# Harvard, Peking University Team Combines NGS, Linkage Analysis to Detect Aneuploidy, SNVs for PGD

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***Premium***

NEW YORK (GenomeWeb) – Over the last couple of years, researchers have demonstrated that a next-generation sequencing-based approach to detect chromosomal aneuploidies in embryos before they are implanted as part of an in vitro fertilization cycle can increase the success of IVF at a reasonable price.

Using the same approach for preimplantation genetic diagnosis — detecting disease-causing point mutations — has been more cost prohibitive due to the depth of sequencing coverage needed to reliably detect those mutations.

Now, a research team from Harvard University, the Third Hospital at Peking University, and the Biodynamic Optical Imaging Center at Peking University has developed a technique that combines low-coverage whole-genome sequencing for aneuploidy detection with targeted deep sequencing and linkage analysis to identify SNVs.

In a study published in the *Proceedings of the National Academy of Sciences* today, the team reported on two couples undergoing IVF. In each case, one partner carried a monogenic disease-causing single-nucleotide variant and the researchers used PGS/PGD to select chromosomally normal embryos free of the SNV. Both couples had healthy babies.

Sunney Xie, a professor of chemistry and chemical biology at Harvard University and the senior author of the study, told GenomeWeb that that the two cases were part of a clinical trial of the technique being conducted at Third Hospital. Xie is collaborating with Jie Qiao of Peking University Third Hospital and Fuchou Tang of Peking University.

The new technique builds on the team's single-cell amplification technique, called MALBAC for multiple annealing and looping-based amplification cycles, that was developed by Xie's Harvard laboratory.

The team initially began conducting a clinical trial of the MALBAC single-cell sequencing PGS method at Peking University Third Hospital and last year reported its first healthy baby born as part of that trial, in

which 24 couples had enrolled at the time.

In that trial, the researchers have been using MALBAC to amplify the genomes of single cells from embryos and then sequence them at low coverage to screen for chromosomal abnormalities. They also assessed the ability of the approach to detect single-gene disorders that one parent was either affected by or a known carrier for.

Throughout that testing, Xie said, the researchers wanted to figure out how to make the SNV detection more accurate while keeping costs down, so they developed an NGS-based method that incorporates linkage analysis, which helps verify the SNVs.

The new technique is dubbed MARSALA, for mutated allele revealed by sequencing with aneuploidy and linkage analysis. It incorporates single cell analysis using MALBAC to determine aneuploidy status via low-coverage whole-genome sequencing and adds steps for SNV calling and verification.

To perform MARSALA, the researchers first biopsy trophoectoderm cells from a blastocyst and amplify them using MALBAC. Next, they use PCR primers to target the known disease-causing SNVs that are carried by one of the parents and could be potentially passed along to the child. The whole-genome amplified product is then mixed with the PCR-amplified targeted region and sequenced to between 0.1x and 2x coverage.

That depth of coverage is sufficient for calling SNVs in the targeted region, since it was pre-amplified, Xie said. In addition, the low sequencing depth would help keep the cost down, although Xie declined to specify how much the procedure would cost.

Then, to eliminate any false positive or false negative SNV calls, the team incorporates a linkage analysis that relies on a set of SNP markers for the disease allele, a principle similar to STR analysis.

"It's hard to detect one specific point mutation in a single cell without making any mistakes," Xie said. "Even the best method will have false postives and false negatives, yet such mistakes cannot be tolerated for IVF."

To develop the set of SNP markers, the researchers have to analyze samples from the couple undergoing IVF, as well as from the parent who passed on the disease allele to one of the partners. They sequence the genomes of the couple and the affected parent to 2x coverage, which the researchers reported is sufficient to detect the disease-causing SNV as well as a panel of SNPs that could be used for verification in the embryo.

In one case, the husband had hereditary multiple exostoses, characterized by bony spurs or lumps in the bones, a condition that he inherited from his father. Genetic diagnosis of the husband identified a frameshift point mutation in the EXT2 gene, which is known to cause the disease.

To perform PGD for that couple, the researchers first analyzed the husband's father, sequencing his genome to 2x coverage and calling SNPs located within 1 megabase of the disease-causing mutation. The researchers then came up with a set of 10 SNPs that were linked to the disease-causing SNV.

Next, they analyzed the embryos for implantation. Instead of relying on just one base call to determine

whether the embryo indeed had the pathogenic SNV, the researchers relied on the set of 10 SNPs, choosing the embryo without the disease-associated haplotype.

"Multiple SNPs close to the target mutation can increase the accuracy of identifying the mutated allele significantly, even if the direct mutation calling is unsuccessful," the authors wrote.

The IVF cycle produced 18 embryos, seven of which were affected, leaving 11 available for transfer. The researchers selected an unaffected, euploid embryo for transfer and the wife gave birth to a healthy baby.

In a second case, the wife carried an X-linked recessive disorder and had previously had an affected son who suffers from X-linked hypohidrotic ectodermal dysplasia, which is characterized by hair, sweat gland, and teeth abnormalities. Genetic diagnosis identified a point mutation in the EDA1 gene, which is known to be associated with the disorder.

When the couple underwent IVF and PGD in order to have a second child, the researchers analyzed the affected son, rather than the mother's affected parent.

In this case, the researchers looked at SNPs within 3 megabases of the disease-causing mutation, focusing on heterozygous SNPs in the wife's mutated X chromosome and SNPs in the X chromosome of the affected son and unaffected husband. They chose 10 SNPs connected to the disease-causing mutation. In this case, the IVF cycle produced four embryos, two of which had the disease-causing mutation. One of those two embryos also had a significant chromosomal abnormality. One of the two healthy embryos was implanted in the woman, and she gave birth to a healthy baby.

To further validate the protocol, the researchers also used Sanger sequencing of the PCR products to verify that the embryos identified as carrying disease-causing mutations did in fact harbor those mutations. Sanger sequencing and the MARSALA method were concordant for all cases but one, where Sanger sequencing did not detect the disease-causing mutation. The authors attributed the discordance to Sanger sequencing's lower sensitivity.

In addition, for both couples, the researchers confirmed that the fetuses were chromosomally normal and did not carry the disease mutations by analyzing amniotic fluid cells with Sanger sequencing, a SNP array, and karyotype analysis. At birth, umbilical cord blood was collected and confirmed to be free of the disease mutation and the pediatricians confirmed both babies as disease-free.

One potential limitation of the technique is that it will not detect de novo or unknown mutations. In addition, it not only requires blood samples from the couple but also from the parent of the affected partner who passed along the disease variant, or from an affected child.

However, Xie said that if a family member is not available, haplotype phasing of a few sperm cells and a few second polar bodies can be performed so that "the haplotype of both parents of the embryos can be accurately phased."

Alternatively an embryo that is created during the IVF process that ends up with the disease causing SNV could also be used as a proxy for an affected family member to choose the SNPs for the linkage analysis.

In both of those cases, the "MARSALA method can be applied to the blastocyst stage embryos with information deduced purely from the IVF couple only," he said.

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