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Genetic-Driven Dieting May Drive Weight Loss in the Future

With New Year's resolutions to hit the gym and eat healthier still top of mind, experts say the next big advance in achieving a healthier weight will be to use an individual's genetic data to customize diets and physical activity plans. According to a review published by a National Institutes of Health working group in the January issue of *Obesity*, the biggest challenge to realizing "precision weight loss" is the need for better analytical tools to uncover the relationships between genetics, behavior, and intentional change in weight.

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Researchers Eye Extending NIPT for Subchromosomal Analysis

Non-invasive prenatal testing (NIPT) is rapidly being adopted as an aneuploidy screening test in high-risk pregnancies. Its benefits include its high sensitivity and its ability to reduce the need for invasive testing. NIPT uses massively parallel sequencing of cell-free DNA in maternal plasma to detect fetal trisomies in chromosomes 13, 18, and 21.

While some are working towards evaluating the benefits of screening wider populations of women with NIPT, others are interested in expanding the scope of testing to include selected subchromosomal abnormalities. But, the evidence base remains limited for this use.

There are no serum-based tests that specifically screen for subchromosomal abnormalities. Individually, these rearrangements are rare, but combined have an estimated, combined incidence close to Down syndrome, researchers say. There are some concerns that widespread NIPT use could actually decrease detection of other pathogenic rearrangements—detectable with microarray analysis from invasively derived samples—given the declining numbers of invasive procedures performed.

A few previous studies demonstrated the potential of extending NIPT to detect fetal deletion and duplication syndromes, but questions remain about the fetal DNA fraction and the depth of sequencing needed for accurate results. Two recent studies provide additional data to fill this gap.

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Genetic-Driven Dieting May Drive Weight Loss in the Future, *Continued from top of p.1*

"I think within five years, we'll see people start to use a combination of genetic, behavioral, and other sophisticated data to develop individualized weight management plans," said work group lead Molly Bray, Ph.D., a geneticist and professor of nutritional sciences at University of Texas, Austin, in a statement.

Bray envisions that in the future, it will be possible for patients to submit saliva samples for gene sequencing, in combination with wearables to collect information about the patients' environment, diet, activity, and stress. A computer algorithm could then take this information and provide patients with specific recommendations to achieve their target weight.

> *"To advance the field of precision weight loss, the combination of an individual's genotype, along with the unique underlying pathophysiology it suggests, should be used to develop dietary and physical activity recommendations that target the metabolic derangements specific to each person."*
>
> —Molly Bray, Ph.D.

The review grew out of a workshop convened by the NIH back in 2014 titled "Genes, Behaviors, and Response to Weight Loss Interventions." It identifies both what is genetically known about obesity and body size, as well as needed future directions for research.

Roughly 150 loci have been identified that are associated with obesity and body size. It is estimated that genetics may account for about half of variance in individuals' body size, while environmental influences, such as diet and activity, account for the other half. But, despite this evidence for a genetic component to regulation of body mass/composition, only a limited number of genes have been identified that are tied to body weight change in response to changes in the environment, the review found. Said another way, experts believe that the genetic determinants of weight change response (genes tied to weight loss, maintenance, and/or regain) may differ from known genes associated with BMI and obesity.

To address these gaps and enable precision medicine in behavioral weight loss to become a reality, the work group offers some future directions for research.

- Genome-wide studies and randomized controlled trials can identify novel genetic loci for intentional weight loss, maintenance, and regain. This will aid in identifying baseline genetic characteristics that will permit tailored treatment guidelines (i.e., identify and target subgroups within obese populations genetically prone to respond well to a specific weight loss intervention).
- Assess whether these genetic discoveries and targeted interventions can be implemented in a clinical setting to motivate behavior change and adherence to weight loss interventions.

"To advance the field of precision weight loss, the combination of an individual's genotype, along with the unique underlying pathophysiology it suggests, should be used to develop dietary and physical activity recommendations that target the metabolic derangements specific to each person," writes Bray and her colleagues. "The success of such interventions would rely not only on an understanding of the pathophysiological mechanisms linking genotype and weight, but also on the ability to communicate a personalized strategy to patients and motivate behavior change."

