‘Mass spectrometry, Sebum, PD and Joy’

ERIG 2020

Perdita Barran

Mass Spectrometry in the UK (and Manchester)

John Dalton Table of relative atomic weights

1805

Edmund Frankland Theory of Atomic Valance

1852

JJ Thomson Nobel Prize for the Electron, Builds mass spectrograph with Aston

1870

Ernest Rutherford
Mentored by Thomson. Discovered the Nucleus & the Proton

1907

Metropolitan-Vickers
Worlds first MS company

1940

James Chadwick Discovered the Neutron & commissioned 1st commercial Mass Spectrometer 'MS1'

1946

John Beynon Collaborates with MV to develop the MS8

1955

1981

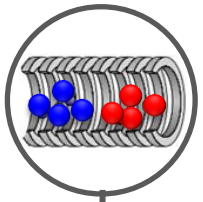
Development of Fast Atom Bombardment **Micky Barber**
First commercial Q-TOF instruments manufactured in UK

1996

The Q-TOF

The Orbitrap Makarov publishes first paper in Manchester

2000



TWIM-MS
Developed in UK

2007

What is Mass Spectrometry?

- Fundamental separation technique that is central to many areas of science
- Used in hospitals, in war zones and on the Philae landing craft.
- Measures *m/z* which can provide molecular identity (with accurate mass + isotope distribution + fragmentation pattern) and also can quantify how much sample is present absolutely and relatively.
- In short what is there and how much
- Relies on sample prep, the formation of ions and the measurement of their mass and the recording of this information.

To be successful, this needs well looked after machines and operators (and good collaboration)

Mass Spectrometry @University of Manchester today

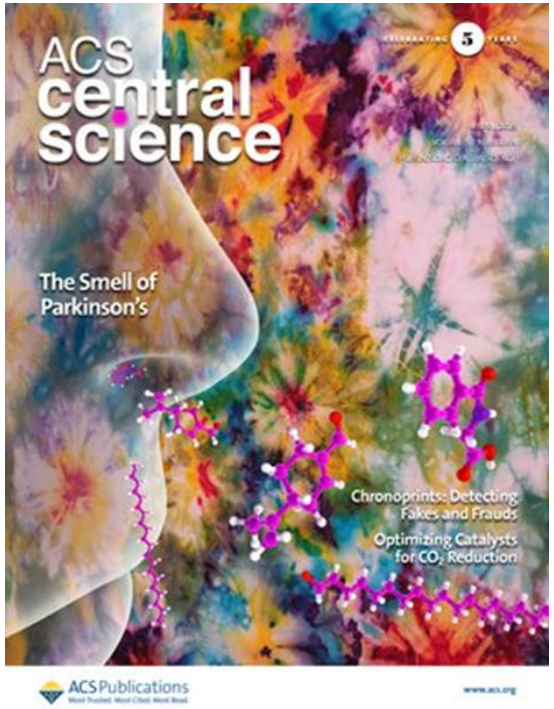
- The University of Manchester has several MS research facilities in many buildings
- The Michael Barber Centre for Collaborative MS is the flagship research facility housed within the Manchester Institute of Biotechnology
- It comprises six academics PIs (Barran, Trivedi, Mills, Lockyer, Pitt, Buckley)
- It possesses 25 mass spectrometry platforms supported by a team of skilled research professionals (five permanent experimental officers, one technician)
- We have an ion mobility and technology development focus
- The MBCCMS supports the research of more than 40 groups internally and externally as well as many industrial collaborators.

Department of Chemistry

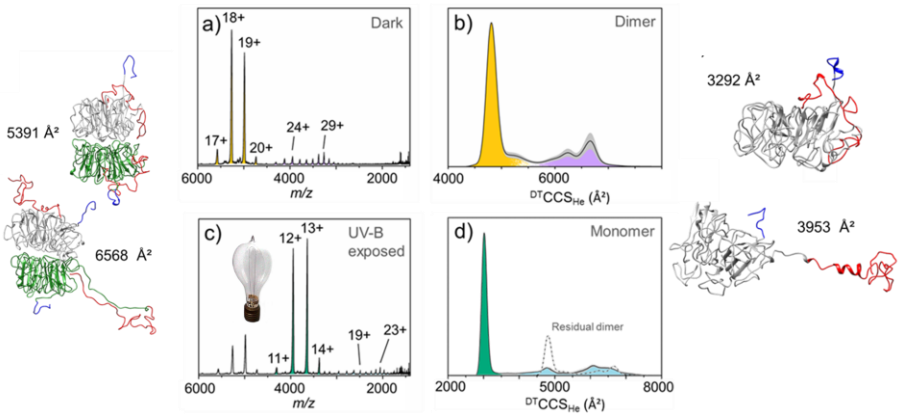
Professor Perdita Barran twitter: @perditab

Mass Spectrometry and Biophysical Chemistry

Professor Barran is Chair of Mass Spectrometry and Director of the Michael Barber Centre for Collaborative Mass Spectrometry at the Manchester Institute of Biotechnology. Her teams research interests include: Biological mass spectrometry; Instrument and technique development; Protein structure and interactions; Dynamic and Disordered Systems; Parkinson's disease Diagnostics; HDX-MS; Proteomics; and Molecular modeling.



Photoactive Proteins – switching the light on



Research Themes

To train people to play with molecules

To **exploit** IM-MS to provide information on the heterogeneity of protein and protein complexes

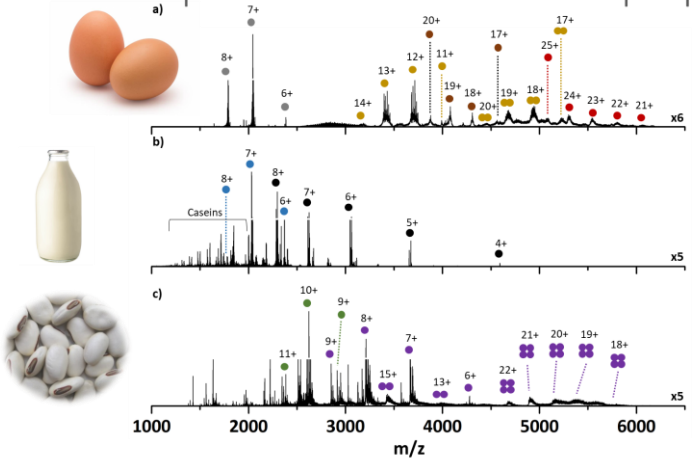
To **develop** instruments & new methods

To **relate** findings to biological activity/function, to health and for biotechnological use

To work **fast** and with **minimum** sample preparation

And to **diagnose** Parkinson's Disease from sebum

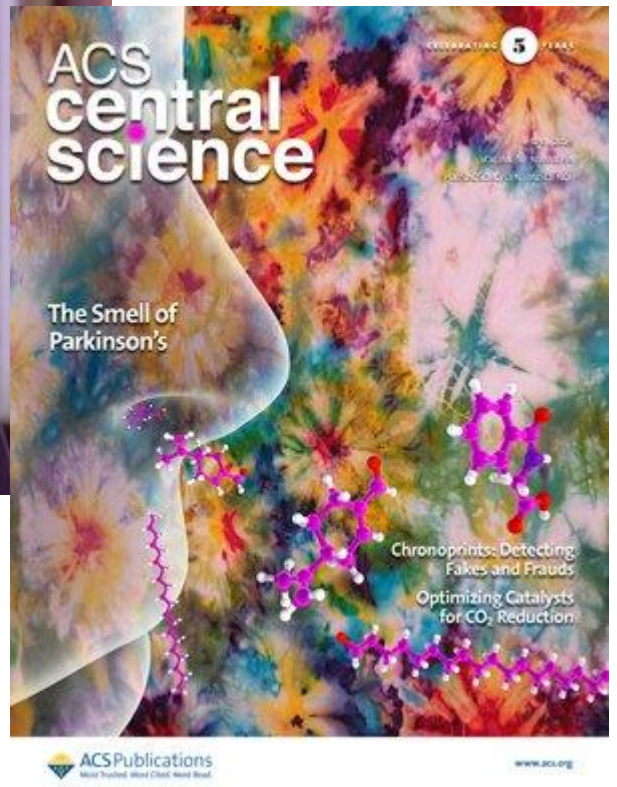
Native MS of protein rich food - no sample prep



And then there was JOY!!!!

“The woman who can smell Parkinson's Disease”

‘From Nose to Diagnose-tics’



Trivedi, Sinclair, et al.

TEDx PaloAlto
x = independently organized TED event

https://youtu.be/IbmJ9eZpMJw

20180018 Patent Pending

www.thelancet.com/neurology Vol 15
February 2016

1982 — 1994 — 2009 — 2012

T-Shirts

SEBUM

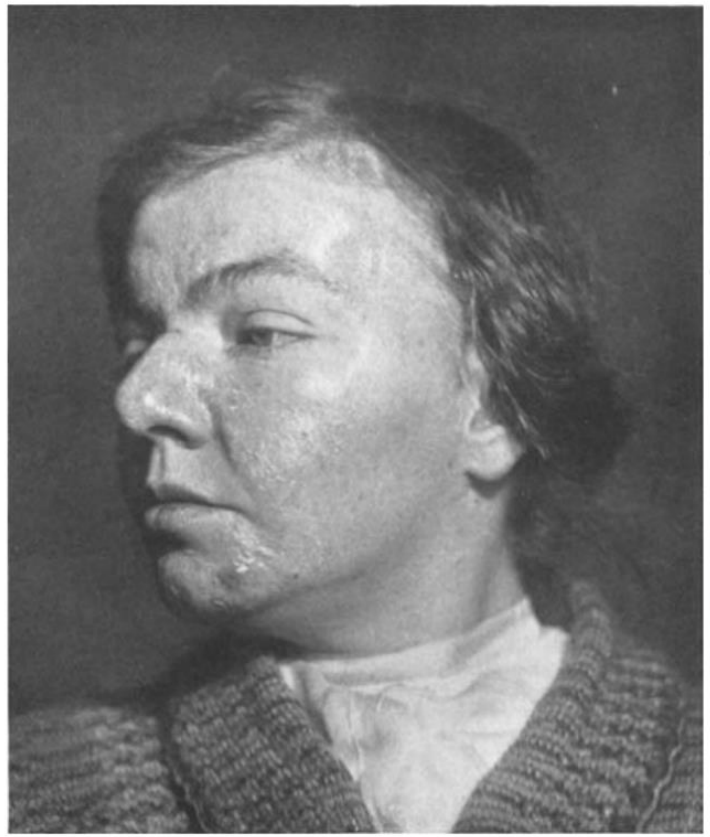


FIG. 3. To show the shiny seborrhoeic facies in a young patient presenting manifestations of Parkinsonism probably not due to encephalitis lethargica.

In 1927 David Krestin reported *seborrheic facies* as a cutaneous manifestation of Parkinsonism.

