



A 01

**GENOMICS APPLIED TO THE MANAGEMENT OF HIGH-RISK AML/MYELODYSPLASTIC SYNDROMES**

*R. Docking<sup>1</sup>, I. Bosdet<sup>2</sup>, R. Moore<sup>1</sup>, Y. Butterfield<sup>1</sup>, S. Chan<sup>1</sup>, M. Jadersten<sup>1</sup>, L. Yang<sup>1</sup>, S. Young<sup>2</sup>, R. Coope<sup>1</sup>, R. Kirkpatrick<sup>2</sup>, A. Mungall<sup>1</sup>, Y. Zhao<sup>1</sup>, I. Birol<sup>1</sup>, R. Holt<sup>1</sup>, S. Jones<sup>1</sup>, M. Marra<sup>1</sup>, A. Karsan<sup>1</sup>,*

*<sup>1</sup> Canada's Michael Smith Genome Sciences Centre, Vancouver, BC; <sup>2</sup> BC Cancer Agency, Vancouver, BC*

The objective of this project is to transition genetic-based AML testing to a genomics platform. Acute myeloid leukemia (AML) and high-risk myelodysplastic syndromes (MDS) are rapidly fatal without proper treatment- either bone marrow transplantation or aggressive chemotherapy. The choice of treatment is dependent on chromosomal and molecular abnormalities present in leukemic cells of the patient. Presently, karyotyping is used to identify common translocations and copy number changes that define risk status, coupled with Sanger-based sequencing of several genes. Based on emerging clinical evidence, it is clear that several other genes will need to be examined for mutations in the next 2 to 3 years to better stratify patients to the correct treatment up front.

We are developing assays to test for multiple gene mutations and fusions by genome-wide sequencing, coupled with targeted sequencing of specific AML regions of interest. In a retrospective study, we obtained full transcriptome (WTSS) sequences for 89 patients using the Illumina HiSeq 2000, in addition to matched exon-capture and whole-genome sequences for some patients. Additionally, a targeted gene panel constructed using the RainDance platform has been designed. An in-house bioinformatics pipeline has also been developed to determine the presence or absence of previously-characterized SNVs, indels, and structural rearrangements. Results comparing the four approaches in terms of coverage, sensitivity, specificity, and clinical utility will be presented.



A 02

## IDENTIFICATION OF A NEW CAUSATIVE GENE FOR ATAXIA WITH OCULAR MOTOR APRAXIA

H. Racher<sup>1</sup>,

<sup>1</sup>Alberta Children's Hospital, Calgary, AB;

Ataxia with ocular motor apraxia (AOA) is a condition characterized by progressive cerebellar ataxia in addition to the inability to make normal voluntary horizontal eye movements. Two well known forms of AOA include AOA1 and AOA2 caused by mutations in *APT*X and *SET*X genes respectively. In this project, we studied a Hutterite patient with early onset AOA and hypoplasia of the cerebellar vermis (without "molar tooth sign"). A SNP array was performed on this patient, which revealed large regions of homozygosity on chromosomes 10 and 13. No significant regions of homozygosity were identified on chromosome 9 where *APT*X and *SET*X reside. Next, whole exome sequencing was performed. A total of 17799 variants were identified. Filtering out known variants from SNP databases, including only the two regions of homozygosity and examining only novel variants in coding sequence, reduced the list to only 1 variant; a missense mutation in the 90kDa centrosomal protein (*CEP90*) gene. *CEP90* encodes a pericentriolar satellite protein that is a core component of the human centrosome, crucial for mitotic spindle pole integrity (Andersen et al., 2003, Kim and Rhee, 2011). Subsequently, this patient's similarly affected younger brother was also found to be homozygous for the *CEP90* mutation. In addition, 3 other Hutterite families whose children were affected with a variety of neurological/cerebellar disorders including AOA in some and classic Joubert syndrome (JSRD) in one patient were also found to share the same mutation in *CEP90*. *CEP90* is known to physically associate with the pericentriolar satellite proteins PCM-1 and BBS4 (Kim et al., 2004). Preliminary protein interaction studies also suggest that *CEP90* interacts with TMEM237, a gene recently associated with JSRD (Huang et al., 2011). *CEP90* thus represents a new causative gene for a form of ciliopathy-related AOA.



