Presenter's Name: Foroutan, Aidin

Additional Author(s): Haghshenas S, Bhai P, Levy MA, Kerkhof J, McConkey H, Niceta M, Ciolfi A, Pedace L, Miele E, Genevieve D, Heide S, Alders M, Zampino G, Merla G, Fradin M, Bieth E, Bonneau D, Dieterich K, Fergelot P, Schaefer E, Faivre L, Vitobello A, Maitz S, Fischetto R, Gervasini C, Piccione M, van de Laar I, Tartaglia M, Sadikovic B, Lebre AS

Abstract Title: Clinical Utility of a Unique Genome-Wide DNA Methylation Signature for KMT2A-Related Syndrome

Abstract:

Background: Wiedemann-Steiner syndrome (WDSTS) has been described as a syndromic condition in which intellectual disability (ID) is associated with hypertrichosis cubiti, short stature, and characteristic facies. WDSTS is caused by pathogenic variants in KMT2A gene, which encodes a histone H3K4 methyltransferase enzyme that regulates chromatin mediated transcription. Interpretation and classification of rare KMT2A variants can be challenging. A genome-wide DNA methylation episignature for KMT2A-related syndrome could allow functional classification of variants and provide insights into the pathophysiology of WDSTS.

Methods: Genome-wide DNA methylation of peripheral blood from a cohort of 60 individuals was performed using the Illumina EPIC arrays. From 60 patients, 56 carried KMT2A intragenic variants (missense, nonsense, indel or splice site changes, including variants of uncertain significance (VUS)) and four patients had only a clinical diagnosis of WDSTS or Kabuki syndrome. From 60 patients, 41 with pathogenic KMT2A variants were used as training case samples for finding the significant differentially methylated probes (DMPs) and the remaining 19 were later mapped to the discovered methylation signature. DMPs were identified using R, comparing the 41 training cases with 82 age and sex matched controls from our EpiSign Knowledge Database (EKD). The robustness and sensitivity of the selected DMPs were tested using unsupervised machine learning algorithms, including hierarchical clustering and multidimensional scaling, as well as 41 rounds of leave-1-out cross validation. To further check the sensitivity and specificity of the selected DMPs, a supervised support vector machine (SVM) classifier was constructed to classify the WDSTS episignature distinct from 38 other neurodevelopmental disorders and congenital anomalies (ND/CAs) available in our EKD.

Results: The changes in the methylation status driven by KMT2A pathogenic variants involve global reduction of methylation in various genes. A support vector machine classifier was developed enabling classification of VUS in KMT2A gene. KMT2A episignature also enabled confirmation of diagnosis in patients with clinical presentation of WDSTS or Kabuki without known genetic variants. When assessed against other episignature disorders available in our EKD, WDSTS classifier was demonstrated to be highly sensitive and specific.

POSTER PRESENTATIONS 1
1B: EPIGENETICS

Presenter's Name: Haghshenas, Sadegheh

Additional Author(s): Rius R, Foroutan A, Levy MA, Kerkhof J, McConkey H, Tchan M, Riley LG, Thorburn DR, Fleming M, Christodoulou J, Sadikovic B

Abstract Title: Biallelic YARS2 variants are associated with a specific DNA methylation episignature

Abstract:

Introduction: Overlapping clinical features of rare genetic disorders and the frequent ambiguity of conventional genetic tests, such as variants of uncertain significance (VUSs) make clinical diagnosis challenging. In recent years, DNA methylation episignatures, which are genome-wide DNA methylation patterns detectable through peripheral blood, have been applied as stable and reliable biomarkers and have been used in a clinical setting. Here, we describe a DNA methylation episignature for the YARS2-related mitochondrial disease, associated with biallelic YARS2 variants, typically characterized by myopathy, lactic acidosis, and sideroblastic anemia.

