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Computational Pipeline Can Analyze 1,000 Genomes a Day

A genome computational pipeline has achieved the remarkable throughput of 1,000 genomes, a speed that will enable population-scale genomics. As part of the Intel Heads In The Clouds Challenge, GenomeNext and Nationwide Children's Hospital (Columbus, Ohio) were challenged to analyze a complete population dataset compiled by the 1000 Genomes Consortium in one week. The 1000 Genomes Project is the largest publicly available dataset of genomic sequences, with whole-genome and whole-exome samples from 2,504 individuals from around the world.

All 5,008 samples were analyzed on GenomeNext's genomic sequence analysis platform, operated on the Amazon Web Services Cloud and powered by Intel processors. The system achieved "unprecedented throughput" with as many as 1,000 genome samples being completed per day. The analysis of 1,000 genomes generated result files close to 100TB. Not only was there a high-degree of correlation with the original analysis performed by the 1000 Genomes Consortium, but additional variants were potentially discovered during the analysis.

"The successful completion of this proof-of-concept not only sets a groundbreaking timeframe for the analysis of a massive quantity of genomic data, but demonstrates the utility of the GenomeNext solution, eliminating the sequence analysis computational bottlenecks, enabling researchers and clinicians to keep pace with processing the magnitude of genomic data analysis required for population-scale genomics" said James Hirmas, CEO of GenomeNext, in a statement.

For more information on how improved genome analysis tools are going to accelerate adoption of sequencing-based technology in clinical settings, please see the Special Focus section on page 8.

Consumer Preferences to Shape Genetic Test Adoption

As genomic testing permeates more medical disciplines, including even well care, patient preferences for testing are expected to increasingly shape testing choices. Professional practice guidelines, physician practice habits, and patient desires will intersect and together drive testing volumes.

Continued on page 2

Consumer Preferences to Shape Genetic Test Adoption, *Continued from bottom of p.1*

As technical hurdles related to performing genetic testing, and even sequencing, have largely been overcome, translational research is shifting to examine how these results affect not just health outcomes, but the practice of routine medicine, including well care, and the psychosocial effect this data has on patients and families. *DTET* examined some recently published studies to gain insights into emerging data on patient preferences for genetic/sequencing testing.

Parents Interested in Sequencing Newborns

The majority of new parents support genetic testing of healthy newborns, according to a study published online Dec. 4, 2014 in *Genetics in Medicine*. While no testing was actually performed—this study simply posed a hypothetical scenario to parents—the researchers say that there is broad support, regardless of demographics.

Parental interest was not significantly associated with age, gender, race, ethnicity, level of education, being a first-time biological parent, or family history of genetic disease.

The researchers surveyed 514 parents, following a brief genetics orientation, regarding their interest in receiving genomic sequencing for their healthy newborn within 48 hours of birth (July 2012 to December 2013). Parents were presented with a choice to take part in a research study that would test many or all of the genes in their baby and were told that they would receive the results.

Parents reported being not at all (6.4 percent), a little (10.9 percent), somewhat (36.6 percent), very (28.0 percent), or extremely (18.1 percent) interested in testing. Married participants and those with health concerns about their infant were significantly less interested in newborn genomic testing. Otherwise, parental interest was not significantly associated with age, gender, race, ethnicity, level of education, being a first-time biological parent, or family history of genetic disease.

"We were amazed at the number of parents enthusiastically interested," says co-author Robert Green, M.D., from Boston Children's Hospital (Massachusetts). "We recognize that hypothetical situations are different from actually taking blood and there have been clear examples where hypothetical scenarios have overstated interest. … But, with MedSeq and BabySeq we will actually be doing it as big projects to understand the impact. … Sequencing healthy individuals is new territory."

Over the 2-year study period, none of the parents surveyed about genomic newborn screening (NBS) refused routine state-mandated NBS. Approximately 98 percent of parents of the 4.3 million newborns born each year in the United States participate in NBS, which in most states is administered without formal consent, but with some provisions for opt-out, although most women report not being aware of NBS.

Women Desire Discussion, Testing of Breast Cancer Risk

Another study found a "marked unmet need" for discussion about genetic testing between clinicians and women diagnosed with breast cancer. The study, published online April 6 in the *Journal of Clinical Oncology*, found that more than one-third of women strongly desired genetic testing, but one in five reported undergoing testing. While these testing rates may correlate with appropriate use of testing based on risk, the researchers uncovered a low rate of discussion and testing among minorities, suggesting some women desiring, and possibly needing testing, are not able to access these services.

Clinician Practice Preferences Affect EGFR Testing

Nearly one in four patients with advanced lung cancer in Europe, Asia, and the United States are not receiving epidermal growth factor receptor (EGFR) test results before starting treatment, according to an abstract presented at the European Lung Cancer Conference (Switzerland; April 15-18). In some cases, patients aren't being tested. Sometimes results are not returned before treatment begins. But, some clinicians, worrisomely, are making treatment decisions disregarding test results.

"There is incomplete implementation of guidelines for identification and treatment of EGFR mutations in non-small cell lung cancer (NSCLC)," writes lead author James Spicer, M.B., Ph.D., Guy's & St. Thomas' Hospital Trust (United Kingdom). While laboratories can directly address some barriers, such as turnaround time, education of oncologists is needed to bring greater "concordance" between practice preferences and guidelines, he says.

Guidelines call for EGFR mutation testing prior to initiating treatment of advanced NSCLC due to better outcomes with targeted therapies, such as tyrosine kinase inhibitors. To assess compliance with these guidelines, the researchers surveyed 562 oncologists from 10 countries between December 2014 and January 2015.

