G2 INTELLIGENCE
A DIVISION OF PLAIN LANGUAGE MEDIA

DIAGNOSTIC TESTING & Emerging Technologies

New Trends, Applications, and IVD Industry Analysis

May 2018

INSIDE THIS ISSUE

www.G2Intelligence.com

Profound Discrepancies Seen Between DTC Genetic Results, Confirmatory Testing

Raw data from direct-to-consumer (DTC) genetic tests have an "alarmingly high" false-positive rate, according to a study published March 22 in Genetics in Medicine. Additionally, the study found that misinterpretation of variant risk, coming from both DTC companies and third-party interpretation services. Together, these inaccuracies could have potential clinical impact, highlighting the importance of confirmatory clinical-grade testing for DTC raw variants, as well as results interpretation by health care professionals with genetic training, the authors say.

Continued on page 2

CDC Receives Funding to Expand Laboratory Harmonization Efforts

The FY 2018 omnibus appropriations bill, passed by Congress in late March, included a little noticed provision that could ultimately bring significant changes to the laboratory industry—$2 million for the U.S. Centers for Disease Control and Prevention (CDC) to expand its laboratory test harmonization efforts.

The bill "recognizes that certain clinical laboratory tests need harmonization to ensure that accurate results are available for correct patient care" and provided the money to the CDC's Environmental Health Laboratory to improve standardization for hormone testing, including specifically, thyroid stimulating hormone, testosterone, and estrogen.

The CDC will provide materials to and monitor laboratories and manufacturers across the country to improve the accuracy and precision of these hormone tests, ultimately, improving diagnosis for polycystic ovary syndrome, hypothyroidism, chronic kidney disease, and osteoporosis.

"We at [the] American Association for Clinical Chemistry [AACC] are thrilled that Congress has provided this funding for an expand-

Continued on page 9

Profound Discrepancies Seen Between DTC Genetic Results, Confirmatory Testing, *from page 1*

"While having access to raw genotyping data can be informative and empowering for patients, this type of information can also be inaccurate and misinterpreted," says lead author Stephany Tandy-Connor, from Ambry Genetics (Aliso Viejo, Calif.).

> *"The misinterpretation and potential inaccuracy of the raw data pose substantial risks to individuals who obtain this type of information from a DTC company."*
>
> — Stephany Tandy-Connor

For years concerns have been raised about the accuracy and methods of DTC testing. Central to these concerns are DTC tests' lack of comprehensive variant assessment. For example, 23andMe's genetic health risk test reports on one variant in the LRRK2 and GBA.3 genes linked to Parkinson disease. However, experts say there are additional known pathogenic variants in these two genes as well as additional genes clinically associated with Parkinson disease (like SNCA and PARK2/PARKIN.4) that 23andMe does not report on. Additionally, some DTC companies provide customers their raw genotyping data that patients can provide to third-party interpretation services for a fee. The comprehensiveness of that analysis may not be known or understood by the patient. In contrast, the authors of the Genetics in Medicine study say, clinical diagnostic tests are generally comprehensive—examining the full coding sequences of all known genes associated with a disease.

"Many of the DTC genetic testing laboratories use a form of single-nucleotide polymorphism genotyping array for their assay. This particular methodology is analogous to spot checking an individual's DNA with coverage at only specific preselected sites. This is not comprehensive full-gene sequencing nor does it include gross deletion or duplication analyses, which are both routinely part of clinical diagnostic testing.

The present study investigated the concordance between confirmatory clinical test results and variants identified in the raw data by DTC genetic testing of 49 patients referred to Ambry Genetics between January 2014 and December 2016. Medical geneticists/genetic counselors and oncologists accounted for the majority of confirmatory test orders (40.8 percent and 20.4 percent, respectively).

Testing of cancer genes comprised 87.8 percent of the orders, with submissions made for 26 unique variants, including four located within deep intronic regions that Ambry categorized as "well beyond the analytical range of most clinical laboratories." Confirmatory testing was performed by Sanger sequencing for single-site analysis (44.9 percent) or next-generation sequencing analysis with Sanger confirmation for samples received for multigene panels.