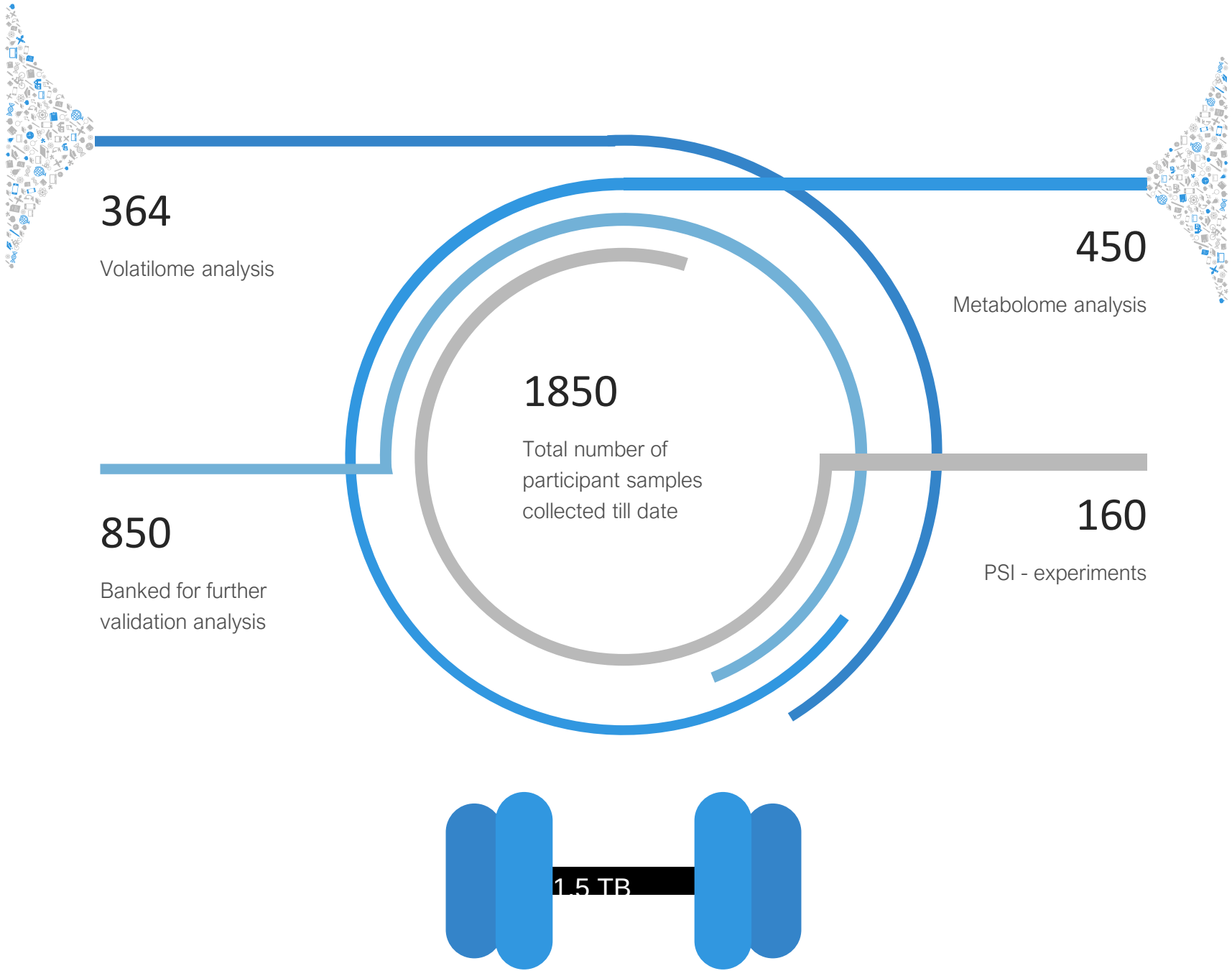
His work was largely ignored.

Recruitment sites for sebum collection

28 across the UK and 4 in the Netherlands

Current approaches for biomarker discovery

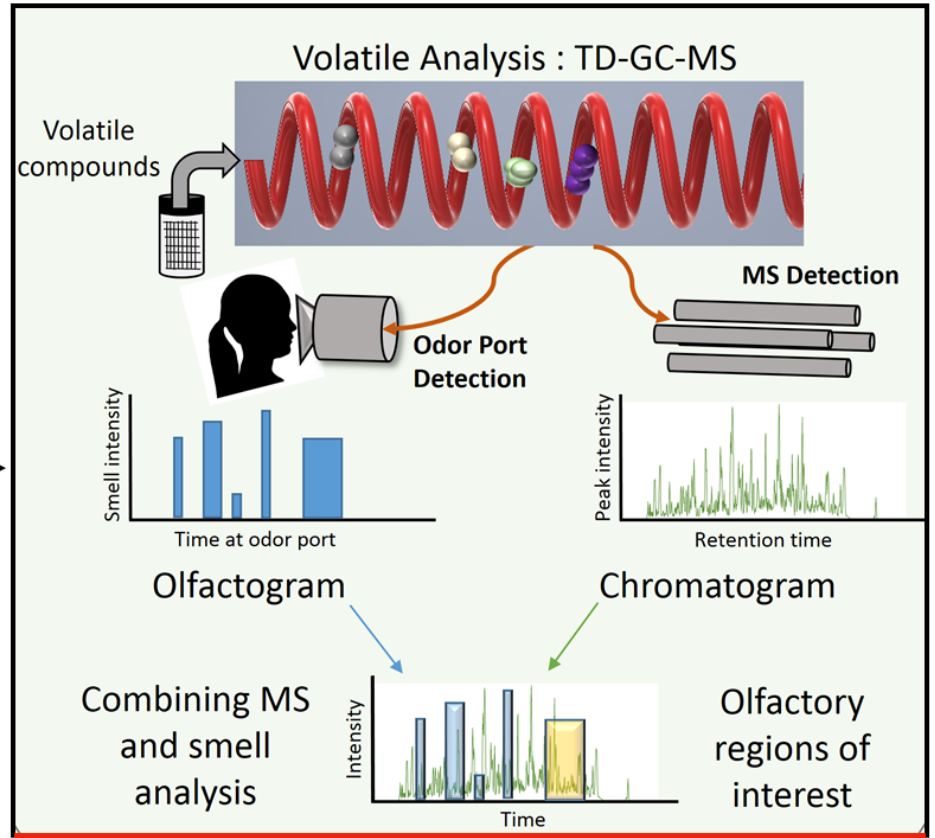
- **Nose2Diagnose Trial** Clinical lead - Monty Silverdale, Science lead - Perdita Barran, Supersmeller Joy Milne.
- **TD-GC MS** - analysis of volatile components directly from gauze, coupling with ODP Catlin Walton-Doyle, Drupad Trivedi
- **LC-MS** – metabolomics of sebum following novel extraction method Eleanor Sinclair, Drupad Trivedi
- **DIA** – ambient analysis of sebum directly from skin swabs Depanjan Sarkar


364
Volatilome analysis
450
Metabolome analysis
1850
Total number of participant samples collected till date
850
Banked for further validation analysis
160
PSI - experiments
1.5 TB

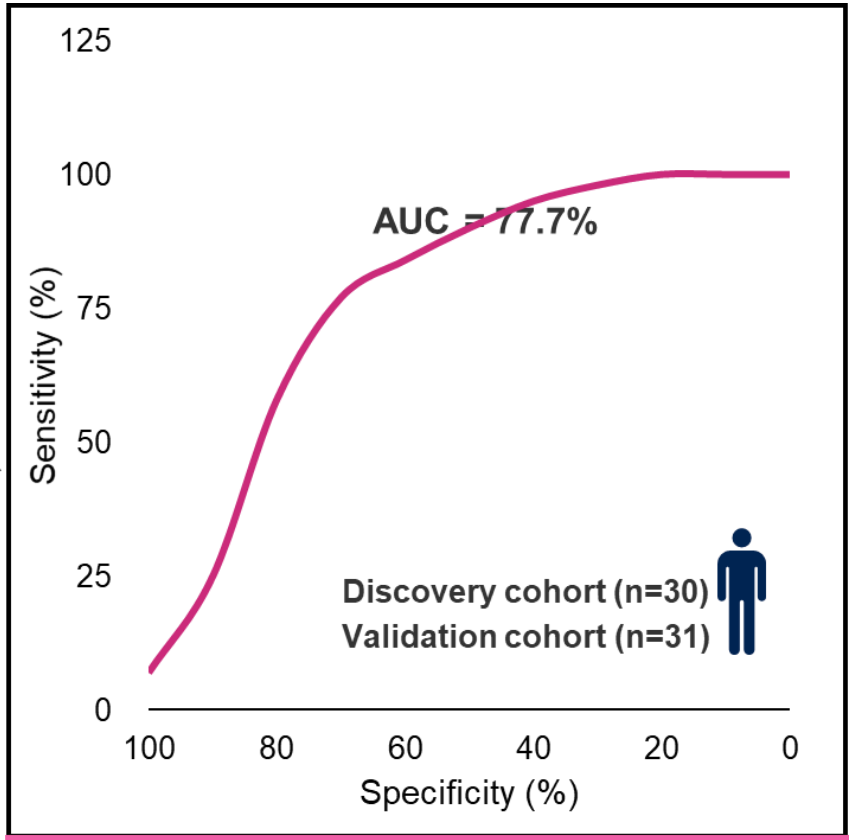
Clinical trial to find out what Joy smelt

