G2 INTELLIGENCE

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Competitive Intelligence & Analysis for an Expanding Global Market

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New ENCODE Grants to Expand Inquiry Into the Role of Genome's Functional Elements in Disease

The National Human Genome Research Institute (NHGRI) recently announced the latest round of grants for furthering research on the functional elements of the human genome as part of the ongoing ENCyclopedia Of DNA Elements (ENCODE) project. The grants, worth $30.1 million, were awarded to seven ENCODE production centers and six ENCODE computational analysis groups as well as a data coordination center and a data analysis center.

Together the awards will continue efforts by the ENCODE consortium to expand its investigation of the functional elements of the genome to a much larger number of human cells and tissues, in part through tapping into the power of comparative genomic analysis.

"These grants build on the momentum of recently published ENCODE findings," said Elise Feingold, Ph.D., program director for ENCODE in NHGRI's Division of Extramural Research. "These grants, part of a set of grants that will be awarded over a four-year period contingent on the availability of funds, will allow us to build on those results and take the next significant steps in deepening our understanding of the entire human genome."

Once experimentally verified, ENCODE data is freely and publicly available.

For more information on the ENCODE project and how it will affect future development of diagnostics, please see the *Special Focus* section on page 8.

As Diagnostics Companies Focus on Controlling Costs, Cepheid Takes Unique Approach, Acquires Plastics Company

Cepheid (Sunnyvale, Calif.) announced plans at the end of September to acquire an unnamed plastics molding company. Analysts say they have not seen another acquisition like this one in the diagnostics industry but that it makes sense given the widespread focus on cost cutting.

"Folks are constantly looking to control costs as they are under pressure from customers with tight budgets, health care cost containment efforts and tightening reimbursement, competition, and the device tax kicking in next year," says Jeffrey Frelick, a senior diagnostics and life science tools analyst for Canaccord Genuity (Boston). "Cost cutting is front and center. [Diagnostics companies] are continuously looking at options, not just as part of a five-year plan."

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▲ **Cepheid Acquires Plastics Company,** *from page 1*

Cepheid previously announced that it had revised its third-quarter revenue downward, missing Wall Street estimates primarily due to intermittent interruptions in the supply of Xpert cartridge parts. In a statement, the company said that it is addressing the parts availability issue, but it expected to exit the quarter with more than $5 million in backorders that will be fulfilled by the end of October.

"Demand for our Xpert family of molecular tests continues to grow at a fast pace while we simultaneously scale-up our next-generation manufacturing operations intended to support our anticipated cartridge demand," said John Bishop, Cepheid's CEO in a statement at the time. "During the third quarter, parts issues arose as we were developing higher capacity production tools and processes, limiting our ability to manufacture at the volumes necessary to meet growing demand."

Calling the Cepheid acquisition "unique," Frelick tells *DTTR* that most diagnostics manufacturers are looking to address cost reduction through plant automation, increasing production volume, and cutting deals on materials and with suppliers, but that "everyone takes a different approach." He says that Alere (Waltham, Mass.) has relied on Asian manufacturing, while OraSure (Bethlehem, Pa.) has incorporated more automation into its plants. But Frelick adds that it is fair to expect that diagnostics manufactures may relook at their manufacturing in light of the Cepheid acquisition.

"Just like evaluating whether to develop or buy [a test] it is the same approach on the manufacturing side. It may be costly year one, but may save money years two to five. It also depends on the product—is it plastic, metal, or biologics?"

Specifics of the acquisition are scant, but company officials told analysts at a Sept. 27 meeting that they will be bringing some molding operations in-house at both its U.S. and European facilities and that the unnamed plastics company will cost Cepheid in the midteens of millions of dollars. The acquired firm must complete an audit, so the acquisition is not expected to close until the first quarter of 2013.

"The acquisition is certainly tied to their [supply] problem. They are moving to multicavity molds to produce more at lower costs," says Frelick. "Does the acquisition alleviate that? Yes, but I wouldn't say they will never have a [manufacturing] problem again, it just might be a different problem."

Does Purchase of Complete Genomics Foreshadow Greater Activity in Chinese Cross-Border Acquisitions?

The September announcement that China-based BGI-Shenzhen plans to buy Complete Genomics (Mountain View, Calif.) has brought attention to questions surrounding the current financial viability of sequencing services as well as the outlook for additional cross-border acquisitions.

"Chinese companies acquiring Western companies is still relatively unique here in China and that is significant on its own merit," says Greg Scott, founder of ChinaBio, a Shanghai-based life science consulting firm. "But in terms of the Complete acquisition specifically, it is significant because it brings in different technology. To date BGI has been using all Illumina equipment."

BGI dominates the sequencing landscape, both in China and internationally. But secrecy shrouds the finances and operations of BGI and many speculate it wouldn't have been able to achieve its current capacity without continued government support. The central, provincial, and local Chinese government offices all have programs to fund scientific research and development (R&D). Scott said that a ChinaBio survey found 162 government programs funding new drug development efforts, which includes sequencing technology.

