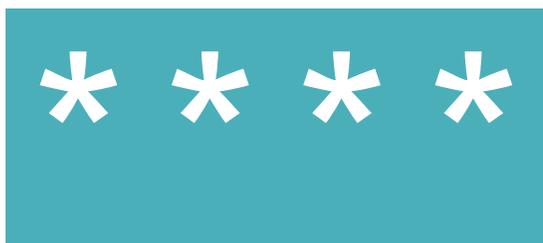
| | |
|--------------------------------------|-----------|
| Research (jointly funded with NICHD) | \$200,000 |
|--------------------------------------|-----------|

| | |
|-----------|----------|
| Education | \$28,000 |
|-----------|----------|

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|------------|----------|
| Management | \$36,000 |
|------------|----------|

| | |
|-------------|-----------|
| Fundraising | \$180,000 |
|-------------|-----------|

In 2003, FRAXA raised more than twice the amount we raised in 2002, which means that we can now fund significantly more research. The 2003 increase was thanks to a growing number of individual donors – more than 3000 in all! Our task for 2004 is to spread the word and broaden our base of support so that we can, in turn, accelerate the pace of Fragile X research.

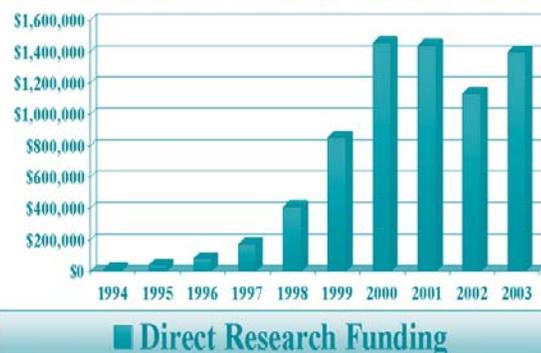


Four Stars for FRAXA!

FRAXA has again received a 4-star rating from Charity Navigator, the largest independent evaluator of charities in the United States. Receiving four out of a possible four stars indicates that FRAXA excels, as compared to other charities in America, in the area of strong fiscal management. Visit www.CharityNavigator.org on the Web for a nice set of charts rating specific aspects of our organization. Guidestar, www.Guidestar.org, is another site featuring further details on FRAXA and other national charities.

Figures are rounded to the nearest \$1000 from financial statements audited by Anstiss & Co, P.C., CPA.

FRAXA Funds Research



FRAXA dollars spent on research grants and fellowships

FRAXA Grants and Fellowships Awarded in January 2004

If you would like to explore the entire portfolio of FRAXA funded research, past and present, please visit our website, www.FRAXA.org. Each FRAXA investigator has a page devoted to his or her research.

These descriptions are written by the investigators and edited for a general audience by Katie Clapp.

Specific Tests of the mGluR Hypothesis

PETER VANDERKLISH, PhD

Scrrips Research Institute, San Diego, CA; \$50,000

Dr. Vanderklish became fascinated by Fragile X after attending last year's Banbury meeting. Banbury conferences are sponsored each spring by FRAXA through a grant from the National Institute of Mental Health (NIMH).

Funded thanks to Andrea and Damon Shelly, who hosted a Christmas luncheon to benefit FRAXA in December, 2003.

Our Previous Work

Consistent with the mGluR theory (see box at right), we observed that stimulation of mGluRs leads to elongation of dendritic spines. These changes in dendritic spine shape are dependent on protein synthesis and resemble those that occur in the Fragile X brain. Interestingly, multiple lines of evidence indicate that LTD and spine elongation are mechanistically linked; that is, that longer, thinner spines express the depressed synaptic state. Thus, altered synaptic plasticity and morphology (shape) may result from the same translation-dependent process that, once induced, is not properly limited in the Fragile X brain. As the saying goes, form follows function.

Our Current Project

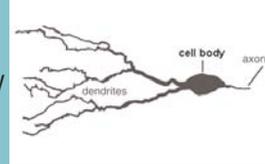
Currently, we are testing three predictions of the mGluR theory:

1. Mark Bear and Kim Huber have already shown that LTD is enhanced in mice lacking FMRP. We predict that mGluR-induced spine elongation should be exaggerated in these mice. We are using live-cell imaging techniques to test this possibility and the ability of candidate pharmacological therapies for Fragile X, such as Ampakines and MPEP, to correct any imbalances.
2. We have evidence that two modes of translation initiation operate in dendrites (CAP-dependent and IRES-dependent), and that stimulation of mGluRs primarily activates just one of these (CAP-dependent). The mGluR hypothesis predicts that lack of FMRP increases CAP-dependent translation; we are testing to see if this is true.

r e s e a r c h

Life imitates the movies

DNA Our genes are made up of DNA. Think of this as the master copy of a movie locked away in a Hollywood vault (neuron's nucleus). DNA is too valuable to be allowed to travel outside the nucleus.

