Cleveland Clinic
Robert J. Tomsich Pathology & Laboratory Medicine Institute

Clinical and Translational

Pathology Innovations

A Publication of the Robert J. Tomsich Pathology & Laboratory Medicine Institute | Fall 2013

Feature Stories:

Also in This Issue:

Strategies for Appropriate Test Utilization: The Right Test for the Right Patient at the Right Time

By Gary W. Procop, MD, MS

There is a great opportunity for pathologists and other laboratorians to take a leadership role in decreasing unnecessary phlebotomy and potentially iatrogenic anemia, increasing patient satisfaction and decreasing overall costs by developing and implementing strategies to reduce inappropriate laboratory testing.

Unnecessary testing presents patient satisfaction and safety issues. The more tests performed, the greater the potential for error. From a patient satisfaction standpoint, it stands to reason that fewer phlebotomies would be associated with greater satisfaction. Finally, overutilization of laboratory testing creates unnecessary financial burdens for hospitals, patients and third-party payers in this ever-tightening era of healthcare reform.

Addressing this issue at Cleveland Clinic was a substantial challenge, given the sheer size of this tertiary care medical center, the volume of laboratory testing and the complexity of our patient population. The keys to our success are summarized below.

Keys to Our Success

- A multidisciplinary Test Utilization Committee, with individuals representing many areas of the organization.
- An open, transparent and collaborative process.
- Team members focused on optimal patient care, improving the patient experience, decreasing phlebotomy and reducing costs.
- Participants are more interested in improving patient care than reducing costs.
- Collaborative meeting with mutual respect, acceptance, and healthy and collegial debate and innovation.
- Rational, evidence-based initiatives.
- Good project management with regular results reporting with shared success.
- Leadership support.
- Top down support with bottom up teambuilding.
- Inclusion of high-level partners from information technology.
- The ability of IT to rapidly respond to change requests.
- ‘Pre-selling’ initiatives with the opportunity for feedback.
- Anyone affected by a decision should be involved in the decision.
- A willingness to learn and change.
- Recognizing you don’t have to win every battle to win the war.

Our efforts are led by the Test Utilization Committee, whose members truly adhere to Cleveland Clinic’s “Patients First” principle. We would never compromise the quality of care for cost savings. However, we recognized that there was substantial waste in the system and that better utilization of these resources could also contribute to enhanced patient care by bettering the system as a whole.

The Same Day Duplicate Test Reduction Initiative

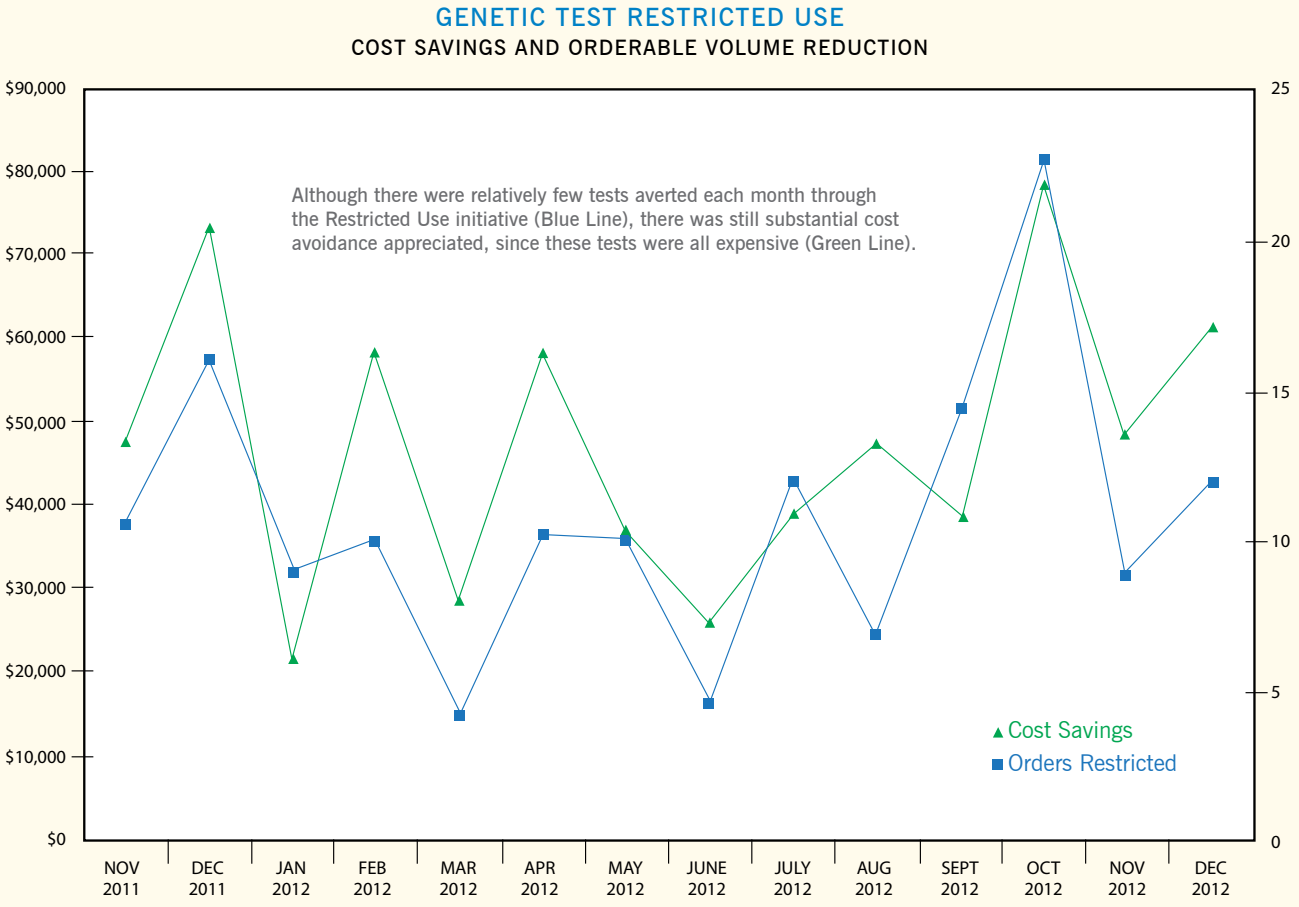
The advent of CPOE systems allows for the opportunity to interact with physicians at the point-of-test entry, so as to assist with optimal ordering. Such methods can be used to guide physicians to the correct test, when the test selection is complex and it can be used to notify the physicians of duplicate test orders. Our initial foray into the use of best practice alerts (BPA) had mixed results. The BPA designed was essentially a “pop-up” window that notified the physician that the test they were trying to order had already been ordered that day. These studies provided evidence that a hard stop option should be explored to eliminate or drastically reduce unnecessary, duplicate testing.

