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Ebola Test Development Continues, Despite Declines in Cases

Despite declines in current cases in Africa, interest remains high for development and validation for a rapid, point-of-care diagnostic test for Ebola. The federal government is funding test development, researchers are publishing results of proof-of-principle and field validation studies, and companies are continuing the process of securing regulatory approval for rapid tests.

In late September, OraSure Technologies (Bethlehem, Penn.) announced it received $7.2 million of continued funding from the Biomedical Advanced Research Development Authority for "clinical and regulatory activities" necessary to secure U.S. Food and Drug Administration (FDA) approval for its OraQuick Ebola Rapid Antigen test, which previously received emergency use authorization (EUA) from the FDA. The company simultaneously announced the U.S. Centers for Disease Control and Prevention is purchasing an additional $1.5 million worth of the test for further field testing. The antigen-based, fingerstick test is read visually and does not require refrigeration or instrumentation, making the test potentially, much more widely available than the current polymerase-chain reaction-based testing.

Expanded Purpose for Existing EUA Assays

The polymerase chain reaction- (PCR-) based assays currently granted EUA were intended for use with blood samples. However, recent evidence suggests the first case of transmission of the virus through the semen of survivors following extended periods after active infection.

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Efforts Expand Whole-Genome Sequencing for Rare Diseases

Multiple efforts are expanding the focus of sequencing, particularly whole-genome sequencing (WGS), for improving diagnosis of rare diseases. Likened to finding the proverbial needle in a haystack, identification of a single, rare, disease-causing mutation can bring families some semblance of closure—a genetic cause for the condition, if not a treatment option.

Families and medical experts are hopeful that a spate of recent announcements expanding access to sequencing-based evaluations for rare and un-

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Ebola Test Development Continues, Despite Declines in Cases, *Continued from top of p.1*

"The potential for sexual transmission of Ebola in West Africa has become a critical concern and the urgent need to screen survivors for the presence of Ebola virus RNA in semen has been realized," writes James Pettitt, from the National Institutes of Health, in a Sept. 15 paper published in the *Journal of Infectious Diseases*. The small study found that the EUA PCR assays perform equally well using semen instead of originally intended whole blood and plasma samples.

The researchers assessed the performance of the Ebola Zaire (EZ1) real-time- (RT-) PCR (TaqMan) assay using six seminal fluid samples spiked with live Ebola virus and six spiked whole blood samples. The EZ1 and Major Groove Binder quantitative RT-PCR assays were "demonstrably suitable," the authors say, and could be used to begin epidemiological studies of long-term survivors. They add that, in the future, determining the assays' performance for urine and saliva would also be "desirable."

New Test Development Continues

While expanded use of existing PCR-based assays is necessary, extensive efforts are underway to bring a rapid, POC Ebola test to the clinical setting.

> *"These findings suggest that the test could have had an immediate effect on patient care and infection control in our facilities by reliably detecting patients… whom would be expected to be highly infectious."*
>
> —Nira Pollock, M.D.

"Accurate rapid diagnostic tests, particularly those that can be completed at the point of care, could move the timing and place of diagnosis closer to the community in which a patient with suspected Ebola virus disease is first seen, halting transmission chains and allowing better allocation of scarce health care resources," writes Nahid Bhadelia, from Boston University, in an editorial published online June 26 in *The Lancet*.

In the same issue of *The Lancet*, researchers present the field validation results of the Corgenix (Colorado) ReEBOV Antigen Rapid Test kit. The dipstick immunoassay, which received World Health Organization and FDA EUA, had 100 percent sensitivity and 92 percent specificity in both POC and reference laboratory testing in adults and children with suspected Ebola in Sierra Leone (Feb. 3 to Feb. 20, 2015).

Testing was performed on samples of plasma or whole blood (collected by either fingerstick or venipuncture) and two independent readers scored results. Results were compared to clinical real-time PCR results (altona Diagnostics GmbH; Germany) tested in a Public Health England field reference laboratory. Additionally, the rapid diagnostic and PCR testing was performed on 284 specimens collected from many clinical sites throughout the country.

