PEDIATRIC AND MATERNAL-FETAL DIVISION NEWS
Volume 34 | Issue 2 |July 2015

AACC Better health through laboratory medicine.

FROM THE MIND OF THE CHAIR

Dear Friends,
A lot is going on in preparation for the upcoming AACC annual meeting…..

First and most important!!! Please congratulate all of our fellow members who have and continue to contribute to the success of this division. As a token of recognition for all of the historical effort, the division will be honored with an achievement award. However, the annual meeting excitement doesn't stop there. In case you are looking for real fun and entertainment in Atlanta, plan to show up at the PMF, Industry, Informatics and Translational Divisions joint mixer. We have a new time and date for it: Sunday evening!! Furthermore, if that isn't enough for your mind and soul, we have scheduled a "pediatric-maternal-fetal hot topic" session on Tuesday afternoon. To round it up, get moving and join us on a poster walk on Wednesday afternoon. Please refer to the newsletter content to learn more about these and other items of interest.

TABLE OF CONTENTS

In addition, and keeping with the tradition of stimulating young minds, we will raffle five division memberships to the Society for Young Clinical Laboratorians during the upcoming meeting. On this note, don't miss the chance to read an important and up to date article written by our fellow, Dr. John Mills on "Variants of Uncertain Significance".

Please stay in touch and let us know your thoughts and suggestions. If you are planning to attend the AACC Annual Meeting in Atlanta, please join us at one of the sessions mentioned above. We are here to help you make a difference.

Best,

DC

David Carpentieri, MD
Chair, AACC PMF Division

THE ABC'S OF PEDIATRIC LABORATORY MEDICINE:

V IS FOR VARIANT OF UNCERTAIN SIGNIFICANCE

John R. Mills, PhD
Clinical Chemistry/Clinical Molecular Genetics Fellow, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

The use of large scale sequencing technologies continues to expand into the practice of medicine. Genetic testing has evolved from looking for a few select abnormalities to broad large scale whole exome and whole genome sequencing (WES and WGS, respectively) resulting in discovery of an increasing number of novel variants. Due to the expanding role of sequencing in clinical practice and increased complexity of analysis, the American College of Medical Genetics and Genomics (ACMG), Association for Molecular Pathology (AMP), and College of American Pathologists (CAP) have put forward efforts to standardize how findings are reported and how this information is communicated in order to properly direct follow up care appropriate for the predicted impact of the genetic information on health and quality of life. The recent ACMG standards and guidelines recommendation proposes an updated classification system for interpretation of sequence variants. (1). Due to variation in the usage of terms defining mutations and polymorphisms, the ACMG has recommended these be known collectively as "variants" with the following modifiers being incorporated– "pathogenic", "likely pathogenic", "uncertain significance", "likely benign" and "benign" to describe variants as they relate to Mendelian diseases. This new classification approach is thought to be more stringent than what most laboratories currently employ, meaning more variants are likely to be categorized as variants of "uncertain significance" (VUS). A VUS is a finding where there is not sufficient clinical or scientific support to unequivocally classify a variant as "pathogenic" or "benign". It is important to recognize that classification of a variant as having "uncertain significance" is a default category where the variant cannot be reasonably categorized as benign or pathogenic.

The ACMG has outlined a comprehensive system for categorizing variants as "benign" or "pathogenic" that utilizes population data, computational/predictive analysis, functional studies, segregation information, de novo status, allelic data and other sources of data. If a variant does not fulfill criteria as either "pathogenic" or "benign", or the evidence is conflicting, the variant defaults to a VUS. The inability to classify all variants as "benign" or "pathogenic" relates to several different gaps in our current knowledge. The identified variant may not have been encountered or reported previously. The variant could have a known pathogenic role; however it could have highly variable expressivity or incomplete penetrance in relation to the clinical presentation. There may be conflicting data in regard to the variant in relation to its segregation with the disease, or the studies may be limited or ambiguous. Potentially misleading, there may be significant publication bias; literature may be prone to overrepresentation of severe cases of disease associated with a specific variant. This could lead to inappropriate variant classification. Another problem classifying variants is that two identical variants may be interpreted and

