P12 - EVOLUTIONARY HISTORY AND GENE EXPRESSION OF ATAXIN-3 PARALOGS

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Introduction: Ataxin-3 gene (ATXN3; 14q32.1) encodes a ubiquitously expressed deubiquitinating enzyme, with homologues among metazoans, plants and protozoans. In humans, ATXN3 may present a (CAG) n expansion, responsible for Machado-Joseph disease (MJD/SCA3). A highly conserved gene copy, ataxin-3 like (ATXN3L; Xp22.2) encodes a protein shown to cleave ubiquitin substrates in vitro more efficiently than ATXN31. Interestingly, a study on another ataxin gene, ATXN1, demonstrated that its copy, ATXN1L, alleviated the neuropathology in mice2. Thus, it is of upmost importance to understand whether ATXN3 copies can have a functional role in disease pathogenesis.
Methodology: To identify ATXN3 copies, we retrieved highly homologous sequences in 33 primates. We explored ATXN3 paralogs through the analysis of interspecific sequence diversity, evolution rates and selective constraints in the reconstructed phylogenies. For ATXN3L, which displays a conserved reading frame, we analysed protein domain conservation and mRNA expression by performing qPCR in 16 human normal tissues.
Results: Our results suggested three independent retrotransposition events: 1) in Haplorrhini (~63 MYA) for ATXN3L; 2) before the Platyrrhini-Catarrhini split (~43 MYA) for ATXN3L2 (LOC100132280; 8q23.2 in humans); and 3) in Cercopithecidae (25-30 MYA) for ATXN3L3 (LOC699321; chr11 in Rhesus monkey). ATXN3L seems to be under selective constraints throughout primate evolution as the parental ATXN3, whereas ATXN3L2 gained premature stop codons that likely turned it into an inactive copy. ATXN3L3 appears as a younger retrocopy with predicted alternative reading frames. Finally, we confirmed that ATXN3L is expressed in human placenta, testis and brain (cortex and substantia nigra).
Discussion: Phylogenetic analyses support the functional relevance of ATXN3L; however, further studies will be important to assess if ATXN3L expression in brain is conserved in non-human primates and detect the presence of endogenous protein ex vivo.
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P13 - MINDDS-WINGS FEDERATED PLATFORM IMPLEMENTATION: A SAFER APPROCH TO DATA ACCESS

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Introduction: Research using biomedical data generated in hospitals/ biomedical health institutions has contributed to considerably increase in knowledge concerning neurodevelopmental disorders (NDDs) and their association with rare genetic variants. However, more could be achieved by pooling data from multisite studies together to create meta-cohorts. A major barrier is the sensitive/private nature of clinical/ genomic data, due to strict legislation access, e.g. GDPR, making it hard to share information. A way forward is to implement systems that make specific features derived from personal, clinical, genomic and phenotypic data visible without transferring complete data sets, thus avoiding compromising individual's data security/privacy. MINDDS-WiNGS is a federated online data platform that overcomes these constraints - instead of physically moving data for analysis among institutions, analysis is carried out in situ through a federated network with world-wide accessibility (https://doi.org/ 10.1101/2022.06.23.497325).
Methodology: MINDDS-WiNGS federated platform (FP) has two main components: MINDDS-connect, a means to make selected data elements findable via online search site for assembling virtual meta-cohorts; from which controlled access can be granted to WiNGS for genomic analysis/research of genetic variants. AIM: Installation in Centro Hospitalar Universitário de Santo António (CHUdSA) is ongoing, joining WiNGS network.
Results/Discussion: Variants have been accessed via WiNGS as Standard Variant Calling Files (VCFs) and stored in Cardiff University. Access to the platform is monitored internally, by an administrator and one or more principal investigators (PI). Registered users are assigned to a PI with access to the information associated with it. In clinical institutions data can be stored either on local servers or be cloud-based, with access to data limited to a user's PI; but requests can be made to access another PI-restricted information. The compilation of patient's clinical, genomic and phenotypic information is achieved by filling in meta-data fields. The MINDDS-WiNGS FP allows data visualization and analysis without the need to have a centralised database, promoting multidisciplinary research across multiple and international borders.
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P14 - ACTIVITY INDUCED GENES EXPRESSION IS IMPAIRED IN POLYGLUTAMINE SPINOCEREBELLAR ATAXIAS

