Chromosome triple play

By Lev Osherovich, Senior Writer

Researchers from Cyprus have developed a rapid, noninvasive diagnostic for Down syndrome that detects methylated fetal DNA in the blood of pregnant women.¹ The team's leader has founded a new company—**NIPD Genetics Ltd.**—to commercialize the technique, which will compete with DNA sequencing-based methods from **Sequenom Inc.** and **Artemis Health Inc.**

Currently, the risk of Down syndrome and other birth defects caused by chromosomal duplication is assessed by measuring the thickness of an embryo's neck by ultrasound and screening for blood-borne fetal proteins.

These methods pose no risk to the embryo but are not definitive. Moreover, ambiguous results often need to be confirmed with an invasive and potentially dangerous procedure such as sampling cells from the amniotic fluid or chorionic villus of the placenta to obtain and count fetal chromosomes.

The method devised by Philippos Patsalis, chief executive medical director of **The Cyprus Institute of Neurology and Genetics**, combines the ease of blood-borne protein screening with the accuracy of chromosome counting without the need to sequence fetal DNA.

"We have something much more simple and lower cost compared with next-generation sequencing," said Patsalis. "Our method can be easily introduced throughout the world without the need to purchase expensive sequencing equipment."

Methylation information

Patsalis' method exploits the fact that fetal cells that leak into the mother's blood in minute quantities contain DNA that is methylated at different regions than the mother's DNA.

The Cypriot team used a mAb that recognizes methylated DNA to selectively immunoprecipitate fetal DNA from maternal blood samples and then used real-time quantitative PCR to amplify and detect the fetal DNA (see **Figure 1, "Detecting Down syndrome"**).

The group found 8 regions along chromosome 21 that were hypermethylated in fetal DNA compared with maternal DNA. The researchers hypothesized that in samples taken from women carrying fetuses with Down syndrome, compared with samples from women carrying healthy fetuses, an extra copy of chromosome 21 would cause a higher yield of methylated DNA.

Indeed, a statistical model combining detected levels from all 8 hypermethylated regions correctly predicted all 14 cases of Down syndrome in a set of 40 blinded samples.

Data were published in *Nature Medicine*.

The next step is "to carry on a large-scale clinical study with 1,000 samples," said Patsalis. "In this study we will be able to assess the accuracy in other ethnic backgrounds" beyond the 40 Cypriot patients tested.

Patsalis also hopes to expand the method to detect hypermethylated markers on other trisomy-prone chromosomes.

He has patented the findings and launched NIPD Genetics to streamline the protocol and develop a kit for diagnosing Down syndrome. The company has raised \$2.5 million in series A funding from undisclosed investors.

Down for the count

Medical geneticists told SciBX that the Cypriot team's method is promising but proving the technique's accuracy will require testing in large and diverse populations and at various times during pregnancy.

"Patsalis can nicely distinguish the methylation patterns between fetal and maternal DNA," said Michael Watson, executive director of the **American College of Medical Genetics**. He said the method offers the ease and low cost of currently used blood-protein screening techniques but its utility in a real-world clinical setting remains unknown.

In particular, Watson was concerned about the possibility of false negatives. He noted that about 5% of patients with Down syndrome have extra chromosomes in only some of their cells. In this situation, termed mosaicism, trisomic fetal DNA would be even more rare than usual, and it's unclear whether Patsalis' method would be sufficiently sensitive to make the correct call.

Watson also was concerned that fetal DNA from previous pregnancies or from twin fetuses could potentially confound PCR-based detection.

Because the analysis of the 40 pregnancies was retrospective, Watson said it remains unclear how early the method could be used during pregnancy.

Dennis Lo, director of the Li Ka Shing Institute of Health Sciences at **The Chinese University of Hong Kong**, said his biggest concern is the robustness of the Cypriot team's method of isolating, detecting and interpreting the fetal DNA. He noted that immunoprecipitation and quantitative PCR can have variable yields and require rigorously validated statistical analysis, whereas interpreting DNA sequencing data is more straightforward.

"I think it would be very difficult to obtain highly reproducible results with an immunoprecipitation step," said Lo. "Furthermore, the readout step of using real-time quantitative PCR would also be expected to have a lower precision than using massively parallel sequencing. I would also be interested to know the robustness of the prediction equation."

Lo has developed sequencing methods to analyze fetal DNA for a range of chromosomal abnormalities including Down syndrome² and

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—Philippos Patsalis,
The Cyprus Institute of
Neurology and Genetics

Figure 1. Detecting Down syndrome.

Papageorgiou *et al.* have devised a method for noninvasive, prenatal detection of trisomy 21, the chromosomal abnormality that underlies Down syndrome.

A blood sample from a pregnant woman [a] contains mostly her DNA but also trace amounts of fetal DNA, which is hypermethylated (Me) compared with the mother's DNA. The amount of fetal DNA in the sample is increased by immunoprecipitation with a mAb that recognizes methylated DNA [b]. The DNA is then amplified and analyzed by real-time quantitative PCR [c]. Because of the extra chromosome, the yield of amplified DNA is higher from women carrying a fetus with trisomy 21 than from women with genetically normal fetuses.

The technology has been licensed to **NIPD Genetics Ltd.**, a new company that is developing noninvasive, prenatal diagnostics for Down syndrome and other chromosomal abnormalities. **Sequenom Inc.** and **Artemis Health Inc.** are developing DNA sequencing-based diagnostic technologies to detect Down syndrome.

has licensed his techniques to Sequenom.

Sequenom also is developing an RNA-based, noninvasive, prenatal Down syndrome test that it hopes to bring to market in 2012.

"The potential advantage of the method by [Patsalis' team] is that it is possibly cheaper than current sequencing-based methods," said Lo. "However, this advantage might be rapidly diminishing with the continual reduction in sequencing costs." Thus, Lo thinks sequencing could soon be done as cheaply as Patsalis' method.

Lo added that sequencing-based approaches can be used as early as 11 weeks into a pregnancy.

Another player in the Down syndrome testing space is Artemis Health, which holds a license to sequencing-based technology developed by Stephen Quake, professor of bioengineering at **Stanford University**, for prenatal detection of Down syndrome.

Sequenom and Artemis have not disclosed pricing information for their future products.

Both Watson and Lo noted that sequencing provides a more complete picture of various chromosomal abnormalities that lead to birth defects than the more focused approach favored by Patsalis.

For example, genomic sequencing can reveal variations in the copy number of relatively small genomic regions that have been implicated in autism spectrum disorders (ASDs) and forms of mental retardation besides Down syndrome.³

Watson also noted that the FDA has increased its scrutiny of prenatal

diagnostics. The agency "has run a series of meetings indicating that they'll be entering the regulation of laboratory-developed testing," said Watson. He thinks that because prenatal diagnostics can influence the decision of whether to continue with a pregnancy, they will be high on the FDA's priority list for regulation.

In the U.S. Congress, meanwhile, Sen. Orin Hatch (R-Utah) is planning to introduce a legislation that would impose a distinct regulatory pathway for *in vitro* diagnostics including Down syndrome tests.⁴

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COMPANIES AND INSTITUTIONS MENTIONED

American College of Medical Genetics, Bethesda, Md.
Artemis Health Inc., Menlo Park, Calif.
The Chinese University of Hong Kong, Hong Kong, China
The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
NIPD Genetics Ltd., Nicosia, Cyprus
Sequenom Inc. (NASDAQ:SQNM), San Diego, Calif.
Stanford University, Stanford, Calif.