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BOOK OF ABSTRACTS

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A 01

CROSSTALK BETWEEN HISTONE MODIFICATIONS AND DNA METHYLATION IN PATIENTS WITH INTELLECTUAL DISABILITY DUE TO JARID1C MUTATIONS

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The X-linked gene, *JARID1C*, encodes a H3K4 demethylase. Mutations in this gene cause intellectual disability (ID). We hypothesized that *JARID1C* mutations would dysregulate DNA methylation at specific genomic targets.

Method: A genome-wide approach was used to analyze sodium bisulfite modified genomic DNA from white blood cells of patients with known *JARID1C* mutations (n=13). The Illumina Methylation 27 Microarray with probes for 27,578 CpG sites covering >14,000 genes was used. DNA methylation profiles of patients were compared to sex- and age- matched controls. Differentially methylated CpG sites were identified using the Mann-Whitney test (absolute methylation difference >17% and p-value cut-off <0.05) with correction for multiple testing.

Results: Differential methylation analysis identified 17 genes with loss of CpG methylation. For 5 genes demonstrating the most significant loss of methylation in patients, the array findings were validated by pyrosequencing. Bioinformatic analyses showed that the DNA methylation alterations co-localized with the expected types of histone modifications in the target genes. CHIP-qPCR and expression array analyses, using lymphoblastoid cell lines from the same patients, are in progress.

Conclusion: Genes that function in epigenetic regulation play an important role in normal neurodevelopment. However, the molecular mechanisms by which multiple hierarchical epigenetic marks drive normal development are not well understood and the critical genomic targets are largely unknown. Therefore, disorders caused by mutations in genes that apply or remove epigenetic marks at specific genomic targets provide a unique opportunity to study the pathophysiology of epigenetic dysregulation in human disease. Our study has identified a specific pattern of DNA methylation alterations in patients with *JARID1C* mutations. These data demonstrate the functional specificity of these epigenetic regulators and also the cross-talk that occurs between histone modifications and DNA methylation in chromatin-mediated transcriptional regulation.

A 02

HOW DO YOU KNOW YOUR GENETIC TEST RESULTS ARE CORRECT? EQA SCHEME RESULTS SHOW THE NEED FOR CONTINUED QUALITY IMPROVEMENT IN LABORATORY DIAGNOSTICS

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Objective: Studies of the reliability of molecular genetic testing have indicated a significant level of inaccuracy in laboratory reports, arising from errors in sample identification, genotyping or interpretation. The European Molecular Genetics Quality Network (EMQN) aims to raise and maintain the quality of Diagnostic Clinical Molecular Genetics Testing by providing international External Quality Assessment (laboratory Proficiency Testing) schemes.

Methods used: In 2009 EMQN provided 25 disease specific and 3 technique specific EQA schemes for over 400 laboratories in 42 countries. The scheme participants received DNA samples with mock clinical referrals and the participating laboratories were asked to perform their routine analysis and interpret the results. The laboratories' reports were marked by a group of experts. The participants received a report on their own performance, which they could compare to that of their peers.

Results obtained: Over 1500 reports were evaluated from laboratories. The standards of genotyping accuracy were high but significant error rates were found and methods of reporting and interpreting data were varied. The overall genotyping error rate was 5.5% across all the schemes with numbers fluctuating greatly between the individual schemes.

Conculsion: The 5.5% error rate found in the 2009 EQA round indicates a clear need for interlaboratory comparisons to measure current standards of lab testing proficiency and to encourage laboratories to raise their technical and reporting performance.