Takeaway: While still several years away, experts are optimistic that future improved understanding of genes tied to weight loss, maintenance, and regain will be identified and exploited to target intervention efforts. G2

Researchers Eye Extending NIPT for Subchromosomal Analysis, *Continued from bottom of p.1*

Rearrangements Less than 6 Mb Difficult to Detect

Standard NIPT can detect the majority of large chromosomal rearrangements (greater than 6 Mb), but negative results cannot rule out the possibility of smaller rearrangements, according to the conclusions of a study published in the Jan. 7 issue of the *American Journal of Human Genetics*. The authors say that given this possibility of undetectable smaller rearrangements, NIPT screening tests should not be expanded to include subchromosomal assessment.

"The lack of an independent method for determining fetal fraction, especially for female fetuses, leads to uncertainty in test sensitivity, which currently has implications for this technique's future as a clinical diagnostic test ."

—Kitty Lo, Ph.D.

This inability to detect small rearrangements is due to current practical limits in sequencing depth and reporting of fetal fraction—the proportion of circulating fetal DNA compared to maternal DNA.

The U.K. study sequenced 31 maternal blood samples collected from a cohort of women undergoing invasive procedures for clinical indications. Fetal conditions were determined by conventional karyotyping, fluorescence in situ hybridization, microarray, or molecular techniques. The included samples had fetal copy number variants (CNVs) ranging in size from less than 3 Mb to 42 Mb on different chromosomes. There were also three cases of unbalanced translocations.

The test samples were first sequenced at 12-plex (24 samples to a flow cell)—the same read depth as the group's standard in-house pipeline for aneuploidy NIPT. If no anomaly was detected, re-sequencing at a higher depth across multiple additional 1.5-plex runs (three samples per flow cell) was performed until the CNV was detected. The group developed a calling pipeline based on a segmentation algorithm for the detection of these rearrangements in maternal plasma.

The researchers found that standard NIPT methodology for aneuploidy can be used to detect the majority of pathogenic chromosomal rearrangements detectable by standard karyotyping. This was doable using a refined bioinformatics pipeline and no extra sequencing costs. Using 12-plex sequencing, 15 of 18 fetal subchromosomal abnormalities larger than 6 Mb were detected (sensitivity, 83 percent; specificity, 99.6 percent). With a read depth to 120 million reads, test sensitivity increased to 94 percent for CNVs larger than 6 Mb and to 93 percent for CNVs larger than 1.5 Mb.

However, for samples with CNVs less than 6 Mb, five of 13 were detected. Of these, three had duplications for which the mother was a carrier, but the current bioinformatics pipeline and standard depth of sequencing could not determine whether the fetus also carried the CNV. The analysis pipeline was also tested on a set of 534 maternal blood samples with no known chromosomal abnormalities that had been sequenced with at least four million reads. Three false positives in two samples resulted in a specificity of 99.6 percent.

"The lack of an independent method for determining fetal fraction, especially for female fetuses, leads to uncertainty in test sensitivity, which currently has implications for this technique's future as a clinical diagnostic test," write the authors led by Kitty Lo, Ph.D., from the University College London in the United Kingdom. "Given that a significant benefit of using NIPT to screen for aneuploidy is the increased safety secondary to the reduced need for invasive testing, extending NIPT to include screening for subchromosomal rearrangements stands to reverse some of

this benefit whilst not offering comprehensive detection of pathogenic rearrangements. The costs and benefits of extending NIPT to this indication should be seriously considered prior to routine implementation."

Considering Fetal Fraction, Maternal Relocations

Fetal fraction is recognized to affect test sensitivity, and negative results cannot be considered conclusive without reporting of fetal fraction. A second study, published Nov. 24, 2015, in the *Proceedings of the National Academy of Sciences*, addressed both of these challenges and said that detection of fetal subchromosomal abnormalities is a "viable extension" of NIPT.

"Because the fetal concentration of DNA is lower at a gestational age of 12 to 16 weeks, … deeper sequencing than 3.5 million reads is likely required for accurate detection of subchromosomal abnormalities."

—Ai-hua Yin

The China-based group investigated expanding NIPT using a semiconductor sequencing platform (SSP) in women carrying high-risk fetuses (fetal structural abnormalities detected on ultrasound). They analyzed plasma from 1,456 pregnant women undergoing an invasive diagnostic procedure (amniocentesis or chorionic villus sampling) and estimated fetal DNA concentration based on the size distribution of DNA fragments. Subchromosomal abnormalities were validated with array comparative genomic hybridization.

