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Diagnostic Testing & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

Issue 10-14/October 2014

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With Rapid Expansion of NIPT Use Come Calls for More Informed Testing Choice

The rapid uptake in noninvasive prenatal testing (NIPT) is unprecedented in molecular testing and represents an early success story for migrating sequencing-based testing into the clinical setting. As the number of women of advanced maternal age continues to increase, NIPT for cell-free fetal DNA has emerged as an increasingly preferred screening tool for the detection of aneuploidy in these high-risk patients.

Now that NIPT is gaining traction, data is emerging on the tests' performance in actual clinical practice as well as some concerns regarding the need for improved counseling prior to testing.

As might be expected, a review of the University of North Carolina (Chapel Hill; UNC) Prenatal Diagnosis unit's initial experience with NIPT showed an increase in NIPT uptake accompanied by a significant decline in amniocentesis procedures, according to a case study published in the September issue of

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How Molecular Information Is Changing Diagnosis

As clinicians work to discover effective means to incorporate molecular information into clinical diagnoses, recent publications show that several distinct models are beginning to emerge. At the center of these efforts are innovative ways to better integrate complex molecular data with phenotypical presentation during the diagnostic process.

DTET examines two models—one in oncology and one in genetics—that provide insight into how some institutions are working to accelerate the effective transition of sequencing-based testing into clinical care. In the first case, Moores Cancer Center (La Jolla, Calif.) describes its experience of establishing a molecular tumor board that incorporates the expertise of basic scientists and bioinformaticians into the clinical decision process. In the second case, German-based researchers have developed a software algorithm that can integrate targeted exome sequencing results with a structured set of phenotypic descriptors in order to increase the efficiency and scalability of medical genetic diagnoses.

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▲ **How Molecular Information Is Changing Diagnosis,** *from page 1*

A molecular tumor board can provide multidisciplinary expertise that allows oncologists without advanced genomic training to incorporate molecular profiles of complex cancer care, according to a study published this summer in the *Oncologist*.

The molecular tumor board initiated at Moores included clinicians, basic scientists, geneticists, and bioinformatics/pathway scientists. Because molecular abnormalities "do not segregate by histology," the experts represented multiple cancer types. The team was initiated in December 2012 and built on the "long-standing tradition" of oncology tumor boards. One-hour molecular tumor board meetings were held every two weeks with 25 to 40 people in attendance. Discussions focused on medical history, radiological findings, and pathology, including molecular profiling results. A consensus was reached for choice of agents most tailored to the patient's specific aberrations.

Molecular test results discussed by the panel included FoundationOne (Foundation Medicine; 33 tests), ResponseDx (Response Genetics; two tests) Molecular Intelligence (Caris Life Sciences; three tests), and full-exome sequencing (Champions Oncology; one test). At the time of publication, 34 patients' cases had been presented representing breast cancer (n = 16), gastrointestinal cancers (n = 8), head and neck cancers (n = 4), and lung cancers (n = 2). Patients had received a median of three prior therapies in the metastatic setting, with prior or anticipated progression.

"The attendance of basic scientists and bioinformatics and pathway analysts [at the molecular tumor board] was crucial so that the therapeutic suggestions could be optimally informed by the results of molecular interrogation of the patients' tumors."
—Maria Schwaederle, Pharm.D.

The median total time from physician order to receipt of molecular diagnostic test results was 27 days. There was a median of four molecular aberrations (mutations, rearrangements, deletions, amplifications, or insertions) detected per patient, with a range of one to 14. A total of 74 genes were involved, with 123 distinct aberrations.

The median time between receipt of molecular diagnostic results and the molecular tumor board presentation was 24 days. Three of the 11 evaluable patients treated on the basis of their molecular diagnostic results achieved a partial response (progression-free survival) despite having previously progressed on therapy. Four of the 11 patients had stable disease, while another four had progressive disease. The authors say the difficulty of treatment management informed by molecular diagnostics is witnessed by the nine patients who could not be treated because they were ineligible for an appropriately targeted clinical trial, logistically or financially could not enroll, or had inactionable aberrations.

"This experience could be of significant importance to oncologists who are increasingly faced with advanced molecular diagnostic data, yet have minimal training in genomics," write the authors, led by Maria Schwaederle, Pharm.D. "The attendance of basic scientists and bioinformatics and pathway analysts was crucial so that the therapeutic suggestions could be optimally informed by the results of molecular interrogation of the patients' tumors."

