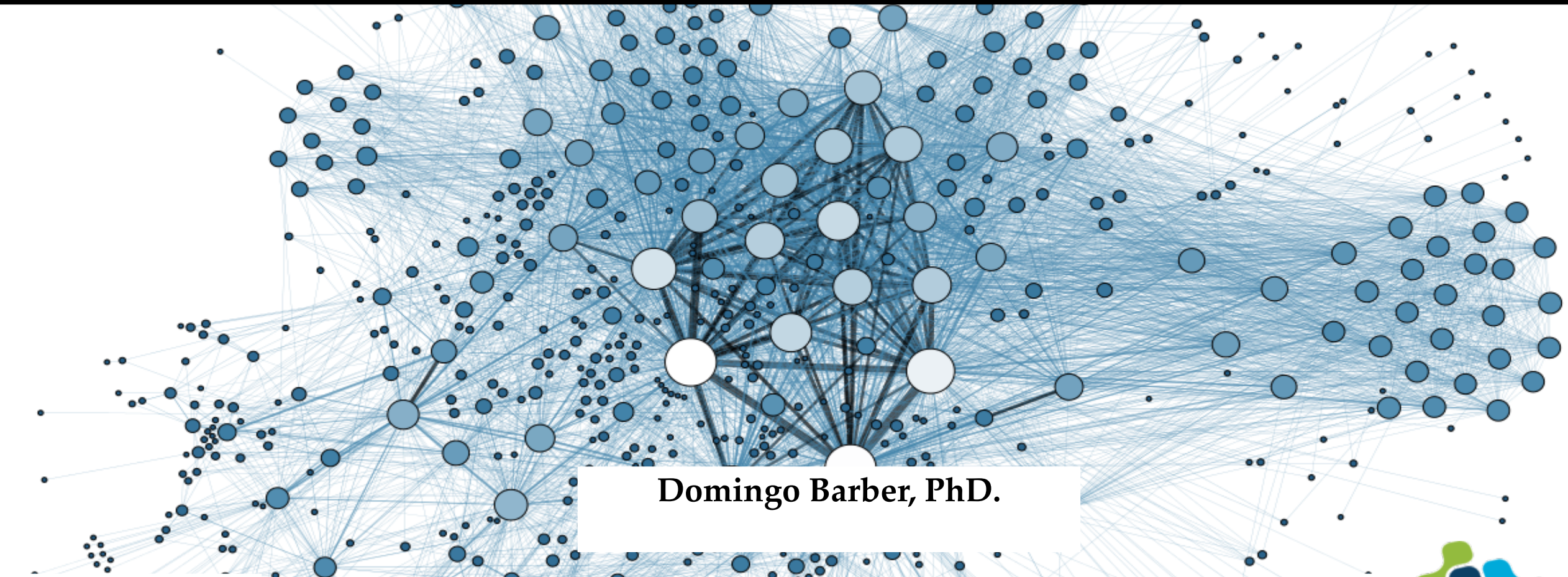
BIG DATA ANALYSIS: METABOLOMICS



Domingo Barber, PhD.

CEU Universidad San Pablo

ARADyAL

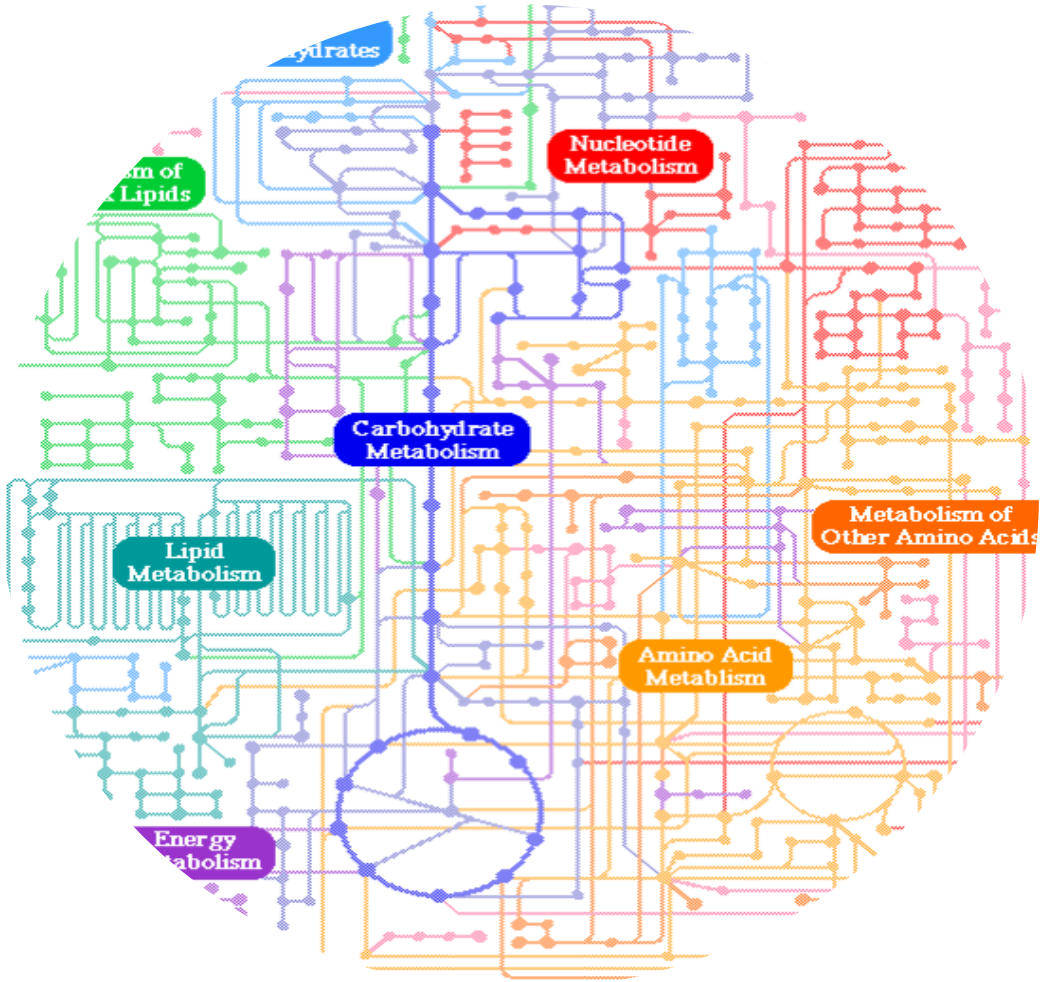
Metabolites

- Metabolites are the molecules of the metabolism
  - It is estimated there are around 300,000 metabolites in humans
    - Specific metabolites can be potential biomarkers of diagnosis or prognosis, or targets for treatment
  - The metabolome is the entire set of metabolites in an organism
- Metabolomics is the science that studies the metabolome

Dumas, ME., *Mol Bio Syst* (2012)
Fiehn, O., *Nat Biotechnol* (2000)

Metabolomics

- Metabolomics was the last “omic” technique developed for biomedical research
- Any phenotype can be described by the collection of metabolites and their levels in the organism
- As a result, any pathology would present specific alterations of some metabolites
- The best example of this is glucose for the diagnosis of diabetes

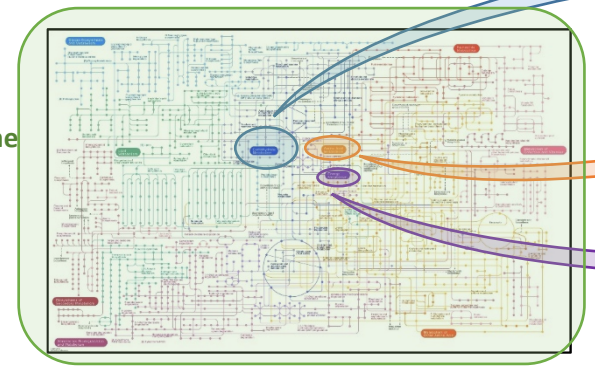


Villaseñor, A., *Clin Exp Allergy* (2017)
Mastrangelo, A., *Curr Top Med Chem* (2014)