Methods: DNA was extracted from peripheral blood of patients with the YARS2-related mitochondrial disorder and control individuals and methylation levels were measured using Illumina Infinium EPIC bead chip arrays. Controls matched by age, sex, and array type to the patient cohort were selected using R MatchIt package. Probes differentially methylated between the case and control groups were selected by linear modeling using R minfi package. Hierarchical clustering was performed by Ward's method on Euclidean distance and multidimensional scaling was performed by scaling of the pair-wise Euclidean distances between samples. Support vector machine was constructed using R e1071 package.

Results: Eleven patients with a confirmed clinical and genetic diagnosis of the YARS2-related mitochondrial disorder were used for DNA methylation episignature detection and model construction. Mean methylation differences between 11 case samples and 55 matched control samples and p-values were obtained and 257 probes were selected as the identifying episignature of the syndrome. The robustness of the detected probes in differentiating case samples from control samples were confirmed by unsupervised models. The identified probes were then used in constructing a support vector machine with high sensitivity and specificity in classifying patients suspected of having the YARS2-related mitochondrial disorder.

Discussion: In this study, a highly sensitive and specific DNA methylation episignature for the YARS2-related mitochondrial disorder was identified. This expands the list of rare genetic disorders with a known episignature. Moreover, this is the first mitochondrial disease associated with an episignature, which paves the way for the detection of episignatures for other mitochondrial disorders in the future.

Presenter's Name: Larsen, Frederikke

Additional Author(s): Good HJ, Shin AE, Zhang L, Asfaha S

Abstract Title: DNA hypomethylation inhibits tuft cell-derived colitis-associated cancer

Abstract:

Introduction: Colorectal cancer is the second leading cause of cancer death in Canada. A major risk factor for the development of colorectal cancer is chronic inflammation leading to colitis-associated cancer (CAC). We previously described a CAC mouse model in which tumors arise from DCLK1+ tuft cells following loss of the tumor suppressor adenomatous polyposis coli (APC) and induction of colitis. Interestingly, epithelial cells in colitis and CAC display DNA methylation changes, but the effect of these epigenetic changes on colonic tumorigenesis is not known. Thus, we hypothesize that inhibition of DNA methylation in DCLK1+ tuft cells reduces colonic tumorigenesis. We investigated this hypothesis by inhibiting DNA methylation by genetic and pharmacologic means.

Methods: We crossed Dclk1-CreERT2/Apcf/f mice to DNMT1f/f mice to delete the DNA methyltransferase DNMT1 specifically in DCLK1+ tuft cells. We then induced CAC in Dclk1/Apcf/f and Dclk1/Apcf/f/DNMT1f/f mice by administering three doses of tamoxifen followed by 2.5% dextran sodium sulfate (DSS) for five days. Fourteen weeks later, we assessed colonic tumor number and size. Lineage tracing from DCLK1+ cells was also examined in colonic tissues from all mice. In a separate cohort of Dclk1/Apcf/f mice, we induced CAC and treated the mice with six doses of the DNA de-methylating drug 5-AZA-2'-deoxycytidine (5-AZA) or vehicle. Ki67 immunostaining was additionally performed to assess cellular proliferation in the colon.

Results: Deletion of DNMT1 in DCLK1+ cells significantly inhibited the number and size of colonic tumors. Treatment of mice with 5-AZA similarly reduced the overall number of mice with tumors, as well as, the number and size of tumors per mouse. Interestingly, 5-AZA treatment was associated with reduced colonic proliferation as assessed by Ki67 staining of epithelial cells, and quiescent DCLK1+ cells that did not lineage trace. Deletion of DNMT1 or treatment with 5-AZA additionally reduced the number of lineage tracing events detected upon exposure to low dose DSS.

Discussion: Our findings demonstrate that DNMT1 loss or 5-AZA both inhibit CAC and tuft cells stemness. Furthermore, 5-AZA appears to exert its anti-tumor effects by reducing proliferation. Our data demonstrates that loss of DNMT1 or treatment with 5-AZA plays an important role in colitis-associated tumorigenesis.

