Clinical Utility of a Large Exome-Based Panel in the Genetic Diagnosis of Autism Spectrum Disorders and Intellectual Disabilities

Abstract: Background: Autism Spectrum Disorders (ASDs) and Intellectual Disability (ID) are heterogeneous, highly comorbid neurodevelopmental disorders inherited in a multifactorial fashion. To date, over 800 genes have been implicated in ASD and ID-related phenotypes, with 97% of pathogenic variants found in coding regions. Genetic variants have been reported in at least 25-50% of ID cases and ~20% of ASD cases. Trio-based studies are reported to increase the molecular diagnostic rate to ~30-40%. Objectives: In early 2017, PreventionGenetics released an exome-based ‘large panel’ including 1,905 genes as part of a comprehensive approach to genetic testing for patients with ASD and ID. Methods: Patient’s DNA is captured using Agilent Clinical Research Exome v2 hybridization probes and sequenced using Illumina’s NovaSeq 6000. Variants are filtered computationally to the 1,905 gene list before annotation and analysis via VarSeq (www.goldenhelix.com). For trio-based studies, de novo variants in any clinically relevant gene are also considered. Patient reports are limited to variants considered relevant to the reported phenotype. Results: A definitive molecular diagnosis was reported in ~13% of cases, while the remaining cases (i.e. ~87%) had a variant(s) of uncertain significance that may explain the patient’s clinical features. Diagnostic rates were higher with trio-based studies (~19% in trios vs. ~6% in singletons). Conclusion: The ASD-ID comprehensive panel leverages the advantages of our PGxome™ workflow and has increased clinical sensitivity compared to smaller NGS panels. This test also provides a reasonable alternative to whole-exome sequencing for patients with neurodevelopmental disorders.

AuthorNames: Diane Allingham-Hawkins, PreventionGenetics, LLC; Greg Fischer, PreventionGenetics, LLC; Moumita Chaki, PreventionGenetics, LLC; Ben Dorshorst, PreventionGenetics, LLC; Gina Londre, PreventionGenetics, LLC; Rachel Vanneste, PreventionGenetics, LLC; Jerry Machado, PreventionGenetics, LLC; Robert Steiner, PreventionGenetics, LLC; James Weber, PreventionGenetics, LLC
De novo heterozygous missense mutations in DDB1 cause a novel disorder of transcriptional regulation manifesting as hypotonia, intellectual disability and dysmorphic features

Primary Category: Clinical Genetics

Secondary Category: Clinical Genetics

Abstract: Background: The DNA damage binding protein DDB1 is part of the CUL4–DDB1 ubiquitin E3 ligase complex which is involved in the regulation of transcription, proteasome degradation, cell-cycle progression, replication and DNA damage response. Loss-of-function mutations in CUL4 and in the gene encoding DDB1–CUL4 substrate receptor PHIP1 have recently been identified to cause phenotypes of syndromic intellectual disability with hypotonia and obesity. Objectives: We report four unrelated individuals with de novo heterozygous missense mutations in DDB1 identified by whole exome sequencing. The individuals were ascertained through Matchmaker Exchange and have a remarkably similar phenotype comprising moderate hypotonia, mild to moderate intellectual disability, and strikingly similar facial features with wavy eyebrows, lateral extension of the palpebral fissures, mid-face hypoplasia and a small nose. The older affected child has truncal obesity. Conclusions: The phenotypes caused by mutations in CUL4, PHIP1 and DDB1 have overlapping features of obesity, hypotonia, intellectual disability and dysmorphism, suggesting that this is a new family of conditions mediated by disruption of the ubiquitin ligase pathway.

AuthorNames: Marjan M. Nezarati, North York General Hospital; Kristin Kernohan, CHEO; Kym Boycott, Department of Genetics; Susan White; Elizabeth Bhoj; Paul Lockhart; Taila Hartley, Care for Rare; Dong Li; Jaime Barea; Simon Sadedin
Lexigene?: Bridging the communication gap through an online English-French-Spanish genetic counselling lexicon

Primary Category: Clinical Genetics

Secondary Category: None

Abstract: Rachel Vanneste, a,b, Mireille Cloutier, c, Priscila D. Hodges, Julie Hathaway, e, Nevena Krstic, Guillaume Sillon, g. a. Canadian Association of Genetic Counsellors, ON b. Prevention Genetics, Marshfield, WI, USA c. Children’s Hospital of Eastern Ontario, Ottawa, ON d. The University of Texas Health Science Center at Houston, Houston, TX, USA e. St. Paul’s Hospital, Vancouver, BC f. University of South Florida, Tampa, FL, USA g. McGill University Health Centre, Montreal, QC

Background. To facilitate the provision of genetic services in the three most commonly spoken languages in North America, an English-French-Spanish lexicon of genetics-related terms was created by genetic counsellors. Objectives. To provide genetic health professionals, trainees in bilingual training programs and medical interpreters a tool to facilitate the translation of genetic terminology and to improve communication with patients. Design/Method. The original French-English Lexigene? was funded through the Canadian Association of Genetic Counsellors (CAGC) and was published online in 2011. In 2016, funding through the National Society of Genetic Counselors (NSGC)’s Audrey Heimler Special Project Award was used to include Spanish. In May 2017, www.lexigene.com became trilingual, allowing individuals to search for genetics-related terms in either English, French or Spanish and find the equivalent term in the other languages. Results. The tool was piloted by 18 genetic counsellors and geneticists. All respondents ranked the tool as “easy” or “very easy” to use and 89% indicated they would use the tool in their practice. Additionally, 80% of the words that respondents searched for were present in Lexigene?. Finally, the qualitative feedback provided was used to improve the website’s functionality. Conclusion: This online tool boasts ~3600 translated terms related to genetics, with more being added regularly. With the aid of Lexigene?, providers and interpreters have enhanced their genetics vocabulary and in turn provide better service through accurate communication.

Author Names: Rachel Vanneste, Prevention Genetics, LLC; Mireille Cloutier, Children’s Hospital of Eastern Ontario; Priscila Hodges, The University of Texas Health Science Center at Houston; Julie Hathaway, St. Paul’s Hospital; Guillaume Sillon, McGill University Health Centre; Nevena Krstic, University of South Florida
Whole exome sequencing identifies Neu-Laxova syndrome as a cause of recurrent cystic hygroma with severe intrauterine growth restriction

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: A non-consanguineous Chinese couple was referred to the Genetics Clinic for positive prenatal screening for trisomy 21 in three pregnancies. Prenatal ultrasound findings included: cystic hygroma, subcutaneous edema, intrauterine growth restriction, hypoplasia of the cerebellum, renal anomalies, and arthrogryposis. Fetal examination showed several common features: severe growth restriction, micrognathia, arthrogryposis, and rocker-bottom feet. Neuropathologic examination revealed: delayed brain development, congenital agenesis of the corticospinal tracts, and hypoplasia of the hippocampus, cerebellum and brainstem. Conventional cytogenomic analysis did not reveal an underlying genetic cause. Exome sequencing identified a homozygous c.1A>C (p.Met1?) variant in the translation initiation codon of the PHGDH gene in all fetuses. Biallelic mutations in the PHGDH gene cause Neu-Laxova syndrome 1 (NLS1). Approximately 70 cases have been identified; however, prenatal diagnosis is challenging and there are few reports which describe the fetal pathology. Apart from micrognathia, the typical facial features, such as hypertelorism and ocular proptosis, were not appreciated on fetal examination. Interestingly, each pregnancy screened positive for trisomy 21 based on first trimester increased nuchal translucency or cystic hygroma. Although edema is a common finding in NLS1, it is usually identified later in pregnancy. While NLS1 remains a rare condition, it may be a cause of recurrent increased nuchal translucency/cystic hygroma when testing for aneuploidy is negative, even in the absence of the classic facial phenotype. This finding provides further support that cystic hygroma represents a heterogeneous group of disorders and that exome sequencing is shedding light on the underlying genetic diagnoses in this group.