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Introduction: Introduction: Polyglutamine Spinocerebellar ataxias (SCAs) are a group of 6 incurable genetic disorders, caused by an expansion of the trinucleotide cytosine-adenine-guanine in their causative genes, which produces a protein with an expanded glutamine region. This project focuses on the study of Spinocerebellar ataxia type 2 (SCA2) and type 3 (SCA3) (1), which are rare dominantly inherited disorders that primarily impair the cerebellum therefore leading to motor ataxia. Activity-induced inhibitor of death (AID), are a group of pro-survival 9 genes which were found to be neuroprotector in several neurological disorders, including stroke, glaucoma, AD, HD, and ALS (2).
In this project, we aim to investigate about the relevance of the expression of AID genes for cerebellum function and whether their expression levels are impaired in SCA2 and SCA3.
Methodology: Wildtype (WT), SCA2 and SCA3 transgenic animals were divided into 2 groups: unstimulated and motor stimulated group. The cerebellum was collected to analyze transcription and translation levels of the AID, and the whole brain was collected do detect the cell type expressing the proteins.
Results: We found that the phosphorylation of the main transcription required for AID gene expression was decreased in transgenic animals in comparison with age-matched WT animals. Accordingly, transcriptional analysis of AID genes showed that AID1 and AID2 that had their expression 6.3 and 3.3-fold increased, respectively, and 4.4 and 3.1-fold increased, respectively. However, this induction was impaired both in SCA2 and SCA3 mouse models. Moreover, immunostaining showed which neuron type were expressing AID genes.
Discussion: AID1 and AID2 are differentially expressed upon stimulation in WT mice, whereas there is decreased in both SCA2 and SCA3 disease models. As well as the translation and transcription factors that regulate the transcription of these genes in impaired in models of both disorders. Finally we found that these genes are expressed in a cell-type specific manner.
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P15 - NOVEL MECHANISMS CAUSING FAMILIAL HYPERCHOLESTEROLAEMIA: FUNCTIONAL CHARACTERIZATION OF VARIANTS IN THE REGULATORY REGIONS OF PCSK9 GENE

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Familial hypercholesterolemia (FH) is a worldwide highly prevalent genetic lipid disorder, characterized by increased LDL cholesterol levels in circulation, leading to premature atherosclerosis and cardiovascular events.
Among the other main causative genes of FH, PCSK9 is a major modulating gene in cholesterol homeostasis, and PCSK9 gain of function variants cause FH.
When intracellular levels of sterols decrease, PCSK9 is positively regulated by Sterol Regulatory Element-Binding Proteins 2 (SREBP2) and Hepatic Nuclear Factor 1α (HFN1α), while LDLR transcription is only regulated by SREBP2. Statins induce the expression of both SREBP2 and HFN1α, which activate PCSK9 transcription, and LDLR transcription cannot compensate for this effect, diminishing the beneficial effects in some patients. The current challenge is to understand the physiological regulation of PCSK9 expression at the transcriptional and post-transcriptional levels.
Based on the lack of understanding of mechanisms behind the regulation of PCSK9 expression, 3 variants in the PCSK9 promoter region, close to the SRE motif, and 14 in the 5' untranslated region (5'UTR), were studied. To this purpose, the promoter and 5'UTR regions of PCSK9 (-650 to -1) were cloned into pGL4.10[luc2] plasmid containing the Firefly luciferase (Fluc) reporter gene. After directed mutagenesis to obtain the variants in the study, they were transfected in HepG2 and Huh7 cell lines, and luciferase activity was determined by the Dual-Luciferase Reporter Assay System in a GloMax Luminometer. To simulate cholesterol starvation, 24 h hours after transfection, cells were treated with a medium supplemented with 0.1% FBS, and, after 18-24h, cells were treated with mevastatin to activate transcription.
In HepG2 and Huh7, c.-346G>C showed a decrease in promoter activity to 26% and 30% of the wild type, respectively, while c.-353G>T showed an increase to 148% and 178% of the wild type. These results indicate that c.-353G>T can possibly lead to an FH phenotype, while c.-346G>C can result in cases of hypocholesterolemia. The remaining constructs are currently undergoing research.
Expanding our understanding of how these variants affect PCSK9 synthesis through the activation of transcription factors could lead to an opportunity for personalized therapy in patients carrying these variants along with other pathogenic variants in causative genes.
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P16 - EFFECTS OF METHANOLIC AND AQUEOUS EXTRACTS OF CARICA PAPAYA LEAF ON MIRNA-MEDIATED TRANSCRIPTIONAL REGULATION OF FETAL HEMOGLOBIN