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A 03

IDENTIFICATION OF PATHOGENIC HEXOSAMINIDASE A VARIANTS IN NON ASHKENAZI JEWISH POPULATIONS

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Objective: To characterize at the molecular level Hexosaminidase A variants in clinically identified Tay-Sachs carriers from diverse ethnic backgrounds

Methods: Sequencing of the fourteen exons and flanking intronic regions of the HEXA gene was performed for Tay-Sachs carriers identified through positive biochemical testing

Results: Among the four Tay-Sachs carriers from different ethnic backgrounds (Nova Scotia, French-Canadian (Quebec), and Sephardic Jew) identified through biochemical testing, sequencing of the entire HEXA coding region permitted to detect two novel variants. One of these, a nonsense variant, is predicted to be disease causing and was ascertained in a carrier with a positive family history. The other variant characterized is located within an intronic region and is predicted to affect splicing. This latter variant was identified in two sibs with unambiguously positive biochemical Tay-Sachs carrier testing results. Neither sib was carrier of an Hexosaminidase A pseudo-deficiency allele.
HEXA sequencing of the fourth sample did not permit to uncover a novel or potentially pathogenic HEXA variant. This fourth sample was obtained from a patient with a “grey zone” Hexosaminidase A enzymatic result.

Conclusion: Sequencing of the entire HEXA coding region is a cost effective and efficient procedure for molecular diagnosis of non-Ashkenazi Tay-Sachs carriers who are more likely to present with rare variants. This technique offers biochemically identified Tay-Sachs carrier couples access to a complementary and reliable prenatal diagnosis testing method.

A 04

MILD PRESENTATION OF SMITH-MAGENIS SYNDROME IN A MOSAIC FEMALE WITH DELETION OF 17p11.2 AND DUPLICATION OF 17q22-q24

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Chromosome 17 has numerous segmental duplications and repeat elements along both the short and long arms that make it susceptible to chromosomal recombination. For example, the region of 17p11.2p12 is associated with Smith-Magenis syndrome (SMS; OMIM #182290) when the deleted region involves the retinoic acid-induced 1 (*RAI1*) gene and hereditary neuropathy with liability to pressure palsies (HNPP; OMIM #162500) when the peripheral myelin protein -22 (*PMP22*) gene is deleted. Similarly, there have been numerous reports of unbalanced translocations involving duplication of the region of 17q21 to the terminus. These clinical entities have all been described with specific phenotypes such as craniofacial anomalies, behavioural disturbances, variable mental retardation and brachydactyly in SMS, muscle weakness and peripheral neuropathy in HNPP and growth impairment, profound mental retardation, hyperlaxity of the limbs and craniofacial abnormalities in distal duplication 17q.

We report a female with a *de novo* chromosome 17 derivative with a 7.4 Mb deletion of chromosome region 17p11.2 to 17p12 as well as a duplication of 12.35 Mb region at 17q22 to 17q24, who was referred for genetics evaluation for developmental delay, peripheral neuropathy, brachydactyly and dysmorphic features. The derivative was present in approximately 12% (3/50 cells) of peripheral blood lymphocytes based on FISH studies and was initially detected by comparative genomic hybridization microarray. To our knowledge, this is the fourth confirmed case of mosaicism involving deletion of 17p11.2 region and the lowest level of mosaicism reported in lymphocytes presenting with features consistent with the SMS phenotype.

A 05

AFFYMETRIX 2.7M HIGH-RESOLUTION GENOME-WIDE SNP ARRAY IN CLINICAL PRACTICE: PRELIMINARY EXPERIENCE

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Pathogenic copy number variants (CNVs) are found in 5-15% of individuals with intellectual disability (ID) using different array CGH platforms. It is expected that arrays with higher resolution genomic coverage detect CNVs more accurately and allow identification of smaller CNVs. We applied Affymetrix Cytogenetics Whole-Genome 2.7M Array to assess CNV detection in 33 ID cases that contain 42 pathogenic CNVs (ranging from 45 Kb - 5.4 Mb) previously detected and confirmed by other array platforms and/or FISH or Q-PCR. 41/42 positive CNVs were detected with 85% confidence level when CNV size cut-off was set to 100 Kb. However, the detection rate dropped to 60% of positive CNVs (25/42) when the confidence level was increased to 90%.