reported differently across laboratories. This relates to differences in classification systems, as well as to variable access to large data sets which impact classification. This leaves the door open for the opportunity for misclassification and potential patient harm. Another major challenge interpreting a VUS is that the categorization of a variant may change over time as more definitive information becomes available. For instance, new variant frequency data from large population studies has led to the re-categorization of many VUS to "likely benign" or "benign". It is critical that the clinical laboratory have the ability to update reports and effectively communicate changes to both patients and providers as VUS are re-categorized.

One solution broadly appreciated by the clinical genetics community and funding mechanisms within the NIH is the establishment a central, standardized database built around high quality data that provides consistent interpretation of variants. The NIH funded ClinGen project, which is dedicated to "building an authoritative central resource that defines the clinical relevance of genomic variants for use in precision medicine and research" has been supporting the expansion of ClinVar, an open access, archive of data documenting the relationships between genetic variations and phenotypes that provides supporting evidence (www.clinicalgenome.org) (3). The benefit of procuring databases with stringent variant classification has been demonstrated by Myriad Genetics. When Myriad Genetics began offering hereditary cancer genetic testing, approximately 40% of variants were categorized as VUS. However, in recent years, as the number of well-documented variant-disease relationships have grown, the rate of variants categorized as "uncertain significance" has dropped to 2.9% for BRCA1/2 mutations and 6.6% for Lynch syndrome genes (4). However, it is important to realize that this represents an exception rather than the norm, as the vast majority of variants discovered using WES/WGS are categorized as VUS.

A key issue in utilizing genetic testing in the medical management of patients relates to knowing how to effectively use the evidence generated. Categorizing a variant as "pathogenic" should coincide with sufficient evidence enabling confident use of this information in the clinical decision. The impact of improper categorization of variants as "pathogenic" can be severe as this designation often results in changes to clinical management (2). Variants classified as "likely pathogenic" can be used for clinical decision making when used in the context of other clinical evidence. In contrast, a VUS should not be used in clinical decision making; rather efforts should be focused on finding stronger evidence to re-classify the VUS as either "pathogenic" or "benign". A variant which is "likely benign" can be utilized in combination with other clinical data to conclude that the variant is not disease causing. The designation of a "benign" variant is backed by strong empirical evidence such that it can be excluded as the cause of the disorder.

Confounding genetic counseling and patient management, a VUS may be identified in a gene that is unrelated to the clinical presentation or initial intent of the genetic test and thus be considered an incidental finding. Incidental findings are unintentionally discovered findings which have the potential to impact the patient. Incidental findings can be categorized as those that are "actionable", "non-actionable but clinically relevant" and those that are of "uncertain significance". An "actionable" incidental finding occurs when the findings can be addressed with therapeutic intervention or preventative medicine. A clinically relevant but non-actionable finding may include discovery of a pathogenic variant where no treatment is available or alternatively the discovery of a single autosomal recessive allele (carrier status) where carrier screening (i.e. Fragile X Syndrome) may be recommended. The incidental findings may also be a VUS making interpretation and genetic counseling especially challenging.

The detection of variants and incidental findings in the context of prenatal screening has become more common due to the development of genetic tests which provide a more comprehensive evaluation of the fetal genome. Since 2013, chromosomal microarray analysis (CMA) has been the recommended initial genetic test following the detection of a fetal structural abnormality. CMA provides a higher resolution method to detect small deletions, duplications and copy number variations compared to its predecessor, standard karyotyping. In a multicenter clinical trial, CMA outperformed traditional karyotyping in detecting pathogenic abnormalities , in addition, detected pathogenic genetic defects in 6% of fetuses found to have a normal karyotype (5). A number of studies have demonstrated that CMA will detect VUS at a higher rate of 2-3% of all pregnancies tested (6). Given that WES/WGS is poised to become a mainstay, it seems probable that such testing will be available clinically for prenatal genetic testing particularly in cases with fetal structural abnormalities or in families with a history of inherited genetic conditions of unknown origin. This will expand the depth and resolution of genetic information well beyond what is currently available with CMA, karyotyping or selective sequencing of a few select genes. While one benefit of this approach will be a more comprehensive analysis of fetal health, until the concept of prenatal sequencing matures there will be a disproportionate jump in the discovery rate of VUS compared to variants categorized as “benign” or “pathogenic”.