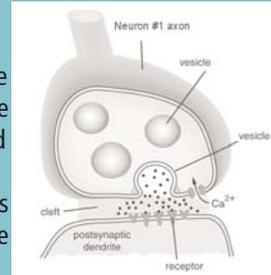


mRNA DNA is transcribed into messenger RNA, which can travel outside the cell body along dendrites to the synapses, the sites of cell-to-cell communication. MRNAs are like movie prints that travel to your local movie theatres (i.e., synapses).

protein Each mRNA provides the code for a protein. Just as a movie can be shown many times a day at a theatre, each mRNA can be translated into its protein many times a minute.

synapse This is where the show goes on ... the site where neurons exchange signals. A synapse has two parts: the signalling neuron's axon and the receiving neuron's dendrite.

When Neuron #1 spits out a message, receptors on dendrites of Neuron #2 are poised to receive it. Many kinds of receptors coat dendrites, but we are especially interested in mGluRs, metabotropic glutamate receptors, because growing evidence indicates that group 1 mGluRs function improperly in Fragile X.



Life Beyond the Movies

Imagine that watching a movie causes the theatre to change its size and shape. In neurons, when proteins are synthesized from mRNAs, one result is synaptic plasticity: a synapse changes its size and shape, depending on how much activity it is getting. One form of synaptic plasticity is LTP (*long term potentiation*), in which a synapse grows stronger and larger in response to high-frequency activity; another is LTD (*long term depression*), in which it shrinks and weakens in response to low-frequency activity. These changes underlie learning and memory.

The mGluR Theory of Fragile X

Several years ago, Drs. Kim Huber, Steve Warren, and Mark Bear found in Fragile X mice an excess of one kind of LTD. Lack of a single protein, FMRP, causes Fragile X syndrome. In the normal brain, stimulation of mGluRs leads to translation of many mRNAs into proteins at dendrites, including FMRP. Recent work suggests that FMRP normally suppresses translation of some of these mRNAs. In the Fragile X brain, however, FMRP is not present to put the brakes on protein production and as a result, too much mGluR-LTD occurs. Scientists believe that LTD is one of the primary mechanisms of learning and memory. The "mGluR theory" of Huber and Bear proposes this as the cellular basis for cognitive impairment and other symptoms of Fragile X.

update

3. Finally, we are testing whether preponderance of long, thin, and presumably lower efficacy, synapses in the Fragile X brain leads to compensatory changes in neurons. Recent research has shown that neurons adapt to deficits in net input by lowering firing thresholds and altering a number of intrinsic properties. If long, thin spines reduce net synaptic input, we would expect to see such changes, and they could underlie a number of symptoms of Fragile X. We are characterizing the intrinsic properties of neurons in the cortex and hippocampus of mice lacking FMRP. If differences are found with respect to control animals, this system could provide a testbed to see if potential therapeutic drugs (such as Ampakines and MPEP) can restore basic neuronal properties to their normal state.

Audiogenic Seizures and Effects of mGluR5 Agonist MPEP in the Fragile X Mouse

ROBERT BAUCHWITZ, PhD

Principal Investigator

QI JIANG YAN, PhD

 Postdoctoral Fellow

Columbia University, \$107,000 renewal

Dr. Bauchwitz's work on seizures in Fragile X mice suggests that mGluR5 antagonists may effectively treat a range of symptoms of Fragile X.



Robert Bauchwitz

As described above, mGluR receptors have been implicated in the abnormal neuronal responses observed in Fragile X syndrome. To address whether reversing mGluR signaling alterations might ameliorate the effects of Fragile X, we have tested a drug, MPEP, which specifically blocks one type of group 1 mGluR receptor (mGluR5).

Using MPEP, we have been able to reverse the susceptibility that Fragile X mice have to audiogenic seizures, which are triggered by very loud sound.

We have also shown that the degree of rescue is equivalent to that found by placing a copy of the human FMR1 gene into the genome of Fragile X mice, suggesting that MPEP can compensate for lack of FMRP in protecting against audiogenic seizures. We are also testing the effects of mGluR5 antagonists in other learning and behavioral assays.

Finally, we are producing new mouse models of Fragile X which might give more robust learning deficits comparable

to those observed in humans. If that is the case, we will use such animals to further assess the effectiveness of mGluR antagonists and other compounds on Fragile X cognition.

Metabotropic Glutamate Receptor Function in Fragile X Syndrome

ROBERT WONG, PhD

SUNY Downstate, NY
\$46,000

Like Dr. Vanderklish, Dr. Wong began his Fragile X studies after last year's Banbury meeting. He is investigating how seizures



Abraham Chuang, Ph.D., Robert Wong, Ph.D., and Riccardo Bianchi, Ph.D.

are generated in Fragile X neurons. More generally, he is looking at how synapses are modified to enable learning and memory and how this process is impaired in Fragile X.