Types of strategy in metabolomics

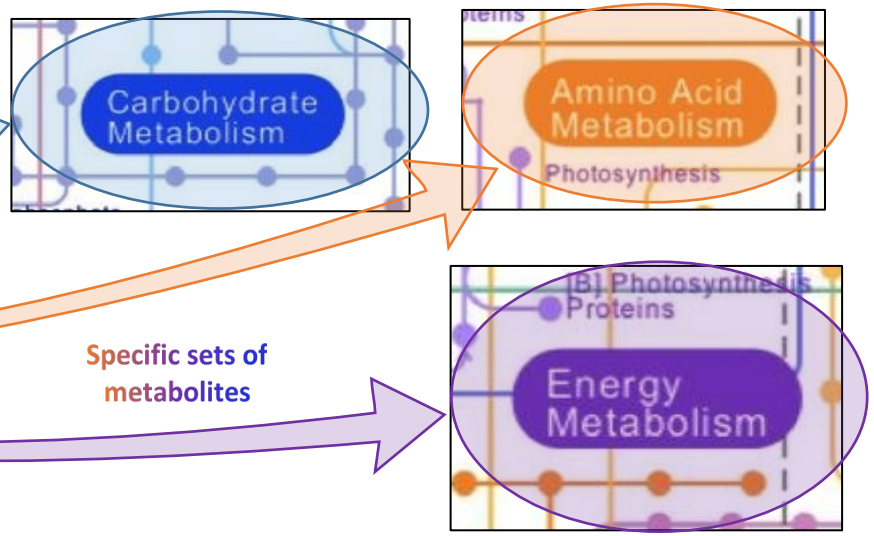
Untargeted
(metabolic fingerprinting)

- ✓ Studies differences in metabolite relative abundances between groups
- ✓ Ideal for finding novel biomarkers
- ✓ Understand biological processes
- ✓ No previous knowledge or hypothesis needed



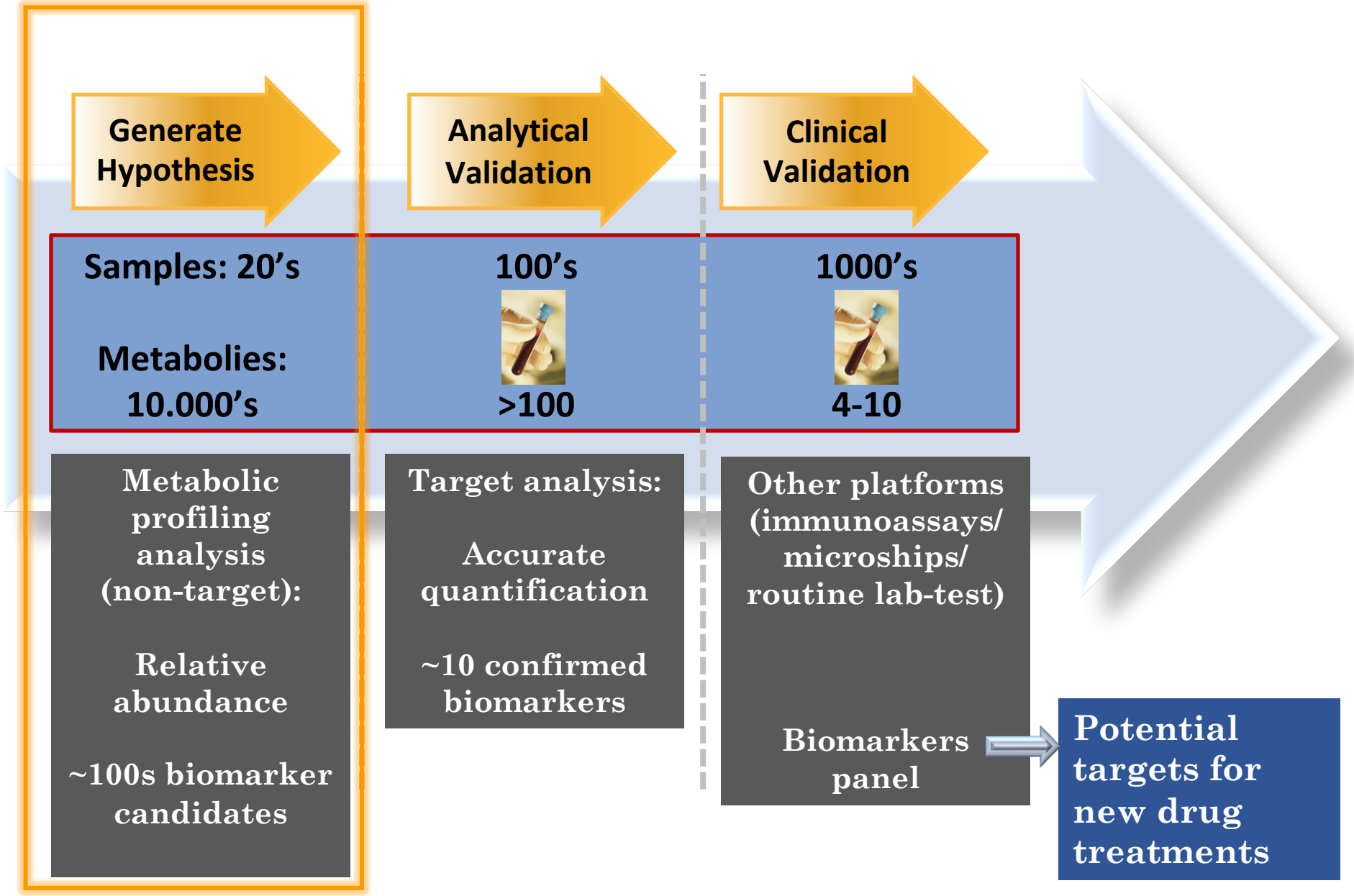
Targeted

- ✓ Accurate quantification of a limited set of metabolites
- ✓ Needs previous knowledge



Villaseñor, A., *Clin Exp Allergy* (2017)

Workflow for biomarker discovery in metabolomics

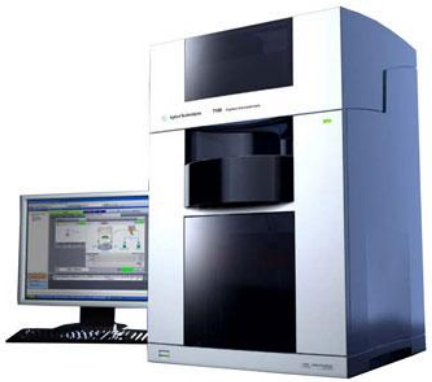


MS related techniques used in metabolomics

Separation technique

High Performance Liquid Chromatography (HPLC)

Gas chromatography (GC)



Capillary electrophoresis (CE)

