Physiological Measures and Novel Sputum Biomarkers to Distinguish Subjects with Mild to Moderate COPD from Asymptomatic Current Smokers, Former Smokers and Never-Smokers

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Karsta Luettich, Patrick Vanscheeuwijck, Nveed Chaudhary, Julia Hoeng, Manuel Peitsch

Philip Morris International R&D, Philip Morris Products S.A.
(part of Philip Morris International group of companies)

Philip Morris International is the sole source of funding and sponsor of this project.

Objective

Identification of **physiological measures** and **sputum biomarkers** to enhance early detection of COPD in asymptomatic smokers

Study Description

Non-interventional, parallel-group, case-controlled study to:

Primary Objective

- Identify a biomarker or panel of biomarkers for the differentiation of subjects with mild to moderate COPD (GOLD stage I and II), asymptomatic current smokers, former smokers and never-smokers using gene and protein analyses in biological samples

Secondary Objective

- Determine optimal methods of biomarker analysis in sputum, nasal and blood samples
- Compare physiological measurements and quality of life (QoL) across the study groups

NCT01780298; Approved by The Black County Ethics Committee, UK

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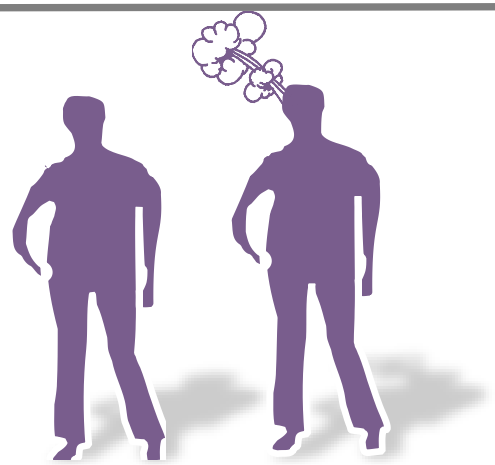
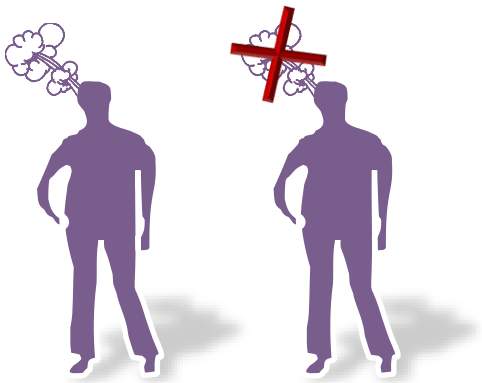
Overarching Scientific Questions

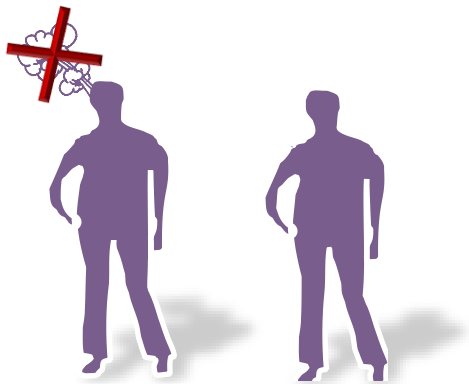


What is the biological impact of smoking?

What is the COPD-specific biology in smokers?



Which of the smoking effects are reversible?



Which of the smoking effects are irreversible?

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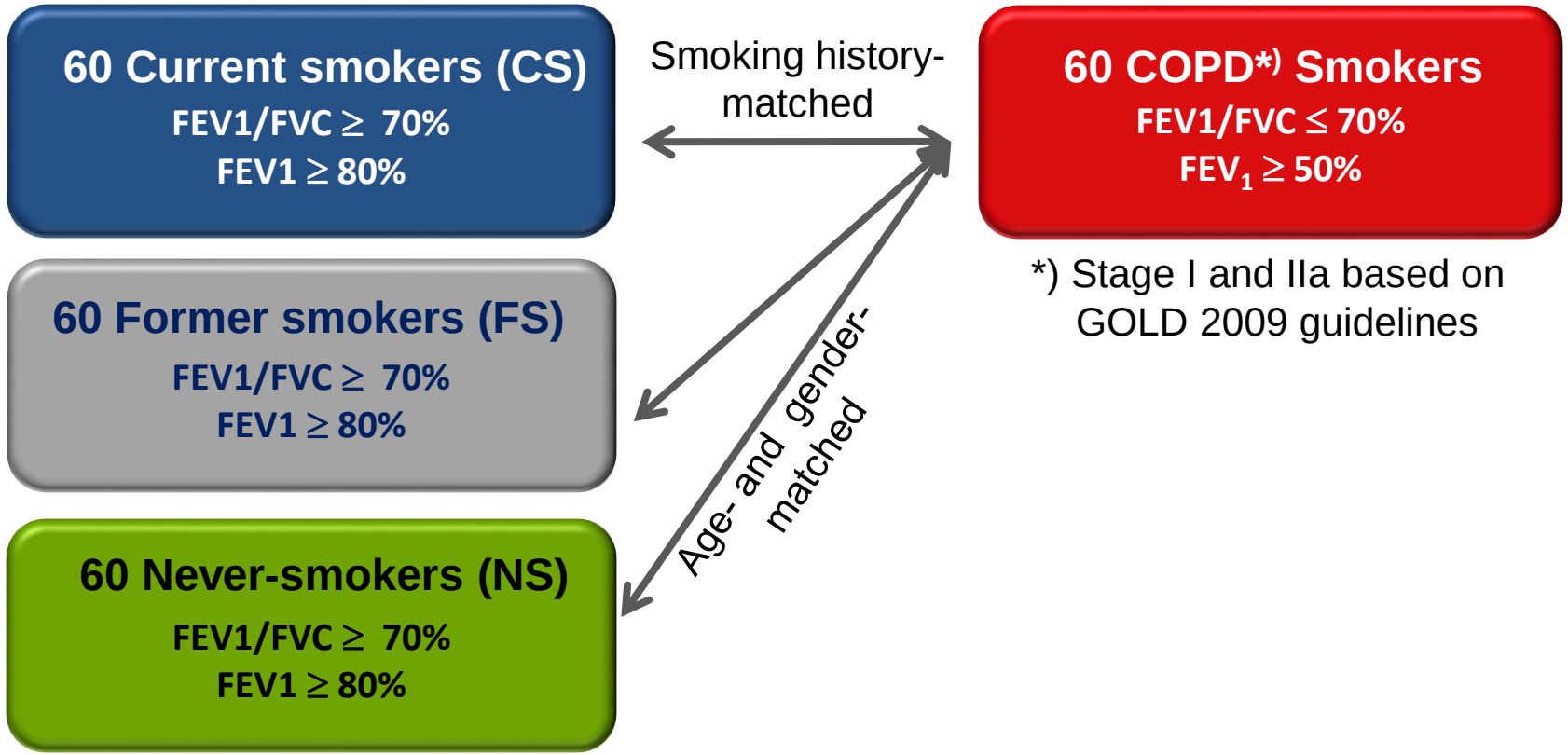
Study Population Overview

≥ 10 pack-year smoking history

≥ 10 pack-years smoking history and have not smoked for at least 1 year



*) Stage I and IIa based on GOLD 2009 guidelines

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Demographics

	Never-smokers (NS)	Former smokers (FS)	Current smokers (CS)	COPD smokers (COPD)
Age [years]	55.2 (7.3)	56.6 (7.0)	55.2 (7.0)	57.4 (7.0)
Gender [Male/Female]	35/25	35/25	35/25	35/25
BMI [kg/m^2]	26.5 (3.7)	27.2 (3.5)	27.6 (3.6)	26.3 (3.7)
Smoking history [Pack-years]	0	28.2 (13.6)	33.5 (14.5)	44.8 (21.9)
Heart rate [bpm]	63.2 (10.4)	64.8 (10.2)	68.4 (10.1)	69.7 (9.6)
Respiratory rate [breaths per min]	15.7 (2.3)	15.2 (2.1)	15.7 (2.1)	14.8 (2.1)
FEV1 [% predicted]	111.8 (13.9)	108.3 (12.4)	100.8 (11.8)	75.6 (18.1)
FEV1/FVC [%]	73.8 (5.9)	72.1 (4.1)	70.4 (5.4)	54.5 (8.8)