The group previously published a study showing the viability of SSP for NIPT and says that SSP offers the potential advantages of lower cost, faster sequencing, and a smaller machine footprint, making them easier to deploy in clinical laboratories. The fetal DNA concentration was determined from the proportion of Y-chromosome sequences in maternal plasma. In a training data set, the researchers observed a correlation between the fetal DNA fraction estimated by the Z score of Y chromosome and the reads ratio of two DNA fragment size regions: 130–140 basepairs (bp) and 155–175 bp. In the prospective cohort of 1,476 women, 66.7 percent of samples with a gestational age of 12 to 16 week had a fetal DNA fraction greater than 10 percent, compared with 95 percent of samples with gestational age greater than 20 weeks.

"Because the fetal concentration of DNA is lower at a gestational age of 12 to 16 weeks, … deeper sequencing than 3.5 million reads is likely required for accurate detection of subchromosomal abnormalities," write the authors led by Ai-hua Yin, from the Maternal and Children Metabolic-Genetic Key Laboratory at Guangdong Women and Children Hospital in China. "The current cost of sequencing a human genome with 0.5× coverage by single reads, approximately the coverage in our study at 3.5 million reads, is comparable with the cost of chromosomal microarray analysis while avoiding an invasive procedure. This depth allowed excellent detection of deletions/duplications greater than 5 Mb in size in our study. Deeper sequencing allows for finer resolution at an additional cost."

Several false positives in the full sample were due to low estimated fetal DNA fraction or samples in which a fraction could not be "reliably calculated." However, the majority of false positives (35 of 55) were due to deletions and/or duplications present in maternal DNA. This, the authors say, points to the necessity of reflex follow-up testing to rule out maternal DNA subchromosomal abnormalities, before a fetus can be diagnosed.

Takeaway: Despite eagerness to expand NIPT for screening for subchromosomal rearrangements, questions remain about the depth of sequencing and determination of fetal fraction needed to ensure accuracy of results.

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New Technologies to Enable Clinical Single Cell Analysis

Personalized medicine is driving the need for isolation of rarer target cell populations, including for the enrichment of circulating tumor cells (CTCs), hematopoietic stem cells, and circulating fetal cells from blood. Current molecular analysis, while increasing biological understanding of cancer and other diseases at the DNA level, often assesses "averaged" cellular populations—derived from millions of cells across a tumor population—rather than individual cells. But, scientists believe that hard-to-detect low-level "subclones" are really the drivers of relapse or disease progression.

> *"While bulk tissue genomic analysis across large populations of tumor cells has provided key insights into cancer biology, this approach does not provide the resolution that is critical for understanding the interaction between different genetic events within the cellular hierarchy of the tumor during disease initiation, evolution, relapse, and metastasis ."*
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> —Quin Wills, D.Phil.

A review of single-cell genomics in cancer, published in the Oct. 15, 2015 issue of *Human Molecular Genetics*, explains that ~1,000X sequencing (which is "way beyond" the depth seen in most studies) would be required to detect 99 percent of mutations carried by a one percent tumor-mass subclone analyzed at the bulk level, whereas with single-cell analysis only ~200 cells are required to reliably detect one percent tumor-mass clones.

"While bulk tissue genomic analysis across large populations of tumor cells has provided key insights into cancer biology, this approach does not provide the resolution that is critical for understanding the interaction between different genetic events within the cellular hierarchy of the tumor during disease initiation, evolution, relapse, and metastasis," writes Quin Wills, D.Phil., from University of Oxford in the United Kingdom, in the review.

The challenge remains in isolating those 200 cells, in a manner that has high throughput, is affordable, and preserves the cell's biological integrity. A flurry of activity advancing microfluidic technology to improve cell sorting is bringing the possibility of single cell analysis closer to a clinical reality.

Sorting Methodology Enables Further Analysis

Cell sorting is often used to enrich or purify cell samples into well-defined populations. Cell sorting serves as the first step in many diagnostic and therapeutic applications, such as the enrichment of hematopoietic stem cells for autologous patient treatments.

Experts predict that cell sorting on microfluidic devices will emerge as the platform for the next generation of cell sorters. Among the platform's advantages are accessible fabrication, low reagent consumption, small footprints, and improved safety/contamination risk due to elimination of aerosols.