AuthorNames: Danielle Bourque, Children's Hospital of Eastern Ontario; Mireille Cloutier, Children’s Hospital of Eastern Ontario; Kristin Kernohan, CHEO; Eric Bareke, Department of Human Genetics, McGill University; David Grynspan; Kym Boycott, Department of Genetics; Jean Michaud, Children's Hospital of Eastern Ontario; Care4Rare Canada Consortium, Children's Hospital of Eastern Ontario Research Institute
Who is opting-in: How proband age impacts requests for secondary findings

**Primary Category:** Molecular Genetics

**Abstract:** Rachel Vanneste§, Diane Allingham-Hawkins§, Ben Dorshorst, Gregory Fischer, Jerry Machado, Robert Steiner, Jiabin Zhang, James L. Weber §Co-presenting authors
PreventionGenetics, LLC, Marshfield, WI Background: Whole exome sequencing (WES) is a powerful tool in the diagnosis of genetic conditions that can also provide information regarding secondary findings. The American College of Genetics and Genomics (ACMG) issued recommendations that pathogenic and likely pathogenic variants in 59 genes should be reported unless patients opt out. At PreventionGenetics, opt-in options are available for four categories: ACMG 59; other predispositions for Mendelian disorders; carrier status for recessive conditions; and pharmacogenetic information. Objective: To assess the impact of proband age on opt-in choices. Method: PGxome® WES orders received July to December, 2017 were reviewed and opt-in choices were compared across age categories. Results: Probands 3 months of age were less likely to opt-in for any secondary findings. The most common opt-in was ACMG 59 (53% for 3 months to 80% for > 18 years). Older probands also opted-in for pharmacogenetic information most frequently (36% for for 3 months to 50% for > 18 years). A similar trend was noted for other predispositions for Mendelian disorders (26% for for 3 months to 43% for >6 - Conclusion: Probands older than 18 years were more likely to opt-in for ACMG and pharmacogenetic information, although differences were noted across all categories. Offering choices with respect to the types of secondary findings returned allows patients and providers to tailor the results received to their personal needs and preferences.

**AuthorNames:** Rachel Vanneste, PreventionGenetics, LLC; Diane Allingham-Hawkins, PreventionGenetics, LLC; Jerry Machado, PreventionGenetics, LLC; Ben Dorshorst, PreventionGenetics, LLC; Greg Fischer, PreventionGenetics, LLC; Robert Steiner, PreventionGenetics, LLC; James Weber, PreventionGenetics, LLC; Jiabin Zhang, PreventionGenetics
Exome sequencing in the neonatal or pediatric intensive care unit: Experience with a two week rapid-assay and analysis of added benefit over comprehensive panels

Primary Category: Molecular Genetics

Secondary Category: None

Abstract: Kristin D. Kernohan1, Taila Hartley1, Arran McBride1, Wendy Mears1, Matthew Lines2, Michael Geraghty2, Julie Richer3, Sarah Sawyer3, Melissa Carter3, Christine Armour3, Pranesh Chakraborty2, Kym M. Boycott1,3, David A Dyment1,3,* 1 Children’s Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, Ontario K1H 8L1 2 Children’s Hospital of Eastern Ontario, Department of Pediatrics, University of Ottawa, Ottawa, Ontario K1H 8L1 3 Children’s Hospital of Eastern Ontario, Department of Genetics, University of Ottawa, Ottawa, Ontario K1H 8L1 Rare genetic disease contributes significantly to the mortality and morbidity of newborns admitted to the neonatal intensive care unit (NICU) or pediatric intensive care unit (PICU), though most do not receive a timely genetic diagnosis during their hospital admission and early life. This represents a missed opportunity as these children may not receive the appropriate medical or surgical management to maximize outcomes, or have appropriate surveillance plans initiated to minimize the later burden of disease. Next-generation sequencing technologies provide an efficient means to rapidly interrogate a patient’s genome. Here we report our experience with initiation of a two-week rapid-exome pilot in 10 trios, and exome analysis of 12 NICU/PICU patients previously assessed by a comprehensive Mendelian disease panel testing in which we identified additional diagnoses in 5/12 (42%) patients. Our data further support the benefits of trio exome sequencing over targeted panels for this patient population, and highlights the logistical approaches to accomplish this in a clinically-relevant timeframe.

AuthorNames: Kristin Kernohan, CHEO; Taila Hartley, Care for Rare; Arran McBride; Wendy Mears; Matthew Lines, CHEO - Metabolics; Michael Geraghty, CHEO - Metabolics; Julie Richer, Department of Genetics; Sarah Sawyer, CHEO; Melissa Carter, The Hospital for Sick Children; Christine Armour, Department of Genetics; Kym Boycott, Department of Genetics; Pranesh Chakraborty, Newborn Screening Ontario; David Dyment
XRCC1 gene polymorphisms and chromosomal instability in esophageal cancer patients

Primary Category: Molecular Genetics

Secondary Category: Cytogenetics/Microarray

Abstract: Jagjeet Kaur1, Vasudha Sambyal1 and Kamlesh Guleria1 1Human Cytogenetics Laboratory, Department of Human Genetics, Guru Nanak Dev University, Amritsar PUNJAB, (INDIA) Background: Faulty DNA repair due to mutations or polymorphism in XRCC1 (19q13.2), which plays a predominant role in both single strand break repair and base excision repair, may lead to genomic instability and cancer. Objectives: The present study evaluated three polymorphisms of XRCC1, p.Arg399Gln, p.Arg194Trp, p.Arg280His and chromosomal instability in esophageal cancer (EC) patients from Punjab, India, their association with esophageal cancer risk and tumor aggressiveness. Material and Methods: Cytogenetic analysis and genotyping for Arg399Gln, Arg194Trp and Arg280His polymorphisms by PCR-RFLP method was done in 215 esophageal cancer patients (89 males and 126 females) and 265 controls (95 males and 170 females). Results: The Arg399Gln polymorphism AA genotype and recessive model (OR=0.57, 95%CI= 0.33-0.98; p=0.038) was associated with a decreased risk of esophageal cancer. No significant difference was found in genotype and allele frequencies for other two polymorphisms. Patients with GA genotype of Arg399Gln polymorphism had higher frequency of chromosomal aberrations than controls. Patients had higher frequencies of structural and numerical aberrations along with some constitutional aberrations as compared to controls. Conclusion: The XRCC1 Arg399Gln polymorphism was associated with an decreased risk of esophageal cancer. Specific chromosomal anomalies related to tumor progression in patients indicated poor prognosis. Identification of inherited genomic changes and their association with chromosomal instabilities might help in identifying subjects with aggressive tumors and selection of suitable therapy for personalized medicine.

AuthorNames: jagjeet kaur, GURU NANAK DEV UNIVERSITY, AMRITSAR; vasudha sambyal, GURU NANAK DEV UNIVERSITY AMRITSAR; kamlesh guleria, GURU NANAK DEV UNIVERSITY, AMRITSAR
Mutational Screening of Hepcidin gene in Hereditary Hemochromatosis with H63D Homozygous/Heterozygous Patients in Pakistan

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: ABSTRACT Background- Hereditary hemochromatosis is an autosomal recessive ailment characterized by p.H63D and pC282Y mutations in HFE gene. Its prevalence is restricted, signifying the probability of other genetic modulators being entangled in iron regulation. Objective- We aimed to screen the mutations in Hepcidin gene involved in Hereditary Hemochromatosis with H63D Homozygous/ Heterozygous patients in Pakistan. Method- In this work, we screened for hepcidin gene polymorphisms in 100 ?-thalassemic (major) patients, exhibiting H63D homozygous and heterozygous traits along with intricate clinical and hematological afflictions by PCR-RFLP. Results- Our results manifest a A>G and G>A polymorphisms at hepcidin splicing acceptor site associate in 4 H63D heterozygous patient exhibiting elevated serum ferritin level along with hepatomegaly, splenomegaly and HCV reactivity. Moreover, it was perceived that this polymorphism will disrupt the posttranscriptional activity of the hepcidin pre mRNA and correspondingly lead L98V and S30C modification in one of the patient which has a stabilized effect of -0.01 and -0.27 kcal/mol on protein structure. Conclusion- Our statistics recommend that analysis for these polymorphisms could be of great concern in order to elucidate the decrease hepcidin expression in hemochromatosis patients. Hence, our results suggest that a mutation in the hepcidin splicing enhancer site endorse dysregulation of iron metabolism.

