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March 2015

TOP OF THE NEWS
High-Res Sequencing Data IDs Outbreak Clusters 1
Evidence Needed for Cost-Effectiveness of Sequencing 1

INSIDE THE DIAGNOSTICS INDUSTRY
Sequencing Rapidly Emerging for Epidemiology, Outbreak Control 5

EMERGING TESTS
Metabolomic Markers Improve Cardiovascular Risk Prediction 4
With Technology Advances, Saliva Tests Hold Greater Promise 9
- Proteomic Assessment for Autism-Related Markers (p. 9)
- Gene Expression Assay for Preemie Feeding Readiness (p. 10)

Test Improves Prediction of Knee Arthritis Progression 11

G2 INSIDER
C. Diff Testing Transitioning as Cases Rise 12

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High-Res Sequencing Data IDs Outbreak Clusters

Whole genome sequencing (WGS) was able to improve discrimination of subclusters of patients as part of an epidemiologic investigation of a 2014 Salmonella outbreak linked to contaminated cucumbers, according to a study published Feb. 20 in *Morbidity and Mortality Weekly Report*.

A multistate cluster was detected in August 2014 through PulseNet, the national molecular subtyping network for foodborne disease surveillance. The infections, Salmonella enterica serotype Newport infections, were indistinguishable using pulse-field gel electrophoresis (PFGE). But, WGS was able to further characterize PFGE pattern JJPX01.0061 isolates from a subset of 58 clinical specimens taken from the total 275 sickened patients in 29 states and the District of Columbia.

WGS results identified 12 distinct illness subclusters across four states, ranging in size from two to six cases. A primary group of genetically-related isolates was seen from cases in Delaware, Maryland, Ohio, Pennsylvania, and Virginia. Phylogenetic analysis also showed that an additional group of highly-related isolates from patients in New York was distinct from the primary phylogenetic group.

"Advanced molecular detection methods, including WGS, might improve discrimination of subclusters during outbreak investigations," write the authors led by Kristina Angelo, D.O., from the U.S. Centers for Disease Control and Prevention. For more information on how high-resolution data generated from next-generation sequencing (NGS) is being used for pathogen identification, outbreak detection, and infection control efforts, please see Inside the Diagnostics Industry on page 5.

Evidence Needed for Cost-Effectiveness of Sequencing

The price of whole genome sequencing (WGS) and whole exome sequencing (WES) is approaching the point where the cost-effectiveness for the technology may trump conventional testing strategies for the evaluation of children with neurodevelopmental disorders (NDDs), according to a study published in *Science Translational Medicine* Dec. 3, 2014. While this is not the first study to show that WGS and WES can improve the diagnostic yield over traditional molecular methods, this is one of the first studies

Continued on page 2

■ Evidence Needed for Cost-Effectiveness of Sequencing, *Continued from bottom of p.1*

to pinpoint a price linked to the cost-effectiveness of sequencing. The cost-effectiveness estimates and the time savings to diagnosis associated with WGS/WES lead the authors to conclude initial diagnostic evaluation of children with NDDs should include trio WGS or WES testing.

"WGS and WES provided prompt diagnoses in a substantial minority of children with NDD who were undiagnosed despite extensive diagnostic evaluations," the authors summarize. "Preliminary analyses suggested that WES was less costly than continued conventional diagnostic testing of children with NDD in whom initial testing failed to yield a diagnosis."

So called diagnostic odysseys for an underlying cause of NDD are notoriously lengthy and costly, and frequently fail to yield a definitive diagnosis in more than half of patients. Primary tests currently used for etiologic evaluation of NDD include neuroimaging, karyotype, array comparative genome hybridization and/or single-nucleotide polymorphism arrays, and phenotype-driven metabolic, molecular, and serial gene sequencing studies. Follow-up invasive tests, including biopsies and cerebrospinal fluid assessment, can lead to a small number of additional diagnoses.

"Preliminary analyses suggested that WES was less costly than continued conventional diagnostic testing of children with NDD in whom initial testing failed to yield a diagnosis."

—Sarah Soden, M.D.

