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Novel Tests May Earlier ID Those at Risk of Suicide, Postpartum Depression

Clinicians have long sought a way to identify which patients are at greatest risk for potentially disastrous consequences resulting from suicidal thoughts or postpartum depression. But finding an objective measure to assess psychiatric states, without asking a patient directly if they're suicidal, has proven challenging. Now, two new studies are providing hope that simple blood tests could hold the answer as to which patients could most benefit from early intervention—prior to exhibiting outward symptoms. The two studies highlight the tremendous ongoing work to identify reliable biomarkers of psychiatric conditions.

Continued on page 9

Universal Drug Testing at Delivery Aids Infants

Universal maternal drug testing at the time of admission for delivery improves the identification of infants at risk for developing withdrawal syndromes due to illicit drug exposure in utero. The greater-Cincinnati region believes this testing protocol could serve as a model for other communities with a high-prevalence of prescription opiate abuse and with conducive laws.

The switch to universal drug testing began in 2013, following pilot programs, in an attempt to combat a growing epidemic of neonatal abstinence syndrome (NAS), resulting from babies born addicted to drugs. In 2013 alone, according to the Ohio Department of Health, there were nearly five admissions per day for drug-dependent babies. Ohio experienced a 760 percent increase in the number of babies diagnosed with NAS from 2004 to 2013. The department says the average cost to treat a NAS baby is nearly $58,000 per hospitalization (an average length of stay of 15 days), totaling $97 million in 2013. Drug testing enables the hospital to identify and admit drug-exposed babies, who otherwise would likely be sent home before NAS symptoms appear.

"Universal testing is designed to help the family, the mother and the infant," says Scott Wexelblatt, M.D., medical director of regional newborn services at Cincinnati Children's Hospital Medical Center. "One of our

Continued on page 2

Universal Drug Testing at Delivery Aids Infants, *Continued from top of p.1*

main goals with this program is to identify and implement the best practice of care for this group of infants."

Twenty percent of infants exposed to opioids detected by universal screening would not have been identified using risk-based screening, according to results from the program's pilot phase, published in the *Journal of Pediatrics* in November 2014. The program is currently operational in 18 hospitals in the greater-Cincinnati region, including hospitals in Kentucky and Indiana. According to the Greater Cincinnati Health Council some hospitals have chosen to obtain informed consent for the testing, while others have made it part of their nursing policy.

"Risk-based screening involves profiling and it still won't catch everybody," Wexelblatt tells *DTET*. "Universal testing is cleaner and actually goes over better ."

Currently, Cincinnati's universal maternal screening is conducted using a urine-based immunoassay, with confirmatory mass spectroscopy-based testing. Current mass-spec confirmatory testing volume at Cincinnati Children's (received from 10 of the 18 participating hospitals) is 20 to 40 samples per day. Confirmatory testing was recently switched from meconium samples to umbilical cord samples, to ease collection for nursing staff. Wexelblatt says they are still fine-tuning the cutoff values in the immunoassay to improve the appropriateness of mass spec testing, which is costly. The hospital is essentially "eating" the cost of the testing (immunoassay ranges from $7 to $13 and mass spec from $50 to $160). While the expense is a "concern," given the flat fee hospitals receive for a delivery, participating hospitals believe it constitutes the "best care" for the infants.

> *"Risk-based screening involves profiling and it still won't catch everybody. Universal testing is cleaner and actually goes over better ."*
>
> —Scott Wexelblatt, M.D.

Wexelblatt does field inquiries from hospitals in other areas considering such a program. He says that there are two keys to success. First, universal testing must be implemented regionally; otherwise, he says, word gets out and drug users will go to another hospital to deliver. Secondly, he says, it only works in cities and states that don't use the test results punitively. Unlike in Tennessee, Alabama, and South Carolina where women caught using illegal drugs during pregnancy can be prosecuted, Ohio does not criminally charge women if they test positive. This difference, keeps the universal testing program legal.

In 2001, the U.S. Supreme Court ruled that pregnant women cannot be subject to "warrantless and nonconsensual" searches. In the case *Ferguson v. City of Charleston*, No. 99-936, women's positive test results were turned over to law enforcement and they were prosecuted for drug offenses and/or child neglect. At the time testing was initiated in South Carolina (1988), the program was driven by an increase in cases of cocaine use among pregnant women. The Supreme Court ruled that drug testing fits in the "closely guarded category of constitutionally permissible suspicionless searches" if there are "protections against the dissemination of the test results to third parties."