A 03

### IMPLEMENTATION OF A CLINICALLY-COMPLIANT DIAGNOSTIC HIGH THROUGHPUT SEQUENCING PIPELINE

*R. Docking<sup>1</sup>, I. Bosdet<sup>2</sup>, R. Moore<sup>1</sup>, Y. Butterfield<sup>1</sup>, S. Chan<sup>1</sup>, L. Yang<sup>1</sup>, S. Young<sup>2</sup>, R. Coope<sup>1</sup>, R. Kirkpatrick<sup>2</sup>, M. Hirst<sup>1</sup>, A. Mungall<sup>1</sup>, Y. Zhao<sup>1</sup>, I. Birol<sup>1</sup>, R. Holt<sup>1</sup>, A. Karsan<sup>1</sup>.*

*<sup>1</sup> Canada's Michael Smith Genome Sciences Centre, Vancouver, BC; <sup>2</sup> BC Cancer Agency, Vancouver, BC*

We have implemented a rigorously QA/QC controlled and CAP-compliant diagnostic sequencing pipeline incorporating BRCA1/2 amplification and Illumina sequencing. The pipeline has the capacity to sequence thousands of samples per year with a shorter reporting turnaround than was previously achieved by our in-house capillary-based Sanger sequencing pipeline.

Target regions are amplified using a Raindance platform and bar-coded libraries are constructed. Post-PCR size selection is performed followed by sequencing on the Illumina HiSeq2000. Barcoding enables multiplexing to a high degree with each lane able to accommodate >50 samples. At this level we still ensure all target bases are covered at a minimum of 100X and at this coverage we observe no false negative calls and no false positives. All pathogenic variants are confirmed by Sanger re-sequencing prior to result reporting. Our validation data and coverage statistics are presented.

A dedicated, stand-alone clinical bioinformatic analysis pipeline has been implemented. This is based on BWA alignment and SAMtools variant calling with some customization. A report detailing any un-callable bases and the variant rates for each position is generated automatically, allowing rapid spot filling if necessary.

The cancer gene panel has been expanded from BRCA1/2 to 14 genes and this clinical implementation is underway, test data will be presented.



A 04

**CONGENITAL HEART DEFECT IN A PATIENT WITH A WT1 MUTATION: A CASE REPORT**

*R. Jobling<sup>1</sup>, M. Friedberg<sup>2</sup>, R. Grant<sup>3</sup>, S. Bowdin<sup>4</sup>,*

*<sup>1</sup> Division of Clinical and Metabolic Genetics, <sup>2</sup> Division of Cardiology, <sup>3</sup> Division of Haematology/Oncology, <sup>4</sup> Division of Clinical and Metabolic Genetis, Hospital for Sick Children, Toronto, ON*

Mutations in the WT1 gene are a known cause of multiple pathologic phenotypes; Denys-Drash Syndrome, Frasier Syndrome, WAGR and Meacham syndrome. As understanding of the role of WT1 in organogenesis has increased, it has become clear that it plays an important role in the developing heart. WT1 has been found to regulate epithelial-mesenchymal transition in the embryonic epicardium, which gives rise to multiple cell types in the heart. Animal models with absence of WT1 are known to develop severe cardiac defects. More recently human fetuses have been described with minimal or absence WT1 expression and major cardiac malformations. We present a patient with transposition of the great arteries, ventricular septal defect and urogenital abnormalities, who was diagnosed at 2 years of age with a Wilms tumor. The patient was found to be heterozygous for a WT1 mutation previously reported in patients with Wilms Tumor. We will also present our review of the cardiac defects in a cohort of patients with WT1 mutations seen at our centre. Given the recent advances in our understanding of the role of WT1 in the developing embryo, these data may represent an expansion of the phenotype of WT1 mutations.



