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
1 Karhilas PJ. Response of chronic cough to acid-suppressive therapy in patients with gastroesophageal reflux disease. Chest 2013

P13
THE OVERLAPPING PREVALENCE OF CHRONIC MUCUS HYPERSECRETION (CMH) AND CHRONIC COUGH (CC)
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10.1136/thoraxjnl-2014-206260.163

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 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 at 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 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
CL Shovlin, FS Govani, IJ Milet, E Thomas, MD Jones, A Giess, L Game. Imperial College, London, UK
10.1136/thoraxjnl-2014-206260.164

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 x 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.

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

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

P15
DIRECTIONAL NEXT GENERATION WHOLE TRANSCRIPTOME SEQUENCING OF PRIMARY HUMAN PULMONARY MICROVASCULAR ENDOTHELIAL CELLS
CL Shovlin, D Patel, FS Govani, A Giess, MD Jones, L Game, IJ Milet. Imperial College, London, UK
10.1136/thoraxjnl-2014-206260.165

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.