The increased detection of VUS using NGS in adult and pediatric populations poses challenges but additional difficulties arise in the case of prenatally detected VUS. While guidelines have addressed reporting variants for adults and pediatric populations, there has been less guidance for the same discoveries in the prenatal setting –an expanding area of complex testing given the emergence of next-generation sequencing (NGS) of cell-free fetal DNA. In pediatric or adult patients, in addition to genetic information, the phenotype is also considered when categorizing variants. In cases of evaluating a variant discovered during fetal screening the phenotype will typically be unclear and thus in this regard classifying a variant in a fetus doesn’t necessarily equate to finding the same variant in the adult or pediatric population. The presence of a variant in a fetus with a structural abnormality may lend itself to confirming pathogenicity of the variant. However, it must be considered that the finding could be coincidental and not causal. Furthermore, a variant inherited from a phenotypically normal parent does not necessarily provide strong evidence that the variant is likely to amount to a “benign” variant for fetus or child.

As the role of comprehensive, in-depth sequencing permeates through medicine, the prevalence of VUS is all but guaranteed to increase. It is important to understand the classification system used for variants and how these relate to the likelihood that detection of the variant will impact patient management. Support of projects such as ClinVar is imperative to ensure accurate, thorough and efficient classification of variants across institutions. The greatest challenge ahead for NGS will be to figure out what to do about ensuing expansion of VUS.

References

1. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-23.
2. Henderson LB, Applegate CD, Wohler E, Sheridan MB, Hoover-Fong J, Batista DA. The impact of chromosomal microarray on clinical management: A retrospective analysis. Genet Med 2014;16:657-64.
3. Rehm RL et al. ClinGen-The Clinical Genome Resource. NEJM 2015;372(23):2235-42.
4. Eggington JM, Roa BB, Pruss D, Bowles K, Rosenthal E, Esterling L, Wenstrup RJ. Current variant of uncertain significance rate in brca1/2 and lynch syndrome testing (mlh1, msh2, msh6, pms2, epcam). Presented American College of

Medical Genetics and Genomics Annual Meeting 2012.
5. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. N Engl J Med 2012;367:2175-84.
6. Hillman SC, McMullan DJ, Hall G, Togneri FS, James N, Maher EJ, et al. Use of prenatal chromosomal microarray: Prospective cohort study and systematic review and meta-analysis. Ultrasound Obstet Gynecol 2013;41:610-20.

2015 Annual Meeting and Clinical Lab Expo: Meeting Highlights

July 26-30th in Atlanta, Georgia

Sessions of Interest for Members of the Pediatric and Maternal-Fetal Division

Ticketed sessions require additional fees.

Sunday, July 26th:

Opening Plenary: Nucleic Acids in Plasma: A Treasure Trove for Research and Clinical Applications

Next-Generation Sequencing for Inherited Disorders

Interpretation of Toxicology Results in the Pediatric and Geriatric Populations

Monday, July 27th:

Bone Markers Beyond Vitamin D: BAP, Osteocalcin, NTx and P1CP
(Developed in cooperation with PMF Division)

Role of Therapeutic Drug Monitoring in Pediatric Cancer Chemotherapy

Interpreting and Establishing Reference Intervals in Neonatal and Geriatric Populations

Circulating DNA Diagnostics for Prenatal, Cancer and Autoimmune Disease Assessments

Tuesday, July 28th:

Pediatric Toxicology: Kids are Not Little Adults

Wednesday, July 29th:

Anti-Mullerian Hormone (AMH): Ovarian Reserve and Beyond

A Revolution in Reproduction: How Emerging Technologies are Personalizing Procreation

Improving Patient Care and Managing Health Care Resources by Ensuring Proper Use of Genetic Testing: The Role of the Laboratory Genetic Counselor

Thursday, July 29th:

Recent Advances in Cervical Cancer Prevention by HPV Vaccination and Screening

Celiac Disease: Its Epidemiology, Pathogenesis and Diagnosis

Please Join Us!