A 05

**MISDIAGNOSIS OF MARFAN SYNDROME IN ADULT PATIENT WHO FULFILLED GHENT CRITERIA**

*S. Dyack<sup>1</sup>, A. Rideout<sup>2</sup>,*

*<sup>1</sup> IWK and Dalhousie University, Glen Haven, NS; <sup>2</sup> IWK Health Centre, Halifax, NS*

We present here an adult patient with a long standing clinical diagnosis of Marfan syndrome diagnosed with Homocystinuria due to Cystathionine Beta Synthase(CBS) deficiency at 48 years of age. He was diagnosed with Marfan syndrome after he developed ectopia lentis (EL). He developed a thrombus in his leg in his mid 20's that required anticoagulation therapy. He fulfilled the revised Ghent criteria for the clinical diagnosis of Marfan syndrome by having (EL), dilatation of his aortic root (AoD) of >4.5 cm and involvement of the skeletal system, including a reduced upper to lower segment ratio of 0.81 and increased arm span to height ratio of 1.08. He fulfilled the 2010 Ghent criteria by having EL and AoD with Z score >2. He also exhibited joint hyper mobility of hips, crowding of his teeth, striae of shoulder, mild scoliosis and arachnodactyly.

Total plasma homocystine was 265.5  $\mu$ moles/L ( range 7.1 to 17.3 $\mu$ moles/L). Methionine was 691 (range 10-50). Bone density was normal. Three previously reported mutations of the CBS gene were found: P78R, K102N and C109R. He was responsive to B6 therapy. Currently with B6 200mg BID, Betaine 3 grams BID, and attention to diet, his total plasma homocysteine is in the 20-30  $\mu$ moles/L range. Methionine is normal or near normal. After treatment, our patient's obsessive compulsive symptoms had significantly abated, by spousal report.

Patients may fulfill Ghent Diagnostic criteria and be misdiagnosed with Marfan syndrome. It is important to distinguish between Marfan syndrome and CBS deficiency as the natural history and treatment differ significantly. CBS deficiency can be associated with normal intelligence in approximately 10% of untreated individuals. It is not clear if this patient's AoD is related to CBS deficiency. In adult patients with ectopia lentis, strong consideration should be given to assessing total plasma homocysteine in the assessment of Marfan syndrome even if the patient fulfills Ghent criteria and has normal intelligence, and should always be done in this context if there is a history of clot formation.



A 06

## IT IS NEVER TOO LATE TO MAKE A DIAGNOSIS

P. MacLeod,

*Vancouver Island Health Authority, Victoria, BC;*

The patient, a retired 72 year old physician was referred for a second opinion regarding a late onset, apparently familial movement disorder. He was initially evaluated by a neurologist who initiated laboratory investigations for the common forms of spinocerebellar ataxia. These excluded SCA 1,2,3,6,and 8. The physical examination documented in a video recording, revealed signs of dysarthria, cerebellar ataxia and a pronounced tremor of head and trunk. There were no signs of a peripheral neuropathy and cognition was intact. The family history revealed that the patient's father also had a late onset movement disorder.

In addition, a paternal aunt and uncle were institutionalized for most of their lives with a diagnosis of schizophrenia. The patient was married and had two adult children. His daughter, aged 41, also has a diagnosis of schizophrenia. This family history is atypical for the hereditary forms of ataxia as well as for schizophrenia. Based on this information, a microarray was performed which revealed a 147 kb deletion at 3p26.2. This specific deletion is diagnostic of SCA 15, and results in the loss of one copy of the [ITPR1](#) gene. The protein functions as a channel that modulates intracellular calcium signaling. A PCR based methodology was used to investigate the patient's daughter who did not show this deletion. Currently there are 31 different forms of autosomal dominant ataxia listed in GeneReviews. Of these, 13 remain without a definitive molecular explanation. At least in the European population, SCA 15 is found in 8-10% of SCA panel negative families. The use of a microarray to study similar families is likely to yield additional information that will assist in the diagnosis and provision of genetic counseling. These observations might also lead to the development of a multiplex PCR technique to expand the utility of the conventional SCA panel.