"The government's investment criteria, if you will, is that they want to see significance [of the technology or company] in the global market that will elevate world opinion on China's performance in the technology fields," says Scott. "China loves being number one. They wanted to build the largest sequencing [enterprise] and they did, in mass anyways."

Despite its mass, questions remain about the utility of that kind of sequencing capacity now.

"BGI has gigantic facilities with hundreds of thousands of pieces of equipment. They are relatively untouchable," says Scott. "Does China and the world need that kind of sequencing capacity. Some have questions about that. Is it overbuilt? Is it actually all in use? I don't have those answers."

Terms of the Deal

In a statement, BGI says that Complete's scientific, technological expertise, and R&D capabilities are complimentary. Additionally, BGI stands to gain a U.S. base through the purchase, although it has reportedly been partnering with U.S.-based researchers.

According to the terms of the deal, BGI will purchase all outstanding shares of Complete Genomics common stock for $3.15 per share in cash (totaling $117.6 million), a 54 percent premium to the $2.04 closing price per share on the last trading day prior to the company's announcement that it was evaluating strategic alternatives to secure needed financial resources, but still well below the $9 price of its initial public offering in November 2010. Complete had not been able to turn its sequencing services profitable. For the 2011 fiscal year ending the company had revenue of $19.3 million but a net loss of $72.3 million.

According to a report in the *New York Times*, the deal, which will close in 2013, will need both antitrust clearance and clearance from a national security review by the Committee on Foreign Investment in the United States, as well as approval from certain governmental authorities in China. Complete Genomics will continue to operate in Mountain View as a separate company.

The Future of Chinese-Western Mergers

"There has been an uptick in cross-border M&A activity starting in 2010 and it really started hitting stride in 2011 and 2012," explains Scott. "Certainly BGI has the wherewithal and it wouldn't surprise me to see continued expansion of their business."

Scott tells *DTTR* that BGI is not the only potential Chinese acquirer.

"We've also looked at [Chinese] diagnostics companies, not sequencing companies per se, and there are companies with the wherewithal to do acquisitions. Most are already active in cross-border alliances and small acquisitions so they are well positioned."

Among the well-positioned companies that ChinaBio identified: In vitro diagnostics manufacturers Shanghai Kehua Bio-Engineering (estimated annual revenue 424 million RMB) and China Medical Technologies (estimated annual revenue 842 RMB), clinical laboratory Kingmed Diagnostics (estimated annual revenue 700 million RMB), and molecular diagnostics company Da An Gene.

MBio Diagnostics Raises $3.9 Million for Push Toward Commercialization of Rapid POC Platform

MBio Diagnostics (Boulder, Colo.) is conducting clinical validation and testing for its first two tests on a novel comprehensive, point-of-care platform. The company recently completed a $3.9 million raise from existing investors to aid with the transition to product commercialization next year. The company had previously raised nearly $20 million in research grants and investments.

"We are turning the corner from development and validation and will be putting effort into commercialization and [supporting] infrastructure," says MBio CEO and co-founder Christopher Myatt, Ph.D. He says the company expects the first approval, a CE mark, in the second half of 2013.

The first product to market will be a CD4 quantification test followed by a multiplex assay for HIV/syphilis/hepatitis C virus serology system.

"What is very unique about our system is that it is a single platform that can run multiple types of testing," explains Myatt. "The CD4 test analyzes cells, replacing flow-cytometry. The same technology can also run an immunoassay."

The platform is based on proprietary optical technology spun out of Precision Photonics Corp., a laser optics company. LightDeck devices, as they are called, are patent-protected fluorescence assay illumination systems that the company says are lower-cost than previous waveguide methods based on an all-plastic design. The device can run a variety of assay formats (immunoassay, nucleic testing) and analyze multiple sample types (blood, saliva, urine) giving MBio systems flexibility with appropriate assay sensitivity. Disposable cartridges for influenza, tuberculosis (TB), and sexually transmitted diseases are being tested globally with partner organizations.

During development the portable system ran as a peripheral for a laptop ensuring that it can be utilized in low-resource and nontraditional settings with results generated in 20 minutes. Myatt says the company anticipates deploying the systems in a variety of venues from Oakland, Calif., to rural Kenya. The immunoassay has been selected by global health organizations, including Foundation for Innovative New Diagnostics and PATH Center for Point-of-Care Diagnostics for Global Health, for field testing (CD4) and a screening study (TB).

While declining to discuss exact pricing or volume expectations, Myatt says that POC instruments are typically priced at a few thousand dollars and tests usually cost a few dollars. The MBio products will be "priced competitively." Additionally, Myatt says that there are 33 million HIV infected individuals and there are 125 million pregnancies a year. "If we are not shipping many millions [of tests], we're not relevant given the scale [of the global health problem]. When, where, and how to do that is what we are planning."