Detection technique

Single quadrupole

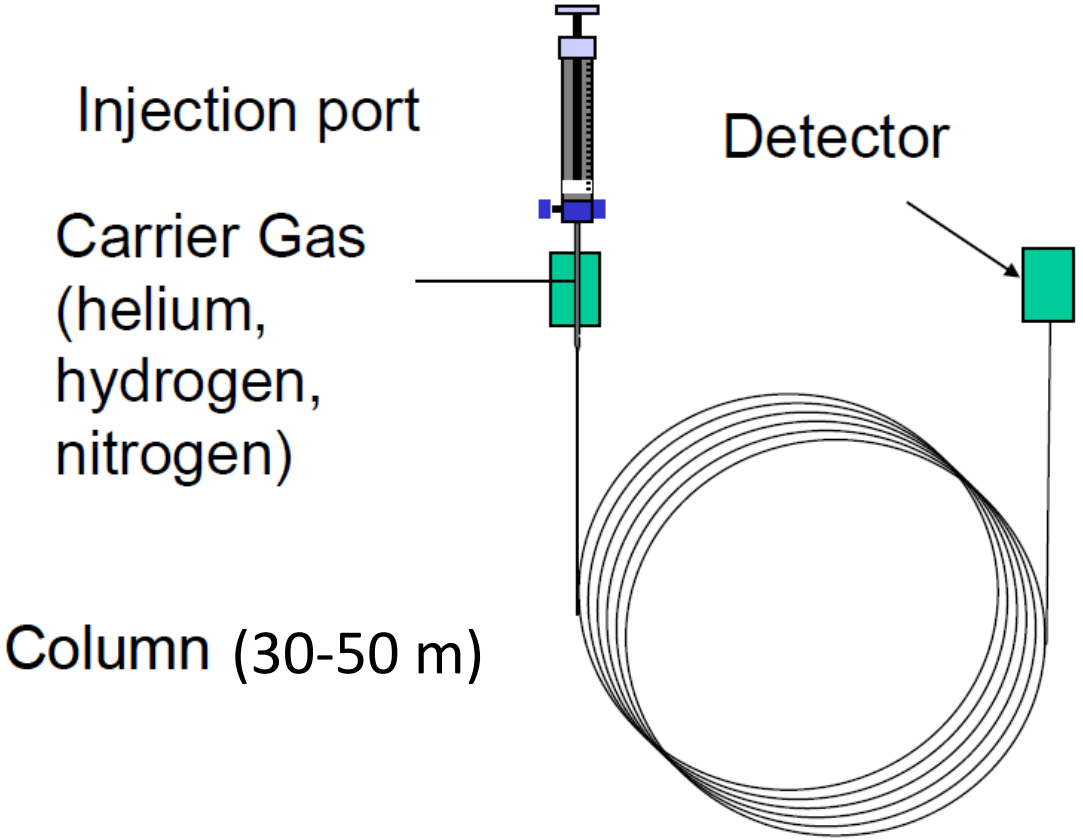
Quadrupole Time of Flight (Q-ToF)

Gives a Retention Time (RT)

Gives a mass/charge ratio (*m/z*)

RT + *m/z*=Describes a specific metabolite

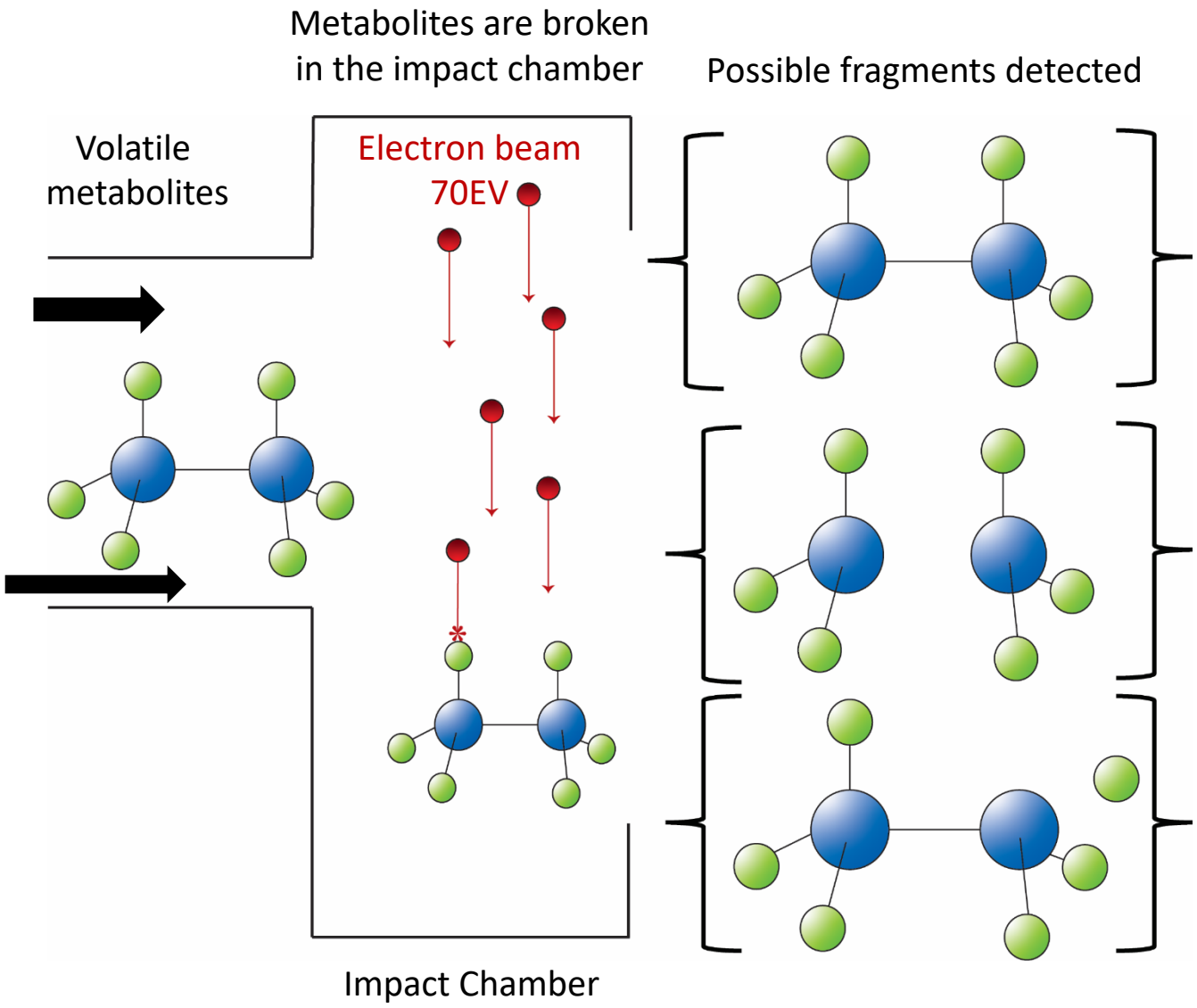
Gas Chromatography coupled to Mass Spectrometry (GC-MS)



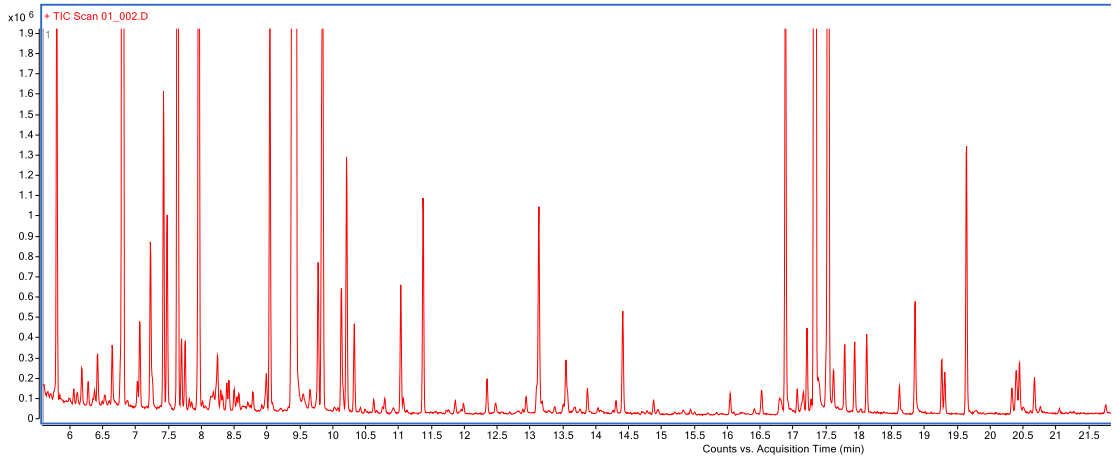
- GC- MS is mainly used to study volatile metabolites
- Metabolites are separated depending on boiling points: The smaller boiling point metabolites exist first

Adapted from Ruperez J.