Lab-on-a-chip formats are emerging that are capable of cellular isolation and processing for downstream interrogation. These miniaturized formats enable sorting of cells based on a wider range of physical and molecular properties than standard flow cytometers, often without antibodies. However, in order for these technologies to achieve potential commercialization, experts say that several "significant" limitations must be overcome. In a review on microfluidics for cell separation, published Feb. 2015 in *Lab on a Chip*,

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lead author C. Wyatt Shields, IV, from Duke University highlights some challenges including: scaling throughput and processing speeds to make processing clinical-scale samples (more than 500 million cells) feasible; ensuring operating pressures don't impact cell function or viability; shrinking instrumentation footprints; reducing the level of technical expertise needed for machine operation; and reducing unit and sample processing costs for clinical application.

Commercialization

Despite the formidable challenges there is much excitement around single-cell genomic DNA analysis, particularly in cancer care, and proof-of-principle studies are beginning to emerge. Yet, to date, trials are small scale and the technology still has to meaningfully enter clinical practice. Experts say that while prototypes exist, the technology requires further investment to ensure affordability, manufacturability, and repeatable performance before commercialization is viable.

"The future of microfluidic cell sorting is promising," writes Shields. "With the large variety of cell sorting devices … microfluidic cell sorting technologies can offer speeds and accuracies in a number of ways that rival current commercial devices in a platform that is more efficient, less cumbersome, and offers a more straightforward standard operating procedure."

DTET spoke to two early-stage companies about their technologies and next steps to bring single cell sorting technologies to the commercial market.

"We think of it like a sandwich. If the meat is the sequencing, one bread is data analysis and the other bread is sample prep," Jose Morachis, Ph.D., CEO of **NanoCellect Biomedical** (San Diego) tells *DTET*. "New [sample prep] technologies like ours are the exciting next frontier in sequencing."

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>
> —C. Wyatt Shields, IV

NanoCellect's WolfCell Sorter platform is based upon microfluidic technology licensed from University of California, San Diego. The company believes the portable, bench-top platform addresses some of the current limitations and accessibility of flow cytometry technology. The platform integrates novel detection algorithms with on-chip sorting that uses a piezoacoustic actuator to gently direct cells into collection channels. The technology reduces shear stress on cells, while the disposable, closed chip eliminates dangerous aerosols and potentially brings flow cytometry abilities to more laboratories, the company says.

The integration of cell-sorting and single-cell positioning will allow diverse downstream applications—including kinetic analysis of secreted particles, genomic sequencing, gene expression, and imaging. Morachis says the company's technology enables advancements in the CTC field, as well as potentially stem cell and CRISPR applications. The cell sorter is currently in beta testing, with a larger release of the $100,000 platform for the research market expected in the summer of 2016.

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Liquid biopsies of CTCs are considered the low-hanging fruit of the single-cell analysis market. While evidence of clinical utility is still necessary to further adoption, experts say the application is already showing signs of "maturing."

Silicon Biosystems (a subsidiary of the pharmaceutical company Menarini Group; Italy), however, has its eye set, not just on the liquid biopsy market, but also on capturing the market for solid tumor samples that are currently "discarded" by molecular labs for insufficient content of tumor cells—currently estimated to be as high as 20 percent to 30 percent of samples.

"We demonstrated we can recover it for NGS analysis even if the sample only has 5 percent tumor cells," Raimo Tanzi, Silicon Biosystems' chief commercial officer, tells *DTET*.

Molecular analysis from formalin-fixed paraffin-embedded (FFPE) samples characterizes all DNA present, including DNA from tumor cells, stromal cells, and infiltrating lymphocytes. Thus, the relative frequency of a given mutation in the heterogeneous mix of DNA molecules is all that can be assessed, while copy number aberration and other genomic instabilities are very difficult to detect, the company says. Its DEPArray system can be used to separate tumor cells from stromal, and other cells in the FFPE section and a "highly pure" collection of tumor cells can be recovered enabling complete genomic analysis.

The company's DEPArray platform utilizes a semiconductor controlled flow chip where individual cells are coupled to single microelectrodes by dielectrophoretic (DEP) forces. The system combines the ability to manipulate individual cells with high quality image-based cell selection. The company says the technology identifies and sorts individual cells, or pools of cells, with 100 percent purity for detailed molecular analysis. Tanzi says the company will launch pure FFPE tumor analysis as a service at its San Diego-based CLIA lab later in the year.

New Technological Approaches

In the last year many academic research groups published studies on novel sorting technologies. Below is a sampling.

