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After Autism Panel, Mount Sinai Set to Launch Exome, Genome Test on HiSeq; Validating PacBio, Proton

by *Julia Karow*

AFTER LAUNCHING a diagnostic autism panel last summer, the Genetic Testing Laboratory at Mount Sinai School of Medicine is preparing to submit whole-exome and whole-genome sequencing tests to New York state's department of health for approval and is working on several additional diagnostic sequencing panels.

While all of these tests run on Illumina's HiSeq, the lab is also validating them on the Pacific Biosciences RS as a second platform, with the aim of eliminating confirmatory Sanger sequencing in the future. In addition, it is considering the Ion Torrent Proton for this purpose.

This year, the lab will put "a big effort" into the launch of its whole-exome and whole-genome sequencing tests, Lisa Edelmann, director of the Mount Sinai Genetic Testing Lab, told *Clinical Sequencing News* last month.

Because the HiSeq has been "well-established" in the lab, she said, it was most suitable for developing clinical tests, even though previous plans had favored the PacBio platform (*CSN 11/16/2011*). Mount Sinai's genomics core facility, which houses the sequencing equipment, had already run "thousands of samples [on the HiSeq], so it made sense to go with that technology," she said.

The core facility shares the Genetic Testing Laboratory's CLIA license, providing it with access to a variety of sequencing platforms. It is currently equipped with three Illumina HiSeq 2000s, two HiSeq 2500s, a MiSeq, a PacBio RS, and an Ion Proton (*IS 1/8/2013*).

The autism panel, the lab's first next-gen sequencing-based diagnostic test, was launched last summer, but the lab has not marketed it widely yet to allow for a pilot phase to gain experience.

So far, 19 patients have received the test, which includes 30 genes and is expected to yield a positive result in about 5 percent of patients with autism spectrum disorder. It is geared at patients who have tested negative in a fragile X test and an array comparative genomic hybridization assay.

Eighteen of the genes in the autism panel are X-linked, 10 genes cause autosomal dominant disease, and the others are associated with autosomal recessive conditions.

The genes are enriched by Agilent SureSelect in-solution hybridization and sequenced on the Illumina HiSeq. According to the test description, several of the genes are at least partially analyzed by Sanger sequencing as well because they are inadequately covered by next-gen sequencing alone. The test has a sensitivity of 97 percent for DNA mutations at the level of a few base pairs but is likely to miss larger genomic rearrangements and DNA insertions. It may also fail to pick up small insertions and deletions.

Turnaround for the test is currently eight to 12 weeks, which Edelmann said is "fairly standard" for next-gen sequencing panels. The test price is approximately $2,800, or about $1,900 for out-of-pocket payments.

Edelmann said the lab is "still waiting to hear back how insurance deals with this" but is hopeful that it will be covered. It accepts almost all types of health insurance for New York state residents.

According to Edelmann, it took the lab about a year and a half to fully validate the autism test – a project conducted in collaboration with Mount Sinai's Seaver Autism Center – in

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accordance with the requirements of New York state's health department. While the department does not have specific guidelines for next-gen sequencing-based diagnostic tests except tumor sequencing (*CSN 11/7/2012*), it expects labs to assess variability within and between runs as well as between lab technicians.

It also required the lab to fully validate its software – in this case, NextGene from Softgenetics. Edelmann said the validation required "a lot of Sanger sequencing" on control samples in order to determine false positive and false negative rates and the best settings for the software.

Right now, the autism panel requires follow-up of clinically actionable variants by Sanger sequencing, which Edelmann said is both laborious and expensive. One way to move away from Sanger sequencing is to use a second next-gen technology, assuming that variants found by both platforms are valid.

For that, her lab is currently validating the autism panel on the PacBio RS against its Illumina test, using the same Agilent SureSelect pull-down library adapted for the PacBio.

This, Edelmann said, "could eliminate a big portion of the Sanger sequencing" and also reduce the turnaround time of the test by a couple of weeks. The validation is expected to be completed by the middle of this year.

The Ion Proton, which the core facility received just recently, could be another possible second platform, she said, but the long reads of the PacBio and its lack of GC-bias "make it a pretty strong technology."