The Test Utilization Committee first identified 10 tests that were deemed never to be needed more than once per day. Although we were allowed to initiate a full electronic stop on these duplicate orders, we were also required to build an alternative avenue for ordering, in the event the attending physician absolutely wanted the repeat test. There were no provider complaints associated with this initiative, so we progressively activated the hard stop clinical decision support tool (CDST) for all tests that the Test Utilization Committee deemed to be appropriate.

In its first full year of implementation, the use of this CDST resulted in the discontinuation of 7,243 unnecessary duplicate orders. The total laboratory cost avoidance (i.e. materials plus labor) was $115,590. Costs associated with providers either performing phlebotomies (i.e. nurse draws) or responding to duplicate test results was not captured, but may be equally significant. This initiative is considered a success, since it is thought to have improved patient care and satisfaction by decreasing unnecessary phlebotomies and decreased costs.

Restricting the Ordering of Genetic Test

Genetic testing has become extremely complex and costly. We were concerned with test ordering patterns, since there were very few individuals who could adequately interpret these tests, yet any intern or resident could order the assay. Therefore, we undertook our second major initiative, which was limiting the individuals who could order complex molecular genetic tests, some of which cost thousands of dollars. We offered “deemed status” to physicians who were knowledgeable about the diseases for which the tests were designed. These individuals could order molecular genetic tests on an outpatient basis, whereas inpatient testing required a consultation with Medical Genetics. This initiative resulted in an annual cost avoidance of $248,923.



A Laboratory-Based Genetics Counselor

We employed a genetics counselor who works diligently on pre-analytic and post-analytic testing issues. She is available for consultations to assist providers in the selection of the appropriate test, screens molecular genetic send-out tests for appropriateness, and interfaces with clinicians when needed. She intervened in 158 test orders in fiscal year 2012-13 that resulted in a cost avoidance of $456,431.

The Future

We are currently assessing the impact of other implemented initiatives, such as Smart Alerts for the regional hospitals and Expensive Test Notifications, both of which are performing well. We are building an “extended hard stop” CDST, which will extend the time period during which a duplicate test will not be allowed. These and other projects will occupy the time of the Test Utilization Committee at Cleveland Clinic in the near future, as we continually strive to improve patient care and prepare for the challenges of health care reform.

Conclusion

The Test Utilization Committee has raised the bar in asking for a quality assessment, “is this test really needed?” Most importantly, we believe we have improved the patient experience, decreased unnecessary phlebotomy for the commonly used tests, improved the use of molecular genetic tests, and decreased healthcare costs. The total annual savings for these initiatives in fiscal year 2012/2013 was $1,362,940. Importantly, our initiatives never interrupted patient care and we would argue has improved it. The entire process has been an enjoyable lesson in team building and enhancing practice within the our healthcare system.

About the Author

Gary W. Procop, MD, MS, serves as Chair of the Department of Molecular Pathology in the Pathology and Laboratory Medicine Institute and Chair of Cleveland Clinic’s Test Utilization Committee.

Dr. Procop completed anatomic and clinical pathology training at Duke University Medical Center, a clinical microbiology fellowship at Mayo Clinic, a medical degree at Marshall University School of Medicine and an undergraduate degree at Eastern Michigan University. He is a Diplomate of the American Board of Pathology in anatomic and clinical pathology, and in medical microbiology, and a Fellow of the American Academy of Microbiology, the College of American Pathologists, the American Society for Clinical Pathology, the Royal Society for Tropical Medicine and Hygiene and the Infectious Disease Society of America.

Dr. Procop has given more than 350 scientific presentations and has more than 120 published manuscripts, one book and 25 chapters to his credit. His primary interests are the practical applications of molecular diagnostic methods for the diagnosis and treatment of infections, infectious disease pathology, mycology and parasitology. Contact Dr. Procop at 216.444.5879 or by email at procopg@ccf.org.

Addressing the Paradigm Shift in Genetic and Genomic Testing

By Felicitas L. Lacbawan, MD, FCAP, FACMG

"Genome: Unlocking Life's Code," the Smithsonian's National Museum of Natural History and National Institutes of Health's National Human Genome Research Institute exhibit, describes in a nutshell what laboratories like ours contribute to patient care.

The Molecular Genetics Pathology (MGP) Laboratory, a section of the Department of Molecular Pathology, is responsive to the clinical molecular diagnostic needs of genetic-based medicine at Cleveland Clinic and the community. Our laboratory strives to provide molecular tests for a full range of indications spanning inherited and genetic disorders to pharmacogenomics including preventive, diagnostic, prognostic, therapeutic or predictive drug (safety and efficacy) use. In most instances, in-house assays are developed and validated and testing methodologies are improved for clinical use.

Our efforts are threefold, we continue to develop tests that are relevant to clinical practice, assist practitioners in molecular test utilization and educate health professionals and the community about the right molecular test used in patient diagnosis, treatment and anticipatory care.