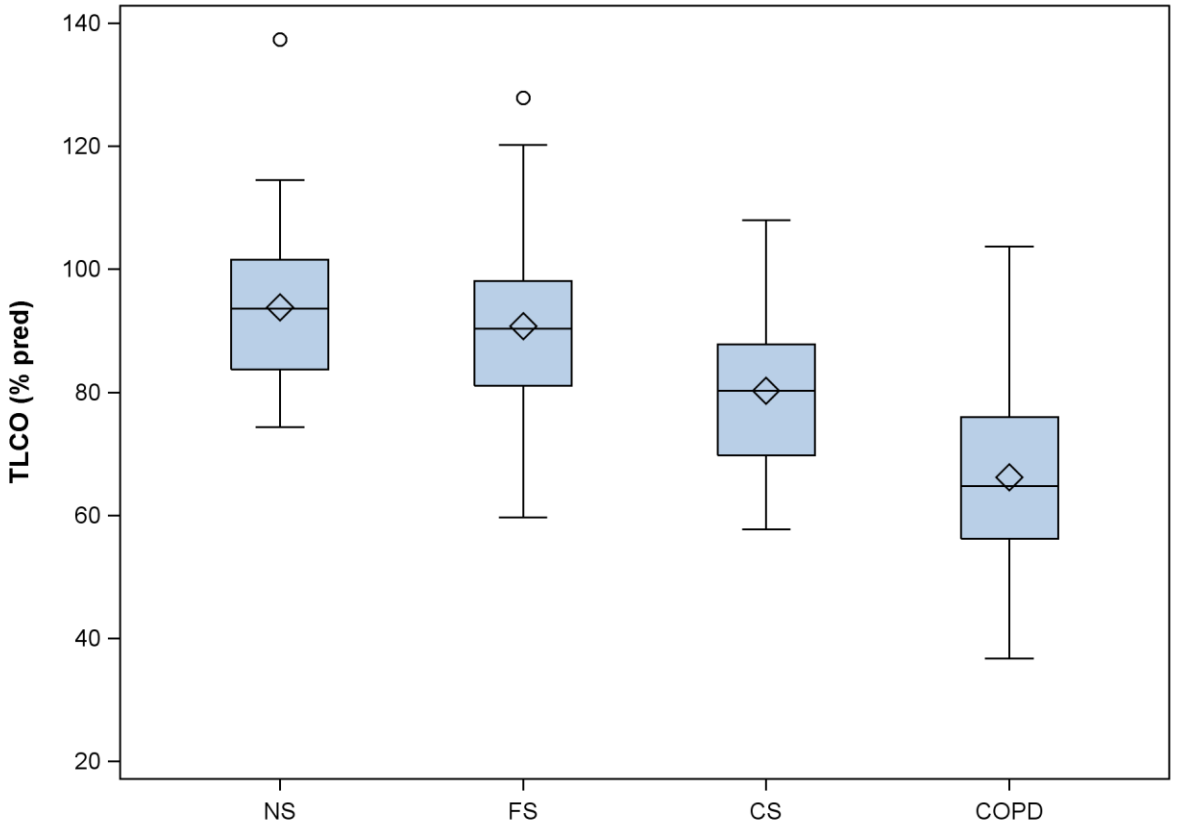
Data are presented as mean (SD) with the exception of gender (n)
BMI: Body mass index; FEV1: forced expiratory volume in 1 second

Gas Transfer Assessment

- Significant differences in TLCO % predicted between COPD and current, former and never-smokers (NS ≈ FS > CS > COPD; p<0.001 and <0.001, respectively)

- No differences in other gas transfer parameters (KCO % predicted, V_A % predicted or V_{IN} % predicted)

- TLCO < 80% predicted seen in some study subjects with normal spirometry including former smokers and asymptomatic current smokers



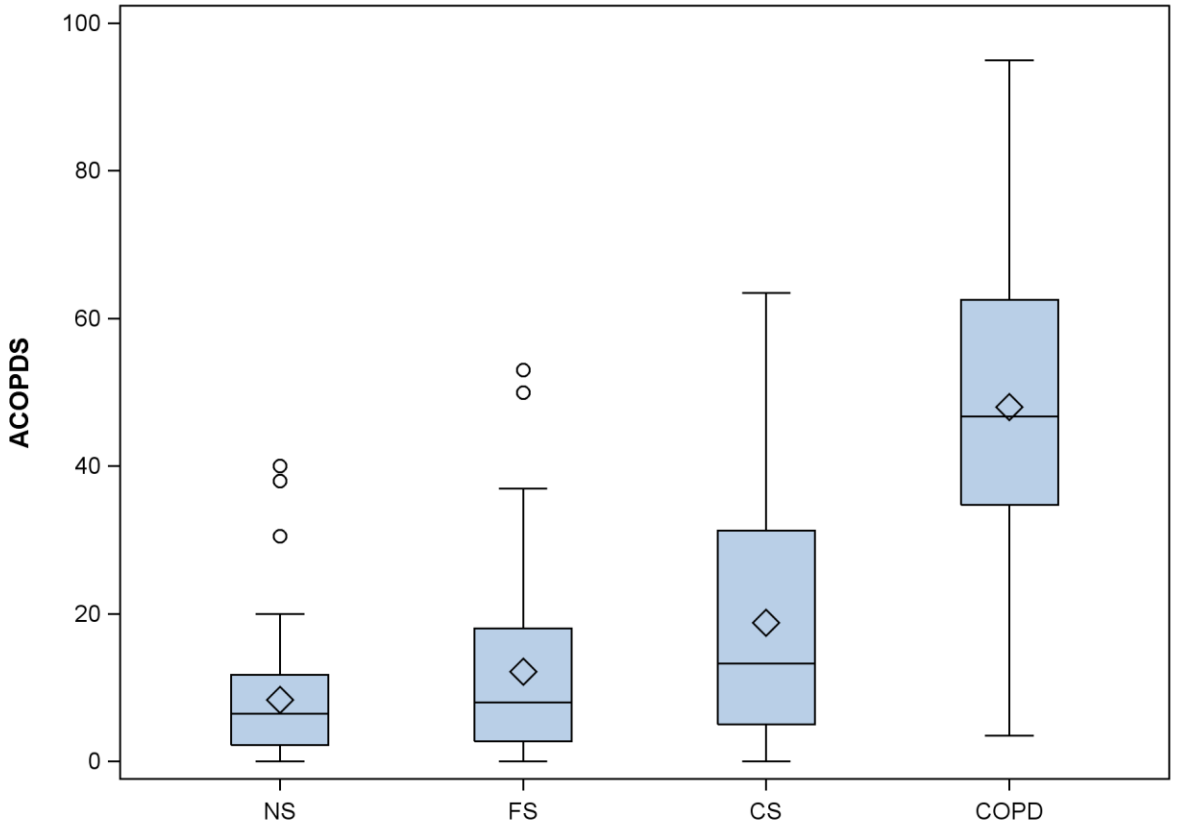
TLCO (% predicted) by study group (NS: never-smokers; FS: former smokers; CS: current smokers; COPD: subjects with COPD). Data are presented as box-whisker plots reflecting the first and third quartile (lower and upper boundary of box, respectively), median (line), mean (open diamond) and minimum values above the lower fence/maximum values below the upper fence (lower and upper whisker, respectively) for the corresponding study group. Outliers are represented by open circles.