Supporting ImageTable:
http://www.xcdsystem.com/CCMG/abstract/File1898/10_SupportingImageTable_0215035003.docx

AuthorNames: saba irshad, Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore
Title: A Clinical Strategy for Streamlining Cancer Predisposition Syndrome Identification in Pediatric Oncology Patients

Abstract: Title: A Clinical Strategy for Streamlining Cancer Predisposition Syndrome Identification in Pediatric Oncology Patients Authors: Leora Witkowski1, Anita Villani2, Anna Pan2, Kalene van Engelen2, Bailey Gallinger2, Harriet Druker2, Catherine Goudie2, David Malkin2, William D. Foulkes3 1Harvard Medical School Genetics Training Program, Harvard University, Cambridge, Massachusetts, USA 2Division of Hematology-Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada 3Division of Medical Genetics, McGill University Health Centre, Montreal, Quebec, Canada Background: Over 10% of children diagnosed with cancer have an underlying cancer predisposition syndrome (CPS) due to a germline pathogenic variant in a cancer predisposition gene. Many challenges limit the likelihood of a physician identifying CPSs, and technological advances in cancer genomics are rapidly outpacing the knowledge of most clinicians. To address this issue, we have developed the McGill Interactive Pediatric OncoGenetic Guidelines (MIPOGG), an educational electronic application that includes 90 tumour-specific decisional algorithms designed to identify pediatric cancer patients at increased likelihood of having a CPS. Objectives: We sought to evaluate the performance of MIPOGG in identifying patients with a CPS at the time of initial tumour presentation. Design/Methods: We retrospectively collected clinical, family history, and tumour details on 175 patients in Ontario with a CPS and a cancer diagnosed between 2000 and 2017. We applied MIPOGG to all patients to determine the tool's sensitivity in appropriately identifying those who require genetic assessment. Results: Of 175 patients, MIPOGG correctly identified >95% of patients with a CPS at primary cancer diagnosis. Over 85% of patients were diagnosed with cancer prior to their CPS recognition, with a mean time to genetics consultation of 4.1 years. Over 2/3 of patients had an unremarkable family history and were identified by MIPOGG through other criteria. Conclusion: The MIPOGG prediction tool was successful in identifying patients at the time of initial tumour presentation. Its use in cancer centers would reduce time to genetics referral, thereby improving quality of care for patients and their families.

AuthorNames: Harriet Druker, The Hospital for Sick Children; Leora Witkowski, Harvard University; Catherine Goudie, The Hospital for Sick Children; Anita Villani, The Hospital for Sick Children; Anna Pan, The Hospital for Sick Children; Bailey Gallinger, The Hospital for Sick Children; Kalene van Engelen, The Hospital for Sick Children; David Malkin, The Hospital for Sick Children; William Foulkes, McGill University Health Centre
Integrated Commercial-Academic Partnership Approach for Delivery of Technological Innovation to High-Throughput Clinical Diagnostic Genetic Services

Primary Category: Molecular Genetics

Secondary Category: Biochemical/Metabolic Genetics

Abstract: Genetic healthcare depends upon accessible, cost effective and high quality genetic testing. Large-scale commercial laboratories enable access and throughput for effective service delivery. Academic medical centers are well positioned to enable content-specific (disease, tissue, or test type) test development, which often requires combined clinical, technological and research expertise. Industry-academic partnerships can optimize diagnostic genetics services by integrating commercial scale with specialized clinical and research expertise. London Health Sciences Center (LHSC) Molecular Genetics Laboratory is an Ontario provincial referral genetic laboratory for a number of genetic disorders. As part of a collaborative effort involving clinical, scientific and laboratory expertise we developed custom NGS gene panel-based technology that enables simultaneous sequencing and copy number assessment and that outperforms the classic gold standard of Sanger sequencing and MLPA at sub-exon resolution. As the provincial clinical center of excellence for neurology genetics, LHSC applied this technology to develop NGS-based gene panels for epilepsy, Charcot-Marie-Tooth and mitochondrial genome sequencing. Using thousands of clinical specimens tested in the LHSC clinical laboratory, we demonstrate sensitivity and specificity that is superior to Sanger sequencing and MLPA testing combined, as part of a cost-effective clinical pipeline. LabCorp and Dynacare (owned by LabCorp), two of the largest laboratories in the USA and Canada respectively, commercialize these tests by providing market access, billing and insurance support, high throughput, and high quality clinical testing. Analytics, informatics and clinical reporting are provided by LHSC’s experts. LabCorp’s tele-genetic counsellors are available to support non-genetics ordering physicians with comprehensive pre- and post-test genetic counselling. This industry-academic partnership represents a novel, effective, scalable, and industry-leading quality for delivery of genetic testing services.

AuthorNames: Han-Xin Lin, London Health Sciences Centre (LHSC); Bekim Sadikovic, University of Western Ontario; Jennifer Kerkhof, LHSC; Alan Stuart, LHSC; Hilary Racher, Impact Genetics; Franny Jewett, Impact Genetics; Jaime Jessen, Impact Genetics
Male with mosaicism for maternally inherited supernumerary ring X chromosome: new clinical case and literature review

Primary Category: Clinical Genetics

Secondary Category: None

Abstract: Spencer S. Zwarych[1] Carla M. Holinaty[2], Reena Ray-Sisk[3], Kellie A Davis[4]. 1 Medical Student, University of Saskatchewan, Saskatoon, SK, Canada 2 Department of Academic Family Medicine, University of Saskatchewan, Saskatoon, SK, Canada 3 Pathology and Laboratory Medicine, Royal University Hospital, Saskatchewan Health Authority, Saskatoon, SK, Canada 4 Division of Medical Genetics, University of Saskatchewan, Royal University Hospital, Saskatoon, SK, Canada Supernumery ring X chromosomes are commonly reported in Turner syndrome females but are rarely seen in males. The ring is often found as a mosaic cell line, which results in variable phenotype between male patients, making it difficult to counsel both patients and their families on possible clinical outcomes and recurrence risk. The literature is extremely limited with fewer than ten reports published to date. Common features reported include facial dysmorphism, developmental delay, urogenital anomalies, and limb anomalies. This report describes an additional patient who is a male, born to term to a non-consanguineous Punjabi couple. Prenatally, there were no concerns. Post-natally, the infant presented with hypotonia, hypospadias, a congenital midline cervical cleft, and a midline capillary hemangioma on the forehead. Array CGH showed an ~27.4Mb duplication for the region spanning Xp21.1 to Xq11.2. Chromosome analysis showed a mosaic karyotype with a normal male cell line and a second cell line having the ring X chromosome. FISH using a probe for XIST mapping to Xq13.2 showed the normal pattern of hybridization on the X chromosome but absent on the ring X chromosome. Maternal cytogenetics testing demonstrated low levels of the ring X chromosome in the peripheral blood with paternal testing showing a normal male karyotype. The goal of this study is to contribute to the literature for this disorder through description of the phenotypic spectrum of disease and providing insight into its prognosis.

AuthorNames: Spencer Zwarych, College of Medicine, University of Saskatchewan; Kellie Davis, Department of Medical Genetics, Alberta Children's Hospital
ID: MMG006
Genetic diagnostic testing for Inherited Cardiomyopathies in Ontario: Considerations for offering multi-gene tests in a publicly-funded health care setting

**Primary Category:** Molecular Genetics

**Secondary Category:** Clinical Genetics

**Abstract:** Background Inherited cardiomyopathies (ICs) are the leading cause of sudden death in young adults. Given their marked clinical and genetic heterogeneity, the content and clinical utility of IC multi-gene panels have been topics of continuous debate. Our genetics diagnostic laboratory has been providing the clinical diagnostic testing for ICs since 2012; we began by testing 9 genes, and expanded our panels to include 45 genes in 2015. Methods We developed an NGS-based assay for the simultaneous analysis of up to 45 IC genes that includes a protocol that minimizes the amount of Sanger sequencing required to confirm variants identified via NGS, which significantly reduces the cost and time required to perform our tests. Furthermore, we assessed the impact of the panel expansion on variant detection, turn-around-time (TAT) and relative costs in a cohort of 993 individuals. Results Our assay enabled the panel expansion and led to a considerable reduction in the test cost and TAT. However, only a marginal increase in the diagnostic yield was observed, while the rate of inconclusive findings increased significantly. Conclusions Our findings suggest that strategic oversight and ongoing evaluation of system impacts are required to optimize clinical utility of multi-gene tests in a publicly-funded health care setting.