Ion source in GC-MS: Electron Ionization (EI)



Typical GC-MS chromatogram of a plasma sample

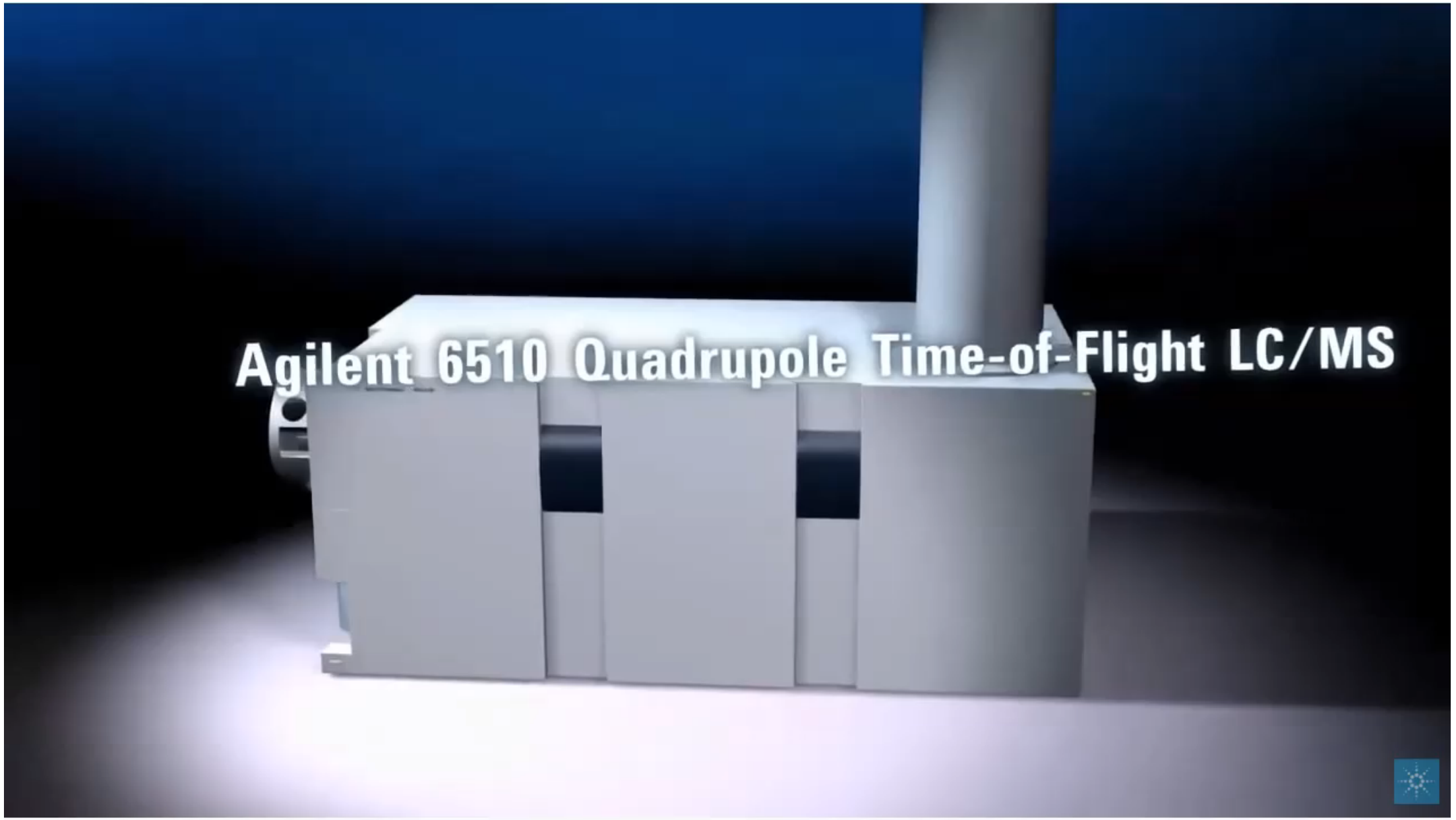


Advantages & Disadvantages of GC-MS

- ✓ Good separation of complex mixtures
- ✓ High precision
- ✓ Robust
- ✓ Ideal for volatile samples such as exhaled breath
- ✓ Existence of extensive databases (used since 70s)

- x Complicated sample treatment in order to make compounds volatile (Derivatization)

Liquid Chromatography coupled to Mass Spectrometry (LC-MS)



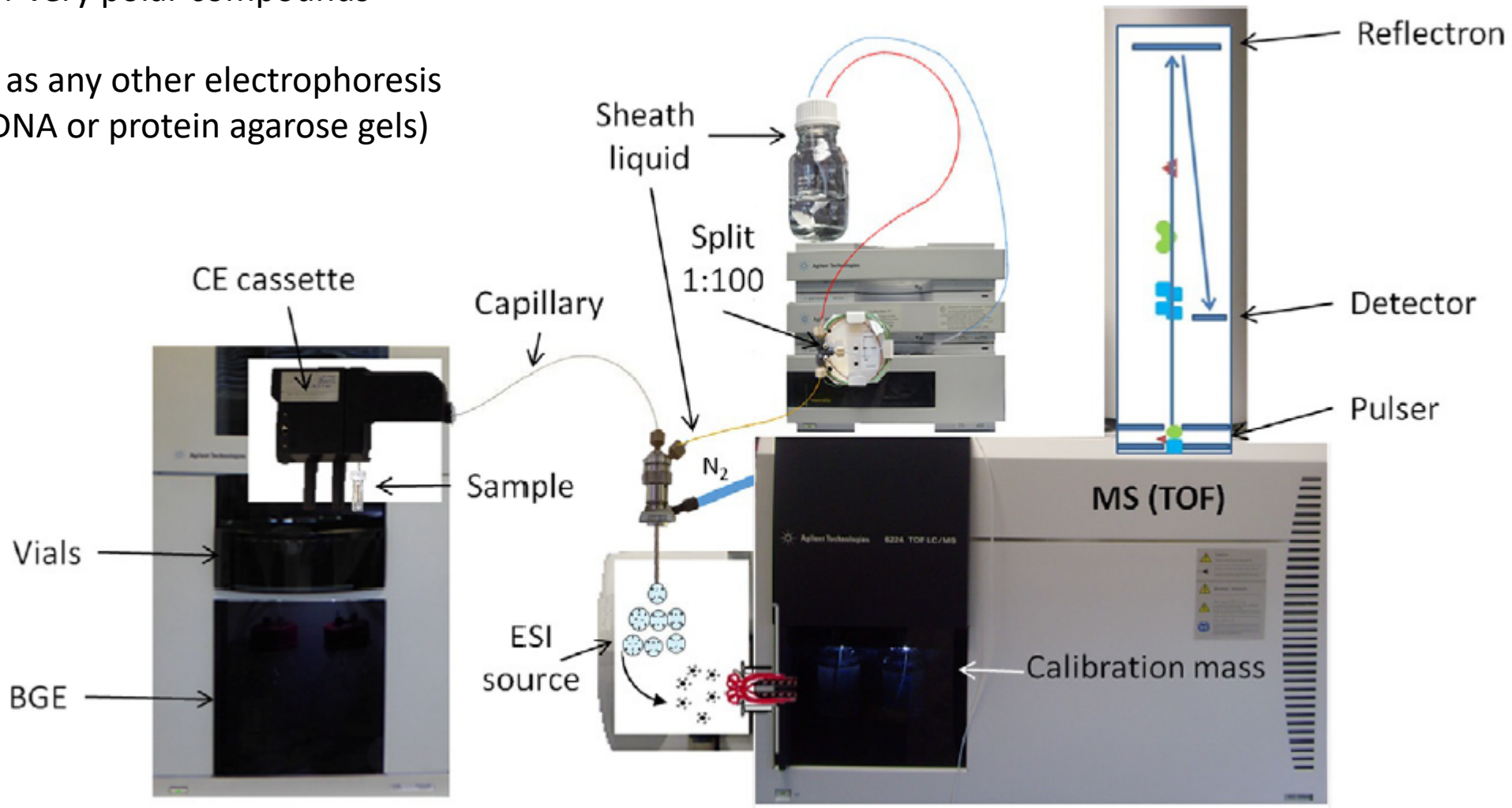
Advantages & Disadvantages of LC-MS

- ✓ Great coverage of metabolites detection (from small polar molecules (Aa) until big non polar molecules (triglycerides)
- ✓ High mass accuracy
- ✓ Robust