- **Parallel Microfiltration Method** (PMF) is a screening method that utilizes cell squishiness to classify cancer cells. The researchers, from University of California, Los Angeles, say cancer cells are generally two to five times squishier than normal cells, with pliability similar to that of Jell-O. PMF enables simultaneous measurements of cell mechanotype across multiple samples by using uniform air pressure to drive cell suspensions through porous membranes. The relative "deformability" of a cell sample is quantified by the fraction of sample retained above the membrane, with stiffer cells blocking the pores and squishier cells allowing more of the cell-and-liquid mixture to pass through. The team found that drug-resistant human ovarian cancer cells are softer than their drug-sensitive counterparts, and that more-invasive cancer cells are softer than less-invasive ones. (The technology was described in a study published Dec. 2, 2015 in *Scientific Reports*.)
- **Acoustic Separation Method ("Acoustic Tweezers")** works by using tilted-angle standing surface acoustic waves to apply pressure to a continuous flow of blood. Based

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on their size and weight, cancerous cells are forced out of the stream into a different channel, where they are collected. The power, intensity, and frequency used to generate the waves in this study are gentle (similar to common ultrasounds), the researchers say, helping to preserve the biocompatibility of the CTCs. The researchers, from Pennsylvania State University, report a throughput rate 20 times higher than previously achieved with similar devices, along with a separation rate of more than 83 percent. The researchers also say they found a way to separate the fluid-containing part of the device from the more expensive ultrasound-producing piezoelectric substrate, making "disposable" acoustic tweezers a cheap possibility. (The technology was described in a study published in April 2015 in the *Proceedings of the National Academy of Sciences*.)

- **Microstructure-Constricted Filtration and Pneumatic Microvalves** combine in this integrated microfluidic device to separate cells based on size and deformability. The researchers, from Northwest A&F University in China, say the technology can separate cancer cells from blood samples with more than 90 percent cell recovery and 80 percent purity. In a test of mesenchymal-like cancer cells and epithelium-like cancer cells (with different deformability) the technology demonstrated a "high" selectivity and recovery rate, the authors say. The high viability of the target cells benefits the ability to conduct downstream analysis. The device can also capture and release cells repeatedly, which improves its scalability. (The technology was described in the Aug. 2015 issue of *The Analyst*.)
- **Thermoresponsive NanoVelcro** is a postage-stamp–sized chip with nanowires that are 1,000 times thinner than a human hair, the researchers say, and are coated with antibodies that recognize CTCs. When 2 milliliters of blood are run through the chip, the tumor cells stick to the nanowires like Velcro. To separate the cells from the chip without damaging them, the purification system raises and lowers the temperature of the blood sample to capture (at 37 degrees Celsius) and release (at 4 degrees Celsius) circulating tumor cells at their optimal purity. The "mild" changes in temperature, the authors say, allow minimum disruption to the cells' viability and molecular integrity. The UCLA-based researchers were able to successfully conduct mutational analysis of the purified CTCs from lung cancer patients sorted by this system. (The technology was described in the January 2015 issue of *ACS Nano*.)

Microfluidic Cell Sorting Technologies

Types of Sorting (Based on Cell Preparation)	Physical Principles (Used For Sorting Mechanism)
Fluorescent Label-Based Sorting - relies on fluorescent probes or stains to identify cells by type.	■ Electrokinetic Mechanisms ■ Acoustophoresis ■ Optical Manipulations ■ Mechanical Systems
Bead-Based Sorting - depends on particles of a specific material, size, and surface-binding capacity to capture target cells, but can manipulate groups of cells simultaneously and holds promise for isolating tumor cells from unmodified biological fluids	■ Magnetophoresis ■ Acoustophoresis ■ Electrokinetic Mechanisms
Label-Free Sorting - relies on the physical differences in the properties of cells (size, shape, density, elasticity, polarizability, and magnetic susceptibility) and generally requires the least amount of preparation	■ Acoustophoresis ■ Electrokinetics ■ Magnetophoresis ■ Optics ■ Passive Cell Sorting

For more information see Shields' review "Microfluidic Cell Sorting: A Review of the Advances in the Separation of Cells from Debulking to Rare Cell Isolation" published in *Lab on a Chip*, Feb. 16, 2015.

Takeaway: A flurry of recent papers testing new methods and applications for single cell sorting attest to the excitement building around the clinical potential for the technology, including in the areas of CTC liquid biopsies.

HbA_{1c} Overtesting Common in Stable, Diabetic Patients

More than 60 percent of adults with stable and controlled type 2 diabetes receive too many glycated hemoglobin (HbA_{1c}) tests, according to a study published online Dec. 8, 2015 in the *British Medical Journal*. The researchers say this excessive testing leads to potential overtreatment, waste of health care resources, and increased patient burden in diabetes management.