The Economics of WGS/WES For NDD

The current study, undertaken at Children's Mercy (Kansas City, Missouri), involved 100 families with 119 children affected by NDDs (intellectual disability, global developmental delay, and autism) who received diagnostic WGS and/or WES of parent-child trios over a 33-month period. Rapid WGS was reserved for families with symptomatic infants and children in intensive care units (50-hour protocol turnaround with the research-use-only STAT-Seq test, versus 16 days for WES in ambulatory patients). The researchers found a definitive molecular diagnosis of an established genetic disorder in 53 of 119 affected children (44.5 percent) overall. However, rapid WGS yielded diagnoses in 73 percent of families with acutely ill children (11 of 15), while testing of children in ambulatory care yielded diagnosis of 40 percent of the 85 children for whom traditional methods failed to yield a diagnosis (33 by WES and one by staged WES followed by WGS). The researchers noted that while they intended to test parent-child trios, they found that in practice, an average of 2.55 individuals per family were tested.

The researchers found that the cost of prior negative tests in the ambulatory patients was $19,100 per family (range of $3,248 to $55,321). The nonacute, ambulatory clinic patients were older and had received a much longer period of subspecialty care and considerable prior diagnostic testing (an average of 13.3 prior tests/panels), compared to acute patients which had on average, seven prior diagnostic tests with a mean total cost of $9,550.

The researchers calculated that at a cost of up to $7,640 per family, sequencing would be cost-effective, assuming a rate of diagnosis of 40 percent and an average charge for prior testing of $19,100 per family. If the average uptake of testing was assumed to be 2.55 per family (instead of the intended 3.0), sequencing would be cost-effective up to $2,996 per individual tested.

"Although $2,996 is at the lower end of the cost of clinical WES today, next-gen-

eration sequencing continues to decline in cost," write the authors led by Sarah Soden, M.D., from Children's Mercy. "Furthermore, the cost-effectiveness estimates reported herein excluded potential changes in health care cost associated with earlier diagnosis."

Additionally, the authors note there is a benefit to increased speed to diagnosis. A diagnosis could have been made 77 months earlier if WGS or WES had been performed at initial symptom onset. Among the 11 families receiving 50-hour WGS, the fastest times to final report of a confirmed diagnosis were 6 days to 10 days, although cases of recently described or previously undescribed genetic diseases and in patients whose phenotypes were atypical for the causal gene, time to diagnosis took longer.

More Evidence Needed

Experts say that up to now the focus has been on the challenges and opportunities of implementing next-generation sequencing (NGS) in clinical practice. Given mounting evidence of the feasibility of using the technology, the focus is shifting towards the economics of sequencing—including the cost-effectiveness of clinical sequencing.

David L. Veenstra, a member of the Institute of Medicine's Roundtable on Translating Genomic-Based Research for Health, argues in a Feb. 12 discussion paper on the cost-effectiveness of clinical sequencing, that future research on cost-effectiveness must look beyond just cost savings and must incorporate the value derived from "improving patients' lives as efficiently as or more so than current clinical practice."

The Canadian Agency for Drugs and Technologies in Health (CADTH) recently released a rapid response report reviewing existing evidence of the cost-effectiveness of NGS. The agency could find one published systematic review analyzing the cost-effectiveness of NGS in the literature and one health technology assessment and one systematic review in the grey literature.

While the report cited evidence that the costs associated with Sanger sequencing were reported to be approximately $500/Mb versus $0.50/Mb using NGS, the agency concluded that given "the distinct lack of robust published data" there is not "sufficient information to make an informed analysis" regarding the cost-effectiveness of NGS.

"The question of the cost-effectiveness of NGS remains unclear," the CADTH writes. "All studies identified in this report have concluded that there is a lack of powerful economic investigations published to date. This is a result of the complexity of analyzing the costs associated with all processes subsequent to the sequencing run itself. In addition health-care costs related to the high proportion of ambiguous variants detected using NGS techniques remain unknown."

To fill the evidence void, Veenstra recommends both large observational studies of the direct and indirect costs of clinical sequencing implementation in the near and intermediate term and modeling studies of the long-term clinical and economic impacts of clinical sequencing for both intended and incidental findings.

Takeaway: As comprehensive NGS-based tests, including WGS and WES, make their way into clinical practice, there are increasing calls for demonstration of the cost-effectiveness of the approach. Emerging evidence indicates that in the case of NDD, which is typically associated with long diagnostic odysseys, the price of WGS/WES is reaching the point where it may be cost-effective for the initial evaluation of cases. G2

Metabolomic Markers Improve Cardiovascular Risk Prediction

Metabolite profiling has identified four new biomarkers that improve the prediction of the risk for heart attack or stroke within the next 15 years, according to a study published online Jan. 8 in *Circulation*. Phenylalanine and three measures of fatty acids independently predict future cardiovascular events and could, the authors say, provide a low-cost means of improving risk assessment.