**Author Names:** Olga Jarinoa, Department of Genetics
Next generation sequencing to determine a cause of familial intracranial aneurysms

Abstract: Ru C. Guo,1 Emma C. Hitchcock,2,3 Harwood Kwan,2,3 Steven Jones,2,4 Patrice Eydoux,2,5 Gary J. Redekop,1 William T. Gibson2,3 1Division of Neurosurgery, Department of Surgery, University of British Columbia 2Department of Medical Genetics, University of British Columbia 3BC Children’s Hospital Research Institute 4BC Cancer Agency Michael Smith Genome Sciences Centre 5Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada Intracranial aneurysms (IA) are a disease in which cerebral artery walls weaken, forming a sac-like bulge that may rupture and cause subarachnoid hemorrhage. A subset of IA patients have a familial form, where two or more members of a family are affected. Our group conducted whole exome sequencing in members of five different families (n = 19) affected with neuroimaging-confirmed IA. We found 38 variants across four genes that overlapped in at least 4/5 families. However, none of these were thought to be potential candidates due to lack of evidence linking them to vasculature. When looking at variants that overlap in at least 3/5 families, 116 variants were found across 32 genes. To determine association with IA formation, the identified subset of genes were filtered down via manual annotation with disease-association and literature-search terms that were known to be relevant to vascular formation and maintenance (e.g. “angiogenesis”, “saccular aneurysm”). Three top candidate genes were obtained: ASTN2, HSPG2, and ITGB4. A rare variant in SERPINA1 was excluded after functional testing revealed normal circulating protein levels and activity against trypsin. A larger participant cohort is desirable in order to identify reproducible disease associations. We have since begun collaborating with three neurosurgeons, which has expanded our cohort to an additional 86 individuals, including 32 families with two or more affected first-degree relatives. Confirmation of emerging candidates will require establishment of a causal relationship between different rare variants in the same gene and relevant vascular pathologies. We intend to do this through functional studies in zebrafish, whose transparent embryos enable easy visualization and study of vasculature.

AuthorNames: Ru Guo, University of British Columbia
A multiparous woman with Marfan syndrome without aortic complication: a case report and review of the literature

Abstract: Background. Marfan syndrome (MFS) is an autosomal dominant disorder caused by mutations in the FBN1 gene. There is a marked clinical variability in this condition and patients are at high risk of life-threatening aortic dissections. During pregnancy, the risk of aortic dilatation and dissection is further increased. Pre-pregnancy aortic root dilatation is a risk factor for dissection, but dissection may also occur without significant dilatation. Objectives. Characterize the case of a woman with MFS who had multiple pregnancies with a normal aorta and review the literature for the risk of aortic complications in pregnant women with MFS and known associated protective or aggravating factors. Design/Method. We reviewed the medical chart and family history of a woman with MFS and multiple pregnancies, and the medical literature for studies reporting aortic complications in pregnant women with MFS. Results. We reported a woman with MFS who went through nine term pregnancies and whose aortic root diameter remained normal, despite a family history of aortic complications. In the 14 studies reviewed, the risk of aortic complication in the peripartum and the postpartum period is 3.9%. The rate of aortic complications in women considered at lower risk (aortic root diameter < 40 mm) was 1.7%. Conclusions. This case exemplifies the variability of the aortic phenotype in MFS. Further research is needed to identify factors that will lead to better stratification of aortic risk in women with MFS, to provide more personalized pre-pregnancy counseling and peripartum care for women with MFS considering a pregnancy.

AuthorNames: Marjolaine Champagne, University of Montreal; Anne-Marie Laberge, Medical Genetics, Dept of Pediatrics, CHU Sainte-Justine
Familial congenital hiatal hernia and short stature segregating with duplications in chromosome 2q34 and 9q22.31q22.32

Primary Category: Clinical Genetics
Secondary Category: Cytogenetics/Microarray

Abstract: Background: Congenital hiatal hernia (CHH) is a rare anomaly that is usually sporadic. The underlying genetic cause is poorly understood but rare reports of familial hiatal hernia suggest a genetic basis in some cases. Objective: We report on a family with three affected family members with a hiatal hernia, including two brothers and their mother. All three have a duplication at 2q34 and a duplication at 9q22. These individuals have additional features including short stature, microcephaly, and mild learning disability or developmental delay. Method: Cytogenetic investigations in all affected family members were performed and included CGH array in the proband and FISH in family members. Results: All three affected family members had the same chromosome duplications. The result of the array in the proband was: arr[hg19] 2q34(212,110,269-212,745,135)x3, 9q22.31q22.32(96,226,955-97,320,408)x3. This resulted in a 634.87kb interstitial duplication at 2q34 and partially overlaps the OMIM gene ERBB4 and a 1.09 Mb interstitial duplication at 9q22.31q22.32 that overlaps twelve RefSeq genes including BARX1. Conclusion: Congenital hiatus hernia is a rare congenital malformation for which the underlying genetic etiology remains poorly understood. To date, there have been at least 20 cases of familial hiatus hernia, with 3 pairs of siblings reported. One patient was described in DECIPHER with an inherited chromosome 9q22.31q22.32 microduplication, hiatus hernia and intellectual disability. We report on a family with hiatus hernia, review the cytogenetic imbalances and involved genes, and discuss a gene of interest, BARX1, as a candidate for this anomaly.

AuthorNames: Caitlin Chang, Department of Medical Genetics; Mary Ann Thomas, Department of Medical Genetics, Alberta Children's Hospital; Patrick Ferreira; Bob Argiropoulos, Department of Medical Genetics
ID: TMG035
Whole genome sequencing to discover genes underlying methylmalonic aciduria

Primary Category: Biochemical/Metabolic Genetics
Secondary Category: Molecular Genetics

Abstract: Whole genome sequencing to discover genes underlying methylmalonic aciduria
Authors: Lina Sobhi Abdrabo, David Watkins, Jacek Majewski, Jean-Baptiste Riviere and David S Rosenblatt Department of Human Genetics, McGill University, Montreal, Quebec, Canada

Background: Inborn errors of cobalamin metabolism are rare genetic disorders that give rise to both hematological and neurological manifestations. Patients with these disorders have elevations of methylmalonic acid and/or homocysteine in blood and/or urine. Objectives: This study aims to discover novel genes and/or causal variants in a cohort of undiagnosed patients with increased methylmalonic acid levels, who have been studied previously using a next generation sequencing panel for cobalamin genes. Design and Methods: DNA was extracted from cultured fibroblasts from 26 patients who had been referred because of elevated levels of methylmalonic acid in blood and/or urine. All cell lines had low levels of [14C]propionate incorporation, a test of methylmalonyl-CoA mutase (MCM) function. Patients were stratified according to their level of propionate incorporation. Whole genome sequencing (WGS) was performed on all the samples. Results: WGS confirmed all the variants previously found on the gene panel. Preliminary analysis showed that none of the patients had a second causal mutation in any single gene known to be involved in cobalamin metabolism. Eight patients had one or more variants in the coding region of other disease causing genes. One patient had two novel presumed pathogenic mutations in PCCA. Review of the clinical records showed that this patient had propionic aciduria in addition to cobalamin-responsive methylmalonic aciduria. Conclusions: These studies demonstrate the diagnostic yield from WGS analysis of patients with undiagnosed methylmalonic aciduria.

AuthorNames: Lina Sobhi Abdrabo, McGill university Health Center Research Institute; David Watkins, McGill University; Jacek Majewski, McGill University and Génome Québec Innovation Centre; Jean-Baptiste Rivière, McGill University Health Centre; David Rosenblatt, McGill University
Validating and optimizing NGS-based diagnostics to improve diagnostic yield and clinical utility

Primary Category: Clinical Genetics
Secondary Category: Molecular Genetics

Abstract: Utility of Whole Exome Sequencing (WES) in clinical diagnostics has been limited by the non-uniform sequencing coverage across exons, leaving a substantial proportion of the regions with shallow coverage that prevents accurate variant detection. We evaluated a WES assay that is specifically designed for clinical use, enables wide breadth of coverage resembling high-coverage gene-panel based assays, and provides high sensitivity in variant detection. We performed WES capture experiments using an assay with boosted clinical content, namely xGen Exome Research Panel (IDT) assay that was spiked-in with custom designed clinical content. Sequencing was performed using an Illumina NovaSeq sequencing system and data was down-sampled to 100M reads. Performance of the WES assay was demonstrated by using reference samples with high-quality variant calls (The Genome In a Bottle Consortium and Platinum Genome samples for SNVs and INDELs and Coriell samples for assessing Del/Dups and complex genetic variants). In clinically associated CCDS genes, the assay achieved high average sequencing depth (183x) and coverage (99.7% of regions covered >20x). Sensitivity to detect SNVs was 0.998, and for INDELs 0.97. Sensitivity to detect 1 exon deletions and duplications was 0.93 and 0.99 for 5 exon deletions and duplications. The assay was observed to provide a uniform coverage over difficult-to-sequence regions (e.g. the RPGR gene) and GC-rich 1st exons. Our results demonstrate that WES assay with boosted clinical content provide high sequencing coverage and allows high variant calling sensitivity for different genetic variations, which makes it well-suited for clinical diagnostics of inherited disorders.

