NEWS

Studies map gene expression across brain development

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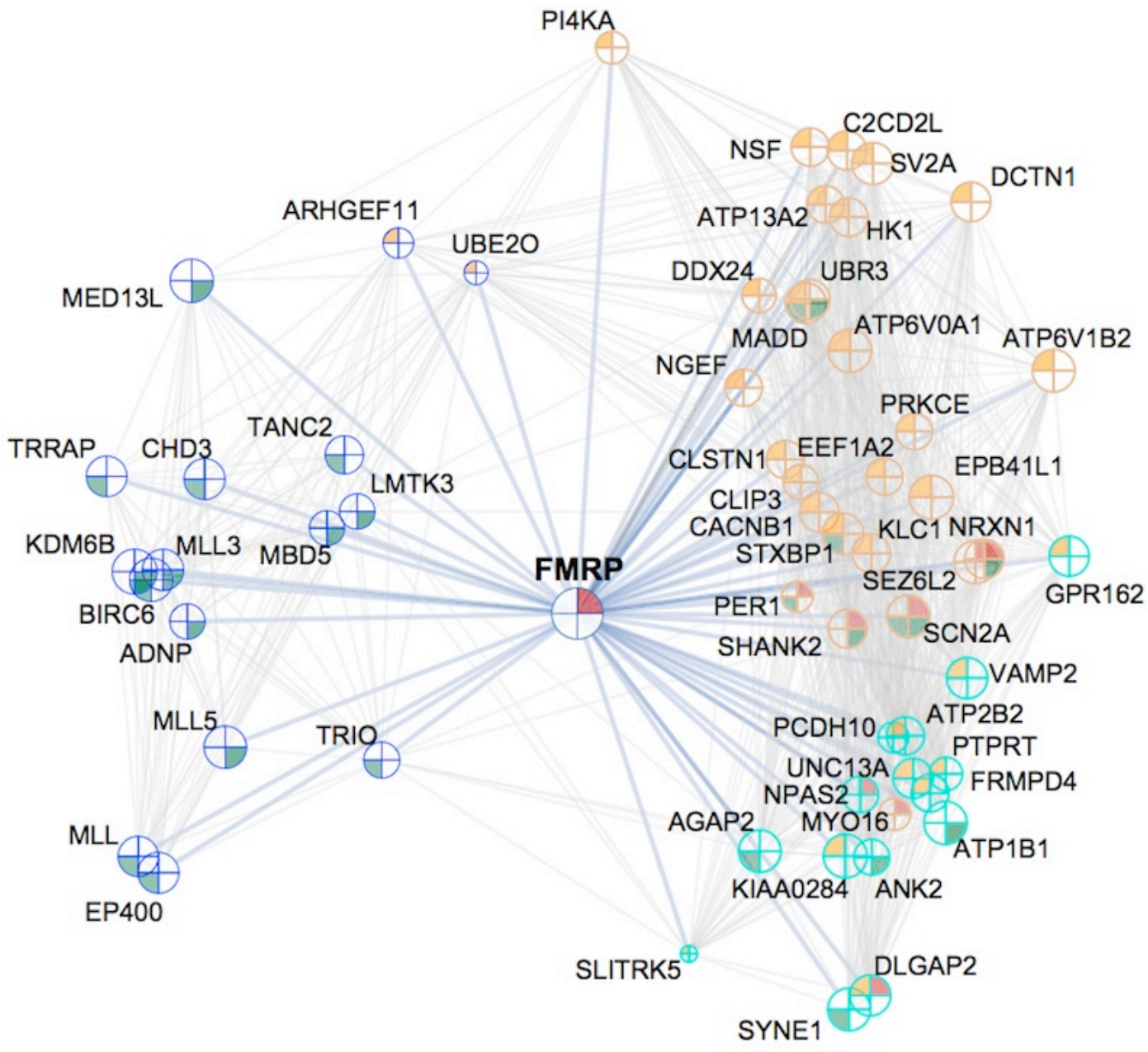

Now that genetic studies have implicated several hundred genes in autism, researchers are turning their attention to where and when in the healthy young brain these genes are expressed. The first two studies to tackle these questions appear today in *Cell*.

One report, led by **Matthew State** at the University of California, San Francisco, analyzed nine genes that **sequencing studies had strongly linked** to the disorder[1]. These genes tend to be

expressed together in certain layers of the cortex in the fetal brain, the study found.

The second study, led by **Daniel Geschwind** at the University of California, Los Angeles, took a broader approach, looking at the expression patterns of hundreds of autism-linked genes[2]. Some of these genes tend to be expressed together in networks related to the workings of the **synapse**, or junction between neurons. Other networks are involved in turning genes on or off.

Despite using different methods, both studies found clusters of autism genes that are important during mid-fetal development, and for the function of neurons that produce the **chemical messenger** glutamate. These so-called 'glutamatergic neurons' mediate excitatory signals in the brain.

"It's remarkable that, despite these huge differences in how we approached the problem, we converged on the same time period and on glutamatergic neurons," Geschwind says. "The themes that are emerging from these analyses are very, very resonant with each other. It's a good thing when that happens in biology."