G2 INTELLIGENCE
Inside the Diagnostics Industry

Luminex's Growth Fueled by R&D Investments Paying Off, Increased Focus on Biodefense Segment

Jeremy Bridge-Cook, Ph.D.

Amy Altman, Ph.D.

Multiplex manufacturer Luminex (Austin, Texas) has seen compounded annual growth of more than 25 percent since 2007. In the past 18 months the company has acquired EraGen Biosciences and Gentura Dx and has launched two CE-marked tests, the xTAG Gastrointestinal Pathogen Panel and NeoPlex Newborn Screening Assay, in Europe, with U.S. launches expected soon.

The company is on track to meet its 2012 revenue goals of $205 million to $215 million and expects to see continued growth in the coming years. Jeremy Bridge-Cook, Ph.D., Luminex's senior vice president of research and development, and Amy Altman, Ph.D., Luminex's vice president of biodefense, recently spoke to *DTTR* about what is driving the company's growth, its pipeline and recent acquisitions, and renewed investment in biodefense.

What is driving the fast-paced growth Luminex has experienced over the past four to five years?

Jeremy: Our financial reporting is broken out into two segments: the technology and strategic partnerships segment and the assay and related products segment. There are different drivers for each segment. In the technology and strategic partnerships area we've made, over the last four to five years, some fairly significant investments in research and development (R&D) that led to the launch of two instruments, our MAGPIX and FLEXMAP, as well as our 500 plex capabilities with our magnetic beads. We feel those investments will help continue to drive a strong, competitive, and differentiated position in the market for our partners to remain at the forefront of multiplexing by using our technology for their products.

With respect to the assays and related products segment, the growth in that segment has been even higher. There we've also made very significant R&D investments in new assay products. xTAG Respiratory Viral Panel (RVP) has been driving growth for the last four to five years in that segment as well as continued growth in our cystic fibrosis product line. But in the future, while those assays will continue to do well, we are going to be adding to them with launches of our xTAG Gastrointestinal Pathogen Panel (GPP) and also in the newborn screening area our NeoPlex product. Both of those have already been launched in Europe and we anticipate they will be launched in the United States in 2013. We also have some longer-term potential growth drivers such as the launch of the system we acquired through GenturaDx and also our biodefense segment.

How has the market strategy for Luminex changed in light of recent acquisitions?

Jeremy: Through our products like RVP and GPP we're a well-established leader in the molecular diagnostics marketplace when it comes to multiplex products. The acquisition of EraGen Biosciences, which took place a little over a year ago, created a new area for our business in the real-time PCR, low-plex segment of the market. That acquisition has done very well for us and we're very happy with how it is evolving. But the one thing that EraGen was lacking was an instrument platform of its own. So when we acquired GenturaDx, it was consistent with our

initial focus on molecular diagnostics for the assay part of our business, and it provided a very competitive platform for us to build our EraGen assay franchise, using their MultiCode real-time PCR chemistry, on a proprietary instrument that we think is going to be very highly differentiated in the market. The two acquisitions are very complimentary.

The second thing is that the GenturaDx acquisition allows us to expand our range of solutions and the range of markets we can address. We can enter space down-market, if you will, to provide solutions to more distributed, lower-volume hospitals. Importantly, it moves us into the sample-to-answer system, the "black box" realm as well. We can build on our core strength of multiplex molecular diagnostics while adding more solutions with [features] like real-time PCR, which gives us lower plex and quantitative solutions; ease of use, which gives us the ability to satisfy customers who want a greater degree of automation; and lower volume, which enables us to move into a whole new category of customers.

Can we expect more M&A activity from Luminex in the future?

Jeremy: At a high level we always consider M&A to be an important strategic consideration. We have talked publicly about our interest in solutions that could support our long-term strategic initiatives. One example of M&A focus would be in assay menu. The broader and deeper the menu is, the more useful the platform is to the customer. We continue to search for good fits going forward.

"The GenturaDx acquisition allows us to expand our range of solutions and the range of markets we can address. We can enter space down-market, if you will, to provide solutions to more distributed, lower-volume hospitals. Importantly, it moves us into the sample-to-answer system, the 'black box' realm as well."
—Jeremy Bridge-Cook, Ph.D.

What trends will be driving biodefense research in the coming years?

Amy: One thing that we've seen with the U.S. Department of Defense (DOD) over the past few years is a movement away from straight environmental surveillance to complementary environmental surveillance along with diagnostics under this umbrella of biosurveillance. The U.S. government is very concerned about emerging global infectious diseases as well as intentional biothreat attacks. What they are really trying to do to capitalize on the dollars that are available is to find common platforms that can do both, which is another reason why Luminex is well suited to this space.

How will the biodefense contract Luminex received from DOD be used to further R&D in the pipeline?