- x Identification of compounds in a non targeted study is very complex
- x Laborious data treatment

Capillary electrophoresis coupled to mass spectrometry (CE-

- Ideal technique for very polar compounds
- Separation Works as any other electrophoresis experiment (e.g. DNA or protein agarose gels)



Godzien, J; et al *The Handbook of Metabolic Phenotyping* (2019)

Advantages & Disadvantages of CE-MS

- ✓ Small volume required
- ✓ Sample preparation is minimal for polar samples such as urine or tears
- ✓ Separation of ionic and polar compounds

- x Lower sensitivity than LC-MS: Injection of nanolitres of sample
- x Complex identification: Only possible by using commercial standards and in Ion Source fragmentation

LC-MS, GC-MS and CE-MS are complementary

Liquid Chromatography coupled to Mass Spectrometry (LC-MS)

Metabolites in solution with a full range of polarities

Gas Chromatography coupled to Mass Spectrometry (GC-MS)

Volatile metabolites

More analytical techniques = More information

Capillary Electrophoresis coupled to Mass Spectrometry (CE-MS)

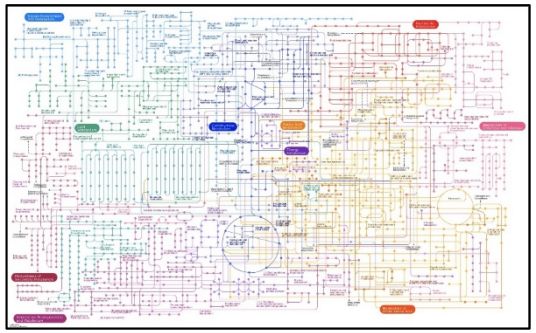
Polar metabolites

Types of strategy in metabolomics

Untargeted
(metabolic fingerprinting)

- ✓ Studies differences in metabolite relative abundances between groups
- ✓ Ideal for finding novel biomarkers
- ✓ Understand biological processes
- ✓ No previous knowledge or hypothesis needed

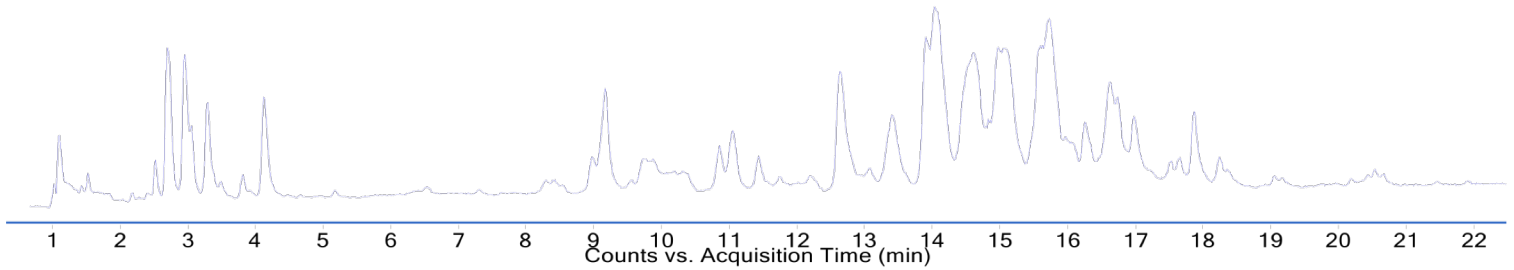
Whole metabolome



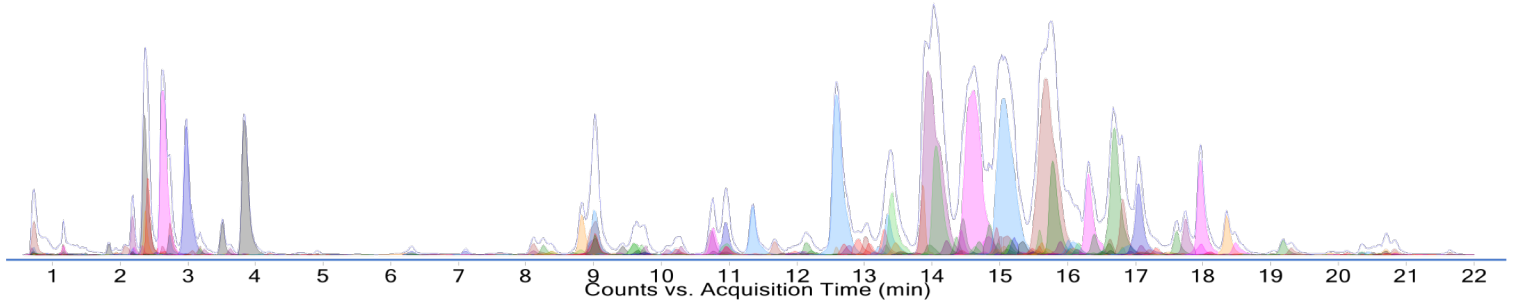
Villaseñor, A., *Clin Exp Allergy* (2017)

Data pre-processing

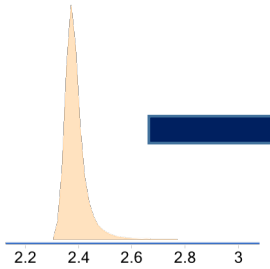
- Typical metabolic profile



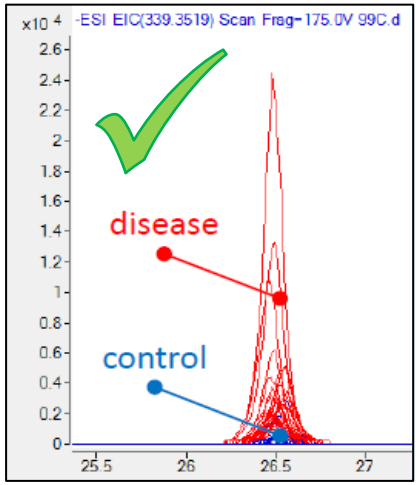
- Underneath, the profile contains metabolites



- Each metabolite is characterized by:



Mass: 543.3390
RT: 2.37
Area: 61109428

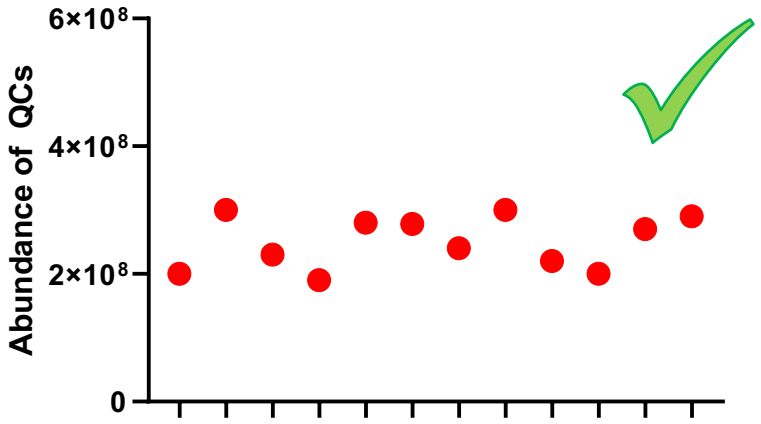


Assessing quality of data measurements: Filtering unreliable metabolites

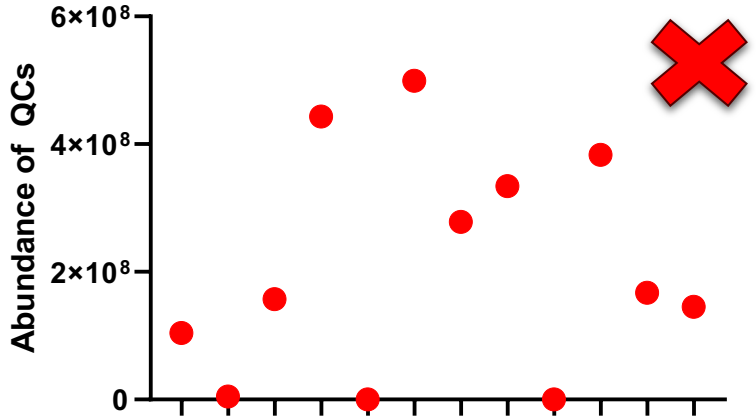
- Different filters are applied to assure the quality of the data:
  - Remove features in the blanks
  - Remove metabolites that have high coefficient of variation (>30%) on Quality Control samples (QCs)
  - Remove metabolites that are not present in a sufficient number of samples (depends on experimental setup)