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Introduction: Hemoglobinopathies are genetic blood disorders resulting characterized by abnormal hemoglobin that disrupts oxygen transport and leads to diverse health complications. Raising fetal hemoglobin (HbF) levels is one possible therapeutic approach. But the current main therapy based on the use of hydroxyurea (HU), faces limitations related to cost and safety, especially in underserved areas. To overcome these constraints, researchers are investigating natural alternatives like Carica papaya (CP) leaf extracts to boost HbF production with fewer side effects.
Methodology: We conducted a comparative study using aqueous (AECP) and methanolic (MECP) CP leaf extracts to evaluate their impact on HbF regulation in K562 myeloid cells. Gene expression changes were assessed using RT-qPCR after 24-hour exposure to varying extract concentrations (0,05 µg/ml, 0,5µg/ml and 5µg/ml).
Results: The transcriptional analysis of hemoglobin genes (HBA, HBB and HBG1) as well as that of its epigenetic regulators (HDAC1, HDAC2, HDAC3, DNMT1 and DNMT3b), transcription inhibitors (BCL11A and KLF1), transcription activators (MYC and HIC2) and miRNA-mediated regulators (miR-30a-3p, miR-29C-3p and miR-148b-3p) has revealed distinct expression patterns in K562 cells exposed to AECP when compared to MECP. While the expression levels of some genes were affected by one extract, other genes were affected by the other extract and some remained unaffected suggesting that the concentration and specific composition of AECP or MECP has a different impact on hemoglobin expression.
Discussion: The differential effects of MECP and AECP exposure on hemoglobin related genes expressed in K562 cells indicates that complex regulatory mechanisms underly their action. The concentration-dependent responses observed emphasize the need for further research to comprehend their implications in hemoglobinopathies. Overall, this study highlights the potential of extracts of CP leaf as modulators of HbF gene expression in K562 cells. Further investigation into their gene modulation mechanisms is warranted.
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P17 - FUNCTIONAL CHARACTERIZATION OF TWO APOB VARIANTS FROM EXON 29 FOUND IN INDIVIDUALS WITH CLINICAL DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction: Familial hypercholesterolemia (FH) is an inherited lipid disorder characterized by increased levels of LDL cholesterol. About 5-10% of FH cases occur due to variants in the APOB gene, but these alterations can be a more common cause of FH than expected since most of APOB variants identified is still unknown their effect on the metabolism. The majority of the variants are missense but there are a few nonsense variants and small indels in exon 29 identified in individuals with hypercholesterolemia phenotype that can cause FH.
The aim of this project was to functional characterize APOB variants from exon 29 identified in individuals referred to the Portuguese FH Study to assess if these are the genetic cause of disease.
Methodologies: LDL from index cases and relatives was isolated through sequential ultracentrifugation. ED-LDLR was purified from HEK293 cells transfected with the pcDNA3.1-EC-LDLR-His plasmid by affinity chromatography. Purified ED-LDLR fragments were coated onto 96-well plates and incubated with the different APOB variants.