Amy: The contract we received from the DOD Defense Threat Reduction Agency (DTRA) is to develop a small point-of-care diagnostic instrument. The instrument is sample-to-answer and it is intended to run a protein assay or immunoassay and a molecular assay within the same sample and on the same cartridge.

The diagnostic being developed can be leveraged in other areas of biodefense such as environmental surveillance. The ability to detect a protein and a nucleic acid from the same sample is very unique in this space. When an official makes a biothreat call that there is a biological event, this can be a high-consequence, high-

regret decision, meaning you don't want to shut down an airport or subway unless you have high confidence in the test results you used to make that call. By having a technology that allows you to do a multiorthogonal analysis, say a molecular assay and a protein assay for anthrax, you essentially have two different tests and it greatly enhances the accuracy of your call.

How are the clinical diagnostics and biodefense segments complimentary within Luminex?

Amy: There is a lot of intersection between biodefense and our core clinical diagnostics business. We've been very deliberate in our biodefense business segment to make sure all of the projects we take on are all aligned with corporate strategy. . . . We can leverage these DOD investments in cutting-edge technologies in our commercial business, both in clinical diagnostics and in biodefense research.

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—Amy Altman, Ph.D.

One way Luminex has an advantage over other companies in the government space is that we have a very strong, robust, commercial business. Companies funded purely through government contracts often end up feeling the brunt of budget cuts, especially with the impending cuts we are hearing about in the DOD. With a company like Luminex, the government benefits by being able to leverage a very strong manufacturer of highly innovative technology, and as a company we benefit from that investment by being able to accelerate some R&D, some innovation that we can then leverage into our commercial business.

For example, the DOD is greatly concerned about the health of their soldiers, especially those deployed into areas where endemic disease can be very debilitating. They need effective ways to diagnose and treat those illnesses. One of the powers of multiplexing is that you can test for so many different agents at once. An estimated 40,000 soldiers during Desert Storm were affected by diarrheal disease, with many of them unable to perform their duties for many days. Since there are so many organisms that can cause diarrheal disease and symptomatically you are unable to distinguish between the organisms it is important to find the cause quickly so that therapeutic care can be administered and the soldier can get back to duty sooner. Our Gastrointestinal Pathogen Panel, which can test for bacteria, viruses, and parasites, would be able to identify the disease causing agent and maybe get that soldier back on the field earlier, which is important to the government in terms of being effective when deployed.

Luminex by the Numbers

2011 total revenue: $184.3 million

CAGR rate 2007 to 2011: 25.2 percent

Instruments shipped to date: Over 9,000

Publications on Luminex technology: 14,000

510(K) FDA cleared products using Luminex technology: 65

Recent CE IVD European launches: xTAG Gastrointestinal Pathogen Panel and NeoPlex Newborn Screening Assay

ENCODE Highlights Complexity of Functional Genetic Variants in Non-Protein Coding Regulatory Networks

The second chapter of scientists' efforts to understand the human genome has been revealed. The Human Genome Project provided the first glimpses of the human genome—identifying 3 billion base pairs responsible for 21,000 protein-coding genes. But it turns out that snapshot was incomplete. The protein-coding genes revealed by the Human Genome Project, and at the center of genomewide association studies (GWAS) ever since, account for less than 3 percent of the genome.

Early this fall the ENCyclopedia of DNA Elements (ENCODE) consortium revealed the next chapter of the genome—results of a multiyear, international effort to create a blueprint of the functional elements of the genome. The billions of base pairs in between the protein-coding genes, the regulatory network, are responsible for the complex control of gene expression. ENCODE was able to identify biological function for 80 percent of the human genome. While GWAS studies of protein-coding regions have begun to reveal associations between genetic variants and disease, the ENCODE findings will give researchers crucial tools to better understand the root genetic functional causes of disease.

"GWAS have in recent years become the workhorse of the field and have identified thousands of DNA variants associated with hundreds of complex traits (such as height) and diseases (such as diabetes). But association is not causality and identifying those variants that are causally linked to a given disease or trait and understanding how they exert such influence has been difficult," writes Inês Barroso, Ph.D., joint head of human genetics at the Wellcome Trust Sanger Institute in the United Kingdom, in an overview of ENCODE published in *Nature* on Sept. 5. "Furthermore, most of these associated variants lie in non-coding regions, so their functional effects have remained undefined. . . . These results imply that sequencing studies focusing on protein-coding sequences (the exome) risk missing crucial parts of the genome and the ability to identify true causal variants."

ENCODE by the Numbers

- 442 researchers in 32 laboratories worldwide
- Used 147 cell types, six extensively
- Mapped 4 million regulatory regions
- Identified biochemical activity in 80 percent of the genome
- Generated 15 trillion bytes of raw data requiring 300 years of computer time to analyze

Barroso goes so far as to call for reinterpreting previous GWAS findings in light of ENCODE findings.

