

PMSF Conference Sunday Morning Research Panel

Disclaimer: I wrote this review based on his notes taken during the 2010 Phelan-McDermid Syndrome Foundation Conference. It will have errors, misinterpretations and inconsistencies. I take full responsibility for these shortcomings. I apologize in advance to the panelists and hold them blameless for the errors that I have surely introduced. – Andy Mitz, parent of a child with Phelan-McDermid Syndrome.

During the 2010 Phelan-McDermid Syndrome Foundation (PMSF) Conference, the Foundation was very fortunate to host researchers interested in Phelan-McDermid Syndrome from top laboratories. The panelists were (from the audience's left to right): Dr. Yasunari Sakai, a researcher from the laboratory of Dr. Huda Zoghbi at Baylor College of Medicine. Dr. Huda Zoghbi is very famous for her research on the causes of Autism. Dr. Sakai is applying mathematic modeling to understand the proteins of the synapse. Dr. Joseph Buxbaum is the Director of the Seaver Autism Center at Mt. Sinai School of Medicine in New York and has been publishing scientific research papers on Autism for nearly 10 years. Dr. Sergiu Pasca works with Dr. Ricardo Dolmetsch at Stanford University. Dr. Dolmetsch is a member of the Foundation's Interim Scientific Advisory Board and a pioneer in the generation and study of iPS (stem cells induced from a person's own body). Dr. Andreas Grabruecker recently completed his Ph.D. under Dr. Tobias Boekers, perhaps the foremost authority on the three Shank proteins. Dr. Grabruecker helped develop a new method for studying the movement of proteins to the post-synaptic density where the Shank proteins operate. Currently, Dr. Grabruecker is a post-doctoral researcher at Stanford University studying the other side of the synapse (the pre-synaptic terminal) to learn how the two components develop and operate together.

The format was selected by Geraldine Bliss, moderator and Chair of the Research Support Committee. She used Questions and Answers to guide the researchers in their presentations. Geraldine Bliss asked each panelist to address her list of prepared questions. The panelist used Power Point slides to explain their research and its importance to the PMS community. The notes below are paraphrased from the presentation. The microphone was passed in one direction, then the other direction.

Question #1: What does your Laboratory do?

Answers:

Dr. Sakai

If you look at the stars at night, there are many millions. Certain stars stand out and can be used for orientation and guidance (navigation). These stars can then be used to locate and map the other stars in the sky. There are many, many proteins in the synapses of neurons. These must all interact with each other properly for the synapse to work. We can draw "star maps" of how the proteins interact, then see which proteins can be used for orientation and guidance (like stars used for navigation). Shank3 is a

protein that has many interactions. Dr. Sakai showed a slide of his very complex map of which proteins interact with Shank3 and how they interact with each other. Studying how diseases change this map is a unique window on disease. For example, someone missing Shank3 may have almost the same map as someone missing a different gene. If that happens, we have found a new disease that mimics Phelan-McDermid Syndrome. Maps like this may also someday explain why most children with Phelan-McDermid Syndrome do not develop any language, but some do.

Dr. Buxbaum

Modern science has developed a new and promising approach to finding drugs to treat genetic diseases. Although very new, this method is very promising. Dr. Buxbaum presented a slide with (most of) these steps:

- Find patients with a genetic problem
- Identify the gene involved
- Modify the genetics of a mouse to remove the same gene (“knock-out mouse”)
- Make lots of these knock-out mice
- Use a battery of tests (behavior, drugs, brain recordings, chemistry, etc.) on the mice to find out exactly how they differ from normal (“wild type”) mice
- Find chemical compounds that help the modified mice seem more like wild type mice.
- Translate the chemical compound into a drug for testing
- Test the drug on patients
- If necessary, use the results to guide further testing in mice

Dr. Buxbaum explained that this method can find a candidate drug in just a few years. That is amazing, since right now there are no drugs at all that specifically target any form of Autism. [The science advisors of the Research Support Committee remind us that it will probably take 10 years to develop and bring to market a safe and effective drug.]

