ABSTRACTS OF INVITED LECTURES

RNA SEQUENCING APPLICATIONS IN FORENSIC GENETICS

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The application of RNA profiling in forensic genetics has experienced tremendous growth and development in the past decade. The earliest studies, and main applications, have been applied to body fluid and tissue identification, using tissue-specific RNA transcripts and, principally, reverse transcription endpoint PCR and subsequent capillary electrophoretic (CE) separation. Several markers have been identified for the forensically most relevant body fluids and tissues and the method has been successfully used in casework. The introduction of Massively Parallel Sequencing (MPS) methodology has provided several benefits that continue to advance the field. Specifically, RNA sequencing permits a more quantitative nuanced approach to gene expression analysis compared to CE since transcripts are counted via the number of reads, resulting in a digital gene expression profile that is amenable to sophisticated statistical methods. Additionally, more targets can be tested in the same sample and RNA sequence variation within transcripts can be interrogated and used for high-resolution assignment of body fluids to donors in mixed in body fluid/tissue samples. This presentation will give an overview on forensic transcriptome analyses and applications, including whole transcriptome sequencing (WTS) as well as targeted MPS approaches. Using data from the authors' laboratories, detailed examples will include RNA biomarker selection, body fluid and organ tissue identification and, via the recent development of an improved high-resolution MPS assay, the assignment of DNA donors to body fluids via coding region SNPs. Recent development in other applications will be briefly mentioned including the potential for determination of the age of stains, the age of the donor, the post-mortem interval and to aid post-mortem death investigations.

SINGLE-CELL PROFILING OF THE HUMAN M1 AND DLPFC IN ALS AND FTLD

Sebastian Pineda[1], Erica Cook[2], Hyeseung Lee[3], Brent Fitzwalter[3], Shahin Mohammadi[3], Luc Pregent[2], Bjorn Oskarsson[4], Jaimin Shah[4], Ronald Petersen[4], Neil Graff-Radford[4], Bradley Boeve[4], David Knopman[4], Keith Josephs[4], Michael DeTure[2], Melissa Murray[2], Dennis Dickson[2], Myriam Heiman[3], **Veronique Belzil[2]**, Manolis Kellis[1]

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are two devastating and fatal neurodegenerative conditions. While distinct, they share many clinical, genetic, and pathological characteristics, and both show selective vulnerability of layer 5b extratelencephalic-projecting cortical populations, including Betz cells in ALS and von Economo neurons (VENs) in FTLD. Here, we report the first high-resolution single-cell atlas of the human primary motor cortex (M1) and dorsolateral prefrontal cortex (DLPFC) and their transcriptional alterations in ALS and FTLD across 75 individuals, including 17 control samples and 58 sporadic and C9orf72-associated ALS and FTLD patient samples. We identify 47 transcriptionally distinct cellular subtypes including two Betz-cell subtypes, and we observe a previously unappreciated molecular similarity between Betz cells and VENs. Many of the dysregulated genes and pathways are shared across excitatory neurons, with Betz cells and VENs being the most transcriptionally affected. Our results suggest that transcriptional similarity between Betz cells and VENs underline the cell-specific vulnerability observed in ALS and FTLD, and explain their concomitant diagnoses.

NEWS FROM THE NEOLITHIC

Joachim Burger

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The transition from foraging to a sedentary and agricultural way of life is the decisive step in human history, without which most of the human populations living today would not exist. This breathtaking cultural transition began 11,000 years ago and has been called the "Neolithic Revolution". It has been researched for over 100 years, in the last 17 years also with the help of palaeogenetic data. Here I present our recent analysis of high-quality palaeogenomes from predominantly Neolithic contexts. For the first time, we have explicitly modeled the population history of late hunter-gatherers and early farmers. In this way, we have created a "demogenomic model" of human populations in southwest Asia and parts of Europe between 30,000 and 7,000 years ago. The study provides new insights into pre-Neolithic population dynamics during the Late Ice Age in Europe and Anatolia. It also clarifies basic demographic processes that led to the differentiation of early Neolithic populations (and present-day Western Mediterraneans). But what do we learn from this for the Neolithic itself?

IMPACT OF OMICS ON FORENSICS

Angel Carracedo

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Genomics is revolutionizing many areas of forensic science at a similar extend as it is happening in clinical medicine and in a similar way as clinical genetics is moving towards genomics medicine; fforensic genetics is slowly transitioning into forensic genomics. The evolution of next generation or massively parallel sequencing is allowing the use of genomic, transcriptomic, and epigenomics to address various forensic questions that cannot be answered, or only in a limited way, using classical forensic genetics approaches. Forensic genetics has directly benefited of the advances in genomics and new applications as the determination of external physical characteristics, the geographic origin of samples or the estimation of the age from minute biological material are now possible. Human transcriptome data of different tissues generated are allowing the identification of RNA markers to determine the cellular source of crime scene samples. This is forensically relevant for reconstructing the course of events that may have happened at the scene of crime and to support the use of DNA at the activity level of evidence interpretation. Non-human genomic and transcriptomic are useful in different forensic contexts and forensic microbiome is now an important emerging field. Proteomics has also some important specific applications. Integration of data is challenging but key for the progress of the field. Forensic toxicology and forensic pathology are also benefiting of the advances in genomics with a special impact in the genetic diagnosis of cardiac sudden death and the so-called molecular autopsy is essential complement to classical autopsy**.**

THERAPEUTIC TARGETING OF THE FOXO3 CIS-REGULOME IN ANAPLASTIC THYROID CANCER

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Anaplastic thyroid cancer (ATC) is the most aggressive and deadliest human cancer (median survival: 4 months). Metastatic ATC is uniformly fatal. ATC is a rare disease (800-1000 U.S. cases/year) that develops in less than 2% of differentiated thyroid cancers (DTCs). Most DTC patients have excellent prognosis in response to surgery, thyroid stimulating hormone suppression, radioiodine and sometimes radiation therapy; and even metastatic disease is often curable. Several mutations have been found to contribute to the striking transformation of DTCs to ATC. However, therapies based upon these mutations have only improved outcomes in specific cases. We previously found that FOXO3, a transcription factor (TF) with an established tumor suppressor function in DTCs, becomes an oncogene in ATC, reflecting increased nuclear retention arising from dysregulated AKT signaling. However, the spectrum of FOXO3 transcriptional targets and downstream pathways, and the epigenetic mechanisms underlying these effects remain unclear. Therefore, our goal is to identify the ATC-specific cis-regulome of FOXO3, discover the epigenetic mechanisms recruited by FOXO3 to regulate these target genes, and investigate the therapeutic utility of agents rationally selected based on their targets' involvement in FOXO3 actions and for their ability to kill ATC cells in vitro and in vivo. Our overall hypothesis is that due to increased DNA binding, FOXO3 becomes a master TF critical for establishing the ATC phenotype, making ATC cells specifically vulnerable to pharmacological agents targeting FOXO3-regulated mechanisms, genes and pathways. We are combining multi-omics with epigenetic pharmacology to define the ATC-specific FOXO3 cis-regulome, to investigate the role of FOXO3-induced chromatin decondensation and reconfiguration in enabling and augmenting the expression of oncogenic gene networks, and to determine the utility of pharmacological agents targeting FOXO3-regulated mechanisms. Our multiomics-supported, mechanistically informed approach may identify key ATC vulnerabilities and validate the therapeutic potential of their targeting with focus on combinations of clinically relevant agents to ultimately transform the therapy of ATC, the deadliest of cancers.

MOLECULAR BASIS OF EPIGENETIC CONTROL: EPIGENETIC REGULATION BY POST-SYNTHETIC DNA AND RNA MODIFICATIONS

Julie Cunningham

Mayo Clinic, Rochester, Minnesota, USA

Post translational modifications of DNA and RNA are critical components regulating chromatin and RNA processes. In this presentation, I will present what we know about the chemistry underlying these modifications, some thoughts on how they may have evolved, and touch upon how perturbations of these mechanisms can impact health.

NON-INVASIVE PRENATAL TESTING FOR THE BETA-HEMOGLOBINOPATHIES USING NEXT-GENERATION SEQUENCING AND PROBE CAPTURE

Henry Erlich

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The presence of fetal DNA in maternal plasma and the massively parallel and clonal features of Next Generation Sequencing (NGS) have made non-invasive prenatal testing (NIPT) a reality. The analysis by NGS of fetal DNA in maternal plasma has been applied to the diagnosis of chromosomal aneuploidies but the NIPT of autosomal recessive diseases has been more challenging. We have applied NGS to the NIPT of the autosomal recessive diseases, sickle cell anemia (SCA) and β-thalassemia, by using a capture probe panel that covers 4 Kb of the β-globin gene and linked SNPs as well as >450 genomic polymorphisms used to estimate the fetal fraction. The hybrid capture method is well suited to the analysis of the small DNA fragments present in the maternal plasma. The fetal fraction is estimated by counting paternally transmitted sequence reads in the plasma library for alleles present in the fetus but absent in the mother. If the mother and father's mutations differ, the paternally transmitted mutation can be detected qualitatively as a sequence in the plasma that is absent in the mother but the mother's transmitted allele is determined by quantitative analysis of the sequence read proportions in the plasma. If the maternal and paternal mutations are the same, as in a pregnancy at risk for SCD, the fetal β-globin genotype is inferred by counting sequence reads corresponding to the mutation and wild type alleles. The observed proportions are compared to those expected for each of the three possible fetal genotypes (Mut/Mut; Mut/WT; WT/WT) to infer fetal genotype. The expected values are calculated based on the fetal fraction estimate. An algorithm assigns probability values to each of the potential fetal genotypes. We have used the bioinformatic strategy of in silico size selection for the maternal plasma reads to increase the fetal fraction. We have also used haplotype information, when available, to consider the observed ratios at linked SNPs to help predict the fetal genotype at the mutation site. This probe capture/NGS system promises to provide a robust non-invasive test for sickle cell anemia and β-thalassemia and represents a model approach for other autosomal recessive diseases.

EPIGENOMICS OF INFLAMMATORY BOWEL DISEASE

William A. Faubion

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Inflammatory bowel disease is a chronic intestinal inflammatory condition affecting 1 in 300 individuals in the developing world for which there is insufficient insight into pathogenicity or precision therapeutics. While there are genetic traits associated with disease, the rise in incidence over the last 3 decades supports an environmental influence to disease susceptibility and behavior. We will review environmental signals received by particular immune subsets (FOXP3+ CD4+ lymphocytes) that couple to epigenetic complexes regulating immune responses in the human IBD, Crohn's disease.

SINGLE-CELL MULTIOMIC ANALYSIS TO IDENTIFY CELL-SPECIFIC TRANSCRIPTIONAL DEPENDENCIES IN CANCER AND COVID-19 INFLAMMATORY RESPONSE

Alexandre Gaspar-Maia

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The human body is composed of an estimated 37 trillion cells that live harmoniously among their neighbors. An equilibrium between differentiated cells, progenitor cells and stem cells has to be achieved at any point of development to enable homeostasis and proper tissue organization. However, in cancer, a single cell can lead to the downfall of an entire organism. To ensure that all the signals and processes are coordinated, cells need to regulate the expression of genes using a multitude of factors (transcription factors that bind to the DNA itself) and chromatin regulators (proteins that surround the DNA). Novel single cell technologies have enabled us to define both the expression and the chromatin landscape. Here, we will discuss how single cell multiomic analysis has enable us to define cell-specific transcriptional dependencies in cancer and in inflammatory response upon COVID19 infection.

3D GENOMIC STRATEGIES TO UNDERSTAND COMPLEX TRAIT GENETIC ARCHITECTURE

Struan F. A. Grant

Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA

We are employing cutting-edge 3D genomic approaches to facilitate understanding of genetic loci for common complex disease. There is a significant need to discover and validate new genetic targets that influence such traits to advance therapies to prevent and treat disease. We are focused on the functional significance of genome wide association study (GWAS) signals associated with various complex traits. While numerous GWAS efforts have been successful in discovering key genetic variants, this approach only reports genomic signals associated with a given phenotypic trait and not necessarily the precise localization of culprit effector genes. Approaches are now emerging to make these determinations; however, they typically suffer from low-resolution and inaccuracies. Using bone mineral density (BMD) as an example, we recently published our high-resolution genome-wide 'variant to gene mapping' efforts, where we integrated RNA-seq, ATAC-seq and chromatin conformation capture (promoter-focused Capture C) in primary human osteoblasts to implicate culprit effector genes for osteoporosis, including validating two novel effector genes, EPDR1 and ING3. ~30% of GWAS signals were found to reside in enhancers with direct physical contact with genes expressed in osteoblasts, totaling 86 leads - many being novel and warranting functional follow-up. However, this also means that many GWAS loci remain to be resolved, so in order to uncover additional aspects of the genetic architecture of bone density determination we are now studying temporally specific roles that are dependent on the stage of differentiation Crucially, our pipeline does not involve large sample sizes, but rather uses primary healthy human cells to triangulate key enhancers coinciding with, and signposted by, putatively causal variants. The ultimate aim is to provide the community with new, high value targets to aid in understanding mechanism, and eventually therapies, for common complex diseases.

POPULATION GENETICS, KINSHIP PRACTISES AND SOCIAL ORGANISATION IN PREHISTORIC SOCIETIES OF EUROPE

Wolfgang Haak

Max Planck Institute for Evolutionary Anthropology, Department of Archaeogenetics, Leipzig, Germany

The continuously growing record of ancient human genomic data reveals ever more detailed insights into the population history of prehistoric societies. By focussing on regional transects, closing spatial and temporal gaps, and through a proper integration of the archaeological context, archaeogenetic studies now exceed simplistic migration scenarios and can provide nuanced accounts of genetic and cultural transformations. Increasing numbers of intra-site studies add further details by shedding light on kinship practices and forms of social organisation in prehistoric societies. I will present a selection of recent case studies from various regions in Neolithic and Early Bronze Age Europe, which further advance our understanding of prehistoric societies and the formation of the European gene pool.