"We found that it was feasible to perform the test in the restricted so-called red zone; that inter-operator agreement at the point of care was high; [and] that results of POC fingerstick testing were consistent with the results of the rapid diagnostic test performed on venipuncture blood samples," writes senior author Nira Pollock, M.D., from Partners In Health (Boston). "These findings suggest that the test could have had an immediate effect on patient care and infection control in our facilities by reliably detecting patients with cycle threshold values of up to 26.3, all of whom would be expected to be highly infectious."

Takeaway: Despite waning attention being paid to the Ebola outbreak, academic researchers, industry, and the government remain committed to development and validation of a rapid, point-of-care Ebola virus assay. G2

Efforts Expand Whole-Genome Sequencing for Rare Diseases, *Continued from bottom of p.1*

diagnosed diseases can bring an end to years of unfulfilled diagnostic odysseys. In the United States, it has been estimated that 30 million people suffer from a rare disorder.

Seven additional clinical sites (Baylor College of Medicine, Duke Medical Center, Columbia University, Harvard teaching hospitals, Stanford Medical Center, University of California at Los Angeles Medical Center, and Vanderbilt University) have been added to the original Undiagnosed Diseases Program (UDP) at the National Institutes of Health's Clinical Center in Bethesda, Md. in mid-September. The NIH plans on using $145 million in Common Fund support over the next seven years for the expanded Undiagnosed Diseases Network (UDN). All applications for evaluation within the network will go through an online patient application portal, rather than through each individual clinical site. Sequencing will be performed at two core sequencing facilities—Baylor and HudsonAlpha Institute for Biotechnology (in partnership with Illumina).

The original UDP has enrolled more than 800 undiagnosed patients for one-week evaluation since its launch in 2008. Over the seven years, 25 percent of those have received some level of clinical, molecular or biochemical diagnosis, the NIH says. Of 160 patients who were admitted during the first two years of the service, diagnoses were made in 39 patients. Diagnoses included two previously un-described disorders, 23 rare or ultra-rare diseases in 28 patients, and nine common conditions, including fibromyalgia and multiple myeloma. The UDP estimates that approximately half of its diagnoses were made directly from agnostic genetic testing, such as whole exome sequencing (WES). The remaining diagnoses were made using targeted biochemical, radiologic, and molecular studies advised by rare disease specialists.

Rare Diseases Turn to Crowdfunding

The Rare Genomics Institute (RGI), a nonprofit that connects patients and families to a network of rare disease experts and an online crowdfunding mechanism, launched ten separate crowdfunding campaigns to enable patients to access WES in the hopes of finding a genetic cause of disease. The campaigns are part of Amplify Hope, an RGI study funded by the John Templeton Foundation, which provides crowdfunding training to families affected by rare disease and tracks the overall reach and scientific impact of the campaigns.

RGI reports that it has helped over 250 families to date. The majority of patients working with RGI who could not receive insurance coverage or obtain grant funding (83 percent) chose to crowdfund the cost of DNA sequencing. The organization says that all of these families who started crowdfunding reached their goal, with 64 percent of families exceeding their crowdfunding target. Crowdfunding partners for Amplify Hope include Ambry Genetics and Baylor Miraca Genetics Laboratories.

The hope is that with the additional clinical sites, more patients will be seen (50 per year per site by summer 2017) and sharing of variant information among the network sites will up the diagnostic yield. The principal investigators of the expanded network are looking to WGS and WES to provide "better answers" to these families who often go on decades-long, "diagnostic limbo."

"Although undiagnosed conditions present an array of challenges for clinicians, once identified, they may ... lead to new, generalizable medical insights," said James Anderson, M.D., Ph.D., from the NIH, in a statement.

HudsonAlpha, one of the core UDN sequencing sites, says it will sequence at least 1,600 genomes over the next three years while Baylor will sequence an equivalent number of exomes. The diagnostic yield of the two methods will be compared. HudsonAlpha says it hypothesizes that WGS will increase the diagnostic success rate 25 percent more than WES.

Efforts to improve diagnosis of rare diseases are occurring abroad as well. Chinese firm WuXi AppTec, which provides next-generation sequencing solutions to academics, the life science industry, and medical institutions, announced in September that WuXi NextCODE and its WuXi Genome Center, China's

only CLIA-certified clinical genomics lab, will provide sequence-based testing for cases of unresolved diagnosis at Children's Hospital of Fudan University (CHFU). CHFU sees 2.3 million patients annually.

