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Alarm Over Autism Test

A hypothesis that maternal antibodies can impair fetal brains stirred plenty of doubts—and that was before the researchers set out to turn it into a test for predicting autism

JACKIE MURPHY DIDN'T WORRY THAT HER son Fintan was a late talker, at least at first. Her other two children had been slow to say their first words, so it was only when the former California nurse noticed that her 20-month-old wasn't responding to his name, or even reacting to loud noises, that she became concerned. "One day, I dropped a toy xylophone behind him and he didn't even flinch," she says. "That's when I knew something was wrong."

Fintan didn't have a hearing problem—he had autism, his mom finally learned after more than 6 months of searching for a diagnosis. A few months later, Murphy enrolled Fintan in the Autism Phenome Project at the MIND Institute at the University of California (UC), Davis, a long-term assessment of children, as many as 1800, aimed at teasing out subtypes of the complex disorder. Murphy also became a research subject, donating a blood sample.

One of the project's researchers, Melissa Bauman, soon informed Murphy that her blood had tested positive for antibodies that react to fetal brain proteins. Bauman asked her to donate more blood for studies exploring the provocative idea that some of Murphy's antibodies had slipped through the placenta and into Fintan's developing brain, affecting its maturation. At that point, Murphy says, she and her husband made a big decision: Fearing that the immune proteins in her blood would harm another baby, they decided that she would not again get pregnant.

Many more women could face a similarly difficult choice. In July, immunologist Judy Van de Water and her team at UC Davis, which includes Bauman and Daniel Braunschweig, bolstered the hypothesis that maternal antibodies cause some autism with two studies, including one showing autismlike symptoms in monkeys injected with such antibodies. And women may soon be able to check whether they have the suspect antibodies: California company Pediatric Bioscience announced that it is moving forward with a new diagnostic test, based on patented antibody screening techniques licensed from Van de Water and UC Davis.

According to a press release issued by the company at the time, a positive result on the test, estimated to cost roughly $800 and be ready in about 18 months, could tell a woman that she has a "99 percent likelihood" of having a child with autism if she

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Looking for answers. Immunologist Judy Van de Water (*far right*) suspects that antibodies carried by Jackie Murphy (*middle*) may have contributed to the autism of her son Fintan (*far left*; Murphy's son Tiarnan, in green, does not have autism).

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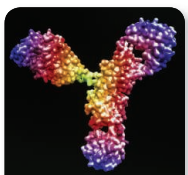
became pregnant. It could also tell parents if the child they just had is likely to develop autism, especially if there are already signs of a developmental delay. Such a scenario, says company President Jan D'Alvise, would let parents enroll a child in early interventional therapies, even before symptoms develop. Already, "people are e-mailing me, saying, I would like to get the test, where can I get the test," Van de Water says.

Pediatric Bioscience's announcement, however, has alarmed many autism researchers. "This is very, very premature—this research has come out of one group, and basically one study. I'm amazed that they're going ahead at this point and trying to commercialize a test," says autism researcher George Anderson of Yale University. He and others say that Van de Water's data are too preliminary, and her statistics too weak, to support such clinical uses. They are skeptical of the mechanism she has proposed for how maternal antibodies could damage the fetal brain. And before any antibody test for autism is launched, they say, her results need to be extensively replicated. "This whole thing could be a house of cards," warns Thomas Wassink, a geneticist at the University of Iowa in Iowa City who studies autism.

Perhaps the most controversial aspect of the proposed test is that some already pregnant women might decide to abort their babies on the strength of the results. Van de Water emphasizes that Pediatric Bioscience plans to limit the "intended use" of the maternal antibody test to women who are not currently pregnant. The company is planning to develop and conduct the test exclusively, she says, thus allowing for control over its use and proper counseling for families. Van de Water is also seeking validation of her group's initial studies, with three large prospective trials under way, but she nonetheless feels compelled to push ahead with Pediatric Bioscience on the test. "The community in autism is a very frustrated group of people. They feel like we've done a lot of research but none of that is impacting our lives right now. … If you wait until I have thousands of samples that's kind of late," she says.