Takeaway: More metropolitan areas with a high prevalence of opioid abuse could consider universal maternal drug testing at the time of delivery. This testing strategy, while costly to hospitals, improves care for drug-exposed infants. G2

Genetic Risk Score Aids in Parkinson's Diagnosis

A new, noninvasive model significantly outperforms any single classifier in identifying patients with Parkinson's disease, according to a study published Aug. 11 in *Lancet Neurology*. The model is able to discriminate patients without evidence of dopaminergic deficit, typical of Parkinson's disease, from those patients with aetiologically typical disease, which the authors say, could improve detection of preclinical disease.

For complex progressive diseases such as Parkinson's disease, preclinical diagnosis and low error rates in diagnosis are crucial both not only for clinical care, but also for correctly classifying patients participating in clinical trials.

"Unlike dopamine transporter positron emission tomography [DAT] scanning, our model is portable and can be administered remotely at a fraction of the cost: our model costs around US$100 per sample versus DAT scanning, which can cost thousands of dollars per patient," write the authors led by Mike Nalls, Ph.D., from the National Institute on Aging (Bethesda, Md.). "This model might be useful as part of a diagnostic path towards more accurate preclinical detection of Parkinson's disease."

"This model might be useful as part of a diagnostic path towards more accurate preclinical detection of Parkinson's disease. "

—Mike Nalls, Ph.D.

The researchers participating in the Parkinson's Disease Biomarkers Program and Parkinson's Progression Marker Initiative developed a model for disease classification using data from the Parkinson's Progression Marker Initiative study (n= 367 patients with Parkinson's disease and 165 controls). Factors selected to contribute to the model included: olfactory function, disease-specific genetic risk score (based on 30 risk factors replicated in one or more studies), family history of Parkinson's disease, age, and gender. The model was then tested using data from 825 patients with Parkinson's disease and 261 controls from five independent cohorts.

Each of five factors included in the model made significant contributions to the content informing the integrative predictive model (smell score, 63.1 percent of the explained variance; genetic risk score, 13.6 percent; family history, 11.4 percent; gender, 6.0 percent, and age 5.9 percent). The researchers found that using the discovery data, the model correctly distinguished patients with Parkinson's disease from controls with an area under the curve (AUC) of 0.923 and with high sensitivity (0.834) and specificity (0.903) at its optimum AUC threshold (0.655). External validation showed good classification of Parkinson's disease, with AUCs ranging from 0.894 to 0.998 across the different cohorts. Furthermore, four of 17 participants who had scans without evidence of dopaminergic deficit and who the model classified as having Parkinson's disease, converted to Parkinson's disease within 1 year.

The authors say that in the future the accuracy of the model can be improved by identifying more disease-specific biomarkers and genetic risk loci. Current resequencing of known loci are expected to generate more accurate estimates of genetic risk.

"Ninety-three percent (N=28/30) of the genetic risk variants that we used to create our genetic risk score are from genome-wide association studies and are therefore probably surrogates for true functional variants because of the inherent nature of these imputation-based studies," write the authors. "Identification of the true functional variants within loci would improve our algorithmic classification of Parkinson's disease."

Takeaway: An algorithm may improve identification of patients with Parkinson's disease, even prior to clinically significant disease.

Inside The Diagnostics Industry

Shift to Gene Panels to Assess Hereditary Cancer Risk Forges Ahead; Evidence of Clinical Utility, Cost-Effectiveness Emerges

A virtually unlimited number of genes tied to hereditary cancer risk can be simultaneously assessed in commercially available tests given both advances in technology and the Supreme Court's two-year old ruling overturning gene patents. By screening multiple genes in parallel, research has shown that diagnostic yields are on the rise and time to results are down, with the added benefit of not adding much incremental cost for delivery of additional information.

> *"The train has left the station and is unlikely to return. It is therefore critical that we assess the clinical utility of such testing."*
>
> —Elizabeth Swisher, M.D.

