

Mosaicism adds to challenge in molecular diagnostics

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Dr. Biesecker

That was illustrated by a session on somatic overgrowth syndromes, which are caused by genetic mosaicism, at the 2015 Association for Molecular Pathology meeting, where Leslie Biesecker, MD, presented on the underlying pathogenesis of such syndromes. “My key message,” Dr. Biesecker tells CAP TODAY, “was that genetic and molecular testing has become confused over the years. Diseases caused by changes in DNA are often inherited but they aren’t always inherited. And when they are not inherited they can be just as important.” Cancer is primarily a disease of noninherited somatic mutation, notes Dr. Biesecker, who is chief of the Medical Genomics and Metabolic Genetics Branch at the National Human Genome Research Institute.

But there is no reason to think this phenomenon is limited to cancer, he adds. “And in fact it is probably a contributor to many diseases. Now that we have newer tools, we as clinicians and clinical pathologists need to be thinking about noninherited ways that genetic change can cause disease. And one is mosaicism.”

Marilyn M. Li, MD, a professor of pathology and laboratory medicine at the University of Pennsylvania, spoke in the session about the use of NGS to detect mosaic genetic alterations underlying overgrowth syndromes. Only in recent years, she says, has there been an understanding that somatic mutations may cause diseases other than cancer. “These genetic diseases can be seen in postnatal or prenatal onset. . . . We would like to have people be aware of somatic mutations that can cause genetic diseases,” says Dr. Li, vice chief of the Division of Genomic Diagnostics and director of cancer genomic diagnostics at Children’s Hospital of Philadelphia.



Dr. Li

The greater sequencing power of NGS “allows us to see mosaic mutations that we probably previously missed that are present at only low levels and only in some tissues,” Dr. Li says.

Josh Deignan, PhD, a co-moderator of the session, noted challenges that arise from the unusual properties of mosaic mutations. “We are now being forced to think more about which sample we’re going to test, whether it’s blood or tissue or buccal cells,” Dr. Deignan said in an interview. “We can obtain DNA from any of these sources fairly easily these days, and yet the allele frequency for a given variant may be different in each sample. We now have to begin to predict and try to understand exactly what that might mean when we write our reports and communicate these types of results to clinicians.

“Is a known pathogenic variant still pathogenic if its allele frequency is only 10 percent? What if it’s only 10 percent and in only one tissue but not any others?” asks Dr. Deignan, who is an assistant professor of pathology and laboratory medicine at the University of California, Los Angeles.

Dr. Biesecker defines a mosaic as an organism with heterogeneous genotypes in cells that originated from a single fertilized egg.

In his AMP talk, he debunked what he calls a fairy tale—that all the body’s cells have the same DNA. He cited a Danish study that sequenced 10 trios to high depth and found five to six mosaic variants in the blood of each person (Besenbacher S, et al. *Nat Commun.* 2015;6:5969). And because blood is just one of many tissues, the true number of mosaic mutations per person is much higher. “Mosaicism is the norm, not the exception,” Dr. Biesecker said.

“It is not a question of whether or not an individual is mosaic; everyone is a mosaic,” he said in an interview. “The real question is where mosaic alterations are in each individual and whether they happen to hit a gene that causes a phenotype, such as *AKT1*, which causes Proteus syndrome.

“Some early mosaics were recognized by dermatology because mosaicism causes patterns in the skin that are visible to the naked eye.”

German dermatologist Alfred Blaschko described some of the earliest mosaics in the late 19th and early 20th century. Mosaic patterns depend on when in embryonic development a mutation arises. An excellent diagram can be found in *Trends in Genetics* (Campbell IM, et al. 2015;31[7]:382–392). Campbell, et al., write that accumulation of genetic variants during development “results in an organism composed of countless cells, each with its own unique personal genome.”

Proteus syndrome is the prototypic mosaicism. “It is far from the most common,” Dr. Biesecker says. “It is important because of its historical significance and the story about Joseph Merrick,” known as “Elephant Man.” Dr. Biesecker and his colleagues chose to study it because it has clear visual evidence of the affected parts of the body. They sequenced the genomes of 29 patients with Proteus syndrome; 26 had an activating somatic mutation in the oncogene *AKT1* (Lindhurst MJ, et al. *N Engl J Med.* 2011;365[7]:611–619). Work in vitro showed that tissues and cell lines from these patients harbored admixtures of mutant alleles that ranged from one percent to about 50 percent.

Proteus syndrome is caused by somatic mosaicism for a mutation that is lethal in the nonmosaic state; it cannot be passed on. If the *AKT1* mutation occurred in the egg or sperm cell, the zygote would not be viable. Other mosaic conditions can exist in the constitutional state, that is, a person can have the mutation in both somatic cells and gonadal cells (also called gonosomal).

In Proteus syndrome, detection in blood is not reliable, as seen in work by Dr. Biesecker and his colleagues. “Essentially all blood samples from patients with the disease have zero mutation,” he says. “That’s the key. It tells you, if you want to do molecular diagnosis on a patient with suspected Proteus syndrome, don’t draw and test blood. It is very unlikely for the mutation to be there even if the patient has the disease.” Visibly affected tissue is a much better sample.

