**MOLECULAR MARKERS OF EARLY STAGE COPD IN SERUM, SPUTUM, AND NASAL EPITHELIUM FROM A CASE-CONTROL STUDY**

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COPD is a multifactorial disease of emphysema, chronic bronchitis, and small airway obstruction mainly caused by pro-kem exposure to tobacco smoking. COPD takes an average 20 years to develop, and while its key pathophysiological stages are relatively well described, its onset is as of yet poorly characterized. In addition, its considerable heterogeneity has made the identification of biomarkers that could be leveraged in diagnosis, patient stratification, treatment or risk prediction of disease development.

Unlike the other clinical studies on COPD, our COPD Biomarker Study focused on the early stage disease and smokers who do not show reduced FEV1 measurement. The primary is yet poorly characterized. In addition, its considerable heterogeneity has made the identification of biomarkers that could be leveraged in diagnosis, patient stratification, treatment or risk prediction of disease development.

**Study Description**

The study was conducted in a non-interventional, observational case-control study design in the United Kingdom, approved by a UK National Health Services (NHS) Ethics Committee (The Black Country Ethics Committee) and in compliance with Good Clinical Practice guidelines. The study has been registered on ClinicalTrials.gov with identifier NCT01790226.

**Study Endpoints**

- Nasal Transcriptionomics
- Sputum Proteomics
- Serum Lipidomics

**Nasal Transcriptionomics**

Nasal epithelium for mRNA analysis was collected from all study participants using a Rhinoprobe® plastic curette during clinical visits. RNA was extracted, processed and hybridized to Affymetrix Human Genome U133 Plus 2.0 Arrays by AMRX Applied Bioscience A/S (Aarhus, Denmark). Following hybridization, data from 108 samples were available for analysis by quantitative, microarray-based statistical analysis as previously described (3).

**Sputum Proteomics**

**107 proteins** were found to be differentially expressed in sputum of current vs. never-smokers and 13 proteins showed differential abundance in the sputum of COPD smokers compared to healthy current smokers (FDR adjusted p-value < 0.05).

Functional association-based analysis for current vs. never-smokers indicates clusters of mucin/ trefoil proteins, complement and peptidase regulators among the over-abundant proteins and clusters of down-regulated antibodies and plasma proteins (C). The differentially expressed proteins in COPD patients vs. current smokers were also seen to be significantly (differentially expressed when comparing COPD with former smokers, while no significant differences were noted when comparing never with former smokers). Interestingly, for most of these proteins differential expression appears to be already present in current smokers, and thus reflects an acceleration of the smoke exposure effects in COPD patients (D). (E) Shown trend for up- and down-regulated protein group).

**Conclusions and Perspectives**

On the transcriptomic level, biological network analysis demonstrates the impact of cigarette smoking on several cellular processes incl. cell stress, cell fate and inflammation which is at least partially reversible upon smoking cessation. Early stage disease development appears to further affect these processes.

Proteomics analysis shows that the abundance of several mucin/trefoil proteins and the protease-antiprotease balance is altered by cigarette smoking and that these alterations are ‘restored’ upon smoking cessation, which could further indicate potential biomarkers. Cigarette smoking also increases the levels of several bioactive, inflammatory lipids which are partially only partially restored when smoking is stopped.

The affected RNA, protein and/or lipid molecules could be further explored as biomarkers with utility in product assessment studies. Specific molecular changes observed in patients with early stages of disease could be further examined with respect to their potential to indicate disease risk.

**References**