As a result of all of these factors, there has been a noticeable uptick in clinical adoption of multigene panels to assess hereditary cancer risk. Yet, despite research heralding the effectiveness of these multigene panels' appearing in the literature, some clinicians are asking whether adoption is occurring prematurely, before there is a good understanding of the consequences of panel-based testing on clinical management of the patient and other potentially affected relatives.

"Many cancer genetics experts have again urged caution, characterizing the use of multigene testing in the clinical setting as premature. Yet thousands of women and their physicians are ignoring this advice, ordering a wide selection of multiplex tests daily," writes Elizabeth Swisher, M.D., from University of Washington, Seattle in an editorial in *JAMA Oncology* on Aug. 13. "The train has left the station and is unlikely to return. It is therefore critical that we assess the clinical utility of such testing."

Given the relatively mature understanding of the risk posed by BRCA1/2 mutations, many are viewing testing for hereditary breast and ovarian cancer (HBOC) as a "tracer condition" that can demonstrate how "early and rapid" adoption of genetic testing influences treatment decisions. In the case of BRCA1/2 testing, evidence indicates that significantly more women diagnosed with breast cancer are receiving genetic counseling and testing, and among those that complete testing more and more are evaluated using multiplex assays, rather than single gene tests. Even Myriad Genetics (Salt Lake City), the company that was built upon patent protected BRCA testing, has shifted to its myRisk test. The company says that the 25-gene panel identifies more mutation carriers than its BRCAAnalysis or colorectal cancer Colaris tested, combined—an increase in mutation detection by 40 to 50 percent.

"The shift to panels is a mega movement, driven for good reasons," Robert Nussbaum, M.D., the chief medical officer at molecular testing company Invitae, tells *DTET*. "Between the wear and tear on the patient and the expense of sequencing single genes, such testing would be completely prohibitive."

Panels for Hereditary Breast, Ovarian Cancer

Now, emerging evidence is showing that identification of more mutation carriers translates into changes in clinical decision-making behavior. Multigene panel testing to assess HBOC risk is likely to change clinical management for substantially more patients than BRCA1/2 testing alone, according to a study published Aug. 13 in *JAMA Oncology*.

Inside The Diagnostics Industry

In the study, more than 1,000 individuals seen at three large academic medical centers were identified as appropriate candidates for HBOC evaluation based on established criteria. The enrolled participants all lacked BRCA1/2 mutations, yet commercially-available multigene panel testing was performed (the 29-gene Hereditary Cancer Syndromes test by Invitae was used at two sites, while the 25-gene MyRisk test from Myriad Genetics was used at the third site). The clinical actionability of detected mutations was assessed using the National Comprehensive Cancer Network (NCCN) established gene-specific management recommendations to measure impact on clinical decision-making.

"If you overdo it with the number of genes, you won't know the clinical significance of all of the variants and it adds to the burden of the clinician and the genetic counselor."

— Robert Nussbaum, M.D.

The researchers found that 40 of 1,046 BRCA1/2-negative patients harbored deleterious mutations, most commonly in moderate-risk breast and ovarian cancer genes (CHEK2, ATM, and PALB2) and Lynch syndrome genes. The authors say that the 3.8 percent prevalence of additional mutations represents a substantial (greater than 40 percent) increase in diagnostic yield of risk-associated mutations compared with BRCA1/2 testing alone. The vast majority of the mutations were deemed clinically significant, driving additional disease-specific screening and/or prevention measures, beyond those decisions based on personal and family history alone. Furthermore, additional changes to familial testing would be considered for almost three-quarters of patients with detected mutations. As expected, the most common mutations were seen in genes associated with low or moderately increased breast cancer risk (40 of 63). Management change (increased screening or preventive surgery) would be recommended for only 10 of 40 of these cases. However, nearly one-third of mutation-positive patients (20 of 63) harbored mutations in high-risk genes tied to NCCN management guidelines and all of these mutations would change case management.