"Using the emerging global standard for genomic data in medicine, we can move beyond static gene panels to employ the full power of the genome to address rare diseases, genetic and genetics-related disorders," said CHFU's President Guoying Huang in a statement.

Takeaway: Beyond the potential for genetic discovery, use of WES and WGS sequencing through expanded collaborative efforts is raising the hope of improved diagnostic yield for rare, undiagnosed diseases.

Geochemical Assay Proving Useful as Real-Time Marker of Bone Change

Changes in calcium isotope ratios may signal bone loss and can be used as a near-real time, rapid test for monitoring both common bone conditions, like osteoporosis, and rarer conditions, like multiple myeloma (MM), according to an abstract presented at Goldschmidt 2015, a geochemistry conference, (Czech Republic; Aug. 16-21). The proof-of-principle has been validated in a series of small studies on bedridden individuals, NASA astronauts in space, rats, and patients with MM.

This effort is a collaboration between biomedical researchers and geochemists, who have developed "extremely accurate" ways of measuring calcium isotope ratios. The relative ratios of the light calcium isotopes ($\delta^{44/42}Ca$) in bone can be determined using mass spectrometry. The rate of change of bone mass can be calculated by measuring the ratios of the two isotopes in blood or urine, as the light calcium isotopes are absorbed from the blood during bone formation and released into the blood during bone destruction.

"The big advantage of these measurements is that they show what is happening in the bone, whereas traditional bone health measurements, such as DXA scans, show what has happened," explains lead author Ariel Anbar, Ph.D., from Arizona State University (ASU), in a statement. Markers of short-term changes in bone metabolism may enable earlier disease detection and evaluation of treatment efficacy.

The researchers piloted the marker in bed-bound individuals prone to bone loss due to unloading and then evaluated urine samples from 30 astronauts (who experience rapid bone loss in space because of zero gravity conditions) before, during, and after space travel. Overall, the researchers found a systematic shift toward excretion of lighter Ca isotopes during

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Variant Identified With Large Effect on Bone Density, Fracture Risk

Researchers identified a novel, low-frequency, non-coding genetic variant that is associated with large effects on bone mineral density (BMD) and fracture risk in individuals of European ancestry, according to a letter published online Sept. 14 in *Nature*. What's more, the study, the largest population genome sequencing effort to date, demonstrates the value of whole-genome sequencing (WGS) in large populations to identify variants tied to complex traits and disease in the general population.

In the current population-based study the researchers conducted WGS in 2,882 participants from UK10K, whole-exome sequencing (3,549 participants in five cohorts), deep imputation of genotyped samples using a combined UK10K/1000 Genomes reference panel (26,534), and de novo replication genotyping (20,271).

A low-frequency non-coding variant near a novel locus, EN1, had an effect size four-fold larger than the mean of previously reported common variants BMD (which only account for a small fraction of BMD-related genetic variance) and a three-fold larger effect than those previously identified for fracture. EN1 was also associated with a significantly decreased risk of fracture (cases = 98,742 and controls = 409,511).

"These findings provide evidence that low-frequency, non-coding variants have large effects on BMD and fracture, thereby providing rationale for whole-genome sequencing and improved imputation reference panels to study the genetic architecture of complex traits and disease in the general population," write the authors led by Hou-Feng Zheng, from McGill University in Canada.

Inside The Diagnostics Industry

Experts Say Lab Industry Needs to Prepare for Adoption of Do-It-Yourself, Smartphone-Based Diagnostics

Like all other industries, the laboratory industry will feel the effects of the digital revolution. In some cases, digital technology will make operations more efficient and cut human errors by eliminating the need for written requisitions, faxes, and manual entry of test results. However, laboratories will be affected in previously unimagined ways too. Futuristic fantasies are rapidly becoming realities and within a few years experts predict patients will be able to perform clinical-grade testing in their homes through their smartphones.

At-home, smartphone-based testing will hasten the co-occurring trends of moving testing towards the patient and the move to using only micro-samples of blood, urine, and saliva. Through wirelessly enabled, handheld devices and smartphones with cameras and/or small, plug-in accessories, patients will be able to perform diagnostic testing (is it a viral cause of cold-like symptoms?) and monitor an expanded array of chronic conditions, much as diabetics have monitored their own glucose.