Test Development

In addition to standard-of-care tests, the MGP laboratory actively promotes translational research and seeks opportunities to collaborate with clinical and research experts in areas of interest. Tests to be in-sourced and developed are prioritized drawing from consultative meetings with clinical specialists, current test utilization patterns in the Clinic, and the commercial availability of DNA/RNA-based clinical laboratory tests. For each molecular test, considerations include clinical utility, ease of test adoption and test validation. In addition to offering tests for more common genetic disorders, the MGP laboratory recognizes the value of developing tests for select rare conditions for which testing is not widely available. Bringing molecular tests in-house not only facilitates access to laboratory experts but translates to significant cost savings from test send-outs, shorter turn-around time and potentially more timely diagnosis and treatment for reduced length of hospital stay and decreased disease morbidity.

In recent years we have witnessed the trajectory of the increasing number and complexity of genetic and genomic tests made available to clinicians and patients. Single gene testing for Mendelian disorders has evolved to gene panel testing. The debut of whole exome sequencing for diagnosis of cases with likely genetic etiology holds considerable promise. The MGP laboratory is positioned to parallel the said trajectory, albeit in a carefully planned and responsible manner. As we actively roll out a number of single gene laboratory tests (LDTs) in the coming months, next-generation (massive parallel) sequencing for multiple genes in a panel will follow. We hope to sustain growth and build capacity, as it is our goal to offer whole exome sequencing in-house in the future.

Test Utilization

The MGP laboratory promotes and supports proper utilization of nucleic acid-based tests in the molecular diagnosis of specific disorders in patients from various specialties of medicine. Ordering the right test to confirm patient diagnosis, to predict the risk, to determine the prognosis, and to optimize drug therapy is an integral part of clinical practice.

MGP has an active role in defining best clinical practice. Genetic test review is performed daily for all molecular test send-outs. Test indications are verified by the laboratory genetic counselor. For more complex tests, as in gene panels, review of the electronic medical record is done to assess the appropriateness of the test with the collaboration of the ordering clinician. Review by clinical geneticist/molecular genetic pathologist staff in the laboratory provides guidance on how best to pursue molecular diagnostic testing. Depending on the clinical presentation and the type and frequency of mutations in the gene(s)

for the disorder being considered, tiered testing may be recommended. Monthly assessment of molecular test send-outs captures a complete picture of molecular test utilization that will guide the laboratory on future test development. Moreover, MGP helped develop the system and infrastructure that integrates pharmacogenetic testing into clinical practice for the Personalized Medication Program led by the Center for Personalized Health as a new enterprise-wide initiative. MGP will continue to collaborate with Centers of Excellence or specialty clinics like the Clinic for Adult Autoinflammatory Disorders of the Orthopaedic & Rheumatologic Institute.

A team of laboratory and clinical experts aim to develop acceptable evidence-based algorithms for disease-related gene testing. Efforts to streamline and standardize test ordering and utilization *(see related story, "The Right Test," on page 2)*, and patient consenting are actively being pursued. The logistics of DNA extraction, storage, retrieval and reporting are currently in place to support future initiatives related to tier-testing and next-generation sequencing.

Education

The MGP laboratory participates in the training and education of students, residents, fellows and house staff to promote proper utilization and interpretation of molecular tests. The new ACGME-accredited Fellowship Program in Molecular Genetic Pathology was granted three-year accreditation. The inaugural fellow joined the department in July 2013. The program will be an overarching, well-rounded fellowship training that covers the fundamentals of human genetics, cytogenetics, molecular hematopathology, molecular oncology as well as molecular microbiology. It emphasizes integrative and evidence-based Molecular Genetic Pathology practice with laboratory management as an integral part of every rotation. With well-qualified faculty members who are authorities in various subspecialties of pathology and genetics, the program will ultimately graduate fellows who will assume a key role in a world-class clinical laboratory that offers molecular diagnostic testing.

As clinical laboratories employ massive parallel sequencing and variants of unknown significance (VUS) continue to mount, so is the demand for appropriately trained molecular genetics professionals. They will serve as an interface between the test performing laboratories and the clinicians in the interpretation of test results, especially given the ease of access to online information by patients. They will advance the critical role of the molecular genetic pathologist as a health team member catering to an integrated approach of care for patients.

Of the possible 25,000 genes that make up the human genome, more than half (14,361 genes) have been described in the Online Mendelian Inheritance in Man. Though only 3,888 defined phenotypes have molecular bases (as of September 10, 2013), increasing number of disorders are becoming well delineated and mutations or variants in their associated genes may have implications for diagnosis, prognosis, therapeutics/drug therapy stratification or risk assessment. There is no doubt that more genetic and genomic tests, if not whole exome or genome sequencing, will become an integral part of everyday health care.

Confounded by the limited time health providers have for each patient, mustering and mastering the different genetic conditions and tests made clinically available becomes a daunting task. Add to this the costs of molecular testing, the increasing complexity and highly technical methodology used in testing, the best definitive diagnostic test for the patient can only be realized by effective communication and close collaboration of the clinician and the laboratory. These are the best answers to the growing demands for responsible genetic testing in the belt-tightening economic realities for health care. So, when your clinic schedule is too tight and there is less time to dive deeper into the current literature on a genetic or genomic test, you can count on the MGP laboratory to help you arrive at the right test for your patient.

Informed Consent for Genetic Testing, Genetic Test Ordering and Test Result Interpretation

As we bring molecular tests in-house, we are reminded of the foremost participants in every genetic test being ordered – the patient and his or her family. As health professionals serving well-informed, internet-savvy patients, it is our responsibility to be able to provide acceptable explanations of the genetic or genomic tests we order on behalf of the patient.

The genome carries the blueprint of who we are – from the color of our eyes to the dosage of a drug we should take. The Human Genome Project has resulted in an explosion in the number of complex, DNA-based tests. The fast-paced discovery of disease-causing mutations in genes is allowing us to catch up with new medical knowledge. With the many specialists that one visits in order to arrive at a diagnosis, each specialist may order genetic tests relevant to a patient's clinical manifestation before referring the patient to a clinical geneticist.