The researchers found that 81 percent of oncologists report requesting EGFR mutation testing prior to first line therapy in stage IIIb/IV NSCLC patients. However, 23 percent of oncologists do not consider EGFR mutation subtypes in making treatment decisions. Mutation test results were available before initiating first line therapy in 77 percent of tested patients, although there were significant differences between countries (51 percent in France to 89 percent in Japan). Insufficient tissue, poor performance status, and long turnaround time were cited as barriers to testing.

"The arrival of a new group of targeted EGFR inhibitors for the treatment of lung cancer ... has brought with it a new requirement for diagnostic laboratories to implement genetic testing," says Spicer in a statement. "The new skills and investment required to deliver this new molecular pathology have understandably taken time to become universally available. Furthermore, the new clinical data underlying these developments has mandated a change in clinical practice."

"This study suggests that discussions regarding the actual risk of a hereditary syndrome are critical, particularly in vulnerable populations," writes lead author Reshma Jagsi, M.D., D.Phil., from University of Michigan, Ann Arbor. "Discussions are essential to help patients at higher risk to access testing while also helping patients at lower risk to appropriately avoid testing without leaving lingering worry."

Given that the discovery of genetic mutations has implications for immediate treatment decisions, future monitoring, and possible repercussions for close relatives, the researchers surveyed 1,536 patients being treated for nonmetastatic breast cancer from 2005 to 2007 about their preferences and experiences with hereditary risk evaluation. Patients were identified through population-based registries with oversampling of minority patients to ensure sufficient representation (17 percent black; 39 percent Latina). Nearly one-third (32 percent) reported a family history of breast or ovarian cancer in a first degree relative.

The researchers found that among this diverse population of breast cancer patients 35 percent of patients reported a strong desire for genetic testing, 28 percent reported discussing testing with a health care professional, and 19 percent reported undergoing testing. Of the 493 patients who expressed a strong desire for testing, 43.4 percent failed to have a genetic testing discussion with a health care professional, despite their desire. Minority patients (blacks and Latinas) were significantly more likely than whites to have an unmet need for discussion, even when controlling for other factors. Among long-term survivors, those with an unmet need for discussion worried significantly more about recurrence (48.7 percent versus 24.9 percent in those without an unmet need).

Among patients who did have testing, reasons for testing included perceived physician recommendation (65.2 percent) and patients' desire for information relevant to family members (53.6 percent). Those who did not receive testing cited reasons including: physician recommendation (64.9 percent), personal choice (8.9 percent), and financial expense (7.0 percent).

"The infrequency of relevant discussion in breast cancer decision making that we observed in our 2006 cohort is concerning," write the authors. "These findings are even more relevant today given the exponential growth in news about genetic risk and rapidly increasing access to an expanded array of available genetic tests."

Takeaway: Emerging evidence demonstrates there is substantial interest among the general public for genetic testing for a range of conditions. In the future, patient desire will be a strong driver for genetic testing. G2

Labs Look to Lean Engineering to Transform Operations

With reimbursement challenging revenue in already constrained budgets, laboratories are increasingly looking for financial stability through cost savings achieved with added operational efficiencies. For many laboratories committed to developing long-term strategies, rather than short-term fixes, lean engineering principles are successfully transforming operations.

"As we were considering pilots in various areas, I was struck that perhaps more so than in health care in general, the laboratory is most like manufacturing, where lean had a lot of success."

—Michael Kanter, M.D.

Lean, a management system first pioneered by Toyota Motor Corporation in the 1990s, is a production system based on the principles of continuous process improvement, elimination of waste, and worker engagement. Today the concept of lean is synonymous with identifying and removing waste from operations, while promoting value to the customer, explains Bohdan Oppenheim, Ph.D., a systems engineering professor at Loyola Marymount University (Los Angeles) and developer of the university's graduate certificate program in Lean Healthcare Systems.

Managers and directors from Kaiser Permanente Southern California have been participating in Loyola's graduate certificate program and are bringing lessons learned to Kaiser's Southern California regional laboratory, which performed 59.1 million tests in 2014.

"We started to be intrigued in lean as an organization by its potential to increase quality and reliability and decrease costs," Michael Kanter, M.D., regional medical director of quality and clinical analysis at Southern California Permanente Medical Group (SCPMG), tells *DTET*. "As we were considering pilots in various areas, I was struck that perhaps more so than in health care in general, the laboratory is most like manufacturing, where lean had a lot of success ."

"[Lean] involves a long-term philosophy of investing in the development of teams of individuals and equipping them with the tools necessary to identify and reduce waste/defects within a system," writes Susan Novak-Weekley, Ph.D., a director at SCPMG Regional Reference Laboratories in North Hollywood, Calif., in a June 2014 commentary on the role of lean in the future of clinical microbiology laboratories, published in the *Journal of Clinical Microbiology*. "Lean eschews the idea of quick-fix solutions to serve short-term goals in favor of a more sustained approach to process improvement."

Staff participation, experts say, is imperative to both eliminate waste and improve quality of care. This long-term commitment is seen through a labor management partnership [(L+M)P] that is in place for the whole Kaiser organization, Novak-Weekley explains, not just within the laboratory. One Kaiser group participating in the certificate program identified 139 best practices that would streamline operations in clinical laboratories, potentially generating savings of $15 million.

"If the culture of lean is not guided and nurtured appropriately, laboratorians quickly learn that most of these initiatives will eventually disappear," writes Novak-Weekley, who says that reasons for failure include: not obtaining the complete support of leadership, failure to create a blameless environment, fears relating to job security, and focus on cost reduction rather than quality improvements.

Takeaway: By taking a long-term view of the need to collaboratively work to eliminate waste and improve quality, lean doctrine can be successfully applied to lab operations.