"Unimpeded by geographical boundaries, smartphone-linked wearable sensors, point-of-need diagnostic devices, and medical-grade imaging, all built around real-time data streams and supported by automated clinical decision–support tools, will enable care and enhance our understanding of physiological variability," predicts Eric Topol, M.D., from the Scripps Translational Science Institute, in a review published April 15 in *Science Translational Medicine*.

The National Institute for Health defines mHealth as the use of mobile and wireless devices to improve health outcomes, healthcare services, and health research.

Defining the Trend

Despite all of the excitement and investment ($2 billion in venture capital in digital health in the first half of 2015, according to Mercom Capital Group), mobile health (mHealth) remains in its infancy and lacks a universal definition. mHealth is a subset of the broader term digital health that includes price-comparison platforms and even physician rating sites. Clinical laboratory-type smartphone testing represents an even smaller, more nascent subset of mHealth, which can include health and wellness smartphone applications too. (This article will focus on user-enabled clinical laboratory-type testing on smartphones.)

mHealth is feasible today because of the convergence of personal computing power with mobile connectivity.

"The first supercomputer, the Cray-1, cost $5 million, weighed 5 tons, and used as much electricity as twenty homes and it could not even play a song, a game, or make a phone call. iPhones are 100 times more powerful than the Cray-1 and we carry them in our pocket or purse," says author John Patrick, D.H.A., formerly the vice president of Internet technology at IBM.

By all accounts smartphone-based testing is in the very early days, but enthusiasm for its potential is great. Consumer do-it-yourself testing, like all new things, experts say, will

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have nonuniform adoption initially. Some patients with chronic diseases, the "worried well," and some clinicians will enthusiastically embrace the convenience and real-time benefits of the technology, while others will be distrusting of the quality and utility of patient-generated data. High-quality evidence to support adoption is still needed and regulatory issues and business models are still being framed. Yet, experts are convinced, this movement will be upon us rapidly.

What does this mean for laboratories?

"Too often, studies of mHealth technology have been designed to answer the question 'How can 'these technologies' fit into existing systems of care?'" says Topol, who is the Gary and Mary West Endowed Chair of Innovative Medicine at Scripps. "The more appropriate question is 'How can systems of care be altered to best take advantage of 'these technologies?'" … The implementation of mHealth technologies only as adjuncts to existing systems of care likely will lead to results that fall well short of demonstrating their true impact."

"If I was a lab director, I'd say, it has happened to all of the other industries. I probably shouldn't ignore it. I can't fight it, so how can I participate?"

—John Patrick, D.H.A.

Now is the time for laboratories to develop a framework for ensuring these test results are used clinically and to define a role for themselves given the anticipated evolution of the industry. With high enough quality of data, payers' interest in these smartphone-based tests will grow as testing moves to "least-cost" settings.

"New innovations in the diagnostic world have lived within the reference laboratories," Chris Wasden, Ed.D., executive director of the Sorenson Center for Discovery & Innovation at University of Utah, tells *DTET*. "Diagnostics were performed in a clinical environment, processed by large organizations in batches, and results were sent to a clinician for interpretation. It hasn't been very consumer-oriented, but now all pressures are driving towards a consumer-centered model."

While the initial inclination is for laboratories to view do-it-yourself, smartphone-based testing as a competitive threat, experts say that laboratories really need to look for the opportunity.

"This is an unstoppable phenomenon," Patrick predicts. "Is health care immune? No. If I was a lab director, I'd say, it has happened to all of the other industries. I probably shouldn't ignore it. I can't fight it, so how can I participate?"

Industry leaders are beginning to envision what participation might look like.

"We serve as expert advisors in diagnostic testing. As clinical laboratory medicine expands to the point-of-care in professional health care settings and into the medical home, we will continue to be consulted as domain experts in the field," Sharon Geaghan, a professor of pathology at Stanford University, told the American Academy of Clinical Chemistry in an April 2014 interview on home-based specimen collection. "In addition to the analytic testing and interpretation of test results from home-based collection kits, there are new professional opportunities offered by an increase in home test collection.

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Laboratorians are essential to successfully implementing self-collection programs and designing community-based or patient-centered recruitment efforts to increase participation in … screening programs."

David Dean, associate director at Cambridge Consultants (developers of the home-testing Flow Health Hub), says that by pushing "smart point-of-care" tests to the patient, laboratories could free up capacity to do more innovative, high-value, complex testing that they may otherwise not have the capacity to perform. Other experts say that traditional laboratory and diagnostics companies' experience with the regulatory environment makes them a valuable partner to technology companies from outside of health care.

