



**BREAKTHROUGHS
IN UNDERSTANDING.**

A compilation of selected
presentations and highlights

from the SECOND ANNUAL
**SIGNATURE GENOMICS
SCIENTIFIC MICROARRAY CONFERENCE**

June 18–20, 2009 / Spokane, Washington

Bettering Patient Lives

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GENOMICS**

Welcome

Fostering a stimulating and friendly environment for all professionals who use array CGH in their practice is the overall goal of the Signature Genomics Scientific Microarray Conference. Our vision is that, with active participation, this annual gathering will become the premier resource for those engaged in clinical molecular cytogenetics. This year's conference focused on prenatal array testing, genetic counseling, and technical laboratory matters.

This booklet is a compilation of selected presentations given this year. It is being distributed to attendees and other scientific professionals who were unable to attend. Signature Genomics is very excited to host this gathering of outstanding health care providers who are driving this revolution in genetic diagnostics. We hope to see you at next year's event to be held June 17–19, 2010 in Spokane, WA.

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Background

It was clear from the discussions at this year's Signature Genomics Scientific Microarray Conference that array-based comparative genomic hybridization (array CGH) is radically changing clinical practice.

In the past, clinicians using even high-resolution karyotype analysis have been limited to a resolution of only about 5 megabases (Mb). Today, array CGH platforms available to any clinician can analyze the genome to a resolution of a few thousand base pairs (kb).

Such high-resolution analysis now makes it possible to quickly and easily evaluate the whole genome, including those regions known to be involved in cytogenetic abnormalities.

Such advances have made it possible to identify and define increasingly small and more complex genetic aberrations, and to find answers to long-standing questions about rare and common syndromes, by elucidating mechanisms behind phenotypes, identifying new lesions, and revealing previously unknown interrelationships between genes, the environment, and clinical presentation.

At the same time, array CGH findings are raising new and intriguing questions that challenge both clinicians and researchers.

At this year's conference, more than one hundred scientists and clinicians from the United States and abroad met to discuss the latest advances in the use of array CGH and how array CGH is being used to answer an increasingly wide variety of research and clinical questions.

As in the past, the conference provided a unique forum where physicians and genetic counselors could share their clinical experience with researchers working on the latest technology.

The presentations at the conference described how array CGH is helping clinicians to identify new syndromes, to clarify the genetic cause of known syndromes, and, most importantly, to provide their patients and their families with more accurate diagnoses.

Finding Answers For Patients: Mosaic 20p11.2 Deletion

Dr. Patricia Gail Williams, an associate professor of pediatrics who sees patients at the University of Louisville Weisskopf Child Evaluation Center in Louisville, Kentucky, says “from the developmental pediatrician’s standpoint” the advent of array CGH “is a huge advance.”

In an interview at the conference, Williams noted that in the past the typical evaluation of a child with developmental delay relied primarily on a high-resolution chromosomal analysis and fragile-X DNA testing.

But too often these tests failed to provide answers, she said.

“Now, when we get microarray comparative genomic hybridization, we’re coming back with answers for parents about what is going on,” she said. “It makes a huge difference.”

In her presentation, Dr. Williams described the evaluation of a 15-month-old female with a *de novo* mosaic 20p11 deletion who presented with panhypopituitarism, hypoplastic pituitary gland and ectopic posterior pituitary gland, cleft lip and palate, kyphosis, heart defects, craniofacial anomalies, seizure disorder, variable muscle tone and global development delay.

The child had been delivered by cesarean section after a full-term pregnancy that had been complicated by maternal hypertension and tobacco use. Decreased fetal movement had been detected as well.

20p12 deletions have been associated with Alagille syndrome and the Jagged 1 (*JAG1*) gene.

Williams reported that oligonucleotide microarray analysis showed a single-copy loss of 13 BAC clones from the short arm of chromosome 20 (20p11.2), indicating a 5.4 Mb deletion that encompassed 23 genes.