The researchers found that 40 percent of variants reported in DTC raw data were false positives and 16 of 17 of these false positives were in cancer-related genes. These false positives were seen in a variety of genes, including BRCA1/2, CHEK2, TP53, ATM, MLH1, and COL3A1.

DTET

Lori Solomon, Editor

Glenn S. Demby, Contributing Editor

Catherine Jones, Contributing Editor and Social Media Manager

Barbara Manning Grimm, Managing Editor

David van der Gulik, Designer

Randy Cochran, Corporate Licensing Manager

Myra Langsam, Business Development

Michael Sherman, Director of Marketing

Jim Pearmain, General Manager

Pete Stowe, Managing Partner

Mark T. Ziebarth, Publisher

Diagnostic Testing and Emerging Technologies (ISSN 2330-5177) is published by G2 Intelligence, Plain Language Media, LLLP, 15 Shaw Street, New London, CT, 06320.
Phone: 888-729-2315
Fax: 855-649-1623
Web site: www.G2Intelligence.com.

In addition, eight variants in five genes (ATM, BRCA1, BRCA2, COL3A1, and COL5A1) that were reported in the "increased risk" category in DTC raw data or by a third-party interpretation service were classified as benign, common variants at Ambry Genetics as well as several other clinical laboratories, based on their presence in in publicly available population frequency databases.

"The misinterpretation and potential inaccuracy of the raw data pose substantial risks to individuals who obtain this type of information from a DTC company," writes Tandy-Connor and colleagues. "It is crucial that clinical confirmatory testing be performed on any variants reported in the raw data provided by a DTC company prior to any changes in medical management to confirm the presence of that variant in the individual as well as an accurate classification."

Takeaway: This study provides some of the first evidence of the inaccuracies of DTC genetic testing based on raw genetic data and variant classification.

Fecal Profiling Could Personalize Diets with Irritable Bowel

Fecal profiling of volatile organic compounds (VOC) may predict response to dietary interventions in patients with irritable bowel syndrome (IBS), according to a study published in the March issue of *Clinical Gastroenterology and Hepatology*. The authors say that VOC profiling is a low-cost, noninvasive tool that may enable personalized treatment options for IBS patients.

While gut microbiota may be related to pathogenesis and severity of IBS, microbiota is currently an unrealistic diagnostic target or marker of treatment outcome in practice because of the costs of its measurement, the authors say. However, some evidence shows that the fecal metabolome can provide insight into the pathophysiology of IBS.

In this study, 93 patients were randomized to both a diet intervention (sham or a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols diet [LFD]) and supplement (placebo or probiotic). Fecal samples were collected at baseline and end of the 4-week study period. VOCs were analyzed using gas chromatography.

The researchers found that VOC patterns showed clear separation between-responders and non-responders to both LFD and probiotic at both baseline and end-of-treatment. There were 15 features in the VOC profiles at baseline that predicted response to the low-FODMAP diet with a mean accuracy of 97 percent. At baseline, there were 10 features that classified response to probiotic with a mean accuracy of 89 percent. Results were similar with end-of-treatment analysis.

"In addition to the predictive nature of the baseline VOC patterns, the end-of-treatment models may provide additional insight into the pathophysiology of symptom generation and mechanisms underpinning dietary intervention." write the authors led by Megan Rossi, from King's College London (United Kingdom).

Takeaway: Fecal profiling of VOCs is a low-cost, noninvasive tool that may enable personalization of dietary treatment options for IBS patients.

Genetic Risk Score Could Improve Diagnosis of Atrial Fibrillation

A genetic risk score (GRS) may identify patients at highest risk for developing atrial fibrillation (AF), according to a study published March 13 in *PLOS Medicine*. The authors say that patients with a high GRS were three times more likely to be diagnosed with AF through use of ambulatory cardiac rhythm monitoring in the two weeks following presentation of symptoms, compared to those with a low GRS.

> *"The ability to combine common single nucleotide polymorphisms [SNPs] into an AF GRS with the ability to differentiate a greater than 3-fold increased risk of AF in a population on a prospective basis may be useful."*
>
> — Evan Muse

AF is the most common heart rhythm disturbance and substantially increases the risk of stroke. Efforts to better diagnose subclinical, asymptomatic AF could lead to initiation of preventive measures that would reduce stroke risk.