Sampling of mHealth Diagnostic Technologies

Cambridge Consultants: The Flow Health Hub enables clinical laboratory testing at home and can alert the patient's doctor if intervention is necessary. The Flow Health Hub consists of a reader and a set of color-coded, disposable cartridges capable of testing on blood, urine, and saliva. The user selects the appropriate cartridge (the blood one has an integrated lancet for a fingerstick test), takes a sample, and inserts the cartridge into the reader. The device uses microfluidic technology and can perform standard tests, such as lipid, metabolic, renal, and electrolyte panels. While still in the "concept" phase, the company is talking to potential partners.

LabStyle: In early September, the company was granted a patent that broadens the claims behind previously granted intellectual property for the Dario blood glucose monitor to now include testing of other bodily fluids through audio jack connections on smart phones and other digital devices.

OJ Bio: The company says its Xtalline device will be out next year and its first test will be in the area of infection control, measuring the protein inflammation marker CRP, which is currently being recommended as a front-line screen to guide appropriate antibiotic use. While pricing is not yet firm, the handheld reader will be priced similarly to over-the-counter blood pressure readers ($30 to $100), while the disposable chip will likely be priced around $2, depending on the application.

Scanadu: While the company's first ScanFlo product measures blood pressure, temperature, etc., Scanadu is now testing a low-cost, urinalysis platform that uses a smartphone to return test results on liver, kidneys, urinary tract, and metabolic functions. The ScanaFlo-Urine measures up to 12 reagents on iPhone-ready test strips. When the reagents react with urine, the results show up as colors on the stick and the iPhone app uses these colors to analyze the urine and report results. Scanadu came out of the X Prize Foundation's Qualcomm Tricorder competition and shortly after completed a record-breaking Indiegogo campaign that closed in July 2013 and raised almost $1.7 million in a month. Earlier this year the company raised $35 million in Series B financing.

Regulation

Back in February, the U.S. Food and Drug Administration (FDA) issued a guidance to clarify the subset of mobile apps it intends to regulate. Analysts say that the guidance offered the budding mHealth industry some needed clarity. It says approval will be needed for accessories to medical devices and accessories that transform mobile platforms into a regulated medical device.

"Regulation can be an obstacle, but it can save lives," says Patrick. "Think about it. If there is the slightest hiccup or bug on an iPhone, it is a big deal online. Shouldn't we expect the same high expectations for how a medical device should work?"

For smartphone-based laboratory tests, experts say there is now no question that FDA approval is needed. The only question is which pathway—reliance on a predicate device or filing a premarket submission for a novel device.

"The problem is that the FDA requires a predicate device to avoid a lengthier, costlier, approval process," Wasden tells *DTET*. "But, predicate devices don't exist. A few years ago we didn't have mobile phones acting as super-computers. We didn't have advanced microfluidics like we have today. We are doing things differently."

Takeaway: Despite concerns over security and the quality of patient-generated mHealth data, experts expect the technology to become more widely commercially available in the next several, so laboratories must act to become valued partners in adoption of this industry-altering technology.

■ Geochemical Assay Proving Useful as Real-Time Marker of Bone Change, *Continued from p.4*

unloading or space travel. The isotope ratio was further assessed (in collaboration with the Mayo Clinic) in 71 adult patients diagnosed with MM or asymptomatic precursor diseases. Patients with active disease had statistically significant lower mean $\delta^{44/42}$Ca than those with non-active disease.

"Physicians treating osteoporosis and other calcium disorders of bone, including multiple myeloma, have very few tools at their disposal to quickly determine whether the treatments they're providing are actually making a difference," says Scott Parazynski, M.D., former NASA astronaut, and current professor at ASU. "It's tremendous to see a sophisticated geochemical assay being translated into what could become a really significant medical diagnostic tool."

Takeaway: Shifts in the ratio of Ca isotopes may be an effective marker of short-term changes in bone composition and with further validation could play a role in evaluating progression of bone conditions and related treatment decisions. G2

Universal Screening Bests Family Hx to ID of Familial Hypercholesterolemia in Children

Family history alone is inadequate for identifying children with familial hypercholesterolemia (FH), according to a study published Sept. 15 in the *Journal of the American College of Cardiology*. A universal cholesterol screening program may be effective for earlier identification of these children with FH, who are at 100-fold elevated risk for cardiovascular complications in early adulthood without timely intervention.