Lung Sound Analysis (Stethographics)

- Significant differences in inspiratory crackle rate between COPD and current smokers and between current and never-smokers (p=0.0030 and p=0.0042, respectively)

- Presence of increased “pendelluft” in COPD smokers compared with asymptomatic smokers, particularly with deep breathing

- Rank score of combined lung sound parameters (total acoustic score) significantly higher in COPD compared with current smokers (46.75 *vs* 13.25; p<0.0001)



Stethographics-based total acoustic COPD score (ACOPDS) by study group (NS: never-smokers; FS: former smokers; CS: current smokers; COPD: subjects with COPD). Data are presented as box-whisker plots reflecting the first and third quartile (lower and upper boundary of box, respectively), median (line), mean (open diamond) and minimum values above the lower fence/maximum values below the upper fence (lower and upper whisker, respectively) for the corresponding study group. Outliers are represented by open circles.

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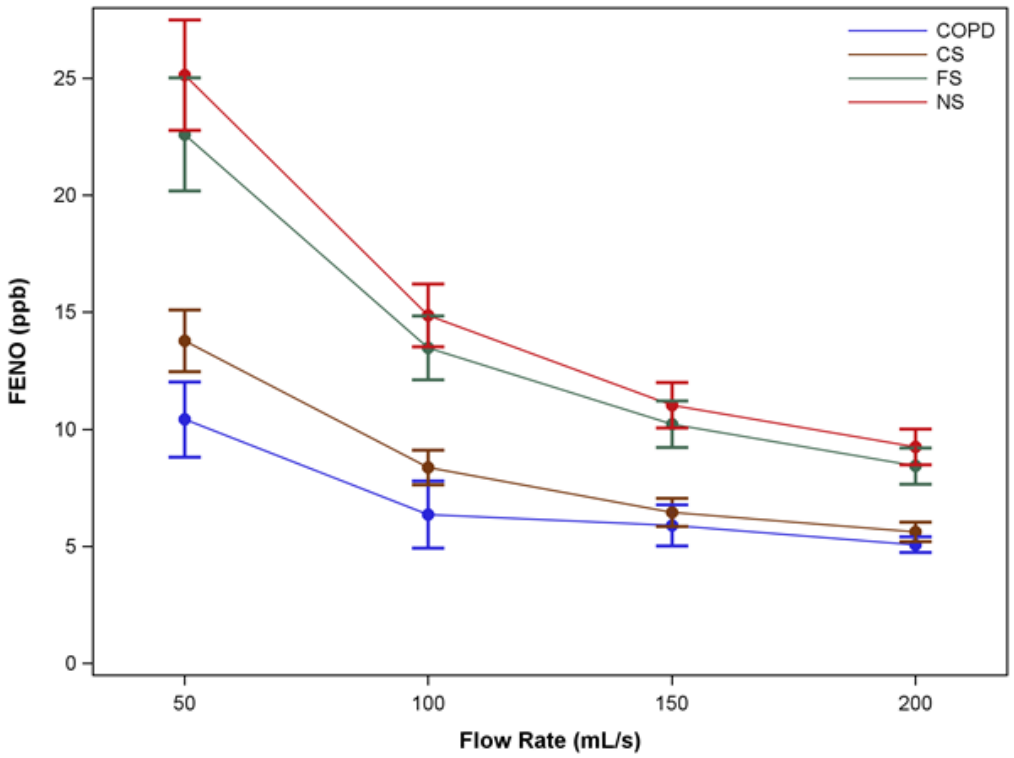
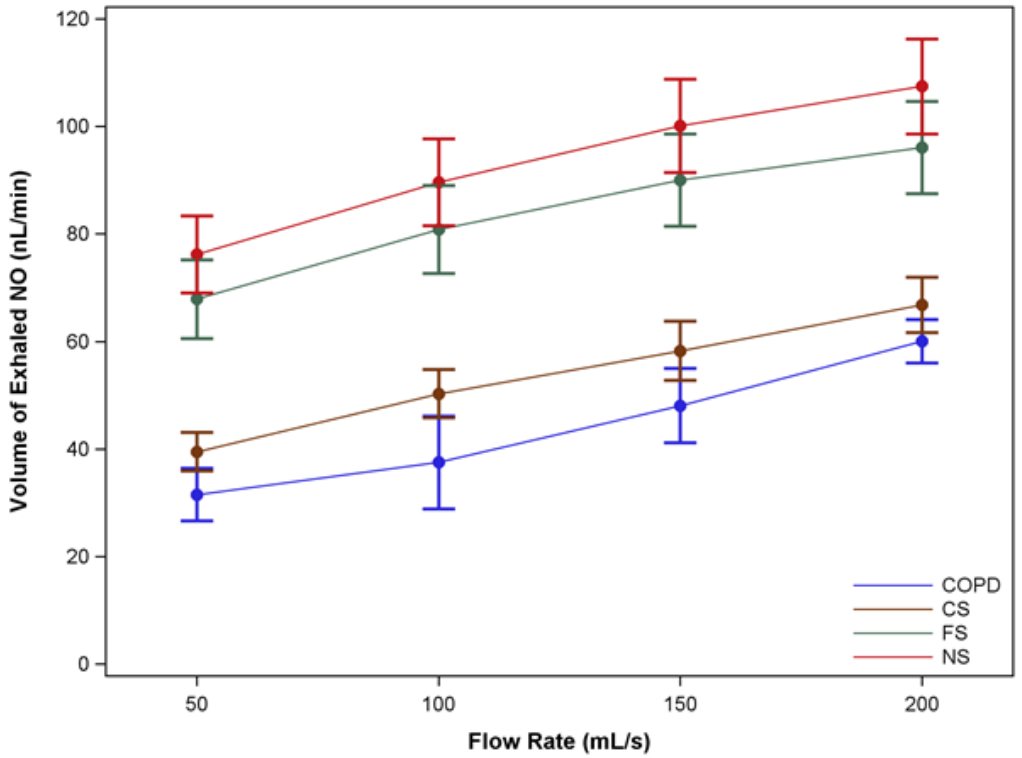
Fractional Exhaled Nitric Oxide





Fractional exhaled nitric oxide was slightly lower in COPD smokers than in disease-free smokers, and in current compared with former smokers ($p > 0.05$)