The study included 903 patients aged 40 years of age or older presenting with symptoms of AF and at least one clinical risk factor for AF. Participants subsequently underwent genetic testing and ambulatory cardiac rhythm monitoring for two weeks. The AF GRS was based on the weighted contribution of 12 single nucleotide polymorphisms (minor allele frequency of more than 5 percent) that previously have been determined to be associated with AF at a genome-wide significance level.

Over the two-week study period, approximately 9 percent of the patients had an AF episode. Participants in the highest quintile of AF GRS were more than three times more likely to have had a recorded AF event than participants in the lowest quintile, even after adjusting for age, sex, smoking status, body mass index, hypertension, diabetes mellitus, heart failure, and prior myocardial infarction.

"The ability to combine common single nucleotide polymorphisms [SNPs] into an AF GRS with the ability to differentiate a greater than 3-fold increased risk of AF in a population on a prospective basis may be useful," write the authors led by Evan Muse, from the Scripps Translational Science Institute in La Jolla, Calif. "Such a panel of SNPs could be assayed at low cost and be used in conjunction with an evaluation of a patient with possible AF."

Takeaway: This prospective validation of a GRS may enable tailoring of diagnostic strategies in patients presenting with AF symptoms.

Pap Test Fluids May Contain DNA Useful for Endometrial, Ovarian Cancer Diagnosis

Cervical fluid samples gathered during routine Papanicolaou (Pap) tests can potentially be used for screening test women for endometrial and ovarian cancers, according to a study published March 21 in *Science Translational Medicine*. While the PapSEEK test needs validation in a large, prospective study, the authors are hopeful that the test's ability to detect DNA mutations associated with gynecological cancers can lead to earlier detection and, hopefully, improved outcomes.

Previous studies have shown that endometrial and ovarian cancers shed cells that collect at the cervix, thus making DNA samples feasible to be collect during routine Pap testing.

In the present study, researchers from Johns Hopkins University analyzed 1,958 samples collected from 1,658 women, including 658 endometrial or ovarian cancer patients and 1,002 healthy controls. Pap brush samples were obtained from 382 endometrial cancer patients and 245 ovarian cancer patients. Additionally, samples using a Tao brush, which extends further into the cervical canal and collects cells closer to where the cancers could originate, were collected in 123 endometrial cancer patients and 51 ovarian cancer patients studied. Analysis of samples examined mutations in 18 genes, which are highly or commonly mutated in endometrial or ovarian cancers, and aneuploidy using a polymerase chain reaction–based, multiplex test.

The researchers found that in Pap brush samples from endometrial cancer patients, 81 percent were positive, including 78 percent of patients with early-stage disease. The sensitivity of Pap brush samples in ovarian cancer patients was lower—33 percent, including 34 percent of patients with early-stage disease. For healthy controls, the PapSEEK test was positive in 1.4 percent of women (specificity, approximately 99 percent).

Intrauterine sampling with a Tao brush increased the detection of malignancy compared to the endocervical sampling with a Pap brush to 93 percent of patients with endometrial cancer and 45 percent of patients with ovarian cancer. There were no false positives (specificity, 100 percent).

The authors say the cost of a PapSEEK test would be more than the cost of a Pap test, but comparable to other screening modalities including colonoscopy and mammography.

Takeaway: Researchers are hopeful that fluids obtained during routine Pap testing may contain DNA shed from endometrial and ovarian cancers, thus enabling earlier detection of these gynecological cancers.

Artificial Intelligence Merges Histology, Genomics to Predict Survival

A deep learning computational approach can predict survival with brain tumors directly from digital histological images and genomic biomarkers, according to a study published online March 12 in the *Proceedings of the National Academy of Sciences*. The so-called survival convolutional neural networks (SCNNs) show accuracy that surpasses the current clinical paradigm for predicting the overall survival of patients diagnosed with glioma, the authors say.

Human assessments of histology, while an important tool in cancer diagnosis and prognostication, are highly subjective. Machine learning or artificial intelligence has emerged as an important image analysis tool for medical imaging.