"These new biomarkers can help to better assess the complex molecular processes behind the development of cardiovascular disease," said lead author Peter Würtz, Ph.D., in a statement. "The improved prediction of cardiovascular risk also suggests cost savings in healthcare by advanced biomarker profiling."

Profiling metabolic status, the researchers say, may provide insights into the underlying mechanisms associated with atherosclerosis formation. The international group of researchers used a high-throughput nuclear magnetic resonance (NMR) spectroscopy platform to qualitatively identify biomarkers tied to incident cardiovascular disease over long-term follow-up. NMR spectroscopy allows for assessment of over 200 metabolism-related markers from a single blood sample. Biomarker discovery was conducted using data from the FINRISK study (n=7,256; 800 cardiovascular events) with validation occurring using data from the SABRE study (n=2,622; 573 events) and British Women's Health and Heart Study (BWHHS; n=3,563; 368 events).

"These new biomarkers can help to better assess the complex molecular processes behind the development of cardiovascular disease."

—Peter Würtz, Ph.D.

The researchers found that 33 out of 68 lipid and metabolite measures tested were significantly associated with incident cardiovascular events, after adjusting for age, sex, blood pressure, smoking, diabetes, and medication. Four metabolites emerged as associated with future cardiovascular events when analyses further adjusted for routine lipids. Specifically, higher serum phenylalanine and monounsaturated fatty acid levels were associated with increased cardiovascular risk, while higher omega-6 fatty acids and docosahexaenoic acid levels were associated with lower risk.

A risk score, incorporating these four biomarkers, was then developed. Risk reclassification was improved with the addition of the biomarker risk score among individuals who did not experience a cardiovascular event. Net reclassification improvement for the whole study population was 7.6 percent in SABRE and 5.3 percent in BWHHS. However, there was a substantial upclassification with inclusion of the biomarker score for persons classified in the intermediate risk range.

"Novel biomarkers for risk prediction are primarily needed for persons in the intermediate risk range, for whom treatment decisions are most challenging," Würtz writes in the study. "The four biomarkers proved particularly helpful in correctly reclassifying individuals in the 5 percent to 10 percent risk grey zone."

Takeaway: Identification of biomarkers through metabolomic analyses may improve individual's risk classification for future cardiovascular events. The researchers are optimistic that this high-throughput biomarker profiling may be a cost-effective means of improving cardiovascular disease prevention.

Inside The Diagnostics Industry

Sequencing Rapidly Emerging for Epidemiology, Outbreak Control

Much as polymerase chain reaction (PCR) revolutionized microbiology 30 years ago, the application of next-generation sequencing (NGS) to the field of infectious disease will improve surveillance efforts, outbreak determination, and infection control activities, in addition to tailoring treatment decisions, thus improving both individual care and global antibiotic stewardship.

"As the genomes of bacteria and viruses are between one thousand to one million times smaller than the human genome they can be sequenced and analyzed more rapidly (in less than a day) and cheaply (for around £50 per genome) bringing the insights of pathogen genomics within reach of the budget and time frames in which clinical and public health microbiology services operate."

—Leila Lusheshi, Ph.D.

Microbiologists and laboratorians will play a central role as NGS is employed for epidemiological, infection control, and patient care purposes. There is noticeable momentum toward the common use of NGS in molecular epidemiology by both public health agencies and hospital microbiology laboratories. The molecular data gleaned from these NGS-based surveillance efforts are informing the understanding of pathogen transmission, shifts in pathogen virulence or microbial sensitivity, and infection control practice.

While use of NGS for individual patient diagnosis remains rare outside of translational research endeavors, experts surveyed by *DTET* believe that within the next five years whole genome sequencing (WGS) of pathogens will be well-integrated into routine public health surveillance and investigation of health care-associated outbreaks, particularly in large hospitals.

"This is the year NGS and infectious disease jump to another level," George Weinstock, Ph.D., from the Jackson Laboratory for Genomic Medicine (Farmington, Conn.), tells *DTET*. "There are a number of examples in the literature where sequencing can detect one base differences in three million base genomes. This incredible sensitivity allows public health organizations [in multiple locations] to determine if clusters of E. coli from bad lettuce are the tip of the iceberg of a nationwide incident or coincidental local outbreaks."