The mosaic deletion was expressed in 83% of cells.

Williams said a literature review identified one other report from 2005 describing a case with a similar 20p11.22 deletion, though the size of the deletion was not provided in that report.

In her presentation, Williams reviewed the clinical features of a number of case reports of 20p11 deletions described in the literature and reviewed the function of a number of the genes in the area that might play a role in the disorders described in her and the earlier cases.

The clinical features of her patient and the other patient reported with the 20p11.22 deletion suggest that this area of 20p has a critical role in neurodevelopment, although the known disorders associated with the genes in that region include other phenotypes, including thrombophilia, dystonia and corneal abnormalities.

At least some of the genes in the area are also known to be associated with neurodevelopment and midline field defects, including holoprocerephaly, said Williams, but further cases will need to be identified and characterized in order to determine the role of these genes in embryogenesis.

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—Patricia Williams, MD

Solving Puzzles: Mosaic Monosomy 7

Another case in which array CGH provided the answer to a difficult clinical case was presented by Danielle LaGrave, MS, CGC, a genetic counselor for both the Cytogenetics and the Maternal Serum Screening laboratories at ARUP Laboratories, an enterprise of the University of Utah in Salt Lake City, Utah.

In this case, LaGrave and colleagues were asked to conduct an array CGH analysis on a 3-month-old Hispanic male, who was born prematurely with multiple congenital anomalies, including ambiguous genitalia, bilateral club feet, small kidneys with pyelectasis, and hyperpigmentation.

Prenatal cytogenetic testing from cells obtained by amniocentesis had found the fetal chromosomes to be normal 46,XY.

But the pregnancy was complicated by intrauterine growth retardation, decreased fetal movement and fetal bradycardia, prompting an emergency cesarean section at 27 weeks and 4 days gestation.

After delivery, the infant was found to have low cortisol levels, a small thymus on chest x-ray, absolute lymphopenia, thrombocytopenia and chronic anemia.

The infant's course was complicated by repeated episodes of bradycardia, apnea and sepsis.

Additional cytogenetic analysis on cord blood and buccal cells, and diepoxybutane (DEB) analysis for Fanconi anemia, did not find an explanation for the child's condition.

To try to sort out what was going on, Dr. Carol Booth, the geneticist caring for the child, and her team ordered array CGH to be performed on a blood sample sent to ARUP. ARUP used a 44K oligo-array platform (Human Genome build hg18) in the performance of the testing.

Array CGH demonstrated a complete loss of one chromosome 7, with a pattern consistent with a high-level mosaic monosomy 7, LaGrave said.

"That's not something you expect to see in a live-born," LaGrave noted in an interview after her presentation.

"We would never have guessed monosomy 7," she said, but array CGH was able to pick up the monosomy because, in addition to looking at cells from the lymphoid lineage in the blood, array CGH analyzes the cells from myeloid lineage.

"That's why conventional cytogenetics didn't pick it up, but array CGH did," she said.

Monosomy 7 is the most common chromosomal abnormality seen in childhood myeloid disorders and is associated with both quantitative and qualitative defects in multiple peripheral blood cell lines. The possibility exists that the monosomy 7 seen by array CGH is caused by the loss of one chromosome 7 in the bone marrow and was a pre-malignant change restricted to that tissue.

LaGrave and her colleagues were able to find one report describing a similar case in medical literature, a case of male pseudohermaphroditism, congenital adrenal hypoplasia, thrombocytopenia and anemia. That child had 46,XY in blood and lung tissue but 45,XY,-7 in bone marrow.

LaGrave speculates that the patient in her study may be a case of monosomy rescue in which the embryo was conceived 45,XY,-7 but soon thereafter a duplication occurred that created a disomic cell line—monosomy rescue—resulting in a viable mosaic.