AuthorNames: Christine Davies, Blueprint Genetics; Miko Valori, Blueprint Genetics; Tero-Pekka Alastalo, Blueprint Genetics; Ville Kytola, Blueprint Genetics; Pertti Salmenperä, Blueprint Genetics; Matias Rantanen, Blueprint Genetics; Massimiliano Gentile, Blueprint Genetics; Samuel Myllykangas, Blueprint Genetics
Functional analysis and structural modeling of a novel variant in the transcription regulatory domain of SIM1 identified in an individual with hyperphagic obesity and Prader-Willi-like features

Primary Category: Molecular Genetics

Secondary Category: Cytogenetics/Microarray

Abstract: Background. Single-minded homologue 1 (SIM1) is a transcription factor with several physiological and developmental functions. Haploinsufficiency of SIM1 is associated with early onset obesity with or without Prader-Willi-like (PWL) features. Exome sequencing was performed on a 31-year-old man presenting with intellectual disability, obesity (BMI 47.4), and impulse control disorder which revealed a novel missense variant in SIM1 (c.2144G>T; p.G715V). Objectives. Previous studies have identified several disease-associated variants that fall near the p.G715V variant within the C-terminal domain of SIM1. Our aim was to functionally characterize the novel p.G715V variant and develop an ab initio hybrid model for the full-length protein. Design/Method. In response to environmental and cellular signals, SIM1 will heterodimerize with the aryl hydrocarbon receptor nuclear translocator (ARNT). We therefore examined p.G715V variant stability and activity in a doxycycline-inducible stable cell line transfected with an artificial reporter construct and either ARNT or ARNT2 as a partner protein. Results. Functional testing of the p.G715V variant revealed a significant reduction in SIM1-mediated transcriptional activity. We also generated the first hybrid protein model for full-length SIM1 to show the predicted spatial relationship between p.G715V and other previously described variants in this region and identified a putative mutation hotspot within the C-terminus. Conclusions. Significant clinical heterogeneity has been observed in patients with SIM1 variants and the penetrance of putative pathology causing alleles is still being examined. Through modeling and functional analysis we were able to show that the p.G715V variant in SIM1 is likely pathogenic and clusters with other previously reported variants.

AuthorNames: Patrick Blackburn, Mayo Clinic; Thomas Caulfield, Mayo Clinic Florida; Margot Cousin, Mayo Clinic; Nicole Boczek, Mayo Clinic; Adrienne Sullivan; Eric Klee, Mayo Clinic; Jay van Gerpen, Mayo Clinic Florida; Murray Whitelaw, University of Adelaide; Paldeep Atwal, Mayo Clinic Florida
Value of whole exome sequencing vs. gene panels in clinical genetics

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: Value of whole exome sequencing vs. gene panels in clinical genetics William P. Pirjamalia, Shengnan Wua, Harry Gaoa aFulgent Genetics, Temple City, California, USA

Objectives. Clinical genetic testing has experienced a shift from single gene to large gene panels, and most recently whole exome sequencing (WES) and whole genome sequencing, largely as a consequence of dropping costs, turnaround time, and diagnostic value. As a result of this shift, clinicians are faced with the challenge of determining the most appropriate course of genetic testing for their patients. Here we review three cases where WES led to the identification of a diagnostic variant. These cases are presented to demonstrate the diagnostic utility of WES, discuss and contrast to gene panels, and to provide a review of the specific variants identified.

Design/Method. Retrospective case reviews of three diagnostic whole exome sequencing analysis performed at Fulgent Genetics. Results. In each of the three patients we identified a diagnostic pathogenic/likely pathogenic variant (CDC42, TRIM8, ASXL3). Only CDC42 can be found as part of a gene panel, whereas TRIM8 and ASXL3 are available through WES. The variants identified in TRIM8 and ASXL3 were previously unreported in the literature; Fulgent Genetics is currently the only lab to offer these as clinical tests. Conclusions. These cases illustrate the value of WES and provide some background to discuss the approach of selecting panels or exomes when patients present at the clinic.

AuthorNames: William Pirjamali, Fulgent Genetics
Deletions of Variable Size Are Commonly Identified in Hearing Loss Patients in a Diagnostic Laboratory: Diagnostic Yield of 110 Cases

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: Next-generation sequencing (NGS) has become a popular tool used to aid in the diagnosis of genetically heterogeneous disorders such as hearing loss. Targeted NGS panels focus on subsets of genes associated with phenotypes, which increases coverage, specificity, and interpretation efforts. We reviewed the last 110 cases send for targeted panel testing for hearing loss either using Comprehensive Hearing Loss panel (158 genes) or Non-Syndromic Hearing Loss panel (93 genes). Majority of the analyses (106/110) were performed as PLUS analyses combining both NGS sequencing analysis and deletion/duplication analysis utilizing NGS data. We have previously shown that our Deletion/Duplication analysis can identify both small deletions/duplications (1-5 exons) as well as larger copy-number variants. The diagnostic yield in these hearing loss patients was 29%. The most commonly implicated genes were GJB2 (16% of diagnostic cases) and STRC (13% of diagnostic cases). Many patients had already had previous genetic testing for GJB2 explaining the lower proportion of genetic diagnoses in this patient set compared to some of the non-screened series. Genetic findings were consistent with autosomal recessive form of hearing loss in 75% of cases, autosomal dominant form in 22% and X-linked form in 3% of cases. Interestingly, 28% of cases with genetic diagnosis had either a heterozygour or homozygous deletion explaining the patient’s phenotype or being identified as heterozygous with a second disease-causing allele in the same gene.

AuthorNames: Eveliina Salminen, Blueprint Genetics; Meenakshi Mahey Kumar, Blueprint Genetics
Analytic Validation of Oligonucleotide-Selective Sequencing Panels for Clinical Diagnostics of Inherited Eye Disorders

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: INTRODUCTION: Genetics diagnostics of ophthalmology patients has been affected by poorly validated genetic tests, lack of transparency, and testing solutions that are not optimized for maximal diagnostic yield. Our goal was to develop a high quality next generation sequencing (NGS) platform, transparently and comprehensively validate its quality and performance, and report here our experiences with hundreds of patients suffering from retina disorders. METHODS: We developed and validated a set of NGS tests (>350 genes), with traceable sample sets, for detecting single-nucleotide variants (SNVs), insertions and deletions (INDELs) and deletions and duplications (DELDUPs). Our assay targeted the coding exons the splice regions, and selected deep intronic variants. We also utilized this platform to diagnose hundreds of patients during 2016-17. RESULTS: We demonstrate, on average, 0.993 sensitivity, 0.999 specificity, 0.992 positive predictive value for detecting SNVs and 0.97 sensitivity for detecting INDELs of 1-220 bases. We also demonstrated 0.93 sensitivity to detect single exon deletions from NGS data. Repeatability and reproducibility of the assays were 0.994 and 0.998, respectively. 99.4% of the target regions were covered with over 20x sequencing depth, mean coverage >200x. We also demonstrate full coverage of the RPGR ORF15 region and 100% sensitivity to detect complex mutations within the ORF15 region. Using ACMG guidelines for variant classification a diagnosis was established in 47% of retina patients, a likely diagnosis in 23%, and 30% were considered negative. Approximately 5% of patients were tested positive for a copy number variation or a non-coding disease causing variant. CONCLUSIONS: Our results demonstrate the analytic validity of the developed tests and show that the technology is well-suited for clinical diagnostics of inherited eye disorders. It also demonstrated a cost-effective diagnostic tool to simultaneously diagnose various types of mutations from SNVs to copy number variations.

AuthorNames: Meenakshi Mahey Kumar, Blueprint Genetics; Tero-Pekka Alastalo, Blueprint Genetics
De novo missense variants in RAC3 cause a novel neurodevelopmental syndrome

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: Gregory Costain, Bert Callewaert, Heinz Gabriele, Tiong Y. Tand, Susan Walkere, Meaghan Snella, Björn Mentenb, Arnaud Vanlanderb, Sarah Vergultb, Susan Blasera, Robin Z. Hayeemsa, Christian R. Marshalla,e, Julia Orkina, Stephen W. Scherera,e, Shoshana Wodaka, David Chitayata,f, M. Stephen Meyna,g aThe Hospital for Sick Children and University of Toronto, Toronto, Ontario, Canada bGhent University, Ghent, Belgium cEberhard Karls University of Tübingen, Tübingen, Germany dVictorian Clinical Genetics Services, Melbourne, Australia eThe Centre for Applied Genomics, Toronto, Ontario, Canada fMount Sinai Hospital and University of Toronto, Toronto, Ontario, Canada gUniversity of Wisconsin, Madison, Wisconsin, United States Background: RAC3 is an understudied member of the Rho GTPase family that is expressed in the developing human brain and functionally linked to key developmental processes. De novo missense variants in the homolog RAC1 were recently implicated in causing developmental disorders. In the RAC subfamily, transforming missense changes at certain shared residues have been observed in human cancers and previously characterized in experimental studies. Objectives: The purpose of this study was to determine whether germline dysregulation of RAC3 is associated with human disease. Methods: We combined exome and genome sequencing with genotype/phenotype aggregation database searching. Results: We rapidly identified five individuals with severe intellectual disability and structural anomalies on brain MRI who had de novo monoallelic germline missense variants altering certain shared residues of RAC3. In silico protein modeling and other lines of evidence support constitutive activation as the underlying disease mechanism. Conclusions: Identification of RAC3 as a new disease gene provides further insights into human neuronal migration and neurodevelopment.