STONE AGE ENCOUNTERS: NEANDERTAL ANCESTRY OF EARLY HUMANS IN EURASIA

Mateja Hajdinjak[1,2]

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Our closest evolutionary relatives, the Neandertals, appeared in the European fossil record around 430,000 years ago (~430ka). Before their disappearance, Neandertals lived throughout Europe, western and central Asia, and the Near East. The ancestors of all modern humans emerged in Africa at least ~300ka and, sometime between ~100ka and ~50ka, a subset of those humans migrated out-of-Africa and spread across the world. However, by ~40ka Neandertals disappeared. The reasons of Neandertal disappearance, the extent to which they overlapped with modern humans, and the nature of interactions between the two hominin groups have been intensively debated for decades. Comparisons between the Neandertal genomes and the genomes of modern humans showed that Neandertals contributed ~2% to the genomes of all people living outside of sub-Saharan Africa. Based on the size of Neandertal segments in modern human genomes, the time of this admixture was narrowed to between 58ka and 52ka, and most likely in the Near East. However, where, when and how often these two groups came into contact was not well understood. Despite genomic data being recovered from more than 6,500 ancient humans to date, genome-wide data of individuals close in time when modern humans could have met some of the last Neandertals are still extremely sparse. Through the combination of minimally destructive sampling, decontamination with mild hypochlorite solution and hybridisation captures, we obtained genome-wide data of sixteen new and improved coverage of further nine previously published modern humans from western Eurasia older than 30ka. In addition to shedding light on the genetic diversity of past populations, these data allow us to start reconstructing the fine-scale dynamics of the interactions between late Neandertals and early humans in Eurasia. Our data suggest that small groups of first humans to arrive in Eurasia interacted intimately with Neandertals, having had close Neandertal ancestors, and were eventually either absorbed into their populations or became extinct. Moreover, our new data further raise the possibility of Neandertal populations becoming assimilated in more numerous human populations that arrived later, indicating a far more dynamic population history and turnovers during this time period than previously appreciated.

A FORENSIC GENOMICS APPROACH FOR THE IDENTIFICATION OF SISTER MARIJA KRUCIFIKSA KOZULIĆ OF RIJEKA

Charla Marshall[1,2,3], Kimberly Sturk-Andreaggi[1,2], Erin M. Gorden[1,2], Jennifer Daniels-Higginbotham[1,2], Sidney Gaston Sanchez[1,2], Željana Bašić[4], Ivana Kružić[4], Šimun Anđelinović[5,6], Alan Bosner[7], Miran Čoklo[8], Anja Petaros[9], Timothy P. McMahon[1], Dragan Primorac[3,5,10,11,12,13,14,15,16,17], **Mitchell M. Holland[3]**

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Sister Marija Krucifiksa Kozulić (1852-1922) was a nun who dedicated herself to helping the poor and less fortunate. In light of her generous and virtuous life, Sister Kozulić is being considered for sainthood by the Vatican. However, this process could not proceed without the identification of her remains, which has required the skills of many experts, including pathologists, anthropologists, and molecular biologists. This presentation will highlight the efforts made to support the identification of Sister Kozulić with a forensic genomics approach; targeting both mitochondrial and nuclear DNA markers using capture and massively parallel sequencing (MPS) methods. Sister Kozulić was buried in a tomb in Rijeka with her biological sister, Tereza Kozulić, along with the commingled remains of other nuns from the Society of Sisters of the Sacred Heart of Jesus. In search for Sister Kozulić amongst the many skeletal remains recovered from the tomb, mitochondrial genome (mitogenome) sequencing was performed on femoral samples using capture and MPS methods developed for degraded DNA. The obtained mitogenome sequences revealed DNA averaging less than 100 bp in length that is typical of historical samples. The results revealed two individuals sharing the same H1 haplotype with an uncommon 13327 G/A heteroplasmy. This finding was taken as a preliminary indicator of the biological sisters' remains, who were the only known maternal relatives buried in the tomb. Additionally,

one of the haplotypes exhibited a private 12337 C insertion at ~30% frequency, which distinguished the two haplotypes from one another. Due to the uniqueness of the haplotype, combined with the rarity of the shared heteroplasmy, it was assumed that these remains represented the sisters. The next step was to perform autosomal DNA testing on the remains, along with a buccal swab from a known paternal niece, to evaluate their genetic relationship. The reference sample of the now-deceased niece was collected in 2011, and the extracted DNA was low quality. Due to the degree of DNA degradation, kinship analysis was attempted after MPS of identity informative SNPs and autosomal STRs using the Precision ID Identity SNP and GlobalFiler NGS STR panels. Although only partial SNP and STR profiles were obtained from the sisters, comparison of their genotypes with that of the paternal niece supported the expected kinship scenario. Based on the SNP and STR results, the likelihood ratio (LR) exceeded 93,000 for a full sibling relationship between the presumed remains of Marija and Tereza versus being unrelated, with a posterior probability of 98.1% when considering the degrees of relatedness tested. In addition, the findings were >574,000 times more likely in favor of the sisters as 2nd degree relatives of the niece than from individuals who were unrelated. Given the supported kinship relationship with a known family reference, the two sets of skeletal remains are believed to belong to Sisters Marija and Tereza Kozulić. In the absence of direct reference samples from the two sisters, it is not possible to identify which set of remains belongs to Sister Marija. Nonetheless, the findings have allowed for the Vatican to move forward with the beatification process. The points of view in this abstract are those of the authors and do not reflect the views of their respective agencies. In addition, this publication in no way reflects an endorsement of products, instruments, or software.

Citation:
A forensic genomics approach for the identification of Sister Marija Crucifiksa Kozulić. C Marshall, K Sturk-Andreaggi, EM Gorden, J Daniels-Higginbotham, SG Sanchez, Ž Bašić, I Kružić, Š Andelinović, A Bosner, M Čoklo, A Petaros, TP McMahon, D Primorac, MM Holland (2020), Genes, 11, 938-950

MASSIVE EPIGENETIC REPROGRAMMING TRIGGERED BY A SINGLE GENETIC ALTERATION

Haojie Huang

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SPOP is a Cullin3-based E3 ubiquitin ligase adaptor (substrate-binding) protein. Whole genome and exome sequencing studies including The Cancer Genome Atlas (TCGA) invariably show that SPOP is the most frequently mutated gene in human primary prostate cancers. In addition to the discovery that prostate cancers with SPOP mutations have highest androgen receptor (AR) transcriptional activity among all genotypically distinct subsets of prostate cancer, this subtype of tumors are reportedly associated with genome-wide DNA hypermethylation in prostate cancer although the underlying mechanisms were elusive. Recent studies from Dr. Haojie Huang's laboratory at Mayo Clinic have shown that SPOP binds and promotes polyubiquitination and degradation of histone methyltransferase and DNMT interactor GLP. SPOP mutation induces stabilization of GLP and its partner protein G9a and aberrant upregulation of global DNA hypermethylation in cultured prostate cancer cell lines and primary specimens of patients. Genome-wide DNA methylome analysis shows that a subset of tumor suppressor genes (TSGs) including FOXO3, GATA5, and NDRG1, are hypermethylated and downregulated in SPOP-mutated prostate cancer cells. DNA methylation inhibitor 5-azacytidine effectively reverses expression of the TSGs examined, inhibits SPOP-mutated PCa cell growth in vitro and in mice, and enhances docetaxel anti-cancer efficacy. Together, these findings identify the GLP/G9a-DNMT module as a mediator of DNA hypermethylation in SPOP-mutated prostate cancer. These data also suggest that SPOP mutation could be a biomarker for effective treatment of prostate cancer with DNA methylation inhibitor alone or in combination with taxane chemotherapeutics.

RECOVERY OF NUCLEAR DNA FROM HAIR SHAFTS ASSOCIATED WITH THE ROMANOV FAMILY

Jodi Irwin

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To optimize the recovery of probative DNA from shed hair, an extraction protocol targeting ultrashort DNA fragments was applied to hair shafts found in items associated with the Romanov family. Published mitochondrial DNA genome sequences of Tsar Nicholas II and his wife, Tsarina Alexandra, made these samples ideal to assess DNA extraction techniques and evaluate the types of genetic information that can be recovered from aged hair. Using this method, the mtGenome of the Tsarina's lineage was identified in hairs that were concealed in a pendant made by Karl Fabergé for Alexandra Feodorovna Romanov. In addition, to determine if the lock originated from more than one individual, two single hairs from the locket were extracted independently and the autosomal SNP data used to assess relatedness. Testing of hairs found in a second artifact, a framed photograph of Louise of Hesse-Kassel, Queen of Denmark and maternal grandmother of Tsar Nicholas II, revealed that the hair belonged to a woman who shared Tsar Nicholas' maternal lineage, including the well-known point heteroplasmy at position 16169.

ARCHAIC AND MODERN HUMANS IN ISLAND SOUTHEAST ASIA

Mattias Jakobsson

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Multiple lines of evidence show that modern humans interbred with archaic Denisovans. Denisovans were initially thought to have a simple shared demographic history with modern humans, through a single admixture event with the ancestor of Australasians, but later work suggest that Denisovan ancestry can also be detected in varying degree across Australasians and at lower levels in East Asian, South Asian, Siberian and Native American populations. I will discuss recent finds of additional admixture between Australasians and Denisovans distinctively in Island Southeast Asia and the Philippines. In total, 118 ethnic groups of the Philippines including 25 diverse self-identified Negrito populations were investigated and we show that some groups, for instance the Ayta Magbukon, possess the highest level of Denisovan ancestry in the world ~30-40% greater than the level in Australians and Papuans. This finding is consistent with an independent admixture event into Negritos from Denisovans. Together with the recently described fossils claimed to be a new species (Homo luzonensis), we propose diverse archaic groups inhabiting the Philippines prior to the arrival of modern humans, likely genetically similar to Denisovans. Altogether, our findings unveil a complex intertwined history of modern and archaic humans in the Asia-Pacific region, where distinct Islander Denisovan populations differentially admixed with incoming Australasians across multiple locations and at various points in time.

DYNAMIC ENHANCER-PROMOTER INTERACTIONS MEDIATE CHEMORESISTANCE IN PDAC

Steven A. Johnsen

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Pancreatic ductal adenocarcinoma (PDAC) displays a remarkable propensity towards therapy resistance. However, molecular epigenetic and transcriptional mechanisms enabling this are poorly understood. We integrated epigenome, transcriptome, nascent RNA and chromatin topology data and identified a novel subgroup of enhancers that mediate transcriptional reprogramming and chemoresistance in PDAC. These Interactive Hubs (iHUBs) display characteristics typical for active enhancers (H3K27ac enrichment) in both therapy sensitive and resistant states but exhibit increased interactions and production of enhancer RNA (eRNA) in the resistant state. Notably, deletion of individual iHUBs was sufficient to decrease transcription of target genes and sensitize resistant cells to chemotherapy. Moreover, targeting either eRNA production or signaling pathways upstream of iHUB activation using clinically tested small molecule inhibitors decreased eRNA production and interaction frequency, and restored chemotherapy responsiveness in vitro and in vivo. Thus, our findings identify iHUBs as important regulators of chemotherapy response and demonstrate their targetability in sensitization to chemotherapy.

MULTI-OMICS TO MECHANISMS: THE ROAD TO MICROBIOME-DRIVEN PRECISION MEDICINE

Purna C. Kashyap

Mayo Clinic, Rochester, Minnesota, USA

The current treatment paradigm of one-size fit all does not consider interindividual variability in an individual's exposome including diet, lifestyle and environment and genetics including host and microbial genes, which underlie the pathogenesis, susceptibility, and outcomes of most chronic diseases to varying levels. While there has been a significant interest in host genetics since with sequencing of the host genome, there is increasing realization that microbial genes also contribute to several pathophysiologic mechanisms. To move towards a personalized medicine approach, we need to consider nonlinear contributions from patient genetics, microbiome and exposome. In my presentation, I will discuss the contribution of the microbiome in the pathophysiology of functional gastrointestinal disorders like irritable bowel syndrome, in the context of host omics and physiological responses, as well as environmental factors. This approach can facilitate personalized treatment strategies by providing more meaningful mechanism-based stratification.

DEEP THINKING: WHERE MACHINE INTELLIGENCE ENDS AND HUMAN CREATIVITY BEGINS

Garry Kasparov

Office of Garry Kasparov, New York, New York, USA

As our machines become capable of more complex tasks, they are evolving from tools to partners—if we use them wisely. There is little doubt that the combination of human plus machine is the key to unlocking the power of artificial intelligence. The key is finding ways to work together, to develop processes that get the best from both. Instead of being afraid of automation encroaching into the intellectual world, we must embrace the potential to discard rote cognitive tasks to focus on the uniquely human elements of life: leadership, creativity, and the pursuit of happiness. Much as a telescope extends human vision, artificial intelligence, or augmentedintelligence, as Kasparov prefers it, will extend our mental abilities. Also like a telescope, we must point our powerful new AI tools in the right direction, ambitiously and imaginatively.

PHARMACOGENOMICS IN CLINICAL PRACTICE: LESSONS FROM INDIVIDUALIZED MEDICINE CLINIC

Adrijana Kekić

Pharmacy Clinical Practice, Mayo Clinic Arizona, Phoenix, AZ, USA

Pharmacogenomics combines sciences of clinical pharmacology and human genomics to predict drug response phenotypes. This presentation will highlight current use of PGx in a clinical practice, its benefits and limitations, and future direction.

THE FUNCTIONAL IMPACTS OF GENE FLOW BETWEEN ARCHAIC AND MODERN HUMANS

Janet Kelso

Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

The genomes of archaic and ancient modern humans offer a unique window into their histories. However, the sequencing and analysis of DNA from ancient humans is complicated by DNA degradation, chemical modifications and contamination. Recent technological advances have made it possible retrieve and sequence DNA from bones and other remains found at archaeological excavations, and we have been able to reconstruct the genomes of several Neandertals. We have also identified, based on their genome sequences, Denisovans, a previously unknown extinct Asian hominin group related to Neandertals.

By comparing these archaic genome sequences to the genome sequences of ancient and present-day modern humans we have shown that gene flow between archaic and modern humans occurred at multiple times, and that this gene flow has shaped the genomes of both Neandertals and of modern humans. For example, the ancestors of some modern humans interbred with Neandertals and Denisovans such that all present-day people outside of Africa carry approximately 2% Neandertal DNA, and that some populations, largely in Oceania, also carry DNA inherited from Denisovans. This introgressed DNA has been shown to have both positive and negative outcomes for present-day carriers: underlying apparently adaptive phenotypes such as high altitude adaptation, as well as influencing immunity and disease risk. In recent work we have identified Neandertal haplotypes that are likely of archaic origin and determined the likely functional consequences of these haplotypes using public genome, gene expression, and phenotype datasets.

