OPINION, **Q&A**

Q&A with John Constantino: Solving the biomarker conundrum

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The most common forms of autism — those that don't occur as part of a rare genetic syndrome — are strongly heritable. But despite this distinctly biological basis, the condition's variability has stymied the search for **biomarkers**, which might be used to improve diagnosis, track prognosis and identify novel therapies.

Part of the problem may be that the biological factors responsible for whether a person has autism differ from those that influence the condition's severity, says **John Constantino**, professor of psychiatry at Washington University in St. Louis, Missouri.

In a commentary published in April in *Molecular Autism*, Constantino lays out a novel way to distinguish between **different types of biomarkers**. To identify those that reflect autism's origins, he says, researchers will have to look at the behavioral traits that precede and predict a diagnosis — and they will have to train their sights not just on autistic people, but on the general population.

Constantino spoke with *Spectrum* about the "biomarker conundrum" for autism, and how researchers can do better.

***Spectrum*: How do researchers traditionally define and search for biomarkers in autism, and what's the conundrum in how that has played out?**

John Constantino: Biomarkers link a biological signature — for example, variation at the level of genes and DNA, brain signatures from neuroimaging or serum markers — to a neuropsychiatric trait or outcome. An outcome could be an autism diagnosis, or it could be autism severity. But a conundrum is: Are you relating your biomarker to a cause of autism or to an effect of autism?

Based on our work with identical twins and families, we think researchers may sometimes assume that their biomarkers relate to causes of autism when actually those biomarkers signal effects of

autism, and that biomarker associations therefore might not always be what they seem.

S: How has your research homed in on this potential problem?

JC: The story goes back to a **paper we published in 2017**. Colleagues at Emory University had found that among infants and babies who have a sibling with autism, social visual disengagement strongly predicted autism. When we analyzed this biomarker among twins in the general population, we learned that social visual disengagement is extremely highly inherited — and that what predicted autism in later-born siblings was also present in some of the typically developing children. That suggested that social visual disengagement is highly genetic and may be necessary but not sufficient to cause autism.

So we started thinking about what else might combine with social visual disengagement to produce autism. We found three other predictors that could be identified before autism's core signs manifest: autism-like traits transmitted from parents to children, signs of attention deficit hyperactivity disorder (ADHD) in the babies, and subtle impairments of motor coordination. These four factors were not only highly heritable, but they were all independent from one another — no overlap.

S: Why is that important?

JC: If multiple things are inherited but there's no correlation between them in an epidemiological sample, they're very likely tapping different sets of genetic factors. We investigated these four factors in the general population and found that, combined, they determine 60 percent of a baby's [autism-like behaviors]. We also showed that, collectively, they account for a similar level of recurrence of autism within families. So we're probably missing additional factors that would account for the other 40 percent. But we think that certain combinations of these inherited predictors, especially when they are severe, will cause autism. That's the model of autism that we want scientists to consider.

S: Can you elaborate a bit on the model of autism you are proposing?

JC: The idea is that these factors act as levers. They can occur in different permutations, and they are present in the general population. There are little kids walking around with low social visual engagement, but if they don't have extreme atypicalities in any of the other factors, they may not have autism. The kids who have autism seem to be the ones with combinations of extremes.

If we could measure all of these markers, or levers, very early, before autism manifests, and nudge them a bit, we could potentially move a child off of a trajectory that leads to autism. It could be, though, that these combinations of developmental liabilities are themselves effects of earlier developmental forces that play themselves out in utero (rather than after birth), and then we're going to have to take a different approach to intervention.

S: What happens after autism manifests, according to this model, and what does that say about biomarkers?

JC: That's the second part of the story. In identical twins, when one twin has autism, 95 percent of the time the other twin has it too. But we were surprised to learn that they can have remarkably **different levels of autism severity**, with coefficients of correlation suggesting that only 5 to 10 percent of the cause of severity is shared between two identical twins. One prior report noted this, but the findings weren't commonly incorporated into thinking about inheritance in autism, and it wasn't common for twin studies to quantify severity in standardized ways. Whenever a pair of identical twins reared together is different for a trait of interest, one has to trace the difference to non-shared environmental factors.

S: What might those be?

JC: Three things can make a pair of identical twins turn out different from each other. One is measurement error. Another is a highly influential event that occurred in one twin but not in the other — for example, a perinatal stroke, or child abuse. The third thing is what's called stochastic influences — random, minor events that in a typically developing person would have zero effect but in someone with a neurodevelopmental condition [might have] much higher consequences. It's like a puff of wind at the wrong time, and your brain goes in direction X instead of direction Y for reasons we don't understand. We think stochastic influences can be very influential in brain development when your brain is vulnerable because of a condition such as autism. What we have learned from studies of identical twins is that a child's autism can be more or less severe almost by chance, and differences in severity can accrue over time as kids get older.

S: How would you advise researchers who are searching for autism biomarkers?

JC: First, we should differentiate inherited, familial autism from autism caused by de novo mutations. De novo mutations are not inherited, and there is a difference in the types of autism engendered by such mutations in that they are predominantly **associated with intellectual disability**, whereas inherited autism is much more commonly (not exclusively) associated with intellectual development in the typical range. An appeal to the field is to separate inherited (multiplex) from sporadic (simplex) cases in study samples, recognizing that they represent potentially distinct pathways to autism and that inherited autistic syndromes are the most common in the population.

Also, when trying to link a biomarker to the cause of inherited forms of autism, it may be most informative to study the association with predictors of autism before the condition manifests. A biomarker's relationship with autism may change after autism is apparent. In parallel, we have to study traits predictive of autism not only in families affected by autism but in families in the general population.

Finally, when conducting a case-control study comparing children with autism and typically developing children, the controls have all the same levers of developmental liability — they're just not as atypical in relation to that trait. Many unaffected controls carry substantial levels of one or more of the traits that confer autism risk — perhaps at the 70th percentile rather than at the 95th percentile. But if you’re not measuring them or controlling for them, you are by definition compromising statistical power to distinguish cases from controls on the basis of measurements of biomarkers that are principally related to those underlying traits.