Author Names: Gregory Costain, The Hospital for Sick Children; David Chitayat
Outcomes of epilepsy panel testing: a retrospective review

Primary Category: Clinical Genetics

Abstract: Epilepsy is a common and heterogeneous disorder with acquired, multifactorial and monogenic causes. The advent of next-generation sequencing (NGS) has made the diagnosis of the rare, single-gene, forms of epilepsy possible. Here we assess the use of comprehensive “panels” to diagnose individuals with epilepsy. A total of 94 patients were followed by the regional Pediatric Neurology clinic and had undergone gene panel testing between 2014 and 2017. Parents were not included in the initial testing. The gene panel sequencing was performed by commercial laboratories and they ranged in size from 10 to 447 genes. Panel selection was based on availability and clinical presentation. For all panels, the diagnostic rate was 16% (15/97). Those with a confirmed diagnosis showed a degree of intellectual disability and/or a syndromic presentation. Sixty-four individuals (68%) had at least 1 variant of unknown significance (VUS). Parental testing was subsequently requested for these individuals to aid variant interpretation. A direct change in management was observed in a few instances (for example, GLUT1 deficiency) and the diagnosis-positive families benefitted from an informed genetic counseling and prenatal testing options. This work shows the benefits of gene panel testing for epilepsy.

Author Names: David Dyment; Alison Eaton, University of Calgary; Sunita Venkateswaran, Children's Hospital of Eastern Ontario; Hugh McMillan, Children's Hospital of Eastern Ontario Research Institute; Sharon Whiting; Erick Sell; Daniela Pohl; Asif Doja
Development of OncoScan SNP Array for clinical analysis of pediatric solid tumours

Primary Category: Cytogenetics/Microarray

Abstract: Michelle Axford1, Anthony Arnoldo2, Paul Thorner2,3, Gino Somers2,3, Cynthia Hawkins2,3, and Mary Shago1,3. Division of Genome Diagnostics, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto Division of Pathology, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto

Background. Medulloblastoma, glioma, and neuroblastoma are amongst the most common solid tumours in children. Recurrent gains or losses of distinct chromosomal regions are prognostic in these tumours. Our clinical algorithms for testing these tumours include genomic microarray analysis on fresh frozen tumour samples. However, most microarray platforms cannot be reliably used for copy number/ SNP analysis of formalin fixed paraffin embedded (FFPE) tumours.

Objective. To validate the use of the Affymetrix OncoScan SNP array platform for the assessment of copy number and heterozygosity in FFPE tumours. Methods. OncoScan microarray analysis was performed on 25 tumour samples and compared to previous results of analysis on the gold standard Affymetrix CytoScan HD platform, already optimized in our clinical laboratory. A cancer gene track specifically focusing on pediatric malignancies was developed to enhance detection of the genetic alterations found in pediatric tumours.

Results. Comparison of the OncoScan and CytoScan HD results revealed that 8 of 9 medulloblastomas, 7 of 8 gliomas, and 8 of 8 neuroblastomas have either completely concordant aberrations or display minor discrepancies that would not alter prognostication. Conclusions. The OncoScan array platform provides effective copy number and SNP information in pediatric malignancies. Although OncoScan has a lower resolution than the CytoScan HD platform, the ability to study preserved specimens with challenging fragmented DNA and/or with limited DNA input is essential to assist with treatment stratification of patients with pediatric malignancies.

AuthorNames: Michelle Axford, The Hospital for Sick Children
Whole Exome Sequencing in the NICU

Primary Category: Molecular Genetics

Abstract: Michelle M. Axford1, Meaghan Snell2, Carol-Ann Ryan1, Marianne Eliou1, Miranda Lorent1, Sarah Bowdin2, Nasim Monfared2, D. James Stavropoulos1,5, Ronald Cohn3, Christian R. Marshall1,5, Aideen Moore4, Rebekah Jobling1,2 Division of Genome Diagnostics, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto Division of Clinical Genetics and Metabolism, Department of Pediatrics, The Hospital for Sick Children; University of Toronto, Toronto Department of Pediatrics, The Hospital for Sick Children; University of Toronto, Toronto Division of Neonatology, Department of Pediatrics, The Hospital for Sick Children; University of Toronto, Toronto Laboratory Medicine and Pathobiology, University of Toronto, Toronto Background. Over 20% of neonatal deaths are reported to be the result of a genetic disease. Timely and accurate diagnoses are important for guiding the care of critically ill neonates. This can be difficult, since presenting signs and symptoms can be non-specific, leading to numerous investigations and treatments that are often invasive, empirical, or lack efficacy. Rapid diagnoses would result in fewer invasive investigations, more appropriate care, and a shorter diagnostic odyssey. Objectives. To use WES as a first-line diagnostic test for 20 neonates presenting with congenital disorders, for whom rapid diagnosis is likely to guide management or treatment in a clinically useful time span. Design and Methods. 20 Sick Kids NICU patients were assessed by physicians for inclusion. WES was completed on the proband, with variants of interest followed up by Sanger sequencing on the trio. Research reports were written after Sanger confirmation. Results. Of 17 completed exomes; 5 had a pathogenic or likely pathogenic AD variant reported, 1 had a homozygous pathogenic secondary finding (deafness), but no variant related to the indication, 2 had a pathogenic or likely pathogenic variant reported along with a VUS in an AR gene that fit the phenotype, 3 others had a VUS reported (1 AD disorder, 2 as the only variant for an AR disorder), and 6 had no variants reported. The average number of working days from sample acquisition to reporting was 15. Conclusions. A NICU exome is a rapid, effective first-tier testing strategy for ill neonates with suspected genetic disorders.

AuthorNames: Michelle Axford, The Hospital for Sick Children
Homocysteine concentration in breast milk is higher in a woman affected with classic homocystinuria

Abstract: Background: The kinetics of lactational homocysteine excretion in classic homocystinuria are not well described. Objective: The purpose of this study was to determine whether a woman with classic homocystinuria has an elevated homocysteine level in her breast milk and whether it affects her child’s plasma homocysteine level. Method: A 28-year-old female with classic homocystinuria was treated with protein restriction, methionine-free formula and supplements during her pregnancy. She delivered a healthy boy at term. Plasma and breast milk samples were obtained 12 days post-partum. A breast milk control sample was obtained from an unaffected individual. The patient’s son’s plasma homocysteine was measured on day of life 1 and 5. Homocysteine levels were measured using a competitive immunoassay in the Dimension Vista System (Siemens Healthcare Diagnostics, Marburg, Germany). Breast milk samples were tested pre and post-ultracentrifugation. Results: The patient’s plasma homocysteine was 240 umol/L (reference < 10 umol/L). The patient’s breast milk homocysteine was 110 umol/L pre and 85 umol/L post-ultracentrifugation. The control breast milk homocysteine was 36 umol/L pre and 11 umol/L post-ultracentrifugation. The patient’s son’s plasma homocysteine were 22 umol/L (day 1) and 9 umol/L (day 5). Conclusions: Lactational homocysteine excretion is elevated in classic homocystinuria; however, plasma homocysteine levels are normal in breastfed infants due to their half normal activity of cystathionine beta-synthase.