The Genetic Testing Lab is also working on other diagnostic gene panels, which it hopes to launch in the second half of this year, including a 12-gene panel for Noonan syndrome – to be used prenatally and postnatally – and two panels requested by Mount Sinai physicians for use in pediatric patients, one for limb defects and one for microcephaly. Like the autism panel, these will run on the HiSeq, and possibly on a combination of HiSeq and PacBio in the future.

Next in Line: Exome, Genome

Over the last year, the lab has also been developing a whole-exome and whole-genome sequencing test for undiagnosed patients with a suspected Mendelian disease who have undergone all available testing. The plan is to submit these tests to New York state's department of health for approval by the end of March.

So far, the lab has been validating these tests on the HiSeq, using anonymous control samples, but it might add either the PacBio or Ion Proton as a second technology later, Edelmann said.

Testing will probably start with parent-child trio exome sequencing, followed by whole-genome sequencing if the test comes back negative. "Eventually, when the cost comes down considerably, we might just offer whole-genome sequencing," Edelmann said.

One concern she has about clinical exome sequencing is that it is incomplete, missing certain genes or exons due to technical limitations. "If a patient has a clear diagnosis of a disorder, and there is a set of known candidate genes, you want to be sure that you sequence all of those candidate genes to completion," she said. "If you're not covering the major candidate genes well enough, then you're not doing your job properly."

Using a second technology, like PacBio or Ion Proton, might yield a more complete exome, but until then, there will still be a need for candidate gene panels, she added.

To select results from exome and genome sequencing for reporting to patients, Mount Sinai's department of genetics and genomic sciences plans to form a working group with rotating members, including genetic counselors, clinical geneticists, laboratory directors, postdoctoral fellows, and clinical fellows. These will jointly determine which variants require follow-up by Sanger sequencing, and which ones are likely clinically actionable. "Often you find things that are likely pathogenic or of unknown significance," Edelmann said. "In those categories, you need to research the genes, figure out what's known about them, figure out whether they are likely candidates. That requires a team of people."

Patients or their parents undergoing exome or genome sequencing will be able to state in their consent forms whether they only want to receive results related to the primary diagnosis or if they'd also like to receive incidental findings.

Mount Sinai will not report mutations related to incurable adult-onset disorders, such as Alzheimer's disease, but will include variants in cancer-predisposing genes that could result in increased surveillance or other prophylactic measures. It will also provide pharmacogenomic panels and certain types of carrier information, for example if a mother carries an X-linked disease gene or if both parents are carriers for the same recessive disorder.

In addition to the tests currently in development, Mount Sinai is considering next-gen sequencing-based carrier screening, although for now, "genotyping is much cheaper," Edelmann said. The lab is currently offering carrier screen-

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ing by Illumina genotyping and is looking into Sequenom's MassArray technology for an expanded panel. For a next-gen sequencing test, she said, the Ion Torrent "would be a good system because they have some panels already set up."

Newborn screening is another area Mount Sinai is interested to explore with next-gen sequencing. Edelmann and her colleagues, in collaboration with New York state's department of health, recently submitted a grant application to the National Institutes of Health for a Genomic Sequencing and Newborn Screening Disorders program. In it, they propose to do exome sequencing on newborns and report clinical results, and to study the ethical and psychosocial aspects of such expanded screening. The research would also involve developing techniques for sequencing from blood spots and improving turnaround time.

In addition, they plan to use PacBio sequencing to diagnose trinucleotide repeat disorders as well as to analyze other disease-related genes that are hard to sequence otherwise because they have closely related pseudogenes or are located in repeat regions. The Genetic Testing Lab is already looking into standalone tests for congenital adrenal hyperplasia and Gaucher disease – both for carrier screening and to diagnose newborns – which it plans to develop on the PacBio this year.

Edelmann explained that current newborn screening in New York state mostly involves biochemical and immunological assays. If any of these come back positive, families are referred to one of seven newborn screening follow-up centers, which include Mount Sinai. Follow-up involves both biochemical tests and sequencing-based tests. "Having one test that would do all of those things at one time and diagnose any disease in a newborn is preferable, especially if it could be done in a timely manner," she said.

Tumor sequencing is another area Mount Sinai is interested in for next-gen sequencing, most likely starting with gene panels. As the Genetic Testing Lab focuses on hereditary disease, Edelmann said, these would mostly likely be developed in c ollaboration with the pathology department.