In addition to the potential benefit of the individual patient, the molecular tumor board yielded broader benefits as well. The authors cite improved education to the treating physician and other attendees, as well improved workflow efficiency, including plans for new processes for tumor tissue acquisition and testing as a result of these meetings.

Software Integrates Genomics, Phenotypical Analysis

Integrating targeted next-generation sequencing (NGS) results into phenotype-driven bioinformatic analysis can provide quick and effective differential diagnostics in medical genetics, according to a study published Sept. 3 in *Science Translational Medicine*. The approach, the authors say, combines a structured set of phenotypic abnormalities with genetic variants to substantially improve the ranking of candidate genes, expediting clinical workflow.

A traditional medical genetics evaluation inherently relies on recognizing a characteristic pattern of phenotypical presentations to guide targeted genetic testing for confirmation of the suspected diagnosis. However, diagnosis is complicated by the vast number of diseases and clinical syndromes, which may display in poorly understood phenotypic presentations along with high genetic heterogeneity. Even with extensive workups, fewer than half of patients with suspected genetic disorders are definitively diagnosed. While whole-exome sequencing (WES) is an increasingly promising option to end these diagnostic odysseys, challenges remain with interpretation, hampering the scalability of WES in clinical diagnostic settings.

The Berlin-based researchers used variants in 2,741 of the most well-established Mendelian disease genes (the disease-associated genome [DAG]) to develop a targeted enrichment panel (7.1 Mb), which achieves a coverage of 20-fold or better for 98 percent of bases. The researchers then utilize an algorithm, the Phenotypic Interpretation of eXomes (PhenIX), that ranks variants based on pathogenicity and semantic similarity of patients' phenotype (cataloged in the Human Phenotype Ontology set that uses more than 10,000 structured terms to describe 3,991 Mendelian diseases based on phenotypic abnormalities).

"We suggest that targeting all known disease genes, that is, a disease-associated genome, rather than the whole exome or genome, is advantageous in terms of target coverage, cost per sample, and the ability to provide quick and accurate clinical interpretation of the variant."
—Tomasz Zemojtel, Dr.Sc., Charité Universitätsmedizin Berlin

The researchers found in computer simulations that relying on a variant score only ranks the true gene in first place less than 5 percent of the time, whereas PhenIX placed the correct gene in first place more than 86 percent of the time. The researchers applied PhenIX retrospectively to 52 patients with previously identified mutations (using Sanger sequencing) and known diagnoses. PhenIX achieved a mean 2.1 rank of the correct gene. In a prospective evaluation of PhenIX in 40 individuals without a diagnosis, PhenIX analysis enabled a diagnosis in 11 cases (28 percent; mean rank of 2.4).

"On the basis of our results, we suggest that targeting all known disease genes, that is, a DAG, rather than the whole exome or genome, is advantageous in terms of target coverage, cost per sample, and the ability to provide quick and accurate clinical interpretation of the variants," write the authors, led by Tomasz Zemojtel, Dr.Sc., Charité Universitätsmedizin Berlin.

Among the benefits of this approach, the authors say, is that it allows a complete workup of targeted NGS results in roughly two hours per patient with a competitive diagnostic yield.

Takeaway: Innovative methods, including molecular tumor boards and software algorithms, are being developed both in oncology and medical genetics in order to improve the efficiency and effectiveness of incorporating complex sequencing-based testing results into routine diagnosis and care.

▲ **With Rapid Expansion of NIPT Use,** *from page 1*

Genetics in Medicine. However, the rates of "unclassified," false-positive, and false-negative results reported to UNC were higher than anticipated based on published preclinical trials, the authors say, who also noted decreased success of the test in obese patients.

Over the study period (January to September 2012) the center tested 280 women using NIPT. Screening criteria included patients with a gestational age of at least 10 weeks with advanced maternal age (35 years or older for single pregnancy or 32 years or older for twins), abnormal ultrasound findings, or a positive first- or second-trimester serum screen. The majority of tested patients had advanced maternal age (71.2 percent). Samples were tested at either Sequenom or Verinata Health.

An abnormal noninvasive prenatal test (either positive for aneuploidy or "unclassified" result) was reported in a combined 6.3 percent of patients (13 of 208 women). For detection of trisomies 21, 18, and 13, NIPT had a combined sensitivity of 87.5 percent and specificity of 99.5 percent. Additionally, there was one false-positive result seen for monosomy 18/trisomy 13 with a subsequent normal karyotype and microarray, but this abnormal NIPT result is believed to be related to metastatic maternal cancer detected postpartum.