Perhaps you have recently ordered a genetic test. You may have come across the name of the gene in your readings or at a national meeting or conference, so you decided to check it on your patients. Before ordering the genetic test for a patient, you may want to address the following:

- Is the clinical manifestation(s) of your patient compatible with a phenotype of a specific genotype or does it mimic of the interaction of environmental factors with a normal genotype (phenocopy)?
- Do you know if there is a single gene or a number of genes associated with your patient's clinical manifestation(s) (genetic heterogeneity)?
- Is there a good genotype-to-phenotype correlation in the disorder being tested? Is the detection rate better if a panel of genes is pursued instead?
- If there are several or a number of genes involved, will tier-testing for the deleterious mutations or variants in the most common gene(s) be appropriate?

Since you are convinced that the likelihood of a positive test result is high, your have decided to order the specific genetic test. As you set out to place the order, you are asked to consent your patient for the test.

You may think a genetic informed consent for testing is just added paperwork on top of the pile of things to do in the documentation of the patient's visit. But before you question the need for a genetic consent, you need to understand the reasons why it may be important. The information contained in a genetic test report may reveal a diagnosis that may exist in the family. Results may predict future health risks for progressive genetic conditions for which there is no cure. There may be direct implications for the health of a patient's family members, including parents, siblings and children. Parents may need to be tested for confirmation. In the process, unexpected unknown biological relationships like non-paternity, parental consanguinity or undisclosed adoption status may be revealed. At this stage, it is best to clarify with the patient what the genetic test is, why it is being done, the possible outcome(s), and limitations of the test. Take this opportunity to gauge the patient's expectations of the test and address any concern that the patient may have about the genetic test.

After proper patient consent, the patient sample is sent to the laboratory for a specific genetic test. A couple weeks later, the result becomes available and your patient sees you in your clinic. You review the test results carefully and it indicates no mutation or variant was detected.

- Would you be able to explain the negative result to your patient?
- Are you familiar with the methodology the laboratory used for the test?
- Are the limitations of the test specified and do you understand them?
- Will the test detect all types of mutation or variant for the gene or the panel of genes you are interested in?
- Are there any guidance and/or recommendations provided in the test result report?
- Are there other appropriate test methodologies that are being used for the same gene or gene panel?
- Are you going to pursue another genetic test?

Before answering the last question, or at any stage in the genetic testing process, it may be best to seek collaborators. A clinical geneticist and a molecular genetic pathologist are expert resources who have the same goal as everyone in the health care team – an integrated and comprehensive diagnostic work-up to ensure value-based care for every patient.

Next time, you can ask our MGP laboratory to serve as your expert resource for a genetic test review.

About the Author

Felicitas L. Lacbawan, MD, FCAP, FACMG, is Head of the Section of Molecular Genetics Pathology, Department of Molecular Pathology, Robert J. Tomsich Pathology and Laboratory Medicine Institute, and the founding Program Director for the Molecular Genetic Pathology Fellowship at Cleveland Clinic. She serves as the medical and technical director of laboratory-developed tests for genetic disorders.

At Cleveland Clinic, she leads the section that provides molecular tests for a full range of indications spanning inherited and genetic disorders to pharmacogenomics. Her research includes clinical and molecular genetics of various neurologic and developmental disorders, including presenile dementia, brain and craniofacial malformations, chromosomal anomalies and pharmacogenomics.

She is currently a member of the College of American Pathologists Subcommittee on Biochemical and Molecular Genetics and the American College of Medical Genetics Professional and Practice Guidelines Committee, and also serves as volunteer status at the undiagnosed disorders program at the National Human Genome Research Institute at the National Institutes of Health (NHGRI-NIH). Dr. Lacbawan has previously worked at the NHGRI-NIH, Children's National Medical Center (CNMC), Georgetown University Medical Center (GUMC), and State University of New York Downstate Medical Center (SUNY-DMC) in various capacities as a clinical geneticist and a molecular genetic pathologist. She is also board-certified in clinical and anatomic pathology.

Dr. Lacbawan was a member of the faculty at the University of the Philippines College of Medicine Department of Human Biochemistry, the George Washington University School of Medicine and Health Sciences, the Georgetown University School of Medicine and the SUNY Downstate College of Medicine as a Clinical Professor. She was a recipient of Interagency Personnel Agreement between CNMC and NHGRI-NIH for five years. Dr. Lacbawan was the founding Director of the CLIA-certified molecular diagnostic laboratory at the Medical Genetics Branch, NHGRI and the NYSDOH-certified molecular pathology laboratory at SUNY-DMC.

Dr. Lacbawan received her medical degree from the University of the Philippines College of Medicine, served her residency at SUNY Upstate Medical University, and her fellowship in clinical genetics at the National Institutes of Health. Contact Dr. Lacbawan at 216.445.0761 or by email at lacbawf@ccf.org.

Whole Genome SNP Microarray Testing is Contributing to Advances in Genetics and Diagnostic Medicine

By Shashirekha Shetty, PhD

Evidence-based approaches coupled with technology advancements in the field of genetics are changing how the human genome is being analyzed and interpreted for diagnostic purposes. Chromosomal microarray analysis (CMA) is a recommended first-tier test for diagnosing unexplained intellectual disabilities, dysmorphic features, congenital anomalies and autism.[1-5] This technique compares relative fluorescent signal intensities of two different genomes, a test and a reference, that compete for hybridization to DNA sequences representing the whole human genome in order to detect a gain or loss of genetic material. However, CMA does not detect allelic imbalances resulting in absence of heterozygosity (AOH).

Advances in the field of genetics and diagnostic medicine have resulted in refinement of the existing CMA platform by incorporating single nucleotide polymorphism (SNP) markers to the existing non-polymorphic markers used to detect gains and losses of DNA. The analysis of SNP data provides information about the allelic imbalances associated with AOH. Identifying regions of excessive homozygosity on a single chromosome could suggest uniparental disomy (UPD), which may warrant further clinical and laboratory investigation when observed on chromosomes with known imprinting disorders associated with UPD. In addition, the detection of excessive homozygosity on multiple chromosomes may suggest consanguinity and, therefore, could be useful in determining candidate genes for further testing for autosomal recessive disorders.