"We recognize that our data regarding clinical actionability are based on consensus practice guidelines, rather than actual clinical practice," writes senior author Leif Ellisen, M.D., Ph.D., from Massachusetts General Hospital (Boston). The authors acknowledge the actual clinical effect of multigene testing could vary depending on the number of patients choosing to be tested, clinician adherence to gene-based management guidelines, and how closely patients follow clinician recommendations. While actionability is a critical driver of genetic test ordering, defining actionability is challenging, Swisher writes in the accompanying editorial. The authors use NCCN guidelines as a standardized measure of actionability, but she notes, NCCN recommendations are incomplete for many genes and therefore may "undervalue" the utility of testing based on current gene-based management guidelines.

Determining Ideal Panel Size

The fact that the technology can interrogate nearly limitless numbers of genes does not necessarily mean that such mega-panels are appropriate or desirable. "There are panels and then there are panels," Nussbaum says. "If you overdo it with the number of genes, you won't know the clinical significance of all of the variants and it adds to the burden of the clinician and the genetic counselor."

Inside The Diagnostics Industry

While some appreciate the potential future importance of collection of more genetic data (as it can be reanalyzed as genetic understanding evolves), others view mega-panels that include low- to moderate-risk genes mutations as actually complicating clinical decision-making, since the clinical actionability of these results is less defined.

"There is growing concern that clinicians are falling behind in their understanding of fundamental aspects of genetic testing and their confidence in discussing results with patients, writes co-author Steven Katz, M.D., from University of Michigan, Ann Arbor, in a July 23 Viewpoint published in the *Journal of the American Medical Association*. "An alternative viewpoint is that the use of genetic tests is outpacing the applicability of their findings to cancer treatment decisions." Katz notes a "widening gap" between the availability of expansive genetic testing and the relative importance of results to treatment decisions.

The more genes that are evaluated for mutations ups the likelihood of finding VUS. According to an abstract presented at the American Association for Clinical Chemistry's annual meeting (Atlanta; July 26-30) researchers from the Hermes Pardini Institute in Brazil tested 12 women with a 15-gene HBOC panel. Initial results found no significant clinical mutations in four patients, while VUS were detected in the remaining eight patients. The researchers defined VUS as undescribed missense mutations or described variants with minor allele frequency of less than 0.02. However, lead author Giovana Torrezan reports than in just over a year the status of some of the VUS has already changed. While rechecking database searches on these variants in preparation of the abstract, mutations in PALB2 and CHEK2 have now been reclassified as benign and damaging, respectively. Torrezan said at the conference that this shift in understanding underscores the need for in silico analysis tools to keep databases up to date.

Cost Effectiveness

Given the questions over the clinical utility of test results, it is no surprise that insurers often balk at paying for panel-based tests. While the industry is well aware of the need to generate evidence of clinical utility, there remains scant evidence as to the cost effectiveness of panel-based testing. This summer, though, researchers published findings that use of a sequencing panel that includes genes associated with highly penetrant colorectal cancer and polyposis (CRCP) syndromes, in addition to Lynch syndrome genes, is cost effective as a first-line test. According to a study published June 20 in the *Journal of Clinical Oncology*, the test is likely to provide meaningful clinical benefits using the standard $100,000 per quality-adjusted life years (QALYs) gained threshold.

The researchers developed a decision model to evaluate the cost effectiveness of various sequencing panels in patients with suspected hereditary CRCP syndromes versus the sequential evaluation for Lynch syndrome, as recommended by current guidelines.

The researchers found that evaluation with a sequencing panel that included Lynch syndrome genes plus other genes associated with highly penetrant CRCP syndromes was "highly cost-effective" and led to an average increase of 0.151 year of life, 0.128 QALY, and $4,650 per patient, resulting in an incremental cost-effectiveness ratio of $36,500 per

Inside The Diagnostics Industry

QALY compared with standard care. Sequencing for only Lynch syndrome genes yielded a cost per QALY of $144,200, significantly higher than the accepted $100,000 threshold. The further addition of genes with low colorectal cancer penetrance resulted in an incremental cost-effectiveness ratio of $77,300 per QALY, still cost effective, but less so.

"Despite the promise of next-generation sequencing [NGS], the utility of testing multiple genes with different modes of inheritance and with varying levels of disease penetrance has been questioned based on the argument that the costs of increased surveillance and unnecessary treatments may outweigh the benefits of cancer prevention, as well as the uncertain consequences of the identification of VUS," write the authors led by Carlos Gallego, from University of Washington, Seattle. "Our findings suggest that the use of NGS panels that includes genes associated with highly penetrant CRCP syndromes can translate into better health outcomes and likely provide acceptable value to the heath care system."