Controversial origins

This isn't the first time purported connections between the immune system and autism have ignited controversy. In 1998, the British physician Andrew Wakefield claimed that eight of 12 children with neurodevelopmental delays had experienced a sudden onset of autism symptoms after receiving the measles, mumps, and rubella (MMR) vaccine. In a press conference held in coordination with the publication of a study in *The Lancet*, he hypothesized that the triple dose of vaccines could trigger an immune reaction that damages the brain. The study was later retracted, Wakefield was stripped of his medical license for falsifying the medical histories of the children, and more than a dozen epidemiological studies have failed to find any connection between childhood vaccines and autism. But the link has yet to be dissolved in the public imagination, says Emanuel DiCicco-Bloom, a child neurologist and neuroscientist at Rutgers University in Piscataway, New Jersey.



Also see the Antibodies special section, page 1191.

That history puts an extra burden of responsibility on researchers considering immune hypotheses for autism. It has also made exploring such hypotheses more difficult, notes Karoly Mirnics of Vanderbilt University in Nashville, a neuroscientist who studies immune changes in autism, schizophrenia, and other mental disorders. "Ten years back, you could not get funding" for this type of research, he says. "You could not publish it. You were sort of considered to be on the fringes of science."

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> **—JUDY VAN DE WATER, UC DAVIS**

One place where researchers interested in immune connections to autism have found support is the MIND Institute, which was founded in 1998 by influential California families who had a "big interest" in the MMR hypothesis, Van de Water says. Vaccine research, however, is no longer a focus at the institute, according to Van de Water, and her work does not directly involve vaccines.

An emerging body of evidence unrelated to childhood vaccines suggests that a mother's immune system may play a role in offspring developing autism, says Paul Patterson, a biologist at the California Institute of Technology in Pasadena who studies interactions between the nervous and immune systems. For example, epidemiological studies have shown that several types of infection during pregnancy can increase the risk that a child will develop schizophrenia or autism, he says. Families with autoimmune disorders such as rheumatoid arthritis and celiac disease are also more likely to have children with the disorder. Patterson's own lab has conducted animal studies showing that inflammatory molecules produced by a pregnant mother in response to an infection such as the flu can affect fetal brain development. "It's a very reasonable hypothesis to be out there testing because of the epidemiologic connection between learning disabilities and mothers with a variety of autoimmune diseases," says Daniel Geschwind, director of the Center for Autism Research and Treatment at UC Los Angeles. "It looks like there's some epidemiologic signal there—the question is, is that a causal relationship?"

Van de Water's interest in maternal antibodies was sparked by a 2003 study by researchers at the University of Oxford, which focused on one 38-year-old mother. The woman had two relatively normal children—a girl with high-functioning autism spectrum disorder (ASD) and a typically developing boy. However, her third child, a 6-year-old boy, had appeared fairly normal until about 18 months and then quickly regressed to severe autism, losing all language. When the researchers extracted serum from the mother, they found that some of her antibodies bound to neurons taken from a developing mouse brain, and they suggested that the antibodies could have triggered her son's condition.

Other researchers have pursued the maternal antibody hypothesis over the past decade, but Van de Water's team has taken it the farthest, Patterson says. In 2008, the group expanded on the Oxford study by extracting serum from 61 mothers of children with autism. About 11% of the

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mothers carried antibodies that reacted strongly to a group of unidentified brain proteins in human fetal brain tissue, and the level of reactivity corresponded with the severity of the child's autism symptoms. None of the 102 control mothers showed the same pattern of reactions.

