## S93 TOLL-LIKE RECEPTOR 2 HAS A TUMOUR SUPPRESSOR FUNCTION IN MURINE NON-SMALL CELL LUNG CANCER

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**Background** Lung cancer is the leading cause of cancer related deaths worldwide. Patients typically present with late stage metastatic disease, making curative treatment impossible in the majority of cases. The value of targeting early stage disease has been widely recognised to improve overall mortality. Oncogene induced senescence (OIS) is an innate cell cycle arrest program instigated following the activation of oncogenes, and is a well known tumour suppressor mechanism. OIS is abundant in pre-malignant lesions in murine lung cancer models, however is lost during the progression to malignancy. We have recently identified a regulatory role for Toll-like receptor 2 (Tlr2) in oncogene-induced senescence[1], however the functional relevance of this has yet to be established *in vivo*.

**Methods** To determine the effect of Tlr2 signalling during non-small cell lung cancer (NSCLC) progression, we used mice heterozygous for the loxp-STOP-loxp-$Kras^{G12D}$allele ($Kras^{LSL\text{-}G12D/+}$), allowing lung specific activation of mutant $Kras^{G12D}$ signalling upon intranasal infection with Cre-recombinase expressing adenovirus (Adeno-CMV-Cre). $Kras^{LSL\text{-}G12D/+}$ mice were interbred with $Tlr2^{-/-}$ mice to generate a $Kras^{LSL\text{-}G12D/+}$; $Tlr2^{-/-}$strain. 1.5 x $10^7$ PFU of Adeno-CMV-Cre was delivered intranasally and mice were culled 12 weeks later. Tumour burden, proliferative markers (Ki67) and senescence markers (p21) were assessed by immunohistochemistry.

**Results** Tumour burden was significantly increased in $Kras^{LSL\text{-}G12D/+}$;$Tlr2^{-/-}$ mice in comparison to $Kras^{LSL\text{-}G12D/+}$;$Tlr2^{+/+}$ mice ($p<0.01$). This was associated with an increased proliferative index ($p<0.001$) and reduced p21 staining ($p<0.001$), indicating a reduced inability to undergo senescence.

**Conclusions** We have identified an *in vivo* functional role for Tlr2 in the suppression of murine lung cancer progression. By understanding the mechanisms regulating this early stage tumour suppressor process we may be able to develop biomarkers of early disease to better stratify lung cancer screening approaches.

### REFERENCE

1. Hair P, *et al*. The innate immune sensor Toll-like receptor 2 controls the senescence associated secretory phenotype. *Sci Adv*. 2019 Jun 5;**5**(6).

# Genetic and cellular mechanisms of pulmonary hypertension

## S94 IDENTIFICATION OF NATURAL TARGETS OF NONSENSE-MEDIATED DECAY RELEVANT TO PULMONARY VASCULAR DISEASES

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**Introduction and Objectives** Nonsense-mediated decay (NMD) is a quality- control mechanism that degrades RNA transcripts harbouring premature stop codons and consequently reduces production of truncated proteins. Inhibition of NMD is being evaluated as a therapeutic approach for pulmonary vascular diseases caused by pathogenic nonsense substitutions causing hereditary haemorrhagic telangiectasia (HHT). As this non-specific approach might also affect the expression of transcripts that are naturally regulated by NMD, our aim was to identify exons that are controlled by NMD and the biological processes they are involved in.

**Methods** Primary human microvascular endothelial cells (HMEC) were cultured to confluence in antibiotic-free medium before treatment for 1 hour with 100µg/ml cycloheximide to inhibit NMD, or fresh media. Ribosomal (r)-RNA-depleted total RNA was used to prepare strand-specific whole transcriptome libraries which were sequenced on an Illumina Genome Analyser II, aligned to hg18, counted using custom scripts, and normalized to total valid reads and exon size. Further scripts were written to identify exons present in HMEC treated with cycloheximide but not media-treated HMEC. Separately, blood outgrowth endothelial cells (BOECs) were established from 23 HHT patients with pathogenic nonsense substitutions in *ENG*, *ACVRL1* and *SMAD4*.

**Results** In the cycloheximide and media-treated normal HMEC, there were alignments to 15,756 RefSeq genes, and 113 micro (mi)RNAs. The 419 most differentially expressed RefSeq genes ($p<0.15$), clustered to Gene Ontology (GO) biological process compatible with the observed induction of membrane proteolysis in cycloheximide-treated cells, validating the methodological approach. There were overlaps between miRNAs that were differentially expressed, and their mRNA targets predicted by Targetscan. The approach also identified candidate alternate exons observed only in the cycloheximide-treated HMEC, including 333 alternate first exons, 662 mid exons, 275 terminal exons and 59 exon extensions. Candidate exons that introduced a premature stop codon into transcripts of genes involved in GO biological processes other than protein translation were validated by reverse transcriptase PCR, prior to selection as a panel to quantitatively evaluate NMD inhibition in BOECs from HHT patients.

**Conclusion** Natural targets of nonsense-mediated decay in HMEC were identified. Further investigation should provide new insights into the role of NMD in cellular physiology.

## S95 IDENTIFYING NEW HEREDITARY HAEMORRHAGIC TELANGIECTASIA GENES BY APPLYING A MACHINE LEARNING APPROACH TO SCREEN WHOLE GENOME SEQUENCING DATA

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**Introduction and objectives** Hereditary haemorrhagic telangiectasia (HHT) is a rare autosomal dominantly-inherited disease that causes pulmonary arteriovenous malformations and pulmonary hypertension. Four disease-causing genes have been identified- *ENG*, *ACVRL1*, *SMAD4* and *GDF2*. Here, we

demonstrate an unbiased screening method using whole genome sequencing (WGS) to identify novel genes that may cause HHT.