28 recruitment sites in the UK

Recruitment

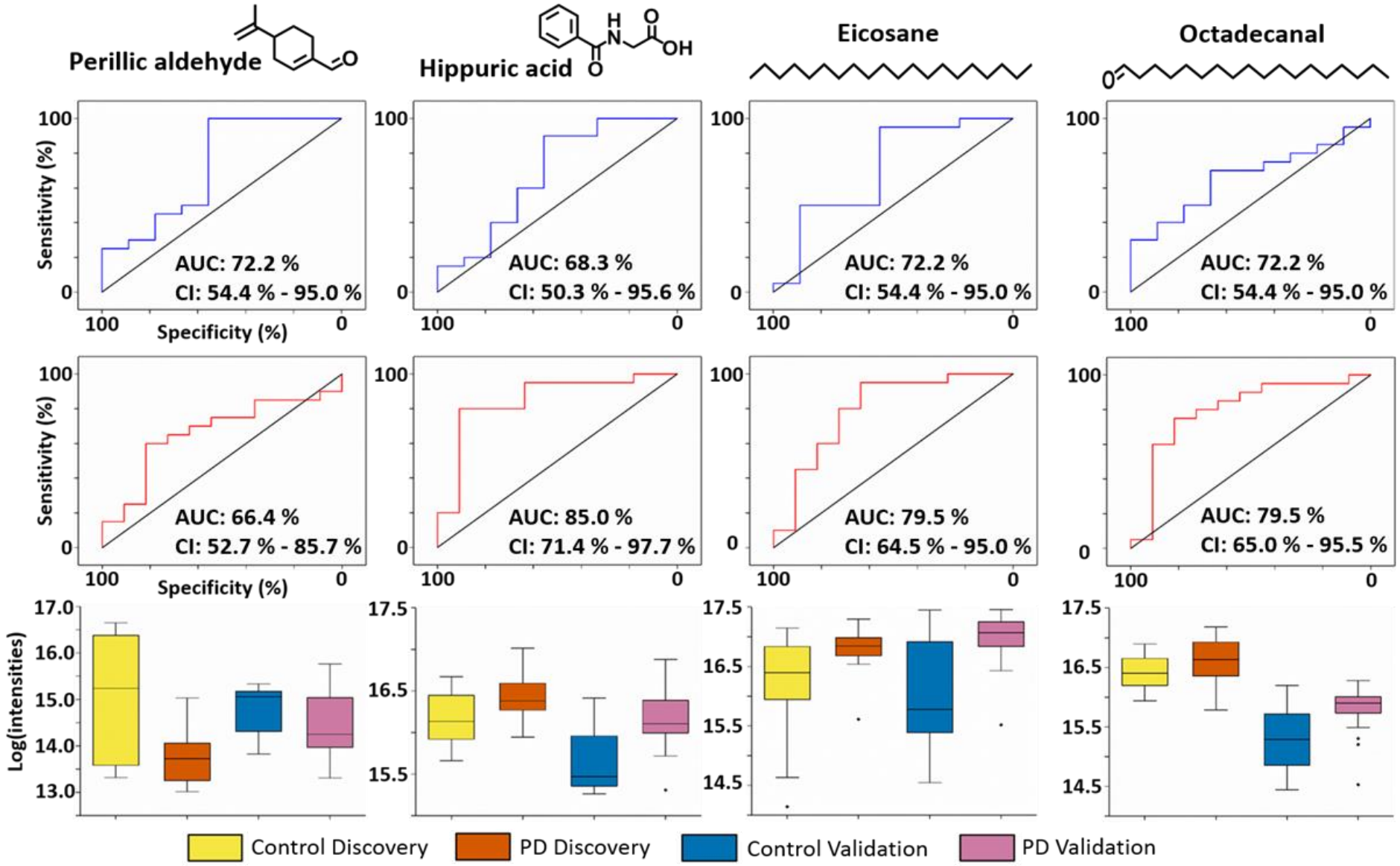


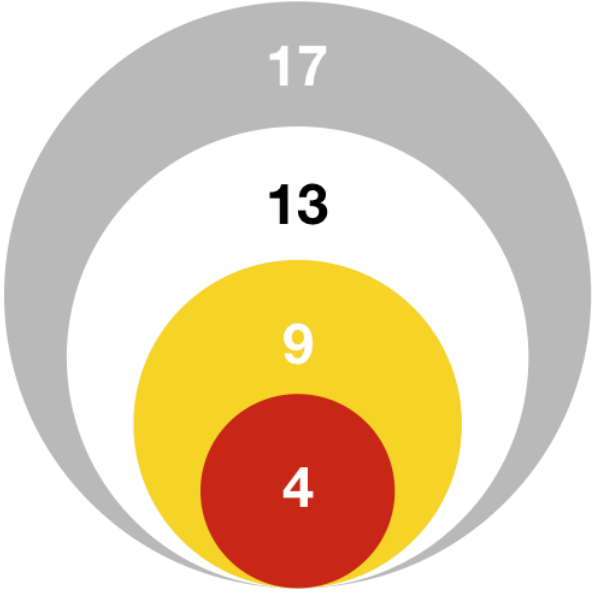
Analysis



Results

Lead Features – TD-GC -MS



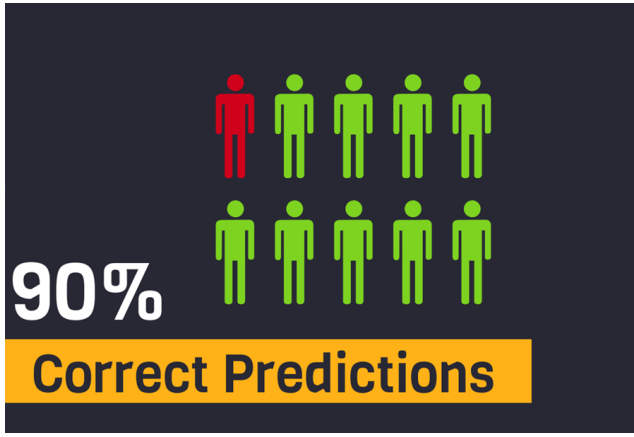


Same expression

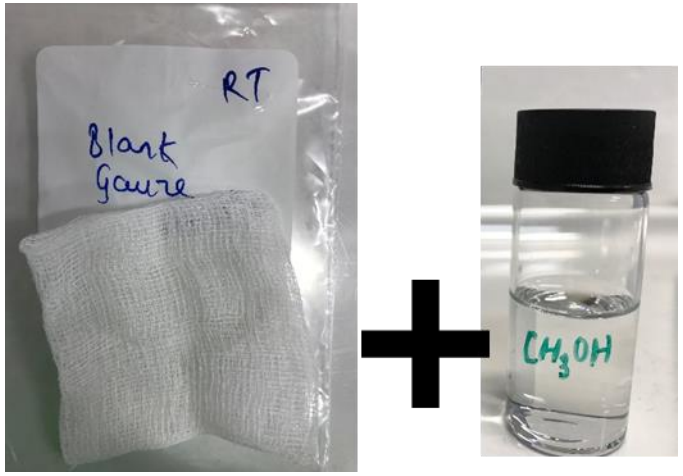
Validated

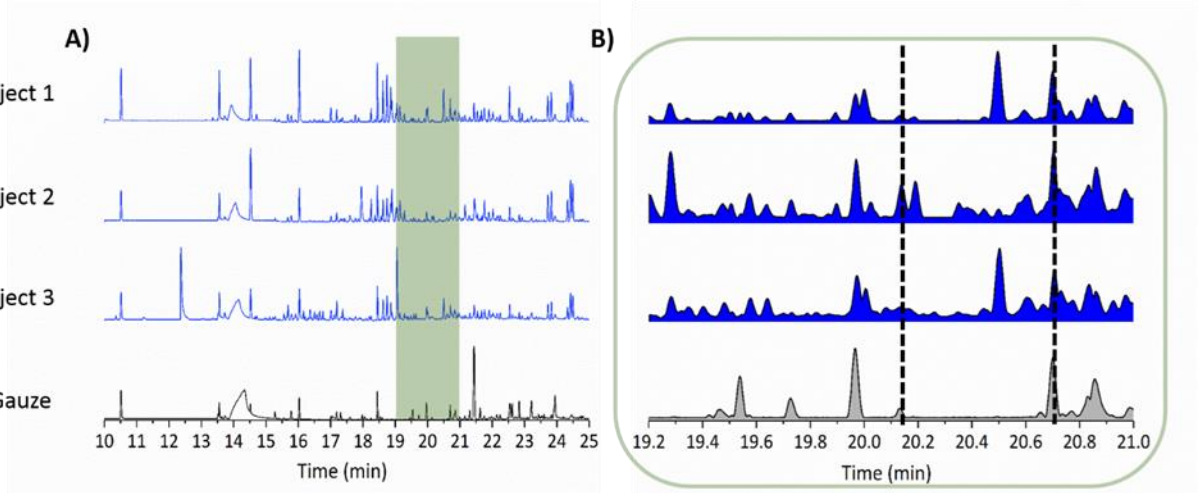
Common

Significant



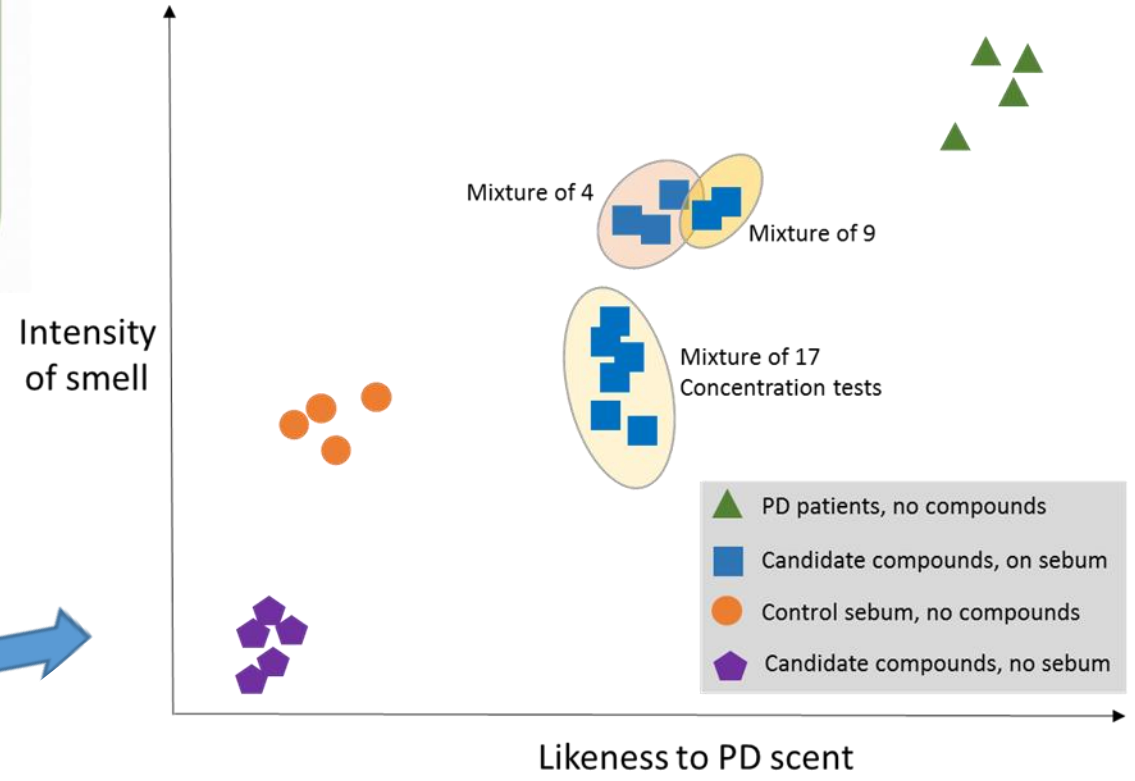
And Joy? Validation by Nose





Via Odour Port

And biomarkers on gauze (and control sebum)