A 07

**THE IMPACT OF FOLIC ACID FORTIFICATION ON THE PREVALENCE OF CONGENITAL HEART DEFECTS IN ALBERTA, CANADA**

*T. Bedard<sup>1</sup>, R. Lowry<sup>2</sup>, B. Sibbald<sup>3</sup>, J. Harder<sup>4</sup>, C. Trevenen<sup>4</sup>, V. Horobec<sup>5</sup>, J. Dyck<sup>6</sup>,*

*<sup>1</sup> Alberta Congenital Anomalies Surveillance System, Calgary, AB; <sup>2</sup> Medical Genetics, Calgary, AB; <sup>3</sup> Alberta Children's Hospital, Calgary, AB; <sup>4</sup> University of Calgary, Calgary, AB; <sup>5</sup> Alberta Health Services, Edmonton, AB; <sup>6</sup> University of Alberta, Edmonton, AB*

**OBJECTIVE:** To determine whether the introduction of folic acid fortification (FAF) in flour and pasta by the Canadian Federal Government in 1998 has had an impact on the prevalence of CHD or subgroups of CHD

**METHODS:** Data sources included the Alberta Congenital Anomalies Surveillance System (ACASS); the Pediatric Cardiology Divisions of the Alberta Children's Hospital (ACH) and the University of Alberta Hospital via the Western Canadian Children's Heart Network's extensive pediatric cardiology database; pathology reports from the ACH; and hospital records. Cases included live births, stillbirths, and terminations of pregnancies, delivered between 1995 and 2002 in Alberta diagnosed with a CHD. Chi square linear trend analyses were conducted for selected CHDs for the total study period 1995-2002. Odds ratios were calculated comparing pre FAF (1995-1997) and post FAF (1999-2002).

**RESULTS:** The total prevalence of CHD remained stable in Alberta during 1995-2002 and when pre- and post FAF periods were compared. There was a 32% increase in atrial septal defects (ASDs), and an approximately 60% increase in cases with an ASD and ventricular septal defect (VSD) in the post FAF period. Significant increases were also noted for these groups during the entire study period. There was a 24% decrease in left ventricular tract obstruction (LVOTO) in the post FAF period as well as during the entire study period. There were no significant changes for the other groups analyzed.

**CONCLUSIONS:** FAF showed no preventive effect on CHD and subgroups of CHD, with perhaps the exception of LVOTO which declined in the post FAF period. However, this has not been supported by the literature, and the significant decline was evident during the entire study period. There were significant increases of ASD and cases with both an ASD and VSD during the study period and the in post FAF period, which may reflect differences in diagnostic and ascertainment practices.



## THE CANADIAN SURVEY ON ECTOPIC NORs

C. Li<sup>1</sup>, J. Chernos<sup>2</sup>, J. McGowan-Jordan<sup>3</sup>, A. Dawson<sup>4</sup>, P. Yang<sup>5</sup>, E. Lemyre<sup>6</sup>, M. Parslow<sup>7</sup>, P. MacLeod<sup>7</sup>,

<sup>1</sup> St. Michael's Hospital, Toronto, ON; <sup>2</sup> Alberta Children's Hospital, Calgary, AB; <sup>3</sup> Hospital of Eastern Ontario, Ottawa, ON; <sup>4</sup> Diagnostic Services Manitoba, Winnipeg, MB; <sup>5</sup> Credit Valley Hospital, Mississauga, ON; <sup>6</sup> CHU Sainte-Justine, Montreal, QC; <sup>7</sup> Victoria General Hospital, Victoria, BC

Ectopic NORs, i.e., stalks and satellites present in chromosomes other than acrocentric chromosomes, is one kind of variant in human chromosomes and its clinical significance has not been well evaluated. A Canadian survey on ectopic NORs was conducted as a nation-wide collaborative work. Both clinical and laboratory information were collected on 21 cases from 14 families with ectopic NORs other than Yqs. There was one case with Xps, three cases with Xqs, one case with 2ps, two cases with 4ps, two cases with 4qs, one case with 5ps, two cases with 5qs, three cases with 8qs, two cases with 12ps and three cases with 21qs. The origin of ectopic NORs was *de novo* in four cases, maternally inherited in five cases, paternally inherited in four cases, and unknown in one case. Short stature, global developmental delay and dysmorphic features were found in all postnatal *de novo* cases. We do not have clinical follow-up information for the prenatal inherited cases, but only one of seven parents who transmitted ectopic NORs to their fetus had a clinical phenotype of immaturity with difficult social relationships. A literature review was conducted, and ectopic NORs were reported in both short and long arms of chromosomes X, Y, 1, 2, and 4, short arms of chromosomes 10, 17 and 18, and long arms of chromosomes 9, 14, 15 and 21. In addition, ectopic NORs were found interstitially in 6p22, 6q15, 7q21.3q22.1, 8q11, 11q21 and 12p11. This survey indicates that there are different clinical consequences for *de novo* versus inherited ectopic NORs, which is in agreement with the reports in the literature. Therefore, it should be mandatory to investigate the origin of the ectopic NORs. Follow-up of those cases is recommended in order to gather more information regarding the nature and consequences of this chromosomal variant.