When no CNV size or confidence level restrictions were applied (confidence 0, segment size 0 Kb), 475 CNV were detected (18 in average/subject, range 6-80). The CNVs on chromosome Y were not counted due to the noisy data on these chromosomes. 21% of CNVs were >100 Kb, 67% were 10~100 Kb while 12% CNVs <10 Kb. More than half of the CNVs within each size group were benign (i.e. common variants reported in at least two studies in the Database of Genomic Variants (DGV), or had no gene content). The proportion of benign CNVs was highest for the <10 Kb CNV size group (70%). In addition to the known positive CNVs, we identified 24 unique CNVs that were not detected previously when a size cut-off of 100 Kb and confidence of 85% were applied. They all contain gene(s) and do not overlap with common CNVs from the DGV, therefore they could have clinical relevance. These CNVs are being followed up by FISH. In light of the recent observation that 9-24% of cases with specific recurrent microdeletions/duplications have a second putatively pathogenic CNV ("two-hit" model Girirajan et al., Nature Genetics 2010), and the validation of the 6.0 Affy array which showed additional pathogenic findings in 3/19 cases with one known pathogenic CNVs detected by lower resolution array (Bernandini et al., EJHG, 2010), we anticipate that high resolution Affy 2.7M Array could reveal further clinically relevant CNVs in patients with previously identified aberrations.

A 06

CLINICAL VARIABILITY AND NOVEL MUTATION IN PATIENT WITH FARBER DISEASE

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Background: Farber disease (MIM 228000) is a rare autosomal recessive condition caused by deficiency of lysosomal acid ceramidase (EC 3.5.1.23). The disease presents classically during the infantile period with a characteristic triad of clinical manifestations: (a) painful joints, (b) subcutaneous nodules, and (c) progressive hoarseness due to laryngeal involvement. All the reported cases in literature had the above features except for neonatal visceral subtype.

Methods: We describe a 2 years old female, product of a non-consanguineous Emirati union, who was quite well till 8 months of age when started to present with failure to thrive, developmental delay with relative sparing of cognitive function, cherry-red spot, painful joint, progressive limitation of joint movement, and hoarseness of voice. She had a sibling, who died with a similar presentation and the nerve biopsy of deceased sibling showed features consistent with Farber disease.

Results: Gene sequencing of *ASAHI* gene analysis confirmed the diagnosis of Farber disease. This patient has two heterozygenous novel mutations; the first one in exon 8 (c.533 T> C) and the other mutation in exon 13 (c.1144A>C). The carrier status of parent has been confirmed. The mother carry the first mutation (c.533 T>C) While the father carry the second mutation (c.1144A>C)

Conclusion: Farber disease is well known for its striking unique triad. However, this patient did not present with subcutaneous nodules, which is one of the distinctive feature of Farber disease.

A 07

TEAM BASED LEARNING IN UNDERGRADUATE GENETIC EDUCATION: A PILOT

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Objective: The goal of innovation in medical education is to encourage higher quality (deeper) learning by focusing on self directed and collaborative approaches using material that has relevance to clinical practice. We describe the pilot of Team Based Learning (TBL) as part of an integrated genetics curriculum and report initial feedback from students.

Method: In 2002, the ICE (ideas, connections and extensions) model was used to develop a set of online cases students worked on in web based chat groups as they received lectures on the relevant material. Further to this, in 2009, a TBL unit was piloted replacing 3 of the 9 remaining lectures. This process included a readiness assessment test, authentic online cases, and web-based genetics resources linked to large classroom sessions built around interactive team exercises. This material was designed and delivered by an interprofessional team of genetics and education experts.

