Autism spectrum disorder (ASD) is a collection of developmental disorders that occur before 3 years of age and include autistic disorder, Asperger syndrome, and pervasive developmental disorder, not otherwise specified. Children with ASD show signs of delays in communication and social interaction along with repetitive behaviors and interests. Today, diagnosis is performed by clinical assessments that involve an examiner observing a patient as he or she performs tasks as well as an interview of the patient’s parents for a detailed developmental history. The current median age at diagnosis is 5.7 years—an age that frustrates families and doctors because some research suggests that an earlier diagnosis can lead to better outcomes.

“Only 20% of children are diagnosed before three,” says Stan Lapidus, founder and CEO of SynapDx, which is developing a commercial assay for ASD and working to shift diagnosis to earlier in life. “There is a clear unmet medical need for which a diagnostic could make a difference.”

What Is the Innovation?

Nearly a decade ago, Dr. Leonard Rappaport, now Chief of Developmental Medicine at Boston Children’s Hospital, asked his colleague Dr. Isaac Kohane, Professor of Pediatrics and Health Sciences Technology at Harvard Medical School, to do something with him on ASD. Kohane, a quant turned biomedical researcher, considered ASD to involve the brain, and since he worked on RNA, he felt he’d have little to contribute. But then he had a realization and went back to Rappaport with a thought metaphor—and a yes.

“Let’s think about Tay-Sachs,” said Kohane, recalling the conversation in a recent interview with Clinical Chemistry. “We think of it as a brain disease but for decades we’ve known that the white blood cells of a Tay-Sachs patient are abnormal looking. It is not a blood disease although in white blood cells we see the same storage granules seen in the brain.”

He was proposing that perhaps, just as in Tay-Sachs, in which echoes of abnormality present in the brain are reflected in the blood, a blood test for ASD could be possible. Lapidus refers to this realization—that RNA from white cells could give insight into the brain—as “remarkable.” The two jumped into the work.

How Does It Work?

Kohane began to study the peripheral blood RNA expression levels of patients with ASD, and he found differences when he compared the levels in patients with those in controls. Those observations both “reassured and worried” him, he says. Reassuringly, he saw differences in markers for nerve development, such as neural growth factors, which he had expected to see. But when he saw differences in inflammatory signals, he worried that the signals he was measuring may have nothing to do with autism.

But in looking through the autism literature, he found research by pathologists who saw activation of macrophages, a ramp-up of inflammation of the brain, in postmortem studies of autistic children. Moreover, spinal tap studies of children, as well as blood studies looking at proteins, also showed increases in inflammation.

“In the epidemiology literature, inflammation has been linked to autism many, many times,” said Kohane.

From these studies, performed way back in 2004, he concluded that autism is heterogeneous and that
multiple signatures could cover a number of etiologies. But it wasn’t until 2012 that his major work was published (1). Why the delay?

“People didn’t believe us,” said Kohane. “They were skeptical because they thought we were measuring something unrelated.” At the time, Kohane explains, most people were looking at DNA’s effect on autism, although of all inherited forms of autism, DNA mutations account for <20%.

“I knew that, using microarrays, if there was a signal there I’d be able to find it,” said Kohane. He went on to do a whole transcriptome analysis to create a 55-gene prediction model (Fig. 1) developed in 1 cohort of 66 males with ASD and 33 controls. This model was then validated in a second cohort of 104 case patients with ASD and 82 controls, to give a sensitivity of 69.2% and specificity of 65.9%.

SynapDx, Lapidus’ company, is now further developing the technology (Kohane is an advisor, but owns no stock in the company). When asked what performance characteristics he would feel acceptable for an ASD diagnostic, Lapidus deflects. “Today’s [test] sensitivity is 20%,” said Lapidus. “Specificity has to be such so that you don’t flood the limited referral resources.”

But specificity is something Lapidus and his company want to improve. SynapDx is currently recruiting 660 children for the largest multicenter ASD study to date, which Lapidus feels will be large enough to give reasonable CIs around their performance characteristics.

Where Can This Technology Fit in the Clinical Laboratory?

Lapidus expects that primary care physicians will one day order this test directly from his company. The fact that ASD lacks an objective gold standard does not scare him.

“You work through it,” said Lapidus, recalling how his company Cytyc successfully introduced the ThinPrep Pap smear back in 1996. “The inter- and intraobserver variability amongst developmental pediatricians is equal or tighter than reading Pap smears.”

Clinical Chemistry asked Dr. Stephen D. Ginsberg, an associate professor of psychiatry, physiology, and neuroscience at the New York University Langone Medical Center, who is also a member of the Tissue Advisory Board for the advocacy organization Autism Speaks, to weigh in on the team’s RNA research. Ginsberg believes that although there may not be a “smoking gun” of a single miRNA that is predictive, he added in an e-mail response that “a panel may be sufficient to significantly differentiate ASD subjects earlier than conventional cognitive and behavioral assessments that are costly and often equivocal.” He also stated that “expression pro-

Fig. 1. The protocol followed for testing and then tuning the prediction model to ultimately generate a model comprised of 55 genes (ASD55).
filing analysis of the blood transcriptome is a step in the right direction for early diagnosis of autism.”

But, he cautions, “one of the major concerns is whether a blood transcriptome approach mimics bona fide changes occurring in vulnerable cell types within the brain.”

For example, this issue is currently debated among those researchers studying Alzheimer disease. Can a blood transcriptome very clearly detail what is happening in the brain, let alone specific areas of it, such as the hippocampus or the temporal neocortex? According to Ginsberg, the jury’s still out.

Moving ahead, Ginsberg says he’d like to see the transcriptome researchers look at next generation sequencing technology, which can be used to assess both coding and noncoding RNA species, which may be “optimally suited to identify alterations that may involve the epigenome as well as the transcriptome.” A recent poster on the use of RNA-Seq to distinguish ASD from other developmental delays by SynapDx suggests they are on the ball (2).

Thinking more holistically, Ginsberg suggests that the blood transcriptome studies might have another effect on the autism spectrum community, alongside the potential for earlier diagnosis. “Blood transcriptome analyses in ASD may prove beyond a reasonable doubt that autism is truly a systemic disorder, and that brain (or gut) involvement is one manifestation of a complex of alterations.”

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