POSTER PRESENTATIONS 1
1B: EPIGENETICS

Presenter's Name: Levy, Michael

Additional Author(s): McConkey H, Relator R, Kerkhof J, Pranckeviciene E, Barat-Houari MA, Bargiacchi S, Biamino E, Bralo MP, Cappuccio G, Ciolfi A, Clarke A, DuPont BR, Elting MW, Faivre L, Fee T, Ferilli M, Fletcher RS, Cherik F, Foroutan A, Friez MJ, Gervasini C, Haghshenas S, Hilton BA, Jenkins Z, Kaur S, Lewis S, Louie RJ, Maitz S, Milani D, Morgan AT, Oegema R, Østergaard E, Pallares NR, Piccione M, Plomp AS, Poulton C, Reilly J, Rius R, Robertson S, Rooney K, Rousseau J, Santen GWE, Santos-Simarro F, Schijns J, Squeo G, St John M, Thauvin-Robinet C, Traficante G, van der Sluijs PJ, Vergano SA, Vos NR, Walden KK, Azmanov D, Balci T, Banka S, Gecz J, Henneman PJ, Lee JA, Mannens MMAM, Roscioli T, Siu V, Amor DJ, Baynam G, Bend EG, Boycott K, Brunetti-Pierri N, Campeau PM, Christodoulou J, Dyment D, Esber N, Fahrner JA, Fleming MD, Genevieve D, Kerrnohan KD, McNeill A, Menke LA, Merla G, Prontera P, Rockman-Greenberg C, Schwartz C, Skinner SA, Stevenson RE, Vitobello A, Tartaglia M, Alders M, Tedder ML, Sadikovic B

Abstract Title: Identification and functional annotation of DNA methylation episignatures associated with Mendelian neurodevelopmental disorders

Abstract:

Introduction: Overlapping clinical phenotypes and an expanding breadth and complexity of genomic associations are a growing challenge in the diagnosis and clinical management of Mendelian neurodevelopmental syndromes. The functional consequences and clinical impacts of genomic variation may involve unique, disorder-specific changes in DNA methylation referred to as episignatures. Episignatures are distinct, highly sensitive and specific biomarkers that have recently been applied in the clinical diagnosis of genetic syndromes.

Methods: Microarrays were used to assess genome-wide DNA methylation in peripheral blood samples from 235 patients from cohorts representing one of 19 genetic neurodevelopmental syndromes. Statistical methods including unsupervised clustering and support vector machine (SVM) machine learning were used to identify the microarray probes with changes in DNA methylation which represented syndrome-specific episignatures. These newly identified episignatures were integrated with and compared with 38 previously identified episignatures.

Results: Nineteen novel episignatures were identified, including examples specific to protein complex, gene, sub-gene, protein domain, and single nucleotides, demonstrating the increasing resolution and specificity of episignatures. Using a multiclass SVM algorithm we were able to differentiate between all 57 episignatures. Genomic assessment showed that in addition to the cohort-specific changes in DNA methylation there is also substantial overlap in differentially methylated probes between some episignatures. The overall distribution of DNA methylation changes across the majority of the neurodevelopmental genetic syndromes analyzed showed enrichment in gene promoters and CpG islands, and under-representation of the more variable intergenic regions. Overrepresentation analysis showed significant enrichment of differentially methylated regions in gene pathways and networks related to neurodevelopment.

Discussion: This study expands the number and spectrum of disorders with detectable DNA methylation episignatures to a total of 57 episignatures associated with 65 genetic neurodevelopmental syndromes, and provides further insight into the molecular etiology of Mendelian conditions.