NGS Improves Resolution

Microbial genomics encompasses the full spectrum of investigation—from pathogen detection, identification, sensitivity testing, and incorporation of metadata into epidemiological tracking. Researchers say that more than 38,000 bacterial and 5,000 viral genomes have been sequenced to date and that pathogen characterization using NGS holds great potential to improve the resolution of the data over traditional microbiological techniques. While PCR is the most widely used molecular method in clinical microbiology, such targeted techniques lack the resolution necessary to determine chains of transmission, where single nucleotides may differentiate cases involved in an outbreak from others not directly involved. Additionally, NGS can provide a "universal" test that can answer questions not only regarding the identity of the pathogen, but also markers of virulence and antibiotic resistance in a single step.

"As the genomes of bacteria and viruses are between one thousand to one million times smaller than the human genome they can be sequenced and analyzed more rapidly (in less than a day) and cheaply (for around £50 per genome) bringing the insights of pathogen genomics within reach of the budget and time frames in which clinical and public health microbiology services operate," writes Leila Lusheshi, Ph.D., from the health policy think tank PHG Foundation (United Kingdom) in a briefing note.

Inside The Diagnostics Industry

NGS Aids Public Health Policy Priorities

Not only is evidence emerging that NGS is technically feasible for infectious disease identification purposes, but it plays a central role in dealing with top public health policy priorities, such as tackling emerging infections and antibiotic resistant bugs.

Agencies such as the U.S. Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA) are incorporating NGS into public health and food safety surveillance through their PulseNet and Genome Trakr networks. As the agencies build their NGS infrastructure both are utilizing a strategy that relies upon decentralized NGS testing in regional laboratory networks with centralized data management using cloud-based technology for cluster determination at the national level.

"Hospitals would be able to routinely monitor the many thousands of resistant isolates identified in their clinical practice."

—Combating Antibiotic Resistance, *September 2014*

The FDA's Genome Trakr Network entered into a $17 million contract with sequencer-maker Illumina (San Diego) back in 2012 to build NGS capacity for food safety testing at its 13 federal labs and 13 state health and university labs. The network is currently sequencing an average of over 800 isolates each month, the agency reports, using MiSeq sequencers. Results are transmitted to a genomic reference database housed at the U.S. Department of Health and Human Services' (HHS) National Center for Biotechnology Information. The real-time uploads enhance the agency's ability to identify potential outbreaks.

While federal agencies are working on translating NGS-based surveillance into routine practice through a series of pilot programs, the President's Council of Advisors on Science and Technology issued a report *Combating Antibiotic Resistance* in September 2014 calling for coordinated efforts to further build national, genomic-based surveillance capacity to combat the threat of growing antibiotic resistant bacteria.

In the report, the council calls for significant investment ($190 million annually) to strengthen national genomic-based pathogen surveillance through building state and local public health infrastructure. The council proposes a national laboratory network for pathogen surveillance (health care, agriculture, and environmental sampling), as well as heightened clinical laboratory capacity at 10 to 20 major health care facilities. The plan calls for ensuring laboratories would be able to receive specimens and relevant metadata; perform genomic analysis; rapidly return information to providers and to relevant public health entities for cluster identification; archive samples; and deposit genomic information and metadata in a publicly-accessible national database. The council estimates that the annual cost for the two laboratory components of the network is roughly $130 million ($80 million for the regional laboratories and $50 million for the hospital-based laboratories).

The council says that by building sequencing capacity, an initial reference collection of antibiotic-resistant pathogens can be developed within three years. Additionally, the council calls for funds to foster the development of new computational methods and tools to aid genome assembly and comparison, metagenomic analysis and interpretation of epidemiologic data into reports for providers, and the development of surveillance and testing standards.

"Ultimately, fully-automated sample handling and data analysis methods should allow the analysis of extremely large numbers of samples," the workgroup writes in the report. "Hospitals would be able to routinely monitor the many thousands of resistant isolates identified in their clinical practice. Surveillance efforts in other settings would become routine and could provide

Inside The Diagnostics Industry

"WGS is now poised to make an impact on hospital infection prevention and control, delivering cost-effective identification of routes of infection within a clinically relevant timeframe and allowing infection control teams to track, and even prevent, the spread of drug-resistant hospital pathogens."