Van de Water's latest work, published in the journal *Translational Psychiatry*, expands the sample still further, to 246 mothers of children with autism. She and her colleagues found that 23% carried antibodies that react to fetal brain tissue, compared with only 1% of 149 mothers who have typically developing children. This time the team pinpointed seven specific proteins that the antibodies target, including ones linked to the proliferation of neurons, neural migration during development, and neural branching (see diagram). "Each works at some stage in the development of a neuron," Van de Water says.

It's not clear why a woman would generate such reactive antibodies. "We may never know," Van de Water says. Some women may be genetically predisposed to produce the antibodies in response to an immune insult such as an infection, or even a vaccine, she suggests. Although that's pure speculation for now, Murphy, who has become a close friend of Van de Water, is already persuaded that a vaccine she received contributed to Fintan's autism. "He's the only one I got vaccinated with the flu shot during pregnancy," she says.

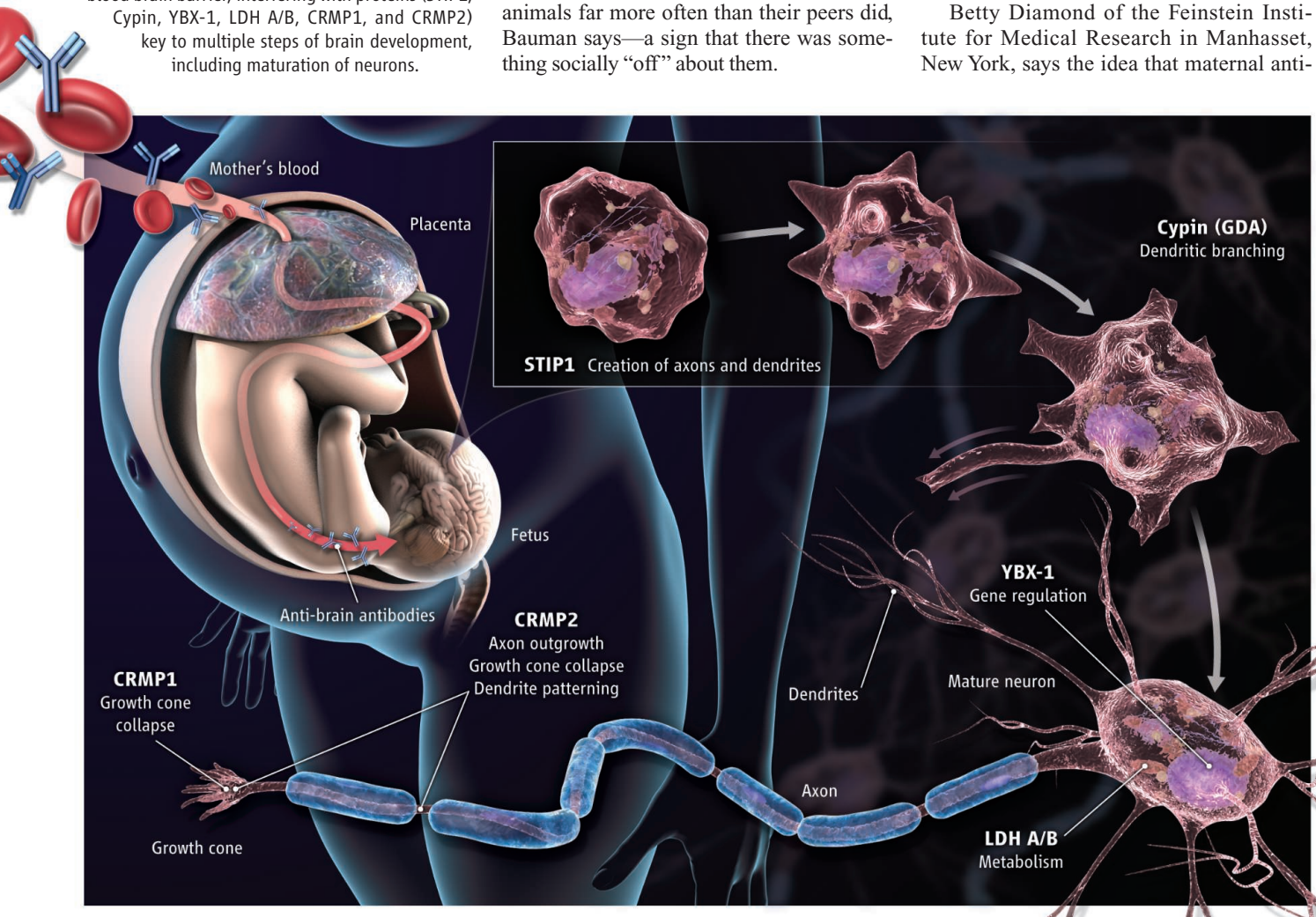
In her other new study, Van de Water teamed up with Bauman and neuroscientist David Amaral, director of the MIND Institute, to link the autism-associated antibodies to symptoms. The team reported that when pregnant rhesus monkeys were injected with these human maternal antibodies, their infants developed behavioral and brain development problems. The researchers say the symptoms resemble some of those seen in people with autism. For example, the young monkeys whose fetal brains had been exposed to the antibodies directly approached unknown animals far more often than their peers did, Bauman says—a sign that there was something socially "off" about them.