THE GENETIC HISTORY AND ORIGIN OF THE BLACK DEATH

Johannes Krause

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High throughput DNA sequencing has revolutionized the field of archaeogenetics in the past decade, providing a better understanding of human genetic history, past population dynamics and host pathogen interactions through time. Targeted DNA capture approaches have allowed reconstructing complete ancient bacterial genomes providing direct insights into the evolution and origin of some of the most infamous bacterial pathogens known to humans such as Yersinia pestis, the causative agent of plague. Ancient Y. pestis genomes spanning over 5000 years of human history from the Stone Age to modern times provide novel insights into the evolution of one of the most infamous human pathogens. They provide direct evidence for the timing and emergence of major virulence factors essential for the transmission of Y. pestis by fleas. The oldest reconstructed genomes of Y. pestis fully capable of causing the bubonic form from the Eastern European Bronze Age provides evidence for prehistoric epidemics in prehistory. Suggesting that the emergence of this form of the disease happened more than 1000 years earlier than previously suggested. Temporal studies of pathogens might thus throw new light on the origin of human diseases and potentially allow predicting and preventing further transmissions and dissemination in the future.

THE ORIGINS, DISPERSAL, AND PATHOGEN HISTORY OF CHICKENS

Greger Larson

University of Oxford, Oxford, UK

There are approximately 80 billion chickens on Earth which makes them by far the most numerous domestic animals. Despite their ubiquity there has been little consensus regarding the timing or location or circumstances of their domestication. In order to understand not just when, where, and how they first became associated with human societies, but also what happened next and how their close proximity to people has driven the evolution of Marek's disease, a highly contagious viral neoplastic disease, I will discuss three recent studies. The first critically assessed the domestic status of chicken remains described in >600 sites in 89 countries, alongside an evaluation of zoogeographic, morphological, osteometric, stratigraphic, contextual, iconographic, and textual data. A second study predicated on the direct radiocarbon dating of >20 ancient European chickens reframes the arrival and dispersal of chickens across Eurasia, and a third study uses an ancient DNA approach to sequence the Marek's virus to understand how decades of vaccines have driven the evolution and virulence of this disease. Combined, these studies establish a new and comprehensive foundation for understanding humanity's most important bird.

GLYCANS AS BIOMARKERS AND FUNCTIONAL EFFECTORS IN CARDIOMETABOLIC DISEASES

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The majority of proteins that evolved after appearance of multicellular life are glycosylated and glycans significantly affect structure and function of these proteins. However, due to structural complexity of glycans and the absence of a direct genetic template, the analysis of protein glycosylation is much more complicated than the analysis of DNA or proteins. Consequently, the knowledge about the importance of individual variation in glycans for both normal physiological processes and diseases is still limited. By generating glycomic data for over 100,000 individuals from some of the best characterized clinical and epidemiological cohorts we enabled glycomics to meet other 'omics. Changes in glycosylation have been observed in numerous diseases, often even before other symptoms of a disease appeared, indicating that they may reflect early steps in the molecular pathophysiology of many complex diseases. Initial data from intervention studies and animal models suggest that reversing changes in glycosylation may decrease the disease risk.

SENSITIZING CANCER CELLS TO DNA DAMAGE-INDUCING AGENTS AND ANTI-TUMOR IMMUNITY

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Spleen-associated tyrosine kinase (Syk) is a non-receptor tyrosine kinase that regulates immunity, cell adhesion, and vascular development. Here we found a new role of Syk in DNA repair regulation. We discovered that Syk is activated by ATM and recruited to DNA double-strand breaks where Syk activates the key enzyme in homologous recombination (HR) to promote resection and HR. Furthermore, Syk upregulation and promotion of resection and HR is a potential mechanism of platinum and PARP inhibitor resistance in ovarian and breast cancer with high expression of Syk. This resistance can be overcome by Syk inhibition or deletion for Syk-high expressing cancer cells. In addition, Syk inhibition could promote anti-tumor immunity by inhibiting M2 macrophages. We propose that Syk is a new target to inhibit HR and sensitize resistant tumors to DNA targeted therapy and immunotherapy.

CROSSTALK BETWEEN HYPOXIA AND EPIGENETICS IN BREAST CANCER

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Hypoxia, a common feature of the tumor microenvironment, regulates various cancer biological processes to drive tumor progression. The hypoxia response is primarily controlled by the transcription factor hypoxia-inducible factor (HIF). HIF enhances thousands of downstream target genes and regulates many hypoxia-induced pathological processes in human cancers, including angiogenesis, metabolism, immune evasion, pH homeostasis, cell survival, maintenance of stem cells, and cell migration/invasion. The transcription activity of HIF is dynamically regulated by multiple epigenetic regulators including p300, JMJD2C, ZMYND8, G9a, GLP, CHD4, and BRD4 in cancer cells. HIF regulators are upregulated in breast cancer and contribute to breast cancer progression by augmenting hypoxia response. Apart from protein-coding genes, HIF also globally induces long non-coding RNAs in human breast cancer cells. These hypoxia-induced long non-coding RNAs represent another layer of mechanism of hypoxia-dependent breast cancer progression. Taken together, HIF and epigenetic regulators are mutually regulated and their crosstalk is crucial for breast cancer progression.

GENOME EDITING, STEM CELLS AND NEANDERTALS

Tomislav Maričić

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Neandertals are our closest extinct relatives and using their genomic sequences together with sequences of thousands of humans living today, we were able to define all genomic positions that are unique to modern humans. Out of those, only around one hundred positions have changed an amino acid and the biological significance of those is largely unknown. Since those changes are shared among all humans, we cannot study them in living humans and have to rely on other model systems, such as pluripotent stem cells (PSCs) and genome editing. This allows us to introduce the Neandertal substitution into a human PSC, and study the effect of the introduced substitution in differentiated tissue of interest, e.g., human brain organoids. In my talk, I will first present methodological improvements in genome editing in which we were able to increase editing efficiency and detect and reduce unwanted editing side effects. Then I will present our study of six amino acid changes in proteins that have key roles in kinetochore function and chromosome segregation. We could show that cells with Neandertal substitutions have shorter metaphase and more chromosome segregation errors when differentiated to human brain organoids, than cells without those substitutions. This suggests that one unique feature of modern humans is that the fidelity of chromosome segregation has improved during neocortex development.

DEVELOPMENT OF NEURONAL CELL DIVERSITY IN THE GUT REVEALED BY SINGLE-CELL TRANSCRIPTOMICS

Khomgrit Morarach, Anastassia Mikhailova, Viktoria Knoflach, Wei Li, Ziwei Liu, Fatima Memic, **Ulrika Marklund**

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As largest part of the peripheral nervous system, the enteric nervous system (ENS) spans the entire gastrointestinal tract and organises into irregular ganglia of intermingled neural subtypes. Owing to these challenging anatomical features, research on ENS composition and development has lagged behind that of the central nervous system (CNS). Meticulous work over decades have identified enteric neural subtypes including motor-, inter- and sensory neurons but the ambiguous molecular markers have hampered elucidation of the full cellular complexity, and thus functionality, of the ENS. We have utilised single cell RNA-sequencing to establish unbiased molecular definitions of enteric neurons in the murine small intestine. Analysis of the myenteric plexus identified 12 enteric neuron classes (ENCs) that were validated in tissue by histochemical detection of unique marker combinations. Further transcriptome analysis of the fetal ENS presented a novel neural diversification mechanism. Unlike in the developing CNS, where spatial patterning of stem cells predominates cell fate decisions, myenteric neuron diversity seems primarily formed via identity conversion of postmitotic neurons. We anticipate that the mapping of enteric neuron classes may help to better define enteric neural circuits, while the developmental blueprint could pave the way for efficient derivation of specific enteric neuron types for the purpose of cell-based regenerative medicine or ENS disease modelling.

CAPTURE AND MPS FOR HUMAN IDENTIFICATION

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In 2015, the Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL) became the first accredited forensic DNA laboratory worldwide to validate massively parallel sequencing (MPS) methods for routine casework. These MPS methods were needed to analyze DNA fragments from chemically treated, historical bone samples that were too degraded for traditional PCR enrichment, averaging only 70 base pairs in size. For example, MPS with hybridization capture (capture-MPS) enabled mitochondrial DNA (mtDNA) analysis of Korean War unknowns that were immersed in formalin baths in the 1950s as a post-mortem preservation treatment prior to burial. STR typing of these samples was not possible, and the success rates for Sanger sequencing of mtDNA were very low (~8%). However, with capture-MPS, mitochondrial genome analysis was successful for approximately 60% of all samples tested. This allowed for comparison of mtDNA between profiles obtained from unknowns and family reference specimens from maternal relatives. While the capability for mtDNA analysis led to hundreds of MPS-based identifications of missing U.S. service members from conflicts dating back to the mid-20th century, unsolved cases still remain. These include cases involving consistent mtDNA profiles, those of service members lacking mtDNA references, and others with hypothesized mutational events between the unknown and the family member's mtDNA sequence. Therefore, SNP capture is necessary to expand the capability for DNA-based identifications. This presentation will broadly cover the SNP capture and MPS methods developed and/or evaluated at the AFMES-AFDIL. These targets include a large SNP panel (850,000 SNPs), two medium SNP panels (25,000 SNPs and 95,000 SNPs), a small SNP panel (5,000 SNPs), as well as whole genome enrichment approaches with some comparisons to untargeted whole genome sequencing.

spore print

Aleksey Matveyenko

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Type 2 diabetes mellitus (T2DM) is one of the major health challenges facing today's society and projected to afflict nearly 1 in 3 people by year 2050. T2DM is associated with an increase in population morbidity/mortality, and more recently, has been shown to exacerbate adverse outcomes associated with COVID-19. The pathophysiology of T2DM is mediated by complex interactions among diverse environmental and genetic susceptibilities, which ultimately culminate in the development of pancreatic islet failure characterized by impaired insulin secretory function. Although underlying genetics contribute to the pathogenesis of T2DM, environmental and epigenetic factors appear to be the primary drivers of this disease. Specifically, recent evidence suggests that disruptions of circadian light/dark and fasting/feeding cycles have contributed to the induction of pancreatic islet failure and an overall increase in the predisposition to T2DM. The aim of the presentation is to describe emerging physiological, genetic and epigenetic insights into the role of the circadian system in regulating pancreatic islet function and failure in health and disease.

PROTEIN MONOAMINYLATION IN BRAIN: NOVEL MECHANISMS OF NEURAL DEVELOPMENT, PLASTICITY AND DISEASE

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Histone H3 monoaminylations at glutamine (Q) 5 [e.g., serotonylation (H3Q5ser) and dopaminylation (H3Q5dop)] have recently been identified as epigenetic markers in neurons. These monoaminylation states appear to play critical roles in the mediation of permissive gene expression supported, in part, by adjacent lysine 4 (K4) methylation and have been implicated in diverse biological and disease processes, ranging from neuronal differentiation to the precipitation of drug-seeking behaviors in adult animals. Our previous work demonstrated that H3Q5ser and H3Q5dop are catalyzed by the Transglutaminase 2 (TGM2) enzyme, both in vitro and in vivo. Here, we discuss the identification of a new class of histone monoaminylation, H3Q5 histaminylation (H3Q5his), which displays dynamic expression in brain in the context of sleep-wake cycles. We further find that H3Q5his, unlike H3Q5ser, electrostatically inhibits recruitment of the chromatin reader protein WDR5 and attenuates MLL1 complex methyltransferase activity on H3K4. Importantly, we demonstrate that H3Q5 monoaminylation dynamics are determined by local concentrations of monoamines, which can be sensed by TGM2. This noncanonical erasure/'rewriting' mechanism suggests a previously unreported biochemical process through which certain post-translational modifications can be established and removed by a single enzyme based upon its sensing of local cellular microenvironments.

THE GENESIS OF THE GENETIC LANDSCAPE IN NORTHEAST EUROPE

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It has been well established that postglacial peopling of Europe has involved three major demic expansions. Hunter-gatherers (HG) and early agriculturalists both from West Asia and pastoralists from the Pontic steppe. In addition, there is a more easterly influence in NE Europe detected in the Iron Age. Here we refine this broad narrative in NE Europe. We first explore the dynamics of western and eastern HGs in the Baltics, suggesting more genetic connections between people associated with different cultures/periods than previously thought. Then we document both abrupt and continuous patterns of genetic change during and after adoption of agriculture in the northern East European Plain. A genetic change with the arrival of steppe ancestry can be seen all over the region, whereas later changes are more subtle and region-specific. Finally, we explore patterns of genetic sharing between Estonians and Finns using large scale biobank datasets and novel methods. Despite substantive differentiation in allele frequencies, the two populations sport unexpectedly many segments of long shared allele intervals dating roughly to around 5th/6th century AD. This shows the importance of relatively recent events for the formation of contemporary populations.

IN THE ABSENCE OF SKELETAL REMAINS: STUDYING ANCIENT HUMAN DNA PRESERVATION IN SEDIMENT

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In 2017, we reported the recovery of Neandertal and Denisovan mitochondrial DNA from Pleistocene cave sediments, suggesting that the analysis of sediment DNA may overcome our dependency on the scarce fossil record for investigating the human past. Yet, this work also raised a number of questions that are important to determine the relevance of this newly discovered source of DNA for future research: How common is ancient human DNA preservation in sediment? What are the temporal limits of ancient DNA preservation in sediment? What is the source of this DNA? Do single samples contain DNA from one or several individuals? Can human DNA move between layers? What are the limits of resolution that can be achieved in the analysis of DNA sequences from sediment? To address these questions, we have undertaken several large studies on different scales. On a global scale, we screened sediment samples from more than 200 archaeological sites, mostly in Eurasia, to assess the preservation of ancient human and faunal DNA. On a local scale, we reconstructed genetic time-series data from archaeological sites based on hundreds of sediment samples. For example, at Denisova Cave in Russia, we detected the DNA of Neandertals, Denisovans and modern humans in 175 out of 728 samples, enabling the reconstruction of the occupational history of the site at an unprecedented level of resolution. In Galería de las Estatuas in Spain, we expanded our search of DNA in sediment from mitochondrial to nuclear DNA, which allowed us to detect a turnover in the Neandertal populations that occupied the site. On a microscale, we analyzed DNA from blocks of sediment that had been impregnated in resin for micromorphological analysis, targeting microstructures derived from bone fragments, coprolites and minerals. Together, the results of these studies show that ancient human DNA is a very small yet frequently detectable component of DNA in Pleistocene cave sediment, that the isolation of the DNA from single individuals is possible from sediment, and that the movement of DNA across archaeological layers is not a common phenomenon. Current efforts focus on the improvement and simplification of methods for the recovery and analysis of DNA sequences from sediment, with the ultimate goal of making sediment DNA analysis a more widely used tool in prehistoric archaeology.