—Beryl Oppenheim, M.B.B.Ch.

early warning signs about potential outbreaks, whatever their origin. Tracking patterns across facilities in the community would show patterns of spread to guide preventive interventions."

Relatedly, HHS has identified the reduction of health care-acquired infections (HAI) as an agency priority goal. Diagnostics, including the potential use of NGS, are key to rapidly diagnosing infections, optimizing treatment as soon as possible, increasing the speed of an individual's recovery and thereby cutting their time in the hospital and associated health care costs. HAIs are receiving increased attention, in part because of payers' crackdown on reimbursements for care necessitated by preventable HAIs and hospital laboratories are looking for technologies that will enable a faster turnaround time for provision of antibiotic sensitivity results to a physician for a suspected HAI.

Case Studies of WGS for Outbreak Investigation

"WGS is now poised to make an impact on hospital infection prevention and control, delivering cost-effective identification of routes of infection within a clinically relevant timeframe and allowing infection control teams to track, and even prevent, the spread of drug-resistant hospital pathogens," says Beryl Oppenheim, M.B.B.Ch., director of infection control at Queen Elizabeth Hospital.

Molecular epidemiology, experts say, encompasses the process of identifying the genetic basis of disease (pathogen variants), transmission (including source and route), and informing prevention pathways supporting hospital infection control.

NGS allows highly accurate, hypothesis-free analysis of multiple isolates for detection in a single test, replacing the need for multiple analyses to identify the organism, its resistance, and virulence. The resolution of NGS is so great, that it can distinguish pathogen strains that differ by as little as one single nucleotide polymorphism. This can speed investigation by public health officials and hospital infection control experts to take effective measures to contain and stop the spread of these pathogens. Real-life case studies are emerging, demonstrating the power of WGS in combination with traditional epidemiological tracking to control outbreaks.

For 18 months, Queen Elizabeth Hospital (England) experienced a "protracted" outbreak of multidrug resistant Acinetobacter baumannii (A. baumannii). WGS was used to determine the relationships between collected isolates. The outbreak involved both civilians and military casualties cared for in multiple wards. The outbreak was caused by A. baumannii pulsotype 27, as determined by pulse-field gel electrophoresis. Genomic DNA was extracted from 114 isolates beginning at week 40 of the outbreak. Genome sequences were determined from 102 isolates. Results revealed that 52 patients and 10 environmental isolates showed genomic similarity to the outbreak reference strain (eight or less single nucleotides differences). WGS determined 18 isolates were not part of the outbreak. Excluding isolates, the authors say, allowed efforts to focus on determining the connections between genetically-related cases, rather than trying to connect all cases.

"By combining WGS and epidemiological data, we reconstructed potential transmission events that linked all but 10 of the patients and confirmed links between clinical and environmental isolates," writes co-author Beryl Oppenheim, in the case study published in November 2014 in *Genome Medicine*. "Identification of a contaminated bed and a burns theatre as sources of transmission led to enhanced environmental decontamination procedures."

Inside The Diagnostics Industry

Challenges to Adoption

While there is mounting evidence that deploying NGS for pathogen characterization and outbreak detection is feasible, experts agree that in order for the technology to be incorporated into routine practice, some additional issues still need to be resolved.

"While the sequencing technology and bioinformatic analysis underpinning the use of pathogen genomics in the management of infectious disease are relatively well established, the integration of these new techniques into the delivery of medical and public health microbiology services is only in its infancy," writes PHG Foundation.

From a practical standpoint Oppenheim says that rapid declines in the cost of NGS and increasing speed to results coupled with significant reductions in the size of the equipment bode well for the future routine adoption of WGS in hospital infection control. But remaining challenges include ease-of-use issues and the skill-level needed to run sequencing tests.

Susan Knowles, senior market development manager for Illumina, tells *DTET* that one of the many reasons public health adoption has "taken off" in the United States is because of the lack of regulatory barriers, as opposed to use of the technology for clinical diagnostics, where FDA approval is needed. Hospitals, she says, are required to investigate potential outbreaks, but whether NGS is used is dictated by each hospital's infection control policy. Though, hospitals, she says, are increasingly aware of the high-quality, high-resolution data achieved with NGS.