Only Verinata reported unclassified results (five of 45 patients). Among these unclassified patients, poor pregnancy outcomes included two unexplained fetal demises and one karyotype-confirmed trisomy 18. NIPT results were not reportable due to a below-threshold fetal fraction in three obese patients.

Over the study period, the number of patients requesting noninvasive prenatal testing increased monthly. Simultaneously, the rate of amniocenteses significantly declined (8.1 percent before NIPT versus 5.3 percent after). There were no significant changes in the rates of chorionic villus sampling (CVS) or first-trimester combined screens with the introduction of NIPT. The authors emphasize that invasive screening (amniocentesis or CVS) or postnatal karyotyping is still recommended as follow-up to abnormal or unclassified NIPT results and with abnormal ultrasound, even with a negative NIPT results.

"Two of these patients with normal NIPT and [ultrasound] anomalies went on to

Predictive Ability of NIPT May Be Overstated

The positive predictive value (PPV) of NIPT may be overstated when results are compared to conventional cytogenetics, according to a study published online Aug. 7 in *Genetics in Medicine*.

Researchers from Quest Diagnostics conducted a review to determine the concordance of results between 109 consecutive cases referred for confirmation of NIPT results with cytogenetic studies (prenatal and/or postnatal studies using karyotyping, fluorescence in situ hybridization, and/or oligo–single-nucleotide polymorphism microarray). All four NIPT providers (Natera, Ariosa Diagnostics, Sequenom, and Illumina) were represented, and positive NIPT results included trisomy 21 (41 cases), trisomy 18 (25 cases), trisomy 13 (16 cases), sex chromosome aneuploidy (16 cases), trisomy 16 (three cases), monosomy 21 (two cases), and one case each of triploidy and microdeletion of 22q11.2. Four cases of negative NIPT results, but with positive ultrasound findings, were also included.

Cytogenetic results yielded a true-positive rate of 93 percent for NIPT trisomy 21 results, 64 percent for trisomy 18, 44 percent for trisomy 13, and 38 percent for sex chromosome aneuploidy. Six cases with positive NIPT results for monosomy 21, trisomy 16, triploidy, or 22q11.2 microdeletion had normal cytogenetic findings. One false negative for NIPT was identified in nonmosaic trisomies 9 and 21. The Quest data, combined with NIPT data from two independent groups, yields a PPV of 94.4 percent for cases positive for trisomy 21 by NIPT but is significantly lower for trisomy 18 (PPV = 59.5 percent), trisomy 13 (44.4 percent), and sex chromosome aneuploidy (37.9 percent). The authors say their dataset was not large enough to differentiate PPV among the four NIPT laboratories.

"The PPV for any test is proportional to the specificity of the assay and the prevalence of the disorder. It is very important to perceive that PPV is not intrinsic to the test," write the authors, led by Jia-Chi Wang, M.D., Ph.D., from the Quest Diagnostics Nichols Institute in San Juan Capistrano, Calif. The authors urge careful interpretation of NIPT results and better education of providers.

"The ability of NIPT to correctly predict a positive result for trisomy 18 and trisomy 13 is less than 60 percent," write the authors. "For this reason, it is crucial that providers and consumers understand that NIPT is fundamentally a screening test and cannot be used as a replacement for invasive prenatal diagnosis."

deliver neonates diagnosed with genetic syndromes, 22q11.2 deletion syndrome and a rare single-gene disorder," write the authors, led by Carmen Beamon, M.D. "This highlights the importance of reinforcing the fact that this technology screens for a limited number of aneuploidies and that patients and providers should not be falsely reassured by normal NIPT results in the setting of anomalies."

Yet follow-up invasive prenatal diagnostic testing is not "universally accepted," the authors say, with only 61 percent of UNC patients with an abnormal NIPT undergoing invasive testing. Other researchers have seen similar findings, raising concerns about pretest counseling, women's understanding of potential NIPT results, and whether or not such testing is understood to be voluntary rather than required, routine testing.

The Need to Better Inform Testing Options

Researchers from the University of California, San Francisco (UCSF), say that although the technology for prenatal testing has evolved rapidly, there is a gap in understanding women's personal interest and preferences for available prenatal tests. The UCSF group says that use of a computerized, interactive decision-support guide can promote preference-based decisions and fully informed choices about prenatal testing options, including the option to forgo testing, according to a study published Sept. 24 in the *Journal of the American Medical Association*.

"Without adequate counseling, this new test [cell-free DNA testing for aneuploidy] may easily come to be viewed as simply another blood test in the large panel of routine prenatal laboratories." —Miriam Kuppermann, Ph.D.