At Cleveland Clinic Laboratories we use Agilent technology comprised of 107,000 oligonucleotide 60mer probes spaced one probe every 40kb across the backbone of the array and one probe every 10kb in targeted clinically significant regions in the genome. For SNP analysis, the array features 60,000 SNP 60mer probes with an effective resolution down to 10Mb. Data for the SNP markers are displayed to demonstrate either homozygosity (AA or BB) or heterozygosity (AB) at every SNP locus. AOH is indicative of hemizygosity if the corresponding CMA data shows a deletion of the region. If the CMA data is normal, AOH signifies homozygosity.

Many abnormal phenotypes in the pediatric population are associated with chromosomal imbalances that can be identified using CMA to detect copy number change (CNC). Thus, whole-genome CMA has replaced chromosome and FISH studies to become the first-tier test for the evaluation of children with unexplained developmental disabilities, intellectual disabilities, dysmorphic features, congenital anomalies and autism. Based on numerous published studies, the yield of pathogenic or clinically significant CNC by CMA is approximately 15-20% in a pediatric population, compared with a yield of 3-5% by standard cytogenetic analysis in the same population. Variants of uncertain clinical significance (VOUS), or clinical significance unknown, are found in less than 10% and could play an important role in the clinical diagnosis. To a great extent, parental and family studies can be helpful in the clinical interpretation of these unknowns, as de novo occurrence of the CNC is more likely related to a pathogenic event.

CMA testing for CNC is recommended as a first-line test in the initial postnatal evaluation of individuals with the following:

- multiple anomalies not specific to a well-delineated genetic syndrome
- apparently nonsyndromic DD/ID
- autism spectrum disorders

This is not a recommended test for adult patients with multiple miscarriages or pregnancy losses and no abnormal phenotype. Chromosome analysis is a better test to exclude possibility of balanced rearrangements that cause pregnancy losses.

Appropriate follow-up is recommended in cases of chromosome imbalance identified by CMA and may include cytogenetic/FISH or other molecular studies of the patient or parents, and clinical genetics consultation.

In addition to detecting CNC, the SNP microarray can also be used to diagnose suspected uniparental disomy (UPD) or imprinting disorder, possible absence of heterozygosity to determine the degree of relatedness by identity-by-descent (autozygosity) and autosomal recessive condition risks.

Additional testing may be necessary to confirm disorders associated with absence of heterozygosity. SNP testing may be used to identify:

- a chromosomal abnormality or micro-duplication/deletion syndrome with a normal karyotype
- the size of a duplication/deletion involved in an unbalanced translocation
- triploidy
- cryptic duplications/deletions in a phenotypically abnormal individual with an apparently balanced karyotype.
- uniparental disomy (UPD)
- absence of heterozygosity to determine the degree of relatedness by identity-by-descent (autozygosity)

The SNP test involves DNA extraction, restriction enzyme digestion, labeling, purification, hybridization, washing, array scanning, analysis and interpretation. DNA extracted from the patient's peripheral blood is digested, labeled and hybridized to the microarray. Following hybridization, the microarray is scanned, and the signal intensities are collected and then compared to a reference in order to determine copy number changes and absence of heterozygosity.

Whole genome SNP microarray testing at Cleveland Clinic utilizes the GGXChip + SNP v1.0 platform, which contains non-polymorphic and polymorphic probes to detect both copy number changes (CNC) and allelic imbalances (SNP probes) within the same array. For CNC analysis, the array is comprised of 107,000 oligonucleotide 60mer probes spaced one probe every 40kb across the backbone of the array and one probe every 10kb in targeted clinically significant regions in the genome. For SNP analysis, the array features 60,000 SNP 60mer probes with an effective resolution down to 10Mb. In the backbone regions the resolution for copy number detection is approximately 120kb and in the targeted regions it is approximately 30kb. In our validation the resolution for AOH was detected at approximately 1.5Mb, but resolution is dependent on SNP probe coverage. For clinical purposes, AOH greater than 10Mb will be reported; however, in chromosomes associated with imprinting disorders,smaller changes will be evaluated further and may be reported.

For a complete list of clinically recognized regions of the genome and imprinted chromosomes please visit www.signaturegenomics.com.[6] There are some limitations to this platform. For example, it is not optimized to detect low-level mosaicism and uniparental disomy of the heterodisomy type. It will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions and balanced insertions), point mutations or imbalances of regions not represented on the microarray.

Interpretation

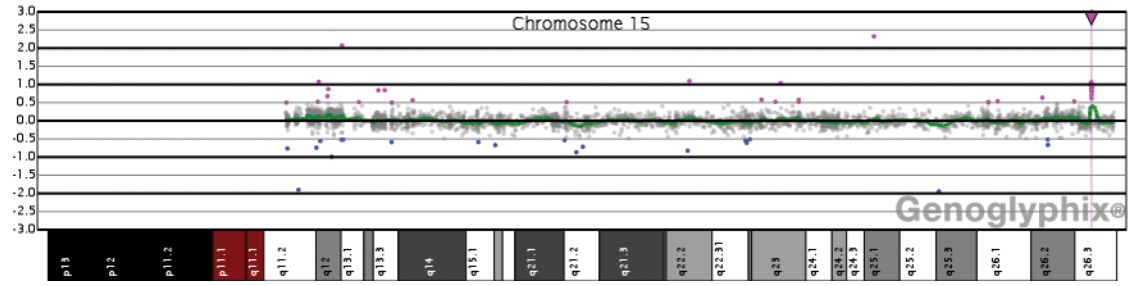
Gains and losses are reported based on genomic content in line with ACMG guidelines for microarray interpretation. Copy number variations (CNV) or CNC devoid of relevant gene content or reported as common findings in the general population may not be reported. A copy number change of uncertain clinical significance may be detected and will be reported per ACMG guidelines in one of three subcategories: uncertain clinical significance, likely pathogenic; uncertain clinical significance, likely benign; or uncertain clinical significance, no classification.