A 09

**LATE ONSET HEXOSAMINIDASE DEFICIENCIES: ADULT PRESENTATIONS OF TAY-SACHS AND SANDHOFF DISEASE**

*P. Hall, C. Anderson, J. Hesemann, A. Studinski, L. Zetzsche, D. Gavrilov, D. Oglesbee, P. Rinaldo, S. Tortorelli, D. Matern, K. Raymond,*

*Mayo Clinic, Rochester, MN;*

**OBJECTIVE:** To present recently identified cases of adult-onset hexosaminidase deficiencies that demonstrate the variability in symptoms, and the difficulties associated with proper diagnosis.

**CASE PRESENTATIONS:** We report nine recently identified patients (by standard enzyme assay using total hexosaminidase activity (not shown in table) and % Hex A) with either late onset Tay-Sachs (6) or Sandhoff (3) disease. These cases demonstrate the variability in clinical presentation and age of onset, even within a family. Non-specific findings such as muscle weakness, cramps, fasciculations and gait disturbance were commonly described. Psychiatric findings were only described with one patient. Patient 1 was diagnosed without delay due to family history.

**CONCLUSIONS:** There is considerable clinical variation among patients with late onset hexosaminidase deficiency with regards to the age at which symptoms present, and their progression. Most patients eventually diagnosed with late onset hexosaminidase deficiencies had been symptomatic for several years before receiving a diagnosis. The identification of late onset cases reported here further support the notion that hexosaminidase enzyme activity should be included in the differential diagnosis of patients presenting with unexplained muscle weakness and gait disturbances in adulthood.



A 10

## EMT CONTRIBUTES TO CISPLATIN RESISTANCE IN OVARIAN CANCER

A. Haslehurst, M. Koti, J. Squire, P. Park, H. Feilotter,

Queen's University, Kingston, ON

The epithelial to mesenchymal transition (EMT) is a process through which a polarized epithelial cell adopts a mesenchymal phenotype. Recently this process has been implicated in drug resistance in cancer, including ovarian cancer. In North America the lifetime risk of developing ovarian cancer is 1 in 71. This disease is often accompanied by a poor prognosis due to late stage diagnosis and resistance to standard chemotherapy, such as cisplatin, resulting in a five-year survival rate of approximately twenty percent. To provide more effective treatment options for patients it is important to understand the cellular processes leading to drug resistance. Working with a cell line model of drug resistant ovarian cancer, A2780 and A2780cis, we were able to identify morphological and phenotypic hallmarks of EMT in the cisplatin-resistant cell line that were not present in the drug sensitive cell line. To determine which genes in the EMT signaling network were being dysregulated, we employed whole genome expression arrays to measure changes in gene expression between the two cell lines. The data revealed upregulation of transcription factors known to function in EMT including *snail*, *slug*, *twist2* and *zeb2*. We have demonstrated that by knocking-down the primary transcriptional regulators of EMT, *snail* and *slug*, the mesenchymal phenotype is largely reversed in the drug resistant A2780cis cells and that they are subsequently resensitized to the effects of cisplatin. Additionally, in a panel of primary human ovarian tumours, classified as either drug sensitive or drug resistant, we are able to show that genes associated with EMT are differentially regulated. We demonstrate that separation of resistant and sensitive primary tumours is possible using an unsupervised hierarchical clustering algorithm and the expression profile of a panel of selected EMT genes. This work strongly suggests that genes associated with EMT may play a significant role in primary cisplatin resistance.