- * If concentration of the samples is different (e.g. in urine samples) normalization of data will be needed

Reliable metabolite

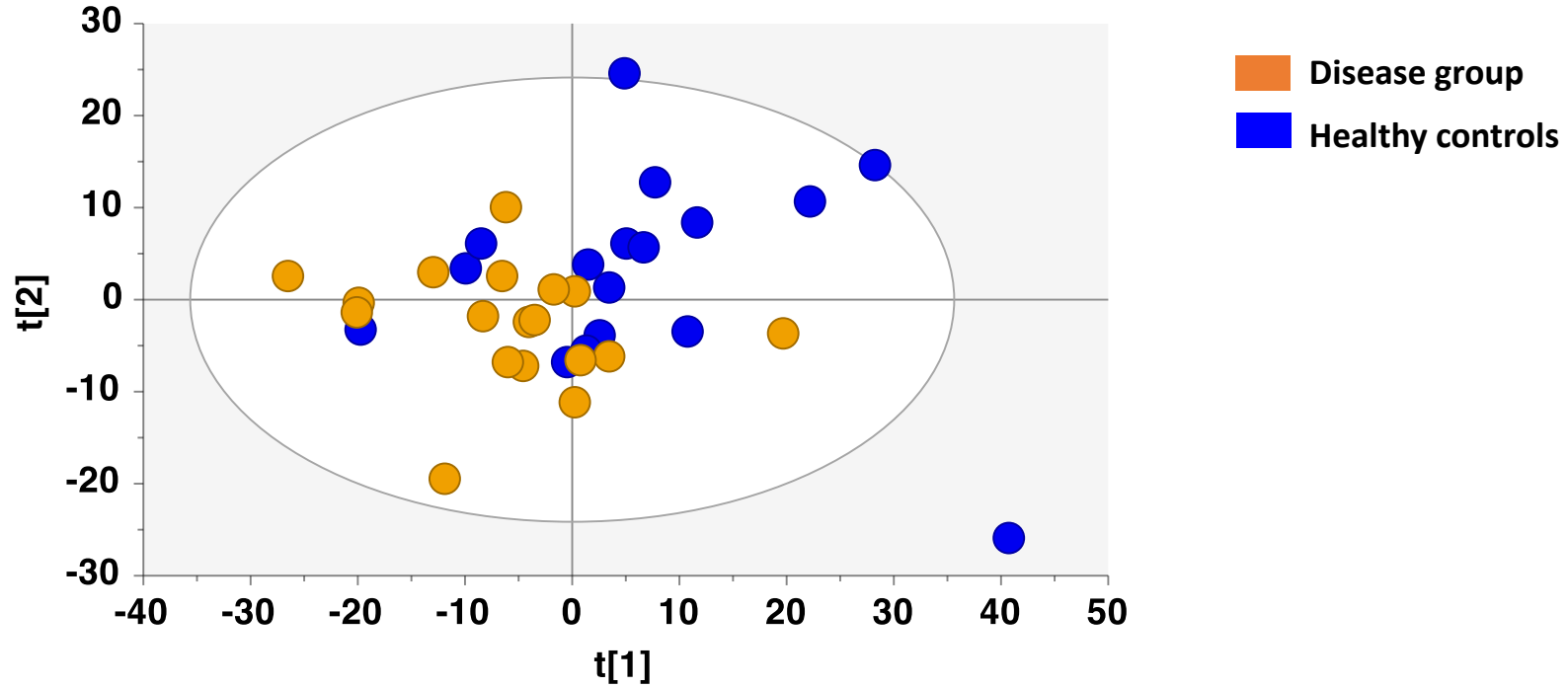


Unreliable Metabolite



Multivariate analysis: PCA

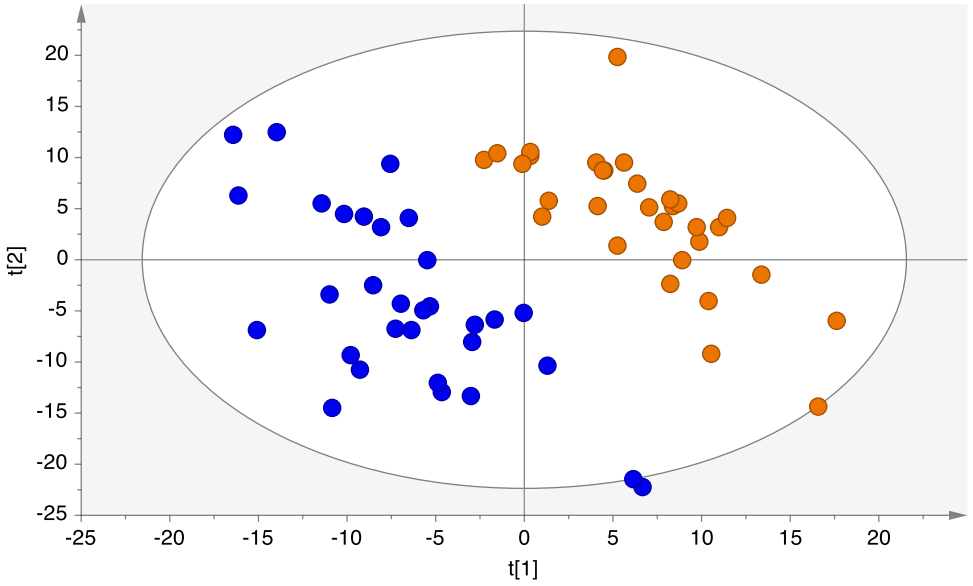
- Principal Component Analysis (PCA) is a blind no supervised analysis
- PCA looks for the similarities between samples
- It is used to observe patterns, clustering and outliers in your samples



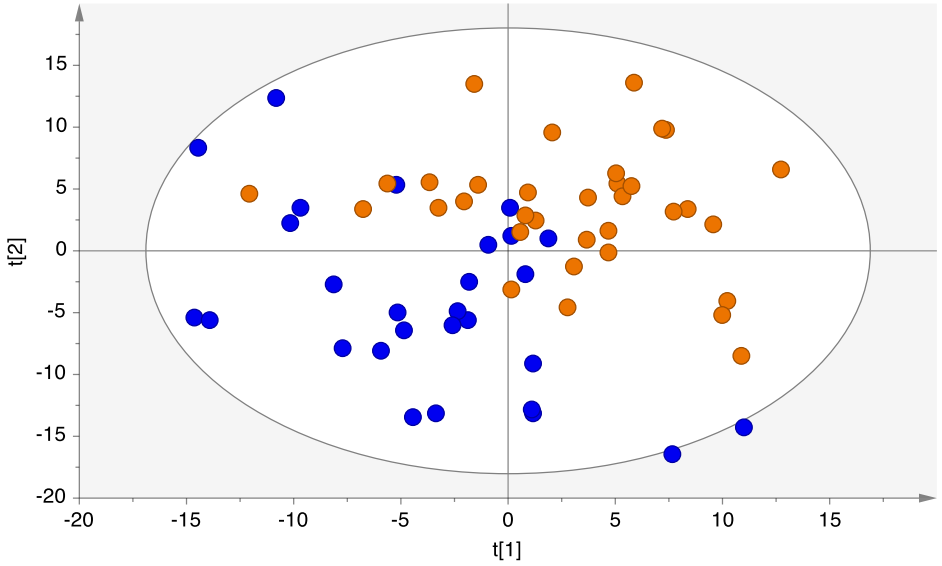
Multivariate analysis: PLS-DA

- Partial Least Square Discriminant Analysis (PLS-DA) is a supervised multivariate analysis
- It identifies if there is a real separation/difference between 2 groups.
- Separation of samples is explained by the first component (x axis, mainly) and the second component (y axis)