METABOLIC REPAIR: A NEW APPROACH TO THE TREATMENT OF ABNORMAL GLYCOSYLATION

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Congenital disorders of glycosylation are a group of genetic disorders that affect protein and lipid glycosylation and glycosylphosphatidylinositol synthesis. For most affected individuals, only symptomatic and preventive treatments are used. Innovative diagnostic approaches using functional glycomics, novel biomarkers, animal models have been developed and our emerging experience with organ transplantation led us to new therapeutic approaches. Most metabolic enzymes demonstrate substrate specificity, including most enzymes of the monosaccharide activation pathways, sugar nucleotide synthesis and transport; pathways, which are very important in the glycosylation process. In most of the cases, one compound is a much better substrate (usually 100- to 1000-fold) than the other structurally similar molecules. The study of inborn errors of metabolism thought us that enzyme specificity is not perfect. Numerous examples exist of “promiscuous” reactions in the context of inborn errors of metabolism, when the concentration of a substrate is highly elevated due to a metabolic block, leading to the production of unexpected metabolites. However, we can also make a use of such a flexibility in treating of inborn errors. The administration of a molecule (e.g., a specific monosaccharide) in high concentration can turn a molecule into a substrate for alternative pathways. In the lack of absolute specificity of metabolic enzymes increasing certain sugar metabolites can activate enzymes of decreased activity leading to suboptimal, but measurable kinetics. Proteins are flexible and amino acid side chains may alter their secondary structure to accept a molecule that is slightly different from the ideal substrate. Emerging metabolic therapies are using this concept in several glycosylation disorders (MPI-CDG, SLC35A2-CDG, SLC35C1-CDG and PGM1-CDG). High throughput drug screens led to unexpected discoveries like the demonstrating the efficacy of epalrestat in altering metabolic flux and enzyme activity in the most common congenital disorders of glycosylation, PMM2-CDG. Here we focus on recent advances in potential therapeutic approaches for CDG.

EPIGENETIC SIGNATURES OF PSYCHOSOCIAL STRESS AND TRAUMA

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The field of social and behavioral epigenetics examines how social and behavioral experiences, such as psychosocial stress, can lead to epigenetic changes. We investigate how psychosocial stress or trauma that is experienced during pregnancy may induce epigenetic marks in future generations. Specifically, we test for associations between maternal stress or trauma, changes in DNA methylation, and health outcomes. Results from two collaborative projects are reported: 1) associations of maternal stress, newborn birthweight, and newborn epigenetic changes in a longitudinal study of mother-infant dyads from the Democratic Republic of Congo (DRC) and 2) investigation of epigenetic signatures of violence trauma in three groups of three-generation Syrian families with contrasting exposures to war violence. DNA methylation (DNAm) data were generated using the Illumina MethylationEPIC BeadChip. In the DRC project, babies at birth (n=66), 6 months (n=7), 1 year (n=34), 2 years (n=31), and 3 years (n=16) were investigated. We identified a signal of epigenetic age acceleration that was significantly correlated with low birthweight and only emerged over time. There was no correlation of maternal stress with newborn DNAm. These results suggest that the impact of low birthweight on adult health may be mediated by epigenetic changes that emerge over the life course. In the Syria project, 45 individuals directly exposed to war violence, 30 prenatally exposed, 15 germ-line exposed, and 45 control individuals were analyzed. Methylation at multiple CpG sites was associated with violence trauma at all three levels of exposure even after strict Bonferroni correction for multiple testing. These results suggest that exposure to violence experienced during pregnancy may impact future generations at the epigenomic level.

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SINGLE-CELL OMICS FOR FORENSIC USAGE

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The separation of individuals from biological mixture and the subsequent genetic characterization and individual identification steps are crucial components of forensic investigation that still pose a significant challenge to the field despite several attempts. Single-cell (multi)omics approaches are an emerging set of tools that are providing an unprecedented level of understanding about cellular identity and processes. In the past few years, several single-cell techniques have emerged that can capture the transcriptome (scRNA-seq), epigenome (e.g., chromatin accessibility, scATAC-seq; DNA-methylation), and genome. These techniques have the potential to solve the challenges that are faced in forensic research, but their adaptation is still lagging behind. Thus, we are exploring the possibility of adapting existing single-cell approaches and develop novel ones for forensic research. First, by using existing scRNA-seq and by developing a novel SNP-based mixture deconvolution bioinformatics pipeline, we succeeded to separate individuals from multi-person blood mixtures according to the individual contributors. In subsequent steps, we were able to determine the sex and biogeographic ancestry (maternal, paternal, and bi-parental ancestry) of the separated individuals and the tissue of origin of the biological mixture. In addition, by comparing the individual SNP profile (from the scRNA-seq) with a reference set (exome-seq), we were able to achieve individual identification of the separated contributors. Next, in order to increase the number of SNPs that can be used for mixture deconvolution and increase the ability of separating more complex mixtures, we tested the possibility of using single-cell scATC-seq and obtained robust separation. At the current state, our novel approach has the potential to identify perpetrators of violent crime from blood mixtures found at crime scenes, while further adaptations may allow moving to other types of biological mixtures. Driven by this success, we are now developing other novel and affordable forensic-specific single-cell methods that will allow determining appearance, ancestry, sex, and other forensically important information.

IMPACT OF DISORDERED METABOLISM ON 3D GENE REGULATION IN DIABETES

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Diabetes mellitus involves systemic alterations in metabolic functions, which contribute to end-organ dysfunction leading to diabetic complications. We previously reported that reduced mitochondrial gene expression and mitochondrial density in type 1 and type 2 diabetic patients were associated with peripheral autonomic neuropathy and delayed gastric emptying. Investigating the underlying mechanisms, we found impaired oxidative metabolism to associate with reduced physiologimarical hypoxic signaling in human and mouse enteric neurons. Genetic and pharmacological manipulations of hypoxia-inducible factor 1 alpha (HIF1A) revealed a role for HIF1A in the regulation of expression of neuronal nitric oxide synthase (NOS1), the source of the gaseous inhibitory neurotransmitter nitric oxide, an established regulator of gastric emptying. Genome-wide analysis of HIF1A binding in conjunction with chromosome conformation capture identified a role for HIF1A in the regulation of target genes including Nos1 by modifying short- and long-range cis-regulatory interactions in part via cohesin recruitment to loop anchors. Pharmacological upregulation of HIF1A levels with a drug approved for use in humans reversed diabetic gastroparesis in female streptozotocin-diabetic mice. In a parallel line of studies, we investigated the role of mitochondrial dysfunction in interstitial cells of Cajal (ICC), electrical pacemaker and neuromediator cells of the gut, which have also been implicated in diabetic gastroparesis. Genetic deletion of the mitochondrial tricarboxylic acid cycle and electron transfer chain enzyme complex succinate dehydrogenase in ICC altered repressive histone and DNA modifications and Kit expression without any deleterious effects on gastric emptying in normal mice but dramatically increased the prevalence and severity of gastroparesis in female but not male diabetic mice. Together, our studies highlight transcriptional and epigenetic mechanisms downstream of impaired mitochondrial function in diabetic enteric neuropathy and gastroparesis.

SOLVING THE MYSTERY OF KASPAR HAUSER

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Molecular genetic identification of historic individuals, also known as Celebrity Genetics, has become a recognized discipline in forensics. These investigations are not only finding interest among a broad audience, the challenging biological material involved often requires the development of alternative technical solutions to yield successful results. Thus, new concepts stimulated by difficult cases have contributed to the field in significant ways. The molecular investigations on remains attributed to Kaspar Hauser perfectly add to this tradition. Kaspar Hauser was a celebrity, the center of curiosity of Germany's Biedermann society, a feature in newspapers and an object of interest to visitors of the city of Nuremburg in the early 19th century. In May 1828, he appeared in Nuremberg seemingly out of nowhere, a lubberly appearance that was barely able to speak and walk. According to his own account, for as long as he could remember, he sat in a small, dark dungeon without ever getting to see anybody else. His only companions were two horses and a dog, all made of wood. While it still remains unclear whether or not his story holds true, the fate of a child growing up in lack of any social contact has been a focus of academic research throughout the past two centuries. As a matter of fact, evidence was brought forward that Kaspar Hauser could have been an abducted prince of the Grand Duchy of Baden, South Germany. In an attempt to shed more light on this assertion, forensic genetic analyses were conducted on samples attributed to him and samples from pedigree members of the House of Baden in the late 1990s and early 2000's. These analyses led to contradictory results. Some of these results were scientifically published, others were only discussed in the media, which left the case unsolved and provided room for speculations. With the emergence of novel forensic genetic methods, including Massively Parallel Sequencing, the case was reopened again in 2019. Old and new samples were investigated using these methods. These analyses not only yielded significant results; they also serve as basis to explain the discrepant data obtained 20 years ago. The new methods and conclusions further provide foreground for the field of forensic genetics and answer some of the questions regarding Kaspar Hauser's descent.

OSTEOARTHRITIS: FROM MOLECULAR PATHWAYS TO THERAPEUTIC ADVANCES

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Being the most common musculoskeletal progressive condition, osteoarthritis is an interesting target for research. It is estimated that the prevalence of knee osteoarthritis (OA) among adults 60 years of age or older is approximately 10% in men and 13% in women, making knee OA one of the leading causes of disability in the elderly population. Today, it is clear that osteoarthritis is not a disease characterized by loss of cartilage due to mechanical loading only, but a condition that affects all tissues in the joint, causing detectable changes in tissue architecture, metabolism, and function. The pathogenesis of OA is primarily determined by the imbalance of pro-inflammatory and anti-inflammatory mediators, leading to low-grade inflammation, which is responsible for cartilage degradation, bone remodeling, and synovial proliferation. The pathogenesis of this degenerative process is not completely understood; however, a low-grade inflammation leading to an imbalance between anabolic and catabolic processes is a well-established factor. The complex network of cytokines regulating these processes and cell communication has a central role in the development and progression of osteoarthritis. In addition, concentrations of both proinflammatory and anti-inflammatory cytokines were found to be altered depending on the osteoarthritis stage and activity. At the moment, biological treatments such as platelet-rich plasma, bone marrow mesenchymal stem cells, and autologous micro-fragmented adipose tissue (MFAT) containing stromal vascular fraction (SVF) are ordinarily used. The cell-based treatment options seem to be the only methods so far that increase the quality of cartilage in osteoarthritis patients. Mesenchymal stem cell (MScs) research offers new opportunities for osteoarthritis treatment as their paracrine effect exhibits clinical improvement in osteoarthritis patients, providing much-needed minimally invasive treatment options. In my lecture, I will present several prospective, non-randomized, interventional, single-center, open-label clinical studies performed at St Catherine hospital where patients with OA, were treated with the intraarticular application of autologous micro-fragmented adipose tissue (MFAT) containing stromal vascular fraction (SVF). After the treatment, dGEMRIC sequencing was used to analyze the

contents of cartilage glycosaminoglycans (GAGs) in specific areas of the treated knee joint as the anionic, negatively charged contrast gadopentetate dimeglumine (Gd-DTPA2−) infiltrates into the cartilage, thus indirectly showing the amount of GAGs in areas of interest at different time points. Our results showed a stable dGEMRIC index in the first 12 months after application of MFAT and a mild decrease in dGEMRIC index after 24 months of application. We believe that molecular changes in the cartilage in patients with OA are mediated by a complex interplay of pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and adipokines. Therefore, we recently launched one of the most comprehensive multi-omic study (Clinical and molecular phenotypization of OA: personalized approach to diagnostics and treatment) on OA patients, aiming to explore cytokines, chemokines, N-glycans, phenylalanine, and miRNA changes in the plasma and synovium before and after intraarticular application of MFAT with SVF in knees of the patients with OA. Simultaneously, we will observe changes in the glycosaminoglycans level (GAG) by using delayed gadolinium (Gd)-enhanced magnetic resonance imaging of cartilage (dGEMRIC), but also, we will perform a standard orthopedic physical examination including KOOS, WOMAC, VAS, CESD-R assessments as well as MRI Osteoarthritis Knee Score (MOAKS).

AFTER MORE THAN 20 YEARS: UPDATE ON THE DNA IDENTIFICATION OF 9/11 VICTIMS

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The terrorist attack on the World Trade Center in New York City on September 11, 2001 took the lives of at least 2,753 individuals on the ground and on the two airplanes. As of March 2022, 1,647 or approximately 60% of the victims have been identified. This mass fatality incident was characterized by severe fragmentation, degradation, and destruction of the human remains, which explains why so many of the victims seem to have disappeared. With only 289 intact bodies, but over 20,000 fragments of human remains, the World Trade Center victim identification became a DNA driven effort. Short tandem repeat (STR) analysis was the main tool for typing muscle tissue and bone samples, as well as ante mortem personal effects and buccal references. The project triggered the development of DNA matching software tools and quality review and anthropological verification procedures. Victim identification took place in several distinct phases and is still ongoing today. Systematic resampling of previously unidentified remains has led to additional identifications, with the most recent ones having been reported in September 2021. The talk will provide an overview on lessons learned during the project and present the most recent developments.

CHARACTERIZING LOCUS SPECIFIC CHROMATIN STRUCTURE AND DYNAMICS WITH CORRELATIVE CONVENTIONAL AND SUPER-RESOLUTION IMAGING IN LIVING CELLS

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The spatio-temporal organization of chromatin is critical for gene regulation. However, simultaneously mapping the structure of chromatin and its dynamics remains a challenge. Conventional fluorescence microscopy in combination with CRISPR/dCas9 labeling has been able to image the spatial distribution and dynamics of loci in living cells, but cannot resolve structural features below ~250 nm. The advent of super-resolution photoactivated localization microscopy (PALM) presented a breakthrough for resolving intracellular structures with up to ~20 nm resolution in fixed cells. However, the motion of chromatin during the long data acquisition time precludes any structural characterization of chromatin in living cells due to motion blurring. Here I will present our correlative conventional fluorescence and PALM imaging approach to quantitatively map time-averaged structure and dynamics of chromatin below the optical diffraction limit in living cells. By employing a repetitive telomere sequence as a well studied model system and by assigning localizations to a telomere as it moves, we reliably discriminate between bound and unbound dCas9 molecules, whose mobilities overlap. Our approach accounts for changes in DNA mobility and relates local chromatin motion to larger scale domain movement. In our experimental system, we show that compacted telomeres move faster and have a higher density of bound dCas9 molecules, but the relative motion of those molecules is more restricted than in less compacted telomeres. Correlative conventional and PALM imaging therefore improves the ability to analyze the mobility and time-averaged nanoscopic structural features of locus specific chromatin with single molecule sensitivity and yields unprecedented insights across length and time scales.