Results: In order to evaluate this innovation we surveyed the class with respect to their preference of learning activity, satisfaction with each activity, clarity of material and required preparation time. General information was also gathered such as previous genetic education, and the use of online resources. The results revealed a range of student acceptance of the methodology which needs to be considered as small group sessions are introduced in favor of a lecture based curriculum. Instructors were impressed by the level of student preparation and in class discussion in the TBL and case presentation sessions and noted the ability to identify and correct student misapprehensions which became apparent during the interactive sessions.

Conclusions: Although these initial results will help inform further development of educational modules, the challenge for future studies will be to explore whether these methods actually result in deeper learning by students, improved knowledge retention and enhanced patient outcomes.

A 08

COMPREHENSIVE GENETIC AND PHENOTYPIC CHARACTERIZATION OF SEVERE OBESITY DISORDERS

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Objective: To discover new genes and pathways involved in human body weight regulation through study of previously uncharacterized rare obesity and lipodystrophy syndromes.

Methods: Subjects are recruited by self-or physician referral and must have either 1) abnormal distribution of body fat (lipodystrophy) or 2) obesity (BM! SDS~3) and ~l of a) mild-moderate intellectual disability; b) ~l major congenital anomalies; c) ~ 2 minor anomalies affecting different systems; d) food-related behavioural abnormalities (hyperphagia, food theft, hoarding, etc.). Detailed genotyping for pathogenic copy number variants begins with Affymetrix 6.0 SNP microarray, or whole-exome sequencing in selected families. Detailed phenotyping includes plasma glucose, insulin, leptin, adiponectin, acylghrelin and desacylghrelin. Total body fat mass is measured via DEXA scan and total fat volume via MRI (Dixon method, allowing the separation of fat signal from water). The combination of fat mass and fat distribution allows calculation of visceral fat mass, a predictor of metabolic risk that is superior to BMJ. Candidate genes are validated using gene expression studies in cohorts of mice exposed to different nutritional states, and specific functional studies as appropriate.

Results: To date. out of a cohort of 22 eligible families, we have found 3 microarray abnormalities (13.6%), consistent with data from other studies of microarray analysis in intellectual disability and congenital malformations. Phenotypic data and data on validation of promising candidate genes will be presented.

Conclusions: Study of families with intellectual disability, birth defects and additional phenotypes such as obesity, hyperphagia and lipodystrophy may be a useful technique to identify major genetic risk factors for these body weight phenotypes. Detailed phenotypic analysis of the affected subjects is required, alongside other *ex vivo* and *in vitro* evidence to validate these candidate genes for their involvement in diseases such as obesity and type 2 diabetes.

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A 09

DE NOVO 10q21.1-q21.3 DELETION IN A CHILD WITH HYPOGLYCEMIA, RENAL TUBULAR ACIDOSIS AND GROWTH HORMONE DEFICIENCY

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Microarray technology has been used increasingly in the investigation of cases with unexplained developmental delay, autism spectrum disorders and multiple congenital anomalies and provides a diagnosis in 15-20% of these group of patients, compared to 3% in using routine chromosome analysis. We present a patient with multiple abnormalities who has been investigated thoroughly since birth and was recently found to have a deletion at 10q21.1-21.3 on microarray analysis.

The patient is a 15-year-old female with a history of autoimmune hepatitis, neonatal hypoglycemia, renal tubular acidosis, growth hormone deficiency and morbid obesity with mild dysmorphic features. Microarray analysis revealed a previously not reported *de novo* deletion of 10q21.1-10q21.3. Thirteen known genes are contained within this region, many of which have an unknown function. Genes of interest include *ZNF365*, a susceptibility locus for nephrolithiasis and *EGR2*, which has been observed in congenital hypomyelinating neuropathy, Dejerine-Sottas neuropathy and Charcot-Marie Tooth disease type 1D. Also contained in part in that region is a candidate tumour suppressor gene, *RHOBTB1*, which has been identified in head and neck cancers. Our patient currently does not have clinical symptoms of neuropathy or neoplasm although it remains unclear if she will be at risk in later life to develop these conditions, as many of these disorders present in adulthood. This case highlights the difficulties in providing anticipatory guidance and counseling regarding late onset conditions such as neuropathy and risk for malignancy detected through microarray analysis especially when the patient is developmentally challenged.