Dr. Pasca

Dr. Pasca explained the creation of induced pluripotent stem cells (called iPS cells). The process starts with a skin sample. Skin cells are grown in a dish, then, undergo “genetic reprogramming”. The reprogrammed skin cells act like stem cells (those normally found in a fetus). With proper care, the iPS cells can be developed into different tissues, including brain cells. The great power of this method is that Dr. Pasca can study cells exactly like those from the brain of a person with Phelan-McDermid Syndrome, starting with just skin cells. Many of the experiments that could only be tried in modified mice can now be done with neural cells which would have the exact genetics of the person with PMS.

Dr. Grabruecker

Dr. Grabruecker explained that when his PhD advisor at the University of Ulm (Dr. Tobias Boeckers) first discovered the Shank protein, he called it ProSAP (proline rich synapse associated protein). Thus, in Europe they call it ProSAP and in the USA they call it Shank. To make matters worse the numbering scheme does not match. ProSAP2 is the same as Shank3. To make it easier for families, he was kind enough to use the “Shank” name for his talk. Dr. Grabruecker explained that his work studied how ProSAP/Shank reaches the synapse. This is very important in understanding how the body regulates (increases or decreases) the amount of Shank protein at the synapse. He also explained that all three Shank proteins work together for normal synapse operation. Work at Ulm studied how the three Shank proteins work together. At Stanford, Dr. Grabruecker is studying proteins in the pre-synaptic terminal. He explained that understanding the operation of Shank3 requires understanding both sides of the synapse. [Shank3 is found only in the post-synaptic terminal, but Dr. Joanna Giza explained in her Saturday lecture that the pre-synaptic and post-synaptic proteins must work properly together, and that various forms of Autism can result from failures on either side.]

Question 2: Why is your work relevant to PMS?

Dr. Grabruecker

There may be many diseases associated with the Shank proteins. Understanding what is in common and what is different will help us develop a fuller understanding of PMS. Likewise, there are three different Shank genes (and thus three Shank proteins). They work together in ways we are only beginning to understand. I studied how the SAM domains of the Shank proteins help them find their way to their normal post-synaptic site. Doing this showed that the zinc level inside the cell is quite important. Now we can see how changing zinc levels can be used to understand or treat PMS.

Dr. Pasca

Right now there is no direct way to study the cells of PMS patients without iPS. Unlike studying animals, genes, or post-mortem tissue, iPS lets you work with live cells from the person with PMS. That allows more specific drug testing and a more disease-specific look at the proteins of the synapse. The process of going from a skin sample to useful neurons takes months.

Dr. Buxbaum

Dr. Buxbaum showed a slide that indicated synaptic transmission at glutamate synapses of heterozygote knockout mice is disturbed more at AMPA receptors than at NMDA receptors. The slope of the field EPSPs as a function of fiber volley amplitude of AMPA receptors (presumably in the hippocampus) is reduced in knockout mouse. That difference between the knockout mouse and the normal (wild type) mouse can be reduced using an experimental peptide. Thus, Dr. Buxbaum’s laboratory is directly applying the drug discovery model to PMS. In addition, Mt. Sinai is using gene chips to study patient genetics. The program is a broad approach for researching Autism in general and PMS specifically.

Dr. Sakai

Dr. Sakai showed that his protein interaction maps include many, if not most, of the proteins important to all forms of Autism. Those proteins include NLGN3, FMRP, TSC1, TSC2 and many others. Shank3 is a major “node” in the map. Because his analysis has shown that Shank3 is an important “guidance” protein for the entire post-synaptic region. His work will undoubtedly be important to understanding PMS.

Question 3: What is unique about your lab's approach?

Dr. Sakai

It was already clear from the slides that Dr. Sakai’s protein-protein interaction maps are unique and Shank3 is important.

Dr. Buxbaum

Dr. Buxbaum emphasized that Mt. Sinai has a very coordinated effort using a wide range of methods. For example, they analyze the genes of individual patients, make knock-out mice and test the physiology and chemistry of those mice. In some cases it is valuable to test genetic or chemical ideas before making a knock-out mouse (which can take months or years). Mt. Sinai has the ability to do rapid testing with genetically engineered fish. Thus, Mt. Sinai has a wide range of tools as part of a coordinated effort to find medicines that might work on PMS and other forms of Autism.