The PHG Foundation says that strategic planning and coordination will be necessary during this transition period to ready for widespread adoption. In addition to addressing systematic concerns (uniform protocols and analyses as well as standardized data sharing methods) and ease-of-use issues (low-cost data management and simplified bioinformatic interpretation and reporting), the economics of integrating the technology into medical care and public health systems must be evaluated.

Experts predict that WGS will be adopted initially for priority applications with specific pathogens. Identifying for which pathogens diagnostic and epidemiologic gaps exist with current microbiological techniques will likely improve care and prevent infections, but developing scenarios for how, when and where it is appropriate to introduce pathogen genomics into microbiology services will ultimately require an economic evaluation of the benefits, expenses, and potential cost-savings.

Technological advancements including culture-free sequencing directly from patient samples will further cut turnaround times, improving the actionability of the results from both a clinical care and public health perspective.

Takeaway: NGS is now poised for meaningful adoption in genomic microbiology, for public health surveillance in the community and in health care settings. With improvements in ease-of-use, particularly for the analysis component, the high-resolution data provided by WGS will improve pathogen detection with a universal test, with results that can inform both case management and infection control efforts.

Clinical Service for Microbiology Sequencing

Illumina (San Diego) and bioMerieux (France) have partnered to launch NGS epidemiology solutions for laboratories over the next four years. The first application, due out later this year, will be an NGS epidemiological service solution for genotyping disease agents. Illumina's high-resolution sequencing platforms, combined with bioMerieux's industry-leading microbiology library (based on culture collection), will provide easily-accessible, highly-accurate information for communities and hospitals to track, prevent, and contain the spread of disease agents, the companies say. The service will deliver a standardized report with a genomic profile of the infectious agents, with sequence-level accuracy and depth of information, including regarding virulence and microbial resistance characteristics. Similar to the federal sequencing surveillance strategy, clients will send the relevant isolates to a designated laboratory equipped with an Illumina sequencing system. The genetic data will be securely uploaded to Illumina's BaseSpace cloud platform for analysis and generation of a customized report.

With Technology Advances, Saliva Tests Hold Greater Promise

Advancements in proteomics and gene expression analysis are providing researchers sensitive enough tools to consider saliva as an ideal, noninvasive sample for a host of new diagnostic tests. Two recent studies highlight the potential of salivary marker analysis to potentially improve the diagnosis of autism spectrum disorder (ASD) and assess feeding readiness in premature babies.

Saliva is functionally equivalent to blood in terms of reflecting the physiological state of the body, according to a review of the emerging use of mass spectrometry for evaluation of salivary biomarkers, published in the January issue of *Clinica Chimica Acta*. It is estimated, the authors say, that saliva contains approximately 2,000 proteins of which 597 are also identifiable in the blood.

"With the fast development of mass spectrometry and proteomic technologies, saliva is a growing area for basic and clinical research with substantial potential for disease diagnosis," write the authors led by Qihui Wang from Sichuan University (China).

Proteomic Assessment for Autism-Related Markers

Protein markers found in saliva can differentiate children diagnosed with autism spectrum disorders (ASDs) from a group of controls, according to results of a pilot study published online ahead of print Jan. 24 in *Autism Research*. ASD diagnosis is currently based on behavioral assessments. But, experts say that the lack of definitive biomarkers hampers accurate diagnosis, treatment monitoring, and reliable prognosis. The emergence of more than 1,000 ASD-associated genes indicates, likely, multiple causes and subtypes, which may be further defined with the addition of protein-level analysis.

"With the fast development of mass spectrometry and proteomic technologies, saliva is a growing area for basic and clinical research with substantial potential for disease diagnosis."

—Qihui Wang

"ASD biological testing is already here in a sense, since gene arrays are being used increasingly, requested by parents and physicians," lead study author Alisa Woods, Ph.D., tells *DTET*. "The challenge is that often the tests do not come up with ASD-associated markers, and even when they do, it is not clear that this information alters treatment.... I think proteomic work needs to be more seriously considered from a basic science perspective, to supplement genomic data."

Woods, along with proteomics expert Costel Darie, Ph.D., analyzed the salivary proteome in six males (aged 5 years to 18 years) diagnosed with ASD and in six neurotypical control subjects without any major medical condition. Samples were processed both individually and in pooled analysis (to amplify the possibility of finding markers for quick initial screening). Using nanoliquid chromatography-tandem mass spectrometry, statistically significant differences were seen in several salivary proteins, including elevated levels of eight salivary proteins and lower levels of two proteins in the saliva of ASD patients, compared with the controls. Most of the proteins found dysregulated in the pooled samples were also found in the individual samples. Furthermore, there was not dramatic individual variability for most of the statistically significant, dysregulated proteins.