We are studying the processes that cause the normal brain to become epileptic. There may be multiple mechanisms involved. We study seizures triggered by the activation of one kind of neuronal receptor, metabotropic glutamate receptors (mGluRs), in hippocampal neurons of mice.

When hippocampal neurons are exposed to chemicals which stimulate only group 1 mGluRs, the neurons fire epileptiform discharges (which trigger seizures). We and others have shown that this occurs only if new proteins are being synthesized.

Our results show that intense stimulation of the glutamate synapses cannot elicit the group 1 mGluR-mediated epileptogenesis in normal mice. Apparently, neurons of normal mice have a mechanism to protect them from seizures. In contrast, using tissue from Fragile X knockout mice, this same stimulation easily and consistently elicited robust seizure activity.

We are testing the theory that in normal mice, the protein FMRP suppresses group 1 mGluR-dependent epileptogenesis by suppressing the translation of one or more proteins which are involved in triggering seizures. Our experiments will evaluate whether the function of group 1 mGluRs is exaggerated in neurons in the cortex of Fragile X knockout mouse. We plan to extend our studies to evaluate whether abnormalities in mGluR function can also affect other basic brain functions involved in learning and memory.

INVESTIGATORS' NOTE:

Fragile X Knockout Mice now available from Jackson Laboratory

The new strain is FVB.129P2-Fmr1^{tm1Cgr}/J.

Visit <http://jaxmice.jax.org> for details.

Pharmacological Rescue of Behavioral Abnormalities in FMRP Deficient Mice by a GABA (B) Receptor Agonist

MIKLOS TOTH, PhD

Cornell University; \$50,000

An exciting aspect of this project is that it evaluates an already-approved drug as a treatment for seizures – and perhaps additional symptoms – in Fragile X.

Fragile X syndrome causes a broad range of symptoms, from cognitive deficiency to anxiety and sensory (tactile, visual and auditory) abnormalities. Some of these symptoms are well reproduced in Fragile X mice; in particular, their response to sound is significantly altered. This includes increased neuronal excitability in the auditory neuronal pathway, audiogenic seizure susceptibility, and increased filtering of sensory input, indicating a functional abnormality in the flow and processing of auditory information. Central auditory processing abnormalities in humans are manifested as inattention, poor listening skill, and difficulty in speech-understanding, which are also typical characteristics of autism, attention deficit disorder and Fragile X syndrome.

The aim of our research is to use drugs to correct neural abnormalities in Fragile X mice. We have found that chronic administration of the GABA(B) receptor agonist baclofen normalizes the defect in sensory information filtering in Fragile X mice, presumably because it suppresses excitability and/or compensates for mGluR activation. We will then test whether other behavioral defects in Fragile X mice can also be corrected by baclofen. By targeting the GABA(B) receptors rather than the glutamate system, these studies may provide an alternative strategy for the treatment of Fragile X syndrome.

CLINICAL TRIAL OF AMPAKINES

Dr. Elizabeth Berry-Kravis is conducting a clinical trial of Ampakines, a new class of experimental drugs. The study is funded by FRAXA at RUSH University in Chicago.

One consequence of mGluR-LTD, which is thought to be excessive in Fragile X syndrome, is fewer than normal functional AMPA receptor proteins. Ampakines work by increasing the activity of AMPA receptors.

Adults with Fragile X are still welcome to participate. See p. 9 or www.fraxa.org for details.

r e s e a r c h

Pharmacological Rescue of the Drosophila Fragile X Model

TOM JONGENS, PhD

University of Pennsylvania; \$70,000

Dr. Jongens first received a FRAXA award in 2002 and this new project builds on the previous work. Dr. Jongens was recently awarded tenure at the University of Pennsylvania.



The Drosophila (fruit fly) genome contains a single gene, called *dfmr1*, that is similar to the human FMR1 gene. In flies, loss of *dfmr1* function leads to behavioral and neuronal defects similar to symptoms observed in Fragile X patients. One behavioral defect displayed by Fragile X flies is the loss of normal circadian rhythms. A normal fly is active for 12-14 hours during daylight and relatively inactive for 10-12 hours at night. If entrained to a light:dark cycle of 12 hours of light followed by 12 hours of dark for several days, a normal fly can maintain a normal pattern of activity in total darkness for up to 3 weeks. But *dfmr1* mutant flies lack this capacity and display an erratic pattern of activity. Similarly, some children with Fragile X have great difficulty sleeping through the night.

Another behavioral change in Fragile X flies is a failure to display immediate recall in a courtship-based learning and memory assay. When placed in a small chamber with an unreceptive female (a previously mated female), normal males learn that their courtship attempts will not be successful and they drastically reduce their attempts. This learning occurs within one hour. These “trained” males remember this negative experience over the next several hours and so they do not court when placed in a new chamber with a receptive (unmated) female. Interestingly, we have observed that the *dfmr1* mutant males learn during the one-hour “training” session with the unreceptive female, but fail to display any memory of this experience, even if they are immediately placed in a new chamber with a receptive female.

