GENETICS

TIME: 14.30 – 15.15

LOCATION: PARSONS ROOM

ROLE OF ALU RNA EDITING IN LONG NON-CODING RNA METABOLISM

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Background: Adenosine to inosine (A-to-I) RNA editing is facilitated by the enzyme adenosine deaminase acting on RNA-1 (ADAR1), which preferentially edits Alu elements. NEAT1, a long non-coding RNA (lncRNA), contains RNA editing sites in its Alu elements and is found in atherosclerotic plaques. However, the role of RNA editing in NEAT1 and its implication in cardiovascular disease remains to be elucidated.
Aim: To analyse the role of ADAR1 mediated RNA editing in the Alu elements of NEAT1 and its implications in cardiovascular disease.
Methods: ADAR1 and NEAT1 lncRNA levels were analysed using qRT-PCR in the peripheral blood mononuclear cells (PBMCs) of patients with stable coronary artery disease (stable CAD, n=49), acute coronary syndrome (ACS, n=58) and healthy individuals (control, n=117). NEAT1 and ADAR1 were silenced in human umbilical vein endothelial cells (HUVECs) and pro-inflammatory conditions were induced using TNF-α for 4 hours. Stability assays were conducted using actinomycin D. Gene expression was measured using qRT-PCR.
Results: Patients with atherosclerotic heart disease showed higher levels of NEAT1 as compared to controls (P=0.0005) with strong association between ADAR1 and NEAT1 lncRNA expression (r=0.78, P<0.001). The silencing of NEAT1 under TNFα-induced conditions decreased the expression of pro-inflammatory cytokine IL8, chemokine MCP-1 and cell adhesion molecules ICAM-1 and VCAM-1 (P<0.05 in all). Silencing of ADAR1 resulted in decreased NEAT1 expression (P<0.001) by reducing its stability (P<0.05).
Conclusion: These results provide novel insights into the role of ADAR1 mediated RNA editing on NEAT1 and show high correlation between NEAT1 and ADAR1 expression in atherosclerotic heart disease.

WHOLE GENOME SEQUENCING REVEALS NOVEL GENETIC VARIANTS IN DNAJB11 AND GANAB ACCOUNTING FOR AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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Autosomal dominant polycystic kidney disease (ADPKD) is a cystic kidney disease and ciliopathy. Genetically, over 90% of cases are caused by mutations in *PKD1* and *PKD2*. Recently, *DNAJB11* and *GANAB* genes have been described to cause atypical forms of autosomal dominant polycystic kidney disease (ADPKD).
We analysed whole genome sequencing data within the Genomic England research environment from patients with cystic kidney disease phenotypes. Using Labkey, in this cohort we analysed heterozygous tiering variants in *DNAJB11* and *GANAB* and used online tools to determine pathogenicity and allele frequency. Variants within *PKD1* and *PKD2* were also analysed in any identified patients.
We identified 9 individuals diagnosed with renal disease, most of them with cystic kidney disease, in whom *DNAJB11* variants were identified, 7 of which had an affected parent. Two patients had previously reported *DNAJB11* variants. Five individuals also had potential pathogenic variants in *PKD1*, *PKD2* or *GANAB*.
An additional 9 individuals diagnosed with renal or liver disease, most of them with cystic kidney disease, were found to have novel *GANAB* variants, 3 of whom had an affected parent. Six of these patients also had pathogenic variants in *PKD1*.

Whole genome sequencing allows the identification of rare genetic variants associated with autosomal dominant polycystic kidney disease. However, the identification of more than one pathogenic allele per family in several cases suggests either oligogenicity or detection of variants of uncertain significance, which require segregation studies and functional validation to determine the precise genetic cause.

INVESTIGATING THE ROLES OF ENDOGLIN AND VEGF IN VASCULAR DEVELOPMENT

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INTRODUCTION: The Transforming Growth Factor beta co-receptor endoglin regulates angiogenesis, inflammation, and wound repair, and endoglin mutations cause the human vascular disease hereditary haemorrhagic telangiectasia (HHT). Vascular Endothelial Growth Factor (VEGF) is a critical angiogenic factor. We hypothesised that the effects of endoglin loss-of-function would be rescued by VEGF inhibition.
METHODS: We obtained a engmu130 mutant zebrafish line in a transgenic (kdrl:GFP) background that labels endothelial cells with GFP. The effect of the mutation and/or treatment for 24hr with the VEGF receptor inhibitor Tivozanib (25-50nM) on vascular development was quantified by lightsheet fluorescence microscopy at 3d post fertilisation (n=8-10/group)
RESULTS: Endoglin homozygous mutants displayed a significantly enlarged dorsal aorta (DA) diameter (wt 22.3µm ± 0.9µm, mut 26.1µm ± 0.3µm, $p<0.01$) and posterior cardinal vein (PCV) (wt 25.4µm ± 0.4µm, mut 29.1µm ± 0.4µm, $p<0.001$). Consequently, blood flow is shunted directly from the DA into the PCV bypassing the intersegmental vessels, recapitulating the phenotype of human HHT.
Treatment with the VEGF inhibitor Tivozanib between 2-3dpf, rescued the phenotype of the endoglin mutants but did not affect the diameter of wildtype animals; DA diameter (wt+Tivozanib 21.8µm ± 0.8µm, mut+Tivozanib 23.2µm ± 0.6µm) and PCV (wt+Tivozanib 24.4µm ± 0.4µm, mut+Tivozanib 26.1µm ± 0.6µm).
CONCLUSIONS: These results indicate the HHT-like phenotype in zebrafish endoglin mutants can be mitigated through modulation of VEGF signalling and implicate VEGF as a possible therapeutic target in HHT.

EXOME SEQUENCING REVEALS DE NOVO AND MATERNALLY INHERITED CNVS IN SEVERE UNEXPLAINED MALE INFERTILITY.

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Severe forms of infertility such as azoospermia and severe oligozoospermia have often genetic causes, with Klinefelter syndrome and *de novo* Y chromosome microdeletions being the most common. However, of all infertile men, 40% have unknown aetiology after being tested for all the known causes. In this study, we explored for the first time the role of *de novo* and maternally inherited copy-number variations (CNVs) on a large scale, sequencing the exome of 160 patient-parents trios, a method principally used for single nucleotide variants (SNVs) studies but often undervalued for CNV detection. The analysis was performed with a novel GATK4-based pipeline that allows CNV identification, plotting and detection of loss of heterozygosity. The analysis revealed two rare *de novo* deletions in two different patients. One deletion occurred on chromosome 11 and partially overlapped a deletion previously reported in an infertile man. The second, affected *NXT2* on chromosome X, a gene evolutionary conserved and highly expressed in testis. A particularly interesting rare, maternally inherited CNV was a duplication in a third patient, larger than 1 Mb, involving *VCX*, a multi-copy gene on the X chromosome already associated to azoospermia when duplicated. These results show that *de novo* and maternally inherited CNVs may play a crucial role in male infertility, however, replication and functional studies are required to further validate the impact of our findings. Furthermore, analysing genome sequencing data will help overcoming the

limitation of the exome data and will give more insight on the role of structural variations to the disease.