Current recommendations call for screening children in the United States between nine and 11 years of age, although the exact form of screening (universal or based on family history) is not unanimously agreed upon. Prospective, epidemiological studies are lacking in children, so Slovenia's experience with universal screening (US) for hypercholesterolemia in 5-year-old children is "useful," experts say.

In the current study, 272 Slovenian children (mean referral age 7.3 years) were identified through US to have elevated total cholesterol (TC; higher than 6 mmol/l or higher than 5 mmol/l plus a family history positive for premature cardiovascular complications). These children subsequently were genotyped for variants in four genes associated with FH (LDLR, PCSK9, APOB [exon 26], and APOE).

The researchers found that more than half of the referred children (57 percent) carried disease-causing FH variants (38.6 percent in LDLR, 18.4 percent in APOB, and none in PCSK9). Among the participants without disease-causing variants, 18.7 percent were carriers of the hypercholesterolemia-associated APOE E4 isoform and 24.3 percent had conditions that remained genetically undiagnosed (a figure in line with other studies estimating polygenic or multifactorial hypercholesterolemia). Interestingly, in patients with no family history, variants in LDLR, APOB, or the APOE E4 isoform were seen in 48.6 percent, 60.0 percent, and 76.5 percent of children, respectively.

"A disease-causing genetic variant for FH was identified in 57 percent of participants referred from the US, with an additional one-fifth identified with the most common

"The primary focus of lipid screening in children should be on how to identify young individuals with genetic causes of dyslipidemia, such as FH."

—Stephen Daniels, M.D., Ph.D.

multifactorial form of hypercholesterolemia, amounting to [more than] 75.0 percent of referred participants carrying a disease-causing or disease-associated genetic variant," write the authors led by Gasper Klancar, from University Children's Hospital in Slovenia. "Comparable diagnostic yield in the adult population with clinical presentation of hypercholesterolemia and our pediatric population without any clinical signs indicates that universal screening may be an efficacious strategy for early detection of FH and prevention of cardiovascular complications."

"The primary focus of lipid screening in children should be on how to identify young individuals with genetic causes of dyslipidemia, such as FH ," writes Stephen Daniels, M.D., Ph.D., from the University of Colorado, in an accompanying editorial. "Even if one accepts that screening has merit, many questions remain, such as ... what is the best age for screening, which lipid measure is most useful, and what cut point would be used to indicate abnormality?"

The authors acknowledge future research will be necessary to determine the optimum timing and size of FH-associated gene panels, but they note that universal testing using next-generation sequencing (NGS) has some advantages including a "significant" cost reduction (approximately 3-fold decrease for the central European region) and faster identification of causative genetic variants (approximately 7-fold reduction in turnaround time based on their lab's experience with NGS versus more "widely used genetic screening methods" (denaturing high-pressure liquid chromatography or high resolution DNA melting analysis and Sanger sequencing).

Takeaway: Universal cholesterol screening in children may be a more effective means of identifying FH in a timely manner, than screening based on family history alone.

Genomic Data Improves Pediatric Cancer Management

Integrative clinical sequencing data can improve clinical management of pediatric oncology cases, just as it is improving adult care, according to a study published Sept. 1 in the *Journal of the American Medical Association*. Potentially actionable findings were found in 46 percent of pediatric cancer patients who had failed standard treatment and results informed clinical action (either change in treatment and family genetic counseling) for 25 percent of these patients. Delays in genomic analysis prevented further clinical actionability.

The authors say that this is the first prospective, pediatric study to assess the feasibility and utility of incorporating multiple, comprehensive sequencing technologies (whole-exome and transcriptome analysis) in cancer care. Studying sequencing in pediatric oncology is complicated by the lower mutation frequency associated with pediatric tumors, compared to adult cases. However, since mutation load increases after relapse, this study focused on kids with relapsed or refractory cancer (n=81), or rare cancer (n=21).