Strong reactions

Van de Water's latest work is "an important step that lots of people have been waiting to see," Patterson says. If she's right, "we're at least doubling" the number of autism cases we can explain, adds Andrew Zimmerman, a pediatric neurologist at the Kennedy Krieger Institute in Baltimore, Maryland. Now, only 15% to 20% of autism cases can be traced to a specific cause, he says—primarily genetic mutations.

But other reactions range from skeptical to sharply negative. Zimmerman himself says that absent replication of Van de Water's clinical results in much larger groups of women, "it isn't clear yet what the [autism] risk to a particular pregnancy is if a mother has the antibodies." He and others also question the maternal antibody hypothesis itself. For example, it assumes that the so-called blood brain barrier, which normally protects the brain from molecules such as antibodies, is permeable enough in fetuses for antibodies to enter and for them to do damage—a fact that has yet to be rigorously established, DiCicco-Bloom says.

Betty Diamond of the Feinstein Institute for Medical Research in Manhasset, New York, says the idea that maternal anti-



Inflammatory hypothesis. Van de Water and colleagues propose that antibodies in a mother's bloodstream travel through the placenta and cross the fetal blood brain barrier, interfering with proteins (STIP1, Cypin, YBX-1, LDH A/B, CRMP1, and CRMP2) key to multiple steps of brain development, including maturation of neurons.

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bodies directly cause autism in some cases is "plausible." In her own lab, Diamond has demonstrated that pregnant mice injected with antibodies made by women with lupus that target DNA-associated proteins and certain cellular receptors on the surface of neurons bear offspring with abnormal brain structures and cognitive problems. And just last month, Diamond published a study of blood from 2700 mothers, which found that roughly one in 10 mothers of children with autism carried what her team calls "anti-brain" antibodies—a ratio roughly four times higher than controls. Preliminary studies suggest that the antibodies targeted proteins different from those that Van de Water has identified, however, and Diamond herself is not ready to claim that these antibodies actually cause autism.

Indeed, Van de Water's dramatic claim that maternal antibodies could play a role in a quarter of all autism cases disturbs many researchers. Because the serum of the tested mothers displayed varying patterns of reactivity to the seven fetal brain proteins—some binding to just one and others to up to five—the researchers added up many different "specific combinations" of reactivity to get their figure of 23%. No specific pattern of reactivity was observed in more than 7% of the mothers of autistic children that they tested, notes Steven Goodman, a biostatistician at Stanford University in Palo Alto. He described the approach as a form of data dredging—sifting data for the largest effects—which at best exaggerates predictiveness, and at worst finds patterns that aren't real.