Inside The Diagnostics Industry

Biocept Believes ctDNA, CTCs Both Hold Promise in Future of Liquid Biopsy

Michael Nall, CEO, Biocept

This year is poised to be a breakthrough year in the adoption of liquid biopsy technology. Biocept (San Diego) is in position to be at the forefront of this emerging market with a growing menu of tests for both circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) on the company's proprietary OncoCEE and CEE-Selector platforms. To date, the company has released commercial tests for breast, gastric and non-small cell lung cancer and has a robust pipeline of additional tests focused on solid tumor biomarkers in colorectal, prostate, and melanoma.

DTET recently spoke to Biocept CEO Michael Nall regarding recent developments at the company and the broader outlook for adoption of the liquid biopsy technology.

How would you characterize the current state of clinical adoption of liquid biopsy technology?

We see liquid biopsy being adopted in three phases. The first phase, where we are today, is using liquid biopsy to fulfill a true unmet medical need—when a biopsy is just not adequate or even possible to obtain. We see this in lung cancer patients most often, but it also could be in metastatic cancer for all types of solid tumors. The most common sites for metastasis are bone, brain, lung, and liver, which are all areas where it would be difficult to obtain a tissue biopsy for molecular profiling. Oncologists really have to have this molecular information to make the appropriate clinical decisions. That is the first phase of liquid biopsy adoption.

The second phase seems to be coming along really quickly. There are some real tailwinds this year, for monitoring. Once these patients have been put on these therapies because of a certain molecular alteration, there is a need to monitor them. Previously molecular alterations were only assessed using tissue biopsies, but you can't turn someone into a pincushion and keep performing biopsies on them all of the time. That is just not practical. But, with blood we can monitor these patients. One of the first areas we see this occurring clinically is for lung cancer patients on tyrosine kinase inhibitors (TKIs), like Tarceva. We can monitor their EGFR status through a blood sample. That's very meaningful for oncologists and we see this occurring. Additionally, you can look for resistance by monitoring certain markers, like T790M. The reason I said there are some tailwinds is that there are some promising drugs in clinical trials and clinicians will need to know that T790M resistance status to move patients to those new therapies.

We see a third phase in the future for identifying recurrence and for a screening assay down the road done with a liquid biopsy. Within five years, I anticipate a lot of movement in this area of screening. We are already starting to see research using CTCs as well as ctDNA and you can find cancer earlier than tumors show up in scans. There is a lot of potential with screening here, but it is going to take investment and proof-of-concept supported by positive clinical trials to get us where we need to go with the technology.

Inside The Diagnostics Industry

There seems to be some debate over the value of CTCs versus ctDNA. Biocept has tests for both. Where do you see the value of each of these methods?

There is a lot of debate when you go to industry conferences, with some people saying you've got to be in CTCs because you need the context of the cell to make sure you are in a cancer cell. But, you also have other people saying with plasma you are more likely to get a result. We think all parts of the cell are important. There is a future in all parts of liquid biopsy.

Using CTCs we have launched assays for breast cancer and lung cancer. But, with ctDNA we can detect mutations like EGFR in a very quick, straightforward, and sensitive way. To me there are true limitations if you go only to plasma. For instance, isn't protein of interest in these cells? That's not something you are likely to do with a plasma-based assay. We are currently collaborating with a couple of partners on microRNA and RNA detection and that is something you are unlikely to get out of plasma. Those are examples of where CTCs are very valuable. Why would you say I don't need one or the other? It's all part of the cell and it is all interesting.

Do you see liquid biopsy opportunities outside of oncology?

We are really focused on oncology today. I think there are many applications in cardiology and, maybe even someday, in Alzheimer's. But today Biocept is focused on cancer and there are plenty of diagnostic targets in the space.

In which sub-areas of oncology do you see the most interest in liquid biopsies?

A lot of what we are doing is in lung. Lung cancer biopsies are exceptionally difficult to obtain. Fine needle aspirate and core-needle biopsy yield very small amounts of tissue. It is not uncommon that there is not enough tissue for molecular testing. There is a true, true need to help these patients, so we are focusing a lot of our efforts there as well as in treatment monitoring. Because of these new agents there is a real need to identify that resistance earlier in treatment. Lung cancer is the low-hanging fruit. In the metastatic, recurrent population for breast and gastric cancer, you run into the same problems. You can't do some tests on bone and you certainly aren't going to do a biopsy with a brain mass. So, in that population there is a real need for this test.

Biocept By-the-Numbers

- Commercialized tests: 9 biomarkers in 3 solid tumor types
- In the pipeline: 8 biomarkers in 3 additional tumor types
- $86 average cost of a blood draw versus $14,600 average cost for tissue biopsy

Stakeholders still struggle with a universal standard for establishing proof of clinical utility. How is Biocept addressing generation of evidence?

We know that with reimbursement there is a high hurdle. We do have reimbursement for most of our tests we do because we use established CPT codes for these mutations. We do get paid today, unlike a company running unique algorithmic assays that must appeal denials with miscellaneous codes. We still need to continue to demonstrate clinical utility, though, to drive adoption and receive adequate reimbursement from payers.

We have taken a first step to establish clinical validity and clinical utility. The papers that have been published by our academic collaborators thus far show a very high correlation between our tests and with tissue, which has been the gold standard. This year we are moving forward with additional studies that take it to the next step—incor-

Inside The Diagnostics Industry

porating patient response to therapeutic choice as an endpoint. It will be important to move clinicians and patients away from tissue biopsy and towards blood-based tests. In addition, we need to prove in a journal a concept that is common sense to everyone—the idea that doing a blood test instead of a surgical procedure can save hundreds of millions, if not billions of dollars for the U.S. health care system. Nobody would argue with the assumption, but we need to prove it.

"This year we are moving forward with additional studies that take it to the next step—incorporating patient response to therapeutic choice as an endpoint. It will be important to move clinicians and patients away from tissue biopsy and towards blood-based tests."