"Clinically unnecessary testing can have detrimental effects for both the patient and the health care system," write the authors led by Rozalina McCoy, M.D., from the Mayo Clinic (Rochester, Minn.) "Excessive tests can cause unnecessary patient discomfort and anxiety, and because of the potential for false positive results caused by expected short-term biological and analytical variability of the HbA_{1c} test, they can increase the risk of further needless testing … and treatment change."

"Excessive tests can cause unnecessary patient discomfort and anxiety, and because of the potential for false positive results caused by expected short-term biological and analytical variability of the HbA_{1c} test, they can increase the risk of further needless testing … and treatment change."

—Rozalina McCoy, M.D.

While redundant HbA_{1c} testing has been previously identified as a problem, previous studies did not differentiate patients by level of glycemic monitoring needed. (Testing is recommended more frequently in those newly diagnosed, with variable glycemic control, receiving intensive insulin treatment, or undergoing treatment changes). The patients in this study had no history that would warrant intensive monitoring.

A national administrative claims database of 31,545 U.S. commercially insured adults (2001 to 2013) was retrospectively analyzed. Participants had non insulin-dependent type 2 diabetes with stable glycemic control. Testing frequency was classified as guideline recommended (2 times/year or less); frequent (3 to 4 times/year); or excessive (5 times/year or more). Changes in treatment were evaluated through pharmacy claims within three months of the index test.

The researchers found that HbA_{1c} testing frequency was excessive in nearly 5.8 percent of patients and was frequent in 54.5 percent. Excessive testing increased the odds of treatment intensification, which the authors called "concerning" given that baseline HbA_{1c} was already less than 7 percent.

"We found that patients over age 65 years and those with high underlying disease burden were more likely to have excessive HbA_{1c} testing," the researchers write. "Current guidelines recommend that patients with significant comorbidities should in fact be treated less intensely and have relaxed HbA_{1c} targets above 7.0 percent, making frequent testing in this population even less useful."

There was significant geographical variability in testing frequency, with the highest prevalence of excessive testing in the Northeast (8.9 percent) and the lowest prevalence in the Midwest region (4.0 percent). Excessive testing rates remained unchanged in 2001 to 2008, but fell significantly after 2009. This may have resulted from the U.S. National Quality Forum's designation of unnecessary laboratory tests as one of nine areas of wasteful or inappropriate care.

Takeaway: Although frequencies have dropped since 2009, excessive HbA_{1c} testing remains a problem in diabetic patients with stable glycemic control for whom guideline-compliant frequencies should be adequate. G2

No Benefit to CA-125 Ovarian Cancer Screening for General Population

The largest-ever study evaluating ovarian cancer screening for the general population showed only a modest reduction in the risk of death after more than a decade of follow-up. The use of changes in CA125 markers over time or transvaginal ultrasound, failed to achieve statistical significance versus no screening in primary analysis, according to a study published Dec. 17, 2015 in *The Lancet*, but the authors were encouraged by the screening methods' greater mortality reductions with longer follow-up periods.

"This late effect was predictable in view of the unavoidable time interval from randomization to diagnosis and then death. For participants who died in the no screening group the median interval from randomization to death was more than 8 years."

—Ian Jacobs, M.D.

Experts say the long-awaited study results don't justify screenings for the general population, but acknowledge more research is needed both in assessing these screening modalities, as well as emerging markers.

This trial, the UK Collaborative Trial of Ovarian Cancer Screening, included postmenopausal women (aged 50 to 74 years) screened at 13 National Health Service Trust centers in the United Kingdom. Women with increased risk of familial ovarian cancer were excluded from the study. Women were randomized to annual multimodal screening (MMS) with serum CA125 interpreted with use of the risk of ovarian cancer algorithm (n= 50,624), annual transvaginal ultrasound screening (USS; n= 50,623), or no screening (n= 101,299). The risk of ovarian cancer algorithm (ROCA) categorized women in the MMS group as normal (annual screening); intermediate risk (repeat CA125 concentration testing in 3 months); and elevated (repeat CA125 concentration testing and transvaginal ultrasound as a second-line test in 6 weeks).