Insurers, including the Centers for Medicare and Medicaid Services (CMS), are not yet convinced of the benefit of paying for panel-based testing, according to a research note published May 28 by senior research analyst William Quirk at Piper Jaffray & Co. CMS published preliminary gap fill rates for sequencing-based panels well below current reimbursement levels calculated using stacking of Current Procedural Terminology (CPT) codes. Of the 21 next-generation sequencing-related CPT codes, which were designed to improve transparency in reimbursement, only four were priced using preliminary gap fill payment. Of the four priced tests, targeted sequencing panels (of five to 50 genes) were priced by only a single Medicare administrative contractor. Quirk called the pricing for the panel "surprising." Quirk cites industry sources saying that there is "little reimbursement traction from private payers" as well, with most tests billed under the new codes denied payment.

"One of the major obstacles to adopting the panels is the perception they are exorbitantly costly," says Nussbaum.

Panel Payment Pricing Still Low

Preliminary release of gap fill pricing shows that payment for panels remains low compared to payments previously derived through stacked gene codes.

Test description	Stacked Equivalent	Preliminary Gap Fill
Hereditary Colon Cancer - Sequencing	$1,963	$796
Hereditary Colon Cancer - Duplication/ Deletion	$1,963	$796
Targeted Sequencing Panel - 5-50 Genes	$2,564	$90
Targeted Sequencing Panel 5-50 Genes plus Gene Expression	$6,852	$90

Source: Piper Jaffray & Co.

Yet, industry watchers are hopeful that momentum is gaining for reimbursing these multigene panels. In mid-August a multi-stakeholder group convened by the Center for Medical Technology Policy (CMTP, Baltimore, Md.) recommended coverage for sequencing panels of 5-50 genomes if they include a subset of constituent genes that are considered to be standard-of-care and medically necessary for the patient. The groups says such reimbursement is necessary to advance personalized medicine for cancer. CMTP focuses on comparative effectiveness and patient-centered outcomes research. The group, which included

Inside The Diagnostics Industry

sequencing testing and technology companies, medical professional societies, patient advocacy groups, and leading health plans, also recommended that payers rely on the College of American Pathologists accreditation program and proficiency testing to assure the analytic validity of sequencing tests, as well as a proposal for payers to cover larger, even more comprehensive panels with preauthorization under circumstances of "extenuating medical need."

In the interim, while payers come up to speed, another strategy is emerging—with companies pricing multigene panels so low they can gain commercial traction through increased access to medically indicated testing with private pay patients. For instance, Invitae (San Francisco), a firm specializing in genetic testing for hereditary disorders, cardiology, hereditary cancer, pediatric genetics, neurology, and hematology, launched a $475 flat fee per indication for its full menu of genetic tests. The patient pay program, the company says, reflects the desire to bring affordable genetic testing "to the masses in a medically responsible way." The pricing is exclusively for patients who register online, pay up front for genetic services themselves, and whose clinician has ordered the testing online. The company envisions patients ordering the test will be those that do not meet coverage policies for testing, those with high-deductible plans, and those not covered by insurance.

"Most of the conversations about reimbursement for genetic testing have focused on economic issues. However, an under-appreciated dimension in using genetic testing for genetic care is an ethical one," Nussbaum previously said in a statement. "For years, many people and their families have not had the benefit of clearly indicated genetic testing due to unwillingness of third-party payers to pay the historically high cost. Now that the genetics market is becoming a generic market—and thanks to the ongoing innovations and cost reductions in sample preparation, sequencing and medical interpretation—we are beginning to see the benefits translated into affordable testing for patients."

Invitae released new pricing for institutional customers and third-party payers, as well. For those payers that include Invitae in their network, the price per indication is $950. For third-party payers with whom Invitae is out of network and non-contracted institutions, the price per indication remains $1,500, the company said in a statement.

Takeaway: Despite awareness of the need to study the effect panel-based testing has on clinical care, panel-based testing for heritable cancer risk will continue to gain momentum, with HBOC testing leading the way.