A 11

**IMPLEMENTATION OF NEXT-GENERATION SEQUENCING IN THE CLINICAL DIAGNOSTIC LABORATORY: A QUALITY APPROACH**

*T. Stockley, P. Ray,*

*Hospital for Sick Children, Toronto, ON;*

Next-generation or massively parallel sequencing (MPS) is an emergent, rapidly evolving technology which allows extensive genetic sequence analysis, with great promise for genetic diagnostics. However implementation of MPS in the clinical diagnostic laboratory setting presents many challenges, due to both 'content' issues related to uniqueness of MPS methodology in comparison to Sanger methods, and 'process' issues in how to implement complex MPS methods in an accredited diagnostic laboratory and return meaningful information from MPS to clinicians and their patients. Our clinical laboratory is currently implementing MPS into the clinical laboratory setting, using a Quality Improvement framework to manage both content and process issues.

**OBJECTIVE:** The goal of this project is to use Quality Improvement framework approach encompassing both content and process assessment and control to ensure successful implementation of massively parallel sequencing in the clinical molecular diagnostic laboratory setting.

**METHODS:** A Quality Improvement framework for clinical laboratory MPS integration was designed to include both 1) Content and 2) Process issues. Content issues included assessing availability and clinical laboratory value of current standards, protocols and guidelines relevant to diagnostics. Content issues were assessed along the entire MPS process, from pre-test consultations and sample intake, to laboratory technical applications, and to post-test reporting to clinicians. Process issues were addressed by use of quality improvement methods, including continuous improvement and cycle of learning, to manage integration of MPS into clinical diagnostic services from intake to reporting.

**RESULTS AND CONCLUSIONS:** Despite promises of MPS for clinical diagnostic use, there are significant gaps in available tools to aid in uptake of MPS for clinical diagnostics. We will present our Quality Improvement approaches for MPS integration into clinical diagnostics.



A 12

**DEVELOPMENT OF A COMPREHENSIVE METHODOLOGY FOR EVIDENCE-BASED EVALUATION OF GENETIC TESTS**

*D. Allingham-Hawkins, A. Lea, E. Houtsmuller, M. McLaughlin, T. Rogstad, S. Levine,*

*Hayes, Inc., Lansdale, PA, USA*

The importance of evidence-based evaluation of genetic tests has received greater attention in recent years.

This has been fueled partially by the explosion in the number of available tests and partially by the realization that the evidence behind many tests is limited. Unfortunately, universally accepted methodology for evaluating genetic tests has not yet been developed. Hayes, Inc., a private healthcare consulting and research company with more than 20 years experience in health technology assessment, launched its genetic test evaluation (GTE) program in early 2008 and has evaluated more than 150 genetic and genomic tests to date. Hayes has developed a detailed and transparent methodology for evaluating genetic tests that combines elements of the ACCE (Analytical validity, Clinical validity, Clinical validity, Ethical, legal and social implications) model together with key elements of the internationally developed GRADE (Grading of Recommendations Assessment, Development and Evaluation) recommendations. Elements of the EGAPP (Evaluation of Genomic Applications in Practice and Prevention) methodology have also been incorporated. The Hayes GTE methodology uses a checklist to grade the quality of individual studies based on study design, outcome measures and study limitations and potential biases. A "toolbox" has then been developed to assist with the evaluation of the overall body of evidence for a test. The toolbox includes detailed guidelines for the elements to consider when evaluating the quality of the body of evidence, using the GRADE recommendations. In addition, special considerations for different categories (monogenic, polygenic and pharmacogenetic) of genetic tests are detailed. Other tools include a flowchart to assess the need for evidence of clinical utility and a decision analysis tool that can be applied when sufficient information is available. The final step is assigning a rating that reflects the strength and direction of the overall body of evidence for the use of that test. Details of the methodology will be presented along, using specific examples to illustrate the evaluation process.



A 13

## INVESTIGATING THE HIGH INCIDENCE OF SANDHOFF DISEASE IN SASKATCHEWAN

*B. Fitterer<sup>1</sup>, P. Hall<sup>2</sup>, N. Antonishyn<sup>1</sup>, M. Gelb<sup>3</sup>, D. Lehotay<sup>1</sup>,*

*<sup>1</sup>Saskatchewan Disease Control Laboratory, Regina, SK; <sup>2</sup>Mayo Clinic, Rochester, MN, USA ; <sup>3</sup>University of Washington, Seattle, WA, USA;*

**OBJECTIVES:** To identify *HEXB* variants present in the northern Saskatchewan population, develop methods to screen for those variants, and determine the carrier frequency among the high incidence communities.