TARGETING EPIGENETIC REGULATOR MUTATIONS IN KIDNEY CANCER

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Clear cell renal cell carcinoma (ccRCC) accounts for ~75% of kidney cancers and is the 8th leading cause of cancer death in the United States. After completion of The Cancer Genome Atlas (TCGA) Project, clinically actionable mutations were identified in virtually every solid tumor. One major exception, however, is RCC, where the current standard of care, checkpoint inhibitor and anti-VEGF therapy, does not take into account that ~50% of RCCs have mutations in chromatin regulators. After first-line therapy, response rates are 20% and there are no FDA-approved therapies that target chromatin regulators, highlighting the need to identify how epigenome regulator mutations can be therapeutically targeted. The epigenome is profoundly disrupted in cancers including ccRCC, including altered DNA and histone methylation patterns that promote oncogenic transcriptional patterns and elevated DNA damage. Aside from the near ubiquitous loss of VHL, the mutational landscape of ccRCC is dominated by loss-of-function mutations in epigenetic regulators, including SETD2, BAP1, and PBRM1. SETD2, the sole factor responsible for trimethylating the histone H3 lysine 36 position, has been firmly linked to poor outcome and the promotion of metastasis. SETD2 and its mark H3K36me3 have been linked to diverse processes ranging from transcriptional regulation, mRNA splicing, nucleosome positioning, and DNA repair, yet exactly how this regulator and its mark drive cancer phenotypes, particularly in ccRCC, remains unknown. Using a combination of engineered cell line models, biochemical methods, and primary patient tumors, coupled with transcriptome/epigenome analysis and interaction studies, we describe novel ways that SETD2 loss-of-function contributes to cancer initiation and progression. We also probe the interplay among multiple regulators of methylation at the H3K36 position to define novel pharmacologic paradigms that may lead to individualized therapies that target SETD2 mutant tumors.

MULTIOMICS REVEALS NEW PATHOBIOLOGIC TARGETS FOR ALCOHOL ASSOCIATED HEPATITIS

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Alcoholic hepatitis (AH) is associated with liver neutrophil infiltration through activated cytokine pathways leading to elevated chemokine expression. Super-enhancers are expansive regulatory elements driving augmented gene expression. Here, we explore the mechanistic role of super-enhancers linking cytokine TNFα with chemokine amplification in AH. Our findings highlight the role of super-enhancer in propagating inflammatory signaling by inducing chemokine expression and the therapeutic potential of BET inhibition in AH treatment. Alcohol-associated liver disease (ALD) in its earliest form is evidenced as hepatic steatosis which may progress to liver cirrhosis. The mechanisms behind this initiating insult are poorly understood and therapeutics to treat ALD are limited. Liver is a specialized organ with cells exhibiting heterogeneity along the porto-central axis. Periportal preponderance of lipid droplet accumulation was noted in human ALD livers compared to other clinical causes of hepatic steatosis. Using single-cell multiomics technology, we studied transcriptional mechanisms across the hepatic lobule that could account for liver zonation of lipid droplets in a murine ALD model. We utilized multiomics data to provide novel insight into HNF4α and PPARα mediated, zone-specific regulation of HSD17β13. We conclude that mechanisms underlying ALD initiate in a zonated manner leading to spatially distinct establishment of hepatic steatosis and provide novel insight into disease pathogenesis.

THE ORIGINS OF HANSEN'S DISEASE (LEPROSY)

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Hansen's disease, also known as leprosy, is caused by the pathogens Mycobacterium leprae and the more recently discovered M. lepromatosis, which is primarily found in Mexico and the Caribbean. Hansen's disease is one of the oldest known human diseases and remains a public health issue today, with over 200,000 new cases reported yearly. Ancient genome analyses show that M. leprae lineages have a most recent common ancestor approximately five thousand years ago. However, the global pattern of genomic variation in M. leprae is not well defined. This is particularly true in the Pacific Islands, where the origins of the pathogen in humans in relation to Colonialism are disputed, and in animals, which are poorly surveyed for this pathogen. To investigate this, we have extracted DNA from 98 formalin-fixed paraffin-embedded biopsy blocks collected between 1992 to 2016 from patients living in the Pacific and from PCR positive samples from 11 species of animals from Brazil. We have also survey small mammals in the Amazon basin of Peru. To date, we have successfully used whole-genome enrichment and next-generation sequencing to generate 12 Pacific M. leprae genomes ranging from 1.6 - 63x depth of coverage. Phylogenetic analyses place these strains in branches 0 and 5, the basal lineages of the M. leprae phylogeny. The phylogeographical patterning and evolutionary dating analysis of these strains support a pre-modern introduction of M. leprae into the Pacific Islands. We have also expanded this work by including time-series samples from patients during treatment and will use both empirical data and modelling to identify ongoing selection. In Peru, the 92 small mammals tested to date have been negative using the RLEP qPCR assay, while initial capture of the Brazilian samples is in progress. This research provides insight into the evolutionary history of M. leprae and the exchanges of this pathogen among species.

GENES, CULTURE, AND HUMAN EVOLUTION

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A commonly-held view is that humans have stopped evolving because we rely on culture to adapt to changing circumstances. However, an alternative view is that culture can also influence human evolution. I will show, by way of examples, that some cultural practices have directly impacted specific genes, while others have indirectly influenced patterns of genetic variation. Furthermore, given that some cultural practices have genetic consequences, we can use genetic analyses to learn more about such cultural practices; as an example, I will discuss a genetic approach to dating the origin of clothing.

INFERRING GENETIC RELATIONSHIPS FROM WHOLE GENOME SEQUENCES - A FORENSIC PERSPECTIVE

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Being able to determine genetic relationships between individuals has long been important in legal medicine and forensic applications, like paternity testing and missing person identification. Current practice typically involves DNA analysis of a small number (around 20) of short tandem repeat markers, which usually is sufficient to establish close relationships. Recent advances in DNA typing technologies have however made it possible to a relatively low cost, with increased sensitivity and with high quality, obtain much more DNA information from a single DNA analysis. This opens up new possibilities for inferring distant relationships but also for introducing new forensic applications such as investigative genetic genealogy. Large DNA datasets may require new methods for the relationship inference and for the assessment of the statistical weight. This presentation will provide an overview of such methods and show how large DNA datasets, obtained from whole genome sequencing, could be used to infer genetic relationships in legal and forensic settings.

REGULATION AND DEREGULATION OF DNA EPIGENETIC MARKS

Natalia Tretyakova

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Reversible DNA methylation allows for the precise activation and inactivation of genes in a tissue-specific manner by mediating DNA-protein interactions and influencing chromatin structure. 5-Methylcytosine (5mC) marks are introduced by DNA methyltransferases (DNMT), while DNA demethylation is initiated by ten eleven translocation (TET) dioxygenases which oxidize 5mC to hydroxymethyl-C (hmC), formyl-C (fC) and carboxy-C. While DNA methylation patterns are stable in healthy somatic tissues, they can become scrambled as a result of inflammation, environmental stress and chemical exposures, contribution to cancer initiation. Recent studies revealed that many other diseases including asthma, Alzheimer's disease, and autism have epigenetic drivers. My laboratory investigates the potential role of DNMT and TET proteins in inflammation-mediated colon cancer and smoking induced lung cancer. We have conducted animal studies to show that chronic inflammation and chronic exposure to cigarette smoke cause dramatic changes in DNA hydroxymethylation, gene expression changes, and aberrant protein expression. Mechanistic studies with recombinant proteins probes potential mechanisms for the observed epigenetic changes, while CRISPR-cas 9 gene editing and RNA interference studies are starting to reveled the functional roles of the affected genes in lung cancer. We employed structure-based design to develop small molecule inhibitors of TET proteins, which can be used as chemical probes to investigate the functions of DNA demethylating enzymes in lung cancer and could comprise initial leads for future drug design. Finally, we are investigating reversible cross-linking between fC in DNA and histone proteins as a potential novel mechanism of epigenetic regulation.

DEVELOPMENTS TOWARDS PERSONALIZED EPIGENOMIC PROFILING IN FORENSICS

Athina Vidaki

Erasmus MC, University Medical Center Rotterdam, Department of Genetic Identification, Rotterdam, The Netherlands

Human genetic variation is a major resource in forensics, but does not always allow us to answer crucial forensically relevant questions. Since the epigenome acts as an interphase between the fixed genome and our dynamic environment, it offers possibilities to address many questions regarding an individual's phenotype. Back in 2017, we proposed that, together with genetic prediction of appearance and biogeographical ancestry, DNA methylation-based ageing and lifestyle habit prediction is expected to increase the ability of narrowing down suspect pools. However, there are still various challenges to be addressed prior to implementing such approach in forensics. During this talk, I aim to present our recent developments towards offering a personalized epigenomic fingerprint. First, to consider the issue of dealing with heterogeneous material, we focused our efforts investigating age-associated patterns of the Y-chromosome, in both blood and sperm. We envision that a future Y-chromosome based age prediction tool would allow us to estimate the age of males from mixed samples, often encountered in sexual assault cases. Secondly, to apply already existing knowledge on lifestyle-associated DNA methylation, we built and thoroughly validated statistical models for the prediction of both tobacco smoking and alcohol consumption habits. In the case of smoking, we also developed a targeted lab method based on next-generation sequencing, which we optimized and tested in a population cohort. Thirdly, to improve DNA methylation detection towards standardization, we developed and validated a novel, patent-pending tool for assessing the initial step of bisulfite conversion, which is currently the golden standard. Finally, to deal with the limited amount of available human biological material, we are currently focusing in developing a new technology, CpGtracer that will soon allow us to simultaneously analyze hundreds of DNA methylation markers from trace amounts of DNA. Overall, while there are still additional considerations to tackle, including privacy, ethical and legal concerns, we are confident that a broadened DNA-based forensic intelligence including epigenomic profiling will soon become valuable, especially during criminal investigations with unknown suspect(s).

CANCER PHARMACOGENOMICS: DISCOVERY AND TRANSLATION

Liewei Wang

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A major challenge facing genomics science is understanding and predicting how sequence variation in noncoding regions of the genome might contribute to variation in gene regulation, variation that could result in variations in various phenotypes either cellular or clinical phenotypes. This challenge is highlighted by the fact that approximately 90% of genome-wide association study (GWAS) single nucleotide polymorphism (SNP) signals map outside of protein coding genes (1-3). A significant advance has been our recognition that many of these GWAS SNP signals locate in the non-coding regions, regulating gene expression through enhancer, so called “expression quantitative trait loci” (eQTLs), SNPs that are associated with variation in gene expression (3, 4). Here, we will present a novel mechanism that we have identified repeatedly for these SNPs using breast cancer clinical trial DNA samples to understand how the SNP might influence drug response, both efficacy and toxicity. What we have found is that the SNP effect on gene expression, leading to different clinical outcome, is dependent on the presence of drugs/hormones mediated though nuclear receptors such as the estrogen and glucocorticoid receptors. This type of SNP-gene expression relationship is often not present or is much less significant at baseline—ie before ligand (either agonists or antagonists) exposure, and occurs only in the presence of a drug or hormone. This type of SNP- gene expression relationship highly depends on the presence of individual endogenous or exogenous compounds, so called PGx eQTL, which can occur often and can be highly significant functionally. As a result, we could potentially take advantage of specific SNP genotypes to manipulate gene expression by exposure to various compounds. These SNPs could be also used as biomarkers associated with various clinical phenotypes.

SIGNAL-DEPENDENT CHROMATIN REMODELING IN METABOLISM AND INFLAMMATION

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In type 2 diabetes (T2D), inflammation induces massive changes in the transcriptome and epigenome of islet endocrine and immune cells, resulting in eventual dysfunction of islets. We identified a novel mechanism of vitamin D-dependent chromatin accessibility dynamics, orchestrated by the balance between two SWI/SNF chromatin remodeling complexes, BRD9-BAF and BRD7-PBAF, in regulating islet dysfunction. In beta cells, the balance between BAF-BRD9 and PBAF-BRD7 determines the VDR-driven anti-inflammatory and pro-survival response. Pharmacologically potentiated VDR signaling by a synthetic ligand in combination with a BRD9 inhibitor can partially restore beta cell function and glucose homeostasis in various T2D mouse models. Tissue specific genetic models further demonstrated the functional role of VDR and BRD9 in beta cell stress response in vivo. Recently, we also identified BRD9 as a modulator for glucocorticoid responses in macrophages. Pharmacologic inhibition of BRD9 potentiated the anti-inflammatory responses of dexamethasone. Mechanistically, BRD9 co-localized at a subset of GR genomic binding sites, and depletion of BRD9 enhanced GR occupancy primarily at inflammatory-related genes to potentiate GR-induced repression. Together, our results revealed the context-dependent function of specific SWI/SNF subunits on VDR/GR activity, and demonstrated the therapeutic potential of targeting bromodomain readers to synergistically enhance NR function.