While most copy number changes observed by chromosomal microarray testing can readily be characterized as pathogenic or benign, there are limited data available to support definitive classification of a subset into either of these categories. In these situations, a number of considerations are taken into account to help interpret results including the size and gene content of the imbalance, whether the change is a deletion or duplication, the inheritance pattern, and the clinical and/or developmental history of a transmitting parent. The continual discovery of novel copy number variations and published clinical reports means that the interpretation of any given copy number change may evolve with increased scientific understanding.

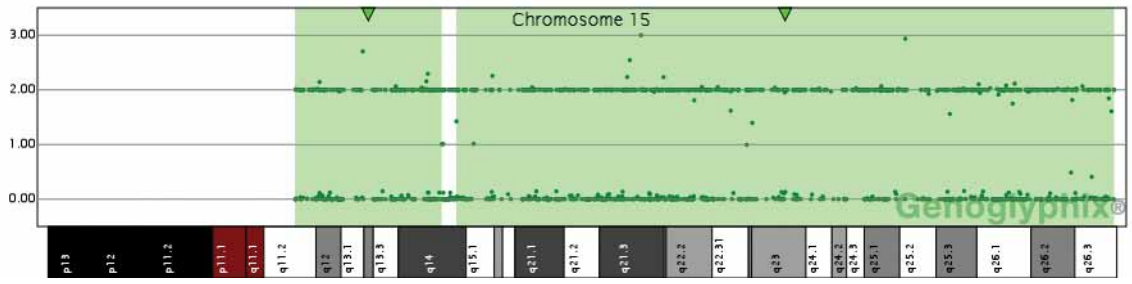
The detection of excessive homozygosity may suggest the need for additional laboratory testing to confirm uniparental disomy or to test for mutations in genes associated with autosomal recessive disorders consistent with the patient's clinical presentation that are present in regions of homozygosity.

Figure 1. Uniparental disomy 15

A: CMA Data, Agilent Array



B: SNP Data, Agilent Array

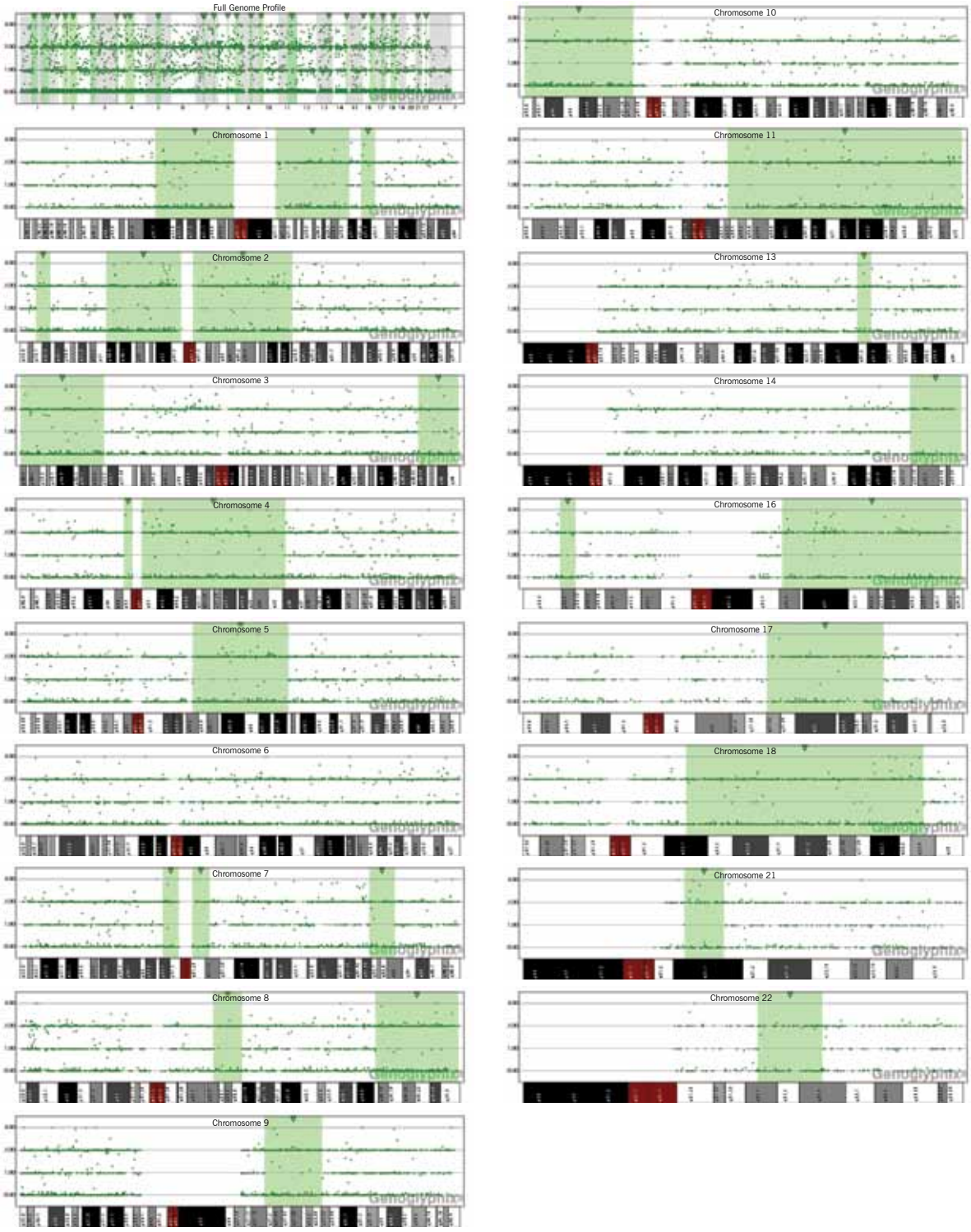


CGH showed normal copy number (1a); however AOH was noted for chromosome 15 (UPD15) (1b) and was confirmed by methylation studies. This patient has been diagnosed with Angelman syndrome.