True biological differences



Fewer biological differences



Disease group

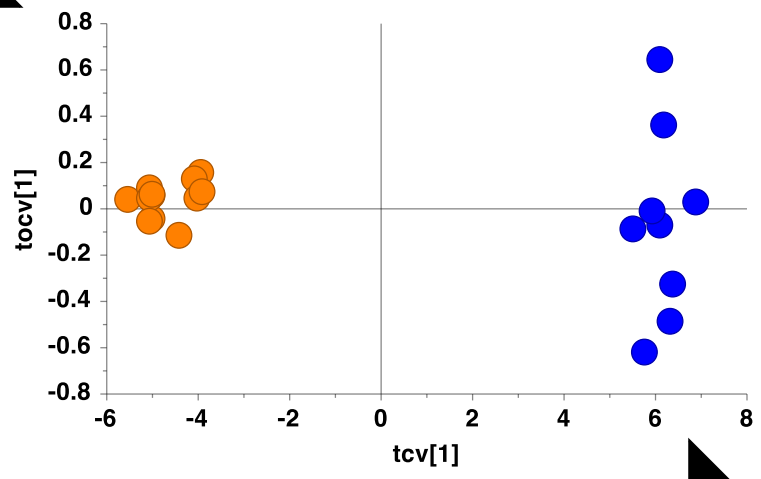
Healthy controls

Multivariate Analysis: OPLS-DA

- Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) is also a supervised multivariate analysis which separates space the variation between samples of 2 groups. **Only used if a good PLS-DA has been obtained.**
- Separation is only based by the first component
- Commonly used for identification of potential biomarkers and prediction of samples

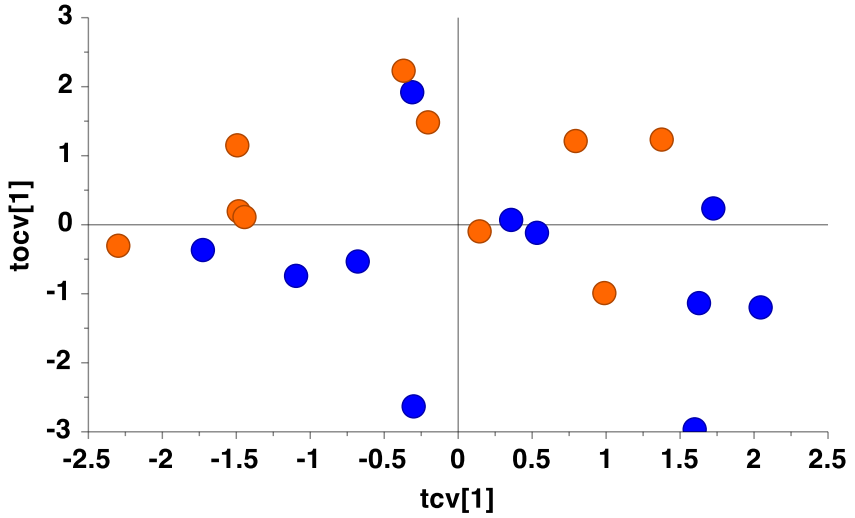
Differences non group related

True biological differences



Differences between groups

No biological differences

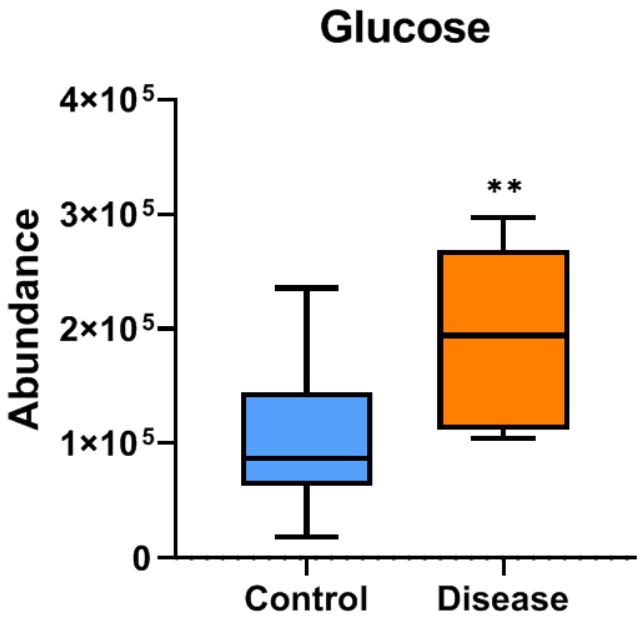


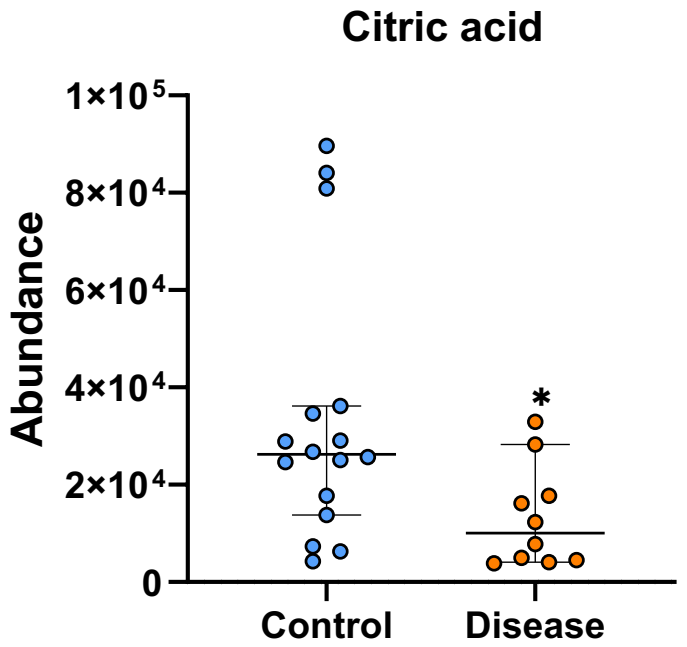
Disease group

Healthy controls

Univariate analysis: ANOVA and non parametric ANOVA

- To detect differences of individual metabolites between groups
- Depending of the number of patients/conditions/animals you use one or another test (t test, U-Mann Whitney, etc.)