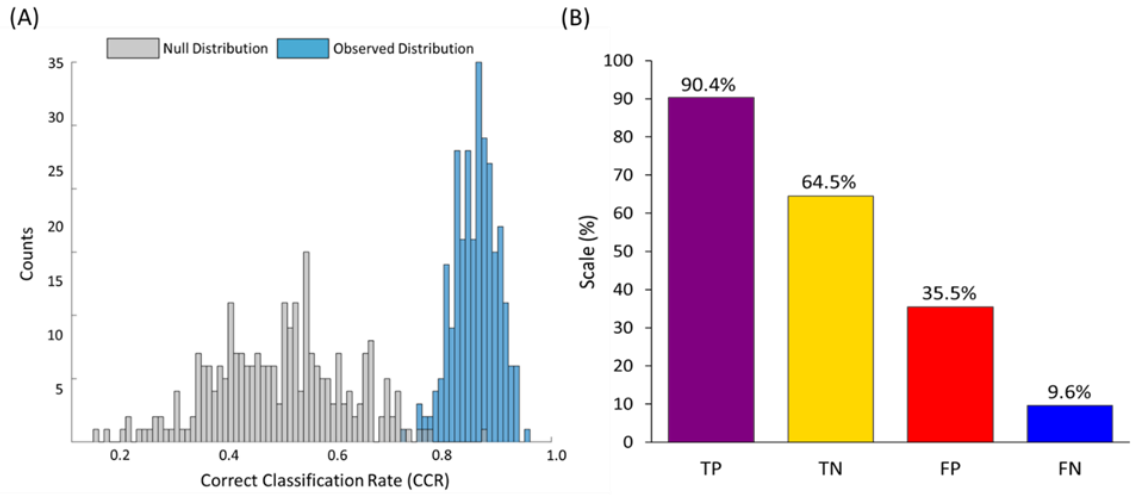
TD-GS MS
129 more participants

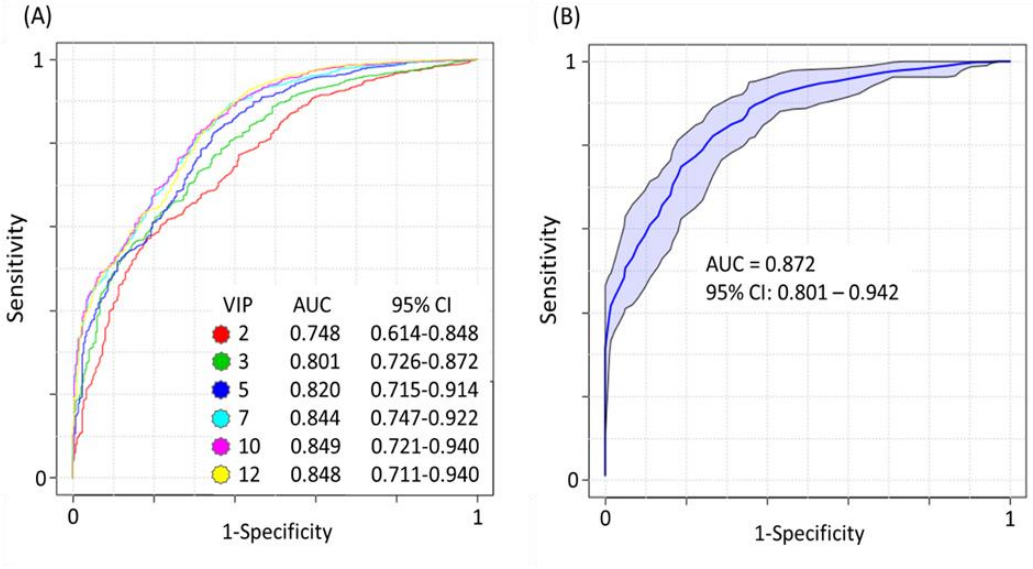
- Use of established method on wider sample set.
- 129 participants – DN (17) PD (83) IC (29)
- Inclusion of QC data
- Reduced data set 520 features
- VIP of 8 shows improved classification to ~84.4 % (from 79%)
- Poor discrimination DN vs. PD

Suggest the odour is phenotypic to PD

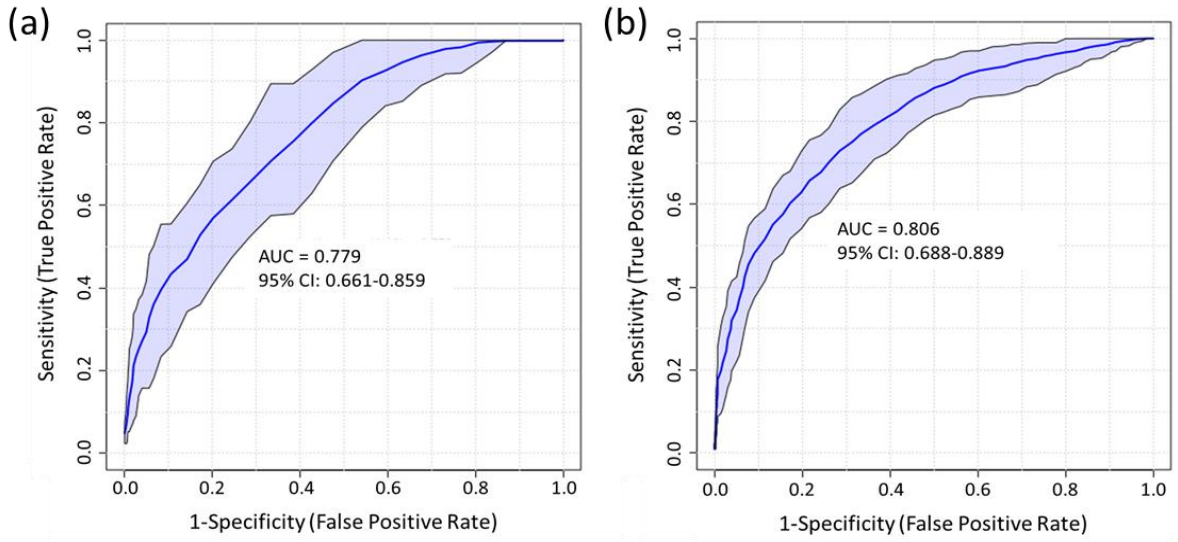
- 90% TP reduced sensitivity (64.5%) due to low(er) number of control subjects
- No match to Eicosane and Octadecanal as pure compounds
- DIA and GC-MS indicates these features are breakdown from larger HC (lipid) moieties.

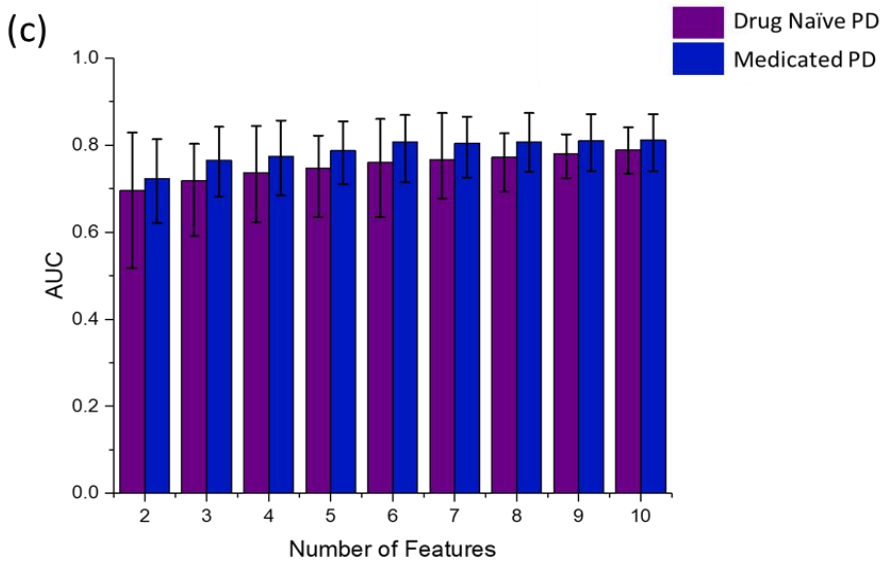
doi.org/10.26434/chemrxiv.12525323.v1
Review ACS Central Science