Event: Pediatric and Maternal-Fetal, Clinical Translational Science, Industry and Informatics Divisions Joint Mixer

Date: Sunday, July 26, 2015
Time: 7:30pm – 9:00pm
Location: Hyatt Regency Atlanta, Embassy C/D
This event is sponsored in part by Waters.

Event: Update and Discussion on AACC's Initiatives in Children's Health

Date: Tuesday, July 28, 2015
Time: 1:00pm – 2:30pm
Location: Hyatt Regency Atlanta, Fairlie

This session will feature three separate presentations:

1) "Newborn Screening Research and Informed Consent" by Carla Cuthbert, Chief, Newborn Screening and Molecular Biology Branch (NSMBB), National Center for Environmental Health, CDC

- Dr Cuthbert will summarize the topic based on a recent meeting in Washington DC targeted to state newborn screening programs, legal and general counsels from State Department of Health, patient advocates, investigators in newborn screening research, patient advocates, representatives from professional organizations/societies and federal partners. The meeting (1) addressed State concerns and the anticipated implications of Section 12 of the Newborn Screening Saves Lives Act Amendment; (2) discussed issues related to broad consent for future use of residual dried blood spots and (3) identified needs to educate the public about newborn screening and their options to participate in newborn screening research.

2) "AACC Position Papers: Newborn Screening and Pediatric Reference Ranges" by Vince Stine, PhD, AACC Director, Government Affairs
   - Dr. Stein will give a brief overview of on-going AACC position papers related to pediatric-maternal-fetal populations and will lead a discussion on those topics.

3) "Glucose Measurements in Neonatal and Pediatric Intensive Care Units" by Brad S. Karon, MD, PhD, Mayo Clinic; Sharon Geaghan, MD, Stanford University; and Alison Woodworth, PhD, Vanderbilt University Medical Center
   - The group will give an overview of regulatory changes, discuss best practices,and will open the floor for a discussion on current issues.

Event: Pediatric and Maternal-Fetal Poster Walk

Date: Wednesday, July 29, 2015
Time: 12:30pm – 1:30pm
Location: Georgia World Congress Center

Poster walks are led by AACC Division subject matter experts and highlight posters selected by the Division for further discussion. The walks are free and limited to 20-30 participants holding a full or daily conference registration

PMF Division Awardees

Please help us congratulate the following winners of these PMF Division awards! Awards will be presented during the Pediatric and Maternal-Fetal, Clinical Translational Science, Industry and Informatics Divisions Joint Mixer on **Sunday, July 26, 2015 from 7:30pm – 9:00pm** at the Hyatt Regency Atlanta (Embassy C/D).

Best Abstract by a Student or Young Investigator:
- Xiaoyi Tian, Institute of Basic Medical Sciences; Beijing, China
- Title: MELPA: A Novel Technology for High-Throughput, Multiplex Genotyping Directly from Dried Blood Spot Without DNA Extraction, With an Application in the Screening for Multiple G6PD Gene Variants at Risk for Drug-Induced Hemolysis

Best Abstract:
- Zhibin Cheng, Institute of Basic Medical Sciences; Beijing, China
- Title: CLIA-PCR: A High-Throughput PCR Technology for Molecular Screening with an Application in Malaria Surveillance for Elimination

Outstanding Contributions to Pediatric and Maternal-Fetal Laboratory Medicine:
- Khosrow Adeli, PhD, FCACB, DABCC, FACB, The Hospital for Sick Kids; Toronto, Canada

THE PMF DIVISION PARTNERS WITH SYCL

The PMF Division is proud to offer five annual Division memberships to the Society for Young Clinical Chemists (SYCL) during the SYCL Mixer scheduled for **Saturday, July 25, 2015 from 5:30-7:30 PM** at the Georgia Aquarium. These donated memberships will allow young professionals an opportunity to share their goals and visions with our group and open our membership up to new members.