The present study explains the SCNN approach, which combines deep learning with traditional survival models to identify survival-related patterns from histology images.

Continued on page 8

INSIDE THE DIAGNOSTICS INDUSTRY

Genotyping to Guide Antiplatelet Selection Feasible, Effective

Despite years of frustration that pharmacogenomic testing for antiplatelet therapy dosing has not been adopted as hoped in the field of cardiology, new evidence is emerging that genetic testing should be employed to guide therapy decisions.

CYP2C19 testing has not been adopted in widespread practice due to conflicting recommendations for its use, as well as uncertainty over its effectiveness its real-world feasibility to inform treatment decisions in a timely matter. Several recent studies are providing evidence to overcome these concerns.

Importantly, the recent studies show that genetic test results are significantly influencing cardiologists' treatment decisions, which may ultimately improve patient outcomes.

Genotyping Improves Patient Outcomes

Patients who receive genotyping to guide the choice of antiplatelet therapy are significantly less likely to experience a primary endpoint event—a composite of myocardial infarction, stroke, cardiovascular death, and major bleeding—compared with patients who did not receive the genetic test, according to the Italian PHARMCLO trial, published March 11 in the *Journal of the American College of Cardiology*, in conjunction with the American College of Cardiology (ACC) 2018 Annual Scientific Session.

Experts say the use of more potent antiplatelet drugs—prasugrel and ticagrelor—involves a fundamental trade-off between decreasing the risk of ischemia, but increasing the risk of bleeding. Experts believe this trade-off can be mitigated by adding genetic information to clinical decisionmaking.

Patients hospitalized for acute coronary syndromes were randomly assigned to standard of care (n=440) or the pharmacogenomic arm (n=448), which included the genotyping of ABCB1, CYP2C19*2, CYP2C19*17 using an ST Q3 system that uses real-time polymerase chain reaction technology at the point of care to provide results within 70 minutes at each patient's bedside. Treatment decisions were informed by a genetic algorithm that was designed to consider the three genes simultaneously, although the ultimate therapy decisions were left to the discretion of the physicians. In the standard of care group therapy decisions were based upon clinical considerations only. The patients were followed up one year.

Genotyping showed that 29.2 percent of patients had at least one copy of the loss-of-function CYP2C19*2 allele and 31.3 percent had at least one copy of the gain-of-function CYP2C19*17 allele. Genotyping significantly changed treatment decisions with clopidogrel was more significantly frequently in the standard of care arm, while ticagrelor was used significantly more frequently in the pharmacogenomic arm. Prasugrel was equally used in both arms. The primary endpoint occurred significantly more often in the standard of care arm versus the pharmacogenomics

> *"These recent studies nicely highlight the potential value of genotyping in personalizing antiplatelet therapy."*
> — Deepak L. Bhatt, M.D.

arm (25.9 percent versus 15.9 percent). Additionally, ischemic and bleeding endpoints occurred significantly more frequently in the standard of care arm.

"This prospective, randomized multicenter study provides evidence that the use of genomic medicine to select P2Y12 receptor antagonists can be successfully incorporated into the clinical care of patients with acute coronary syndromes," write the authors led by Diego Ardissino, M.D., from Azienda Ospedaliero-Universitaria di Parma, Italy. "This may be considered one of the most challenging clinical settings in which to use pharmacogenetic data to guide clinical practice because of the urgency of the situation and the need to start drug treatment promptly. In our case, this was made feasible by the development of a bedside instrument capable of providing genotype results within 70 minutes of blood sampling."

However, the Ardissino had to urge caution, as the trial was stopped after enrolling approximately one-quarter of the planned 3,600 patients. At the ACC meeting Ardissino explained that ethical committees decided to stop the trial because of the lack of in vitro diagnosis certification for the genetic testing instrument used, despite the same committee's initial approval of the study.

Despite the fact they had a far smaller sample than planned, the PHARMCLO trial still showed quite a large beneficial effect on outcomes in the genetic tested group.