AuthorNames: Andrea Yu, Children's Hospital of Eastern Ontario; Matthew Lines, CHEO - Metabolics; Matthew Henderson, Newborn Screening Ontario
Myotonic dystrophy Type 1: expansion sizing in the absence of Southern blot analysis

Abstract: Myotonic dystrophy type 1 (DM1) is one of the most common muscular dystrophies. The disease is caused by a CTG repeat expansion in the 3’ untranslated region of the DPMK gene and has a variable presentation which can be categorized into three phenotypes: mild DM1 characterized by adult-onset, cataracts and mild myotonia; classic DM1, characterized by muscle weakness and wasting, myotonia, balding, cataracts, and cardiac arrhythmia; and congenital DM1, characterized by hypotonia, post-natal severe generalized weakness, respiratory insufficiency, frequently cognitive impairment and early death. A diagnosis of DM1 is confirmed by the presence of 50 or more CTG repeats. Individuals with mild DM1 generally carry 50-150 repeats, those with classical DM1, 100-1000 repeats, and those with congenital DM1 typically >1000 repeats. Note that repeat ranges for each of the phenotypes overlap and caution should be used in predicting phenotype based on repeat size. The standard testing protocol currently employed by the molecular diagnostics laboratory at the Alberta Children’s Hospital includes PCR amplification of alleles in the normal, premutation and mild range (50-150 repeats), followed by triplet-primed PCR (tpPCR) to detect large expansions. Since tpPCR does not provide any information regarding expansion size, Southern blot analysis has historically been used to size expansions in females of reproductive age to predict the risk of children with the congenital form of DM1. With the desire to move away from Southern blot analysis, we evaluated the ability of the current PCR-based protocol to size expanded alleles without a Southern blot or any significant increase in cost or labour. By adding gel electrophoresis after PCR amplification, we found the current PCR protocol was able to amplify alleles up to 500 repeats. Further alterations to the PCR protocol, including buffer, enzyme and PCR amplification conditions, allowed for amplification of ~1000 repeats. High levels of mosaicism make interpretation of results challenging when resolving expanded alleles by gel electrophoresis; however, this is also a challenge for Southern blot analysis. Overall, PCR amplification of expanded alleles in the clinically relevant range was possible without the use of a Southern blot.

AuthorNames: Maksym Misyura; Ryan Lamont, University of Calgary; Jillian Parboosingh, University of Calgary
Rare variants in SUZ12 found in three unrelated families with an overgrowth phenotype

Primary Category: Molecular Genetics
Secondary Category: Clinical Genetics

Abstract: Sharri S. Cyrus, Ana SA. Cohen, Ruky Aghahovbeb, Kristiina Avelac, Brian HY. Chungd, Kit S. Yeungd, Ho Ming Luke, Sanaa Choufanif, Weksberg Rosannaf, William T. Gibsona,b a Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada b British Columbia Children’s Hospital Research Institute, Vancouver, BC, Canada c Department of Clinical Genetics, Helsinki University Hospital, Helsinki, Finland d Department of Paediatrics and Adolescent Medicine, the University of Hong Kong, Hong Kong e Clinical Genetic Service, Department of Health, Hong Kong f Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada Background: The Polycomb Repressive Complex 2 (PRC2) is an epigenetic regulator, with an essential role in gene expression. It is comprised of 4 well conserved subunits namely: EZH1/2, SUZ12, EED and RbAp46/48. Pathogenic variants within EZH2 and EED have been found to cause Weaver syndrome and Cohen-Gibson Syndrome respectively. More recently there has been 1 reported case in literature of a germline mutation within SUZ12 in a proband who had suspected Weaver Syndrome. Objective: To further delineate the landscape of genetic causes of rare overgrowth syndromes Design/Method: In our existing overgrowth cohort, patients who did not have variants in EED or EZH2, underwent whole exome or whole genome sequencing. Candidate variants were validated by Sanger sequencing and where possible parental samples were sequenced. Results: Three probands were found to have rare coding variants within SUZ12. One proband inherited a missense variant from an affected father, while the other two probands have indel variants. These probands have increased growth parameters, mild dysmorphic features with varying other abnormalities. Conclusions: We have identified four additional individuals two of whom are related with SUZ12 variants who have an overgrowth phenotype.

AuthorNames: Sharri Cyrus, Department of Medical Genetics, University of British Columbia
ID: TCG028
Quadruple genetic diagnoses in a neonate with hypotonia

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: Background. It is important to determine an accurate genetic diagnosis to end a diagnostic odyssey, tailor disease management and provide appropriate genetic counselling. For some patients, there is not a single explanation for the clinical presentation. The frequency of multiple genetic diagnoses in patients referred for whole-exome sequencing (WES) is 1.4 - 7.2%, reflecting that complex phenotypes drive providers toward broader testing. Objective. To highlight the potential for multiple genetic diagnoses to underlie even an ostensibly straightforward presentation of neonatal hypotonia. Method. Case report. Results. At consult, the patient was an 11 day old premature male with hypotonia, mild dysmorphisms and cryptorchidism, born to non-consanguineous First Nations’ parents. Rapid, trio-based WES identified three de novo genetic diagnoses: Prader-Willi Syndrome (maternal uniparental disomy (UPD) of chromosome 15), Coffin-Siris syndrome (a likely pathogenic SMARCA4 variant) and Klinefelter syndrome (47,XXY karyotype). Concurrently obtained microarray and methylation testing for Prader-Willi syndrome also demonstrated the diagnoses. The patient is also homozygous for the P479L variant of CPT1A present at increased frequency in circumpolar and BC coastal indigenous populations and causes a form of carnitine palmitoyltransferase deficiency type I. Conclusions. The benefit of WES for rapid diagnosis in clinical practice is evident. Concurrent diagnosis of these conditions directed medical management (G-tube placement and outpatient ventilatory planning, in view of anticipated long-term needs; avoidance of fasting; identification and treatment of hypothyroidism; and consideration of growth hormone therapy). Furthermore, concurrent microarray testing may be unnecessary when WES includes copy number variant and UPD analysis.

AuthorNames: Katherine Blood, Department of Medical Genetics, University of British Columbia; Anna Lehman, University of British Columbia; Laura Stewart, BCCH; Michael Seear, Department of Respiratory Medicine, Department of Pediatrics
Mutations in HNRNPU and childhood neurocognitive dysfunction

Abstract: Background: Recently, intellectual disability and/or childhood epileptic encephalopathy have been linked to mutations in HNRNPU, which encodes heterogeneous nuclear ribonuclear protein U (hnRNP-U). There are occasional cardiac or renal anomalies. This emerging neurodevelopmental syndrome is not clinically diagnosable without comprehensive genomic testing to identify a mutation and rule out alternative or additional diagnoses. The data describing the spectrum of HNRNPU phenotypes and mutations remains limited to fewer than 30 individuals described thus far in the literature. Objective: To augment data on phenotypes associated with rare HNRNPU variants. Design/Method: Case series, ascertained through BC Children’s Hospital’s CAUSES research study. At the time of interim analysis, a cohort of ~200 children had been enrolled and underwent exome sequencing with an aim to identify a genetic diagnosis for a developmental disorder. Results: Two children with de novo HNRNPU (NM_031844.2 (hg19)) mutations were identified. One child harboured a missense variant (p.His457Pro) and the other a frameshift variant (p.Pro200AlafsTer14). In both children, the phenotype was non-specific, and characterized primarily by developmental disability plus fever-triggered seizures in one, which is a recurrent HNRNPU phenotype. Both children had strabismus, and both had minor renal anomalies (nephrocalcinosis in one and unilateral hypoplasia in the other). Conclusions: We report two additional individuals with a putative HNRNPU-related disorder. Epilepsy is a frequent but not constant feature of this disorder, being present in 93% of reported individuals. Mutation type (missense versus haploinsufficiency) does not yet appear to correlate with presence or absence of seizures.

AuthorNames: Anna Lehman, University of British Columbia; Alexandra Roston, University of Alberta; William Gibson, University of British Columbia; Christèle du Souich, UBC; Clara van Karnebeek, BC Children's Hospital / UBC; Tanya Nelson, Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada; Alison Elliott, Department of Medical Genetics; Jan Friedman, Department of Medical Genetics; Harinder Gill, Department of Medical Genetics; Jill Mwenifumbo, Department of Medical Genetics; CAUSES Study, Department of Medical Genetics; Shelin Adam, Department of Medical Genetics
Diagnostic challenges in a patient with global developmental delay, lipodystrophy, periventricular leukomalacia and spasticity

Primary Category: Clinical Genetics

Abstract: Diagnostic challenges in a patient with global developmental delay, lipodystrophy, periventricular leukomalacia and spasticity

Melissa J. MacPhersona, A. Micheil Innesa,b, Lorne Seargeantd, Miao Hec, Walla Al-Hertania,b

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Objective: Present diagnostic challenges encountered in a consanguineous patient with a complex phenotype, followed in Metabolics clinic.

Methods: We report a 4 year old male born to consanguineous parents followed for developmental delay, nystagmus, seizures, spasticity, lipodystrophy and failure to thrive. MRI brain showed bilateral patchy periventricular white matter abnormalities and volume loss with normal 1H-magnetic resonance spectroscopy.