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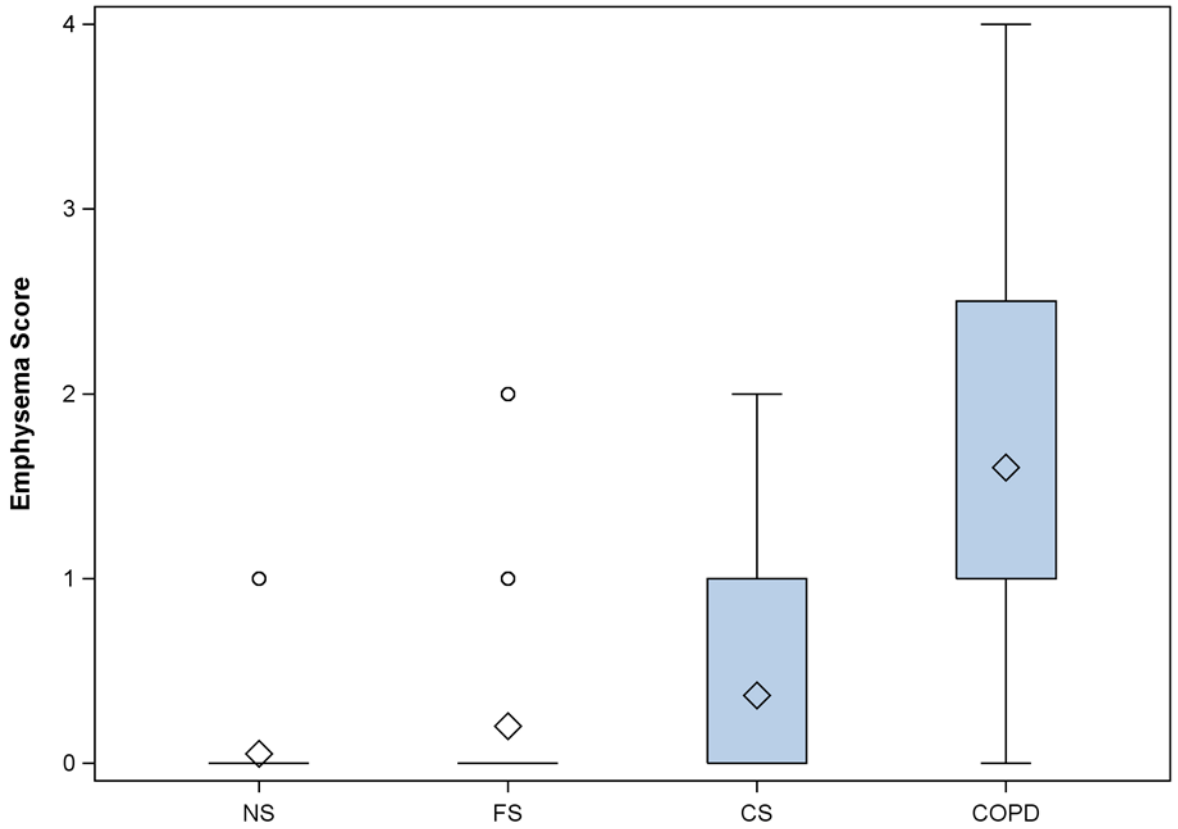
Chest HRCT Imaging

- Significantly higher emphysema scores in subjects with mild to moderate COPD compared with current asymptomatic smokers ($p<0.001$), and in the current smokers compared with never-smokers ($p=0.001$)

- Emphysematous lesions seen in all control groups, with nearly one third of current smokers with normal lung function exhibiting mild to moderate emphysema on CT

- No difference in small airway disease scores between COPD and asymptomatic smokers

- Pulmonary nodules more frequently identified in COPD patients and current smokers than in former and never-smokers



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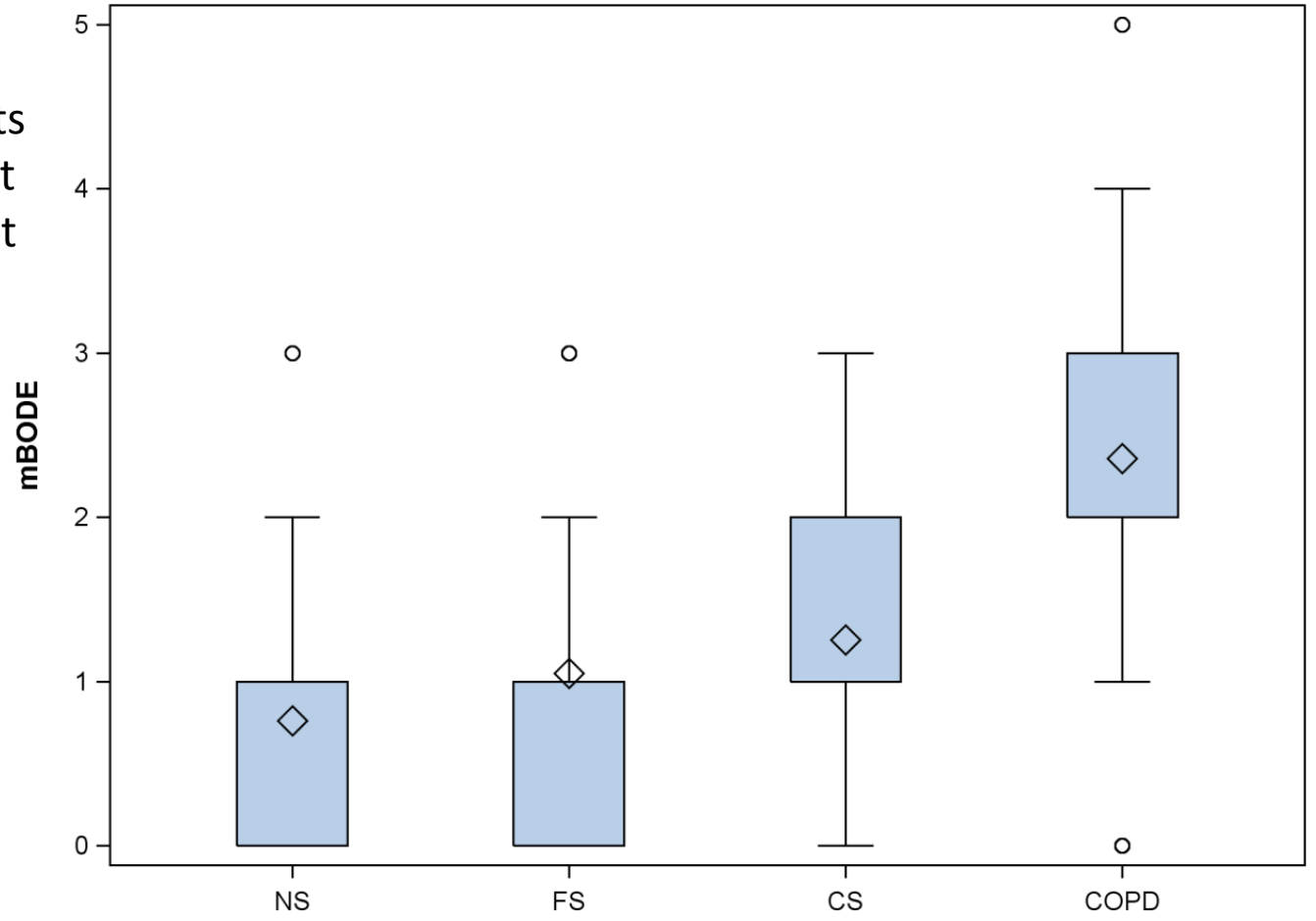
Quality of Life

- Significantly higher modified BODE scores in subjects with mild to moderate COPD compared with current asymptomatic smokers ($p<0.001$), and in the current smokers compared with never-smokers ($p=0.001$)



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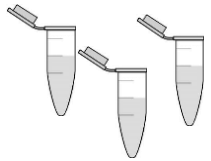
Biomarker Identification

Induced Sputum	Proteomics	Best representation of the inflammatory state of the upper airways. Contains cells of interest and protein mediators	√
Whole Blood (Lymphocytes)	Transcriptomics Proteomics Lipidomics	Identify correlations with data identified from the sputum. Blood represents the ideal and most convenient matrix for large-scale biomarker measurements	√ (T, L)
Nasal fluid	Proteomics	A number of respiratory researchers have shown strong correlations between inflammation in the lung and nose. May represent a convenient matrix for large-scale studies	√
Nasal scrapes	Transcriptomics Proteomics	May represent a convenient matrix for large-scale studies if correlations are seen with the sputum	√ (T)
Nasal lavage	Transcriptomics Proteomics	May represent a convenient matrix for large-scale studies if correlations are seen with the sputum	√ (P)

Induced Sputum Analysis

Proteomics Workflow

Sputum Supernatants



Digestion & isobaric labeling (TMT)