—Michael Nall

Uncertainty remains regarding future regulation of laboratory-developed tests (LDTs). Do you believe this uncertainty affects adoption?

At Biocept we continue to keep an eye on developments and are awaiting final guidance for LDTs. We are engaged with industry consultants and are part of the 21st Century Medicine Coalition. We are trying to develop our strategy as much as possible.

I don't see adoption being affected by this debate at all. It's mainly an internal debate between regulatory bodies and the laboratory industry. I'm not sure how much clinicians are aware of this debate or that it affects their decision-making. That said, this discussion is certainly important to our company. At Biocept we are well suited for the possibility of future regulation. We manufacture the microfluidic device we use for CTC capture and we control that process, which we think will be an advantage if we do need to secure U.S. Food and Drug Administration (FDA) clearance or approval. In addition, once we capture the CTCs we use kits that have been approved by the FDA. We source companion diagnostic kits from Abbott so we are in a way doing an approved assay. We are just doing it on tumor cells out of blood, not tumor cells out of tissue.

In the coming years how do you see cancer testing evolving?

We are going to see a further evolution toward genomics. I anticipate a movement toward broader tumor profiling with diagnosis. At the start, you will need to qualify a patient with a big genomic profile, comparing germline and somatic mutations. From there, there is this huge opportunity in treatment monitoring. More specifically, with liquid biopsy, it makes sense to monitor patients with more targeted assays. In the future, possibly in the five- to 10-year timeframe, there will be a way to make truly personalized assays, based on that tumor's unique genomic signature. These assays will help clinicians monitor their patients for treatment response, resistance, and cancer recurrence.

When it comes to screening, the medical community must do a better job. Looking at lung cancers or other cancers with such a high mortality rate underscores this need for improved screening. Part of the problem is that patients are being diagnosed at such a late stage. Our current mode of screening for lung cancer is problematic. Screening through a blood test for the molecular basis of disease offers an improved opportunity. It's early to say how broad cancer screening will be. In the nearer term we can do a better job screening at-risk populations, say smokers, with a targeted assay. However, in the long term, there are nearly limitless opportunities in the space and the many different ways screening could evolve.

SPECIAL FOCUS: Genome Analysis

Faster Genome Analysis Enabling Clinical Application, Population-Scale Translational Research

Genome analysis pipelines are getting faster. These advances in computational pipelines are going to alleviate the notorious analysis bottleneck that challenges clinical adoption of genome sequencing. To achieve widespread clinical relevance, time to results must be cut significantly and to facilitate the next wave of understanding about the genetic origins of disease, these analysis pipelines must be robust enough to accommodate population-sized datasets of tens of thousands of genomes. Experts believe the technology to overcome these analysis challenges is now entering the marketplace.

As next-generation sequencing (NGS) instruments become more commonplace in laboratories and as these platforms churn out raw data at even faster rates, access to scalable analysis tools becomes even more critical. Optimized analysis workflow solutions become the missing link—able to transform big data into clinically actionable information or scientific discoveries.

To get from raw base pair data to reports of pathogenic variants requires multiple computational steps—alignment, deduplication, realignment, recalibration, and variant discovery. The resulting variant call format then requires tertiary analysis to match variants with clinically relevant information. Current analysis approaches can take weeks to complete and require bioinformatics expertise and computing infrastructure that poses a significant cost exceeding the price of actually generating sequencing data. Taken together these pose daunting challenges for laboratories.

> *"If it takes two days to get through the sequencing and then two weeks of analysis to determine the pathologic variant, that is too long to be relevant for a critically ill newborn."*
>
> —James Hirmas

"We got our first next-generation sequencing instrument in 2010. We had modest computational resources and it was a really overwhelming amount of data coming off of the instrument," Peter White, Ph.D., director of the Biomedical Genomics Core at Nationwide Children's Hospital (Columbus, Ohio) tells *DTET*. "Keeping up with it got worse."

An Automated Solution

To overcome the challenges of analyzing these large amounts of data, White and his team developed a computational pipeline called "Churchill." By applying novel computational techniques, the fully-automated Churchill can analyze a whole genome in 77 minutes. Churchill developers predict that the platform's speed will have a "major impact" in clinical diagnostic sequencing. Churchill's algorithm was licensed to Columbus-based GenomeNext for commercialization as a secure, software-as-a-service.

"Accuracy and speed are extremely important even if you are dealing with one sample," says James Hirmas, CEO of GenomeNext. "If it takes two days to get through the sequencing and then two weeks of analysis to determine the pathologic variant, that is too long to be relevant for a critically ill newborn. "

According to a Jan. 20 article in *Genome Biology*, Churchill's performance was validated using the Genome in a Bottle Consortium reference sample. Churchill demonstrated high overall sensitivity (99.7 percent), accuracy (99.9 percent), and diagnostic effectiveness (99.7 percent), the highest of the three pipelines assessed. The other pipelines tested were

SPECIAL FOCUS: Genome Analysis

Curoverse Launches Open Source Infrastructure Software Pilots

In mid-April, Curoverse (Boston) announced the public beta trial of its Arvados open source software platform for bioinformatics. Curoverse will offer both cloud-based and on-premise solutions to aid in data management, processing, and sharing of genomic data.

Adam Berrey, Curoverse's CEO, tells *DTET* that he is confident, that Arvados provides the right foundation for the future of genome data management. The platform's capabilities are designed to accelerate new scientific discovery, share scientific work, increase the reliability of clinical testing, and lower the costs of operating large bioinformatics computing systems.