A 10

IDENTIFICATION OF A HOMOZYGOUS *STAR* MUTATION IN FOUR MEMBERS OF TWO NEWFOUNDLAND FAMILIES WITH FAMILIAL GLUCOCORTICOID DEFICIENCY

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Objective: To identify the gene and mutation(s) responsible for Familial Glucocorticoid Deficiency (FGD) in affected members of two Newfoundland (NL) families.

FGD is an autosomal recessive disorder caused by resistance to the action of ACTH on the adrenal cortex. Affected individuals develop hyperpigmentation, hypoglycemia, seizures and shock often secondary to stress such as infection or accident.

Patients and Methods: In the 1970-80s four NL children (a brother and sister in one family and two brothers in an apparently unrelated family) were diagnosed with 'Addison's Disease' in childhood (3-8 years of age). Each presented with hyperpigmentation and severe hypoglycemia following a flu-like illness. Subsequent investigations indicated low cortisol, increased ACTH but normal renin and aldosterone levels, and the diagnosis for each child was refined to FGD. Two candidate genes, the ACTH receptor gene (*MC2R*) identified in 1993, and the melanocortin 2 receptor accessory protein gene (*MRAP*) identified in 2005 were sequenced and no mutations were identified. Recently a third candidate gene on chr 8, the steroidogenic acute regulatory protein gene, *STAR*, was identified and sequenced.

Results: The same homozygous mutation in *STAR* (R188C) was identified in all four affected individuals in the NL families. An uncle in one NL family with a very late diagnosis of FGD at age 58 was also homozygous for the mutation. In a larger cohort of FGD patients this mutation, R188C, was homozygous in four affected members of two other families, and the mutation R192C was present in four affected members of a fourth family.

Conclusion: Mutations in *STAR* have previously been identified in patients with Lipoid Congenital Adrenal Hyperplasia with an earlier age at diagnosis and both gonadal and adrenal steroid deficiency. A subset of mutations of *STAR* are apparently responsible for FGD3 with adrenal cortical deficiency only and a variable age at onset.

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A 11

USING HOMOZYGOSITY MAPPING IN CONSANGUINEOUS FAMILIES TO GUIDE DAIGNOSTICS OF HETEROGENEOUS DISORDERS: A PILOT PROJECT

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Within the diagnostic setting, genetically heterogeneous disorders pose a distinct challenge as how to prioritize genes for mutational analysis. Currently, detailed phenotyping may be able to prioritize genes, although this is not helpful for many disorders, is time consuming, and may not be cost effective, provided funding is available at all. Further, in families where the previously known molecular causes have been ruled out, additional candidate genes may not be known or reside in a complex pathway comprised of many members. Here, we show the application of homozygosity mapping by SNP microarray in consanguineous families to guide mutation screening in heterogeneous autosomal recessive disorders as a pilot project at the Alberta Children's Hospital. As a proof of principle case, we have identified the mutation responsible for Leigh disease in three affected siblings of Hutterite descent. Leigh disease is a mitochondrial respiratory chain disorder resulting from mutations in more than 28 genes to date. Mitochondrial-encoded gene investigations did not reveal any mutations leaving at least 15 nuclear-encoded genes as potential candidates. Homozygosity mapping by SNP microarray in two of the three affected family members led us to identify a homozygous insertion at position c.393 in NDUSF4 (p.E132RfsX15), a known Leigh disease gene. Thus, by using homozygosity mapping by SNP microarray in two affected individuals we were able to reduce the number of candidate genes from at least 15 to two and identified the disease causing mutation in the first candidate sequenced. We also present additional families where the principle of `clinical' homozygosity mapping has been used and a cost-benefit analysis of the cases examined to date.