"The recent introduction of cell-free DNA testing has intensified the complexity of prenatal testing decision making, generating concerns about the potential for erosion of informed choice," write the authors, led by Miriam Kuppermann, Ph.D.

The researchers conducted a trial in which prenatal clinic patients (of varying literacy) were randomized to either a computerized, interactive decision-support guide (lasting 45 minutes to one hour) with access to free prenatal testing (n = 357) or usual care as per current guidelines (n = 353). The researchers found that women in the intervention group were significantly less likely to have invasive diagnostic testing and were more likely to forgo testing altogether, although they had higher knowledge scores (more likely to correctly estimate the amniocentesis-related miscarriage risk and their estimated age-adjusted chance of carrying a fetus with trisomy 21).

"This study's finding that women who were randomized to the intervention group were less likely to undergo testing than those who received usual care adds support to the contention that women may not be receiving adequate counseling about their options," conclude the authors. "With the advent of cell-free DNA testing for aneuploidy, it is particularly important that women understand the purpose and potential consequences of undergoing testing, as without adequate counseling, this new test may easily come to be viewed as simply another blood test in the large panel of routine prenatal laboratories."

The authors do caution that while no information on NIPT was included in the decision-support guide nor was the test available to study participants, "the general features of cell-free DNA testing and the conditions for which it screens are similar to the tests covered in this study, and the implications for counseling and informed patient decision making remain the same."

Takeaway: The uptake in NIPT is unprecedented in molecular diagnostics. But with this shifting paradigm in prenatal testing comes concern over understanding of the positive predictive value of commercially available tests, as well as patients' awareness of testing's downstream implications.

MDx Emerging on Mobile Platforms

Most of the conversation regarding shifting test volumes of advanced molecular testing toward decentralized locations has focused on whether this type of testing, including next-generation sequencing-based testing, will be housed in large, reference laboratories or whether the testing will be performed in smaller, local laboratories as the price of instruments declines. But emerging technology companies are making the case that advanced testing can be accurately performed even closer to the patient as a point-of-care (POC) test performed in a clinic, doctor's office, or even in field environments.

"There is a push to explore what one can do with a smart phone and consumer electronic devices, but that is not 100 percent aligned with diagnostic market requirements," says Arjang Hassibi, Ph.D., founder and CEO of InSilixia (Sunnyvale, Calif.), which is developing a POC, highly-multiplexed nucleic acid detection platform. "The hardware in smart phones is more applicable to measure easy physiological signals, like EKG, but the toolbox is very limited. But there is a need to bring in vitro diagnostic tests, both basic metabolites and more complex, to the POC or near patient settings."

InSilixia was a Distinguished Award winner in the Nokia Sensing XChallenge, in which microdiagnostic systems were highlighted. The goal, Hassibi says, should be an automated, portable molecular diagnostic (MDx) instrument costing less than $1,000 (per-test price of less than $50) that can target up to 1,000 nucleic acid (DNA or RNA) targets with high accuracy in an hour.

The goal should be an automated, portable MDx instrument costing less than $1,000 (per-test price of less than $50) that can target up to 1,000 nucleic acid targets with high accuracy in an hour. —Arjang Hassibi, Ph.D., InSilixia

With award money and venture capital investments in tow, many companies will be launching commercialization efforts within the next year. The technology behind these systems could make "bedside" genomics a reality, but company executives are focusing their efforts on finding the ideal markets for these emerging platforms, recognizing that POC success will be biomarker dependent.

"A prostate cancer gene might not need to be known immediately, whereas with an actionable, critical care marker you would want to know sooner rather than later," says Jared Bauer, CEO of ApolloDx, a Salt Lake City-based company developing a multitest, mobile diagnostic platform.

Bringing testing to the site of patient care holds the promise of lowering costs, increasing workflow efficiency, and providing a more personalized care experience.

"The infrastructure needs and cost of sending out a test can be substantial. If you can get the same result at the POC within 10 minutes, you can discuss the results right there and then, and can cut out some of the massive infrastructure requirements," Bauer tells *DTET*. "Physicians have been reduced to clerks. A pediatrician told me he spends a portion of his time everyday making multiple calls to laboratories."

Aside from the potential gains in workflow efficiency, such technology can impact patient care.

Generating actionable MDx results takes too long and is too costly. As an example, Hassibi cites testing options for urinary tract infections, which he says generate 7 million

visits annually. A dipstick test ($30) is approximately 70 percent accurate in identifying infection, but it generates results in half an hour. By contrast, a urine culture may take days ($40 to $100) but can detect E. coli infections with near perfect accuracy. MDx can determine not only E. coli infections but also the resistance profile of strains, with similarly superior accuracy, but DNA analysis (more than $500) could take weeks.