This study is an expansion of the MiOncoSeq program established in adult patients in 2011 and involved 102 consecutive pediatric cases (mean age 10.6 years; May 2012 to October 2014). Participants underwent integrative clinical exome (tumor and germline DNA; more than 150-fold average coverage) and transcriptome (tu-

mor RNA) sequencing. Results were discussed by an interdisciplinary precision medicine tumor board, which made recommendations. Mandatory preenrollment genetic counseling addressed the potential for incidental genetic findings. Mandated disclosure was required for findings that directly influenced the current cancer management strategy, but patients or parents could choose whether to receive incidental results associated with high risk of hereditary cancer syndromes in patients and other family members (89 percent agreed).

"Thirty-six percent of patients exhibited a driving gene fusion, which indicates a potential role of including RNA sequencing . . . in the work-up of individuals with cancer."

—Rajen Mody, M.B.B.S.

The researchers found that 91 of the 102 patients had adequate tumor tissue for sequencing. Forty-two patients had actionable findings that changed their cancer management (15 of 28 with hematological malignancies and 27 of 63 with solid tumors), including change in treatment for 14 patients overall. The authors acknowledge that the lack of a control group limited assessment of whether individualized treatment improved clinical outcomes beyond standard care. All nine patients and families with actionable incidental genetic findings agreed to genetic counseling and screening, including four of these families with no familial cancer syndrome history that would have otherwise precluded referral for cancer genetics counseling.

"Thirty-six percent of patients exhibited a driving gene fusion, which indicates a potential role of including RNA sequencing (transcriptome sequencing), in addition to whole-exome analysis, in the work-up of individuals with cancer," write the authors led by Rajen Mody, M.B.B.S., from the University of Michigan, Ann Arbor. "Furthermore, the presence of actionable germline findings in 10 percent of patients suggests a role for matched normal sequencing and mandatory genetic counseling in the management of cancer when children and young adults are diagnosed."

From a practical standpoint, the median turnaround time (TAT) from study enrollment to case presentation at the precision medicine tumor board was 53 days (longer than expected). The authors say that waiting for bioinformatics analysis, as well as the next tumor board, were the primary causes of the delay, which did impact the providers' ability to take clinical action in some cases. The authors note that TAT improved from a mean of 60 days for the first 51 patients to 48 days for the second half. The actual costs of integrative clinical sequencing were estimated to be $6,000 per patient including supplies, labor, and bioinformatics analysis.

"Although comprehensive sequencing is clearly optimal for discovery efforts, gene panel next-generation sequencing strategies that provide robust depth of coverage (necessary to detect potentially important subclonal events) and that can now be manufactured to cover the majority of known gene-fusion events have significant advantages as a companion diagnostic when the goal of the assay is to promptly assign therapy because results generally can be returned in less than two weeks ," writes co-author Robert Schnepp, M.D., Ph.D., from the University of Pennsylvania (Philadelphia), in an accompanying editorial. "There is clearly no single best technology, and the field will likely adapt a hybrid approach to address practical, clinical, and financial pressures."

Takeaway: Sequencing results can inform pediatric cancer care. Key components to pediatric implementation include genetic counseling, RNA sequencing, and matched tumor-germline sequencing. Advances in pediatric clinical drug trials will further aid actionability of findings. G2

Exome Sequencing Comparable to Microarray for Autism Diagnosis, Higher Yield Seen With Appropriate Patient Selection

Diagnostic yields of whole-exome sequencing (WES) and chromosomal microarray analysis (CMA) are comparable among a heterogeneous sample of children with autism spectrum disorder (ASD), according to a study published September 1 in the *Journal of the American Medical Association*. The combined molecular diagnostic yield of the two technologies was significantly higher in children with more complex morphological phenotypes. The authors say these findings may inform appropriate selection of molecular diagnostic testing for children affected by ASD.

Currently, CMA is the recommended first-tier genetic test for individuals with ASD, with a documented diagnostic yield ranging from 7 percent to 9 percent, while WES is still primarily used in research settings.

In the current study, CMA (for detection of copy-number variants) and WES (for single nucleotide mutation detection) were performed in a heterogeneous group of unrelated children with ASD (all 258 participants for CMA and 95 randomly selected probands with samples from both parents for WES). Targeted-sequencing was conducted in cases of suspected syndromes. The children also underwent detailed assessments to determine the presence of major congenital abnormalities and minor physical anomalies and probands were phenotypically stratified by morphological severity. The complex phenotype was defined as the presence of multiple minor physical anomalies.