**METHODS:** Sanger sequencing was used to examine the exons and flanking intronic regions of the *HEXB* gene from the most recent patient born with Sandhoff Disease. Real-time PCR and MS/MS-based assays were developed to screen for a novel variant and aberrant hexosaminidase activity. The two assays were then compared in a retrospective study of the northern population using residual dried blood spots.

**RESULTS:** Two novel pathogenic *HEXB* variants were identified in patients previously diagnosed with Sandhoff Disease in Saskatchewan. These variants include a single nucleotide deletion in the coding region of exon one and a missense mutation resulting in the loss of a disulfide bond-forming cysteine residue. The deletion was present in four confirmed Sandhoff Disease cases whereas the missense mutation was only found in one patient. As a result of the method comparison other variants were identified with unknown pathogenicity.

The MS/MS-based assay proved to be effective for detecting the infantile form of Sandhoff Disease but unreliable for detecting carriers. The PCR assay, specific for the single nucleotide deletion, accurately detected both homozygous and heterozygous individuals. Upon screening the northern community with the PCR assay, ~1:28 individuals were found to carry the deletion. Carrier status was confirmed by genetic sequencing.

**CONCLUSIONS:** Multiple *HEXB* variants are responsible for Sandhoff Disease in northern Saskatchewan. Special consideration for mitigating the disease in these communities is warranted based on the high carrier frequency. These assays can provide diagnosis and confirmation of Sandhoff Disease as well as the opportunity for prenatal testing.



A14

**A CASE REPORT OF HEMOPHILIA A CAUSED BY A COMPLEX REARRANGEMENT INVOLVING CHROMOSOME X**

*J. Liu, B. Argiropoulos, R. Lamont, R. Perrier, J. Chernos,*

*Alberta Children's Hospital, Calgary, AB*

We report a male patient with hemophilia A and trigonocephaly caused by a cryptic complex rearrangement involving chromosomes X and 1. Southern blot analysis did not identify the common intron 22 inversion which accounts for approximately 48% of hemophilia A patients. The result did show an atypical pattern suggesting a duplication of intron 22 that could be a possible benign variant. Due to the additional clinical features, parallel array CGH testing was performed. This revealed a complex rearrangement involving 3 copy number gains. Two gains were identified on the X chromosome at band q28. The proximal one spanned a 53 kb region from nucleotide 153726875 to 153816719 (NCBI36/hg18) and encompassed exons 14 – 25 of factor 8 (F8) gene. The second gain was more distal and spanned a 219 kb region from nucleotide 153904147 to 154124050. These two gains are separated by a 69 kb region of normal copy number. Both gains were independently verified. The third gain represents a 450 kb region within the short arm of chromosome 1 at p13.1 from nucleotide 116948676 to 117399493. For independent verification, FISH was performed and revealed that the gain of 1p13.1 was inserted in the distal long arm of the X chromosome at Xq28. However, it remains unclear where precisely the insertion location is and how it is related to the two gains described above. Further studies to address this are underway.

Therefore, this patient has a complex rearrangement involving chromosomes X and 1. In light of the patient's diagnosis of hemophilia, this complex rearrangement is most likely perturbing F8 gene structure and function. RefSeq genes of the distal gain on Xq28 and the gain on 1p13.1 are not associated with an OMIM disease. Thus, their contribution to the patient's phenotype is of unknown clinical significance at the present time.



**Prairie Provinces Session Abstract 1**

**SEARCHING FOR PARKINSON'S DISEASE CAUSE: A GENETIC STUDY**

*A. H. Rajput*

Parkinson's disease (PD) is characterized by substantia nigra (SN) neuronal loss and Lewy body (LB) inclusions. It is the second most common neurodegenerative disorder. PD is sporadic in most, but approximately 10% cases have a genetic basis.

Mean age of PD clinical onset is 62 years and age specific incidence increases progressively.

Search for environmental cause has been a difficult, due to long preclinical interval, and numerous possible causal candidates.

Genetic studies have identified familial aggregations with autosomal dominant or recessive patterns. Some such studies did not report on brain histology, while others did not detect LB inclusions. In some families with LB inclusions the age of onset was early and disease progression accelerated compared to sporadic PD cases.