An Overview of ENCODE Findings

The numbers are staggering. It took 442 researchers from 32 laboratories worldwide to map more than 4 million regulatory regions, creating a menu of 1,640 genomewide data sets from 147 cell types to find out what each of the genome's 3 billion bases does. In so doing, the project generated 15 trillion bytes of raw data that required the equivalent of 300 years of computer time to analyze.

ENCODE was organized by the National Human Genome Research Institute (NHGRI) and launched in 2003 with the ambitious goal of identifying all of the genome's functional elements. The project launched initially as a pilot project focusing on developing the methods and strategies needed to produce results and was limited to examining only 1 percent of the human genome. The pilot required funding of $40 million plus approximately $125 million for model organism research and ENCODE-related technology development and model organism research, NHGRI says. Since 2003 the technologies were standardized across the consortium, relying heavily upon next-generation DNA sequencing. By 2007, NHGRI concluded that the technology had sufficiently evolved for a full-scale project in which the institute invested approximately $123 million over five years. In the next phase of ENCODE, which received funding in late September (see page 1), ENCODE will broaden the scope of its catalog with respect to the types of functional elements and cell types studied. It will also develop new tools for more sophisticated analyses of the data.

> ***"By carefully piecing together a simply staggering variety of data, we've shown that the human genome is simply alive with switches, turning our genes on and off and controlling when and where proteins are produced."***
>
> ***—Ewan Birney, Ph.D.***

"We've come a long way," said Ewan Birney, Ph.D., the lead analysis coordinator for the ENCODE project, from the European Bioinformatics Institute (United Kingdom) in a statement. "By carefully piecing together a simply staggering variety of data, we've shown that the human genome is simply alive with switches, turning our genes on and off and controlling when and where proteins are produced. ENCODE has taken our knowledge of the genome to the next level, and all of that knowledge is being shared openly."

This September the ENCODE project culminated in the coordinated publication of a set of integrated or threaded papers including one main paper and five related papers in the journal *Nature*, 18 papers in *Genome Research*, and six papers in *Genome Biology*. Additionally, six review articles were published in the *Journal of Biological Chemistry* and two related papers in *Science* and one in *Cell*. Additionally, more than 100 papers using ENCODE data have been published by investigators who were not part of the ENCODE Project but who have used the publicly available data in disease research.

Among some of the key findings published in September:

- Researchers dispelled the notion of "junk DNA" proving that about 80 percent of the genome is biochemically active. The scientists uncovered 4 million regulatory regions where proteins interact with the DNA, acting as on/off switches to control gene activity.
- Some of DNA bases serve as landing spots for proteins that influence gene activity. Other bases are converted into strands of RNA that are themselves end products that perform gene regulatory functions, not as intermediary messenger molecules. While many other bases are simply places where chemical modifications serve to silence stretches of chromosomes.
- Regulatory switches can be either near or very distant from the gene they regulate. These switching agents act in different combinations in different cell types yielding a more complex, sometimes cell-type specific, network of regulation.
- Coding regions may not have a definitive beginning and end with genes overlapping and having multiple beginnings and ends.

Taken together the findings provide evidence for a much more complex genetic system than initially understood with many disease-linked genetic variants mapped to tracts of non-protein-coding regions in the regulatory network.

"We expect to find that many genetic changes causing a disorder are within regulatory regions, or switches, that affect how much protein is produced or when the protein is produced, rather than affecting the structure of the protein itself," said Mike Pazin, Ph.D., an NHGRI program director working on ENCODE in a statement released at the time of the publications. "The medical condition will occur because the gene is aberrantly turned on or turned off or abnormal amounts of the protein are made. Far from being junk DNA, this regulatory DNA clearly makes important contributions to human health and disease."

> ***"Genome sequencing for clinical use zooms in almost exclusively on coding regions. We have been spending the most time looking in regions with the least amount of information."***
>
> ***—Michael Snyder, Ph.D.***

ENCODE's Impact on Diagnostics

Current and future findings based on ENODE data will certainly impact clinical diagnostics and change the understanding of disease in the future.

"One of the major future challenges for ENCODE (and similarly ambitious projects) will be to capture the dynamic aspects of gene regulation. Most assays provide a single snapshot of cellular regulatory events, whereas a time series capturing how such processes change is preferable, writes Joseph

Ecker, from the Howard Hughes Medical Institute and the Salk Institute for Biological Studies in La Jolla, Calif., in an overview of ENCODE published online in *Nature* Sept. 5. "Additionally, the examination of large batches of cells — as required for the current assays — may present too simplified a view of the underlying regulatory complexity, because individual cells in a batch (despite being genetically identical) can sometimes behave in different ways."

But researchers developing new methods to capture the altered dynamic of gene regulation linked to disease states will have a new tool.

