acid suppression therapy, even in the context of heartburn. However, a larger dataset is required to understand whether those with heartburn might be more likely to respond.

REFERENCES
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P13 THE OVERLAPPING PREVALENCE OF CHRONIC MUCUS HYPERSECRETION (CMH) AND CHRONIC COUGH (CC)

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Introduction There is considerable interest surrounding the role of chronic mucus hypersecretion (CMH) in the development of COPD but varying definitions of CMH have created uncertainty regarding its prevalence. Some studies characterise CMH as chronic phlegm production whilst others as chronic cough (CC) productive of phlegm. By understanding how these symptom groups relate to each other, we may be better equipped to interpret existing data and search for therapeutic targets. We report the prevalence and overlap of CMH and CC over 43 years of adult life from age 20 years within the nationally representative MRC National Survey of Health and Development (NSHD) birth cohort.
Methods The MRC NSHD is a birth cohort study of men and women born during one week in March 1946 within England, Scotland and Wales. CMH and CC presence was determined by completion of the MRC questionnaire on respiratory symptoms in the following years (study member age in years): 1966(20), 1971 (25), 1982(36), 1989(43), 1999(53) and 2009(63). The MRC questionnaire defines CMH as the production of phlegm from the chest on most days for three months of each year and defines CC as cough on most days for three months of each year.
Results 1394 subjects (47% male) completed questionnaires on all six occasions between 1966 and 2009. 398 study members (26.8%) reported either symptom at least once with a majority of CMH reporters concurrently reporting CC (See Table 1). The percentage of CMH reporters concurrently reporting CC increased with age (0.5%/year increase, CI 0.18–0.82, p = 0.001).
Conclusion Most chronic phlegm producers report concurrent CC, and this percentage increases with age. Restricting CMH definition to those with CC, however, misses the substantial proportion of the population who report chronic phlegm

Abstract P13 Table 1 Prevalence of symptoms amongst the 1394 participants providing complete data

		Year (age in years)					
		1966 (20)	1971 (25)	1982 (35)	1989 (43)	1999 (53)	2009 (63)
% of Total Population (n=1394)	Either symptom	6.7%	7.8%	7.0%	9.0%	10.3%	13.3%
	CMH	4.7%	4.4%	3.9%	5.1%	5.7%	9.0%
	CC	4.4%	6.0%	5.7%	7.5%	8.8%	11.0%
	CMH with CC	2.5%	2.6%	2.7%	3.7%	4.2%	6.7%
	CMH without CC	2.2%	1.9%	1.2%	1.4%	1.5%	2.3%
No. of study members with CMH		66	62	55	71	80	125
% of CMH population with CC		53.0%	58.1%	69.1%	71.8%	73.8%	74.4%
% of CMH population without CC		47.0%	41.9%	30.9%	28.2%	26.3%	25.6%

production without CC. The requirement for a chronic cough to define CMH may underestimate the total prevalence of chronic sputum producers and hence those potentially at risk of COPD development or progression.

Basic mechanisms of acute lung injury, interstitial lung disease and PAH

P14 VERY HIGH QUALITY NEXT-GENERATION DNA SEQUENCING DATA FROM HUMAN GENOMIC DNA SAMPLES STORED, AND INTERMITTENTLY DEFROSTED OVER TWO DECADES

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Introduction and objectives Concern about the DNA quality for next-generation sequencing encourages use of dedicated preparative kits. The purpose of this study was to attempt to sequence ten stored DNA samples that had been prepared from human blood using phenol chloroform methods 12–17 years earlier, frozen at -70 C and not subjected to special treatments.
Methods The ten DNA samples that had been defrosted on multiple occasions, were defrosted again for library preparations using the Agilent SureSelect Target Enrichment System for Illumina paired-end multiplexed sequencing. Sequencing was performed on an Illumina HiSeq 2000 instrument for 2×100 base reads. Sequencing data were processed with RTA version 1.7.45 with default filter and quality settings, aligned to human genome build hg18 using CASAVA Eland pair algorithm, and demultiplexed with CASAVA 1.7.
Results All libraries passed stringent quality control steps at each step of library generation. More than 10 Giga bases (Gb) of sequence was generated from each read. High quality scores meant that even the data from the last of the 200 sequencing cycles were usable, with a cycle 200 median Phred score of 25. More than 3.3 million clusters passed filters for each read, and more than 86% of the sequence reads aligned to the human genome. For each sample, approximately 8 million primary sequence reads uniquely mapped to the captured region of interest.
Conclusions Extremely high quality DNA sequences can be obtained using stored DNA samples prepared many years earlier, and not subjected to any special treatments in the intervening years. The findings will be of particular importance to research communities where acquisition of new samples is not always possible.

P15 DIRECTIONAL NEXT GENERATION WHOLE TRANSCRIPTOME SEQUENCING OF PRIMARY HUMAN PULMONARY MICROVASCULAR ENDOTHELIAL CELLS

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Introduction and objectives To improve our understanding of the dynamic interplay between non-coding and coding RNA species, we developed solution-based methods to capture the entire endothelial transcriptome. In contrast to microarrays and CHIP-based methodologies, there was no pre-specification of RNA target sequences, and the identity of the DNA strand of origin was preserved.

Methods Triplicate confluent primary human microvascular endothelial cells (Promocell GmbH) were cultured in the presence and absence of TGF-beta stimuli relevant to pulmonary arteriovenous malformations and hereditary haemorrhagic telangiectasia (HHT). Following rRNA-depletion of total RNA, seven libraries were prepared using Illumina reagents, and 8 pM of each library used for cluster generation and sequencing on Genome Analyser II. Algorithms for aligning reads to NCBI36/hg18 and GRCh37/hg19 included Bowtie, TopHat and Seqmap. Validations were performed using quantitative rt-PCR.