The authors say the findings support the possibility that immunological responses are present in some forms of ASD and could provide a first step toward a diagnostic test for ASD. Woods tells *DTET* larger validation studies are being planned. The published study was partially funded by Shire pharmaceutical company.

Gene Expression Assay for Preemie Feeding Readiness

A separate study, published in the February issue of the *Journal of Pediatrics*, has identified salivary biomarkers related to oral feeding success in premature babies. The markers noninvasively assess the mechanisms underlying the development of oral feeding readiness (a newborn's brain, sensory, and facial development) and may improve clinical decision-making, and possibly cutting length of stays.

"Compared with subjective cue-based feeding algorithms, this approach represents a significant advance to supplement clinical decision making."

—Jill Maron, M.D.

Prior to discharge, infants must demonstrate mature oral feeding skills, according to American Academy of Pediatrics guidelines. However, the determination of oral feeding readiness is a significant clinical challenge, as it is based on subjective assessments. Oral feeding delays and complications increase the risk for poor growth and developmental disabilities and are additionally tied to increased length of hospital stay and millions of dollars in health care expenditure the authors say.

In the present study, the Tufts University researchers utilized whole-transcriptome microarrays to compare saliva pre- and post-oral feeding success in 12 preterm newborns (postconceptional age [PCA] range at pre-success measurement, 33 5/7 to 36 2/7 weeks; post-success range 34 2/7 to 37 3/7 weeks). Gene expression biomarkers were selected from computational modeling (n=15); evidence-based (n=6); and reference markers (n=3). Then, 400 salivary samples were evaluated by reverse-transcription quantitative polymerase chain reaction amplification. Models assessed genes, alone and in combination, controlling for sex and PCA.

The researchers found that advancing PCA and female sex both significantly, positively predicted an infant's ability to feed orally. The five genes most predictive of feeding success represent sensory integration systems (NPHP4, PLXNA1), hunger signaling (NPY2R), facial development (WNT3), and energy expenditure (AMPK). A mature oral feeding pattern was predicted by negative expression of three genes (NPHP4, NPY2R, and WNT3), and positive expression of two genes (AMPK, PLXNA1). The combination of the five genes in addition to PCA and sex, demonstrated "good" accuracy for determining feeding success.

"Ultimately, these models have the potential to allow caregivers to develop individualized treatment modalities based upon each infant's gene expression profile to improve quality of care," write the authors led by Jill Maron, M.D., from Tufts University Medical Center (Boston). "Compared with subjective cue-based feeding algorithms, this approach represents a significant advance to supplement clinical decision making and establishes the foundation for the development of a point-of-care assay."

Maron tells *DTET* by incorporating the results of this low-cost test (approximately $50 to run) into feeding decisions, it would be "realistic" to expect a reduction in one day to three days in the NICU (average daily NICU stay $3,500) per child. First, further validation in a multicenter trial is necessary. In general, though, Maron says, salivary testing is particularly appealing in pediatric populations, where repetitive blood sampling is untenable in very low birth weight infants due to their low blood volume and is undesirable in young children.

Takeaway: While these tests are likely years away from clinical use, saliva represents an important specimen that is increasingly recognized for containing diagnostically significant markers of disease and human development. G2

Test Improves Prediction of Knee Arthritis Progression

Researchers have developed a tool to predict those at greatest risk for primary knee osteoarthritis (KOA) progression that incorporates using both genetic and clinical information, according to a study published online Jan. 7 in *Rheumatology*. By better identifying those likely to experience radiographic progression of joint degradation and the need for total knee replacement (TKR), this test may facilitate earlier diagnosis of joint destruction and enable early interventions intended to slow disease progression.

The clinical outcomes of primary KOA are highly variable," says lead author Francisco Blanco, M.D., in a statement. "Some patients may live for years without suffering significant loss of functional capability or evident radiologic progression. Instead, others may end up disabled or needing prosthetic surgery in a few years. In this, genetics play a key role: rapid progression of knee osteoarthritis is hereditary in 60 to 70 percent of all cases.

