VIEWPOINT

Analyses of gene activity may yield clues to roots of autism

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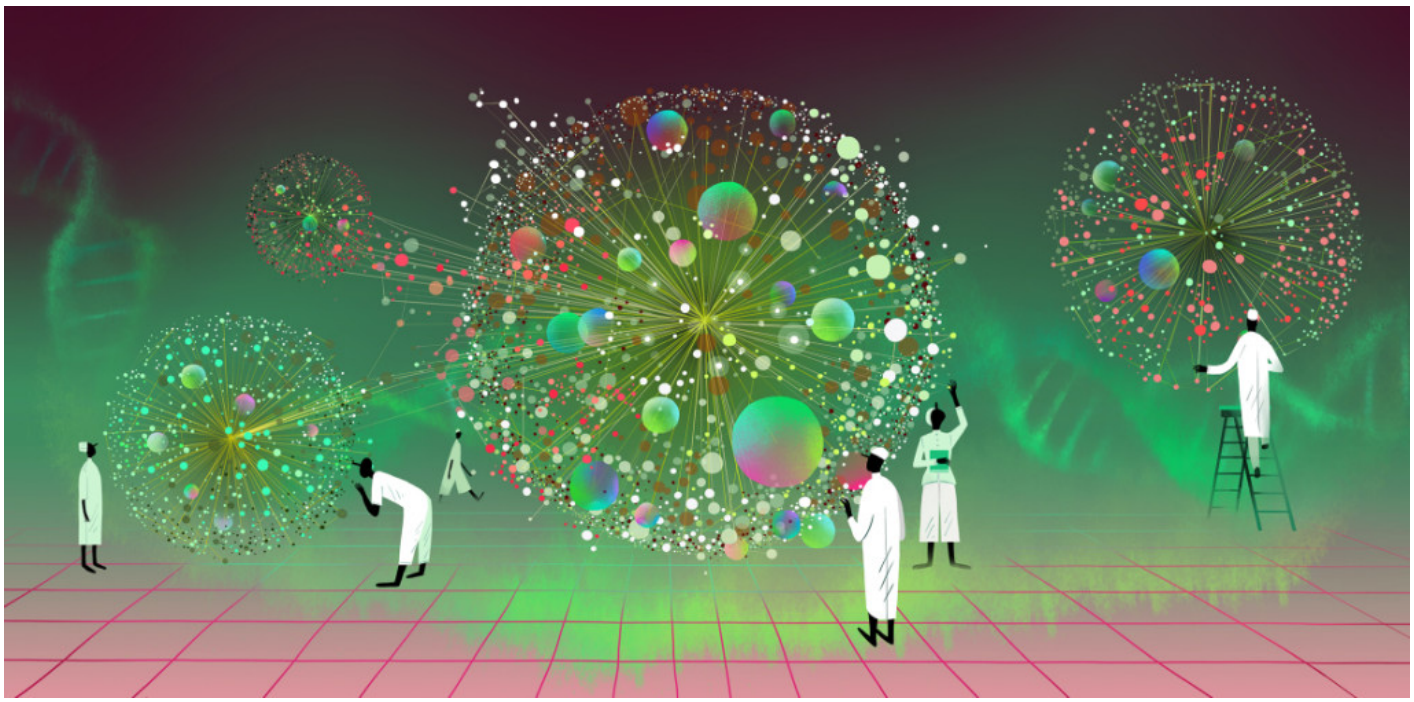

The number of genetic variants implicated in autism is large and growing, but it's increasingly clear that identifying these variants is only the beginning of the quest to understand the biology of autism.

Genes not only store information but also serve as templates for RNA transcripts that then give rise to the proteins that function in a cell. So it is crucial that we understand which genes show differences in their RNA levels, too.

This is particularly important because although autism may affect large numbers of genes, their functions appear to converge on only a few biological pathways. These include the development of

the brain's outer shell (cerebral cortex), the function of neuronal junctions **(synapses),** translation of the genetic code into protein, and the activation of **brain immune cells called microglia**[1].

