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P20 Functional Genomics and Epigenomics

P20.001.D Investigating the meaning of age-related changes in DNA methylation by studying the correlation between epigenetic age acceleration and progressive human appearance traits

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Introduction: DNA methylation markers have been proposed as a predictor of biological age. At the same time, phenotypic aging is a potential model for exploring the molecular mechanisms of aging. By investigating the correlation between epigenetic age acceleration (EAA) and externally visible phenotypes we aim to investigate molecular pathways involved in aging processes and disclose promising targets for antiaging therapies.

Materials and Methods: A cohort of about 1000 individuals of European descent with described physical phenotype and collected lifestyle information will be examined using Infinium® Global Screening Array and Infinium® MethylationEPIC 850K microarray. DNA methylation data will be examined using various age prediction models to calculate EAA. The EAA values will be further correlated with phenotypic traits including hair loss, hair greying, and wrinkles formation as well as with genetic variation.

Results and Conclusion: The study will improve our understanding of the role of interactions between genes, DNA methylation, and EAA in determining age-related appearance traits. We will assess the heritability of the aging rate and measure the importance of environmental factors for accelerated aging. The role of individual CpG and SNP markers will be tracked in enrichment analysis. The study will have practical value in forensics by developing prototype predictive models for specific age-related features based on genetic and epigenetic information, as well as may find practical application in the cosmetic industry by developing products to prevent or slow down phenotypic aging. This research was supported by the grant from the National Centre for Research and Development no DOB-BIO10/06/01/2019.

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P20.002.A A multi-omics approach to study monozygotic twins discordant for amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterised by progressive death of upper and lower motor neurons. The majority of ALS cases are sporadic, while 10% are familial. ALS aetiology is still not completely understood. To investigate genetic and epigenetic factors underlying ALS, we studied a monozygotic twin pair discordant for ALS with a multi-omics approach combining whole exome sequencing with genome-wide methylome- and transcriptome data from whole blood and PBMCs. For methylation, we used the Illumina EPICArray which covers 850,000 methylations sites and the ChaAMP software for the analyses, while for gene expression study, Illumina TruSeq Stranded mRNA sequencing was performed. The results were considered independently and in combination to identify disease-relevant methylation changes and their downstream impact. Twins tested negative for mutations in main ALS-genes. We identified 59 differentially expressed genes (DEGs) (p.adj < 0.1; |log2FC| >1) involved in immune system pathways. After QC, we found 2 differentially methylated probes (pvalue adj ≤ 0.1) in CACNA1G, expressed mostly in brain, and VAX1 genes; while filtering by delta beta (Δβ) values, we identified 2 probes with Δβ ≤ - 0.25 (in an intergenic region and RUSC1-AS1) and 2 probes with Δβ ≥ 0.25 (in AARS and KPNA4). None of them fell into the highlighted DEGs. Finally, mRNA-seq results were compared with larger literature datasets. Further comparative analyses on external epigenetic datasets as well as CNV and SNV analyses on exome data are ongoing to elucidate possible epigenetic and somatic genetic factors that could underlie susceptibility to sporadic ALS.

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P20.003.B Resistance profile and genetic diversity among selected ESBL-producing *Escherichia coli* isolates from urocultures in a portuguese hospital

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Introduction: Antimicrobial-resistant bacteria are contributing to mortality and morbidity worldwide. The Extended-Spectrum β-lactamase-producing *E. coli* is considered one of the great concerns regarding the public health issue. The purpose of this work was to determine prevalence and genetic characteristics of selected ESBL-producing *E. coli* isolates from urinary infections.

Materials and Methods: Twenty cefotaxime/ceftazidime-resistant *E. coli* isolates were obtained aleatory from urocultures in a Portuguese hospital, during June 2017-July 2018. Identification was performed by MALDI-TOF MS. Antimicrobial susceptibility

against 13 antibiotics was analyzed by disk diffusion test. Screening of ESBLs was performed and resistance genes were analyzed by PCR/sequencing. Phylogenetic grouping was also performed by multiplex-PCR.

Results: ESBL-production was detected in 90% of the isolates (18/20), mostly associated with CTX-M-15 (n = 13) and CTX-M-1 (n = 1) enzymes. Tetracycline resistance was associated with *tet*A (n = 5) and *tet*B (n = 3). The most common phylogenetic group among ESBL-producers was B2 (n = 13), followed by D (n = 2), C (n = 1) and A (n = 1). The isolates carrying the $bla_{\text{CTX-M-15}}$ gene were ascribed to phylogroups B2 and D, and the $bla_{\text{CTX-M-1}}$-carrying isolate was typed as phylogroup C. The two ESBL-negative *E. coli* isolates also carried a CTX-M gene (which variant was not determined).