In collaboration with Sean McBride and Tom McDonald at Albert Einstein College of Medicine, we are attempting to identify drugs that ameliorate the two defects described above. Since these studies and others suggest that there is a defect in synaptic plasticity in all Fragile X models, we will test the effect of drugs that are known to alter the activity of neuronal receptors that modulate synaptic plasticity.

u p d a t e .

Already we have tested mGluR antagonists (MPEP and other compounds) and have seen some very promising rescue of the defects observed in the courtship based learning and memory assay, including rescue of short-term memory.

The Role of MicroRNAs in the Pathogenesis of Fragile X

THOMAS TUSCHL, PhD Principal Investigator

ALEXEI ARAVIN, PhD Postdoctoral Fellow

Rockefeller University; \$35,000

More than ten years have passed since it was discovered that Fragile X syndrome is caused by the absence of a single protein, FMRP. Studies have demonstrated that FMRP regulates the translation of mRNAs into proteins by recognizing and binding to numerous mRNAs. This process is crucial for the function of neurons. However, it is not well understood how FMRP recognizes a particular mRNA and how it regulates the mRNA's translation into a protein. Understanding this will help facilitate the development of therapeutic treatments.



Thomas Tuschl



Alexei Aravin

Recent discoveries in the new field of RNA interference (RNAi) have lent insight into how FMRP recognizes its target mRNAs. Like FMRP, the RNAi machinery regulates the translation of numerous mRNAs. The RNAi machinery recognizes its target mRNAs through tiny non coding RNAs termed microRNAs (miRNAs). miRNAs are found in all animals, so they must have a fundamental role in regulating gene expression.

The recent finding that FMRP interacts with the RNAi machinery suggests that FMRP functions with the RNAi machinery to regulate gene expression. FMRP and miRNAs most likely recognize and regulate a common set of mRNA targets in the human brain. The misregulation of these target mRNAs in Fragile X patients probably causes the disease.

Our laboratory pioneered the biochemical investigation of RNA interference. To understand miRNA function, we have developed tools that interfere with miRNAs in human cells. We will use "bioinformatics predictions" in combination with the tools and assays we have developed to identify miRNA targets, and hence possibly new targets of FMRP.

Our laboratory pioneered the biochemical investigation of RNA interference. To understand miRNA function, we have developed tools that interfere with miRNAs in human cells. We will use "bioinformatics predictions" in combination with the tools and assays we have developed to identify miRNA targets, and hence possibly new targets of FMRP.

Flies for Kids: Developing a Genetic Model for the Neuropathology and Behavioral Deficits in Fragile X

BASSEM HASSAN, PhD

Flanders University, Belgium; \$25,000



Bassem Hassan (left) and lab members

Dr. Hassan's decision to go into this field is not only a matter of pure scientific interest, but also personal since Fragile X has touched his family.

In our lab we use the fruit fly, which has proven a powerful tool for unravelling genetic mechanisms. Fruit flies have a single copy of the Fragile X gene, called dFMR1. The fly dFMR1 protein, as with the human protein, is known to interact with other proteins and mRNAs (the intermediate between DNA and protein).

Children with Fragile X display behavioral impairments and anatomical defects in how neurons (brain cells) connect to each other. We have already shown that flies lacking the *dfmr1* gene show behavioral and anatomical defects in their brains. How do these defects occur? The Fragile X protein appears to play a major role in controlling the expression of other genes – many other genes! How, then, can we tell which of these genes are most important in causing the brain defects?

To tackle this question, we checked all genes in flies for the sequences to which the dFMR1 protein binds. We found around 260 such genes. Next we asked which of these genes are not correctly regulated in mutant flies. We found that genes which regulate the shape of cells, *cytoskeleton* genes, were most consistently affected. Next, we asked if playing with the amounts of these cytoskeletal proteins and the amount of dFMR1 could prove a functional relationship between the two. This was the case. It appears that the major problem in the brains of Fragile X flies, and perhaps in the brains of patients as well, is that genes which give neurons their shape and control their connectivity are not present in the right amounts.

The key now is to understand the relationship between the misregulated genes, the defects we see in brain cells, and the behavioral problems of the Fragile X flies. To do that, we have to be able to switch the dFMR1 gene off and back on whenever and wherever we want and ask which brain cells need this protein and when do they need it for normal development and behavior. Using a new trick called "transgenic RNAi," we are testing the requirements for dFMR1 in different neurons at different times and should be able to correlate the genetics with the anatomy and the behavior to paint a detailed picture of how this one gene can have such dramatic effects on brain development.