Ultimately, these findings suggest that, despite multiple genetic causes, we may need to target only a few pathways to effectively treat people with autism.

Because a major source of biological regulation occurs at the level of gene expression, studying patterns of expression can highlight the key pathways in specific tissues such as the brain. This may then point us toward new therapies.

Deciphering data:

'Transcriptomic' studies are those in which researchers quantify gene expression across all genes present in a tissue sample and identify differences between the study group — in this case, people with autism — and controls. This analysis generally reveals values for 10,000 to 20,000 genes, a number too large for the human brain to make sense of on its own.

Network analyses — via software tools that identify biologically relevant patterns from large datasets — are often the key to analyzing all these data.

In one type of analysis, called weighted gene co-expression network analysis (WGCNA), researchers group genes into modules based on the similarity of their expression patterns[2]. They then interpret the functional role of genes within each module, often intuiting the roles by looking at the genes in aggregate.

For example, in modules constructed from brain gene expression data, at least one module is likely to be enriched for genes expressed in neurons and another for **genes in glia**, cells that support neurons. These separate modules help to explain the cell types present in the tissue studied. Once researchers have identified the modules, they can look at whether the expression pattern in a given module differs significantly between people with autism and those without.

Combining the functional information — for instance, what cell types are present and the function of genes in each module — with the expression patterns allows researchers to determine which pathways are altered in autism.

Imperfect analyses:

Although network analyses help us make sense of large gene expression datasets, they have some limitations.

First, a small number of individuals available for study could prevent a researcher from constructing biologically meaningful networks and prohibit useful interpretation of the data.

Second, because network analyses are designed to pick up subtle differences between groups, any systematic differences between cases and controls may lead researchers to incorrect conclusions.

For example, if samples from individuals with autism contain different cell types than those in controls (perhaps due to sampling of slightly different regions of the brain), WGCNA would pick up this difference, and a naïve researcher could incorrectly claim to have found an association with autism.

Accounting for such confounding factors is a key step in enabling researchers to draw conclusions that can be trusted.

What's more, these analyses decipher only gene expression information. They are not designed to crunch data related to genetic variants, DNA modifications that affect gene expression, or information about the proteins themselves. Combining all of these datasets could provide an even more complete molecular understanding of autism. Groups are working on these multilevel analyses.

Finally, network analyses do not provide information on causality. In contrast to DNA, which generally does not change over a lifetime, gene expression levels differ over time. Mutations in DNA are almost certainly primary and may cause the condition, but differences in gene expression, as detected by network analyses, may or may not lead to autism. They might be compensatory — that is, they might result from having autism.

Still, by pointing to pathways of interest, gene expression analyses can provide ideas for new treatments.

Promising pathways:

Given the potential for understanding gene expression using network analyses, it's important to consider what we can expect from this approach. In the past five years, researchers have published a number of studies looking at **gene expression in postmortem brain** samples[3,4]. This work has repeatedly highlighted two main differences in gene expression in the brains of individuals with autism.

One of these differences has to do with alterations in the expression of genes that are active in neurons. Intriguingly, however, the genes that harbor mutations are different from the ones showing altered expression. The neuronal pathways involving genes whose expression is altered in autism represent a promising set of drug targets separate from the genetic variants individuals may harbor.

The other reproducible pathway implicated by network analyses includes genes related to immune

regulation. Many of the genes in this pathway tend to be active in a type of **microglia** that have an anti-inflammatory effect.

This result suggests that an exaggerated anti-inflammatory response occurs in the autism brain. Researchers will need to pin down whether this finding is causal or compensatory.

Network analyses with large sample sizes will undoubtedly improve our ability to identify which genes are driving these expression differences. They may also help us to detect genes whose expression differs only subtly in people with autism.

Taken together, network analyses of gene expression have identified two biologically interesting and testable pathways that can be targeted in therapeutic studies, emphasizing the utility of this approach.

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