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*—Danielle LaGrave,
MS, CGC*

Additional testing is being undertaken to determine whether the mosaic monosomy 7 seen in this child is more likely because of a pre-malignant change or monosomy rescue.

Monosomy rescue can allow both cell lines to survive, but selection favors the survival of the disomic cells in most tissues. LaGrave and her colleagues theorized that in such a case, the persistence of the monosomic cell line would cause congenital anomalies that might vary in severity depending on the timing of the monosomy rescue and the level of mosaicism in different tissues at critical points of development.

LaGrave said the ambiguous genitalia in this male infant might be secondary to fetal adrenal insufficiency rather than a primary defect.

The infant died at 5 months of age after he became hyponatremic and unresponsive to treatment. The parents declined postmortem examination.

Providing Information For Care: 1q42q44 Microdeletions

Blake Ballif, PhD, of Signature Genomics presented research that used array CGH to explain why children with microdeletions of 1q42q44 present with different phenotypes.

Individuals with microdeletions of 1q42q44 typically present with mental retardation, seizures, and numerous congenital anomalies, including microcephaly and agenesis of the corpus callosum.

However, the severity of their problems can vary. Some children, for example, present with seizures and microcephaly, others have microcephaly but no seizures, and still others seizures but no microcephaly.

Previous studies have implicated haploinsufficiency of the gene *AKT3*, one of the three closely related isoforms of the protein kinase B (PKB/Akt), as the cause of microcephaly and/or agenesis of the corpus callosum in individuals with microdeletions of 1q42q44.

But the region responsible for seizures in this group of individuals has not been described.

Using microarray testing, Ballif and his colleagues at Signature Genomics identified more than one hundred individuals with abnormalities of 1q44.

Of these, they characterized 39 by high-resolution oligonucleotide array CGH.

They then correlated the duplication and deletion sizes with clinical presentation.

They identified microdeletions less than 400kb that were contained within or overlapped *AKT3* and that were associated with microcephaly but not seizures.

In one case, this microdeletion was inherited from a mother with microcephaly.

“This finding implicates *AKT3* as the most likely candidate gene for the microcephaly phenotype in individuals with deletions of 1q44,” Ballif said.

The team also defined another region that appears to be critical for the seizure phenotype, an approximately 500kb deletion that contains or partially overlaps six candidate genes.

What we want to do is provide more meaning and understanding; not just say you have this gain or this loss, but also provide information that helps you understand the clinical significance of that abnormality.

—Blake Ballif, PhD

Ballif says the findings are an example of how array CGH is making it possible to understand genotype-phenotype correlations better.

“These data suggest that if your patient has a deletion of one of these specific intervals that it is more likely that they will have seizures or microcephaly,” he said.

The key to unraveling the genetics behind these disorders, however, will continue to be collaboration between the clinicians and the cytogeneticists, Ballif added.

“We have to work hand-in-hand with the doctors who can provide more detail about the patients. This type of work really can’t go forward without the collaboration of multiple institutions, physicians, and laboratories working together,” he said.

Challenging Assumptions: 18q Subtelomere Deletion

Sarah T. South, PhD, assistant professor in the Departments of Pediatrics and Pathology at the University of Utah School of Medicine, described a case that challenges the common clinical assumption that deletions remain stable from generation to generation.

The case involved a 3 ½-year-old female with hypomyelination, ataxia, anal stenosis, a history of growth retardation, and mild developmental delay.

The family history was significant for a mother who had anal stenosis that required rectal dilation as a child, two previous miscarriages, and a nephew with cleft lip and palate. The girl’s three siblings were phenotypically normal.

A lengthy battery of tests had been done, including: fluorescence *in situ* hybridization (FISH) for 22q11.2, FISH studies for Xq22 (Pelizaeus-Merzbacher disease); and studies looking at serum amino and urine organic acids, biotinidase activity, lysosomal enzymes, very long chain fatty acids, screening for mitochondrial disorders, and congenital disorders of glycosylation. All proved normal.