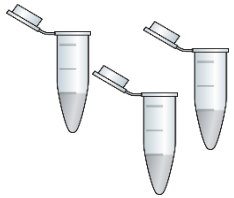
Liquid chromatography

Tandem mass spectrometry

Computational analysis

Transcriptomics Workflow

Sputum Pellets



RNA extraction and QC

RNA processing and labeling

Chip hybridization and scan

Computational analysis

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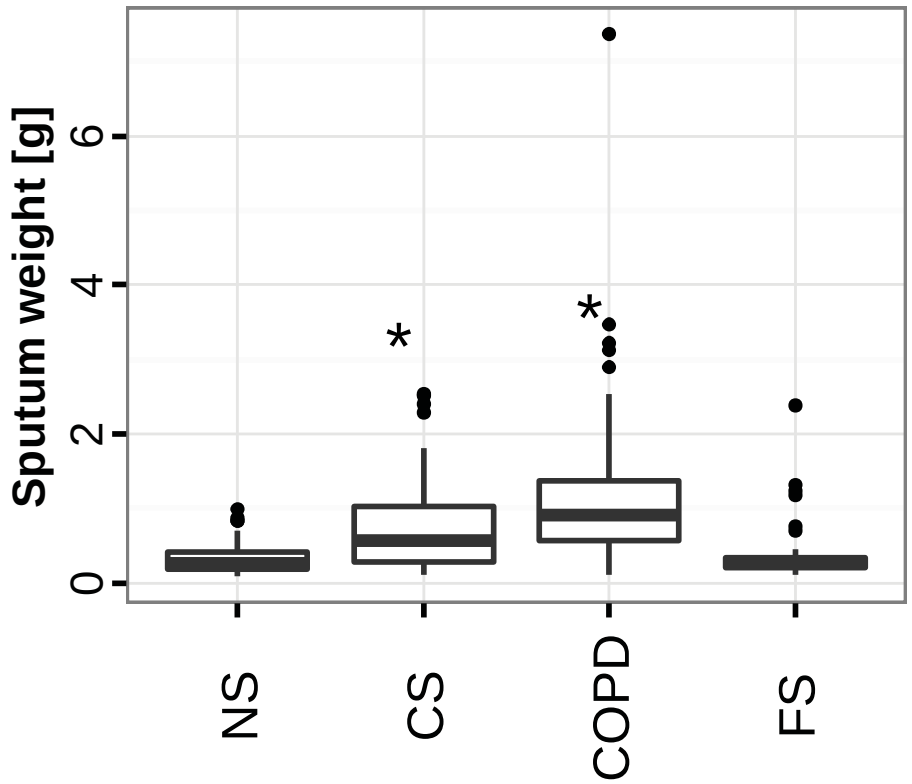
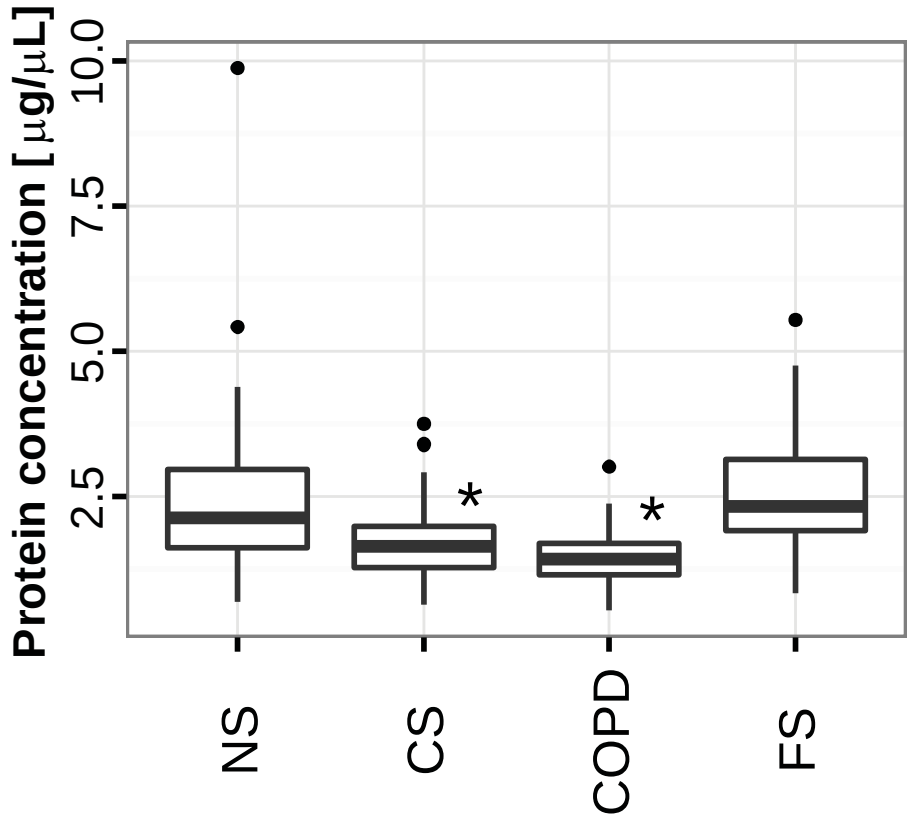
Induced Sputum Proteomics





Boxplot for the measured weights of the induced sputum samples (left) and measured protein concentrations for the sputum supernatants (after protein precipitation; right).
* indicates the p-values < 0.05 compared with NS (Welch t-test)