"Our data suggest that medical evaluation of ASD children may help identify populations more likely to achieve a molecular diagnosis with genetic testing."

—Kristiina Tammimies, Ph.D.

The researchers found that the majority of the participants (69.4 percent) were classified as an essential phenotype (no or few physical anomalies), 19.8 percent had equivocal dysmorphology, and 10.9 percent were complex. The diagnostic yield of CMA was 9.3 percent (24 of 258) versus 8.4 percent (8 of 95) from WES (mean coverage depth of 108×; in 9 ASD susceptibility genes, some of which are known to have variable expressivity and penetrance). Among the children who underwent both forms of testing, the estimated proportion with an identifiable genetic etiology was 15.8 percent (15 of 95 children). The yields were statistically different between the morphological groups, with the combined yield significantly higher in the complex group versus the essential group, indicating a higher burden of de novo events in syndromic children. Additionally, incidental or medically actionable findings were reported for 8.4 percent of the participants.

"Our data suggest that medical evaluation of ASD children may help identify populations more likely to achieve a molecular diagnosis with genetic testing," write the authors led by Kristiina Tammimies, Ph.D., from the Hospital for Sick Children in Canada. "Based on analysis of the combined diagnostic yield of CMA and WES, we estimate that more than 35 percent of ASD children with additional medical and dysmorphology features might be able to receive a molecular diagnosis. In contrast, only 6.0 percent of ASD children without syndromic features received a molecular diagnosis."

Several authors report financial ties to SynapDx, which is developing a blood-based test for ASD, but was not involved in this study.

Takeaway: While genetic testing in children with ASD is expected to increase in the coming years, these findings provide initial evidence that appropriate selection of children affected by complex ASD could substantially improve the diagnostic yield. G2

G2 INSIDER

Nutrigenetic-Based Diet Doesn't Increase Weight Loss, but Adherence May Play Role

Dieting is hard. Some have hoped that genetically tailored diets may make weight loss efforts more effective. But, a small study of 51 obese and overweight adults suggests nutrigenetic-guided diets offer no advantages over standard, balanced diets. However, according to a study published in the September issue of *Clinical Gastroenterology and Hepatology*, genetic insights may identify individuals most likely to benefit from a traditional, balanced diet weight loss strategy.

Previous genome-wide association studies have identified more than 30 loci associated with obesity, but data remains scarce as to how these genetic variations impact weight loss or metabolic profiles. Yet, there are commercially-available, direct-to-consumer tests making dietary recommendations.

In the current study, participants were randomized to either a nutrigenetic-guided diet (balanced, low-carbohydrate, low-fat, or Mediterranean; n = 30) or a standard balanced diet (n = 21). The Pathway FIT test (Pathway Genomics) was used to make diet selection in the genotype-guided therapy (GT) group.

The researchers found that at eight weeks and at 24 weeks there was no significant difference between the groups in the percentage of participants who lost 5 percent of their body weight (35.0 percent for GT versus 26.9 percent for the standard diet). Further, there were no differences in improvements in biomarkers related to metabolic disease (lipid profile or glucose homeostasis). Both groups had difficulty adhering to the diets. However, adherence to the GT diet correlated with weight loss, while adherence to the standard diet was not tied to weight loss.

"The problem with nutrigenetic-based personalized diet therapy is that recommendations to alter dietary intake remain a poor treatment for obesity because of non-adherence," writes lead author Karen Frankwich, from University of California, San Diego. (Several authors report financial ties to Pathway Genomics, which funded the study). "Even when given their nutrigenetic information with guided education regarding their nutrigenetic-based diet, GT participants were no more adherent to their diet than those in the standard therapy group."

Nutrigenetics, the authors say, may still play a role in predicting which individuals would benefit from lifestyle modification and dietary intervention. For those randomized to the balanced diet group based on nutrigenetics, 100 percent of participants lost at least 3 percent of their body weight, compared to 50 percent of participants genotyped to other diets.

The authors acknowledge the likely continued growing interest in genetic-guided therapies for obesity specifically and disease globally. Adoption of nutrigenetics for obesity care must still overcome the practical barriers of cost and non-standard reporting, but the main hurdle for nutrigenetic-guided personalized therapy, they say, is the overall lack of effective remedies for obesity. G2

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