Presenter's Name: McConkey, Haley

Additional Author(s): Kerkhof J, Levy M, Relator R, Rooney K, Foroutan A, Pranckeviciene E, Haghshenas S, Sadikovic B

Abstract Title: Clinical Epigenomic Testing in Canada: Discovery and Clinical Assessment of Episignatures

Abstract:

Introduction: Neurodevelopmental disorders (NDDs) often present with overlapping phenotypic characteristics, making a clinical diagnosis difficult. First-tier genetic testing includes microarray and targeted exome sequencing based on the phenotypic presentation. Approximately 75% of patients do not receive a diagnosis from this testing and must undergo reflex testing, which includes large gene panels, whole exome sequencing or whole genome sequencing. This testing can take months or years and is costly, and patients can be left with no variants detected or a variant of uncertain significance. DNA methylation is an epigenetic mechanism that is involved in chromatin compaction and regulation of gene expression. A set of NDDs exhibit unique combinations of DNA methylation changes at multiple loci across the genome, called episignatures, which can be used to resolve ambiguous clinical cases. EpiSign is a diagnostic test developed by our laboratory used for identification of episignatures in peripheral blood of patients with suspected genetic conditions. This study aims to assess the use of EpiSign in clinic, as well as expand the number of detectable episignatures.

Methods: The EpiSign test is able to detect episignatures through whole-genome methylation analysis, then compares the detected changes to known episignatures of NDDs. A current national trial, called EpiSign-CAN, is in progress with the main goal of obtaining real-world prospective evidence to validate the utility of EpiSign in both the first-tier and reflex setting. This study also includes an exploratory objective of expanding the clinical utility of the EpiSign test through discovery of additional epigenetic signatures.

Results: EpiSign v3 contains 57 episignatures associated with 65 genetic syndromes. These episignatures can be protein complex, gene, sub-gene, protein domain and even single nucleotide-level specificity. This list is expanding, with new disorders being assessed for episignatures. The EpiSign-CAN study is underway and enrolling patients in both the first-tier screening phase of their journey and in the reflex testing stage.

Discussion: The use of EpiSign in Canadian genetics clinics provides an additional strategy for physicians to assess patients with ambiguous clinical presentation or genetic findings. Additionally, it has the potential to impact healthcare resource allocation and provide a more cost-effective approach for the diagnosis of rare disease.

POSTER PRESENTATIONS 1
1B: EPIGENETICS

Presenter's Name: Morin, Amanda

Additional Author(s): Castellani CA

Abstract Title: Epigenomic and transcriptomic profiles in inducible models of mitochondrial DNA copy number and heteroplasmic burden

Abstract:

Bidirectional crosstalk between the nuclear genome (nDNA) and the mitochondrial genome (mtDNA) is required for proper cell functioning and homeostasis. Altering the quantity (mtDNA-CN) or the quality (heteroplasmy) of mtDNA leads to alterations in the nuclear epigenome. Further, mtDNA variation is associated with variability in health and disease and all-cause mortality. Replication of mtDNA is regulated by nuclear-encoded genes, including POLG (DNA polymerase γ) and TFAM (mitochondrial transcription factor A). TFAM knockout models previously developed in the lab have shown an 18-fold reduction in mtDNA-CN leads to variation in nuclear gene expression via CpG methylation. It has also been shown that expression of exonuclease-deficient (D198A) and polymerase-deficient (D1135A) dominant-negative POLG (DN-POLG) mutants results in up to a 5-fold increase in heteroplasmic load and up to 75% reduction in mtDNA-CN, respectively. This project aims to use optimized culture models to assess the ability of mtDNA variation to drive epigenomic and transcriptomic change. We are in the process of creating two tetracycline-inducible expression models using the Flp-In T-Rex293 cell line, a HEK293 cell line derivative with an integrated Flp recombinase target (FRT) site and constitutive expression of the tetracycline operator repressor. These models allow for dose-dependent alteration of mtDNA-CN and heteroplasmic load via tetracycline-inducible control of DN-POLG expression. We will co-transfect this cell line with an expression vector containing a FRT site and a DN-POLG mutant under the control of two tetracycline operators, and a vector expressing Flp recombinase, mediating recombination between the FRT sites in the cell genome and the expression vector. mtDNA-CN and heteroplasmy in DN-POLG stable cell lines will be measured with qPCR and whole genome sequencing, respectively. DNA methylation profiles will be measured using the Illumina Infinium EPIC Methylation Array and gene expression profiles will be measured by RNA sequencing. We anticipate that alteration of mtDNA-CN and heteroplasmy will result in site-specific DNA methylation changes and differential gene expression. These models will allow us to assess the mechanisms mediating the effect of mtDNA variation on health and disease, and will also be used to test hypotheses regarding the potential role of mtDNA as an environmental biosensor which translates vital information about cell state to the nuclear genome.