A 12

A FAMILY WITH MICRODELETION AT 2q14 ASSOCIATED WITH IUGR, OFC AT THE 2ND%, COGNITIVE IMPAIRMENT AND HYPERCOAGULABILITY

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We describe a family where a mother and two of her sons presented with severe cognitive difficulties and head circumferences at or below the 2nd% in the context of a novel microdeletion at 2q14. In addition, the two affected brothers were born with intra-uterine growth restriction and required orthopedic treatment for tight Achille's tendon. The mother had a history of hypercoagulability with multiple thrombotic events requiring the initiation of long-term warfarin prior to her pregnancy with the proband. Three full-brothers without the microdeletion had normal head circumferences and did not have severe cognitive deficits.

Microarray (Baylor) showed a maternally inherited 2 megabase deletion at 2q14.3. The deletion was confirmed by FISH and other siblings were studied using the same FISH probe.

This 2q14 microdeletion identified in this family includes the BIN1 and PROC genes. Haploinsufficiency of PROC causes protein C deficiency thereby explaining the hypercoagulability in this family. BIN1 is a highly conserved gene in mammalian and alternative splicing leads to the expression of 2 isoforms: amphiphysin 2, primarily expressed in muscle, and amphiphysin 2a (amph2a) which is mainly expressed in brain (particularly at nodes of Ranvier). Amph2a forms heterodimers with Amph1 forming a complex which is required in the endocytic recycling pathway and is evolutionarily conserved from worms to mammals. Knock-out mouse for amph1 show cognitive deficits and a higher rate to sudden death due to paroxysmal seizures. Our findings suggest that haploinsufiency of BIN1 may lead to dosage imbalance between amph2a and amph1 and explain our patients' cognitive deficits and microcephaly.

A 13

ARRAY CGH IN REPRODUCTIVE GENETICS

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Reports describing application of array-CGH to study copy number variations (CNVs) in miscarriages have been limited to ~1000 miscarriages worldwide. These initial studies indicate that cytogenetically invisible CNVs exist in 1-13% of miscarriages. However, the origin of the CNVs (*de novo* or inherited), their presence in unrelated controls, or the exact size of the CNVs and gene content remain largely unknown. In addition, detailed obstetrical histories for the couples and pathology findings for the miscarriages were usually not provided.

Using a 0.01Mb resolution array (Agilent) we investigated the possibility that putatively pathogenic CNVs exist in 26 miscarriages with normal karyotypes from couples with idiopathic recurrent pregnancy loss-RPL (Cohort 1) and in 17 sporadic miscarriages with morphological developmental abnormalities as described by embryoscopy (Cohort 2).
In Cohort 1, eleven unique (previously not described) miscarriage CNV were detected and were all parental in origin. In Cohort 2 the unique CNVs were also predominantly parental in origin; only 1/6 unique CNVs was de novo. All unique CNVs were in the 250kb range or smaller. Although our study suggests that the frequency and size of whole-genome array detected *de novo* CNVs in sporadic and recurrent chromosomally normal miscarriages appears to be smaller than those identified in subjects with postnatal developmental defects, several interesting candidate genes were identified. For example, the maternal origin of two CNVs in Cohort 1 was of interest as they involved the imprinted genes *TIMP2* and *CTNNA3,* which are only normally expressed from the maternal copy in the placenta. Screening for these CNVs in additional females with RPL using qPCR detected an additional carrier of the *CTNNA3* containing CNV. In Cohort 2, the recurrence of CNVs containing genes belonging to syntaxin family, which have a role in endosome transport and fusion, is of interest as the dysfunction of these genes may lead to cell death. Our preliminary study shows that array-CGH is useful for detecting and cataloguing CNVs in miscarriages and couples with RPL and suggests that investigating further the involvement of CNVs, particularly those containing genes that are imprinted in placenta (eg *TIMP* and *CTNNA3*), in women with recurrent pregnancy loss would be worthwhile.