The researchers found that at a median follow-up of 11.1 years ovarian cancer was diagnosed in 1,282 women overall (0.6 percent) with 338 (0.7 percent) in the MMS group, 314 (0.6 percent) in the USS group, and 630 (0.6 percent) in the no screening group. Death occurred in 148 of the diagnosed women in the MMS group (0.29 percent), 154 in the USS group (0.30 percent), and 347 in the no screening group (0.34 percent). Screening yielded a mortality reduction over years 0 to 14 of 15 percent with MMS and 11 percent with USS—both statistically insignificant.

However, upon further prespecified analysis, with exclusion of the prevalent cases of ovarian group in the MMS group, the mortality reduction became significant. The authors say the long-term effect of an MMS screening program is about a 28 percent mortality reduction after seven years of screening. Findings from this trial suggest that for 641 women screened annually using the multimodal strategy for 14 years, one ovarian cancer death is prevented.

"This late effect was predictable in view of the unavoidable time interval from randomization to diagnosis and then death. For participants who died in the no screening group the median interval from randomization to death was more than 8 years," write the authors led by Ian Jacobs, M.D., who co-invented ROCA. "The mortality hazard rate in the no screening group seems to increase, whereas in the two screened groups, it levels off. … This finding suggests that the difference in mortality between no screening and screening groups will increase with time and further follow-up."

Takeaway: Ovarian cancer screening using a CA-125 algorithm-dependent strategy is not yet justifiable for the general population of women.

Research/Applied Project Opportunities from the International School of Biomedical Diagnostics at Arizona State University

The International School of Biomedical Diagnostics (ISBD) at Arizona State University (ASU) recently invited companies in biomedical diagnostics and affiliated fields to partner with ISBD on research projects addressing issues in the diagnostics industry. Industry partners will benefit from projects executed by an inter-disciplinary team of students in ISBD's Biomedical Diagnostics online Master's degree program that have significant value to the company's business. "An ideal project is one which is multi-faceted and requires an inter-disciplinary team to address a challenge or problem the company has but [doesn't] have the resources to do at that time," explains Carl Yamashiro, Associate Research Professor, Biomedical Informatics at ISBD. He indicates some of the past projects have provided competitive intelligence to the host company on industry developments and the status of diagnostics in a particular area–which can, for example, allow the company to determine whether they can and should develop a product that will be competitive in that area. Other potential subjects for research projects include regulatory, bioethics and quality systems issues and challenges. Given the online nature of the degree program, laboratory-based research cannot be considered for these projects.

"By partnering with ISBD and ASU on supporting such projects, you are playing a very significant role in educating future leaders in the diagnostics and allied fields, and possibly identifying future colleagues to help drive the long term success of your company," said the ISBD/ASU announcement inviting research proposals. ISBD's Master's degree program "is designed to provide students a broad perspective of the field with a focus on applied research, technology development, reimbursement and regulation and current perspectives in the biomedical diagnostics field."

Many of the students already have professional experience working in laboratories and bring their own expertise to their projects, notes Yamashiro. Additionally, partnering companies also benefit from the breadth of knowledge available to the students from university faculty, several having more than 20 years experience in the diagnostics industry. While the research program operates within the department of biomedical informatics, the program can draw on expertise in the other schools and colleges within ASU in disciplines such as bioengineering, health economics, regulatory science, law and nursing. "[I]f there's a need for expertise in a particular discipline, … [the research team] can go to a resident expert here at the university that can give them advice," says Yamashiro.

Companies whose research proposals are selected will be asked to provide a mentor to work with a team of 3-4 master's students, spending an average of 1-2 hours per week overseeing progress of the research and providing practical advice. Teams will be conducting projects from March 14 - July 8, 2016. Proposals with a description of the scope of work must be submitted by January 29, 2016, to Carl Yamashiro at carl.yamashiro@asu.edu. For more information, contact Carl by email or call (480) 884-0348.

Six Protein Markers May ID Mood Disorders

Serum-based proteomic profiles may be useful in identifying and differentiating mood disorders, according to a study published online Dec. 8, 2015 in *Translational Psychiatry*. While these findings need further validation, psychiatrists are hopeful that such research will help move psychiatric diagnoses away from exclusive reliance on clinical observation of behavioral symptoms and towards empirical use of biologically based test results

A consecutive sample of patients with a confirmed diagnosis of major (unipolar) depression (UP; n=52) or bipolar depression (BP-I, n=46; BP-II, n=49) and controls (n=141) were tested using a blood sample. Initial analysis was based upon a proteomic multiplex profile of 320 proteins utilizing Myriad RBM's Discovery Multi-Analyte Profiling (MAP) platform. DiscoveryMAP is a quantitative multiplexed immunoassay service product, based on Luminex xMAP technology platform that was initially based upon immune mediator and cytokine quantification, increasingly recognized in the underlying neurobiology of mood disorders. Rigorous exclusion criteria were used to eliminate participants with non-specific inflammatory contributions from systemic illness and anti-inflammatory/biotic drug therapy.