Dr. Biesecker drew attention to another diagnostic conundrum posed by a different form of mosaicism, the

conditions caused by mutations in the *PIK3CA* gene—*PIK3CA*-related overgrowth spectrum, or PROS. He calls it “aligning our concepts to biological reality.”

“Mutations in this one gene can cause any of nine clinical disorders, depending on where they are in the body, how much mutation is there, and what kind of mutation. That’s a molecular-clinical diagnostic challenge that this [molecular laboratorian] audience really needs to grasp and appreciate,” he tells CAP TODAY. Like the mutations underlying Proteus syndrome, the mutation in many forms of PROS cannot be detected in the blood. Some conditions can be either mosaic or constitutional, others only mosaic. “How we do molecular diagnosis to make all of this work is a huge challenge to our field,” Dr. Biesecker says.

That a mutation in one gene can cause widely differing phenotypes raises a profound question for medicine, he says: Should we classify disease by phenotype or by molecular attribute? He showed the toe of a four-year-old child on which sat a small lesion. “Just a bump,” he called it. Yet this lesion shows the same visual effect as tissue from patients with Proteus syndrome and displays the same histology as in Proteus syndrome—cerebriform connective tissue nevi. In addition, the cells harbor the same *AKT1* genetic variation.

“Clinicians feel very uncomfortable saying this patient has the same disease as patients with Proteus syndrome,” Dr. Biesecker says. “It pushes the boundary of our comfort zone for classifying patients by mutation. Mosaicism strains the concept of diagnosis.” Yet, he adds, “It is the pathophysiology that matters.”

He cited another disease pair that raises questions about the meaning of a diagnostic entity: MCA CNS dysgenesis syndrome, a severe malformation syndrome entailing maldevelopment of the face, heart, and central nervous system; and paroxysmal nocturnal hemoglobinuria (PNH), which has adult onset. Both conditions are caused by loss-of-function mutations in the *PIGA* gene, germline in the first condition and mosaic in the second. “Does the clinician think those two patients have the same disease?” Dr. Biesecker asked.

“Mosaicism makes everything in genetics more challenging and more interesting,” he concluded. “It also makes reporting more complex.”

Clinical molecular testing for mosaic mutations underlying somatic overgrowth syndromes can be performed in either a genetics laboratory or a cancer genetics laboratory, in Dr. Li’s view. Most important, she says, is that testing “really needs to be in a molecular laboratory where the director understands genetics.” Patients may have an increased risk of cancer but the aberration itself is not cancer; it is more like a benign tumor. “The laboratory director needs to understand how that can happen,” Dr. Li says.

Her laboratory had already been studying NGS for cancer when she saw a publication on NGS for overgrowth syndromes and adopted the method. They set up a targeted panel to detect all reported mutations in genes *AKT1*, *AKT2*, *AKT3*, *PIK3R2*, *MTOR*, and *GNAS* and all coding sequences plus their flanking regions of *PIK3CA* and *PTEN*. Average coverage with the panel is greater than 2,000×. Mutations are confirmed with a second technology.

To evaluate the overgrowth panel, 50 patients with suspected mosaic overgrowth syndrome were tested. Age ranged from prenatal to 35 years. Affected tissue was tested in 35 postnatal cases and one prenatal case, while blood was tested in 13 cases and amniotic fluid in one case. A mutation was detected in 28 of the 50 cases (56 percent). As Dr. Li noted, the detection rate was much higher in affected tissue, 62 percent, than in blood, 33 percent.

Twenty-five mutations were in *PIK3CA*, one in *AKT1*, and two in *PIK3R2*. All mutations have been reported previously as activating mutations in cancer. Mutant allele frequencies varied from 1.5 to 49.2 percent.

Increased sensitivity with the overgrowth panel was illustrated in a patient with a three base pair deletion in the *PIK3CA* gene. Cancer exome sequencing at 167× detected the mutation in affected tissue at a frequency of 4.2 percent (seven of 167 reads), while the overgrowth panel at greater than 2,000× found a frequency of 10.68 percent (223 of 2,088 reads).



Dr. Deignan

Sensitivity can also be enhanced by in vitro culturing of affected tissue, an effect that was particularly pronounced in amniotic fluid cells in the one prenatal case. “You may not get a positive result on direct testing,” Dr. Li says, “but if you culture tissues you consistently see an increase in mutant allele frequency.”

“Our experience demonstrates that NGS technology is highly sensitive for the detection of low-level mosaic mutations and can be used for the diagnosis of these mosaic overgrowth syndromes in both prenatal and postnatal settings,” Dr. Li summed up.

“The more we learn about mosaicism, the more challenging the field of clinical molecular diagnostics becomes,” Dr. Deignan says. “But the more rewarding it becomes as well. Every case has the potential for a spectrum of variation that no one in the world has ever seen before, and as the molecular diagnostic laboratorian, you get to be the first one to try to understand what it means.”

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