Most older individuals show signs of OA. But, OA diagnosis remains based on symptomatic and radiographic presentation. Yet, the clinical course is "highly variable" and mean annual total direct costs for OA patients can be substantial, the authors report. So better prediction of those at risk for rapid progression could aid in optimizing clinical management of the disease by increasing the number of control office visits, and utilizing more expensive imaging evaluations for high-risk patients, while allowing low-risk patients to continue their usual physical activity.

Spanish researchers conducted a retrospective, multicenter study involving 220 KOA patients in the exploratory cohort (180 females and 40 males; mean age 61.3 years) and a replication cohort of 62 KOA patients (47 females and 15 males). Participants had a Kellgren–Lawrence (KL) grade 2 or 3 at the time of primary KOA diagnosis, with "progressors" defined as those whose KL grade increased to 4 or who were referred for total knee replacement within 8 years after diagnosis. Saliva or serum samples were collected for DNA isolation and genotyping using real-time polymerase chain reaction.

Older age at KOA diagnosis was the only clinical variable analyzed that was significantly associated with KOA progression (88 progressors in the exploratory cohort and 37 in the replication group). The researchers identified 23 single nucleotide polymorphisms significantly associated with KOA severe progression in the exploratory cohort. The predictive accuracy of the clinical variables alone was limited, but when genetic variables (eight single nucleotide polymorphisms) were added to the model, prediction significantly improved (area under the curve, 0.66 versus 0.82). The predictive ability for KOA progression of the full model was confirmed on the replication cohort.

Bioiberica Pharma is developing this tool, the Arthrotest, which has been available clinically in Spain since June 2013 (cost roughly $276). The company tells *DTET* that they are developing plans for expanded commercial access in Europe and the United States, with a U.S. replication study planned for this year. Bioiberica funded the study.

Takeaway: By incorporating genetic variants into a model with clinical variables (i.e. age) clinicians may be able to better predict which patients are at greatest risk for KOA progression, thereby personalizing disease management.

G2 INSIDER

C. Diff Testing Transitioning as Cases Rise

The United States is at a "transition point" when it comes to diagnosing Clostridium difficile (C. diff), experts say. Complicating efforts to document the increasing burden of severe C. diff is the current migration of laboratories to more sensitive diagnostic tests for detection of the infection as well as the multitude of diagnostic testing strategies. Two recent studies highlight both the transition of testing approaches, as well as some of the remaining limitations on testing.

Experts say either multistep approaches using polymerase chain reaction (PCR) for the toxin gene(s) or single-step PCR on liquid stool samples have the highest sensitivity and specificity. Yet, testing is unable to differentiate asymptomatic colonization and symptomatic infection, leading experts to remind clinicians that diagnostic testing for C. diff infection should be performed only in symptomatic patients.

According to new data released by the U.S. Centers for Disease Control and Prevention (CDC) in a study published Feb. 26 in the *New England Journal of Medicine* (*NEJM*), C. diff caused almost half a million infections in the United States in 2011. A convenience sample of 37 clinical laboratories across the Emerging Infections Program (EIP) sites submitted all C. diff–positive stool specimens from cases with full medical-record review for culture, with recovered isolates undergoing pulsed-field gel electrophoresis. Isolates also underwent PCR assay. Additionally, all laboratories were surveyed to assess the type of C. diff diagnostic tests that they use. The CDC's incidence and burden estimates are based on use of nucleic acid amplification testing by 52 percent of labs, which was observed across EIP sites.

Researchers, who conducted a separate systematic review of the diagnosis and treatment of C. diff, found that diagnostic approaches are complex due to the availability of multiple testing strategies. The review, published in the Jan. 27 issue of the *Journal of the American Medical Association*, found that even with highly sensitive tests, testing can be time intensive and still provide data of limited utility, including the failure to distinguish between asymptomatic C. diff colonization and symptomatic infection, making test of cure futile.

"I think that the best way to sum it up is that we need to use better methods overall in diagnosing C-difficile and that includes the basics of determining who should be tested," said Clifford McDonald, M.D., from CDC's Division of Healthcare Quality Promotion, on a telebriefing. "If you use a very sensitive test, but are very selective in how you decide it should be used or who to test, it's a very good way to diagnose diseases, in general. If you use highly sensitive tests indiscriminately, you'll end up over diagnosing the infections."

Company References

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Illumina 858-202-4500

U.S. Centers for Disease Control and Prevention 800-232-4636

U.S. Food and Drug Administration 888-463-6332

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