NEANDERTAL GENE VARIANTS AND COVID-19

Hugo Zeberg[1,2]

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Early in the COVID-19 pandemic, it was clear that SARS-CoV-2 infection tend to have drastically different outcome in different people. Whereas some patients suffer only mild disease, others become critically ill. Some risk factors, such as high age and metabolic syndrome, can explain some of the variability but far from all of it. Therefore, host genetic risk factors have been investigated by several large studies, which have been successful in identifying more than 20 risk genetic risk factors influencing the outcome of COVID-19. In this talk I will describe the evolutionary history and the Neandertal origin of two of the major genetic variants influencing the outcome of COVID-1

High school student Future scientist award presentations

Presentation number: FSA 1

DAMAGE TO HAIR CELLS DURING SPINNING IN RELATION TO NUTRITION (VESTIBULOCOCHLEAR ORGAN MODEL)

Filip Bulat

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The sensory receptors of vestibulocochlear organ in humans are hair cells which transmit the stimulus via nerves to the brain. Evolutionary, they are of ectodermal origin. Many diseases affect these cells resulting in degradation and loss of function in vestibulocochlear system. We were interested in knowing how starvation, i.e., malnutrition affects ciliates Paramecium sp. The purpose of the research was to experimentally test the reaction of malnourished Paramecium sp. (starving Paramecium) to spinning, which is a physiological stimulus for the vestibular organ. The same volume of Paramecium culture medium of good physiological condition with optimal concentrations of oxygen in the water was placed in 10 test tubes marked with letters A1 to A5 and B1 to B5. The culture (Paramecium sp.) that was fed was marked group A, while the malnourished culture was marked group B. The test tubes were kept on a rack. The same volume of sample was added to each test tube. Immediately before spinning each tube was tightly screwed and put in a separate small transparent bag tied at the top with the wool string 10 cm long. Bags were evenly spun for 5 minutes. The test tubes were then placed back on a rack and after 10 minutes a sample from the test tube was taken with a pipette and put in a Petri dish on the bottom of which a net for counting Paramecium sp. sized 1cm x 1cm was drawn. The Petri dish was then filled with a sample of Paramecium sp. culture medium to the 3 mm mark. The experiment was conducted in the following order: A1 and B1 were not exposed to trauma, and they represent control samples, while other samples were exposed to spinning: A2 and B2: on the 1st, 4th, and 8th day of the experiment, A3 and B3: on the 1st, 3rd, 5th, 7th, and 8th day, A4 and B4: one spin each day, A5 and B5 two consecutive spins each day. The proportion of dead Paramecium in a given sample volume during 8 days after spinning was measured in relation to the control sample. Compared to control sample in test tube A2, 18,33% specimens die. In test tube B2 20,83% specimens die. In both samples the majority of Paramecium survives and has proper mobility. The total number of live specimens in sample B2, on the eighth day of the experiment, is smaller in relation to the total number of live specimens in sample A2 on the eighth day of the experiment. 40.67% of Paramecium die in sample B3 after eight days, while 28,21% of Paramecium die in sample A3. 43,75% of Paramecium die in sample B4, and 44,83% of Paramecium die in sample A4. Test tube B5 has 50,21% of dead Paramecium, while 42,54% of Paramecium die in sample A5. Results show that better nutrition leads to better resistance to physiological, and especially to extreme stimuli, and the survival of the cells is thus greater. Since there is no data on the subject that was analyzed, it is assumed that better condition of well-nourished specimens results in better resistance to damage; therefore, sensory receptors that have proper nutrition (blood flow) are more resistant to damage and degradation of function in vestibulocochlear system.

Presentation number: FSA 2

THE COMPARISON BETWEEN CURRENT TREATMENTS FOR EARLY ALZHEIMER'S DISEASE

Ante Čolak

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Detailed study and analysis of the accession literature on the basis of which the discussion of the comparison was made. Aducanumab is effective in removing β-amyloid plaque, but as an anti-AD drug it is not effective in preventing or alleviating symptoms. On the other hand, symptomatic therapies are effective in alleviating the cognitive decline caused by neurodegeneration, but are not effective in preventing the development of AD. The first, the PRIME test, showed that aducanumab reduces amyloid PET SUVR findings 15 within 54 weeks of treatment. Due to the promising results, Biogen decided to conduct two new studies: EMERGE and ENGAGE. Moreover, EMERGE met its goal: subjects treated with the highest dose, 10 mg/kg, had a significantly better result (30% better) measured on the Clinical Dementia Rating Scale (CDR-SB). Furthermore, participants in the ENGAGE study improved their cognitive performance by 27% measured by CDR-SB. Current treatments for Alzheimer's disease vary from symptomatic treatments to the drug known as aducanumab which reduces the β-amyloid plaques that are considered to be one of the causes of this neurodegenerative disease. Due to the insufficient improvement of cognitive abilities among patients, aducanumab has proved to be insufficient treatment for cognitive manifestations of the disease. Comparatively, symptomatic treatments, like acetylcholinesterase inhibitors and NMDA receptor antagonists, help patients improve their cognitive capabilities, meaning they are more effective than aducanumab regarding the symptoms. An indirect benefit of the discovery of aducanumab is shaping the course of Alzheimer's research.

Presentation number: FSA 3

THE IMPACT OF DISINFECTANTS ON DIFFERENT TYPES OF BACTERIA AND BACTERIAL RESISTANCE

Mei Đulabić Chalfe

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The aim was to determine how everyday and ubiquitous disinfectants affect different types of bacteria and whether different cosmetic ingredients in some antibacterials such as hand gel affect the growth of different types of bacteria positively or negatively. Recognize if the resistance of different bacteria to the same disinfectants differs. Also, the aim of the study was to determine whether Salmonella's resistance to disinfectants differs from other gram-negative bacteria. Collecting swabs from 7 places then applying diluted solutions of these swabs to various selective and differential nutrient substrates (Plate Count agar, Mac Conkey agar, BD Salmonella Shigella Agar). Placing Petri dishes in an incubator and calculating the CFU before and after using the disinfectant. Gram-negative bacteria were on average more susceptible to disinfectants than other bacteria. The most effective disinfectant was 70% ethanol alcohol. The least effective disinfectant was antibacterial wipes. Salmonella was more resistant to disinfectants than other gram-negative bacteria. The main conclusion of the study is that all types of bacteria have shown some kind of resistance to the disinfectants used, which coincides with the read literature and hypothesis.

Presentation number: FSA 4

THE INFLUENCE OF 21 CENTURY ON HIGH SCHOOL STUDENTS' MENTAL HEALTH

Robertina Filković

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This research is carried out to confirm the fact that social networks and school have negative influence on adolescents' mental health. The fact is proven by two hypotheses: 1. Social networks have negative influence on high school students' mental health. 2. The pressure made for better academic accomplishment leads to higher number of suicidal high school students. The research is carried out with two questionnaires. First questionnaire, which was carried out at the end of the January, was solved by 374 high school students. Second questionnaire is carried out to establish how much does school affect students' mental health. It was carried out in mid-April and was solved by 238 students. Results from first questionnaire show that today many high school students have symptoms of mental illnesses. The main cause is technology. According to answers social networks cause development of anorexia nervosa and bulimia nervosa. On average, school success is important to examinees and among those who said that school success is very important 23 think about suicide and 14 resort to self-harm. Depression symptoms mostly appear at the examinees who engage with sport. The results show that high school students are getting suicidal, and they are finding help in illicit substances. They are isolating themselves and their social life and academic success are failing. To reduce the number of high school students who are suffering from mental illnesses, parents and students' education is needed.

Presentation number: FSA 5

REVIEW OF MICROPLASTICS AND NANOPLASTICS EFFECT ON HUMAN HEALTH AND EPIGENETICS MODIFICATIONS

Lucija Glavičić Marović

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This essay aims to review the current body of knowledge on the effects of microplastics and nanoplastics on human health. The additional objective is to discuss the possible impact of microplastics and nanoplastics on human epigenetic modifications. An electronic search of published articles was conducted in PubMed and Google Scholar using the following keywords: “microplastics human health”, “microplastics epigenetic”, and “microplastics epigenetic modification human”. Microplastics can pose risks to human health in three ways: as physical particles, chemicals, or microbial pathogens found in biofilms on microplastic particles. The most important entry route to the human body is ingestion. Only small microplastic particles can be absorbed (<150 μm can penetrate the gastrointestinal epithelium, <100 nm can penetrate the dermal barrier, <10 μm can be absorbed through the alveolar epithelium). Potential harmful pathways to human health include gut and lung inflammation, oxidative stress and cytotoxicity, translocation, metabolism and energy homeostasis, neurotoxicity, reproductive toxicity and carcinogenicity. Prenatal and neonatal exposure to certain chemicals forming plastics (bisphenol A, phthalates, and others) can cause not only epigenetic changes, but also genetic and morphological changes. The potentially harmful effects are determined mainly by levels of exposure, concentration and individual susceptibility. Growing evidence suggests that increased exposure to microplastics can increase incidence in gut and lung inflammation, obesity, immunological disorders, neurological diseases, cardiovascular diseases and cancer. The increasing exposure of humans to microplastics calls for further research to understand its impact better.

Presentation number: FSA 6

INFLUENCE OF NATURAL AND COMMERCIAL DISINFECTANTS ON BACTERIAL GROWTH AND DEVELOPMENT

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The aim of the research was to determine which of the agents used has antibacterial properties and which disinfectant is the most effective when it comes to bacterial growth inhibition. An experiment was conducted comparing the effects of commercial disinfectants (soap, antibacterial soap, antibacterial hand gel) and natural disinfectants (aqueous clove extract, aqueous garlic extract, lavender oil, immortelle oil) on bacterial growth and development. Samples were taken from the classroom handle. Swabs were inoculated into tripton soy broth, after incubation decimal dilutions were made which were then inoculated on nutrient agar. After incubation of nutrient agar, colonies were counted. The following parameters were changed in the experiments: incubation time of tripton soy broth and nutrient agar, type of disinfectant and volume of disinfectant. The results showed that lavender oil was the most effective in inhibiting the growth of bacterial colonies, while antibacterial hand gel was the least effective. Disinfectants are more effective when placed in a larger volume (1 ml) than in a smaller one (0.2 ml), and pentanol-based antibacterial soap inhibits bacterial growth better than regular sodium benzoate-based soap. In conclusion, all disinfectants used showed antimicrobial properties. In further research, it would be desirable to investigate the disinfectant effect of lavender oil on specific species of bacteria and compare the results with other natural disinfectants. The impact of the active substance on soap effectiveness could also be researched in the future.

Presentation number: FSA 7

EFFICIENCY AND FUNCTION OF SUNSCREEN

Nika Adriana Marijanović

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Sunlight consists of different spectrums of radiation such as visible light, ultraviolet (UV) and infrared light. Light is measured in wavelengths (λ), and the unit of measurement is nanometer (nm) and millimeter (mm). Different light radiations in the spectrum have different wavelengths. Sunlight can have positive and negative effects on human health. Negative effects can overshadow the positive effects with a lack of caution when exposed to sunlight for longer periods of time. Some of the consequences can be burns, premature skin aging, hyperpigmentation, allergic reactions and more serious conditions such as skin cancer. These kind of skin damages caused by the sun are mainly due to UV rays. There are many different products on the market that can protect our skin from damaging rays. The sun protection factor rating (SPF) system for sunscreens is based on the level of UVB protection offered by the product. SPF multiplied by natural skin protection in minutes determines the maximum length of time you can expose your skin to the sun without the risk of UVB-induced skin damage. However, SPF protection depends on the use of the right amounts (2 mg/cm^2) of the product, therefore 60% of the time allowed by SPF is recommended. The aim of this experiment was to examine the effectiveness and quality of different sunscreens. The hypothesis of this research was that sunscreens with a higher factor will provide better cell protection. For this experiment, a total of 6 different sunscreens was used, of which 3 with a factor of 50 and the other 3 with a factor of 30. The MTT test, a standard test that measures the survival (viability) of cells after a treatment was used in the study to measure cell survival after exposure to UVB radiation. The best cell protection was provided by Eucerin 30 cream with a viability of 111.35%, followed by Vichy 50 (101.39%), the third Avon 50 (98.59%), followed by Eucerin 50 (95.64). %) and with lower viability Nivea 30 (79.3%) and Clarins 30 (78.02%). After all the information gathered from this experiment, I can conclude that the sunscreen Eucerin 30 provides the best protection against UV radiation, and Clarins 30 the worst, even though it is the most expensive product. I have also noticed that creams with a thicker composition also have a better effect. Spray sunscreens such as Nivea 30 and Clarins 30 provided the weakest protection.

Presentation number: FSA 8

INFLUENCE OF EUTROPHICATION ON CETINA RIVER MICROORGANISMS

Frane Marušić

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The aim of the study was determining the effects of anthropological eutrophication on microorganisms in the Cetina River. Methods used were the following: smear method, incubation, rapid KOH test (determination per gram), flow cytometry and morphological characterization of bacteria. Compared to the data from the water sample collected five kilometers before the mouth of Cetina, quantitative data from the flow cytometer showed a decrease in the total amount of bacteria at the mouth of the river Cetina, where the city of Omis is located, but also a marked increase in bacterial predators (HNF) and cyanobacteria. Comparing these data with other research, it was found that eutrophication is a possible impact on the differences observed in the entire aquatic ecosystem and the food chain of the Cetina River. On the other hand, the characterization of bacteria showed that there is no significant difference between the types of bacteria at the stations, which leads to the conclusion that eutrophication does not affect the diversity of bacteria at the Cetina river. Humans have a great impact on the ecosystem of the Cetina river and are one of the causes of significant changes in the community of microorganisms. Although eutrophication is not yet strongly expressed in the Cetina River, it is necessary to protect Cetina so that more negative consequences do not follow, such as the development of infectious diseases, algae blooms, and degradation of recreational opportunities.

Presentation number: FSA 9

S IS FOR STIRRING THE SENSES (NOT THE MUSHROOM SOUP)

Nika Miličević

V. Gymnasium, Zagreb, Croatia

The aim of the research is to determine whether the application of different types of synesthesia will improve the performance of three tasks related to memory, concentration, and visual perception in high school students. The intention is also to compare the effects of synesthesia exercises in 1st and 3rd-grade students and to compare the results of girls and boys in the experimental groups. The study involved 200 high school students: 100 1st grade students and 100 3rd grade students with equal proportions of boys and girls. Students were divided into control and experimental groups and solved tasks for examining memory, concentration, and visual perception in separate classrooms. The first experiment examined whether colors help with grapheme memorization, the second whether drawing and eating candy help concentrate-, and the third experiment whether sound and touch improve visual perception. Each experiment lasted 60 seconds. The results were processed by a two-way t-test in Excel. Processing the results, it turned out that the differences between the 1st-grade groups were not, and between the 3rd-grade groups were statistically significant, and that the 1st-grade girls in the concentration and visual perception experiments had higher results than the 1st-grade boys. Synesthesia was found to help 3rd graders solve tasks, but not the 1st graders. It turned out that only older boys were significantly better in all experiments compared to younger 1st graders and that 1st-grade girls were significantly better only in memory and concentration experiments than 1st-grade boys.