Presenter's Name: Nagano, Tyler

Additional Author(s): Castellani CA

Abstract Title: Effect of CRISPR Induced Mitochondrial DNA Variation on the Nuclear DNA Epigenome and Transcriptome

Abstract:

Introduction: Mitochondrial DNA copy number (mtDNA-CN) is associated with several age-related chronic diseases and is a predictor of all-cause mortality. A previous study by Castellani et al. identified a role for mtDNA-CN variability in the regulation of nuclear gene expression via nuclear DNA (nDNA) methylation. What remains unknown are the underlying biological mechanisms controlling the effect of mtDNA-CN on nDNA gene expression. In this study, we hypothesized that site-specific differential nDNA methylation and differential gene expression resulting from in vitro reduction of mtDNA-CN would uncover shared genes and biological pathways important in the mechanisms mediating the effect of mtDNA-CN on disease.

Methods: To test this hypothesis, we generated epigenome and transcriptome profiles for three independent human embryonic kidney (HEK293T) cell lines harbouring a mitochondrial transcription A (TFAM) heterozygous knockout using CRISPR-Cas9, and matched control lines. Methylation data was generated using the Illumina Infinium Methylation EPIC BeadChip, RNA sequencing was performed using the Illumina HiSeq 2500 instrument and the data was analyzed to call differentially methylated sites (DMS), differentially methylated regions (DMR), and differentially expressed genes (DEG). Finally, we integrated the analyses and performed functional enrichment to determine genes and pathways which may facilitate mtDNA-CN effect on nDNA gene expression.

Results: Our results identified 4205 DMS associated with mtDNA-CN at epigenome-wide significance ($p<1\times10^{-7}$). 228 DMR associated with mtDNA-CN ($P<1.17\times10^{-5}$) and 164 DEG were also identified ($p<3.59\times10^{-6}$). Enrichment analyses demonstrated that the "neuroactive ligand receptor interaction", "GABAergic synapse" and "Nicotine Addiction" KEGG pathways were overrepresented in the DMS analysis ($p<6.37\times10^{-4}$, $p<1.54\times10^{-4}$, $p<1.42\times10^{-6}$), DMR analysis ($p<4.20\times10^{-3}$, $p<1.91\times10^{-4}$, $p<4.67\times10^{-3}$) and DEG analysis ($p<7.73\times10^{-3}$, $p<1.94\times10^{-2}$, $p<2.12\times10^{-3}$).

Discussion: These findings demonstrate that genes in the "neuroactive ligand receptor interaction", "GABAergic synapse" and "Nicotine Addiction" KEGG pathways may be related to the underlying biological mechanisms which facilitate mtDNA-CNs effect on nDNA methylation. Further, these results suggest that mitochondrial DNA variation signals to the nuclear DNA epigenome and transcriptome and may lead to changes relevant to development, aging, and complex disease.

POSTER PRESENTATIONS 1
1B: EPIGENETICS

Presenter's Name: Relator, Raissa

Additional Author(s): van der Spek J, McConkey H, Kerkhof J, Levy M, Kleefstra T, Butler K, Sadikovic B

Abstract Title: Episignature for Witteveen-Kolk syndrome due to haploinsufficiency of SIN3A gene

Abstract:

Introduction: Witteveen-Kolk syndrome (WITKOS) is a rare neurodevelopmental disorder caused by heterozygous alterations in the SIN3A gene. Common clinical features of this syndrome include pre- and postnatal growth retardation, developmental delay, intellectual disability, hypotonia, behavioral issues, dysmorphic facial features and digital and genital abnormalities. These characteristics highly overlap with other disorders, and severity of their presentation can be variable. From previous studies, it has been established that haploinsufficiency of SIN3A gene is the primary cause of the disorder. There is also an expanding number of genetic disorders, often caused by gene haploinsufficiency, that have been shown to be associated with distinct epigenetic profiles, called episignatures. With SIN3A being a known epigenetic regulator, we hypothesize that individuals with WITKOS due to gene haploinsufficiency will exhibit a unique methylation profile associated with the condition.