"These recent studies nicely highlight the potential value of genotyping in personalizing antiplatelet therapy," Deepak L. Bhatt, M.D., from Brigham & Women's Hospital in Boston, Mass., told Medscape Medical News at ACC. "There are a number of larger ongoing trials, such as TAILOR PCI, that will need to provide greater clarity to the field before we routinely change practice."

Testing is Feasible

In addition to mounting evidence that CYP2C19 genotype–guided antiplatelet therapy strategy is effective, it is also feasible and sustainable in a real-world clinical setting, according to a study published April 3 in *Circulation: Genomic and Precision Medicine*. Additionally, the study found a higher risk of major adverse events tied to use of clopidogrel in CYP2C19 loss-of-function (LOF) allele carriers, suggesting that use of genotype-guided prescribing may improve clinical outcomes.

Clopidogrel plus aspirin remains one of the most commonly prescribed blood thinners, but it is recognized that CYP2C19 loss-of-function alleles impair its effectiveness. Yet, there remains considerable debate about whether CYP2C19 genetic testing should be used clinically to guide antiplatelet therapy selection in patients undergoing percutaneous coronary intervention.

The University of North Carolina Cardiac Catheterization Laboratory implemented a clinical algorithm that recommends CYP2C19 testing in high-

INSIDE THE DIAGNOSTICS INDUSTRY

risk patients and alternative dual antiplatelet therapy (DAPT; prasugrel or ticagrelor) in LOF allele carriers. The study assessed use of the algorithm and subsequent clinical outcomes in 1,193 patients who underwent percutaneous coronary intervention (July 1, 2012 to June 30, 2014).

The researchers found that the median time from genotype order to return of results was 1 day, with 75 percent of results available by the day after the procedure. CYP2C19 genotyping was performed in just under three-quarters of patients (72.8 percent). However, the frequency of genotype testing significantly varied over time. The authors note that it is unclear what specific factors contributed to the fluctuations in fidelity.

Among genotyped patients, just under one-third of patients (30.2 percent) carried one or two LOF alleles. Alternative DAPT was prescribed in approximately 71 percent of LOF allele carriers. Like genotype testing, use of alternative therapy in CYP2C19 intermediate and poor metabolizers varied significantly over time. The authors note that while recurrent clinician education was used, automated clinical decision support within the electronic health record (EHR) system to alert clinicians about the genotype result was not available during the study period.

Clinical outcomes were assessed in 999 patients with available 1-year follow-up. There was a significantly higher (four-fold) risk for major adverse cardiovascular or cerebrovascular in LOF carriers prescribed clopidogrel versus those prescribed alternative DAPT. But, bleeding event rates were similar across groups.

"The feasible implementation and sustainable use of a genotype-guided algorithm … was possible because of several key factors that alleviated logistical barriers," write the authors led by Craig Lee, Pharm.D., Ph.D., from University of North Carolina, Chapel Hill. "Notably, in-house genotype testing with prompt turnaround of results in the EHR, and interdisciplinary collaboration and communication among physicians, clinical pharmacists, and nurses have proven critical."

Takeaway: While several large studies are still ongoing, implementation of a CYP2C19 genotyping strategy to guide antiplatelet selection is feasible and appears to have clinically meaningfully impact on outcomes.

Artificial Intelligence Merges Histology, Genomics to Predict Survival, *from page 5*

Hematoxylin and eosin stain-stained tissue sections are first digitized to whole-slide images. These images are reviewed using a web-based viewer to identify regions of interest that contain viable tumor with certain histologic characteristics. An image sampling and risk filtering technique significantly improves prediction accuracy by minimizing the effects of intratumoral heterogeneity. High-power fields are sampled from these regions of interest and are used to train the neural network to predict patient survival. (SCNNs recognize important structures, like microvascular proliferation, that are used by pathologists in grading and prognosis.) Predictions are compared with patient outcomes to adaptively train the network.

The SCNN approach was validated by predicting overall survival in gliomas using data from the Cancer Genome Atlas. SCNN predictions were highly correlated with both molecular subtype and histological grade and were consistent with expected patient outcomes.