Dr. Pasca

The neural cells made from iPS cells can be placed in a dish using special markers that show the neurons in action. For those participants who stuck around after the talk, Dr. Pasca was able to run his movie demonstrating calcium transport failures in iPS cells from patients with Timothy Syndrome. Having actual neurons from actual patients in a dish for study allowed them to test candidate drugs and directly observe whether the problems caused by the disease are improved by the drug treatment. Being able to visualize the problem (like the case of Timothy Syndrome) allows rapid screening (also called “high throughput” screening) of candidate drugs.

Dr. Grabruecker

Dr. Grabruecker referred to the laboratory of Dr. Tobias Boeckers when he said that his laboratory has the most complete set of knock-out mice and antibodies for testing the presence of the Shank proteins. Dr. Grabruecker showed a slide with both red and green color dyes lit up. He said that these dyes provide accurate measures of which Shank protein (Shank1, Shank2 or Shank3) is at which synapse and in what concentration. Also, Dr. Grabruecker’s work on transport mechanism may someday allow the development of nano-particles to the post-synaptic compartment. Such a targeted drug delivery system

could revolutionize the way drugs are administered. Thus, the unique power of the laboratory was the very special set of tools and the long history and experience studying Shank proteins.

Question 4: What do you think are the most promising directions in PMS research?

Dr. Grabruecker

One hope is simply that many people are now studying Shank3. He pointed to his colleagues and noted that all the approaches: iPS cells, mouse models, high throughput screening and small molecule libraries (to help test drugs) are all together very promising.

Dr. Pasca

The iPS method is very promising both to understand the mechanism of disease and for drug screening. However, they will need more phenotype data. [Our new data registry project is aimed exactly at giving scientist that information.]

Dr. Buxbaum

The most important/promising procedures are 1) study the function of Shank3, 2) find what is different between normal and affected synapses, 3) use the science to select molecules (drugs) that might help, 4) test the molecules with high-throughput screening, 5) find better ways to test whole animal behavior. There is already a candidate drug that may help with Fragile X Syndrome, there is no reason PMS should not be the next in line for new drugs.

Dr. Sakai

Dr. Sakai agreed that all of the methods mentioned by others are very important. He added that his protein-protein interaction maps could lead to a much better understanding of 1) how is PMS similar or different from other forms of Autism and 2) why is PMS more severe in some people than others. He believes that the phenotype severity is more related to protein-protein interactions than to deletion size.

Questions from the audience

Q – How does pruning affect learning?

Dr. Buxbaum. Shank3 is needed to prevent unwanted pruning.

Q – Why is Zinc important? Should our children take Zinc?

Dr. Grabruecker. Zinc is passed from the presynaptic compartment to the post synapse during transmission. It is important for stabilizing the synapse in the interplay among the different Shanks. However, the amount of Zinc involved is tiny and there is no direct way to measure synaptic Zinc. There is no reason to think that taking Zinc will help the synapses.

Q – Why are there so many phenotypes (variations among our children)?

Dr. Pasca. The good news is that relatively few drugs might be able to help many different autism-related diseases.

Dr. Buxbaum. We need more studies of “background” genetics [how the rest of the family genetics influence the missing gene].

Dr. Sakai. The protein-protein maps should help answer why there are so many phenotypes.

Q - Do you want families to come visit your facility?

Dr. Buxbaum. Yes! It takes three days for full evaluation and medical tests (including blood, MRI, EEG).

Q - Do you feel you need to study more variants of Shank3 mutations?

Dr. Buxbaum. Yes. More variants need to be studied.

Q - Do patients/subjects pay for the tests?

Dr. Buxbaum. No.

Q - What is the highest item on your wish list?

All speakers: There are too many cuts in research funding. We need more funding from organizations and we need more pressure on legislators to fund this type of research.

Q - Should people with mosaic deletions come to the Mt. Sinai for evaluation? Do you need though subjects?

Dr. Buxbaum. Yes, by all means. We are interested in mosaic deletions.

Q - What other genes need to be studied?

Dr. Buxbaum – Shank3 is the most important.

Dr. Pasca – We don't know the whole story yet. There are many “modifiers” that could be very important.

Q - Are skin cells the best for iPS?

Dr. Pasca - Right now skin cells work best for us. Blood may work ok, but the methods are still being worked out.