Arvados' data management features help users organize, manage, verify, and track (origin and usage) very large data sets—ranging from terabytes to petabytes. This, Berrey says, aids laboratories in their audibility and compliance checks. Additionally, the data processing capabilities make it easy for users to run "consistently reproducible" complex analytical workflows on the elastic computing infrastructure. Berrey tells *DTET* that even after analysis pipelines are updated, Arvados allows users to roll back to old versions to verify and reproduce test results. The system's collaboration functionality allows for secure sharing of data and analytical pipelines within a lab, between labs, and publicly on the Internet.

"We believe the ideal solution is not to move data, but to move the computation," Berrey says. "Move the question to the data rather than the data to the question."

The Arvados platform was originally designed by the team of Alexander Wait Zaranek, Ph.D., at Harvard University, in order to manage the genomic and biomedical data being collected for research projects. Arvados 1.0 is scheduled for release this summer and the company's full commercial launch will be in the second half of 2015.

the Genome Analysis Toolkit-Queue (using scatter-gather parallelization) and HugeSeq (using chromosomal parallelization). The developers say Churchill's deterministic performance "sets an NGS analysis standard of 100 percent reproducibility, without sacrificing data quality."

"We aren't naive to think that other groups aren't trying to do this and they may achieve comparable speed in the future," James Hirmas, GenomeNext's CEO tells *DTET*. "So the issue is quality. The hidden dark secret of genome analysis tools is determinism and reproducibility."

Churchill divides the genome into thousands of smaller regions and runs them in parallel. While this sounds obvious, development was "challenging." White says that central to Churchill's parallelization strategy is the development of a novel deterministic algorithm that enables division of the workflow across many genomic regions with fixed boundaries or 'subregions.'

"This division of work, if naively implemented, would have major drawbacks: read pairs spanning subregional boundaries would be permanently separated, leading to incomplete deduplication and variants on boundary edges would be lost," White writes in *Genome Biology*. "To overcome this challenge, Churchill utilizes both an artificial chromosome, where interchromosomal or boundary-spanning read pairs are processed, and overlapping subregional boundaries, which together maintain data integrity and enable significant performance improvements."

Churchill's speed is also highly scalable, enabling full analysis of the 1000 Genomes raw sequence dataset in a week using cloud resources. This, the developers say, demonstrates Churchill's utility for population-scale genomic analysis. Churchill identified 41.2 million variants in the set with 34.4 million variant sites in common between Churchill and the 1000 Genomes Project's analysis. The 1,088 low-coverage whole-genome samples had a total analysis cost of approximately $12,000, inclusive of data storage and processing, White says.

Hirmas tells *DTET* that the company's platform is well suited to both clinical laboratories and research entities engaging in large-scale genomic studies. Sequencing, Hirmas explains, is run in batch jobs and it is more economical, depending on instrument size, to run 20 or even 50 samples in a tube. While 50 samples waiting for analysis doesn't meet the thousands of genomes associated with population-scale genomics, 50 genomes may still be problematic for a lab if it takes two weeks to analyze each genome.

SPECIAL FOCUS: Genome Analysis

The provision of fast genome analysis solutions as a service in the cloud is expected to accelerate clinical adoption of whole-exome and whole-genome sequencing and will enable the technology to be adopted by smaller laboratories. Genome analysis as a service eliminates many of the upfront costs and on-going overhead expenses tied to in-house analysis development. Labs can get tests up and running faster without having the outlay of capital investment to procure computer infrastructure. Additionally, labs don't have to assemble hard-to-find bioinformatics teams.

These commercial systems are scalable, meaning laboratories have access to the computational power they need when they have high volumes, but aren't managing the overhead of on-site equipment capacity when testing volumes are low. Finally, despite the uncertainty of added regulation of sequencing-based testing and evolving security policies, GenomeNext and other emerging software-as-a-service genome analysis companies are building their systems to meet security and other laboratory regulations. For instance with these services, clinical laboratories can lock-down their analysis pipeline to meet CLIA and College of American Pathology regulations.

"The unfolding calamity in genomics is that a great deal of this life-saving information, though already collected, is inaccessible," Antonio Regalado writes in MIT Technology Review. *"The risk of not getting data sharing right is that the genome revolution could sputter."*

Population-Scale Data Analysis

While the clinical implications of speedier genome analysis are clear, speed is also paramount to enabling the analysis of more genomes for translational research. It took the 1000 Genomes Project six years to sequence 2,504 individuals, analyze the genomes, and release final population variant frequencies.

The often-heard frustration that sequencing the human genome has not yielded an understanding of the genetic etiology of common diseases as many scientists had hoped for can be addressed, experts say, with larger-scale genomic studies. The next series of breakthroughs in medicine will depend on populational-sized comparisons of hundreds of thousands or maybe even millions of genomes to crack the root genetic causes of disease.

In order to analyze that many genotypes in a meaningful timeframe, networked cloud computers will be necessary to generate enough processing power. But to fully take advantage of the data captured in the growing repository of sequenced DNA data, large amounts of genomic information must be able to be transferred, shared, and re-analyzed in a secure fashion.

"The unfolding calamity in genomics is that a great deal of this life-saving information, though already collected, is inaccessible," Antonio Regalado writes in *MIT Technology Review*. "The risk of not getting data sharing right is that the genome revolution could sputter."

The 'Internet of DNA,' a global network of millions of genomes, was named as one of *MIT Technology Review*'s top 10 breakthrough technologies for 2015. The magazine believes this genome sharing may be achievable in the next two years. Regalado says DNA sequencing instruments will be able to produce 85 petabytes of data this year worldwide and twice that much in 2019. By comparison, all of Netflix's master copies of movies take up 2.6 petabytes of storage.

SPECIAL FOCUS: Genome Analysis

Genome sequencing is "largely detached," he says, from "our greatest tool for sharing information: the Internet." The data from the 200,000 genomes already sequenced are largely stored in disparate systems and when shared, are "moved around in hard drives and delivered by FedEx trucks." Culturally, scientists are often reluctant to share genetic data, in part because of the legal risks surrounding privacy rules and the threat of security breaches.