Results: Metabolic workup including creatine kinase and transferrin isoelectric focusing (TIEF) were negative. A novel homozygous likely pathogenic variant in NOTCH3 (c.5677C>T; p.Arg1893*) was identified but deemed not sufficient to explain the phenotype, so whole exome sequencing (WES) was pursued and revealed in addition to the NOTCH3 variant, novel homozygous missense variants in ISPD (c.733C>G; p.Leu245Val) and in PMM2 (c.657G>C; p.Glu219Asp), both predicted deleterious by in silico tools and classified as variants of uncertain significance (VUS). Despite the normal TIEF, the variants in PMM2 warrant further investigation with N-glycan analysis as many subtypes of CDG are missed by TIEF alone. Similarly further investigations of the ISPD variant is needed given the recent suggestion that supplementation with ribitol is therapeutic.

Conclusion: This case highlights the emerging concept of multiple diagnoses in a single patient and hence the value of early WES in complex phenotypes, especially in consanguineous families; 2. Emphasizes the utility of N-glycan analysis over TIEF to diagnose CDG; 3. Demonstrates the emerging idea of recessive versions of dominant disorders associated with phenotypic expansion; 4. Illustrates challenges in effectively diagnosing patients with rare diseases, especially if there is potential for therapeutics.

Author Names: Melissa MacPherson, Alberta Children's Hospital; Walla Al-Hertani, University of Calgary; Micheil Innes, University of Calgary; Lorne Seargeant; Miao He
Paradoxical ketotic hypoglycemia and persistent biochemical abnormalities in mitochondrial HMG-CoA synthase deficiency

Abstract: Alina Levtova, Daniela Buhas, Paula Waters, Pierre Allard, Beatriz Puisac, Juan Pié, Grant Mitchell, Catherine Brunel-Guittone a Division of Medical Genetics, Department of medicine, CHUM (Centre Hospitalier Universitaire de l’Université de Montréal) and Université de Montréal, Montreal, Quebec, Canada b Department of Human Genetics, McGill University, and Dept Med Gen, Montreal Children's Hospital, Montreal, Canada c Division of Medical Genetics, Department of Pediatrics, CHU Sainte-Justine and Université de Montréal, Montreal, Quebec, Canada d Department of Pediatrics, Sherbrooke University Hospital Centre, Sherbrooke, Quebec, Canada e Unidad de Genética Clínica y Genómica Funcional, Departamento de Farmacología-Fisiología y Departamento de Pediatría, Hospital Clínico Universitario "Lozano Blesa", Facultad de Medicina, Universidad de Zaragoza, ISS-Aragon and CIBERER-GCV02, 50009, Zaragoza, Spain. Background. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHS) deficiency is a rare, but possibly underdiagnosed, disorder of ketogenesis from fatty acids. It classically presents with hypoketotic fasting hypoglycemia, metabolic acidosis, encephalopathy, and transient hepatomegaly. Objectives. The purpose of this study is to describe an unusual presentation and course of mHS deficiency in a six-month old girl. Design/Method. The mode of presentation, clinical course, biochemical findings, and results of clinical exome testing were reviewed. HMGCS2 variants, were evaluated through expression analysis in E. coli. Results. A girl presented at the age of six months with severe hypoglycemia (1.9 mmol/L; reference, 3.0-5.6), metabolic acidosis (bicarbonate 3.9 mmol/L, reference 18-25), lactate 0.9 mmol/L (reference, 0.5-2.2), and ammonia 74 umol/L (reference, 5-55) and mild ketonuria. Plasma acylcarnitines showed multiple elevations (~3-fold elevated C2 and C4-OH, 3-fold elevated C14:1, C16, C18:1, and 2-fold elevated C6, C8, and C10). Urine organic acids also showed a global disturbance. Although the initial decompensation responded well to intravenous dextrose, the baby showed persistent hepatomegaly and abnormal organic acids even when asymptomatic and receiving frequent feeds, possibly due to insufficient total caloric intake. She had a second metabolic decompensation at 9 months. When supplemental gavage feeding was introduced, the chronic abnormalities resolved and she has subsequently had a benign course. Two novel variants in HMGCS2 were identified and shown to be pathogenic: c.1502G>A (p.R501Q) and c.437T>G (p.M146R). Conclusions. This report extends the known biochemical, clinical, and molecular spectrum of mitochondrial HMG-CoA synthase deficiency and emphasizes the importance of achieving anabolism over the long term.

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Warsaw Breakage Syndrome: Further Clinical and Genetic Delineation of Disease-Causing Mutations

Primary Category: Clinical Genetics

Secondary Category: None

Abstract: Warsaw breakage syndrome (WBS) (MIM# 601150) is a recently recognized autosomal recessive cohesinopathy, characterized by severe prenatal and postnatal growth restriction, microcephaly, developmental delay, cochlear abnormalities and sensorineural hearing loss. Only seven cases have been reported in the English literature, and thus the known information on the phenotype and genotype of this interesting condition is limited. Here we provide the clinical and molecular phenotypic data of five unrelated patients carrying novel bi-allelic mutations in the DDX11 gene, identified via trio whole exome sequencing. Of those, we evaluated the unclear effect of the missense variant p.Arg378Pro (R378P) on DDX11 function. We also performed molecular studies of the recombinant DDX11-R378P protein expressed in 293T cells as well as assessed the subcellular localization in HeLa cells, and the results revealed a decreased level of intact DDX11-R378P protein and poor nucleolar localization of the DDX11-R378P protein, respectively. These results indicate that R378P has a damaging effect on DDX11 expression and is associated with WBS phenotype. Moreover, the clinical and cytogenetic data of the seven reported patients were collected for further delineation of the WBS phenotype, and a detailed comparison of the 12 patients is provided. Interestingly, we noted that elevated chromosomal breakage was an inconsistent finding in WBS, unlike the cohesion defects such as premature chromatid separation and division. We also concluded that the cardinal clinical features in WBS (microcephaly, growth retardation and cochlear abnormalities) are almost universally present. This report further extends the knowledge of the clinical and molecular characteristics of WBS.

AuthorNames: Ebba Alkhunaizi, University of Toronto; David Chitayat; Fowzan Alkuraya, Faisal Specialist Hospital and Research Center; Robert Brosh Jr, NIH; Karen Chong, Mt. Sinai Hospital; Sanjay Kumar Bharti, NIH; Mary Ann George, Sick Kids Hospital; Ghada Abdel-Salam, National Research Centre; Ranad Shaheen, Faisal Specialist Hospital and Research Center
Tumor-first testing of BRCA1 and BRCA2 to identify germline and somatic variants in ovarian cancer

Primary Category: Clinical Genetics

Secondary Category: Molecular Genetics

Abstract: Tumor-first testing of BRCA1 and BRCA2 to identify germline and somatic variants in ovarian cancer Tara J. Spencea, Tong Zhang, Natalie Stickleb, Suzanne Kamel-Reida,c, Patricia Shawd,e, Tracy L. Stockleya,c,d aAdvanced Molecular Diagnostics Laboratory, Princess Margaret Cancer Centre, University Health Network bPrincess Margaret Genomics Centre, Princess Margaret Cancer Centre, University Health Network Departments of cClinical Laboratory Genetics and dPathology Laboratory Medicine Program, University Health Network eDepartment of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON

Objectives: Due to recent approval of PARP inhibitor treatment for women with high grade ovarian serous cancer and either a somatic (tumor) or inherited (germline) BRCA1 or BRCA2 mutation, testing of formalin fixed, paraffin embedded (FFPE) tumor tissue for BRCA1 and BRCA2 is now required in Canada. A ‘tumor first’ approach would maximize the number of women eligible for PARP inhibitors, as testing of FFPE tumor tissue will identify both inherited and somatic BRCA mutations. There is an urgent need to evaluate the tumor-first approach for BRCA including ability to detect germline and somatic changes, quality metrics, and copy number variants (CNVs). Design/Method: DNA from 50 FFPE tumors, from known germline BRCA carriers with ovarian cancer, were sequenced using a custom NGS hybrid capture library on the Illumina NextSeq. Quality control included assessment of percent Q30, duplication rates and mean target coverage. Data analysis used a custom bioinformatics pipeline with alignment to hg19 (BWA-MEM, GATK) and discovery of single nucleotide variants and indels by VarScan2. CNVs were assessed using CNVkit and an internally derived normal reference panel. Results: Of 23 cases analyzed to date, reported germline variants in BRCA1 (n=16) or BRCA2 (n=7) were detected in tumor tissue in all cases. Quality metrics important to ensuring variant quality from FFPE testing were defined, and CNV assessment on FFPE evaluated. Conclusions: A tumor first approach identified 100% (n=23) of the known germline BRCA1/BRA2 mutations in testing of FFPE tumor tissue. This method effectively enables detection of somatic and germline BRCA mutations to maximize PARP inhibitor eligibility in ovarian cancer patients.

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