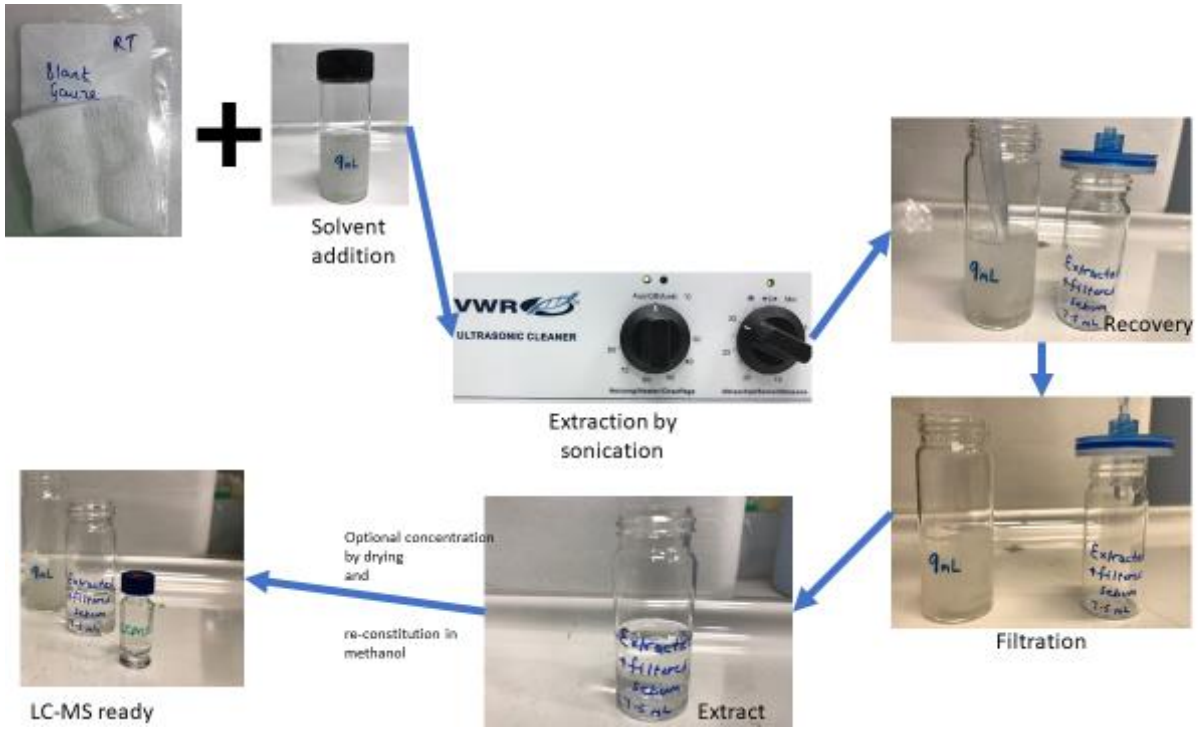




LC-MS on Sebum







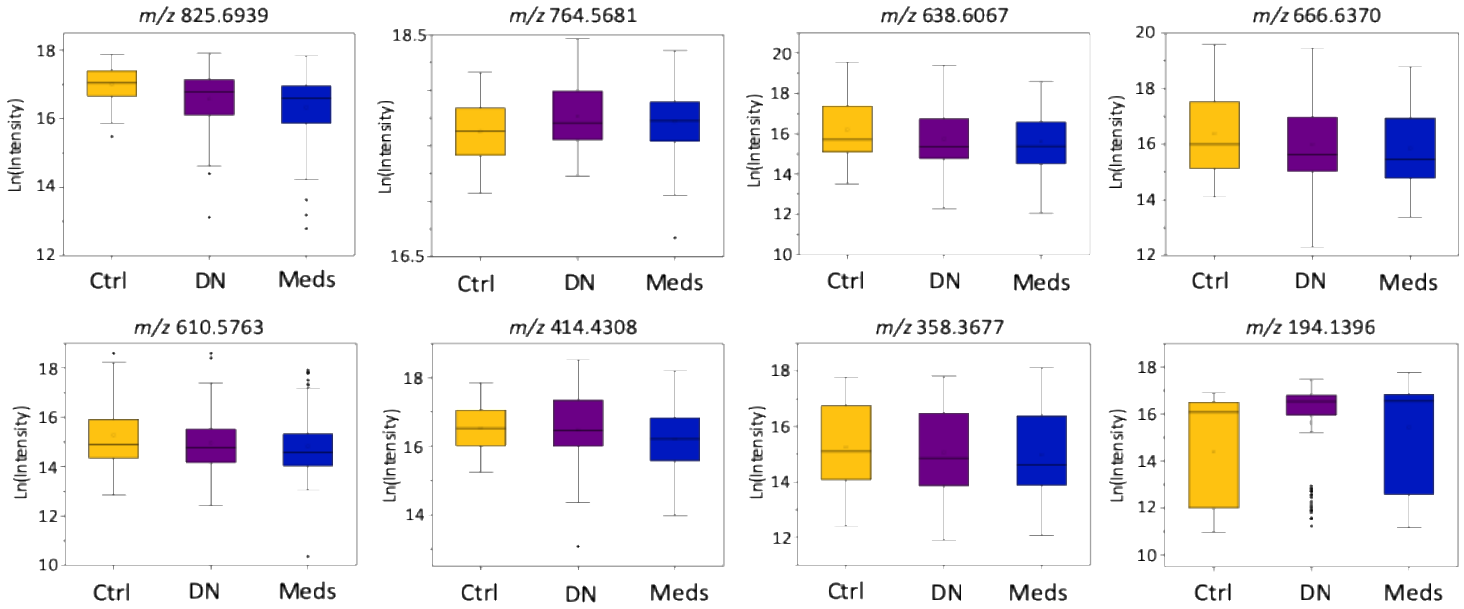
- 435 participants, 274 patient samples
- 80 drug naïve PD, 138 medicated PD and 56 well matched control subjects
- Classification accuracy >70% PLS-DA used for model
- From '~9000' features top 10 gave ~80% accuracy
- Some metabolites distinguish stage
- 10 putatively identified with VIP>1 to distinguish between DN and PD

doi.org/10.26434/chemrxiv.11603613.v2 Nature Communications

LC-MS VIPs that distinguish IC, DN, PD – towards stratification of disease progression

Feature	Putative Annotation (Accurate mass & MS/MS fragmentation)	Expression Drug Naïve PD (Fold Change)	Expression Medicated PD (Fold Change)
m/z 825.6939	TG(50:5)	↓ (0.77)	↓ (0.64)
m/z 764.5681	HexCer(36:2)	↑ (1.15)	↑ (1.10)
m/z 666.6370	Cer(42:0)	↓ (0.60)	↓ (0.47)
m/z 638.6067	Cer(40:0)	↓ (0.61)	↓ (0.47)
m/z 610.5763	Cer(38:1)	↓ (0.63)	↓ (0.48)

Measured Feature	Database Matches (accurate mass)	Formula	Expression Drug Naïve PD (Fold Change)	Expression Medicated PD (Fold Change)
m/z 414.4308	FA(26:0) Methyl pentacosanoate	$C_{26}H_{52}O_2$	↑ (1.23)	↓ (0.84)
m/z 358.3677	FA(22:0)*	$C_{22}H_{44}O_2$	↓ (0.81)	↓ (0.78)
m/z 194.1396	FA(8:0) L-Cladinose Metaldehyde†	$C_8H_{16}O_4$	↑ (1.74)	↑ (1.78)
m/z 550.6277	-	-	↑ (1.33)	↑ (1.10)
m/z 368.4242	-	-	↓ (0.15)	↓ (0.14)

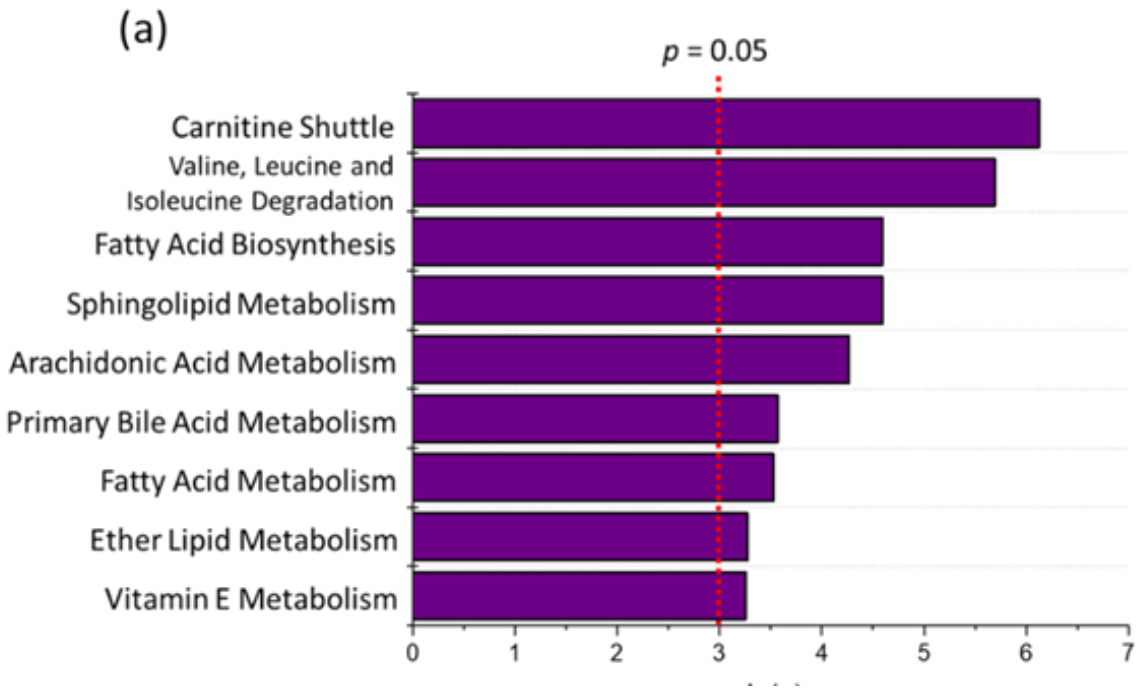


TG (Triacylglyceride); HexCer (Hexosylceramide); Cer (Ceramide)

FA (Fatty acid)

Pathway Analysis

Drug Naive

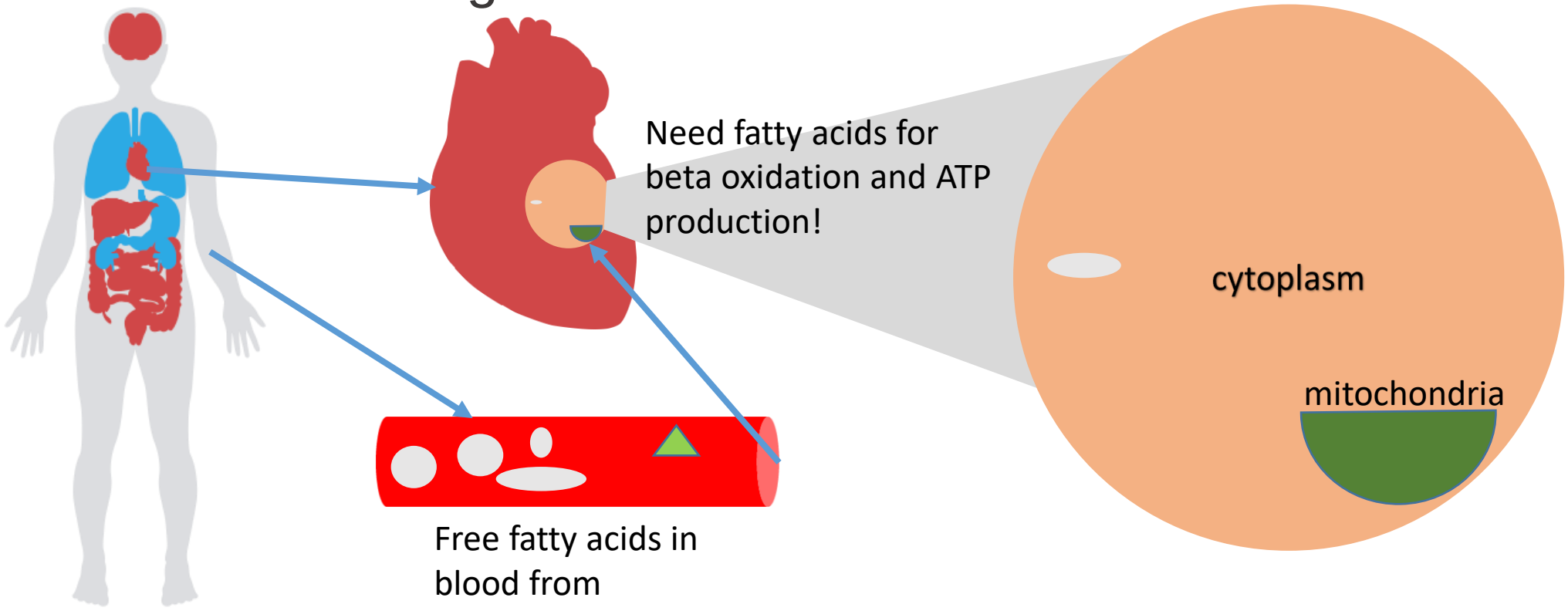


PwP

Hypothesis:

Our results indicate a significant shift of endogenous metabolome in PD, causing an enhanced effect reflected on lipid-like molecules that are captured on the skin sebum.