Patient privacy policy is slowly evolving to reflect the genomic and Internet era. In late March, the National Institutes of Health (NIH) issued a position statement on use of cloud computing services for analysis of controlled-access data. The agency decided, "In light of the advances made in security protocols for cloud computing in the past several years and given the expansion in the volume and complexity of genomic data generated by the research community, the NIH is now allowing investigators to request permission to transfer controlled-access genomic and associated phenotypic data obtained from NIH-designated data repositories ... to public or private cloud systems for data storage and analysis."

Comfort with genomic data sharing will also grow with the development of enhanced security measures, such as advanced encryption methods. According to a March 23 *Nature News* article, significant progress has been made using homomorphic encryption to analyze genetic data. At the iDASH Privacy & Security Workshop (San Diego; March 16) groups demonstrated that they could find disease-associated gene variants in about ten minutes using the method.

In homomorphic encryption, data is encrypted on a local computer and then the scrambled data is uploaded to the cloud. Computations can be performed directly on the encrypted data in the cloud and an encrypted result is then sent back to a local computer, which decrypts the answer. The cloud-based computational pipeline would never 'see' the raw data, but the cryptographers say, the scheme gives the same result as calculations on unencrypted data. Early versions of the technology were hampered by the extended time analysis took on encrypted data.

While the cryptographers at the workshop acknowledge that the encrypted technologies are still slower than analysis pipelines using raw data, they are "encouraged." Five teams demonstrated homomorphic encryption schemes that could examine data from 400 people within about 10 minutes, and could pick out a disease-linked variant from among 311 spots where the genome is known to vary. It took 30 minutes to analyze 5,000 base pair stretches (a little larger than the size of a typical gene), while for larger stretches of sequence data—100,000 base pairs, or about 0.003 percent of the overall genome—analysis was not always possible, or took hours, and consumed up to 100 times more memory than computing unencrypted data, *Nature News* reports.

"The same calculation that took a day and a half in 2012 now takes us five minutes to do," Shai Halevi, Ph.D., a researcher in cryptography and information security at the IBM Thomas J. Watson Research Center (Yorktown Heights, N.Y.), tells Nature News. "Now is the time to ask, is this fast enough to be usable?"

Takeaway: The industry is poised to dramatically reduce the amount of time needed to analyze a genome from weeks to hours. This profound improvement in speed, in combination with enhanced data sharing, management, and security methods, will accelerate both clinical adoption of whole-exome and genome testing, as well as the ability to conduct large-scale genome analysis for translational research studies.

Genomic Testing Making Inroads in Cardiology

Adoption of pharmacogenomic testing has trailed behind optimistic projections, particularly in the field of cardiology. But now two recent studies published in *The Lancet* by the Thrombolysis in Myocardial Infarction Study Group are demonstrating that genetic information can improve risk assessment for coronary artery disease and can identify patients most likely to benefit from preventive statin therapy. Furthermore, pharmacogenomic testing can identify atrial fibrillation patients who are at greater risk for bleeding events early in treatment and would benefit from novel anticoagulant therapy instead of widely-prescribed warfarin.

> *"Clinical, biochemical, and imaging parameters have been used to stratify cardiovascular risk and potentially to tailor therapy. Our present analysis suggests that genetics might also have such a role."*
>
> —Jessica Mega, M.D.

"There are many conditions for which genetic testing can fit very nicely into routine cardiology practice," Jessica Mega, M.D., lead author of both studies, tells *DTET*. "Cardiologists are personalizing medicine all the time for patients, by taking into account other variables like diabetes status, creatinine levels, or age. There has been this hesitation around genetic information, but I believe that is eroding."

Genetics IDs Cardiac Risk, Those to Benefit From Statins

A genetic risk score identifies individuals at increased risk for heart attack or death from coronary artery disease, according to a study published online March 4 in *The Lancet*. The researchers say the composite score further identifies those individuals who will derive the most clinical benefit from initiating statin therapy.

"Clinical, biochemical, and imaging parameters have been used to stratify cardiovascular risk and potentially to tailor therapy," writes Mega, who is currently on leave from Harvard University to work on a project with Google X. "Our present analysis suggests that genetics might also have such a role ."

The Brigham and Women's Hospital-based researchers conducted a meta-analysis of 48,421 individuals (3,477 events) participating in five different trials. A genetic risk score was developed based on 27 previously identified single nucleotide polymorphisms associated with heart disease. Individuals were stratified into low (quintile 1), intermediate (quintiles 2 to 4), and high (quintile 5) risk groups.

Participants classified as having intermediate or high genetic risk had more than a 30 percent and 70 percent increase in the risk of cardiovascular death or a heart attack, respectively, compared to the lowest risk group. Those with the highest risk though, showed the greatest benefit from statin therapy. Benefits were seen in both primary and prevention of recurrent cardiovascular events.

"Debate continues about the use of statins in people at lower risk of coronary heart disease events, especially in primary prevention populations, which is driven by concerns about safety and cost-effectiveness in an extremely broad population," write the authors. "For that reason, an understanding of the absolute risk reductions achieved with statin therapy in different subgroups could be useful."

Genetic Variants Raise Warfarin Safety Concerns

Patients with genetic polymorphisms in CYP2C9 and VKORC1 are more likely to experience early bleeding upon initiation of warfarin treatment, according to a study published online March 11 in *The Lancet*. Pharmacogenomic testing can identify atrial fibrillation patients with these variants tied to bleeding risk and can allow clinicians to tailor therapy selection and improve safety outcomes.