The researchers found that six of the 320 proteins analyzed—growth differentiation factor 15 (GDF-15), hemopexin (HPX), hepsin (HPN), matrix metalloproteinase-7 (MMP-7), retinol-binding protein 4 (RBP-4) and transthyretin (TTR)—showed statistically significant differences between the patients and the healthy controls. The protein levels were higher in BP-I compared to all other patient groups and controls. MMP-7 was significantly different in mood disorder patients (BP-I, BP-II, and UP) versus controls; MMP-7, GDF-15, and HPN were significantly different in bipolar cases (BP-I and BP-II) versus controls; and GDF-15, HPX, HPN, RBP-4 and TTR proteins were all significantly different in BP-I versus controls.

"The results of this feasibility study support the possibility of developing a diagnostic test using the discovered biomarkers, which need to be validated, to help facilitate accurate diagnosis and rapid treatment initiation with improved clinical outcomes," write the authors led by Mark Frye, M.D., from the Mayo Clinic (Rochester, Minn.). "A proteomic-based differential diagnosis as an advanced decision making tool or companion diagnostic to guide evidence-based algorithms for mood stabilizer versus unimodal antidepressant therapy would have great clinical impact."

Takeaway: The discovery of six protein markers capable of distinguishing mood disorder patients, particularly BP-I, from healthy controls is a promising development in moving psychiatric diagnoses towards one driven by biomarker-based testing.

G2 INSIDER

Monthly Lab Testing Not Necessary With Common Acne Medicine

Monthly laboratory testing may not be necessary for patients taking the standard doses of oral isotretinoin (13-cis retinoic acid; first marketed as Accutane) for the treatment of acne, according to a review and meta-analysis published online Dec. 2, 2015 in *JAMA Dermatology*. While the drug is known to adversely affect cholesterol and triglyceride levels, these changes usually occur early in the course of treatment and few effects are severe or high-risk. Thus, the authors say, less frequent laboratory monitoring may be cost saving and reduce patient discomfort and inconvenience.

The drug package insert recommends baseline fasting lipid and hepatic panels with repeated testing at weekly or biweekly intervals until "the response has been established," but there are no specific suggestions for ongoing monitoring. In 2004 the American Academy of Dermatology published a consensus statement recommending periodic monitoring of serum triglyceride and cholesterol levels, as well as transaminase levels. But, dermatologists' actual testing patterns are not known.

The current meta-analysis included 26 clinical trials (1,574 patients) of oral isotretinoin (lasting at least four weeks) in patients aged 9 to 35 years with acne vulgaris. Laboratory values for lipid levels, hepatic function, and complete blood cell count were evaluated. The study's key findings were that isotretinoin is associated with a statistically significant change in the mean value of several laboratory tests (white blood cell count and hepatic and lipid panels), but that the mean changes across a patient group did not meet a priori criteria for high-risk or grade 2 abnormalities. Furthermore, the proportion of patients with laboratory abnormalities was low.

Most laboratory test abnormalities were detected early (typically between 6 and 8 weeks) during the treatment period and there were no substantial late adverse effects (up to 20 weeks) of isotretinoin therapy for the parameters measured—triglyceride and total cholesterol levels. (Later laboratory data were not available for other laboratory parameters.)

"A decrease in the frequency of laboratory monitoring for some patients could help to decrease health care spending and potential anxiety-provoking blood sampling," write the authors led by Young H. Lee, M.D., from Sharp Rees-Stealy Medical Center in San Diego. "At our institution, we perform a lipid and hepatic panel at baseline and after two months of isotretinoin treatment, with more frequent monitoring dictated by baseline abnormalities and medical history."

In an accompanying editorial lead author Kanade Shinkai, M.D., Ph.D., from University of California, San Francisco, suggests that despite the evidence and call for reduced monitoring, dermatologists may be limited in their willingness to accept new recommendations as a result of isotretinoin's "notorious reputation" for drug-induced effects as noted by the "unusually high number of legal proceedings related to this particular drug."

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