Uniparental disomy: Uniparental disomy (UPD), the presence of two copies of a chromosome or chromosomal region from a single parent, is clinically relevant when it involves loci that undergo genomic imprinting. There are two types of UPD: isodisomy, in which the two parental copies are identical; and heterodisomy, in which the parental copies are derived from both parental homologs and are therefore not identical. SNP microarrays will detect isodisomy but not heterodisomy, since only isodisomy or segmental isodisomy results in homozygosity. UPD is the most likely explanation for an absence of heterozygosity that is restricted to a single chromosome, especially if the region is very large. Several chromosome regions are imprinted and lead to an abnormal phenotype in the presence of UPD derived from a particular parent *(Figure 1)*. UPD in other regions of the genome, even if covering a large region, is generally not considered to be a pathogenic finding in and of itself (see recessive disease discussion below).

Identity by Descent (IBD) and Consanguinity: The observation of multiple AOH regions, also known as long continuous stretches of homozygosity (LCSH), present on multiple chromosomes is generally assumed to reflect inheritance of these regions by descent from a common ancestor. This type of homozygosity is referred to as "identity by descent." A single or a few small isolated stretches can be the result of a founder effect in an isolated population. Presence of especially long stretches on multiple chromosomes suggests the possibility of a more direct biological relationship between the parents (i.e., parental consanguinity) *(Figure 2)*. A consanguineous relationship refers to the sharing of a common ancestor and the term consanguinity is generally used when individuals are second cousins or closer. However, there are other explanations for a relatively high level of identity by descent. For example, a high overall level of homozygosity can result from unusual recombination or segregation patterns during meiosis. It may also be observed for a distantly related couple who have multiple common ancestors. The latter circumstance may occur, for example, in individuals from an isolated population that arose recently from a small founding group or in populations where cousin marriages are common. For the above reasons, the SNP data themselves are not diagnostic of a specific degree of parental relatedness.[7-8] Therefore, SNP data must be interpreted in the context of additional family and social history information and the clinician must determine whether it is appropriate to pursue the question of parental consanguinity for individual families.

Figure 2. Parental Consanguinity



Multiple AOH regions were identified, consistent with parental relatedness OR identity by descent.

Autosomal recessive disease risk: Regardless of whether AOH results from uniparental disomy (UPD) or identity by descent, homozygosity anywhere in the genome raises the possibility of recessive conditions. Our reports alert the physician to the increased possibility of these conditions for regions of homozygosity greater than 10Mb. The referring physician can use this information in conjunction with clinical features and family history to determine whether mutation testing of individual genes is warranted.

Triploidy: Triploidy can be seen prenatally and appears at an appreciable frequency in miscarriages, but it is extremely rare postnatally. The three types of triploidy are 69,XXX; 69,XXY; and 69,XYY. With aCGH, 69,XXY and 69,XYY triploidy can be detected, but not 69,XXX. The SNP Microarray allows for the detection of all types of triploidy due to the capability of detecting four genotypes (AAA, AAB, ABB, and BBB) rather than the normal three genotypes (AA, AB, and BB).

Genetic Counseling: A referral to a clinical genetics professional is often appropriate for individuals and families undergoing whole genome microarray testing. This may be valuable both before and after testing. Families should be aware of the possibility of a result of uncertain clinical significance and the need for parental blood samples to help interpret the change. Families should also understand that findings of AOH may require additional testing of the proband before a diagnosis can be made. Clinical geneticists can guide testing strategies and further evaluate the patient in light of the test results. In some cases it may be important to discuss the potential for discovery of parental consanguinity. Genetic counseling can also elicit a thorough family and social history, which can be critical in the interpretation of the SNP array results.

The failure to detect evidence of uniparental disomy does not exclude the clinical diagnosis of an imprinting-associated disorder. UPD may be of the heterodisomy type, which is not detected by the array, and mechanisms other than UPD can cause the disorder.[9] Similar to the copy number-only CMA, the CMA copy number + SNP cannot detect balanced rearrangements and may not be capable of detecting low-level mosaicism. It also does not detect point mutations, small deletions or insertions below the resolution of the assay, or other types of mutations such as epigenetic changes. Finally, test results are sometimes of uncertain clinical significance, and studies of additional family members may be required to assist with interpretation.

References

1. Miller DT *et al*. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am J Hum Genet*. 2010;86:749-764.
2. Shaffer LG *et al*. Working Group of the Laboratory Quality Assurance Committee of the American College of Medical Genetics. *Genetics in Medicine*. 2007;9:654-662.
3. Kearney HM *et al*. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genetics in Medicine*. 2011;13(7);680-52.
4. Manning M *et al*. Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genetics in Medicine*. 2010;12:742-745.
5. Stavropoulos J and Shago M. CCMG guidelines for genomic microarray testing. Approved by the board of directors, Canadian College of Medical Geneticists. 2010.
6. SignatureChip OS Disorders Tested. Clinically recognized regions of the genome assayed by the SignatureChip OS(v4)/ Signature PrenatalChip OS(v4). Signature Genomicsfrom PerkinElmer, Spokane, WA 99207 USA. (www.signaturegenomics.com).
7. Kearney HM *et al*. Diagnostic implications of excessive homozygosity detected by SNP-based microarrays: consanguinity, uniparental disomy, and recessive singlegene mutations. *Clin Lab Med*. 2011;31(4):595-613.
8. Papenhausen P *et al*. UPD detection using homozygosity profiling with a SNP genotyping microarray. *Am J Med Genet A*. 2011;155A(4):757-68. ACMG guidelines.
9. Tucker, T *et al*. Uniparental disomy: can SNP array data be used for diagnosis? *Genetics in Medicine*. 2012;14:753-756.

About the Author

Shashirekha Shetty, PhD, was appointed to the Department of Molecular Pathology in 2011 and serves as Section Head of Molecular Cytogenetics and Technical Director of the Microarray Core.

Before coming to Cleveland Clinic, Dr. Shetty was Medical Director at ARUP Laboratories in Salt Lake City, Utah. She previously served as Assistant Director of the Cytogenetics laboratory at Alberta Health Services in Alberta, Canada; and Cytogenetics Consultant for Strollery Children's Hospital, Capital Health, in Alberta.