Few Genes Included in HBOC Panels Show Enough Evidence of Elevated Risk

A study published June 4 in the *New England Journal of Medicine* evaluated the clinical validity of 11 commercially available multigene testing panels for HBOC.

In total the panels covered more than 100 genes (range per test, three to 97) with breast cancer specifically mentioned as an indication for 21 of these genes. Currently, the authors say, only variants in BRCA1, BRCA2, and TP53 confer a high risk of breast cancer. Existing evidence only puts PALB2 and PTEN in the moderate risk category (genes for which fully deleterious mutations confer a risk of breast cancer that is two to four times as high as that in the general population). Other genes with sufficient evidence of conferring moderate risk include CHEK2, ATM, and NF1. While mutations in STK11, CDH1, and NBN show "clear evidence" for an association with risk of cancer, risk estimates are "too imprecise" for categorization of the magnitude of risk. The authors caution that risk estimates for PTEN, STK11, and CDH1 are based on studies of selected and thus, may be "seriously overestimated." The authors say other currently tested genes have "insufficient evidence" and "caution against their use" in such panels.

■ Novel Tests May Earlier ID Those at Risk of Suicide, Postpartum Depression, *Continued from top of p.1*

RNA Test Can Predict Suicide Risk

The combination of blood-based RNA biomarkers in a simple questionnaire app can predict which psychiatric patients are at the greatest risk of suicide, according to a study published online Aug. 18 in *Molecular Psychiatry*. The researchers had previously shown in a proof-of-principle study that blood-based gene expression biomarkers could predict future hospitalizations due to suicide in male bipolar disorder patients. The present study broadens validation of an improved set of markers across major psychiatric disorders (bipolar disorder, major depressive disorder, schizoaffective disorder, and schizophrenia) and combines the quantitative markers with an app-based questionnaire to achieve greater than 90 percent accuracy in identifying which patients are most likely to attempt suicide.

The multi-step study first identified changes in gene expression associated with progression from no suicidal ideation (SI) to high SI states among 37 participants of a longitudinal psychiatric cohort (*n*=217). Expression changes were assessed using both absent/present (reflecting on/off of transcription) and differential expression (more subtle gradual changes in expression levels). Candidate markers were then prioritized using the group's Convergent Functional Genomics approach. Next, these top biomarkers were validated using blood from a demographically matched cohort of suicide completers from the coroner's office (*n*=26 male violent suicide completers with samples collected within 24 hours of death). Lastly, the top biomarkers were tested in an independent cohort of psychiatric participants for prediction of suicidal ideation (*n*=108) and in a follow-up cohort of psychiatric patients (*n*=157) for prediction of hospitalizations due to suicidality.

"We propose that the widespread use of such risk prediction tests as part of routine or targeted health care assessments will lead to early disease interception followed by preventive lifestyle modifications and proactive treatment."

—Alexander B. Niculescu III, M.D., Ph.D.,

The researchers found that biomarkers for suicidal ideation only are enriched for genes involved in neuronal connectivity and schizophrenia, while biomarkers also validated for suicidal behavior are enriched for genes involved in neuronal activity and mood. Among the top biomarkers for suicidal behavior, 14 percent were tied to psychological stress response, and 19% for involvement in programmed cell death. The best individual biomarker across psychiatric diagnoses for predicting suicidal ideation was SLC4A4, with an area under the curve (AUC) of 72 percent. It performed even better for patients with bipolar disorder, predicting suicidal ideation and future hospitalizations with an AUC of 93 percent and 70 percent, respectively.

Two new 22-item clinical questionnaire-based apps, one for affective state and one for suicide risk factors (life events, mental health, physical health, stress, addictions, and cultural factors) predict suicidal ideation across psychiatric diagnoses (AUC range of 85 and 89 percent, respectively). Integrating the top biomarkers and the clinical questionnaire information into a universal predictive measure (UP-Suicide) showed broad-spectrum predictive ability across psychiatric diagnoses with an AUC of 92 percent, which improved to 98 percent for bipolar disorder.

"We propose that the widespread use of such risk prediction tests as part of routine or targeted health care assessments will lead to early disease interception followed by preventive lifestyle modifications and proactive treatment," writes lead author Alexander B. Niculescu III, M.D., Ph.D., from Indiana University in Indianapolis.