Ultimately, DiCicco-Bloom says, Van de Water's studies will need to be replicated in thousands of women from different backgrounds to establish whether the maternal antibodies have any predictive value. That should be fairly easy to do, now that the fetal brain proteins targeted by the antibodies have been identified, notes epidemiologist Ian Lipkin, of Columbia University, principal investigator of the Autism Birth Cohort, a large Norwegian study of more than 100,000 children.

Van de Water agrees that her new work needs to be replicated by independent groups. She adds that she is involved in three large prospective studies now in progress at UC Davis, all funded by the National Institute of Environmental Health Sciences. "I didn't want people to get the impression that this was final," she says of her most recent work. "This was really a discovery paper, and not a clinical validation."

Testy questions

Then why, critics ask, is Pediatric Bioscience already trumpeting a test? Van de Water argues that developing the test in parallel with the research means it will be available sooner for doctors to order. "We'll also know if it's not going to work more quickly," she says. She adds that she is facing competition from other groups, such as Diamond's lab.

Anderson is not persuaded. "They're overstating what they have, and then they're proceeding too quickly," he says. "You don't need to commercialize something to make it available as a research tool."

The researcher's corporate ties add to the unease. "She has a patent, and you could perceive a conflict of interest there," DiCicco-Bloom says. Van de Water emphasizes that her relationship with Pediatric Bioscience has been thoroughly vetted by UC Davis, which allows her to split a 30% share of whatever royalties the company pays to the university for use of the patent with other investigators on the team. As chief scientific adviser to Pediatric Bioscience, Van de Water cannot perform the clinical validation studies herself because that would violate the UC Davis policies guarding against conflicts of interest; she is also prohibited by the university from accepting free or discounted stock in the company, she adds.

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Financial considerations aside, the key to any medical diagnostic test is its positive predictive value, a number that indicates how often a test, if positive, is right, Anderson says. In a disorder such as autism, which has a prevalence in the general population of about one in 88 births, even a few false positives very quickly render a test of limited value, he says. Based on his calculations using Van de Water's data and the prevalence of autism, "I get a positive predictive value of 16.5%, which is pretty bad." At that rate, only one out of six positive tests would actually be correct, he explains.

Goodman says that he came up with the same low positive predictive value after crunching the data provided in Van de Water's paper. "That assumes that you accept these numbers at face value, and I don't," he says. Based on the design of the study and his own analysis of the group's published data, he describes Pediatric Bioscience's claim that a positive result on a test for these antibodies will mean that a woman has a "99 percent" likelihood of having a child with autism as "completely false."

Van de Water and D'Alvise respond that the company's press release was "a bit unclear as written" and that likelihood "was not meant to convey likelihood in the statistical sense, but rather the 99% accuracy with which the study demonstrated specificity of the biomarkers for ASD." They also say that test is not meant to screen the general population but would be for women at higher risk of having autistic offspring, such as those who are older or who already have a child with developmental issues.

The U.S. Food and Drug Administration will not be required to review the accuracy or clinical validity of the Maternal Antibody Related Autism (MAR) test, as the company calls it, before it goes to market. Instead, D'Alvise says, Pediatric Bioscience will set up a blood testing lab under the Clinical Laboratory Improvement Amendments, a set of alternative federal standards that regulates diagnostic tests that only one laboratory has the expertise to run. "Typically this is the way new diagnostic tests get offered before they become mainstream," D'Alvise says.

Van de Water believes her test has predictive power, based on the relatively small population she's tested so far. But she acknowledges that it might not hold up in larger, more diverse populations. "I hope it is a valuable area to pursue—we think it is—but down the road who knows what will happen."

DiCicco-Bloom still isn't convinced that the maternal antibody test will bring much benefit and suggests that it could do more harm than good. "Pretty soon, everybody who worries about autism is going to be getting [tested], some for good reason, some because they're highly educated, motivated people," he says. "The moment that this test is given to physicians, it's not going to be controlled. No medical test ever is."

–EMILY UNDERWOOD

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