"Genome sequencing for clinical use zooms in almost exclusively on coding regions. We have been spending the most time looking in regions with the least amount of information," says Michael Snyder, Ph.D., a senior member of the ENCODE consortium and chair of genetics at the Stanford University School of Medicine in Calif. "The impact of ENCODE is that it reveals the complexity of our genomes. It doesn't push personalized medicine further out; it just shows us the challenge of interpretation. . . . We were probably too optimistic before and more realistic now."

Genetic associations with complex illnesses are tied to these regulatory genes five to 10 times more often than genetic variants tied to mistakes in single genes, Snyder had previously said.

"Genomes are practically identical in most people, but my prediction is that if you get beyond GWAS and the DNA and look for changes in the transcription factory, methylization factory, where nucleosomes sit, and binding sites no two will be identical," says Steven Johnson, Ph.D., an assistant professor from Brigham Young University (Salt Lake City), whose lab aided in some of the ENCODE production work relating to nucleosomes. "It's a whole new way to look at [disease], to classify disease by root cause."

Snyder and his colleagues published a number of papers with the ENCODE consortium, including two that describe their creation and use of RegulomeDB, a database that integrates ENCODE and other DNA data with genetic variations known or thought to be associated with specific diseases. RegulomeDB, combines these data sources into a powerful tool that scores variants to help identify variants with putative regulatory potential and provides testable hypotheses as to their function, allowing researchers to refocus work on variants in the regulatory network rather than strictly looking for associations in protein-coding gene regions.

"What ENCODE did was bring together and present this data in a high-profile way and format so that scientists afield from this could take from it and apply it to what they are working on," says Johnson, who believes epigenetic applications from ENCODE will gradually creep into clinical analysis and diagnostics in the next five to 10 years using a technology based on a combination of chromatin immunoprecipitation and sequencing analysis.

FOBT Kits Mailed to Patients Increase Screening Rates

Mailing fecal occult blood testing (FOBT) kits to patients overdue for colorectal cancer screening is an effective way to improve screening in underserved populations, according to a study published in the September/October issue of the *Annals of Family Medicine*. The study builds upon previous findings in managed care populations indicating that non-visit-based outreach to patients may be an effective strategy in a broad range of populations.

"Our study demonstrated that the direct-to-patient outreach intervention was efficacious even in a health care setting that had already implemented point-of-care clinician-directed electronic clinical reminders to promote appropriate colorectal cancer screening," writes lead author Muriel Jean-Jacques, M.D., an assistant professor at the Northwestern University Feinberg School of Medicine in Chicago. "This finding is in keeping with prior studies that have shown the superiority of patient-directed outreach over clinician-directed reminders."

The National Institutes of Health estimates that approximately 50 percent of eligible adults are not screened for colorectal cancer. Rates are even lower in disadvantaged, racial minority, foreign-born, and uninsured populations. To address these low screening rates the researchers randomly assigned 104 patients to the intervention group and 98 to usual care, which were only referred for screening during clinician visits at a federally qualified health center. The researchers found that significantly more patients who received the mailed FOBT kit completed colorectal cancer screening (mostly FOBT) over one year, compared to the usual care patients (30 percent versus 5 percent).

"There is a major shift in care from primarily visit-based care to providers being responsible for patients not just when they walk in the office door. Practices who have embraced the concept of the medical home and proactive, non-visit-based care are excited about this," Jean-Jacques tells *DTTR*. "There is a lot of discussion about how to reach different racial-ethnic groups and how much to tailor the message. We saw substantial improvements doing nothing other than Spanish-English instructions and having translators available. With tailoring, I would expect even better results."

Clinicians Urged to Use CSF 14-3-3 Protein Test Less

A guideline released by the American Academy of Neurology (AAN) urges more appropriate use of the cerebrospinal fluid (CSF) 14-3-3 protein to diagnose sporadic Creutzfeldt-Jakob disease (sCJD). Given the rareness of the disease, clinicians are discouraged from using a shotgun approach when ordering spinal taps in patients with dementia. The guidelines, published online Sept. 19 in *Neurology*, emphasize that only in patients who have rapidly progressive dementia combined with a physician's strong suspicion of sCJD (as determined by a pretest probability between 20 percent and 90 percent), should the CSF 14-3-3 assay be used.

sCJD, a fatal brain disease in which malformed prion proteins cause a transmissible spongiform encephalopathy, is rare with an incidence of one in 1 million. Yet the 14-3-3 test has been the subject of much debate since sCJD can only be diagnosed precisely with histopathologic examination of samples from brain biopsy or autopsy and the 14-3-3 test lacks the accuracy to diagnose the disease with certainty or to rule it out completely.

Members of the guideline development subcommittee of the AAN reviewed the literature and pooled the data from 1,849 patients with suspected sCJD from nine Class II studies to estimate the diagnostic accuracy of the 14-3-3 in patients with suspected sCJD. In the pooled analysis the researchers found that 14-3-3 protein assays are probably "moderately accurate" in diagnosing sCJD with a sensitivity of 92 percent and specificity of 80 percent, a likelihood ratio of 4.7, and negative likelihood ratio of 0.10.