A standard chromosome analysis, however, identified a terminal deletion of the long arm of chromosome 18, which was confirmed by a subtelomeric FISH probe.

The parental chromosomes appeared normal, but the mother was found to have an 18q subtelomere deletion by FISH. Array CGH of the child and the mother showed a nearly 10-fold expansion of the terminal deletion from mother to daughter.

Neither the child nor the mother had evidence of mosaicism, leading South and her colleagues to hypothesize that the expansion of the deletion occurred in the maternal germ line.

The findings, South said, challenge a number of assumptions. One such assumption is that when parents are phenotypically normal, pure terminal deletions in the child are likely *de novo* and parental studies are not needed. Clearly prenatal testing can be helpful.

The findings also contradict the assumption that deletions remain stable in size through generations and, therefore, family studies can use a marker from within the abnormality, such as

We’re going to learn so much more about how chromosomes behave with this technology. So we have to recognize what our assumptions are and be ready to accept that some of those assumptions are wrong.

—Sarah South, PhD

FISH. Such markers, the findings show, may either underestimate the size or fail to identify a parental deletion as the result of the expansion of the deletion.

And finally, the findings suggest that differences in phenotype between a parent and an offspring may not be caused by such factors as differences in environment or genetic background but because of changes in genetic content caused by the expansion of deletions, South said.

“What this case demonstrated was that the assumption that the deletion size is stable is not true in all cases, and because we’ve been making this assumption for so many years, we don’t know how many cases of this phenomenon we’ve missed,” South said. “So we may have a very rare case report or we may have something that is a little bit more broadly applicable. But until we recognize expansions of deletions are possible, we won’t design studies to identify them.”

South said the findings suggest that clinicians and researchers should be doing more extensive parental array CGH studies in appropriate cases because there may be many phenomena that challenge our assumptions of what is true.

“We’re going to learn so much more about how chromosomes behave with this technology. So we have to recognize what our assumptions are and be ready to accept that some of those assumptions are wrong,” she said.

Overcoming Technical Limitations: Mosaic Lineage-Specific Ring 7

A number of presentations described the use of array CGH to detect and characterize mosaicisms.

Andrea K. Bailes, MS, from Nationwide Children’s Hospital in Columbus, Ohio presented the case of an adopted 15-year-old Caucasian female with learning disabilities, small size, dysmorphic features, and a cleft palate. Previous G-banded lymphocyte chromosome analysis and DiGeorge FISH analysis were found to be normal.

Bailes and her colleagues used the SignatureSelect V2.0 BAC microarray, which revealed the presence of a mosaic loss of the chromosome 7p and 7q termini.

However, FISH analysis using 7p/7q subtelomeric probes on phytohemagglutinin-stimulated, cultured metaphase cells yielded normal results.

Interphase 7p/7q subtelomeric FISH analysis performed on an unstimulated, cultured residual peripheral blood sample was consistent with a loss of both termini of chromosome 7 in 29% of cells.

FISH analysis of a buccal smear using 7p/7q subtelomeric probes demonstrated a loss of both terminals of chromosome 7 in 9% of cells.

Bailes hypothesized that the abnormal chromosome 7 most likely is a ring chromosome. The abnormal chromosome 7 appears to be limited to the myeloid lineages of the blood and absent in T-lymphocytes. However, other tissues not tested may also contain the abnormal chromosome.

Bailes noted that though patients with mosaic ring chromosome 7 have not been reported, rare cases

Microarray analysis may continue to identify additional cases of lineage-specific chromosome abnormalities.

—Andrea K. Bailes, MS

of non-mosaic ring chromosome 7 are found in the literature.

These non-mosaic cases have been associated with growth and mental retardation and abnormal skin nevi.

All the reported cases, however, were detected with standard chromosome analysis, suggesting the abnormality was also present in T-lymphocytes.