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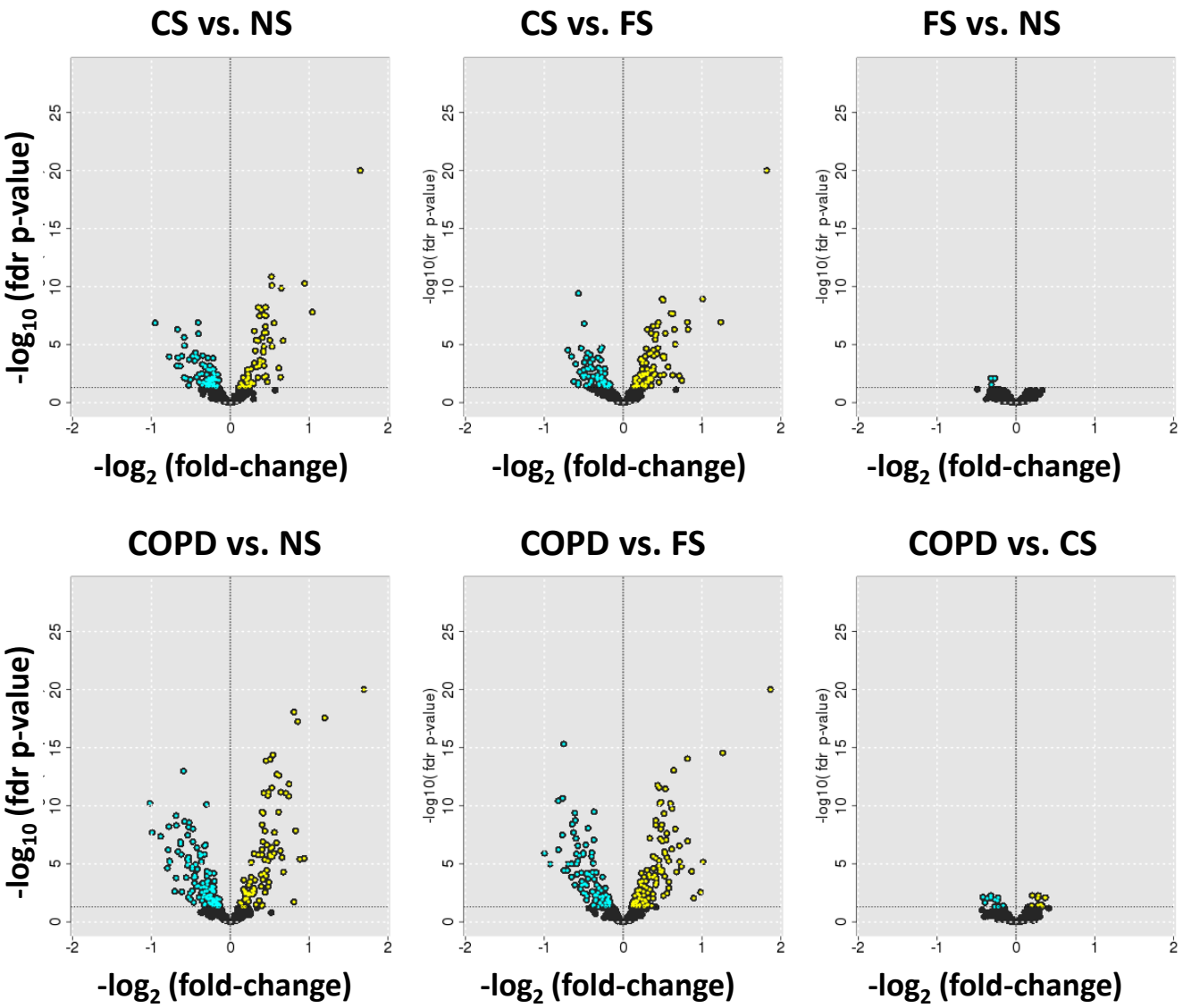
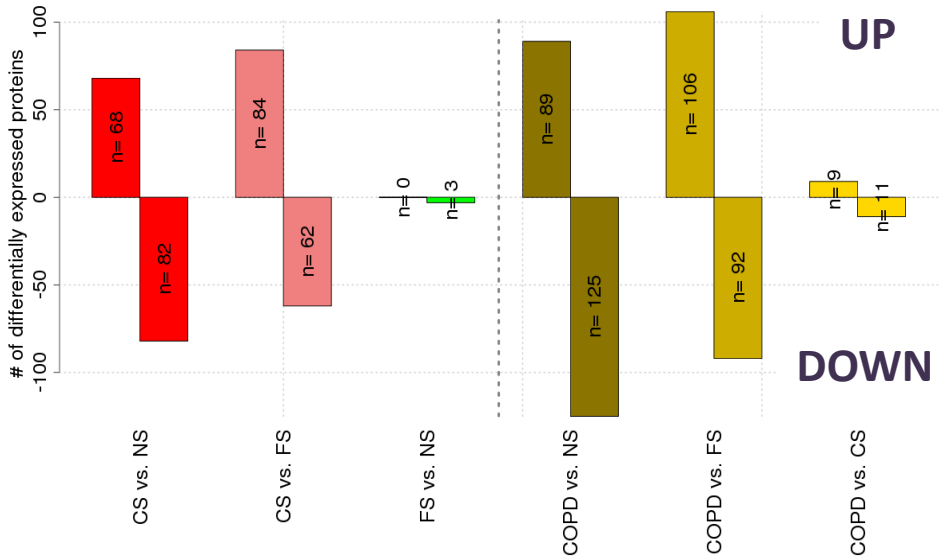
Differentially Abundant Sputum Proteins





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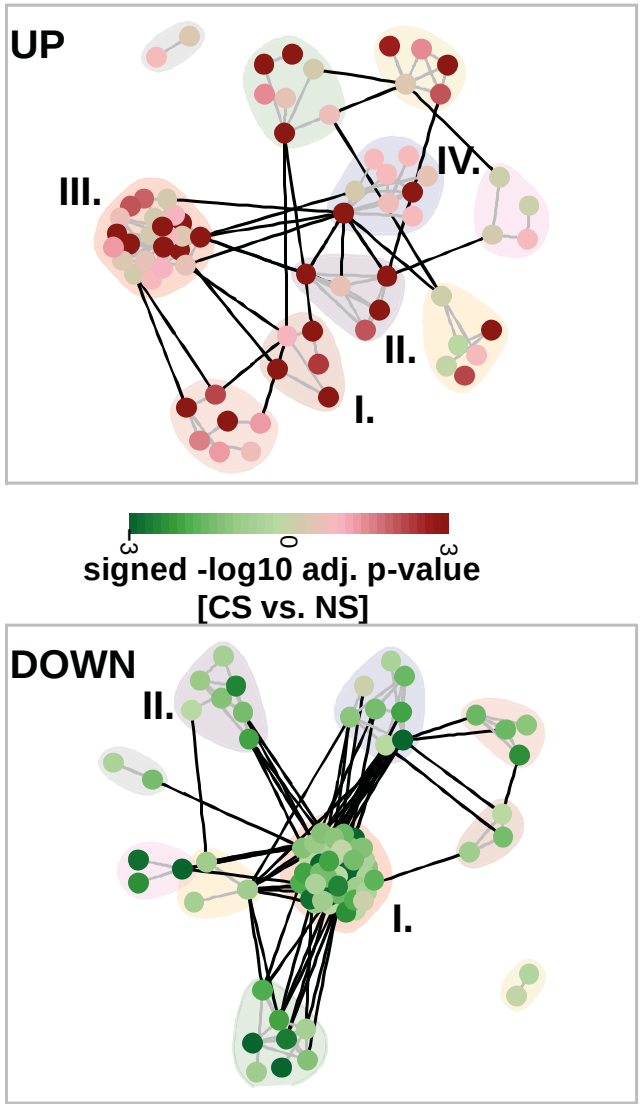
The Sputum Proteome Reflects Smoking

- Mucin/trefoil proteins, xenobiotic metabolism enzymes, antiproteases, and proteins involved in redox processes were increased in sputum of CS compared with that of NS

- Blood plasma-derived proteins and immunoglobulins were decreased in sputum of CS compared with that of NS



I. Mucin/trefoil proteins
II. Xenobiotic metabolism
III. Peptidase regulators
IV. Redox process

I. Blood plasma
II. Immunoglobulins

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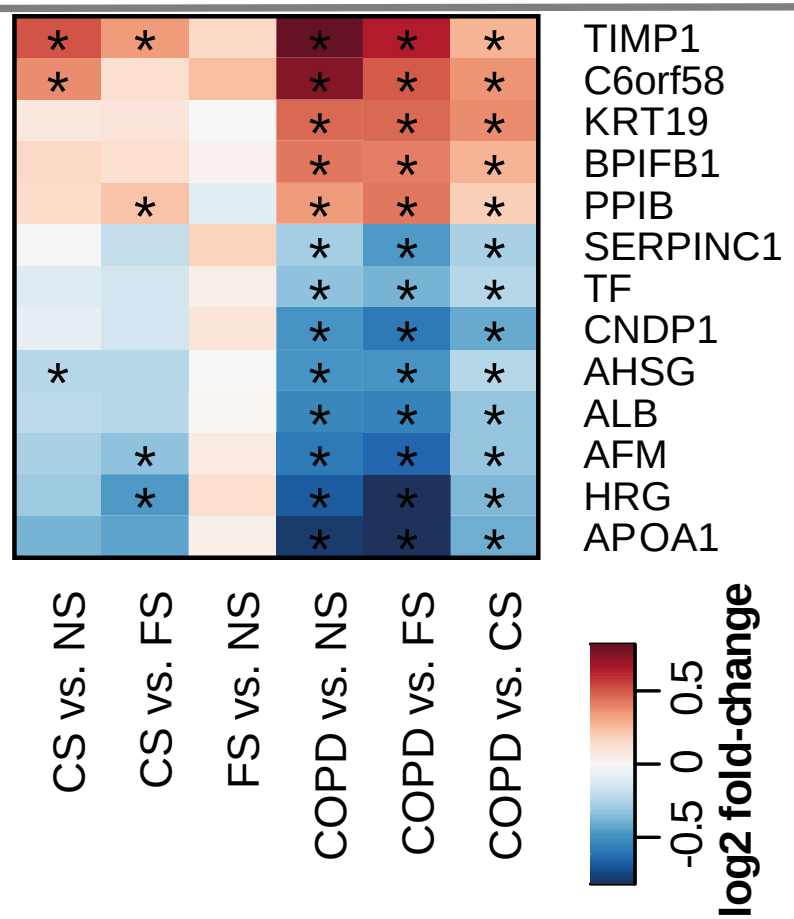
The Sputum Proteome Reflects Disease

- Comparing the sputum proteome of COPD smokers with that of asymptomatic smokers identified 13 differentially abundant proteins including antiproteases and proteins linked to the mucosal immune response

- Smoking alone significantly affects the expression of these proteins; presence of disease appears to “amplify” their dysregulation



Fold-change heatmap for the 13 differentially expressed proteins between the COPD and CS groups. The log2 fold-change is color coded according to the scale at the bottom right corner. “*” indicates statistically significant differential abundance (Benjamini–Hochberg adjusted p-value <0.05).