SPECIAL HONOR!

The Pediatric and Maternal-Fetal Division is being recognized at the 2015 Annual Meeting for helping AACC advance the profession. AACC President David Koch, PhD has recognized the Division for supporting AACC advocacy on behalf of children's health and for leadership in raising awareness of children's health issues through the following activities:

- For more than a decade, the Division has provided annual updates on the NIH National Children's Study and similar initiatives during a forum at the AACC Annual Meeting.
- The Division helped draft language for AACC's position statement, Newborn Screening and Improving Children's Health, published in July 2014.
- Division leaders Dr. Patricia Jones, Dr. Michael Bennett, and Dr. Shannon Haymond participated in AACC's first Congressional briefing held October 14, 2014. They discussed the vital role of clinical laboratory tests in the medical treatment of costly health conditions leading to reduced life expectancies if left unchecked.

Divisions will be honored during the 2015 Annual Meeting at the Divisions Management Group Meeting on Sunday, July 26th at 1:00pm at the Hyatt Regency in Atlanta.

CONGRATULATIONS to all past and present Division Board members on these amazing accomplishments!

EXCERPTS FROM THE LITERATURE

Articles of interest to the Division membership compiled by the Editorial Board. Please welcome our newest member of the Board, **Brenda Suh-Lailam, PhD, DABCC!**

HDL particle number measured on the Vantera®, the first clinical NMR analyzer (VLP)

Steven P. Matyus, Paul J. Braun, Justyna Wolak-Dinsmore, Amy K. Saenger, Elias J. Jeyarajah, Irina Shalaurova, Suzette M. Warner, Timothy J. Fischer and Margery A. Connelly

Clinical Biochemistry 2015, 48: 148-155

In this highlighted article the authors test the performance characteristics of the Vantera analyzer in measuring high density lipoprotein (HDL) particle number in clinical samples. The Vantera is the first NMR analyzer in the clinical

setting. Low HDL cholesterol concentrations have a strong relationship with an increased risk of cardiovascular disease (CVD). Nevertheless, recent efforts to raise HDL cholesterol have not decreased CVD rates. Recent studies have suggested that HDL particle number could be a better predictor of CVD than HDL cholesterol concentrations, because it is a more accurate measure of HDL than HDL cholesterol concentrations. Nuclear Magnetic Resonance (NMR) has been used to measure HDL particle number and size. Furthermore, NMR has also been used to measure other lipoproteins including VLDL and LDL. In the present study, the clinical performance characteristics were determined to be: linearity= 10-65 µmol/L; imprecision CVs=2.0-3.9 %; interference= of 30 substances tested, 3 showed potential interference (acetylsalicylic acid, nicotinic acid and clopidrogrel hydrogen sulfate); method comparison = slope of 1.06, intercept of -1.34 and R^2 of 0.98 when compared to the NMR Profiler. In addition, the article also included reference range, sample stability and collection tube comparison studies as well. Although this article did not focus on pediatric patients, there should be interest in applying this methodology to the pediatric setting in the near future. This could be spurred on by the recent guidelines by the National Heart Lung Blood Institute (NHLBI), which recommend screening of children for dyslipidemia. This is in conjunction with the increasing rates of obesity in the pediatric population seen in recent times. The introduction of NMR into the clinical laboratory is an exciting innovation. Could this technology bring about a revolution similar to what we are seeing with chromatography and mass spectrometry? What other applications are on the horizon for the clinical laboratory NMR?