Bailes and her colleagues speculate that the number of patients with lineage-specific mosaicism may be under-reported because of the technical limitation of standard cytogenetic analysis, which is often limited to the lymphoid lineage. Also, while BAC microarray analysis is thought to detect mosaicism at the 20-30% level, very low-level mosaicism may be missed.

Understanding Cancer: Myelodysplastic Syndromes

Array CGH is proving increasingly valuable in the evaluation of malignancies. The identification of novel cytogenetic aberrations may lead to cancer treatments tailored to the genotype of the tumor—treatments that will be more effective and have fewer side effects.

Myelodysplastic syndromes (MDS) are a heterogeneous group of myeloid neoplasms that lead to bone marrow failure.

More than 10,000 new cases are diagnosed in the U.S. each year. Patients generally develop peripheral blood pancytopenia. But 25% will transform to acute myelogenous leukemia (AML).

“Most people with MDS don’t transform to AML, so why does that happen? What’s going on? Why is there this genetic instability in this group?” Marilyn Slovak, PhD, said in an interview after her presentation.

Currently, a patient’s prognosis is based on the International Prognostic Scoring System, a morphological classification based on the percentage of bone marrow blasts, the presence of peripheral cytopenia and the karyotype.

However, only 50% of MDS patients show recurring cytogenetic aberrations.

In this study, Slovak working with her colleagues at the City of Hope in Duarte, California and Signature Genomics, set out to see if high-resolution array CGH would be able to detect hidden clonal aberrations in patients with a diagnosis of MDS.

In particular, the team sought to see if array CGH could detect aberrations that could predict either overall disease prognosis or AML transformation. They also compared the array CGH analysis with conventional cytogenetic analysis and FISH.

“Understanding the genetics underlying the disease is going to make it easier for the clinicians to provide patients with better treatment options and might reveal ways to block or stop the progression of the disease,” Slovak said.

In their study, Slovak and colleagues analyzed bone marrow samples from 30 patients diagnosed with MDS. The group included all subtypes of MDS and therapy-related myeloid neoplasms or t-MN.

Understanding the genetics underlying the disease is going to make it easier for the clinicians to provide patients with better treatment options and might reveal ways to block or stop the progression of the disease.

—Marilyn Slovak, PhD

Their bone marrow blast count was less than 30% (range 0-30%, median, 8%).

Array CGH identified a number of recurring submicroscopic imbalances in these patients.

“It turned out to be a whole lot more complex than we ever thought it would be,” Slovak told the conference.

In these patients, array CGH identified submicroscopic imbalances including deletions of *RUNX1*, 3q26.2/*EVI1*, 5q32/*TCERG1*, 12p13.1/*EMP1*, 17q11.2/*NF1* and amplifications of 12p and 19p13.3.

These and other findings of the study suggest that a microarray designed to detect the genomic regions implicated in myeloid disorders has the potential to “improve clinical assessment, prognosis and possibly therapy decisions for patients with MDS,” Slovak concluded.

Conclusion

Two strong themes emerged from this year’s Signature Genomics Scientific Microarray Conference.

First, array CGH is revolutionizing our understanding of the human genome and the practice of clinical cytogenetics. With its speed and ease of use, array CGH is bringing to the clinic the ability to analyze and interpret human chromosomes with a resolution that was once available only in the research setting. This ability coupled with the clinical experience of physicians and genetic counselors working with patients and their families is leading to new discoveries and deepening our understanding of cytogenetics.

Second, to make the most of this new technology, collaboration between researchers and clinicians is essential. Researchers can identify lesions, but lesions are meaningless without an understanding of their clinical impact, an understanding that only clinicians working with their patients and their families can provide.

At this year’s Signature Genomics Scientific Microarray Conference, we saw how well such collaboration can work when researchers and clinicians come together to join the science of cytogenetics with clinical practice.

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For more information about this conference, please contact Signature Genomics at 877.SigChip (744-2447) or ssmc@signaturegenomics.com.

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