MGluR-Dependent Protein Translation in Wildtype, FMR1 Knockout, and FMR1 YAC Transgenic Mice

ERIC KLANN, PhD Principal Investigator

LINGFEI HOU, PhD Postdoctoral Fellow

Baylor University; \$57,000

Dr. Klann, who first received a FRAXA grant last year, collaborates with several other Fragile X investigators at Baylor University, the site of one of the federally-funded Fragile X Research Centers created under the Children's Health Act of 2000.

Previous studies indicate that FMRP binds to certain mRNAs and may regulate the translation of these mRNAs into proteins. As explained above, other studies show that mGluR-LTD is enhanced in mice that lack FMRP. Taken together, these two findings suggest the intriguing possibility that mGluR-LTD may be enhanced in Fragile X mice *because of* an increase in the translation of specific mRNAs. We are investigating this possibility.



We have found that several signaling pathways couple mGluRs to the protein translation machinery during mGluR-LTD. These pathways point to candidate mRNAs that may be rapidly translated after the induction of LTD. We have observed that rapid translation of several mRNAs occurs during LTD in normal mice, and that, in contrast, translation of these mRNAs is altered in Fragile X mice. In complementary studies, we have begun to study mGluR-LTD in mice that overexpress human FMRP (YAC FMR1 transgenic mice) to determine whether there are differences in LTD-induced mRNA translation between wild-type mice and YAC FMR1 transgenic mice.

We believe that identifying mRNAs translated in response to mGluR activation, and finding out whether their translation is altered during LTD in FMR1 knockout mice and/or YAC FMR1 transgenic mice, will be helpful in designing therapeutic agents for the treatment of patients with Fragile X.

FMRP Regulates Small GTPase Ras Signaling and Glutamate Receptor Trafficking

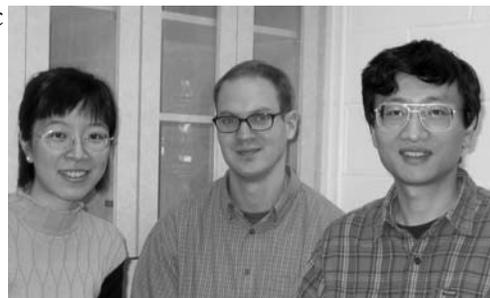
JULIUS ZHU, PhD

University of Virginia \$65,000

Dr. Zhu's team is investigating synaptic plasticity in the Fragile X knockout mouse. They have found that two

particular signaling pathways – small GTPase Ras pathways – are impaired in the knockout mouse. In these mice, they find very few of the AMPA receptors which are normally at synapses. Lack of AMPA receptors results in reduced synaptic plasticity in the Fragile X mice.

This research complements Dr. Elizabeth Berry-Kravis's ongoing clinical trial of



Hailan Hu, Joel Baumgart, and Julius Zhu

AMPAkines,

compounds specifically designed to enhance the activity of AMPA receptors, so that each existing receptor is more effective. Dr. Zhu and his team are using physiological and molecular biological techniques to investigate the defects in Ras signaling and AMPA receptor trafficking in Fragile X mice. They will test whether Ras-GEF (a protein which activates Ras and is regulated by FMRP) can restore normal delivery of AMPA receptors to synapses. Their findings may point to promising targets for the design of new drugs to treat Fragile X.

Generating Human Neurons Carrying the Fragile X Mutation

CLIVE SVENDSEN, PhD Principal Investigator

ANNA BHATTACHARYYA, PhD Postdoctoral Fellow

University of Wisconsin, Madison; \$50,000

Neural stem cells have exciting implications for the potential treatment of many nervous system disorders. Dr. Svendsen, a world-reknown expert in stem cell technology, is growing human neurons that express the Fragile X mutation. These stem cells are generating infinite quantities of neurons in cell culture, in the form of neurospheres, balls of neurons which increase in size. These neurons will be characterized in detail to understand the effects of lack of FMRP. The neurons will be made available to the scientific community.

There are essential differences in biology between mice and humans (not to mention the even greater differences between fruit flies and humans) which make these human neurons invaluable tools to advance studies of Fragile X and evaluate potential treatments. Unlike the mouse and fly neurons, in which the Fragile X gene is deleted (knocked out of the genome), these human neurons contain the actual Fragile X genetic mutation, so that therapeutic strategies aimed at reactivating the gene can be explored.

STUDIES IN SEARCH OF PARTICIPANTS

Please note that there are no direct medical benefits to individuals participating in these studies; rather, they will help advance the Fragile X field.

Clinical Trial – Ampakines

Dr. Elizabeth Berry-Kravis is conducting a clinical trial at RUSH University in Chicago to evaluate a new potential treatment for Fragile X and autism. The compound, Ampakine CX516, may help improve learning and memory in Fragile X. The study is funded by FRAXA.

Adults with Fragile X are welcome to enroll in this study. Prospective subjects should contact study coordinator, Tina Potanos, at 312-942-4036. Tina will explain the details, answer any questions, and set up the schedule for visits to Chicago for those who decide to participate. Dr. Berry- Kravis is also available at the same phone number to answer questions.