In a statement, Niculescu said he believes the apps are ready to be deployed and tested by medical professionals, particularly in emergency department settings, while the biomarkers are undergoing further validation both in women and in people without a psychiatric diagnosis.

Gene Expression Marker May Predict Postpartum Depression

Epigenetic regulation of the oxytocin receptor gene (OXTR) appears to be tied to development of postpartum depression (PPD), according to a study published July 21 in *Frontiers in Genetics*. The hormone oxytocin has long been known to play a role in mood regulation and maternal bonding, but factors contributing to decreased expression of OXTR may contribute to PPD and identifying this variability prior to symptom onset is central to predicting risk of PPD.

"Identification of genetic and epigenetic susceptibility to depression in pregnancy may be one key element in a multidisciplinary approach to reduce the development of PPD and hence the adverse sequelae of depression," write the authors. Jessica Connelly, Ph.D., senior author from University of Virginia, Charlottesville adds in a statement, "We know that women who have experienced depression before pregnancy are at higher risk of developing depression in the postpartum period. However, women who have never experienced depression also develop postpartum depression. These markers we identified may help to identify them, in advance."

"Findings from this study argue for the integrative use of genetic and epigenetic markers in the oxytocin pathway to better understand and predict risk of psychological disorders in the postnatal period, a critical period for healthy mother–infant interaction."

—Aleeca Bell, Ph.D.

Data from women (269 PPD cases and 276 controls matched for age, prior parity, and presence of depressive symptoms in pregnancy) participating in the Avon Longitudinal Study of Parents and Children (date of delivery between April 1991 and December 1992) were analyzed for symptoms of depression (both at 32 weeks gestation and 8 weeks postpartum). Additionally, blood was evaluated for OXTR DNA methylation and genotype (rs53576_GG and rs2254298_A).

The researchers found that no genetic/epigenetic interactions predicted PPD in the total sample. However, there was a significant interaction between rs53576 genotype and methylation in the OXTR gene amongst women who did not have depression prenatally, but developed PPD. Those women with GG genotype showed more than 2.6 greater odds of PPD for every 10 percent increase in methylation level, whereas methylation was unrelated to PPD amongst "A" genotype carriers. There was no such interaction among women with PPD and prenatal depression.

"Findings from this study argue for the integrative use of genetic and epigenetic markers in the oxytocin pathway to better understand and predict risk of psychological disorders in the postnatal period, a critical period for healthy mother–infant interaction," writes lead author Aleeca Bell, Ph.D., University of Illinois at Chicago. "Early identification of susceptibility might allow clinical vigilance for the possible development of PPD, and targeting of preventative interventions. The biologically at-risk women in this study did not display elevated symptoms of depression in pregnancy, but went on to display an increased risk of PPD after birth."

Takeaway: Researchers are moving closer to identifying quantifiable markers that can effectively predict risk of harm from psychiatric conditions. Targeting preventive interventions and therapies at high-risk individuals may ultimately cut rates of suicide and postpartum depression.

Vaginal Microbiome Predicts Risk of Premature Labor

The composition of a woman's vaginal microbial community may predict risk of preterm birth. According to a study published Aug. 17 in *Proceedings of the National Academy of Sciences* a specific bacterial pattern ups the risk of preterm delivery, and the longer the pattern persists, the greater the risk. The authors say the findings have important implications for pregnancy outcomes.

Preterm birth is a sizable problem globally. In the United States alone there are approximately 450,000 babies born prematurely each year. Prior research suggests that at least a quarter of preterm births are associated with occult microbial invasion of the amniotic cavity, most often from the mother's host microbiota. Given this link, the researchers analyzed the microbiomes of 49 pregnant women, 15 of whom delivered preterm. From 40 of these women (in the discovery cohort) the researchers analyzed the microbiota from 3,767 specimens (collected prospectively and weekly during gestation and monthly after delivery from the vagina, distal gut, saliva, and tooth/gum) to characterize temporal changes and community features associated with preterm birth. For the nine women in the validation set, 246 vaginal samples were analyzed.

"We now recognize that our bodies' microbial communities perform many beneficial functions, yet there may be times when the communities get out of whack ."