Methods: We performed genome-wide methylation analysis on DNA samples derived from peripheral blood of 14 patients with SIN3A variants resulting to haploinsufficiency using Illumina EPIC arrays. These cases were compared to controls selected from the EpiSign Knowledge Database by matching age and gender. We performed computational analysis in R to identify significantly differentiated probes distinguishing the WITKOS cases from controls, and assessed the epigenetic signature defined by these probes using hierarchical clustering and multidimensional scaling. We further tested robustness of the identified signature by building a support vector machine classifier and use this model to distinguish the disorder from known episignatures in other neurodevelopmental disorders and unaffected controls.

Results: Our analysis showed a robust, unique episignature in WITKOS patients characterized by 120 differentially methylated probes. We confirmed the specificity of the prediction model based on this signature by testing over 1000 individuals with other neurodevelopmental disorder. Furthermore, sensitivity of the signature was also established by correct identification of 4 patients carrying SIN3A mutations with varying phenotypes and resolution of an unresolved case.

Discussion: Our results reveal a correlation between altered epigenetic profiles and clinical presentation of WITKOS patients. Moreover, the identified signature is proven to be useful for molecular diagnosis of the disorder.

POSTER PRESENTATIONS 1
1B: EPIGENETICS

Presenter's Name: Wang, Honglin

Additional Author(s): Feng B, Chakrabarti S

Abstract Title: Circular RNA along with microRNA-9 moderate EndMT in diabetic cardiac fibrosis

Abstract:

Introduction: Diabetic cardiac myopathy (DCM) is a potent and independent risk factor in developing cardiovascular disease and heart failure. Despite successful management of other cardiovascular risks, diabetic patients still see increased incidents of heart failure. Hyperglycemia damages endothelial cells, causing inflammation and transcriptional derangement, which drive endothelial cells to take on a mesenchymal phenotype (EndMT). Cells with a mesenchymal phenotype then become a source of cardiac fibroblast-like cells that contribute to cardiac fibrosis, a characteristic of DCM. Previous research has shown that the noncoding RNA (ncRNA) MicroRNA-9 (miR9) regulates the expression of various genes related to EndMT. CircularRNAs are a new class of endogenous ncRNA which function by sponging up and therefore downregulating microRNAs. Results of an assay show that six circRNAs, circRNA_12164, circRNA_35663, circRNA_34936, circRNA_40807, circRNA_36135, and circRNA_42623, which are both upregulated in diabetic murine heart tissues and have a binding affinity to miR9. We hypothesize that miR9 along with circular RNAs modulate DCM.

Methods: We induced diabetes in normal and endothelial-specific miR9-overexpressing mice to quantify the downstream effects of hyperglycemia and miR9 on the expression levels of fibrotic genes and the six identified circRNAs using qRT-PCR. CircRNA will be quantified following digestion of linear RNA with RNase. Next, we will attempt to establish a causational relationship between miR9 and circRNAs in human cardiac microvascular endothelial cells (HCMECs). We will transfect HCMECs with mimics of miR9 and identified circRNAs to observe their downstream effects in both hyperglycemic and normoglycemic conditions.

Results: Our preliminary results show that endothelial-specific miR9-overexpressing mice show significantly reduced expression of FN1 and Col1A1 in both diabetic and non-diabetic mice. MiR9 overexpression was able to completely prevent glucose induced FN1 production.

Discussion: Our findings show that miR9 overexpression protects against development of cardiac fibrosis.