Study of Carriers

Maureen Leehey, MD, a movement disorders neurologist and professor of neurology at the University Of Colorado School Of Medicine in Denver, is conducting a study in collaboration with Randi and Paul Hagerman's group in California, Elizabeth Berry-Kravitz's group in Chicago, and Ann Reynolds, at

the Child Development Unit/Fragile X Clinic in Denver.

We have recently found that some Fragile X carriers (particularly men) develop progressive neurological problems after about age 50. These include tremor, balance difficulty, Parkinsonism, and memory problems. We intend to determine why this happens, what these neurological problems are specifically, and how often they occur.

We will evaluate Fragile X premutation carriers over 50 years of age. We will also evaluate persons over 50 that do not have a change in their FMR1 gene (often a spouse) for comparison. We can travel to your home if you live in Colorado or an adjoining state.

Please call Cathlin Rice, Study Coordinator, at 303-315-2389 if you are interested.

Medical Issues in Children

Ann Reynolds, MD, at the Child Development Unit/Fragile X Clinic in Denver, is leading a study comparing medical issues in children with autism, Fragile X, developmental delay and typical development.

WHO: Children 4 to 7 years with autism, Fragile X, developmental delay or typical development

WHAT: Involves sleep and family history questionnaires, and diet and stool diaries

If you are interested, please call: Dr. Ann Reynolds at 303-861-6619.

Improving Parents' Experiences with Diagnosis of Their Children

Elizabeth Taylor is a graduate student in the Genetic Counseling Program at Brandeis University in Waltham, Massachusetts. Elizabeth is conducting a study on parents' experiences with the diagnosis of their child with a genetic condition. Participation in the study involves completing an anonymous online survey and an optional interview.

Please contact Elizabeth Taylor at etaylor@brandeis.edu if you are interested in participating.

Genes and Behavior in Fragile X

Dr. Walter E. Kaufmann, at the Kennedy Krieger Institute in Baltimore is investigating how certain proteins controlled by the Fragile X gene contribute to intellectual and behavioral problems in children with Fragile X. With new laboratory techniques, scientists can now measure proteins in blood samples and determine whether the amount of these proteins relate to certain intellectual and behavioral problems in boys with Fragile X.

Boys aged 3 to 10 who are diagnosed with Fragile X and boys without known learning problems are invited to join this study. Participants will be reimbursed for expenses. For more information, please contact Pia Stanard at 443-923-7617 or Stanard@kennedykrieger.org.

Update from the National Fragile X Foundation

A preliminary agenda for the 9th International Fragile X Conference is now available at www.FragileX.org. While this agenda will be enhanced and updated numerous times in the months to come, the preliminary version gives you a good sense of the comprehensiveness of this event which will range from the latest in molecular research to detailed "How to" sessions dealing with the day-to-day learning and behavior of children with Fragile X. Sessions will also address the very latest information on Fragile X-associated Tremor Ataxia Syndrome (FXTAS).

The registration form and related information about the 9th International Fragile X Conference can be found at www.FragileX.org under the "Conferences & Events" button on our home page. Please mark your calendars now for June 23-27, 2004 in Washington, DC. Deadline for early registration is April 23rd.

The National Fragile X Foundation was pleased to recently cosponsor, along with the NICHD, a recent three-day "Early Intervention Working Conference" in Palm Springs, California. Under the leadership of Dr. Don Bailey from FPG at the University of North Carolina, and Dr. Steve Warren from the University of Kansas, twenty-three of the country's leading early intervention professionals gathered to discuss the state-of-the-art in their field, and to discuss how this knowledge can be applied to infants and toddlers with Fragile X. The NFXF was pleased to play a role in this important enhancement of the body of knowledge regarding Fragile X.

Robby Miller, Executive Director, 1-800-688-8765 or NATLFX@FragileX.org, The National Fragile X Foundation, PO Box 190488, San Francisco, CA 94119

FRAXA FUNDRAISERS

Raising Awareness and Funds for Research

Where does FRAXA get the funds to support the pivotal projects described in this newsletter? FRAXA has no government funding and no endowment. Every dollar is donated by dedicated families and their friends. Over 3000 people donated to FRAXA in 2003 through fundraisers such as those featured here.

As you know, FRAXA is one of the most efficient charities in the world, with only one paid staff and hundreds of volunteers, so your dollars are hard at work advancing research towards our goal: effective treatment and ultimately a cure for Fragile X.

Los Angeles Luncheon

With less than one month of planning, Andrea



Shelly, of Newport Coast, California, organized the first fundraiser in Orange County, California, for FRAXA. The Shelly's seven year-old, Elisabeth, was

diagnosed three years ago with Fragile X, and has been in intense therapy since then, including behavioral modification, speech therapy, occupational therapy, equestrian therapy, swimming and gymnastics. Elisabeth is currently enrolled at UCI CDC, a day treatment program which specializes in teaching children with ADD/ADHD by combining educational intervention with behavior modification strategies.