Lead author Gary Gronseth, M.D., from the University of Kansas Medical Center, tells *DTTR* that if the guideline is heeded, he would expect a "dramatic decrease" in CSF 14-3-3 protein test orders. The authors urge future investigation for the presence of a combination of multiple biomarkers "such as t-tau, p-tau, S-100, or NSE in the CSF is needed in addition to, or in lieu of, protein 14-3-3." G2

Urinary Bone Markers Predict Future Fracture Risk in Premenopausal Women

Higher levels of urinary bone turnover markers measured in young, premenopausal women can predict fracture risk, according to a study published in August in *Menopause*. These findings extend previous research that had shown these markers were predictive of fracture risk in older, postmenopausal women and most likely reflect faster rates of bone loss.

Bone remodeling associated with estrogen deficiency during menopause is associated with such bone loss, placing women at higher risk for fractures, particularly of the hip, wrist, and vertebrae.

This study followed 2,305 premenopausal women 42 to 52 years of age for an average of 7.6 years. Serum osteocalcin (a bone formation marker) and urinary cross-linked N-telopeptide of type I collagen (NTX; a bone resorption marker) were measured at baseline with NTX measured annually as well.

The researchers found that the 184 women who suffered fractures during the study period had significantly higher (10 percent) median baseline levels of NTX. There was a 59 percent greater risk of fracture in women with a baseline NTX greater than the median, compared to women whose NTX levels were less than the median. These findings were independent of age, race, prior fracture history, or bone mineral density. The women whose NTX values were greater than the median at baseline also experienced greater loss of spine and hip bone mineral density. The combination of both low spine BMD and high NTX is associated with almost a threefold increased risk of fracture. Median baseline osteocalcin levels were slightly higher, but not statistically significantly, in women who experienced an incident fracture.

"Urinary measures of NTX are more variable than serum measures of NTX because they must be corrected for creatinine level. However, this variability would bias our findings toward null," acknowledge the authors, led by Jane A. Cauley, Dr.P.H., from the University of Pittsburgh in Pennsylvania. "[But], women may prefer urine samples to blood draws, generating greater clinical acceptance."

The authors also note that new, more sensitive markers of bone formation than osteocalcin are now recognized and that a marker such as bone-specific alkaline phosphatase may have been more strongly associated with fracture. G2

Elevated Levels of Rheumatoid Factor Linked to Long-Term Development of Rheumatoid Arthritis

Elevated levels of the autoantibody rheumatoid factor can be present many years before the clinical manifestation of rheumatoid arthritis (RA) and individuals having elevated rheumatoid factor face up to 26-fold greater long-term risk of developing RA, according to a study published Sept. 6 in the *British Medical Journal*. These findings, the authors say, may lead to revision of clinical guidelines for earlier referral to a rheumatologist based on rheumatoid factor testing, even in the absence of affected joints.

The researchers prospectively studied 9,712 patients in a general population cohort for almost 30 years. All patients were free of RA at study initiation. Blood was drawn at study initiation and completion, and national medical registries were used to identify RA diagnosis.

The researchers found that 183 individuals developed rheumatoid arthritis during the course of the study. There was a 3.3-fold increased risk of developing RA in healthy individuals with a doubling in levels of rheumatoid factor. There was a significant increase in the cumulative incidence of rheumatoid arthritis with increasing rheumatoid factor with hazard ratios for rheumatoid arthritis of 3.6 for rheumatoid factor levels of 25-50 IU/mL, 6 for 50.1-100 IU/mL, and 26 (15 to 46) for >100 IU/mL, compared with <25 IU/mL. Fifty- to 69-year-old women who smoked and had rheumatoid factor levels >100 IU/mL had the highest absolute 10-year risk of rheumatoid arthritis (32 percent).

"A current debate is whether elevated levels of rheumatoid factor, elevated levels of anti-citrullinated protein antibody, variations in the PTPN22 gene, or some combination of these offer the best means of predicting short term (2 to 3 years) risk of rheumatoid arthritis," write the study authors, led by Sune Nielsen, a senior scientist at Copenhagen University Hospital in Denmark. "However, the debate does not include long term risk prediction, simply because good evidence is lacking. Our finding that elevated rheumatoid factor levels are associated with an increased long term (up to 28 years) risk of rheumatoid arthritis provides such data."

Two Tests Studied for Occupational-Related Diseases

Publications of two separate studies indicate growing interest in diagnostic tests able to diagnose or identify those at high risk of occupationally caused disease or injury. Separate groups of researchers have identified a genetic marker indicating those at risk for a vibration-induced white finger disease as well as a biomarker that can distinguish patients exposed to asbestos from those with mesothelioma.