- Carnitine shuttle alters in very early stage PD
- As the disease progresses, fatty acids, beta oxidation and squalene metabolism changes

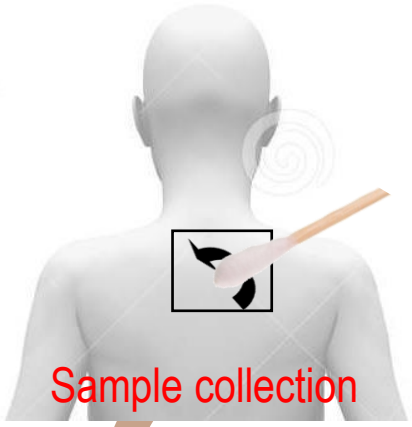


Carnitine shuttle:

1. Activation
2. Transport
3. Oxidised fatty acyl-coA ready for energy metabolism

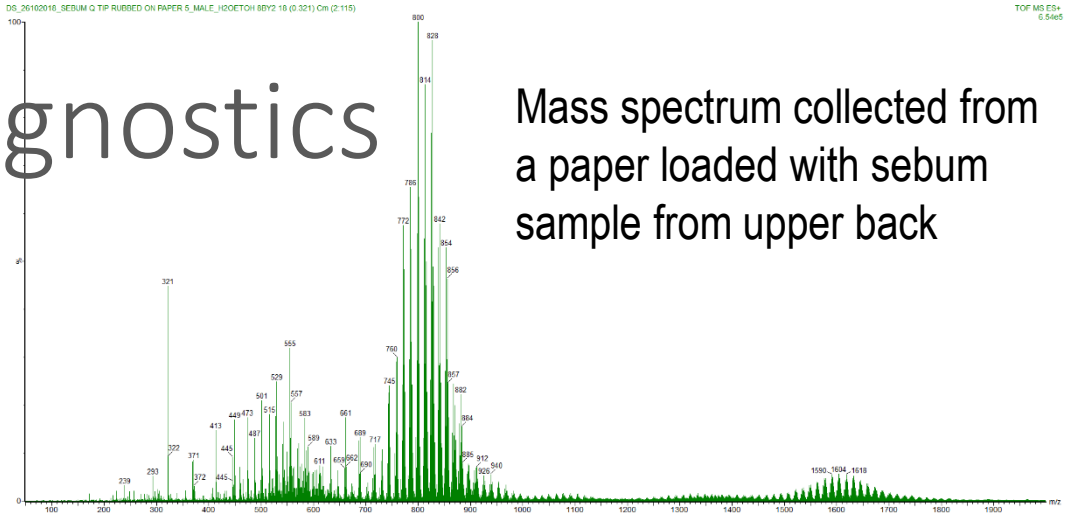
Paper Spray– rapid targeted diagnostics

Depanjan Sarkar



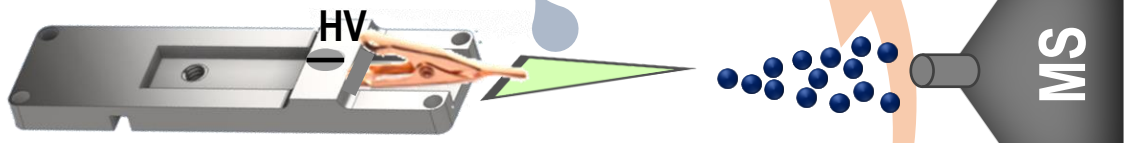
Sample collection

Few Minutes



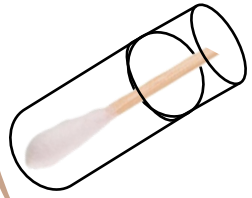
Mass spectrum collected from a paper loaded with sebum sample from upper back

Solvent



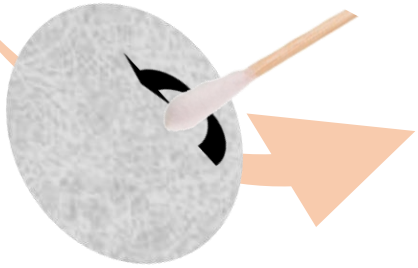
Connect to the paper spray source

Transport

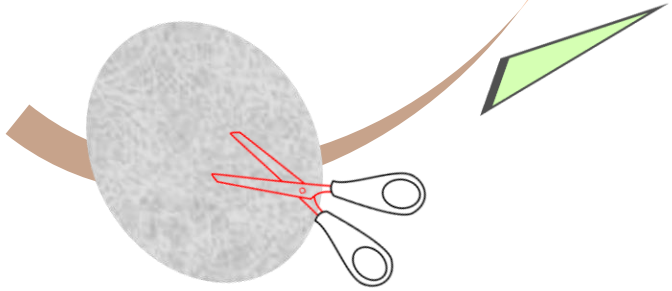


High specificity

2-3 mins



Load (wipe) the sample on filter paper



Cut it into a point

Fast ambient analysis of sebum samples

Analysis of PSI-IM-MS data

Broad Mass spectrum, signature envelope
Many masses made up of a complex signature of ions which include conformers and multiply charged larger species. This is deconvoluted by ion mobility.

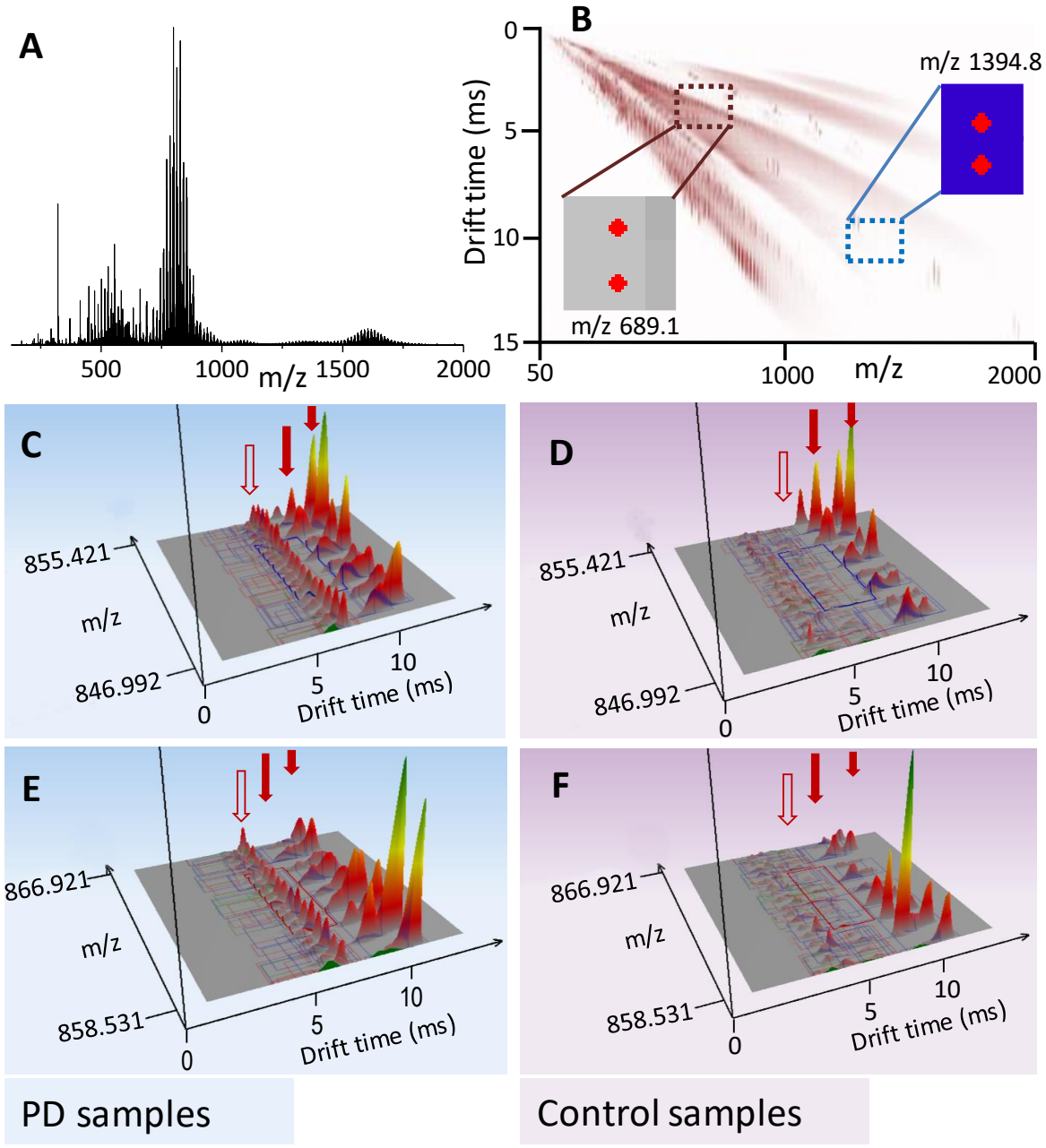
34 people with PD and 30 matched control subjects as a training set and a further 91 samples as a validation set.

~ 4200 features from each individual

Regions of the IM-MS data set that are distinctive are highlighted.
Ridge of features found in PD and NOT in control