The Christmas-themed luncheon event, held on December 18th, attracted close to 100 guests and featured Christmas hand-bell carollers, a silent

auction chock-full of goodies, including a Louis Vuitton limited edition Dalmation pochette handbag, two Baby Phat pastel-pink limited edition cell phones, and an adorable tea-cup Chihuahua, and casual French food catered by Pascal's. Despite the winter date, the event was held outside in Shelly's front tented courtyard with the winter sun warming the guests and the purpose of the event. It raised over \$45,000 for FRAXA research. Shelly plans on holding another event this year.

Dallas/Plano, Texas

David and June Sturgell raised over \$5000 for FRAXA by hosting a party in December in their Plano home. In attendance were Dr. Kim Huber, Professor at the University of Texas at Southwestern and past FRAXA grant recipient, and many friends and parents of Fragile X children. The Sturgells thank all who attended and those who couldn't but generously contributed donations to the party.



In addition, Alexander Sturgell's school, Hughston Elementary hosted a "STOP" fundraiser for FRAXA. STOP -- "Students Thinking about Peers" -- is a Plano school system program which promotes developing leadership skills. Over a two-week period students donated change, raising over \$500. Each day over those weeks, a Fragile X fact was communicated during morning announcements. This was public education at its best!!! Thanks go to all the students, Principal, Mrs. Louann Collins, and Counselor, Mrs. Pam Hart. "Hughston, best in the West, every day, every way."



San Diego Badminton Bash



Cindy and Brendan de Gruchy and their family and friends hosted a Badminton Bash in San Diego. The event not only raised awareness of Fragile X and \$4000 for FRAXA but also

promoted the sport of Badminton. With no experience necessary to play, over 50 players of all ages showed up to give it their best shot. Trophies were awarded to all levels of play with first place ribbons going to all kids with Fragile X.

A New York Holiday Gift

We thank Writers House Literary Agency for their year-end donation to FRAXA in lieu of the usual holiday gift book

to their clients. FRAXA Board member Susan Cohen is an agent there, and her colleagues decided to make the contribution in honor of her son, Julian-whose father, Barry Berg, is also a Writers House author.

In addition to the value of the agency's monetary contribution, the holiday card sent to several hundred clients included a FRAXA brochure which helped spread awareness of Fragile X – and spurred a few authors to make their own contributions as well!

Will you host a Fall Fling event in Fall 2004? If we can organize more than 30 events around the US, the collective impact of that many events, small or large, will help us entice the media to feature stories about Fragile X.

All events are welcome ... yard sales, bake sales, letter campaigns, runs, walks, bike rides, dinner parties in your home, children's events, pizza parties, bowling tournaments. Need help? We have "To Do" recipes for each type of fundraiser and a list of other parents who have run similar events. We can supply brochures, ideas, and even a FRAXA Files CD with a large collection of resources for volunteers.

Hosting an event can be a lot of work but it is fun, rewarding, and worthwhile! Call or email Katie Clapp, (978) 462-1866 or kclapp@fraxa.org

CALENDAR OF EVENTS

MARCH

For those of you who have always wanted to pull a 100,000+lb. Boeing 727 your opportunity awaits on March 27, 2004 at the **2004 Tug of War!** FRAXA's Michigan chapter and Goodwill Industries of SW Michigan are teaming up for a Jet Pull at the Kalamazoo International Airport. Teams will compete to pull the Boeing 727 the fastest for 12 feet. Visit www.goodwillswmi.org/events.htm or contact Denise King at (269) 382-0490.

APRIL

Join the festivities Saturday, April 3, at St. Mary Church in Hockessin, DE, with drinks and hors d'oeuvres, **live band and silent auction**. For tickets, call Jen Nardo at (302) 234-7854 or email Jen9612@aol.com

MAY

The Nebraska Fragile X Families Association presents the **7th Annual Mary Higgins Clark Gala**. Special guest include Mary Higgins Clark, Eileen Naughton, and Senator Chuch Hagel. Founded last year, the Nebraska Fragile X Families Association consists of 26 families with 36 members affected by Fragile X, ranging from 1 year old to 52 years old. They are very excited to be hosting such a major event. For invitations, please call Kelly Randels at (402) 778-5802 or visit www.fragilexnebraska.com. We hope to see you!

On Wednesday, May 19th, Harry Manion will host **FRAXA's 10th Anniversary Celebrity Gala** in Boston. See p. 1 or FRAXA.org.

JUNE

The **8th Annual Patrick's Pals** 3-on-3 Basketball Tournament happens Sunday June 6, at BB&N in Cambridge, MA. Rolling Stone and Men's Journal feature writer Paul Solataroff is 2004 Patrick's Pal of the Year. This tournament is hosted every year in honor of Patrick Vershbow by his lifelong friends.