Presentation number: FSA 10

DIFFERENCES IN THE HUMAN JAW OF THE MODERN HUMAN FROM *AUSTRALOPITHECUS AFRICANUS*

Goran Narančić

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The aim of this investigation is to establish the presence of metric trends in hominid dental evolution by comparing the teeth measurements of gypsum jaw models of modern humans from Western Balkans with the measurements of Australopithecus from Wolpoff's data. The method of measuring the dimensions of the teeth is based on the Lundstrom's method. Mesiodistal (in the direction of the tongue, "from the back to the front") width of tooth's crown and its breadth (from right to left) were measured with a caliper. For the teeth in the same part of the jaw (upper and lower) and of the same type and position (for instance the second incisor or first molar) the arithmetic mean of all measurements for that tooth was calculated and the standard deviation as well. The incisors in the modern human samples were significantly smaller than the ones in Australopithecus, while the difference was significantly larger in the second incisor than in the first one. In Australopithecus the canines are as much as 18% larger than in the modern human sample. The premolars of Australopithecus are significantly greater than those of the modern man, but the second premolar is much smaller than the first one in modern human. The first molar is significantly larger in Australopithecus than in the modern man. There is a clear metric trend of a decrease in teeth size in the evolution of the human jaw on the sample of the people from the Western Balkans and some teeth have shrunk more than others. It is to be assumed that the same trend will keep up in the future if the assumed causes of this trend are true and if the sedentary lifestyle carries on as it is.

Presentation number: FSA 11

COELIAC DISEASE

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The aim of the study was designing a preparation with a therapeutical effect on coeliac disease based on genetically modified lactobacilli in mixture with bifidobacteria. The methods used were genetic modification of selected LABs (e.g. L. gasseri, L. johnsonii L. reuteri, L. acidophilus) caring genes for gluten proteases and preparation of mixture ratios of modified LABs and bifidobacterial BIF with prebiotic (Bifidobacterium longum and Bifidobacterium bifidum) for testing in mice animal model for coeliac disease. The results included genetic modification of LABs: cloning gene for gluten processing proteases; expression vector (such as an RCR – rolling cycle replication, which rapidly synthesizes plasmids - replicon) containing cloned gene; inducible promotor nisin-controlled gene expression system (NICE), transcriptional terminator and ribosome binding site to ensure optimal transcription and translation; cloned genes for glycosylation to enable post-translational modifications and functionality of produced enzymes. Mixtures of LABs and BIF in different ratios together with prebiotic for their metabolic stimulation orally applied in mice should be tested over time tracking the changes of the gut microbiome and gluten degradation, as well as regular blood tests to check the antigen levels. Genetically modified LABs orally inserted into the gut biome could be the best and safest step in the direction of developing a drug for celiac disease. I propose a cloning vector based on a RCR replicon, inserted by electroporation, with a promoter belonging to NICE, which would allow LABs to produce gluten-specific proteases, and would possibly help replenish the gut biome, if they were introduced in a cocktail with bifidobacteria.

Young scientist award presentation

Presentation number: YIA 1

NON-DESTRUCTIVE EXTRACTION OF ANCIENT DNA FROM BONE AND TOOTH ARTEFACTS

Elena Essel[1], Elena I. Zavala[1], Ellen Schulz-Kornas[2,5], Anna Schmidt[1], Birgit Nickel[1], Julia Richter[1], Sarah Nagel[1], Mateja Hajdinjak[1,3], Marie Soressi[4], Matthias Meyer[1]

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In contrast to skeletal remains and sediments, which are widely used sources of ancient faunal and human DNA, human-made artefacts from Palaeolithic sites are not commonly utilized for genetic investigations into the past. We hypothesized that tools and ornaments prepared from faunal bones and teeth may not only preserve DNA from the animals they belonged to, but could also trap DNA from the hominin individuals who made and/or used them. As such artefacts are often too precious for destructive sampling, we developed a non-destructive DNA extraction method that uses a temperature-controlled release of DNA by immersing artefacts in phosphate buffer. We then applied this method to a set of ten Pleistocene bones and teeth that were similar in size and shape to materials typically used for artefact production. Quantitative 3D surface texture measurements conducted before and after DNA extraction showed no substantial surface alterations, in contrast to another method previously suggested for non-destructive DNA extraction. When applied to a set of eleven artefacts from the Châtelperronian layers of the Quinçay site in France (excavated and cleaned more than 30 years ago), our method enabled the recovery of ancient mammalian mitochondrial DNA from two of the samples. DNA sequences were assigned to Cervidae and Elephantidae, in agreement with the morphological identification of the artefacts. Alas, the majority of sequences (70.9 and 98.3%) originated from recent human DNA contamination introduced during and after excavation, hampering our ability to detect traces of ancient hominin DNA that may point to the makers/users of these artefacts. In summary, we present a method for isolating DNA from ancient artefacts prepared from bones and teeth while preserving not only their visual appearance, but also their structural integrity. Moreover, we demonstrate that our method is in principle suitable for identifying the source material of artefacts in cases where morphological identification is difficult. Further investigations would require both, material excavated under cleaner conditions (e.g., using gloves) as well as limited subsequent handling to increase the probability that DNA from the makers/users of the artefacts can be recovered.

Key words: Non-destructive DNA extraction, temperature-controlled extraction, artefacts, ancient DNA

Presentation number: YIA 2

A COMPREHENSIVE PHARMACOGENOMIC MULTI-GENE PANEL ANALYSIS IN CLINICAL PRACTICE, EXPERIENCE FROM ST. CATHERINE HOSPITAL

Vid Matišić[1], Petar Brlek[1], Vilim Molnar[1], Eduard Pavelić[1], Martin Čemerin[2], Kristijan Vrdoljak[2], Andrea Skelin[1,3], Damir Erceg[1,4,5,6], Davor Moravek[1], Ivana Erceg Ivkošić[1,6], Dragan Primorac[1,6,7,8,9,10,11,12,13,14]

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The field of pharmacogenomics is still in its early stages. However, multi-gene panel-based pharmacogenomic tests are readily available for both clinicians and patients. In the Republic of Croatia, single-gene testing has been available for over a decade; however, commercial panel-based tests targeting multiple genes known to influence drug response is a new concept that was implemented in 2018 at St. Catherine Hospital. This cross-sectional study aimed to report the prevalence of actionable pharmacogenetic interventions in patients who had undergone pharmacogenetic testing using the RightMed 27-gene panel. Retrospective analysis of single-center electronic health records was performed, including a total of 319 patients. Patients underwent pharmacogenomic testing by the RightMed panel using a TaqMan quantitative real-time PCR method and copy number variation (CNV) analysis to determine the SNPs in the 27 targeted genes from 2018 until 2022. Actionable drug-gene pairs were found in 235 (73.7%) patients. Relevant guidelines on genotype-based prescribing were available for 133 (56.7%) patients at the time of testing. Based on the patients' genotype, 139 (43.6%) patients were using at least one drug with significant pharmacogenetic interactions, potentially predisposing them to adverse drug reactions or lack of therapeutic response. Two out of three patients in our practice were found to have at least one gene-drug interaction; therefore, the next step in personalized medicine is integrating pharmacogenomic data into patients' electronic health records to optimize drug therapy.

Key words: pharmacogenomics, clinical application, adverse drug reactions

Presentation number: YIA 3

FUNCTIONAL VALIDATION OF GWAS HITS ASSOCIATED WITH IgG GLYCOSYLATION USING CRISPR/Cas9 TRANSIENT EXPRESSION SYSTEM

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Alternative glycosylation of immunoglobulin G (IgG) Fc region has a crucial role in defining pro- or anti-inflammatory effector function of the antibody. Gene network involved in regulation of IgG glycosylation is still poorly understood because glycoyltransferases and glycosydases are not the major players in this regulatory process. In this study we functionally validated gene loci associated with IgG glycosylation in previous genome-wide association studies (GWAS). We utilized established stably transfected cell line FreeStyle™ 293-F with CRISPR/dCas9 fusions for direct regulation of genes targeted with specific sgRNAs. This cell system was designed to secrete IgG molecules so that glycans on IgG can be analysed following gene manipulations. We manipulated 22 GWAS hits, grouped according to glycosylation traits such as galactosylation, fucosylation, sialylation and bisecting GlcNAC. Following gene expression manipulations, IgG glycosylation was analysed. Out of seven genes associated with galactosylation, only MANBA, HIVEP2, TNFRSF13B and EEF1A1 showed change of agalactosylated structures when upregulated using dSaCas9-VPR. Out of six hits associated with fucosylation, only upregulation of TBX21 and TBKBP1 showed significant changes in galactosylation, but no change was observed for fucosylated glycan structures. These results suggest that a different cell model might be preferable to validate different GWA hits, such as Lymphoblastoid Cell Line (LCL) which is known to be rich in fucose glycan structures. Out of five loci associated with bisecting GlcNac, upregulation of KDELR2 resulted in an increase in biantennary glycan structures with bisecting GlcNac. The upregulation of DERL2 and RRBP1 resulted in an increase of digalactosylated biantennary glycans. Out of four GWA hits for sialylation, only downregulation of SPPL3 led to hyperglycosylation with concomitant increase in sialylated and galactosylated structures and decrease in agalactosylated glycans, despite the fact that all genes were successfully up- or downregulated. Overall, these results have proven the functional role of several GWA hits which are not glycosyltransferases but are associated with the IgG glycosylation pathway. Ongoing research on LCL cell line might unravel the exact role of these and other GWAS hits.

Key words: CRISPR/dCas9, IgG glycosylation, FreeStyle™ 293-F Cells, GWAS

Presentation number: YIA 4

MOLECULAR CHARACTERIZATION OF OCULOAURICULOVERTEBRAL SPECTRUM IN A GROUP OF EGYPTIAN PATIENTS

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Oculoauriculovertebral spectrum (OAVS) is the second most frequent malformative disorder of head and neck with highly heterogeneous etiology and pathogenesis. Genetic causes have been brought up due to the existence of familial cases and numerous chromosomal abnormalities have been associated with this spectrum. Retinoic acid (RA) signaling pathway has been implicated in various developmental processes and is essential for craniofacial development. Interestingly, MYT1; the first described candidate gene for OAVS belongs to RA induced transcriptome. The molecular study was carried out on 32 clinically suspected OAVS Egyptian cases with no history of teratogenic insult or numerical chromosomal aberrations using array comparative genomic hybridization, next generation sequencing panel testing, whole exome sequencing and Sanger sequencing of ALX genes in three clinically suspected Oculo-auriculo-frontonasal spectrum cases. Array-CGH revealed dosage anomalies in 6 patients. NGS panel testing revealed missense variants of unknown significance in ZYG11B and HMX1 genes in 2 patients. One missense de novo heterozygous probably pathogenic variant was identified in DGKD gene through WES, performed for a selected trio. This variant was not reported in any databases and is annotated probably damaging by different bioinformatic tools. ALX genes screened were negative for deletions in all patients. Sanger confirmation, segregation analysis followed by functional studies for all variants and genes of interest is highly recommended to reveal their candidacy to OAVS. This research work might represent a strong initiative for future studies based upon the various CNVs and variants of interest detected and may set a paradigm for molecular diagnosis of cases with overlapping phenotypes.

Key words: OAVS, array CGH, NGS, WES, DGKD

Presentation number: YIA 5

A CENTRIFUGAL MICROFLUIDIC SOLUTION FOR THE AUTOMATION OF FORENSIC EPIGENETIC SAMPLE PREPARATION

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For those evidence-producing criminal cases that lack genetic reference material for comparison and are ineligible for or non-producing of database matches, the human epigenome has been suggested as a reservoir of information for female sex typing, monozygotic twin individualization, body fluid identification, behavioral trait prediction, and DNA phenotyping by estimation of human chronological age. In particular, more than 300 research studies have been published suggesting the utility of methylation status at specified genetic loci for approximation of human age. However, the most commonly employed strategies for epigenetic analysis require sodium bisulfite conversion (BSC), a sample preparation step to preferentially deaminate unmethylated cytosines to uracil, leaving methylated cytosines intact and distinguishable for downstream analysis. Unfortunately, conventional BSC techniques are characterized by extensive DNA loss and require time-consuming, labor-intensive workflows with a high propensity for contamination. We propose a microfluidic solution for forensic epigenetic sample preparation that leverages centrifugal force to enable rapid, efficient conversion of forensically-relevant DNA input masses in a closed, automated microCD (μCD) format. Faster conversion rates and increased DNA recovery are possible via the enhanced surface-area-to-volume ratio specific to the microfluidic strategy. The method was designed with multiplexing in mind and assessed with methylation standards by multiple downstream analytical processes, including real-time polymerase chain reaction (RT-PCR), high resolution melting (HRM), and pyrosequencing. Early phase goals of this project included testing the chemistry at the microfluidic scale, adjusting the parameters of the reaction step most commonly associated with DNA loss, optimizing microfluidic architecture, and completing preliminary μCD BSC. Assay characterization was completed with primers targeting age-associated loci, ELOVL2 and FHL2. Our approach enabled reduction of incubation intervals, thereby decreasing the total assay time, with increased DNA recovery and comparable conversion efficiency to a gold-standard method.

Key words: Sodium Bisulfite Conversion, Forensic Epigenetics, DNA Phenotyping, Rotational Microfluidic

Abstracts selected for podium presentation

Presentation number: PP1-MG

MiR-182-5p AND miR-375-3p IN BLOOD PLASMA AS BIOMARKERS FOR PROSTATE CANCER

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With prostate cancer (PCa) being the most commonly diagnosed neoplasm among men and often resembling benign prostate hyperplasia (BPH), biomarkers with a higher differential value than PSA are required. We investigated the expression of certain miRNAs from liquid biopsies as potential epigenetic biomarkers of PCa. The absolute expression of miR-375-3p and miR-182-5p were quantified in blood plasma and seminal plasma of 65 PCa and 58 BPH patients by digital droplet PCR. The sensitivity and specificity of these microRNAs were determined using ROC curve analysis. The higher expression of miR-182-5p and miR-375-3p in the blood plasma of PCa patients was statistically significant as compared to BPH ($p = 0.0363$ and 0.0226, respectively). Their combination achieved a specificity of 90.2 % for predicting positive or negative biopsy results, while PSA cut-off of 4 µg/L performed with only 1.7 % specificity. In seminal plasma, miR-375-3p, and miR-182-5p showed a statistically significantly higher expression in PCa patients with PSA >10 µg/L compared to ones with PSA >10 µg/L. MiR-182-5p and miR-375-3p in blood plasma show higher performance than PSA in differentiating PCa from BPH. Seminal plasma requires further investigation as it represents an obvious source for PCa biomarker identification.