Results More than 2 Gigabases of sequence was generated. Transcriptome-wide profiles were similar between libraries, with sixteen types of RNA species detected including 146 micro (mi) RNA families (47 broadly conserved), and 10,749 protein-encoding mRNAs representing ~5.5% of mapped reads. Alignments to endothelial mRNAs/miRNAs were substantially higher than to gene loci for non-endothelial mRNAs/miRNAs. mRNA exon alignments demonstrated sharp exon boundary delineation, but replicate alignments to non-repetitive intronic regions involved in multi-exon deletions in HHT patients. There was an inverse relationship between alignments depths and qt-PCT cycle thresholds (Ct), where single alignments were detectable, and Ct values of 20 generated by 0.02 nM spiked RNA. Across all experiments in replicate donor/treatment RNAs, for a panel of single open reading frame miRNA genes, RNASeq alignments (gene strand read counts normalised to the total number of valid reads and exon/locus size) explained 72% of the variance of qt-PCR cycle threshold (p < 0.0001). Dynamic whole transcriptome profiling is in progress.

Conclusions These novel directional next generation RNA sequencing methods provide new insights for mutational mechanisms, and a systems approach to dissection of regulatory and target RNA networks relevant to human disease.

P16 **A SYSTEMATIC CHARACTERISATION OF INFLAMMATION IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION**

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Introduction The pathogenesis of chronic thromboembolic pulmonary hypertension (CTEPH) is poorly understood. Idiopathic pulmonary arterial hypertension (IPAH) is associated with systemic and localised inflammation. Distal vasculopathy is seen in IPAH and CTEPH (2 compartment model). Inflammation has also been implicated in CTEPH pathogenesis. We undertook a systematic assessment of inflammatory markers and immune cells to determine the role of inflammation in CTEPH.

Methods We examined the distal tails of 26 pulmonary endarterectomy (PEA) specimens and explanted lungs (5 CTEPH, 11 IPAH). Formalin fixed samples were immunostained with anti-human CD45 (inflammatory cell), CD79a (B cell), CD68 (macrophage) or CD3 (T cell) antibodies. Cell counts were normalised to the area surrounding vessels bounded by airspace structures for perivascular cell counts; to vessel area for media cell counts and per high powered field for lung parenchymal cell counts. Serum was collected from 61 patients pre and 6 months post PEA. Cytokines were measured using a multiplex array (pg/ml; mean±SD).

Results Table 1.

CD3$^+$ cells were less abundant in the media of small vessels (mean 0.03 ± 0.01 mm^2) in CTEPH vs. IPAH (p = 0.02).

Perivascular and parenchymal cell counts showed non-significant reductions of CD3$^+$ cells in CTPEH (p = 0.19 and 0.08 respectively). More CD68$^+$ cells were seen in the parenchyma in CTEPH (p = 0.03). Few CD20$^+$ cells were seen in parenchyma with no difference.

Neovascularisation correlated to CD45$^+$ cells in PEA specimens when normalised to specimen area (r = 0.4, p = 0.01).

Conclusions In CTEPH, most serum cytokines were not elevated sufficiently to suggest systemic inflammation is important in pathogenesis. The change in IL10 and TNFα are possibly due to improvement in cardiac function post PEA as described previously. The relative lack of CD3$^+$ T cells in the media of small arteries in CTEPH suggests that localised inflammation is also less important. The significance of CD68$^+$ cells in CTEPH lung parenchyma needs further assessment.

The correlation of CD45$^+$ cells and neovascularisation in PEA specimens may suggest an association between inflammation and neovascularisation.

Inflammatory signals maybe related to thrombus remodelling and cardiac dysfunction rather than to CTEPH pathogenesis. Isolating these processes is required to expand on this descriptive study.

Abstract P16 Table 1 Cytokine levels pre and post pulmonary endarterectomy

Cytokine	n	Pre PEA pg/ml	Post PEA pg/ml	p
IL2	16	0.22 ±0.35	0.264 ±0.53	0.32
IL4	16	1.74 ±6.4	1.052 ±3.69	0.96
IL5	16	0.99 ±0.44	0.855 ±0.07	0.18
IL6	61	5.09 ±13.3	2.69 ±5.31	0.12
IL7	16	0.13 ±0.00	0.13 ±0.00	n/a
IL8	61	10.42 ±14.37	5.84 ±8.30	0.0022
IL10	61	10.42 ±20.22	3.10 ±4.95	0.0004
IL12	16	0.13 ±0.00	0.74 ±2.45	0.32
IL13	61	1.64 ±2.69	1.65 ±3.67	0.51
TNFα	61	10.75 ±7.91	8.89 ±7.04	0.0087
GM-CSF	16	0.27 ±0.19	0.24 ±0.16	0.47
IFNγ	16	0.49 ±1.00	0.41 ±1.10	0.62

Data presented as mean ± SD. p – Wilcoxon rank-sum test. IL – interleukin; TNFα – tumour necrosis factor α; GM-CSF – granulocyte-macrophage colony-stimulating factor; IFNγ – interferon

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P17 **MOLECULAR COMPLEXITIES IDENTIFIED THROUGH TARGETED GENOMIC SEQUENCING OF THE HHT3 LOCUS ON CHROMOSOME 5**

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