Distinct biomarkers – large phosphatidylcholine and cardiolipin compounds

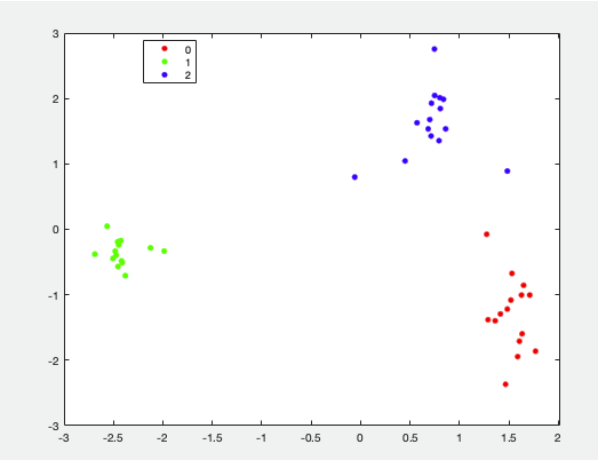
doi.org/10.26434/chemrxiv.12517385.v1



RBD cohort (n=15) – TD-GC-MS

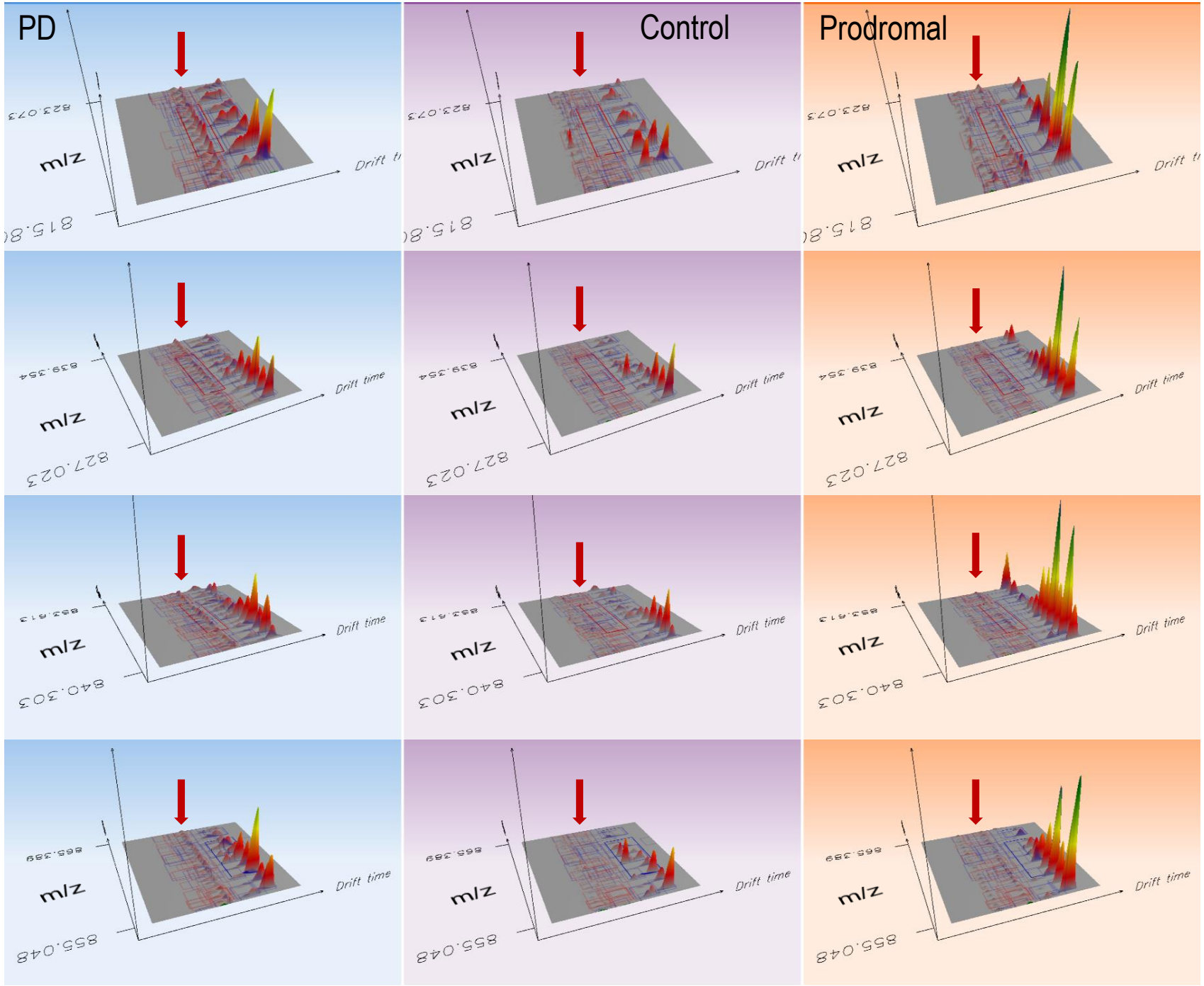
- 206 features from 44 samples
  - Prodromal = 15 samples
  - Controls = 14 samples
  - PD = 15 samples
  - Due to lack of QCs - peaks with missing values in more than 40% data, removed.
  - Remaining missing values, replaced by 0.01
  - Data normalized to total counts and log10 transformed

LOO CV accuracy 71%	Prodromal actual	PD actual
Prodromal predicted	62%	12%
PD predicted	38%	88%



LOO CV accuracy 74%	Control actual	Prodromal actual
Control predicted	83%	33%
Prodromal predicted	17%	67%

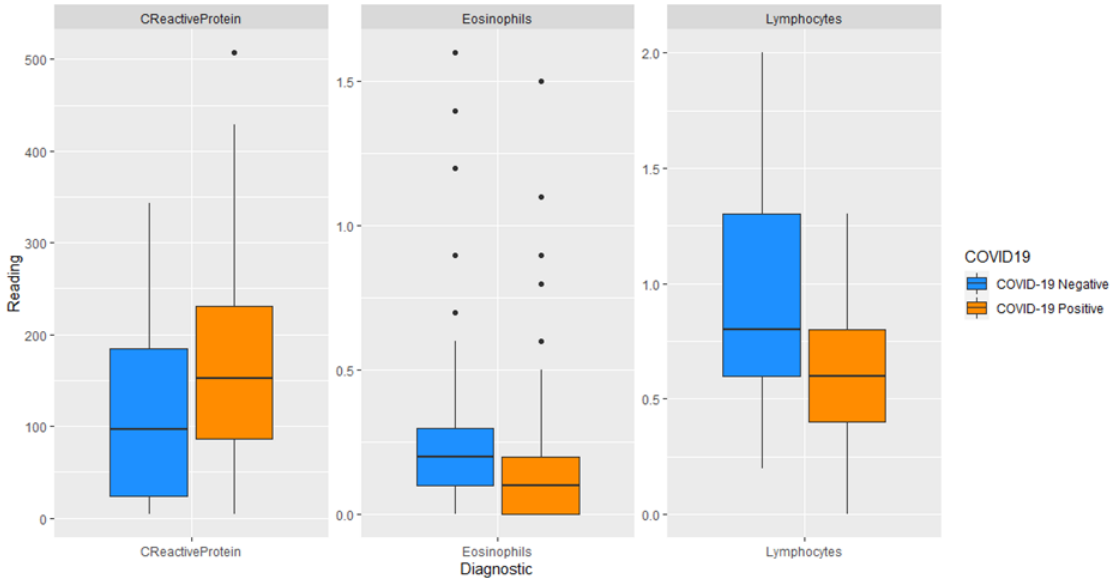
Collaboration with Werner Poewe and Beatrice Heim (Innsbruck)


PD
Control
Prodromal
m/z
Drift time

Repurpose PD test for COVID-19

- Collaboration between UoSurrey (Melanie Bailey + Matt Spick) & UoM
- 67 participant samples, 30 COVID-19 +ve and 37 –ve
- LC-MS method adopted
- Total FA depressing COVID-19 +ve – Dyslipidemia?
- Separation of COVID-19 positive and negative participants
- Levels of C-Reactive Protein (CRP)

significantly higher for COVID-19+ve

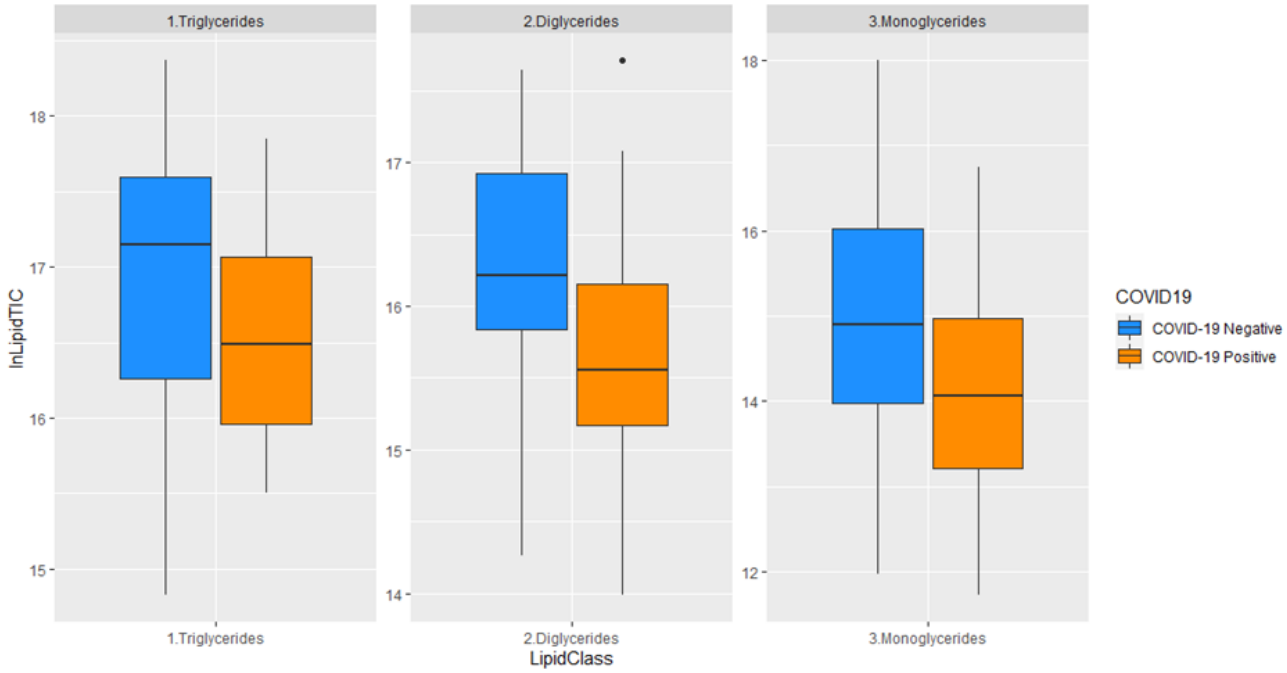


Matt Spick, Katie Longman, Cecile Frampas, Catia Costa, Deborah Dunn Walters, Alex Stewart, Mike Wilde, Danni Greener, George Evetts, Drupad Trivedi, Perdita Barran, Andy Pitt and Melanie Bailey