"The positive economic argument for these mobile platforms is more than just unit and test costs," says Jo-Ann Stanton, Ph.D., the lead of a University of Otago (New Zealand) team developing a portable polymerase chain reaction (PCR)-based DNA sequencing instrument. "Real-time access to diagnostic information means a response can be made at the time and place it is needed."

"It is not a doomsday for the laboratory. . . . Laboratories need to be involved."
—Jared Bauer, ApolloDx

Implications of Mobile Platforms for Laboratories

"Small, portable, low cost DNA sequencers certainly have the potential to bring genomic medicine out of large centralized pathology laboratories and closer to the patient," writes science program lead Leila Luheshi, Ph.D., on the blog of the health policy think tank, PHG Foundation (United Kingdom). "The availability of mobile sequencing might also become particularly important for rapid genomic testing outside large-scale health care facilities, in community medical centers or as parts of mobile screening units."

Luheshi cautions, though, that demonstrating both the analytical validity and clinical utility of these devices is "likely to be long and far from straightforward . . . , [but] caveats aside, it is important to remember that the world's first DNA sequencer in a USB stick is now up and running, and the future of 'mobile genomics' looks bright."

Additionally, experts believe that mobile genomics can improve access in resource-limited markets as well as improve the timeliness of care in developed nations, particularly in the area of infectious disease testing. While the technology is expected to be disruptive, it will not be the demise of traditional laboratories.

"It is not a doomsday for the laboratory," Bauer says. "We are going to market working with the laboratories. Laboratories need to be involved."

DTET conducted a survey of the emerging marketplace to identify mobile platforms that have entered or will enter the commercial realm within the next year.

The **Freedom4** platform utilizes quantitative PCR to identify DNA sequences in real time. The platform was developed over six years at the University of Otago and is being spun out of the university's commercialization office in partnership with the New Zealand company Ubiquitome. Stanton, lead of the Freedom4 device development team, tells *DTET* via e-mail that the device is poised to become a commonly used tool both in and out of the classic health care environment for rapidly detecting suspected viruses or bacteria.

The device weighs the same as a typical laptop but is palm-sized. The Freedom4 has a six-hour battery life and can be tethered to a laptop or connect wirelessly via smart phones or tablets, running custom software, for results analysis and interpretation. The Freedom4 device is currently commercially available for research use only with early access pricing of $10,000 for the remainder of 2014. Per-test pricing and regulatory strategy will depend on the diagnostic development partner, Stanton says. The

open platform has demonstrated analytical equivalence to commercial lab-based platforms and assays for a range of gastrointestinal and respiratory viruses.

InSilixia's **Hydra-1K** platform utilizes a complementary metal-oxide-semiconductor-based DNA analysis technology. The platform uses conventional reagents including polymerases and synthesized DNA oligonucleotides. DNA sequence identification is enabled by a highly-multiplexed amplification and pixel-level DNA capturing and optical detection, all in the same reaction chamber.

Hassibi tells *DTET* that the company's initial focus will be in high-volume infectious disease detection in clinics and other near-patient settings. The company expects its first commercial product to be available after approval through the 510(k) process in late 2015. Hassibi says InSilixia's strategy is to aggregate test volume through a combination of pursuing larger, clinically relevant markets in-house and partnering (through licensing agreements). The Hydra-1K could drastically undercut current diagnostic methods. The company says it can run highly accurate DNA tests in an hour for less than $50 per test on a small reader which costs about $250.

ApolloDx unveiled in September its mobile **ApolloDx Diagnostic Platform**. The simple, 7 ounce analyzer, disposable cartridge, and proprietary smartphone app can generate laboratory-quality, GPS-tagged results, the company says. Bauer says the device is capable of advanced agent detection in human, veterinary, agriculture, and biothreat applications. Within 10 minutes of test strip insertion, quantitative and qualitative results are displayed and securely transmitted and integrated into "almost any" medical or laboratory software system. The CLIA-waived platform will be capable of in vitro and companion diagnostics but will launch initially in the veterinarian and agriculture markets sometime next year.

The experts *DTET* spoke to see a further evolution, down the road, toward direct patient testing, with results linked electronically to providers. Many of the profiled companies are already planning for this as is Atomo Diagnostics (Australia), which is adapting its AtomoRapid platform for self-testing applications in the second quarter of 2015.