The Evaluation of Suspected Child Physical Abuse (BS-L)

Cindy W. Christian; Committee on Child Abuse and Neglect

Pediatrics 2015,May; 135(5):e1337-54

Child physical abuse is a significant cause of pediatric morbidity and mortality with lifelong health consequences for victims and their families. Even though some studies suggest declining rates, child physical abuse is still a public health concern with survivors shown to have poor health outcomes in the long-term. A recent publication by CW Christian and the Committee on Child Abuse and Neglect aimed to provide guidance to clinicians on identifying and evaluating suspected child physical abuse. As the identification of abuse can be challenging, the authors discussed several factors that can aid in the identification of abuse, including: risk factors for child physical abuse, medical histories and physical examination findings that are suggestive of abuse. They also recommended laboratory and radiologic tests that may be used to identify underlying health problems included in the differential diagnosis, potentially contributing to the physical findings. Several categories of laboratory testing were suggested based on the type of injury or condition identified during physical examination. These tests are shown in the table on the following page (table summarizes this and other relevant literature). Severity of injury, injury type, age, and level of development of the child are all factors that determine the extent of diagnostic testing. Testing is most extensive in those with the most severe injury and youngest age. Pediatricians play an important role in the recognition, evaluation, and protection of abuse victims, which may prevent lifelong negative consequences and lead to improved health outcomes in survivors. Laboratorians should be aware of the role of testing in suspected child physical abuse.

Laboratory Tests That May Be Useful in the Assessment of Suspected Physical Abuse and Differential Diagnoses (*summary compiled from the literature, BS-L*):

Type of Injury at Physical Examination	Potential Underlying Health Problems	Laboratory Testing	Comments
Fractures	Bone-mineralization defect	• Calcium, phosphorus, alkaline phosphatase, 25-hydroxyvitamin D, PTH	• Evaluation of bone health could help rule out or identify bone disease such as rickets
	Genetic bone disease	• Skin biopsy for fibroblast culture and/or venous blood for DNA analysis	• Evaluation could help rule out or identify genetic bone disease such as osteogenesis imperfecta
	Scurvy or copper deficiency	• Serum copper, vitamin C, ceruloplasmin	• It is essential to rule out scurvy in at risk children as the associated bone pathology could potentially be confused with physical child abuse
Bruises	Bleeding disorder	• CBC, platelets, PT, INR, aPTT, VWF antigen, VWF activity (ristocetin cofactor), factor VIII level, factor IX level	• Helpful in ruling out a bleeding disorder as the reason for bruising, especially if suspected from the family history or physical examination
Abdominal trauma		• Aspartate aminotransferase (AST), alanine aminotransferase (ALT)	• Useful in evaluation of liver injury
		• Amylase, lipase	• Useful in evaluation of pancreatic injury
		• Urinalysis	• Useful in evaluation of urinary tract injury
Head trauma	• Glutaric aciduria, type 1 (GA1): macrocranium, subdural hematoma, sparse intraretinal and preretinal hemorrhages, frontotemporal atrophy • Hemorrhagic disease of the newborn	• CBC, platelets, PT/INR/aPTT; factor VIII level, factor IX level, fibrinogen, d-dimer • Urine organic acids - screen for GA1	• Subdural and retinal hemorrhages, which in the right context are suggestive of non-accidental injury could sometimes occur in GA1 patients. Ruling out GA1 could help prevent misdiagnosis of abuse in such cases.
Cardiac injury		• Troponin, creatine kinase with muscle and brain subunits	• Useful in the determination of cardiac injury

PMF Division Executive Board:

Chair
David Carpentieri, MD

Chair Elect
Shannon Haymond, PhD

Secretary
Christina Lockwood, PhD

Treasurer
Sihe Wang, PhD

Past Chair
Sharon Geaghan, MD

Members At Large
Angela Ferguson, PhD
Brad Karon, MD, PhD
Jon Nakamoto, MD, PhD
Alison Woodworth, PhD

Webmaster
Olajumoke Oladipo, PhD

Newsletter Editor
Joely Straseski, PhD

Newsletter Editorial Board
Van Leung-Pineda, PhD
Brenda Suh-Lailam, PhD