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The Sputum Proteome Reflects Smoking and Disease

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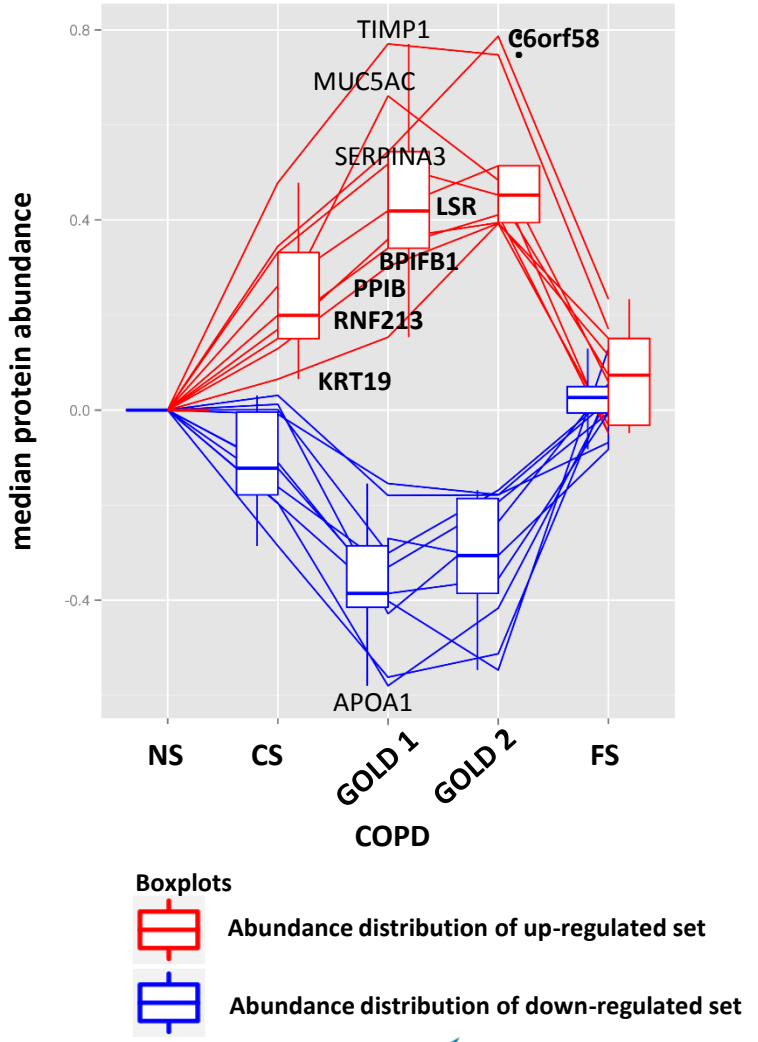

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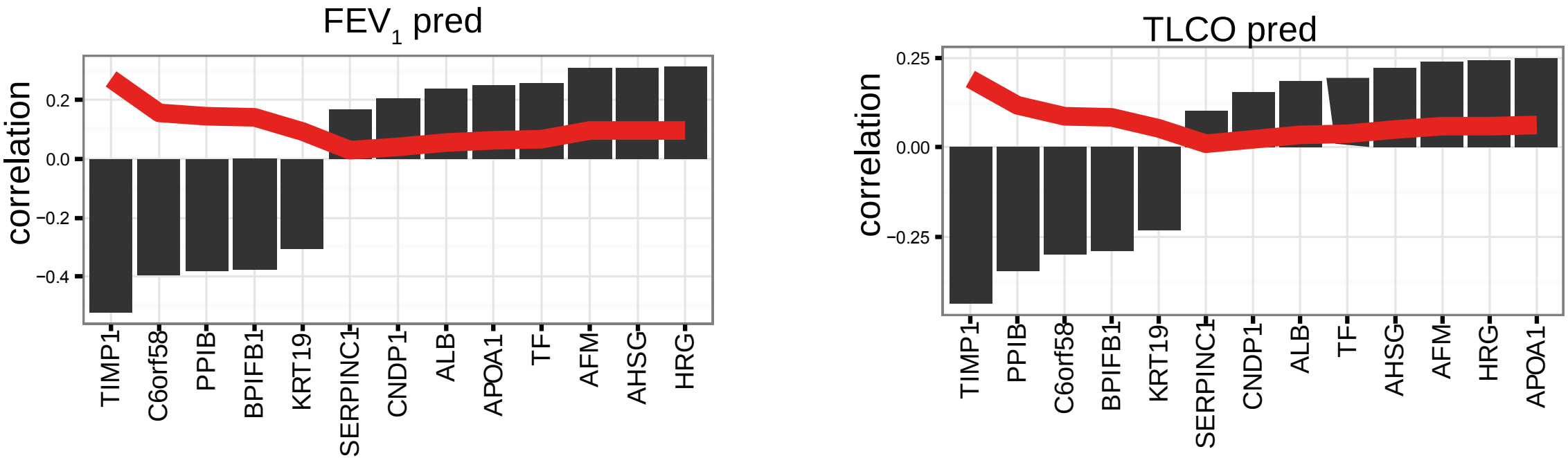
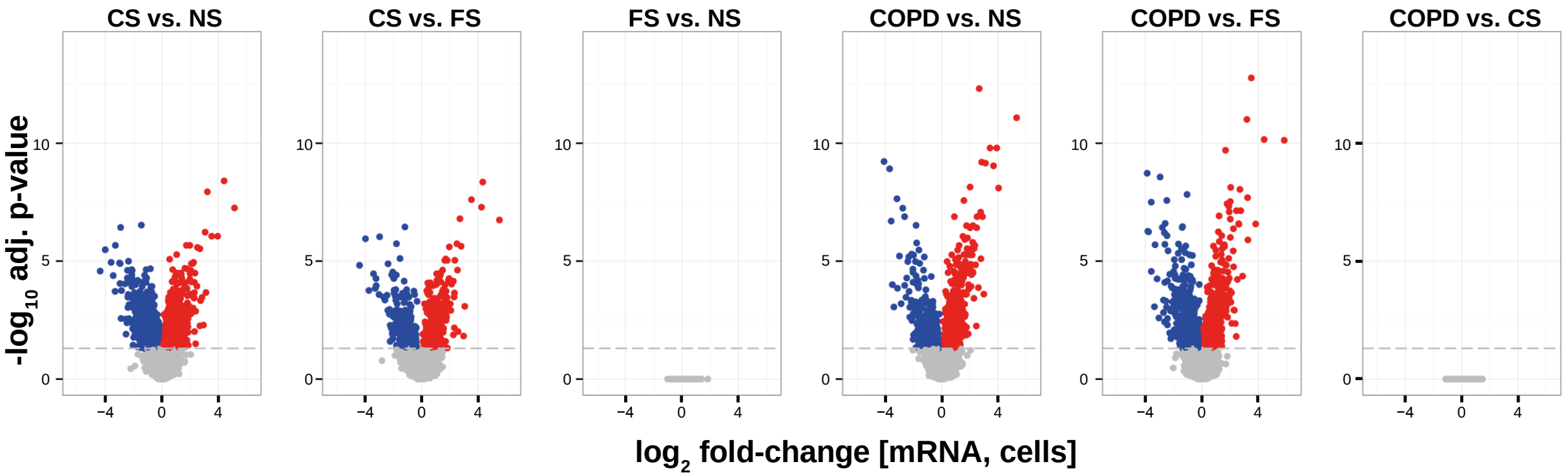
Sputum Proteins Correlate with Physiological Measures



Correlation plots with FEV1% of predicted (left panel) and TLCO predicted (right panel) for the 13 differentially abundant proteins in COPD vs. CS.
The bars show the Pearson correlation coefficient and the red lines the coefficient of determination (R2) for each protein.