doi.org/10.1101/2020.09.29.20203745

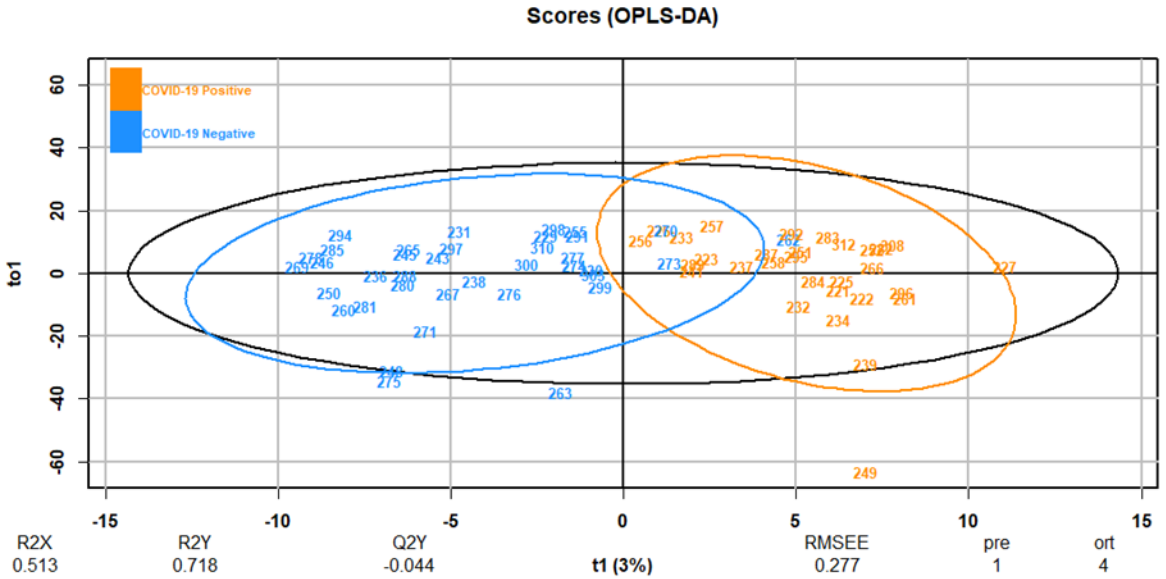
LC-MS Sebum Analysis by lipid class

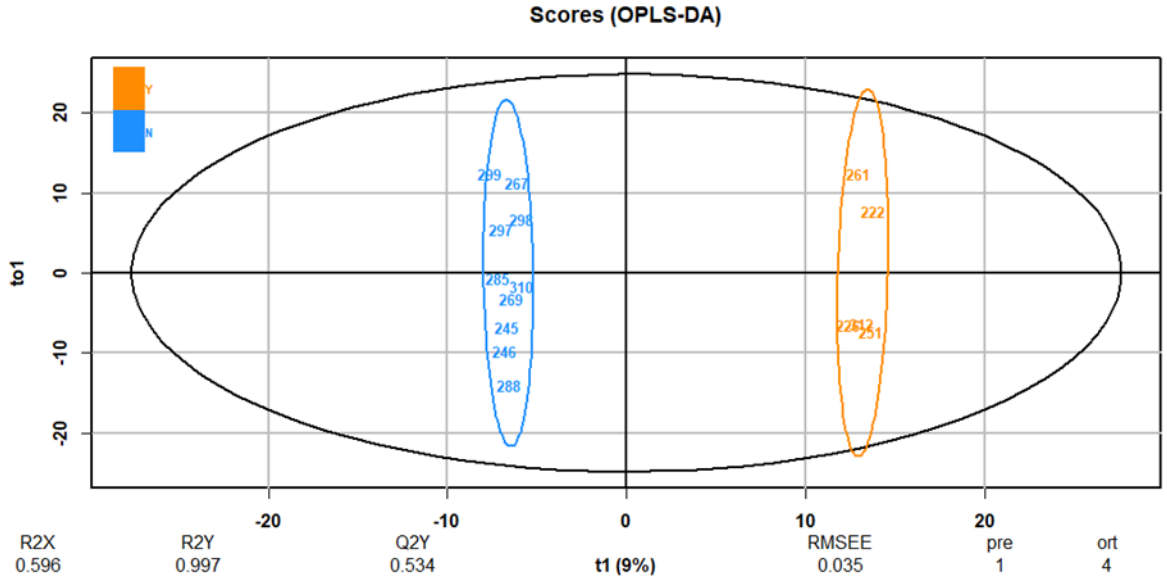
- No individual lipids or features were found that were suitable for univariate analysis of COVID-19 positive versus negative participants.
- At the aggregate level, lipid classes show differentiation; aggregate triglyceride, diglyceride and monoglyceride all depressed for participants with both a positive COVID-19 diagnosis and PCR result.



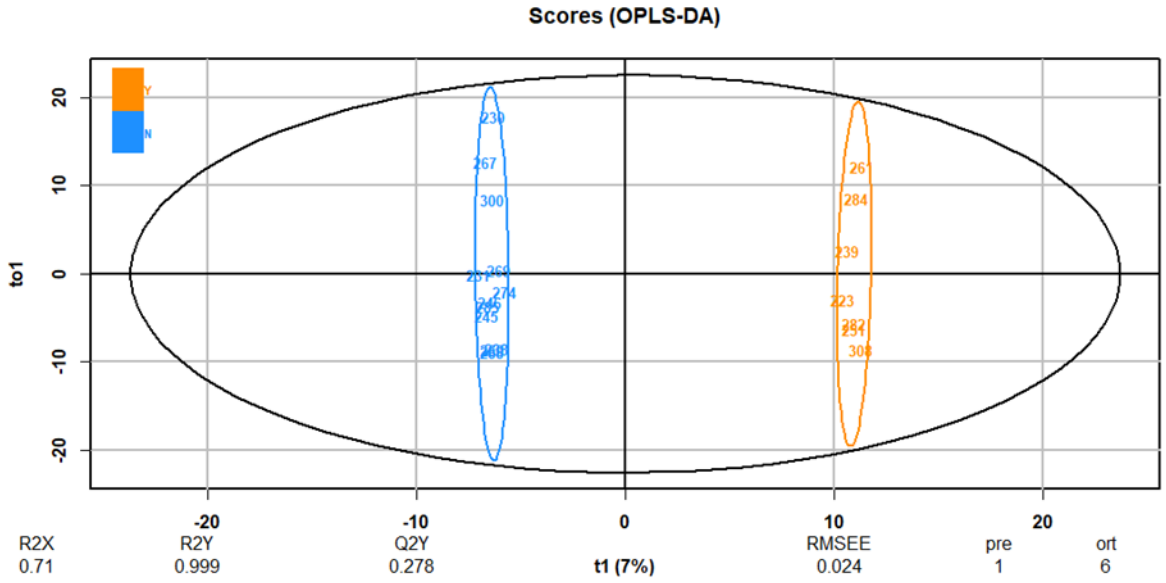
Population level clustering

- Unsupervised PCA and OPLS-DA gives limited separation (age/co-morbidity range too substantial)
- Subset modelling indicates differentiation for high cholesterol, T2Dm, and hypertension





high cholesterol



type-2 diabetes mellitus (T2DM)

Conclusions and Outlook

- Hybrid mass spectrometry approaches can help with biological, medical and biotechnological challenges
- IM-MS is a robust tool for separation and for structure analysis

- There is a distinctive PD odour and this is molecular in nature.
- Run ‘Classic xC-MS’ on 950 samples
- LC-MS of Sebum mapping pathways
- Lipids…..
- Development of paper spray for in field/at home test kit

Acknowledgements

PD work

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30 collecting sites

1800+ patients

3 dogs (Medical Detection Dogs)

THE BARRAN GROUP

MANCHESTER 1824
The University of Manchester

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Camila Lisco
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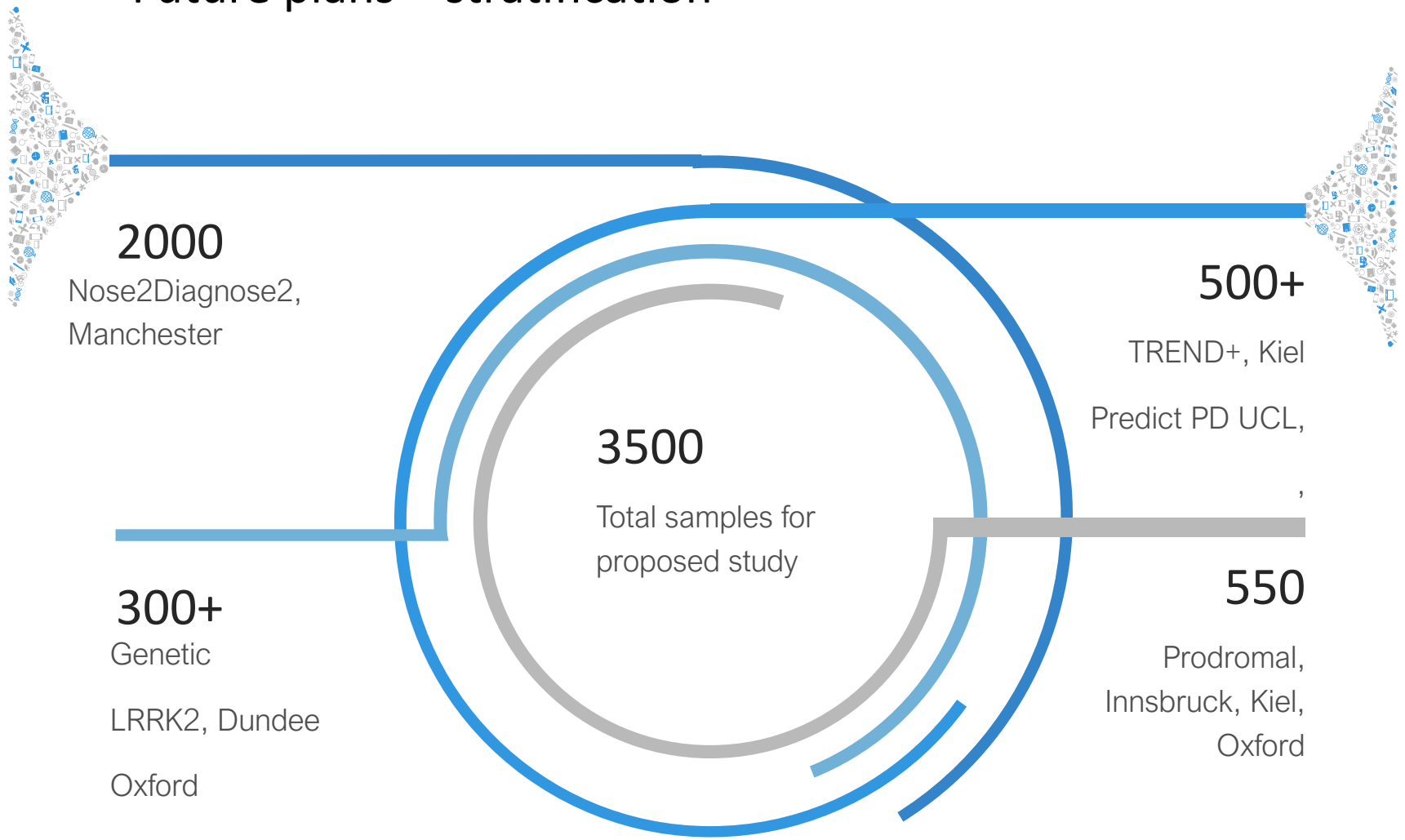
Future plans – stratification



Current approaches for biomarker discovery

TD-GC-MS

- Sample direct from Gauze ~300 features of interest
- Coupled to odour port
- Portable to other labs
- Data requires substantial chemometrics High proportion of (common) fragments with different retention times.
- Poor databases for large volatiles (underivatised compounds)
- Contamination from APs
- Run time ~35 mins

LC-MS

- Many Features (9000, 6000 reproducible)
- New method for sebum extraction
- More features, and better mass accuracy better data bases
- Pathway analysis possible
- Some (more) method development required to retain larger lipids
- Solvent use in LC & extraction
- Contamination from APs
- Runtime ~20 mins

PSI-IM-MS

- Data obtained in ~2 mins 4000 features, 500 statistically significant.
- Distinct signal of intact lipids
- Evidence for unique biomarkers -visual reference
- No cross contamination Requires IM to separate isobaric features
- Requires method development
- Runtime ~5 mins low cost sample collection