Possible Early Biomarker of Mesothelioma Identified

Plasma fibulin-3 levels can differentiate healthy persons with exposure to asbestos from patients with mesothelioma, while effusion fibulin-3 levels can further distinguish mesothelioma effusions from other effusions, both malignant and benign, according to a study published in the *New England Journal of Medicine* on Oct. 11. When further validated, the marker may aid in earlier detection of pleural mesothelioma and to monitor treatment strategies.

The researchers found that plasma fibulin-3 level was significantly elevated in patients with mesothelioma in the two separate geographic cohorts, compared to asbestos-exposed persons without mesothelioma. These elevations were confirmed in a blinded validation with the use of specimens from a third cohort. Plasma fibulin-3 levels discriminated between stage I or II mesothelioma and asbestos exposure without mesothelioma with a specificity of 94 percent and a sensitivity of 100 percent. Effusion fibulin-3 levels were significantly higher in patients with pleural mesothelioma in both cohorts than in patients with effusions not due to mesothelioma.

"Surprisingly, effusion fibulin-3 levels did not correlate with plasma levels. Cavitary levels of fibulin-3 may reflect the biology of mesothelioma more accurately than plasma levels, because an advanced stage of disease was associated with higher effusion fibulin-3 levels," write the authors, led by Harvey Pass, M.D., from New York University Langone Medical Center in New York. "The specificity and sensitivity of fibulin-3 . . . are superior to those of other published markers, and fibulin-3 levels are not influenced by the duration of asbestos exposure. In addition, high levels of fibulin-3 in effusions have a high positive predictive value for the presence of mesothelioma and appear to reflect the prognosis."

The authors encourage prospective validation of the marker and additional research on the prognostic implications of an elevated level to better understand its utility as a treatment monitor as well as determining the number of years prior to clinical onset readings could be useful.

Marker Identified for Risk of Industrial Vascular Injury

The Sirt1A2191G single nucleotide polymorphism (SNP) can be a diagnostic marker for vibration-induced white finger disease (VWF), a widespread industrial injury triggered by the continued use of vibrating handheld machinery, according to a study published Oct. 1 in *Clinical Epigenetics*. Epigenetic mechanisms are increasingly recognized as playing a key role in a number of vascular disorders.

The researchers analyzed peripheral blood samples from 74 patients with VWF (male, 93.2 percent; median age, 53 years) and from 317 healthy volunteers (gender equally distributed, below 30 years of age). Four of 113 potential genetic polymorphisms within the Sirt1 genomic region were assessed.

The Sirt1 SNP A2191G was identified within Sirt1 exon 9, with significantly differing allelic frequencies in the VWF population and the control population. The heterogeneous A/G genotype is significantly overrepresented in the VWF patient population compared to the control population.

"We therefore claim the Sirt12191 A/G genotype to be a risk factor for VWF, and that it may be used as a biological marker to facilitate the identification of risk populations who are being considered for exposure to potentially hazardous hand-held vibrating machinery," conclude the authors, led by Susanne Voelter-Mahlknecht, from the University of Mainz in Germany. "This may prevent thousands of employees from experiencing physical pain and disability, in addition to long-lasting administrative fights for VWF compensation, which frequently end unsettled and in great frustration." G2

G2 Insider

Improvements in Post-Vaccine Serologic Testing Needed to Eliminate Perinatal Transmission . . . While rates of postvaccination serologic testing among infants born to hepatitis B surface antigen (HBsAg)-positive mothers from 2008 to 2011 have increased, strategies to increase testing are still needed to eliminate perinatal hepatitis B (HepB) transmission, according to a study published in the Sept. 28 issue of *Morbidity & Mortality Weekly*.

An estimated 25,000 infants are born to HBsAg-positive women annually in the United States and they carry a significant risk of contracting HepB infection without recommended postexposure prophylaxis (vaccination and hepatitis B immune globulin), according to the U.S. Centers for Disease Control and Prevention (CDC). Following postexposure prophylaxis, the CDC's Advisory Committee on Immunization Practices recommends postvaccination serologic testing (PVST) at age 9 months to 18 months.

"PVST is critical for guiding medical management of infants born to HBsAg-positive women," write the authors, including Emily A. Smith, M.P.H., from the National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention at the CDC. "PVST (anti-HBs and HBsAg) . . . determines if infants are susceptible and should be revaccinated and retested, or are infected and require additional medical care."

Using data from 4,214 infants participating in Enhanced Perinatal Hepatitis B Case Management Projects (EPHBP) in five states, the researchers found that 77 percent of EPHBP-managed infants received PVST and 63.7 percent had reported serologic outcomes. In 9.8 percent of infants PVST was incomplete with anti-HBs only (41 cases) or HBsAg only (371 cases).

"Although rates of PVST have increased, this analysis highlights areas in need of improvement," conclude the authors. "Strategies are needed to increase the rates for overall testing and testing for both anti-HBs and HBsAg, which are required to confirm outcomes."

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