"Bleeding complications are the most important concern related to warfarin therapy because of the narrow therapeutic range and high degree of variability between individuals," writes Mega and internationally-based colleagues. "Although these genetic variants affect the warfarin dose required, their effects on clinical outcomes, namely bleeding, remain debated, and payment for genotyping is controversial."

While the U.S. Food and Drug Administration label for warfarin states that genetic variants in the CYP2C9 and VKORC1 genes can assist in optimizing warfarin dosage, a "conclusive link" between these gene variants and bleeding outcomes remains debated. Newer anticoagulants with lower rates of associated bleeding are now available, but are significantly more expensive than warfarin.

ENGAGE AF-TIMI 48 was a randomized, double-blinded clinical trial in which patients with atrial fibrillation were assigned to warfarin (dosed to achieve a target international normalized ratio of 2.0 to 3.0), or to higher-dose (60 mg) or lower-dose (30 mg) edoxaban once daily. The trial prespecified genetic analysis for a subgroup of patients (n=14,348). In the current study, the authors assessed genotypes and bleeding events for those taking warfarin and edoxaban.

"In cases where there is a plan to use warfarin, genotyping could identify close to 40 percent of patients in whom there is an early increased risk of over-anticoagulation and bleeding with use of standard dosing practices."

Of the genotyped patients, 4,833 were assigned to warfarin and the majority (61.7 percent) were classified as normal responders, while 35.4 percent were sensitive responders and 2.9 percent were classified as highly sensitive responders.

In the first 90 days of therapy, sensitive and highly sensitive responders spent significantly greater proportions of time over-anticoagulated and had increased risks of bleeding with warfarin (31 percent increased risk for sensitive responders and more than 2.6 times greater risk for highly sensitive responders), compared with normal responders. Among participants taking warfarin, 334 had an overt bleeding event in the first 90 days. The researchers say that genotype added "independent information beyond clinical risk scoring."

"Although the increased risk of any overt bleeding was most apparent in the first 90 days, we found a long-term excess of serious bleeding subtypes ... in sensitive and highly sensitive responders receiving warfarin," write the authors. "Heightened response to even small doses of warfarin might, therefore, make some individuals vulnerable to over-anticoagulation and bleeding from fluctuations in diet, drug–drug interactions, and other environmental factors at any time."

Among patients randomized to either dose of edoxaban, genotype was not significantly associated with an increased risk of bleeding. But for those taking edoxaban, bleeding risk was cut in the first 90 days among sensitive and highly sensitive responders, compared with those taking warfarin.

"Warfarin will continue to be used because of low cost and wide availability," the researchers say. "In cases where there is a plan to use warfarin, genotyping could identify close to 40 percent of patients in whom there is an early increased risk of over-anticoagulation and bleeding with use of standard dosing practices. ... This risk could be substantially mitigated by using edoxaban." The study was funded by Daiichi Sankyo, the developer of edoxaban.

Takeaway: Evidence supporting incorporation of genomic testing into routine cardiology practice is mounting. Genomic data can lead to better risk assessment therapy selection to reduce risk of future events, while mitigating safety concerns. G2

Vermillion's OVA1 Cost-Effective in ID'ing Ovarian Cancer Patients

The OVA1 multivariate index assay (Vermillion; Austin, Texas) is a cost-effective strategy for triaging women with pelvic masses prior to surgery, according to an abstract presented at the American College of Medical Quality annual meeting (Alexandria, Virginia; March 26-28). Compared with both the Dearking-modified American Congress of Obstetricians and Gynecologists guidelines (mod-ACOG) and off-label CA-125 biomarker testing, OVA1 both achieves cost-savings and improves referral of women with risk of ovarian cancer to gynecologic oncologists.

OVA1 is a U.S. Food and Drug Administration-approved blood test for pre-surgical assessment of ovarian tumors for malignancy. The protein-based assay uses qualitative serum testing of five biomarkers (pre-albumin, apolipoprotein A1, beta 2 microglobulin, transferrin, and CA-125II) along with a proprietary algorithm to generate a numerical score to stratify patients by risk of malignancy.

The researchers compared the cost-effectiveness of the OVA1 test to evaluate the clinical and cost implications of adopting the assay in clinical practice from the perspective of public payers. Costs were based on reimbursement rates from Centers for Medicare & Medicaid Services Fee Schedules. CA-125, which is frequently used off-label for ovarian cancer diagnosis, is known to be plagued by false negatives and a lack of specificity for detecting early-stage ovarian cancer.

The researchers found that OVA1 was cost-effective from the payer perspective, driven in part by fewer re-operations and pre-surgical CT scans. Overall, the assay resulted in an incremental cost-effectiveness ratio (ICER) of $35,094 per quality-adjusted life year (QALY) gained, superior to the commonly accepted cost-effectiveness threshold of $50,000 per QALY. Compared to CA-125 alone, OVA1 was also cost saving with an ICER of $12,189/QALY gained. Sensitivity analysis showed that ICER was affected by the percentage of patients not referred to a gynecologic oncologist when diagnosed with advanced epithelial ovarian cancer. Appropriate referral is known to considerably improve clinical outcomes.

"The findings clearly demonstrate that the time has come to evaluate retiring off-label use of CA 125-II for ovarian cancer triage and to consider updating the ACOG guidelines to include the more sensitive and cost-effective use of OVA1 for pre-surgical evaluation of ovarian cancer risk," Valerie Palmieri, Vermillion's CEO, said in a statement.

The test was initially launched in 2010 and offered through Quest Diagnostics. However, Vermillion recently launched a wholly-owned subsidiary, ASPiRA Labs, and will begin to transition testing of Quest customers there. OVA1 lists for $1,495.