Conclusions: These findings indicate that the CTX-M-15 enzyme is the main mechanism of ESBL-production among urinary infections isolates in our hospital, being these isolates of the phylogroups B2 and D.

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P20.004.C E6 and E7 HPV16 oncogenes influence gene transcription trough the genome-wide pattern deposition of MBD2,3 components of NuRD nucleosome remodeling complex

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Human papilloma virus (HPV) is the etiologic agent of cervical cancer and the third most commonly diagnosed type of cancer in women worldwide. The nucleosome remodelling and deacetylation complex (NuRD) is a group of associated proteins with ATP-dependent chromatin remodelling and histone deacetylase activities. MBD2/3 proteins from NuRD complex exhibit methyl-CpG-binding domains, which mediate an interaction with methylated DNA. The current study aims to assess the viral oncogenes influence on the MBDs overall binding pattern to CpG islands. ChIP-Seq for MBD2/3 genome wide DNA binding pattern (e.g. promoters, gene control region, transcriptional enhancers, etc.) in untreated and HPV 16 E6/E7 shRNAs treated CaSki cell culture was performed and the results were analysed using Base Space Illumina apps. MBD2/3 proteins were localized at the level of intron, intergenic regions and TSS. After ChIP-Seq peak score analysis, a cut-off of 9 was establish and 54 gene loci were identified. The corresponding genes were further analysed in qRT-PCR and their expression was found to be deregulated. When both oncogenes (E6 and E7) were silenced, we noted an enrichment of MBD2/3 proteins at CDK6, DLC1, NRIP1 gene loci involved in oestrogen receptor (ER) signalling pathway. Another interesting gene loci involved in mRNA processing and cancer growth and metastasis were identified (EIF4G3 and DCP2). Viral oncogenes act synergistically on the gene transcription pattern by interacting with the MBD2/3 proteins of NuRD complex. Epigenetic gene control is a complex phenomenon that is guided by internal, cellular and external factors as well as viral infections. **Acknowledgments: TE39/2020**

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P20.005.D New *cis*-regulatory elements modulate *CFTR* expression

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8% of the human genome is covered with candidate *cis*-regulatory elements (cCREs). Anomalies of CREs at distance from a gene have been identified as being involved in various genetic diseases. Although, more than 2100 variants have been detected in *Cystic Fibrosis Transmembrane conductance Regulator* (*CFTR*) gene, responsible of Cystic Fibrosis (CF) or *CFTR*-related disorders, some patients have an incomplete genotype or present extremes phenotypes. Development of chromatin conformation study techniques identified several long-range regulatory elements of *CFTR* gene. Our aim is to highlight the role and involvement of regulatory elements on the architecture and conformation of the *CFTR* gene.GWAS3D score application allowed us to highlight involvement of four *CFTR* introns in gene regulation, introns 26 (4374 + 1,3 kb), 24 (4095 + 7,2 kb), 1 (185 + 10 kb) et 12 (1811 + 0,8 kb). Introns 1 and 12 have already been described as two main cooperative *CFTR* CREs in intestinal cells. Activity tests in Caco-2 intestinal cells show strong cooperative effects of the four predicted introns on *CFTR* promoter activity. Chromatin immunoprecipitation analyses demonstrate enrichment of a large network of keys transcription factors (TFs) in intestinal cells, such HNF1a, p300, FOXA1/A2, CDX2 and TCF4, in introns 24 and 26 enhancers. In conclusion, two new CREs with cooperative enhancer activities have been identified, enriched with important TFs, redefining the 3D regulation model of the *CFTR* gene in intestinal cells. Ongoing studies of chromatin conformation and CRISPR interference will further characterize the role of these new enhancers.

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P20.006.A Functional characterisation of *GJB2 cis* regulatory elements and WGS of heterozygous patients with NSHL

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Three-dimensional chromatin organization plays a key role on gene expression. Gene regulation depends on *cis*-regulatory elements which can interact with gene promoter by chromatin loop. Alteration of chromatin architecture and/or *cis*-acting elements can lead to *enhanceropathies*. Several unelucidated nonsyndromic hearing loss and deafness 1 (*DFNB1)* cases carrying out only one heterozygous pathogenic mutation on Gap Junction Beta 2 (*GJB2*) gene, led to strongly suggest the presence of distant *cis*-regulation. Thanks to chromatin conformation study, we previously identified several *cis*-regulatory elements which have enhancer action and silencer effect on *GJB2* expression. Analysis of CTCF binding allowed to purpose a *DFNB1* 3D looping model. We identified cooperative effects between enhancers. To confirm an