"SCNN can effectively discriminate outcomes within each molecular subtype, effectively performing digital histologic grading," write the authors led by Pooya Mobadersany, from Emory University in Atlanta, Ga. "Using visualization techniques to gain insights into SCNN prediction mechanisms, we found that SCNNs clearly recognize known and time-honored histologic predictors of poor prognosis and that SCNN predictions suggest prognostic relevance for histologic patterns with significance that is not currently appreciated by neuropathologists."

Takeaway: These results suggest a role for artificial intelligence in precision medicine that will expand the use of computational analysis in the field of pathology.

CDC Receives Funding to Expand Laboratory Harmonization Efforts, *from page 1*

ed CDC harmonization program," said AACC CEO Janet B. Kreizman, in a statement. "Patients and their physicians should be free to think about the clinical implications of test results and not about whether differences in test results are due to different labs performing those tests."

The AACC had been actively working to advocate for harmonization funding by leading efforts to raise awareness in Congress.

Test harmonization enables comparison of test results over time even if patients switch providers or laboratories switch out equipment. Advocates believe standardizing test results will ultimately reduce health care costs and medical errors by eliminating unnecessary follow-up diagnostic procedures and treatments.

To date, one of the most prominent harmonization efforts focused on cholesterol testing, which the CDC began in the 1980s. AACC says the CDC's Lipids Standardization Program generated savings ranging from $338 million to $7.6 billion per year since its inception. But the group says that "very few lab tests" have been harmonized in the same way.

But as medicine moves more towards evidence-based practice, with a focus on clinical use of practice guidelines, harmonization becomes more important.

AACC, with the support of 18 associations (American Association for Clinical Chemistry, American Clinical Laboratory Association, College of American Pathologists), laboratories (ARUP Laboratories, LabCorp, Mayo Medical Laboratories, and Quest Diagnostics), and industry (Roche Diagnostics, Thermo Fisher Scientific) are calling that 2019 appropriations include an additional $9.2 million for CDC to continue to expand harmonization efforts.

Takeaway: The $2 million allocation to the CDC its laboratory test harmonization program may just be the beginning of standardization efforts.

Methods for Reanalysis of Exome Sequencing Emerging

Clinical whole-exome sequencing (WES) offers diagnosis for approximately 30 percent of patients, but it has exponentially increased the number of variants identified—many of which are of unknown clinical significance. The continuous evolution of genomic knowledge necessitates reanalysis of exome sequencing results over time.

Automated bioinformatics pipeline may enable providers to efficiently and effectively characterize annotation changes, supplement existing genomic results with these changes, and filter the results for changes of interest.

Despite recognition that reanalysis with the latest variant databases may enable increased diagnostic yield, reanalysis poses many practical challenges including workflow and time constraints, questions about the effective frequency of reanlaysis, and issues of patient follow-up. There are currently no standards for reanalysis. *DTET* examined reports from several leading institutions that presented their reanalysis findings at the American College of Medical Genetics and Genomics Annual Clinical Meeting (Charlotte, N.C.; April 10-14). These case reports show some emerging solutions for the challenge of reanalysis of exome sequences.

Automated Annotation for Reanalysis of WES Data

Automated bioinformatics pipeline may enable providers to efficiently and effectively characterize annotation changes, supplement existing genomic results with these changes, and filter the results for changes of interest, according to a presentation by Charu Kaiwar, M.D., from the Mayo Clinic.

Kaiwar reported on a process that enters all negative exome cases into a pipeline capable of providing periodic, time stamped differential outputs. The so-called Automated Reanalysis Pipeline performs systematic reanalysis of clinical exomes against continually updated knowledge sources. The annotation changes are traceable back to their original downloaded source. The Mayo researchers report the system is scalable and can be integrated into an existing bioinformatics network.

The final output of the pipeline contains variants flagged using user-defined criteria based on gene-variant-disease associations in public databases, population frequency, and predicted impact of variant on protein function.

The researchers reported on a pilot of 29 previously sequenced patients in the Mayo Clinic Diagnostic Odyssey cohort. The updated re-annotation was performed at an average of 12.5 months after initial evaluation. A total of 87 variants in ClinVar and 599 variants in the Online Mendelian Inheritance in Man (OMIM) filters were flagged for manual screening against phenotypes. Two variants—both single hits in autosomal recessive diseases—were relevant to patient phenotype. The researchers say further evaluation of the genes for copy number variants and other abnormalities are underway.