Still, the research reveals a complex mix of networks, time periods and cell types involved in autism, underscoring the notorious heterogeneity of the disorder.

"Both of these studies are, in a sense, heroic, in terms of the breadth and depth of what they're going into," says **John Allman**, professor of neurobiology at the California Institute of Technology, who was not involved in either study. "They represent just how incredibly difficult it is to address this stuff."

Seed genes:

Geschwind's team and others have previously **analyzed gene expression** in postmortem tissue from people with autism. A downside of that approach is that most of that tissue comes from adults, and none from anyone younger than 2 years old.

In contrast, both new studies tapped into **BrainSpan**, an online database of gene expression data from more than a dozen brain regions across the full span of human brain development, from prenatal stages through infancy, adolescence and adulthood. The resource pulls from postmortem tissue studies done by several nonprofit, government and academic groups.

State's team searched this resource for genes that had passed a high threshold of statistical confidence in **whole-exome sequencing** studies — which sequence the protein-coding portions of the genome — ANK2, **CHD8**, **CUL3**, **DYRK1A**, **GRIN2B**, **KATNAL2**, **POGZ**, **SCN2A** and **TBR1**.

State and his colleagues created a series of networks with each of the seed genes at the center, connected to other genes that are the most similar with respect to the timing and location of their expression in the brain.

Of these networks, the researchers focused on the ones that happen to be enriched with an independent set of 122 'probable' autism genes, defined as those in which one person from the exome studies carries a loss-of-function mutation.

Despite the diverse biological roles of the seed genes, the researchers found them to be surprisingly convergent, meaning that many of the genes are expressed at the same time and place.

The networks that include a disproportionately high number of autism genes tend to be expressed during mid-fetal development (10 to 24 weeks after conception) in the prefrontal cortex. What's more, they are brimming with genes related to glutamate neurons in layers 5 and 6, the deepest layers of cortex.

"We were really amazed at how strong the evidence was for this initial set of genes," State says. "We thought maybe we'd need to start with 50 or 100 genes before we would see an identifiable pattern."

State is careful not to overstate the results, however. As more autism genes are added into the analyses, many other convergence points are bound to pop up, he says. "This is not a unifying theory of autism."

Overall, his results are similar to what **Zoltán Molnár** and his colleagues reported from mouse brains earlier this year[3].

"Looking at how co-changing genes form a network is a very powerful approach," says Molnár, professor of developmental neurobiology at the University of Oxford in the U.K., who was not involved in the new studies.

Mixed modules:

In the second study, Geschwind and his colleagues first used BrainSpan to track the expression of 15,585 genes — essentially every protein-coding gene expressed in the cortex — across early development, from 8 weeks after conception to 1 year of age.

From these data, the researchers identified clusters, or 'modules,' of genes whose expression tends to be synchronized, turning on and off together across early development. Genes within each module have similar functions. Genes in modules 2 and 3, for example, are primarily involved in the transcription of DNA into RNA, whereas those in modules 13, 16 and 17 are involved in synapse

function.

Several of these modules turn out to be enriched in autism genes. For example, the researchers showed that a set of 113 genes implicated by whole-exome sequencing studies of autism are significantly overrepresented in just two modules: 52 of the genes are enriched in module 2 and 61 genes in module 3.

In contrast, a set of 155 genes that the researchers identified from **SFARI Gene** are overrepresented in modules 13, 16 and 17, the study found. (SFARI Gene is a curated database of autism candidate genes sponsored by the Simons Foundation, SFARI.org's parent organization.) Another set of more than 400 genes identified in a **previous study of postmortem autism brains** also turns up in these three modules.

The researchers also found that FMRP, the protein disrupted in the autism-related disorder **fragile X syndrome**, regulates autism genes in modules 2, 16 and 17. This fits with an earlier study showing that many of **FMRP's targets are hit by spontaneous mutations** in children with autism, Geschwind notes.

To confirm that these network associations are autism-specific, the researchers compared autism genes with those involved in intellectual disability, which occurs in about one-third of individuals with autism. They found that a set of 401 intellectual disability genes is not enriched in any of the modules.

This comparison shows the importance and specificity of the autism gene convergence, Allman says. "It's a real strength of the study."

The researchers all agree that these network analyses are bound to be important resources for the field at large.

If a scientist is interested in making a mouse model of a particular autism gene, for example, he or she could refer to the networks to choose the period of brain development and the brain regions to focus on. Geschwind's team has created an interactive network browser that is freely available on his **laboratory website**.

Gene networks are ultimately limited, however, by the postmortem data used to create them, which were heavily concentrated on the cortex. The same types of analyses could be done on other brain areas, such as the cerebellum, and other organ systems, such as the gut, placenta and immune system.

"Autism is an extremely complex disease where the environment is playing on an unfolding genetic program," Molnár says. "We shouldn't ignore some of the systems which might feed into this."

References:

1: Willsey A.J. *et al. Cell* Epub ahead of print (2013) **Abstract**

2: Parikshak N.N. *et al. Cell* Epub ahead of print (2013) **Abstract**

3: Hoerder-Suabedissen A. *et al. Proc. Natl. Acad. Sci. U.SA* **110**, 3555-3560 (2013) **PubMed**