NEWS

Pesticide effect on cells may resemble signs of autism

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A class of fungicides used on crops can produce changes in mouse brain cells that look strikingly similar to those seen in the brains of people with autism.

The findings, published 31 March in *Nature Communications*, support the use of 'transcriptomics' — a rapid method of analyzing gene expression in cells — for identifying chemicals that trigger patterns of gene expression like those seen in autism. The method could flag chemicals that contribute to the condition[1].

"It provides a rational way of finding environmental risk factors," says study leader **Mark Zylka**, associate professor of cell biology and physiology at the University of North Carolina at Chapel Hill.

Scientists have had trouble establishing a causal connection between autism and **exposure to toxic chemicals**. Nailing down links to any specific chemical culprits is especially difficult because of the many possibilities: More than 80,000 chemicals are approved for environmental use in the U.S., says **Amy Kalkbrenner**, assistant professor of environmental health sciences at the University of Wisconsin-Milwaukee.

Epidemiologists need clues from other branches of science to decide which chemicals are worth investigating, says Kalkbrenner, who was not involved in the new study. "The number of chemicals out there is very large, and so it's really important for us to expand our gaze in a way that's thoughtful."

The new approach provides a platform for screening potential chemical contributors to autism as well as testing drugs for the condition. But looking at expression changes in cells is only a first step. To determine whether a chemical ups autism risk, scientists would need to track exposure to the chemical and subsequent autism diagnoses. They would also have to investigate exactly how the chemical might act on the brain, using animal models.

Chemical clusters:

Zylka's team exposed mouse brain cells to each of 294 chemicals found in the environment. These include herbicides, pesticides and molecules used to manufacture plastics. They measured gene expression levels in all of the cell batches. Then a computer program grouped the chemicals based on the similarity of the expression patterns they induced for 5,121 genes.

The researchers compared these patterns with those from 18 studies of gene expression in postmortem brain tissue covering 10 different neurological and psychiatric conditions, including autism.

"I think it's a really creative use of this kind of data," says **Dan Arking**, associate professor of genetic medicine at Johns Hopkins University in Baltimore, Maryland. Arking was not involved in the study, but some of the **autism brain analyses** came from his lab.

One group of eight chemicals, dubbed 'cluster 2,' yields gene expression changes in mouse neurons that are remarkably similar to those seen in the autism brains. The cluster contains four fungicides, an herbicide and three insecticides, including rotenone, which has been tied to Parkinson's disease[2].

The chemicals lower the expression of genes involved in the function of **synapses**, the junctions between neurons, and create an uptick in the expression of genes involved in immune function. They also elevate the expression of genes active in **brain cells known as microglia**. Microglia respond to infection and injury, and shape synapses.

Cause or consequence:

The chemical groupings are different from those seen in previous assays, most of which gauged cell death instead of gene expression or looked at cells other than neurons. The new test may better reflect the subtle, brain-specific effects of the compounds.

"I've always thought that studying the transcriptome gives you a better picture of what is actually going on functionally in the cell or tissue," says **Valerie Hu**, professor of biochemistry and molecular medicine at George Washington University in Washington, D.C., who was not involved in the work.

Whether the cluster 2 chemicals contribute to autism risk in the real world is unclear. The gene expression patterns seen in postmortem autism brains could represent **a compensatory response in the brain** to developmental changes rather than pathways that cause autism.

"We have no idea whether it's cause or consequence," says **Christine Ladd-Acosta**, assistant professor of epidemiology at Johns Hopkins University. "But it's worth following up on these

findings in an epidemiology study."

Cluster 2 chemicals are widespread. Zylka and his team combed through data on pesticide use and residues on foods from the U.S. Geological Survey, the Food and Drug Administration and the Department of Agriculture. They found that the use of several fungicides in the cluster has been increasing over the past 15 years. Conventionally grown spinach, kale and other produce items carry relatively high levels of these chemicals.

Simple screen:

The researchers also identified three mechanisms by which cluster 2 chemicals may interfere with the function of neurons: The pesticides trigger the production of damaging oxygen-containing molecules known as free radicals, impair the function of structures inside cells called mitochondria, and disrupt the internal cell 'skeleton,' causing swelling of the cells. Scientists have previously linked each of these processes to autism and problems with neurodevelopment.

Intriguingly, treating the cells with vitamin E, an antioxidant, blocks free-radical production and cell swelling caused by the fungicide fenamidone.

A drug that stabilizes the cell skeleton has similar effects, as does a third compound, sulforaphane, found in broccoli. Sulforaphane, in particular, partially prevents the gene expression changes seen in fungicide-exposed cells, and a pilot study of this compound showed that it **eases autism symptoms in men with the condition**.

If the results can be replicated, they suggest that gene expression analysis can form the basis for screening potential autism treatments, says Arking. "To me, that's very exciting to have something that you can do *in vitro* that gives us a way to test drugs."

REFERENCES:

1. Pearson B.L. *et al. Nat. Commun.* **7**, 11173 (2016) **PubMed**
2. Tanner C.M. *et al. Environ. Health Perspect.* **119**, 866-872 (2011) **PubMed**