The **AtomoRapid**, which integrates blood collection, blood delivery, and lancing mechanism directly into the test cassette, can accommodate test strips for a wide variety of conditions from celiac disease and allergy to infectious diseases. The device, half the size of an iPhone, has been deployed in South Africa for HIV and malaria testing and will expand to Southeast Asia by the end of the year and East and West Africa in 2015. CE Mark submissions have been made for AtomoRapid HIV, which will be submitted to the U.S. Food and Drug Administration for approval in the second half of 2015, Byron Darroch, Atomo Diagnostics' business development director, says.

"AtomoRapid revolutionizes the way the rapid testing is done in the field, through the engineering out of human errors commonly seen in the use of traditional 'bits in a box test kits,'" Darroch tells *DTET* via e-mail. "Current test kits are made up of up to six components that the user has to then use in the right sequence of steps—something that is not always easily accomplished."

Takeaway: There is a definite trend toward advanced, mobile diagnostics. In the coming year, mobile platforms capable of DNA sequencing, primarily for the detection of infectious diseases, will emerge commercially.

Will Sequencing Penetrate Newborn Screening, Infant Care?

What is believed to be the first healthy, U.S. baby to have his DNA sequenced prenatally was born this summer, according to *MIT Technology Review*. (A handful of fetuses have been sequenced as part of investigation of abnormalities.) The child's father, a graduate student and professional genetics blogger who did the sequencing for the sake of coolness and not medicine, believes sequencing of fetuses will become routine practice.

Will prenatal sequencing become the norm? Not likely any time soon, according to experts *DTET* spoke to. However, sequencing is being discussed as an alternate technology for newborn screening (NBS) tests. Yet employment of the technology for NBS is fraught with ethical considerations, and some believe the notion of sequencing newborns challenges the very intention of NBS—to identify potentially lethal or life-changing conditions that have effective treatments if detected early.

The best interest of the child has been the driver of NBS decisions and has been the focus of early attention on the use of genetic testing in children. Generally, professional societies do not support the systematic genotyping of newborns or young children. The problem, of course, is too much information including identification of conditions that don't manifest until adulthood or genetic variants for which the clinical significance is not fully known.

"The discussion of the possible role for sequencing in NBS must ultimately be about whether or not it improves infant and child health," says Michelle Lewis, M.D., from Johns Hopkins' Genetics and Public Policy Center (Baltimore). "Technology must have a clear benefit for the public's health. NBS is designed as part of state public health programs which serve the population as a whole, which is different from a clinician with an obligation to an individual patient."

The pivotal considerations for use of whole-genome sequencing (WGS) in next-generation sequencing (NGS) include the ethical consideration of what information to report, as well as resource and logistical issues surrounding counseling, follow-up reportingy, and the potential need for additional diagnostic testing and care.

"My personal viewpoint is that it is a pretty bad idea to do WGS in NBS, but targeted NGS panels is a spectacular idea," says Jonathan Berg, M.D., Ph.D., a clinical geneticist at University of North Carolina, Chapel Hill (UNC).

Targeted or staged approaches to WGS are being considered as a possibility to prioritize and filter the number of findings. Agreement remains elusive as to the criteria needed to generate a list of reportable conditions as well as timing of reporting. Some have suggested withholding some results until parents have had more time deliberate the implications, until the child reaches maturity, or until mutations gain evidence of validity and clinical utility.

"A more elegant approach would be a system of screening enabling screening of relevant conditions at a relevant point in time," says Berg, who concedes such a scenario would pose challenges with follow-up and a lack of interoperable medical records.

If results were reported outside of the framework of NBS, when there is a "captive audience," there are also considerations for an expanded role of the health department and the pediatrician or family physician. Others have proposed a two-tiered approach

NBS Then and Now

Sept. 30 marked the 50th anniversary of newborn screening, which began as a recommendation to routinely screen all newborns for phenylketonuria.

The recommended uniform screening panel, devised by the U.S. Department of Health and Human Services' Discretionary Advisory Committee on Heritable Disorders in Newborns and Children, consists of 31 core conditions and reporting of 26 secondary conditions. The number and type of conditions varies by state.

with a "classical" NBS and a WGS screening with additional parental consent, which some fear could create "justice" and access issues.

"There is an urgent need for discussion and some level of international professional and public consensus," particularly surrounding the appropriateness of returning incidental findings, wrote Bartha M. Knoppers from McGill University in Canada, in a commentary piece published in *Science Translational Medicine* in March. "Were NBS programs to incrementally expand their screening panels to introduce WGS, the reception may be discordant, disorganized, and disruptive. The policies of NBS should be discussed prospectively and carefully."