A Saskatchewan Mennonite family had PD in 3 generations. The clinical picture and pathology was similar to sporadic cases. DNA samples including 4 brains from 57 members were analyzed by M. Farrer's laboratory at UBC. All known genetic causes of parkinsonism (2012) were excluded. Exome sequencing was performed and a novel mutation of DNAJC13 was identified. These indicate critical role of neuronal vesicular dynamics in PD.



**Prairie Provinces session Abstract 2**

**TINKERING WITH GENES**

*P. Hull*

Geneticists have mostly been involved in gene identification. Attempts to modify gene function is now a reality. The genetic background to two dermatological disorders as well as current and proposed interventions will be discussed.

Atopic dermatitis is associated with loss of function mutations in the gene *Filaggrin*. The protein product is crucial to normal barrier function of the skin, preventing access by allergens and water loss. Decreased filaggrin in the skin has been conclusively tied to the well-known atopic march with the progression from atopic dermatitis to hayfever and asthma. Severe atopic dermatitis persisting into adulthood has been associated with the homozygous state.

To date all loss of function mutations have been associated with mutations giving rise to stop codons. Drugs have been developed that are able to effectively read through these weak stop codons resulting in restoration of filaggrin expression in vitro. These drugs are now progressing through toxicology and will be in clinical trials within the next few years.

Pachyonychia congenita is a rare keratin disorder associated with a debilitating plantar keratoderma. Several treatments aimed at genetic modification have reached clinical trials. These have included gene silencing with specific SiRNA, gene modulation with rapamycin and gene promoter suppression using statins.



**Prairie Provinces Session Abstract 3**

**NEWBORN SCREENING AND BIOCHEMICAL GENETICS IN SASKATCHEWAN**

*D. C. Lehotay*

Both Biochemical Genetics and Newborn Screening (NBS) started in Saskatchewan in the 1970's with province-wide screening of all newborns with tests being offered for PKU, and congenital Hypothyroidism. The NBS screening program expanded to include close to ~ 30 new tests by tandem MS in 2000, one of the first such initiatives in Canada.

I will review the work and findings of both Biochemical Genetics and NBS laboratories, and describe some of the ways in which the diagnosis of inborn errors of metabolism (IEM) has been impacted by the introduction of tandem MS.

We will discuss some of the features that are unique to Saskatchewan, and characterize the patient population of this province. We will show the incidence, frequency and type of IEM's in SK as determined by the Newborn Screening Program and the Biochemical Genetics laboratories. We will also show quality indicators, turnaround time for NBS, and how diagnosis of IEM has changed as a consequence of expanded NBS.

We will review our findings regarding the incidence and diagnosis of HHH Syndrome, as well as Sandhoff's disease within the Saskatchewan population by both mutation analysis and by the measurement of molecules that are characteristically elevated in these diseases. We will also show the correlation between genetic and biochemical methods of analysis for the diagnosis of these two diseases. MCAD is one of the most common fatty acid oxidation defects. While the incidence of this disease in SK is lower than other provinces, we were able show that while there is no correlation between genotype and clinical phenotype in this disease, there appears to be a correlation between the biochemical phenotype and genotype in MCAD deficiency.

One of the major developments of the last fifteen years in the diagnosis of IEM has been the introduction of expanded NBS. I will review some of the ways in which the findings of such programs around the world has impacted our understanding of IEM.



**Prairie Provinces Session Abstract 4**

**PARTNERSHIPS FOR HEALTH RESEARCH IN ABORIGINAL COMMUNITIES IN NORTHERN SASKATCHEWAN**

*J. Irvine*

Engaging families and communities is important in many situations when developing and initiating genetic research initiatives. This is especially evident when approaching research with First Nations, Metis and Inuit communities as there is a historical sense of mistrust of scientific research in many Aboriginal communities. Several long-standing research partnerships exist with various northern Saskatchewan Aboriginal communities for various genetic conditions. The building of trust relationships between investigators and communities has been key to these partnerships and the development of research collaborative agreements has assisted in strengthening these relationships. Principles include the respective inclusion of community leadership in providing direction of the initiative, the negotiation of community consent over and above individual consent, the consideration of community benefit and risk, and the agreement on roles and expectations in advance.