**Methods** Through the UK 100,000 Genomes Project Data Release 6.0, WGS data were available for 160 HHT participants from 126 families, following Illumina pipeline alignments and variant calling. For the current project, customised scripts were written in Python to extract all variants in HHT patients' variant call files (vcfs, currently for single nucleotide variants and small indels). The variants were then prioritized by characteristics such as allele frequency, deleteriousness, gene location and gene expression profiles, using both stepwise filtering and machine learning feature selection algorithms including LASSO and SVM-RFE.

**Results** A mean of 4,813,192 variants (range 4,726,104 to 5,362,271) were found in each HHT patient. Stepwise filters removed an average of 3,663,003 variants which exceeded an allele frequency of 0.02% in the 1000 Genome Project database, and a further 690 synonomous variants that did not change the genetic code. Excluding variants present in HHT patients where a likely pathogenic variant was already identified through the Genomic Medicine Centres left a residual 501,702 variants. Subsequent stages required novel machine learning algorithms focusing on endothelial cell-expressed variants (defined if present in one of the 11,488 genes with alignments in our RNASeq experiments in primary normal human microvascular endothelial cells); in-house RNASeq changes following BMP9 or TGF-β1 stimulation; and absence or very low frequency in non HHT Participants in the 100,000 Genomes project. Selected variants are being prioritised based on expert input from the HHT PAVM GeCIP Pathway Analyses Subgroup's knowledge of gene coding and untranslated regulatory regions, and detailed functional pathways.

**Conclusions** We have already identified multiple genes with putative damaging variants in patients with unexplained HHT, and are next to focus on variants in genes expressed by other cell types. Similar approaches could also be implemented in other rare diseases.

## S96 IDENTIFYING GENETIC MODIFIERS OF DISEASE SEVERITY USING WHOLE GENOME ANALYSES OF FAMILIES WITH HEREDITARY HAEMORRHAGIC TELANGIECTASIA RECRUITED TO THE 100,000 GENOMES PROJECT

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**Introduction** Why individuals with the same disease-causing DNA variant can have very different phenotypes is a puzzle in many conditions inherited as autosomal dominant traits. To explore, we focussed on hereditary haemorrhagic telangiectasia (HHT) in which approximately 50% of patients develop pulmonary arteriovenous malformations (PAVMs). In turn, these PAVMs can vary in severity dramatically within the same HHT family. With the rare opportunity for whole genome analysis of many HHT families recruited to the 100,000 Genomes Project[1], examination of potential phenotypic modifiers within families with the same HHT pathogenic variant was a relevant and unique question.

**Methods** Data were analysed within the 100,000 Genomes Project Research Data Embassy, following Illumina pipeline alignments and variant calling. Customised Python script was used to identify families with the same pathogenic HHT variant and extract each affected individual's DNA variants. Comparisons between affected family members differing markedly in disease severity were then performed using 3 separate methods: comparison of clinical tiered DNA variants, analysis of newly released copy number variants, and comparison of all single nucleotide variants and small indels in patients' variant call files (vcfs).

**Results** From the initial data set of 193 fully sequenced HHT families taken from Data Release 7 of the 100,000 Genomes Project, we selected those in which one family member had noticeably more severe symptoms recorded by the recruiting Genomic Medicine Centre. In one typical nuclear family with 3 affected members including one with severe PAVMs, within tiered genes of known function, 111 variants were only present in the PAVM-affected patient. Extending to copy number variants identified a further 363 variants that differed between this patient and the less severely affected relatives. Extending to vcf analyses using python script identified 490,225 variants that were only present in the PAVM-affected patient. The combined variant list is being cross-referenced to genomic locations of known gene coding regions and untranslated regulatory regions using *in silico* prediction tools.

**Conclusions** Whilst HHT is an autosomal dominant trait, these data emphasise the potential extent to which an unaffected parent may affect the disease severity through the influence of other inherited gene variants.

### REFERENCE

1. : http://www.genomicsengland.co.uk

## S97 HAEMOGLOBIN CHALLENGE INDUCES DYSFUNCTION IN HUMAN PULMONARY ARTERY ENDOTHELIAL CELLS: POTENTIAL RELEVANCE TO PULMONARY ARTERY HYPERTENSION

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**Background** The link between pulmonary arterial hypertension (PAH) and haemolytic anaemias, such as sickle-cell disease and thalassaemia, is well established. Recent studies have implicated sub-clinical haemolysis and the release of cell free haemoglobin (CFH) in idiopathic PAH. The interaction between CFH and pulmonary artery endothelial cells (PAECs) could induce endothelial dysfunction, a key component of the pathophysiology of PAH.

**Objectives** This study aims to investigate the role of CFH in PAEC dysfunction, defined in terms of intracellular and mitochondrial reactive oxygen species (ROS) generation, altered cell proliferation indices and changes in gene transcription of the ROS-generating enzyme NADPH oxidase-2 (Nox2).

**Methods** Cultured human PAECs (hPAECs) were challenged with 10 μM haemoglobin (Hb) or no treatment (control) for 24 hours. Flow cytometry was used to measure total intracellular ROS (dihydroethidium assay), mitochondrial ROS (MitoSOX assay) and cell cycle profile using propidium iodide. Nox2 gene expression was measured using RT-qPCR. Cell proliferation was measured using the BrdU assay.