Parent's Perspective

The National Institutes of Health last year funded four pilot programs ($25 million over five years) under the Genomic Sequencing and Newborn Screening Disorders research program to evaluate if sequencing of newborns' genomes provides useful medical information beyond current NBS programs. All of the funded programs (Brigham and Women's Hospital in Boston, Children's Mercy Hospital in Kansas City, Mo., University of California, San Francisco, and UNC) will incorporate assessments of the ethical, legal, or social implications of using genomic information in the newborn period into the projects.

Early research is showing that even parents who have experienced a positive NBS result with their infant have reservations about the types of information they would want from WGS as part of NBS, according to an abstract presented at the National Society of Genetic Counselors Annual Educational Conference (New Orleans; Sept. 17-20).

Researchers from Stanford University, led by Shannon Rego, conducted interviews with seven parents who had a child screen positive on NBS since August 2005 to assess their attitudes toward WGS as part of NBS. Parents expressed strong interest in learning about medical conditions in their infant that were treatable or preventable. While several parents also wanted to know about untreatable and nonpreventable conditions, usually citing a desire to

SCID: A Case Study of a New NBS Condition

A newborn screening test for severe combined immunodeficiency (SCID) reliably identifies infants with this life-threatening inherited condition, leading to prompt treatment and high survival rates, according to an article published Aug. 20 in the *Journal of the American Medical Association* (*JAMA*). The researchers also determined that SCID affects approximately one in 58,000 newborns, making it less rare than previously thought.

SCID is the first set of heritable immune disorders included in NBS and is characterized by defective T cell production, which can result in life-threatening infections. Early detection is imperative for optimal treatment and survival. Isolated DNA from infant dried blood spots can be assayed using polymerase chain reaction to detect T cell receptor excision circles (TRECs), a biomarker for naive T cell lymphopoiesis and identifier of SCID. NBS for SCID began in 2008 in Wisconsin and, while added to the national recommended uniform panel for NBS in 2010, is only tested for in 23 states, the District of Columbia, and the Navajo Nation.

Researchers led by Antonia Kwan, Ph.D., from the University of California, San Francisco, examined 3 million tests (January 2008 to July 2013) from 11 NBS programs to evaluate SCID screening algorithms and test performance data.

Screening detected 52 cases. Screening caught all infants with SCID. Cutoffs for referral testing varied (less than 40 TREC/µL in seven programs and less than 252 TREC/µL in three programs). Variations in follow-up practices were also seen across states.

"The TREC assay has proven excellent for detecting disorders with poor T-cell production . . . , but finding additional immune defects prior to onset of recurrent or life-threatening infections will require further methods," write the authors. "Genomic sequencing may be required to detect deleterious mutations in primary immune defects, of which nearly 200 are known."

But some caution that standardization of NBS, including test performance, is needed before the more complicated issues accompanying sequencing can be addressed.

"Before [SCID] screening becomes universal in the United States, agreement is needed on what constitutes a positive TREC screening test, on ensuring referrals to physicians competent to make a diagnosis, and on providing definitive therapy to every infant detected with SCID in every state," writes Neil A. Holtzman, M.D., from Johns Hopkins Medical Institutions (Baltimore), in an accompanying *JAMA* editorial. "The technological advances in newborn screening since [phenylketonuria] screening was first recommended in the United States 50 years ago have been remarkable. However, organizational advances that ensure safe and effective NBS nationwide have not matched them."

be better prepared for the future, the parents who only wanted actionable information believed knowledge of these other conditions "would be a burden." These reservations, the authors say, are more pronounced than those expressed in previous studies.

Takeaway: Routine sequencing as part of NBS or healthy infant care is unlikely to gain traction in the near future due to ethical considerations of which conditions to report, as well as resource limitations, particularly in the public health system. However, as sequencing prices continue to drop, experts do see a role emerging for targeted sequencing panels in NBS. G2

Markers of Aging May Improve Cancer Treatment Selection

Incorporating objective biomarkers of functional age could potentially better predict which elderly adults could tolerate cancer treatment, according to a study published in the *Journal of Clinical Oncology*. Such a measurement could identify those patients most at risk from side effects of cancer treatment, including increased toxicity, functional decline, decreased quality of life, and ultimately, poorer survival.