Dr. Shetty earned her graduate degree and her doctorate, both in microbiology, from the University of Mumbai, Lokmanya Tilak Medical College in Mumbai, India. She has co-authored nearly 100 articles on genetic evaluation of disease states. She completed her advanced training in clinical cytogenetics at the Alberta Children's Hospital in Calgary, Alberta, Canada.

Dr. Shetty is board-certified in clinical cytogenetics by both American and Canadian colleges. Contact Dr. Shetty at 216.636.5844 or by email at shettys@ccf.org.

Genetic Counselor Available for Guidance

Jacquelyn D. Riley, MS, LCGC, our Laboratory Genetic Counselor in the Pathology and Laboratory Medicine Institute, is a key member of our expert team to ensure that every patient received an integrated and comprehensive diagnostic work-up and value-based care. She collaborates with laboratory experts and clinicians in appropriate test ordering and utilization. She partners with the clinical and laboratory genetics staff in the development of policies and procedures with regard to genetic testing and referrals for clinical genetic consultation and provides genetic counseling education to laboratory professionals. Riley can be reached at rileyj2@ccf.org or through Client Services at 800.628.6816.

New Staff

Sanjay Mukhopadhyay, MD
Board Certifications: Anatomic Pathology, Clinical Pathology
Specialty Interest: Pulmonary Pathology
Phone: 216.444.6781
Email: mukhops@ccf.org

Yaxia Zhang, MD, PhD
Board Certifications: Anatomic Pathology, Clinical Pathology, Cytopathology
Specialty Interest: Cytopathology
Phone: 216.444.6781
Email: zhangy7@ccf.org

Li Yan Khor, MD
Board Certifications: Anatomic Pathology, Clinical Pathology
Specialty Interest: Cytopathology
Phone: 216.444.6781
Email: khorl@ccf.org

M. Qasim Ansari, MD
Board Certifications: Anatomic Pathology, Clinical Pathology, Immunopathology, Hematology
Specialty Interests: Autoimmune diseases, Immunology
Phone: 216.445.2056
Email: ansarim@ccf.org

Megan Nakashima, MD
Board Certifications: Anatomic Pathology, Clinical Pathology
Specialty Interests: Hematopathology, Hemostasis
Phone: 216.636.0186
Email: nakashm@ccf.org

Yu-Wei Cheng, PhD
Specialty Interests: Molecular Genetics, Cancer Genetics, Epigenetics
Phone: 216.445.0757
Email: chengy@ccf.org

Kaplan to Receive Travel Scholarship from American Cytopathology Foundation

Erica Kaplan, a cytotechnologist in Clinical Pathology, was chosen to receive a Travel Scholarship from the American Cytopathology Foundation for its 2014 Annual Scientific Meeting in Dallas. Kaplan was chosen for her achievements during her brief career that demonstrate initiative, leadership, commitment, and a keen interest and love for cytopathology. A formal announcement of scholarship recipients will be made in November during the 61st ASC Annual Scientific Meeting in Orlando.

Pathology Innovations Magazine offers information from the medical staff in the Cleveland Clinic's Robert J. Tomsich Pathology & Laboratory Medicine Institute about its research, services and laboratory technology.

Raymond Tubbs, DO
Medical Editor
216.444.2844

Editoral Board:
Thomas Bauer, MD, PhD
James Cook, MD, PhD
John Goldblum, MD
Eric Hsi, MD
Lisa Yerian, MD

Kathy Leonhardt, *Director Marketing and Communications*

Gary Weiland, *Editor*
Ruth Clark, *Designer*
Yu Kwan Lee, *Photographer*

Dr. Bosler to Speak at Lab Leaders' Summit 2013

David Bosler, MD, Head of Cleveland Clinic Laboratories, will speak on future challenges for the laboratory at "Reimbursement Blitz: Building an Action Plan for 2014," the Lab Leaders' Summit 2013 sponsored by G2 Intelligence on Monday, December 9, at the Union League Club of New York.

Dr. Bosler's topic, "What the Lab of the Future Must Achieve: Meeting the Demands of a Restructured Health Care Marketplace," will focus on the era of accountable care organizations (ACOs) and other emerging delivery and reimbursement models that put providers more at risk. In this environment, clinical laboratories and pathologists have the opportunity to play a major role and demonstrate added value by facilitating the integration of clinical information across the continuum of care.

With this and other factors in mind, Dr. Bosler's session focuses on the laboratory of the future, including why facilities must be designed around core principles of flexible configuration and efficient workflow. He will discuss the operational requirements to improve quality, cost and flexible capacity — while also strengthening capabilities in test development and informatics.

Dr. Bosler's talk is a part of the G2 Intelligence program that will explore tactics for fighting back against drastic payment cuts, as well as strategies for coping with continued reimbursement pressures. Visit LabLeadersSummit.com for more information on this year's agenda, program objectives and speaker roster.

Agreement Expands National Presence of Cleveland Clinic Laboratories

Cleveland Clinic Laboratories will now be the main provider of specialized testing and diagnostics services for ACL Laboratories, one of the largest hospital system laboratories in the United States.

As part of the relationship, Cleveland Clinic Laboratories will offer its highly specialized expertise in esoteric testing. ACL Laboratories and their two pathology groups will also be able to confer with Cleveland Clinic Laboratories pathologists for second opinions and subspecialty consultation leading to expert diagnosis.

Laboratory tests are a vital element of diagnosis, treatment planning and monitoring for every disease from the most common to the esoteric. Cleveland Clinic Laboratories offers more than 2,400 tests and provides high-quality, state-of-the-art diagnostic services to physicians and healthcare facilities across the country.

ACL Laboratories, jointly operated by Wisconsin-based Aurora Health Care and Chicago-based Advocate Health Care, provides services to 26 hospitals, two central laboratories and more than 110 system clinic settings and patient service centers. In addition, ACL also provides laboratory services to more than 5,000 outreach clients.

Cleveland Clinic

The Cleveland Clinic Foundation
Pathology Innovations Magazine

9500 Euclid Avenue / LL2-1
Cleveland, OH 44195