Key words: miRNA, prostate cancer, liquid biopsy, biomarkers

Presentation number: PP2-FG

EXPANDED CODIS STR ALLELE FREQUENCIES - EVIDENCE FOR THE IRRELEVANCE OF RACE-BASED DNA DATABASES

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In the US, it is assumed that the most conservative random match probability (RMP) can be estimated using the suspect's self-declared racial reference DNA database. If the suspect's race is not known, the RMP is typically estimated across different race-based CODIS STR allele frequency databases, including African American, Asian, Caucasian, Hispanic, and Native American, and the most conservative RMP estimate is used. In a recent study, we evaluated the relationship between RMP and race based on CODIS STR profiles corresponding to the five race-specific allele frequency databases. Our analyses confirmed that most genetic differences between individuals are only to the slightest extent attributable to racial classification. Approximately 98% of the genetic variation was found to occur among individuals and not between races. We could not distinguish individuals separated by race as distinct genetic clusters based on forensic STR data. Accordingly, RMP values were exceedingly small regardless of the race-based STR allele frequency database, and the values also did not vary significantly when incorporating race-specific reference data. Therefore, our results show that the use of racial information does little to generate conservative RMP estimates. This finding implies that we do not need to include racial information in the US to get conservative estimates of RMP. Therefore, using race as a proxy for a genetic distinction to produce larger (i.e., conservative) RMPs for an individual DNA profile is irrelevant.

Key words: Race, random match probability, population structure, forensic DNA database

Presentation number: PP3-MG

ENRICHMENT OF RARE VARIANTS IN GENES INVOLVED IN MITOCHONDRIAL METABOLISM IN PATIENTS WITH EARLY-ONSET OR FAMILIAL PARKINSON'S DISEASE

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There is a growing body of evidence supporting mitochondrial dysfunction as a mayor driving force in Parkinson's disease (PD) pathogenesis, with the hallmark being the discovery of mitochondrial toxins inducing PD. The goal of this study was to analyze rare variants of genes involved in mitochondrial metabolism in patients with early-onset and familial PD. We collected both nuclear and mitochondrial variants for 204 Croatian, Serbian and Slovenian patients with PD who referred to our center for diagnostic whole exome sequencing. The 204 patients with PD were further selected based on the disease age of onset and their family history. Sequencing was performed using a standardized set of protocols. We used population variation resources in variant annotation, which included an in-housed background population variant frequency estimates based on compilation of over 7000 exomes, as well as Genome Aggregation Database dataset. The cutoff frequency for rare variants was 5% in both the local and gnomAD databases. We excluded variants deviating significantly from Hardy-Weinberg equilibrium. In our study we found statistically significant differences in 16 gene variants between PD patients and the control group. After analysis genes GBA, NPC1, ATP13A2, HTRA2, CP, SOD1, WARS2, CLN6, HEXA, NPC2, SYNJ1, WDR45, PANK2, POLG which are located in nucleus and MT-CO1 gene located in mitochondrial genome had $p<0.05$. After adjustment only nuclear genes still had $p<0.05$, while MT-CO1 gene had padj=0,381. All identified genes have important role in lysosomal or mitochondrial function. We confirmed previous findings that dysfunction in lysosomal metabolism plays an important role in pathogenesis of PD. We found that ATP13A2 mutation is more abundant in patients with early onset PD, not only juvenile form of PD. Mutation in HtrA2 gene results in protein accumulation and destabilization of mitochondrial membrane. WARS2 encodes tRNA synthetize and our study is the first one that links its mutation with early-onset PD. Our study is the first one that identified new gene, WARS2, as a risk factor for early-onset PD and confirmed that dysfunction of lysosomal or mitochondrial metabolism is risk factor for developing PD.

Key words: Lysosomes, Mitochondria, Parkinson Disease

Presentation number: PP4-FG

DEVELOPMENT AND VALIDATION OF A NEW MULTIPLEX METHYLATION-SPECIFIC PCR ASSAY FOR FORENSIC BODY FLUID IDENTIFICATION

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The identification of the body-fluid from which DNA originates, along with the STR profiling, is important in uncovering the context of deposition of biological traces and can aid in reconstruction of events. Several authors have reported MS-SNuPe assays for detecting differentially-methylated CpGs for differentiation between saliva, semen, vaginal fluid, venous and menstrual blood. The aim of our research was to develop and validate a novel multiplex methylation-specific PCR (F-MS-PCR) assay for detection of methylation in 5 body-fluid specific CpGs in a DNA sample. The test consists of PCR amplification of bisulfite-converted DNA from a sample by using 5 pairs of fluid-specific primers, one of which is fluorescently tagged at its 5'-end and specific to bisulfite-converted DNA, and the other is specific to bisulfite-converted but only methylated DNA. Thus, only amplification of the methylated body-fluid specific locus is achieved, the PCR product is detected by CE and the body-fluid is detected. To assess the performance, 27 semen, 21 saliva, 21 venous blood, 21 menstrual and 20 vaginal samples were tested. DNA was extracted by PrepFiler Forensic DNA Extraction Kit, quantified by Quantifiler Duo on 7500 Real-Time PCR System, and bisulfite-converted by using EpiJET Bisulfite Conversion Kit. PCR reactions consisted of: 5 µl Qiagen Multiplex PCR Mix, 1 µl 10x primer mix, 3 µl water and 1 µl bisulfite-converted DNA. PCR products were detected on 3500 Genetic Analyzer and data was analyzed by GeneMapper ID-X software. Validation of the limit of detection, sensitivity, specificity, application in casework, and analysis of old samples and mixtures, was also performed to assess the suitability of the test for routine use in the forensic genetic laboratory. The test showed high specificity (100%, except 96.73% in menstrual fluid) and high sensitivity (100%, except 97.6% in vaginal and 80.7% in menstrual fluid). The lower limit of detection varied between 125 pg (semen) and 1 ng (menstrual blood). It has fewer pipetting steps compared to MS-SNuPe, shorter sample to result time and lower cost. The test has easy interpretation, with presence of a peak indicating detected fluid, and it can be easily applied as a powerful tool in the forensic genetic laboratory.

Key words: Body-fluids, DNA methylation, multiplex PCR, MS-SNuPe, capillary electrophoresis

Presentation number: PP5-MG

THE EFFECT OF HOST GENETICS ON COVID-19 SUSCEPTIBILITY AND SEVERITY: A STUDY OF 16 CODING GENES ON A SUBSET OF BOSNIAN-HERZEGOVINIAN PATIENTS

Lana Salihefendić[1,2], Larisa Bešić[1], Ivana Čeko[1,2], Naida Mulahuseinović[2], Dino Pećar[2], Amra Džuho[1], Selma Durgut[2], Damir Marjanović[1,3], Rijad Konjhodžić[2], Adna Ašić[1]

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The aim of this study was to analyze the effect of patient genetics on the severity of symptoms and susceptibility to COVID-19 infection. 60 COVID-19 patients from the General Hospital of Tešanj, Bosnia and Herezgovina, were recruited in the study, and divided into three groups (n=20 for each group) of patients exhibiting mild, moderate and severe clinical presentation of COVID-19. DNA was isolated from whole blood using QIAamp® DNA Mini Kit. Ion Torrent GeneStudio S 5 platform was used to perform the sequencing of 16 target genes and their regulatory regions, namely HLA-A, HLA-B, HLA-C, ACE2, IL-6, IL-4, TMPRSS2, IFITM3, IL-12, RIG-I/DDX58, IRF-7, IRF-9, IL-1B, IL-1A, CD55, and TNF-α. Selected genetic variants of interest were subjected to confirmatory Sanger sequencing on the SeqStudio Genetic Analyzer System. Our study confirmed that older age, male sex, and cardiovascular, respiratory and metabolic comorbidities are risk factors for severe COVID-19. In addition, we have identified several genetic variants that were significantly more common in severe than in mild and/or moderate groups of patients, including those on the genes IL4, IL1B, TMPRSS2, CD55, DDX58 and IRF7. We have further confirmed that the variant rs2285666 on ACE2 might be protective, as it was more common in moderate and mild than in severe clinical group, but significantly affected by the patient's sex, considering that ACE2 is found on X chromosome and escapes X inactivation in females. The results of this study have further emphasized the importance of personalized approach to each COVID-19 patient, as host genetics plays an important role in response to SARS-CoV-2 infection, including both susceptibility and severity of the clinical presentation. Future studies, most prominently GWAS using larger patient cohorts and appropriately matched controls, are expected to produce even more data on the effect of human genetic variants on the course of COVID-19. This study is offering the first such data, not only for the Bosnian-Herzegovinian population, but for the Western Balkan region as well.

Key words: COVID-19, host genetics, personalized approach, SARS-CoV-2

Presentation number: PP6-MG

MOLECULAR AUTOPSY: IDENTIFICATION, CLASSIFICATION AND REPORTING OF SEQUENCE VARIATIONS IN YOUNG SUDDEN UNEXPECTED DEATH VICTIMS AND AFFECTED FAMILIES

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Sudden cardiac death (SCD) is an important public health issue. In young individuals a significant number of SCDs is caused by inherited cardiac diseases, frequently not detectable during conventional medico-legal autopsies (including histological and toxicological analyses). Therefore, these deaths are referred to as sudden unexplained deaths (SUDs). Next-generation sequencing (NGS) became an indispensable tool in molecular autopsy investigations. Nonetheless, NGS brought new challenges, especially regarding the interpretation of the large number of variants of unknown significance (VUS). Therefore, evaluation and classification of genetic findings is of great importance to establish a causal link between phenotype and genotype and for prevention of the remaining family members. We performed a structural assessment of young sudden death cases aged between 1-50 years including molecular autopsy. Detected sequence variants were assessed according to the ACMG classification standards. In addition, cardiological data of our new centre of sudden death in the young were investigated to evaluate possible genotype-phenotype correlations. We identified 53 rare protein-altering variants (MAF < 0.2%) classified as VUS or worse. 13 % of the cases exhibited a clinically actionable variant (pathogenic, likely pathogenic or VUS - potentially pathogenic) that would warrant cascade genetic screening in relatives. To date, using molecular autopsy in combination with the assessment of family members (n=149), an inherited cardiac disease could be detected in 24 % of the cases. 67 % of the families are still under investigations. Our data reveal that, despite the undeniable advantages, molecular autopsy is not a stand-alone tool. Moreover, multidisciplinary collaboration is crucial for an optimal management of sudden unexplained death cases in order to identify additional relatives at risk.

Key words: Sudden death, Molecular Autopsy, inherited cardiac disease

Presentation number: PP7-MG

CELL LINE MODELS WITH STABLY INTEGRATED CRISPR/DCAS9 FUSIONS FOR STUDYING THE EPIGENETICS OF IgG GLYCOSYLATION

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To study alternative glycosylation of immunoglobulin G (IgG), which is functionally important in in pro- or anti-inflammatory effector function of the antibody, we developed a system for IgG production in the model cell line HEK-293F, which we modified by incorporating the CRISPR/dCas9-based molecular machinery for epigenetic regulation of gene expression. We extended our existing modular system for CRISPR/dCas9 fusions facilitating gene regulation to enable its stable integration into HEK-293F cells, thus creating derived cell lines with integrated machinery for programmed transcriptional control. A key factor for targeting the dCas9 fusions with activators and repressors, the guide RNA (gRNA) was transiently transfected into the derived cell lines on a separate plasmid, that also contains a cassette for monocistronic expression of IgG heavy and light chain. In this bipartite system, the creation of derived cell lines with CRISPR/dCas9 components eliminates the inefficiency of transfection with a large construct, while combining a key element for targeting (gRNA) with the IgG expression cassette on a small plasmid facilitates high transient transfection efficiency while virtually eliminating the background from untransfected cells. We validated the system by up- or downregulating the known glycosyltransferases. Transcriptional up- and downregulation of B4GALT1, responsible for adding galactose to the glycan core, had the expected effect on the abundance of agalactosylated and galactosylated structures, changing the glycosylation profile according to the transcript level. Downregulation of FUT8, encoding a fucosyltransferase, decreased the core-fucosylated structures, while upregulation of ST6GAL1 and MGAT3 increased sialylated structures and those with a bisecting GlcNAc, respectively, in line with our expectations. The system is now being used to further study the role of genes associated with IgG glycosylation in GWA studies. It can also be easily repurposed to serve as a model for other proteins and their posttranslational modifications, with appropriate targeting via gRNA. Finally, to eliminate the frequently raised concern about suitability of the HEK293-F system as a model for plasma cells, we are currently adapting the system for use with lymphoblastoid cell lines (LCLs).

Key words: epigenetics, glycosylation, CRISPR, Cas9

Presentation number: PP8-AG

WEST COUNTRY STORY - A DETAILED INVESTIGATION OF NEOLITHIC & BRONZE AGE INDIVIDUALS FROM SOUTH-WESTERN ENGLAND

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Six thousand years before present (BP), the Neolithic expansion to Britain displaced local hunter-gatherer societies, introducing a radically different, more sedentary lifestyle. Two millennia later, a new migratory wave reached Britain, through the Bell Beaker folk, again transforming the established genetic and socio-cultural landscape of the island. While distinct burial rituals and material artifacts underline the strong cultural differences between the two populations, the Bronze Age practice of reusing more ancient Neolithic tombs renders the differentiation of individuals through archaeological study alone problematic. In order to finely investigate the population dynamics that occurred during the transition between the Neolithic and the Bronze Age in Britain at an individual resolution, we employed a genomic approach on 30 samples (age between 6000-3500 BP) from 7 sites in South-Western England. Our analyses reveal a pattern consistent with a genetic turnover, while signs of admixture between the populations are visible as well. First kin relationships between four individuals provide a direct insight into the makeup of a Neolithic multigenerational family. On three occasions, we find the remains of male Bronze Age individuals buried within Neolithic sites, suggesting a possible trend of burial site reuse. By integrating genetic and archaeological analyses, our study delivers a snapshot of one of the most influential demographic shifts in British history, shedding light on both the individual history of these tombs as well as the region's wider socio-cultural transformation.

Key words: Neolithic, Bronze Age, Migration