Elderly patients, the authors say, are quite heterogeneous in their physical ability to tolerate cancer therapy, but standard methods are lacking to assess this risk, as chronological age and comorbidities are not adequate determinants. In a review published Aug. 20, the authors evaluate potential biologic markers of functional aging, including chronic inflammatory markers and markers of cellular senescence.

The most studied markers are those of chronic, systemic inflammation (including C-reactive protein [CRP], tumor necrosis factor-α, and D-dimer). They are compelling because of their ease of measurement, although potentially not "ideal markers" because they are not independent of the pathology of cancer. A variety of studies have tied these markers to acceleration of the aging process, exacerbation of age-related diseases, as well as an association with clinical measures of frailty, functional decline, and a heightened risk of mortality.

"Several chronic inflammatory markers (interlukin-6, D-dimer, and CRP) . . . may have greater predictive ability among patients without baseline functional impairments, suggesting they may identify prefrail patients that may not otherwise have been identified," write the authors, led by Joleen M. Hubbard, M.D., from the Mayo Clinic (Rochester, Minn.).

In addition to measures of systemic inflammation, circulating markers of cellular senescence, such as $p16^{INK4a}$ and telomere length, have also been studied as potential biologic markers of aging.

"It is anticipated that there will be a combination or panel of markers that will have the best predictive power of end points such as toxicity, functional decline, quality of life, and survival," the authors write. "As with the geriatric assessment, patients would potentially be placed into frailty and/or aging categories (i.e., low-, intermediate-, or high-risk groups) to predict risk for the specific end point."

Hubbard tells *DTET* that a thorough clinical geriatric assessment can take two hours or more, which is increasingly not feasible in routine clinical practices. However, if markers can provide a reliable measure of frailty in seniors, an economic argument can be made for their inclusion in geriatric workups.

Takeaway: Incorporating objective measures of functional aging could become a part of clinical geriatric oncological evaluation in an attempt to better predict which elderly patients can tolerate cancer treatment. G2

G2 Insider

Histopathologic Review Important With Bariatric Surgery Specimens. . . Histopathological review of resections from gastric sleeve resections is necessary as unexpected, clinically significant pathology may be found, according to an abstract presented at the College of American Pathologists annual meeting (Chicago; Sept. 7-10). In a recent case review study, nearly one-quarter of bariatric surgery patients had a clinically significant finding, which the authors say is noteworthy for better informing the discussion of standardizing preoperative workups for these obese patients.

The need for preoperative endoscopy is currently debated for patients readying for bariatric surgery. While often routine practice, few studies have examined the histopathologic changes in the bariatric specimens of morbidly obese patients to determine if preoperative endoscopy findings would likely alter the operative plan.

In the present study, researchers conducted a retrospective case review of 343 bariatric gastric sleeve surgery cases performed at the University of Illinois Hospital and Health Sciences System (January 2009 to August 2013). More than one-half of the patients had histopathologic findings, with 24.8 percent of these findings reaching clinical significance.

The most frequent finding was gastritis (69 percent), including chronic inactive (51 percent of all patients), chronic active (15.4 percent), acute-on-chronic gastritis (2 percent), and associated Helicobacter pylori infection (22.8 percent). Six patients (1.7 percent) had ulcers, with only two of them having a previously documented history of ulcers. Histopathological examination showed that two patients (0.58 percent) had gastrointestinal stromal tumors. Other findings included an unknown recurrence of adenocarcinoma (one patient), one polyp (one patient), a gastric diverticulum (one patient), mucosal and vascular congestion, intestinal metaplasia, hemorrhage, and pyloric stenosis (1 patient). No significant histopathologic changes were seen in one-quarter of patients (n = 85).

This review confirms the importance of examining these specimens histologically, because clinically significant lesions may be found, and follow-up treatment may be necessary.

Experts say that while a higher rate of endoscopic abnormalities in these patients may be seen, the majority of these findings often do not affect the actual operative management, and therefore a selective approach toward preoperative endoscopy is currently called for (2008 American Society of Gastroenterology guidelines) until further data show otherwise.

Rachel Gordezky, M.D., a pathology fellow and lead author of the study, says that one takeaway is that while these specimens are from patients going in for routine, elective procedures, they can commonly contain underlying, unexpected conditions, and the specimens warrant "the same care" in examination as those from patients with known masses.

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 Diagnostic Testing & Emerging Technologies (ISSN 2332-1520) is published by G2 Intelligence, 24 Railroad Street, Keene, NH 03431-3744. Tel: 800-531-1026 or +1 603-357-8101. Fax: +1 603-357-8111. Web site: www.G2Intelligence.com.

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