Differentially Expressed Genes



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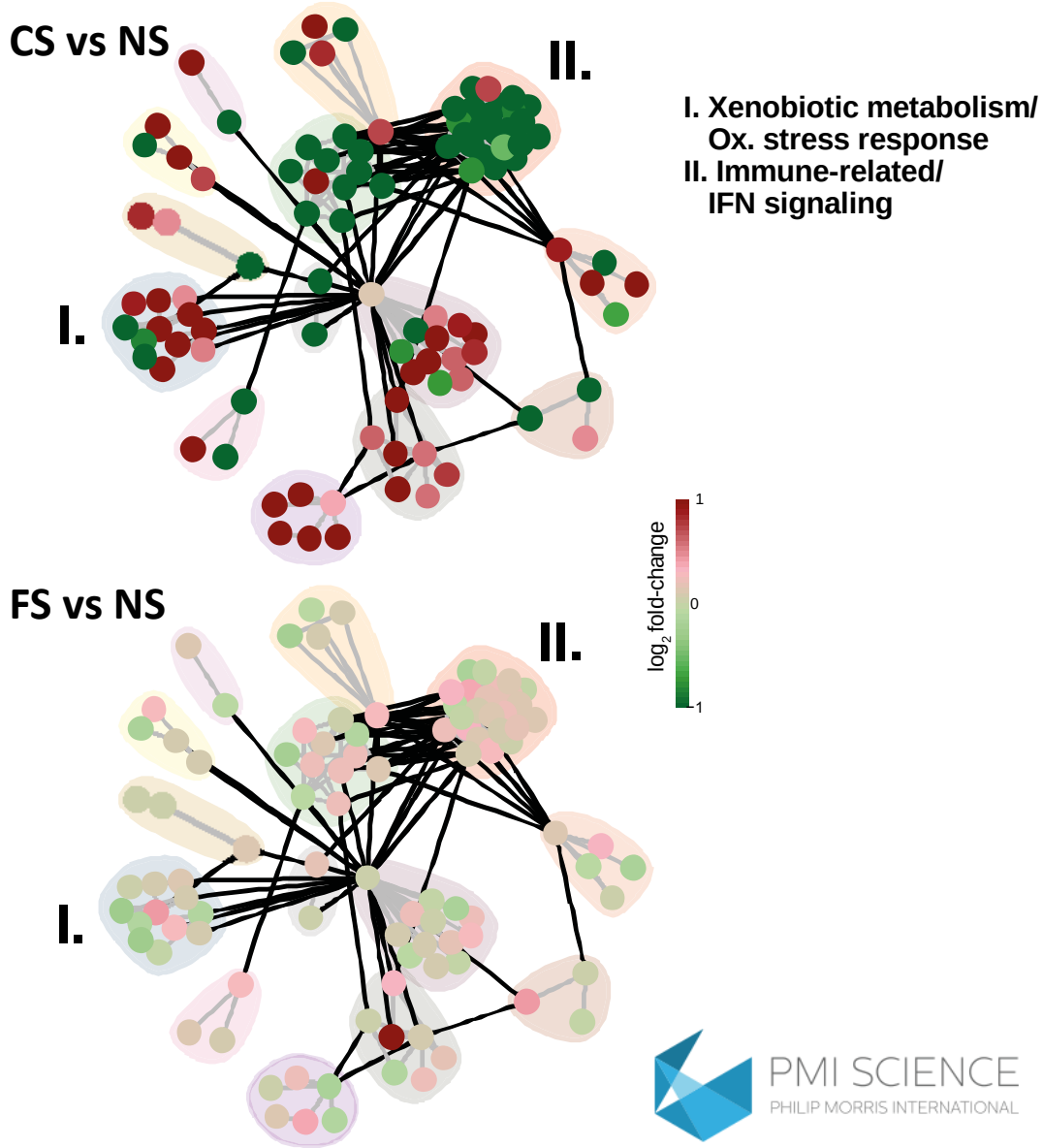
Sputum Transcriptome Reflects Smoking (Pellets)

- Smoking significantly affects the expression of several genes, resulting in
  - Up-regulation of xenobiotic metabolism enzyme-encoding genes and those linked to oxidative stress
  - Down-regulation of genes associated with immune responses and interferon (IFN) signaling
- Cessation largely reverses smoking-related gene expression changes
- COPD status does not further change the sputum transcriptome



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Study Limitations

- Subjects enrolled based on self-reported smoking status and smoking history
- Enrollment of COPD smokers completed first
- Sample size calculated based on known distributions of white blood cell count and inflammatory mediator levels in induced sputum in similar COPD study populations
- Cross-sectional design

Summary and Conclusions

In this non-interventional, parallel-group, case-controlled study we have demonstrated that:

- Physiological measures such as diffusion capacity, CT chest imaging and lung sound analysis allow for differentiation of asymptomatic ('healthy') smokers from subjects with mild to moderate COPD patients (GOLD stage I and II) not identified using spirometry
- The modified BODE QoL assessment allow differentiation of asymptomatic ('healthy') smokers from subjects with mild to moderate COPD patients (GOLD stage I and II) not identified using spirometry
- Sputum analysis detects cigarette smoking-related alterations in the proteome and transcriptome; changes in proteome are further augmented in COPD smokers, allowing for further stratification.
- Sputum proteome analysis can distinguish COPD from asymptomatic smokers with a similar accuracy than the combination of the commonly used parameters FEV1, TLCO% and total COPD score.

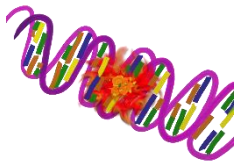
Titz B, Sewer A, Schneider T, Elamin A, Martin F, Dijon S, Luettich K, Guedj E, Vuillaume G, Ivanov NV, Peck MJ, Chaudhary NI, Hoeng J, Peitsch MC. (2015). Alterations in the sputum proteome and transcriptome in smokers and early-stage COPD subjects. J Proteomics. 128:306-20. **PMID: 26306861**

Chaudhary NI, Luettich K, Peck MJ, Pierri E, Febler-Medlin L, Vuillaume G, Leroy P, Peitsch MC. (2016). Physiological Measures in Subjects with Mild to Moderate COPD Compared to Asymptomatic Current Smokers, Former Smokers and Never-Smokers. Manuscript in preparation

Acknowledgements

Queen Anne Street Medical Centre

Respiratory Clinical Trials Team



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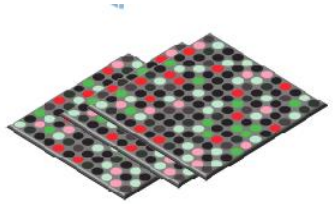

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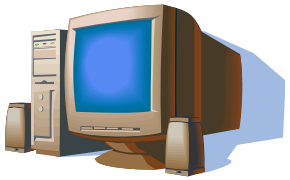

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AROS APPLIED BIOTECHNOLOGY A/S

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