Using Electronic Health Records for Delivering Variant Updates

Changes in the MedSeq genome interpretation pipeline over the course of the study (July 2013 to February 2015) required reanalysis of the first 100 MedSeq genomes (50 healthy individuals and 50 with a cardiomyopathy diagnosis) using the updated pipeline (August 2015).

> *"The findings in this study demonstrate the ability to comprehensively and efficiently interpret human genomes for both screening and diagnosis as well as implement a model for delivering regular variant updates through the electronic health record."*
>
> — Kalotina Machini, Ph.D.

While the initial analysis yielded 1,201 variants, including 189 novel predicted null variants, only 230 unique variants (85 novel null and 59 previously reported) met criteria for return of results to subjects. Reanalysis enabled an additional 22 participants to receive updated results. These updates were delivered directly through the electronic health record through system delivered alerts and included reclassification of variants that were previously reported (n=10), newly identified variants as a result of reanalysis (n=9), and three cases of both.

"The findings in this study demonstrate the ability to comprehensively and efficiently interpret human genomes for both screening and diagnosis as well as implement a model for delivering regular variant updates through the electronic health record," concluded study presenter Kalotina Machini, Ph.D., from Brigham and Women's Hospital in Boston, Mass.

Reanalysis for Rare Disease Identification

The Undiagnosed Diseases Network (UDN) in a National Institutes of Health-funded research program in which patients with previously undiagnosed disease receive access to a cross-disciplinary network of research and clinical investigators.

Clinical evaluation and phenotyping, including genetic analysis with WES, is conducted by the HudsonAlpha Institute of Biotechnology (Huntsville, Alabama). Of the initial 270 cases assigned for genetic analysis, initial analysis included return of 144 clinical reports with primary findings for 127 variants connected to the patients' phenotypes.

Given additional discovery of gene-disease associations in the two years since the UDN began, HudsonAlpha began reanalysis of unsolved cases based on newly published findings both in literature and databases such as the Genome Aggregation Database and ClinVar. Additionally, new tools now enable scans of whole-genome sequencing data for structural variation and detection of repeat expansions.

Using novel secondary analysis methods, the institute reported identifying genomic variations, including mosaicism, repeat expansions, deletions, and translocations, likely to explain disease in an additional 17 patients. Reanalysis also yielded more than 50 candidate variants of interest for further investigation, that will possibly further advance the knowledge base for rare diseases, the authors say.

Reanalysis of Pediatric Patients

Laboratory-initiated reanalysis enables a time and cost effective, as well as a sensitive approach to increasing the diagnostic yield of WES, according to a presentation by Jill Murrell, Ph.D., from the Children's Hospital of Philadelphia (Pennsylvania).

Primary WES analyses was performed on 700 consecutive patients with clinical indications (neurodevelopmental disorders, multiple congenital

anomalies, and immunodeficiencies) for WES testing. The overall diagnostic yield (associated with phenotype) was 30.1 percent.

The researchers developed an automated algorithm for reanalysis that utilized all of the genotype, phenotype, and test interpretation data generated during primary analysis and correlated patient data with terms generated from PubMed, OMIM, ClinVar, and Human Gene Mutation Databases. Identified variants were manually reevaluated.

Reanalysis was performed on 240 of the 443 nondiagnostic WES cases. Thirty-eight novel diagnoses were identified, resulting in a 15.8 percent increase in diagnostic yield. Novel disease genes, which had not been known to be associated with disease at the primary review were identified in 17 of the 38 cases. Reported candidate genes were reclassified as diagnostic in seven cases due to new evidence published in the literature. The researchers say that if reanalysis were performed monthly, approximately five variants for proband-only WES and 0.5 variants for trio WES would require evaluation.

Takeaway: Case reports presented by leading institutions highlight emerging solutions to address the challenge of reanalysis of exome sequences. While methodology varies by institution, all report an increase in diagnostic yield with reanalysis.