"As we are moving towards an accountable care environment, the trend is that more gynecologic oncologists are becoming employees of health care systems and being paid a salary, regardless of the number of surgeries they perform," Palmieri, tells *DTET*. "They are also measured on outcomes, which are based on the need to perform surgery on the right patients. Our test helps the physician determine if the patient is low-risk and can be treated by the local gynecologist, or at high-risk of being malignant and needs referral to a gynecological oncologist. Timing wise, the environment is right for adoption of this test."

Takeaway: Appropriate triage of ovarian cancer patients using a multivariate index assay is cost-effective and will additionally aid survival through improved specialist referral of women at higher risk for malignancy.

In-Field Lactate Testing Could Improve Trauma Patient Triage

In-field lactate testing by emergency responders can significantly improve the triage of trauma patients en route to the hospital, according to a study published in the March issue of the *Journal of Trauma and Acute Care Surgery*. Point-of-care lactate (P-LAC) better predicts which trauma patients will need resuscitative care (RC), compared to traditionally used field measures based on vital signs.

The authors say that more than 1.8 million trauma patients annually may be inappropriately undertriaged, increasing morbidity and mortality, as a result of imperfect prehospital trauma triage guidelines. Lactate, a circulating biomarker of organ perfusion failure, has been shown inhospital to be associated with mortality in patients with sepsis, myocardial infarction, and trauma. P-LAC devices, which can be used prehospital, operate similarly to glucometers, and cost only a few dollars per patient, may improve identification of underlying severe traumatic injury prior to arrival at the hospital .

Both the emergency medical services and emergency department receiving care team were blinded to the lactate results.

Data from nine sites (Level I or II trauma center) participating in the Resuscitation Outcomes Consortium from March 2011 to August 2012 were used to compare prehospital P-LAC measurements with systolic blood pressure (SBP) for predicting the need for RC in trauma patients (SBP between 70 mm Hg and 100 mm Hg) transported by ground emergency medical services. A drop of blood obtained during IV line insertion was placed on a measurement strip for lactate testing using a hand-held device (Lactate Pro; Arkray, Japan).

Both the emergency medical services and emergency department receiving care team were blinded to the lactate results. RC was defined as needed if any of the following occurred within six hours of emergency department arrival: blood transfusion of five U or greater; intervention for hemorrhage including thoracotomy, laparotomy, pelvic fixation, or interventional radiology embolization; or death.

The researchers found that 18 percent of the 387 patients required RC. A P-LAC cutoff point of 2.5 mmol/L was used to yield approximately the same estimated specificity as that of SBP of 90 mm Hg or less (≈ 48 percent). There were significant differences in the sensitivities of P-LAC of 2.5 mmol/L or greater (93 percent) versus SBP of 90 mm Hg or less (67 percent).

Regardless of timing of P-LAC (either less than 15 minutes from 911 call to lactate measurement or greater than 15 minutes) P-LAC sensitivity was better than SBP. While higher P-LAC was tied to higher need for RC, P-LAC less than 2.5 mmol/L had a negative predictive value of 97 percent, compared with 87 percent for SBP greater than 90 mm Hg. P-LAC was also statistically superior to shock index (heart rate / SBP) for predicting RC.

"Given the association between elevated P-LAC and the need for RC, informing the trauma team may result in an appropriate response and a reduced time to definitive care," write the authors led by Francis Guyette, M.D., from University of Pittsburgh in Pennsylvania.

Takeaway: Use of P-LAC testing may improve the triage of trauma patients during transport to the hospital.

G2 INSIDER

Unnecessary Testing Occurs Before Cataract Surgery

More than half of Medicare beneficiaries undergoing cataract surgery have at least one preoperative test, despite published guidelines against this testing, according to a study published April 16 in the *New England Journal of Medicine.* The authors say clinicians' lack of adherence to guidelines results in substantial, unnecessary Medicare expenses.

To assess current prevalence and cost of preoperative testing, the researchers used claims from 440,857 Medicare beneficiaries (66 years of age and older) undergoing cataract surgery in 2011. Tests were classified as preoperative if ordered within 30 days of surgery and included: complete blood count, chemical analysis, coagulation studies, urinalysis, electrocardiography, echocardiography, cardiac stress tests, chest radiography, and pulmonary-function tests, as well as components of standard laboratory panels.

The researchers found that roughly half of all beneficiaries underwent at least one preoperative test (53 percent). Patient characteristics were similar between those having and not having a preoperative test. Compared to the 11-month baseline period, the mean number of tests per beneficiary during the preoperative month increased by 66 percent. While 13 percent of patients underwent one test during the preoperative month, 11 percent underwent two, 10 percent underwent three, 7 percent underwent four, and 13 percent underwent five or more. The 798,150 tests performed during the preoperative month cost approximately $16.1 million. The Medicare expenditures on testing in the month prior to surgery were $4.8 million higher than the mean monthly expenditures during the preceding 11 months, which "strongly suggests," the authors say, that most of the additional tests classified as preoperative tests were in fact being ordered in anticipation of surgery.

There was substantial variation among ophthalmologists in the use of preoperative testing. More than one third (36 percent) of ophthalmologists ordered preoperative testing for 75 percent or more of their patients, while 8 percent ordered testing for every patient. The researchers note a limitation of the data precludes identification of the actual physician (ophthalmologist or other member of the care team) responsible for the excess testing performed. Compared with patients undergoing surgery in ambulatory surgery centers, patients undergoing surgery in hospital outpatient departments had higher odds of testing. Yet, the authors say the ophthalmologist performing the surgery appeared to be a more powerful driver of testing than patient characteristics.

"Our results showed no difference in the prevalence of testing as compared with 20 years ago, before the introduction of guidelines," write the authors, led by Catherine Chen, M.D., from University of California, San Francisco. "This is an important problem, considering that the number of annual cataract surgeries is projected to increase to 4.4 million by 2030, with Medicare paying for more than 80 percent of cataract surgical procedures in the United States." G2

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