—Daniel DiGiulio, M.D.

The researchers found that microbiota community composition remained "remarkably" stable at all four body sites during pregnancy. A distinct vaginal community pattern dominated by a low prevalence of Lactobacillus bacteria with elevated abundances of Gardnerella or Ureaplasma (community state type [CST] 4) was significantly associated with preterm delivery. This finding was similarly seen in the validation cohort in which four of the nine women delivered preterm.

"Of note, if we had collected specimens less frequently than weekly, we would have missed a number of excursions to CST 4 and hence would have been hindered in associating this state with preterm birth," write the authors. The authors also observed that most women experienced a post-delivery disturbance in the vaginal community characterized by a decrease in Lactobacillus species and an increase in diverse anaerobes species, which persisted for up to one year post delivery. While this disturbance was unrelated to gestational age at delivery, the authors say it may have implications for the known increased risk for preterm birth in women whose pregnancies are closely spaced.

"Our data suggest that if the microbiome plays a role in premature birth, it may be something that is long in the making," lead author, Daniel DiGiulio, M.D., Stanford University (California), said in a statement. "It may be that an event in the first trimester or early second trimester, or even prior to pregnancy, starts the clock ticking.... We now recognize that our bodies' microbial communities perform many beneficial functions, yet there may be times when the communities get out of whack."

Takeaway: While the findings need further validation in a larger, more diverse group of women, in the future, testing the vaginal microbiome may identify which women are at risk for premature delivery, which may ultimately be preventable through early intervention with a probiotic treatment. G2

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Isothermal Molecular C. Diff Tests Ideal for Small, Mid-Sized Labs

Two molecular in vitro diagnostic assays that utilize isothermal reactions to detect toxigenic C. difficile are accurate, yet "fast" and "relatively simple to use" compared to most of the PCR-based assays, according to a study published in the August issue of *Diagnostic Microbiology and Infectious Disease*. The data shows that AmpliVue (Quidel) and Illumigene (Meridian Biosciences) can be used in diverse laboratory settings with both adult and pediatric patient populations.

Reliable and quick detection of the presence (or absence) of C. diff toxin genes in stool samples, can result in faster initiation of the proper treatment of patients and initiation of infection control measures to prevent the spread of the disease, especially in health care environments. While there has been growing interest in the sensitivity of molecular assays for the diagnosis of C. diff infections, most of the early U.S. Food and Drug Administration-cleared nucleic acid amplification tests require extraction of genomic material on a separate instrument followed by amplification using thermocyclers. While these are an improvement over the gold standard diagnostic method of cytoxigenic culture, diagnostics involving isothermal reactions may achieve similar performance in a relatively simple test format, compared to polymerase chain reaction- (PCR-) based assays.

In the current *DMID* study, laboratorians at multiple sites (two children's hospitals [median age less than 10 years] and one general hospital [median age 58 years]) evaluated the use of isothermal amplification chemistries for detection of C. diff using fresh specimens. These assays were used in hospital laboratories with low- to medium-volume C. difficile testing (January 2013 to September 2013). All 758 stool samples were tested for the presence of C. diff toxin gene using the Illumigene assay (isothermal loop-mediated isothermal amplification) as the test of record. Remnant stool specimens that were enrolled in the study were deidentified and tested with the AmpliVue assay (isothermal helicase-dependent amplification). Any discordant results were resolved by performing toxigenic culture.

The researchers found that the two assays showed 97.8 percent concordance overall. Following discordant resolution, the combined performance for all three sites for the AmpliVue assay and Illumigene assays were: 96.1 percent sensitivity for both; 99.2 percent and 99.8 percent specificity, respectively; 96.1 percent and 99.2 percent positive predictive value, respectively; and 99.2 percent negative predictive value for both. This study found that in patients less than two years of age the assays performed similarly, suggesting that these kits can be reliably used in this population.

The methods studied here offer several advantages," writes lead author Stella Antonara from Nationwide Children's Hospital (Columbus, Ohio). "They do not require specialized training of technologists, they can be performed on demand without having to batch samples, and the results can be available within hours of receipt of the sample. These assays are ideal for small- to medium-sized laboratories that lack room and capabilities for larger PCR systems due to their small space requirements."

Company References

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