The Fragile X Alliance of Ohio will host their **8th Annual Golf Tournament** on June 28th in Cleveland. Register online at www.fragilexohio.org

JULY

Ron and Amy Watkins will host their **Second Annual FRAXA Dinner** on July 31, 2004 at The Links at Union Vale, New York.

Help FRAXA Through the Combined Federal Campaign

Imagine that FRAXA added a million dollars to its annual budget. Does that sound like wishful thinking? It's not really, because \$1 million is less than one percent of the more than \$100 million raised annually during the Combined Federal Campaign (CFC). The CFC is the sole fundraiser for federal agencies and the military. It is an annual autumn event during which federal and military employees are encouraged to donate funds to the charity of their choice.

Last year, the first time FRAXA was involved, CFC donors designated over \$13,000 dollars for FRAXA with little fundraising on our part. To boost this figure, FRAXA was represented by Frank and Susan Roth and Mary Beth Busby in the Fall of 2003, with keynote speeches or appearances at three fundraising kickoffs and 15 charity fairs. It is too soon to know how much FRAXA's donations will increase in 2004 because of their efforts, but we do

know that hundreds of federal employees learned about Fragile X for the first time.

At one event, Frank talked with more than 50 people who had never before heard of Fragile X. Ninety percent of them asked questions because they knew someone who had children with problems or were going to have children or grandchildren. People want to know about Fragile X. Benjamin Roth, who has Fragile X, was the star attraction at the Bethesda National Medical Center CFC Kick-off, where he shook hands with an Admiral!

More than 3000 charities meet CFC criteria, but many of the other charities do not have the message that we have of "We are just one gene away from a cure," nor are they helping children with a medical condition. Frank, a USDA Forest Service employee, had many friends tell him that they wanted to give to someone they knew or to something that would make a real difference. Others, who knew that the Roths' son Benjamin has Fragile X, did

not know that FRAXA is a CFC charity. FRAXA's CFC number is 0220.

There are CFC events in almost every U.S. city and wherever there is a military or federal installation worldwide, and the event sponsors are always looking for new charities to come speak about their work.

Frank and Susan have agreed to help interested families and supporters get started. They will contact the CFC organizers in your area and get you the information you need. The 2004 campaign will start in the early fall, and planning events and getting our name in front of the CFC organizers will begin in late summer. If you have some time and want to hand out information, give presentations, or just talk with others, please let the Roths know. You can contact the Roths at writeroth@xecu.net. Just think what we can do with a small piece of \$100 million!



Benjamin Roth

FRAXA RESEARCH GRANTS AND FELLOWSHIPS

Deadlines: May 1 and December 1 each Year

FRAXA offers fellowships and grants to encourage research aimed at finding a specific treatment and ultimate cure for Fragile X syndrome:

- Postdoctoral fellowships of up to \$40,000 each per year
- Investigator-initiated grants for innovative pilot studies aimed at developing and characterizing new therapeutic approaches (no funding limit)

FRAXA is particularly interested in preclinical studies of potential pharmacological and genetic treatments for Fragile X and studies aimed at understanding the function of the FMR1 gene. A special RFA has been issued; see www.fraxa.org for details.

FRAXA UPDATE

EDITOR: Katie Clapp, MS

CONTRIBUTORS: Michael Tranfaglia, MD
Mary Beth and David Busby
Recipients of FRAXA
Research Awards
Frank and Susan Roth
Andrea Shelly

DESIGN: Mary Lou Supple

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Fragile X in the News

Cody Randels is the son of Kelly and Ryan Randels. Kelly is chairing FRAXA's May 6th gala in Omaha. Cody, who has Fragile X, will be three years old in March. He walks, runs, and is beginning to talk.



Seth Thomas of Swansea, MA, was in the news! Seth's mom Joanne has almost succeeded in informing everyone in the Swansea area about Fragile X! She has contacted newspapers, the local cable TV station, and area businesses to raise both awareness and funds for research.

PLEASE HELP

FRAXA

in supporting research aimed

at treatment for Fragile X

FRAXA is a national 501(c)(3) tax-exempt organization run by parents of children with Fragile X. Every penny you donate goes to research: FRAXA has specific grants to cover all overhead. Supporters receive this newsletter and are welcome to participate as active volunteers.

Yes, I would like to help FRAXA

- | | |
|---|---|
| <input type="checkbox"/> Member (\$25+) | <input type="checkbox"/> Benefactor (\$500+) |
| <input type="checkbox"/> Donor (\$50+) | <input type="checkbox"/> Research Underwriter (\$1000+) |
| <input type="checkbox"/> Sponsor (\$100+) | <input type="checkbox"/> Named Research Fund (\$5000+) |
| <input type="checkbox"/> Named Research Chair (\$25,000+) | |

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FOUNDATION
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Massachusetts 01950

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