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(SERPINA10: $r=0.85$), periostin (POSTN: $r=0.79$) and nitric oxide synthase 2 (NOS2: $r=0.72$). Correlations in controls were not significant (not shown).

Conclusion Eotaxin-3 gene expression is upregulated in the airway epithelium in eosinophilic asthma and highly correlates with IL-13 signature genes – including biomarker genes POSTN and NOS2.

Please refer to page A190 for declarations of interest related to this abstract.

From bench to lung: scientific advances in respiratory research

S94

ELITE ATHLETES SUSCEPTIBLE TO RESPIRATORY TRACT INFECTION ARE CHARACTERISED BY REDUCED CIRCULATING MEMORY T REGULATORY CELLS, UPPER AIRWAY MICROBIAL DYSBIOSIS AND DYSREGULATION OF SPHINGOLIPID METABOLISM

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Rationale Respiratory tract infection (RTI) is a major issue in athlete health and is the leading cause of training and competition time-loss. The host-defence immunomodulatory factors associated with heightened RTI susceptibility remains unclear.

Objective This prospective study aimed to characterise host immune factors in international athletes exhibiting heightened RTI susceptibility. **Methods/measurements:** Comprehensive clinical and physiological phenotyping was prospectively undertaken in a cohort of 121 elite athletes. Athletes were characterised using objective retrospective electronic medical record analysis as highly susceptible (HS) (≥ 5 confirmed RTI over last 18 months) (N=22) or non-susceptible (NS) (< 2 confirmed infections over last 18 months) (N=23). Peripheral blood lymphocyte population phenotyping of HS and NS athletes was performed by flow cytometry, with validation of findings by mass cytometry. The immune response to microbial stimuli was analysed by peripheral blood mononuclear cell (PBMC) stimulation assays. Further immuno-metabolic phenotyping was performed through 16S rRNA microbial sequencing of oropharyngeal swabs and global untargeted plasma metabolomic profiling. Findings were compared to data from a non-athletic healthy control group (N=10).

Main Results HS athletes had a persistently reduced memory T regulatory cell compartment compared to NS athletes ($p=0.005$) and healthy controls ($p=0.002$) (see figure 1) with a T helper 2 skewed PBMC immune response to microbial stimuli additionally seen in HS athletes. 16S rRNA microbial sequencing revealed a reduced bacterial biomass of the oropharyngeal microbiome in athletes compared to healthy controls ($p=0.032$), with plasma metabolomic profiling showing significant differences in sphingolipid pathway metabolites in HS athletes compared to NS athletes and healthy controls. Immune phenotypic differences were not related to sporting discipline or evidence of underlying asthma or atopy.

Conclusion Elite athletes have evidence of upper airway microbial dysbiosis, with a reduction in circulating memory T regulatory cells, and metabolic dysregulation of the sphingolipid pathway evident in those HS to RTI. Further prospective longitudinal work is needed to explore this novel

Abstract S94 Figure 1 CD4⁺ FoxP3 populations in elite athlete cohort. The CD3⁺ CD4⁺ T cell population identified using CyTOF was subjected to tSNE analysis using Cytobank. Using heat maps overlaid on tSNE plots, 2 FoxP3⁺ populations were identified and termed as FoxP3 P1 and FoxP3 P2 (a). Heatmaps were further used to examine differences in CD4 and Treg markers, with FoxP3⁺ population 2 seen to be CD45RO^{hi}CD45RA^{dim}CD95^{hi} identifying it as a memory Treg subset (b). FoxP3 P2 populations as a total of total CD3+CD4+ T cells were calculated (c) and compared for total athletes (TA), highly susceptible (HS), non-susceptible (NS) athletes and healthy controls (HC)

potential mechanistic link to elite athlete infection susceptibility.

Please refer to page A190 for declarations of interest related to this abstract.

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TRANSCRIPTIONAL SIGNATURES OF BLOOD OUTGROWTH ENDOTHELIAL CELLS FROM PATIENTS WITH PULMONARY ARTERIOVENOUS MALFORMATIONS AND HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Introduction and Objectives Pulmonary arteriovenous malformations (PAVMs) are most commonly caused by hereditary haemorrhagic telangiectasia (HHT). This multisystemic condition, inherited as an autosomal dominant trait, results from a heterozygous loss-of-function variant in *ACVRL1*, *ENG* or *SMAD4*. Heterozygous endothelial cell phenotypes have proved elusive, hindering preclinical testing of potential therapeutic agents. Here, our objective was to define the transcriptional changes occurring in patient-derived blood outgrowth endothelial cells (BOECs).

Methods With ethical approvals (16/ES/0095), BOECs were established from HHT/PAVM patients heterozygous for a pathogenic variant in *ACVRL1*, *ENG* or *SMAD4*, and from healthy volunteers. HHT gene protein production by patient and control BOECs was evaluated by ³⁵S-methionine pulse chase experiments. Single cell qRT-PCR aimed to verify expression and heterogeneity of 48 transcripts in 40 viable (DRAQ7 negative) BOECs per genotype. Long and short RNA libraries were generated from BOECs prior to Illumina HiSeq sequencing of paired-end reads, and alignment to GRCh38. Differential alignments were used to rank transcripts for discovery gene ontology process identifications.

Results 24 BOEC lines were established from patients heterozygous for one of 10 different nonsense (stop gain) pathogenic variants in *ENG*, *ACVRL1* and *SMAD4*, with a median of two donors per genotype. Pulse chase experiments distinguished the genotypes. Blinded analyses of normalised RNA-Seq alignments also identified the source heterozygous HHT genotypes: *ENG* alignments were lowest in heterozygous *ENG*^{+/−} BOECs (Dunn's *p*=0.0089); *ACVRL1* alignments lowest in heterozygous *ACVRL1*^{+/−} BOECs (*p*=0.0040) and *SMAD4* alignments lowest in heterozygous *SMAD4*^{+/−} BOECs (*p*=0.007). By single cell qRT-PCR, 7/48 (15%) genes were expressed in all BOECs, 7/48 (15%) in no BOECs with 34 genes expressed in a proportion of BOECs. Seven genes displayed differential expression patterns between HHT and control BOECs, confirmed by distribution plots of all 16,807 RNASeq Ensembl transcript alignments in BOECs from different donors. Ranking transcripts by differential alignments in *ENG/ACVRL1/SMAD4* compared to control BOECs, identified consistent gene ontology processes enriched compared to equivalent numbers of randomly-selected transcripts.

Conclusions There are reproducible, transcriptional signatures in pulmonary AVM and HHT patient-derived BOECs distinguishable from healthy volunteer BOEC signatures. Common patterns for *ACVRL1*, *ENG* and *SMAD4* BOECs suggest a shared HHT transcriptome phenotype.

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PULMONARY ARTERIOVENOUS MALFORMATIONS – GENETIC VERSUS CLINICAL EVIDENCE OF UNDERLYING HEREDITARY HAEMORRHAGIC TELANGIECTASIA

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Introduction and Objectives Pulmonary arteriovenous malformations (PAVMs) result in early onset but preventable strokes and other complications. Patients know that PAVMs can be a familial condition, most commonly due to hereditary haemorrhagic telangiectasia (HHT). Since the April 2020 NHS National Genomic Test Directory launch, patients with PAVMs are eligible for gene testing only if they already meet a definite clinical diagnosis of HHT, requiring two further Curaçao Criteria from nosebleeds, mucocutaneous telangiectasia, or first-degree relative with HHT. Our goal was to test the validity of this requirement.

Methods We audited ClinVar-listed variants in the major HHT genes, and with ethical approval, case notes of patients with PAVMs who had undergone NHS genetic testing. Tests were ordered predominantly between 2015–2019 through Mainstreaming Genomics initiatives via gene-test panels, or whole genome sequencing through the 100,000 Genomes Project.

Results ClinVar lists 2,804 variants in *ENG*, *ACVRL1* and *SMAD4*, including 909 likely pathogenic/pathogenic variants that diagnose HHT. Most are loss-of-function frameshift, stop-gain and splice site variants (390/645 [60%] for *ENG/SMAD4*; 126/264 [47%] for *ACVRL1*). At least 50% of people with one of these variants would be expected to have PAVMs. At our institution, 124 patients with PAVMs were the first in their family to have a gene test. Of these, 83 (67%) tested positive for HHT, i.e. were found to be heterozygous for a likely pathogenic or pathogenic variant in *ENG*, *ACVRL1* or *SMAD4*. Focussing on the 83 patients with PAVMs and genetically-diagnosed HHT, only 63/83 (76%) met three or more Curaçao criteria. For the remaining 20 patients with PAVMs and a positive HHT gene test, none met the family history criterion for HHT. While 14 (70%) described nosebleeds as an adult, only 3 (15%) had classical HHT telangiectasia, and the cohort included families where pulmonary AVMs (single or multiple) were the only HHT clinical feature across individuals with *ENG* pathogenic variants in two generations.

Conclusions There is a high burden of deleterious variants in HHT genes. Two-thirds of unselected PAVM patients have genetically-confirmed HHT, but of these, 1 in 4 display few if any clinical features of HHT. Wider gene testing is recommended.

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INVESTIGATING THE PRO-FIBROTIC EFFECTS OF GALECTINS IN IPF – A POTENTIAL ROLE FOR GLYCAN-MEDIATED INTERACTIONS WITH INTEGRINS

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Introduction Integrins are a family of transmembrane heterodimer proteins differentially expressed on the cell surface of many lung cell types. Integrin-mediated activation of the key