Significant compounds need to be identified

			Non allergic vs Severe	
No	*m/z* (Da)	RT (min)	% Moderate	*p* value
1	479.3366	21.44	**56.9**	**0.017**
2	553.3793	26.01	**121.2**	**0.004**
3	553.3747	22.23	**47.9**	**0.03**
4	551.3602	22.3	**55.1**	**0.009**
5	557.3327	16.11	12.9	0.537
6	541.3386	19.44	**45.7**	**0.004**
7	531.3102	20.21	**17.3**	**0.017**
8	559.3407	23.76	**77.1**	**0.004**

Identification of compounds in LC-MS and CE-MS

1. First step → Search into specific web databases

   <u>Tentative identification</u>, based only on its mass and RT

CEU MASS MEDIATOR

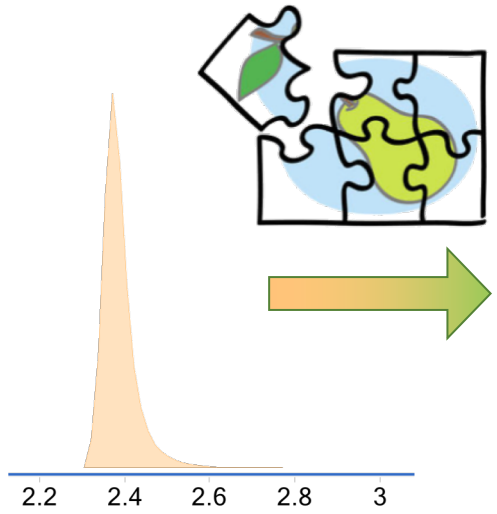
METLIN

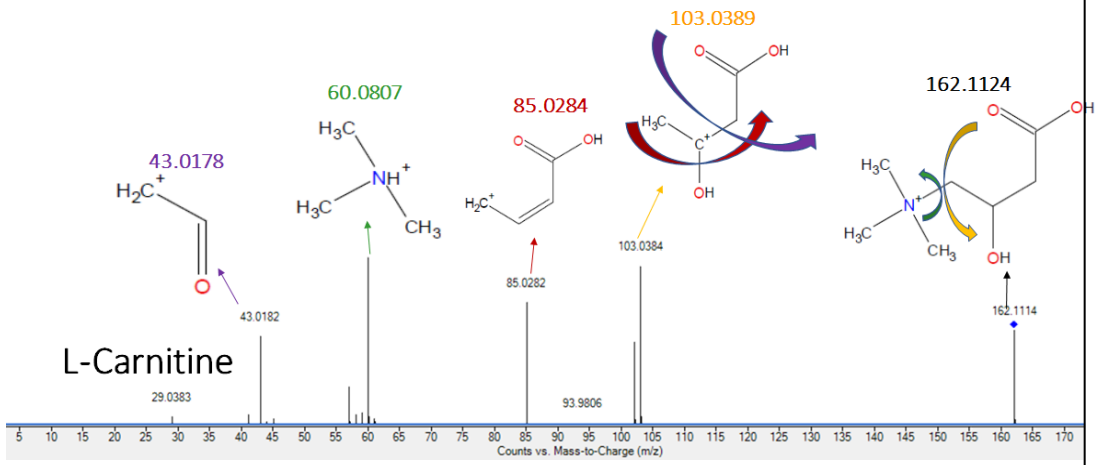
KEGG

LIPID MAPS

hmdb
The Human Metabolome Database

2. Second step → fragment the significant metabolite and puzzle out the structure (i.e. L-Carnitine)

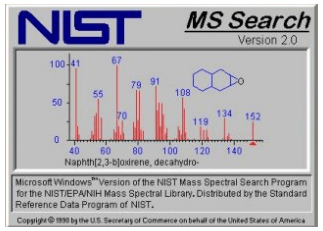




Identification in GC-MS

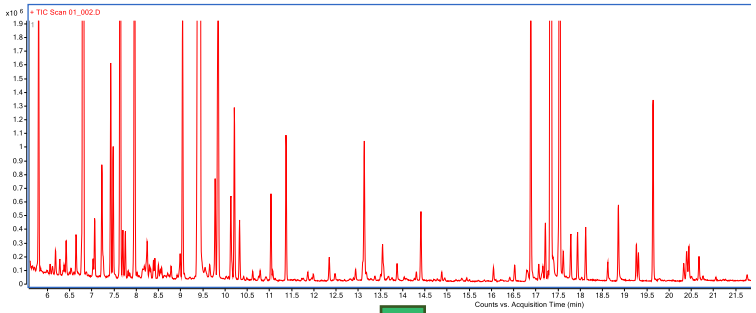
- Contrary to LC-MS and CE-MS identification is performed during data treatment

- Common databases used:

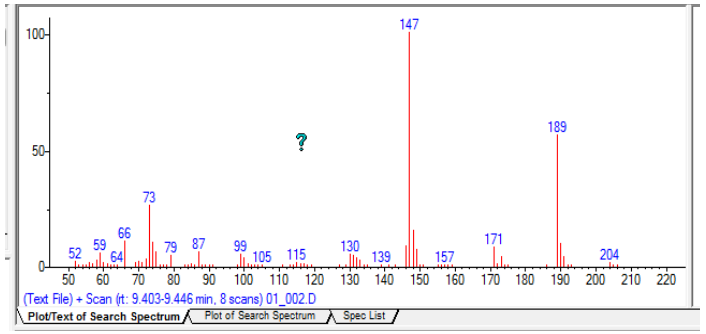
1. 

2000s compounds

2. **Fiehn library**- 1000s compounds

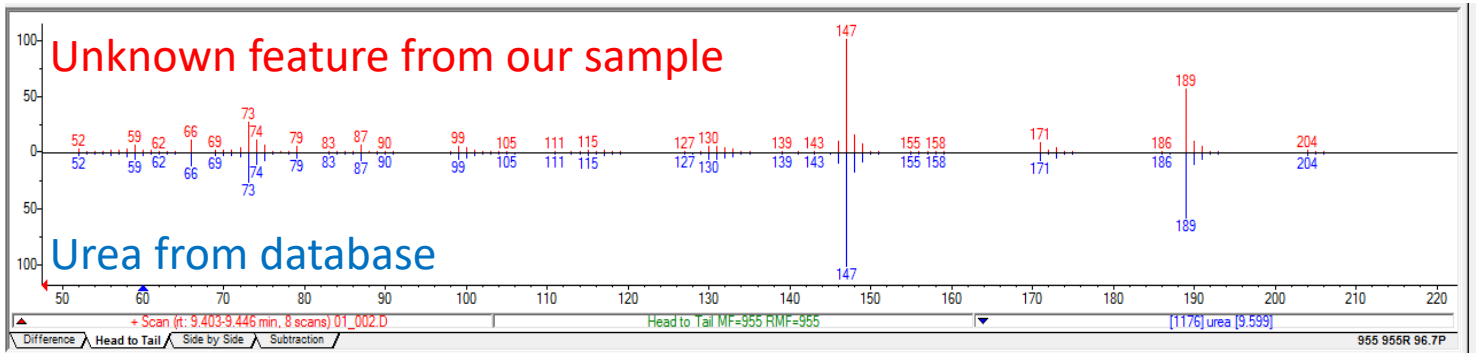


Complete chromatogram



1 spectra at RT 10.32

Is compared with databases



Common problems

Common problems in untargeted metabolomics: Big patient cohort

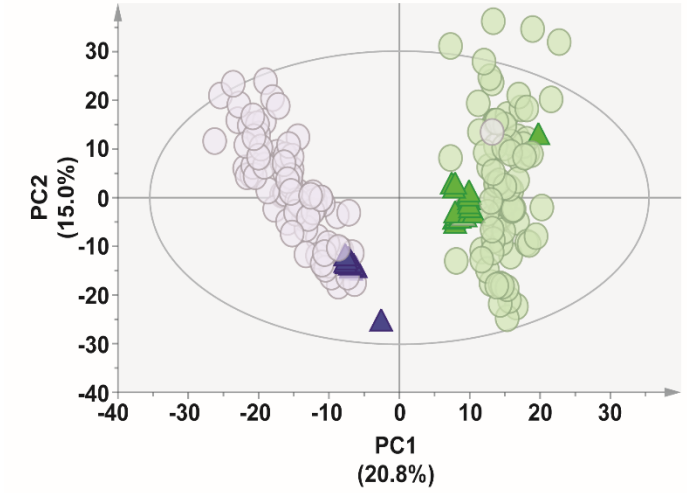
Big cohort of samples may not be able to analyze continuously in a single batch

The quality between each batch can be good but does not necessary that can data can be overlapped

Normalization strategies: Intra-batch and inter-batch normalization

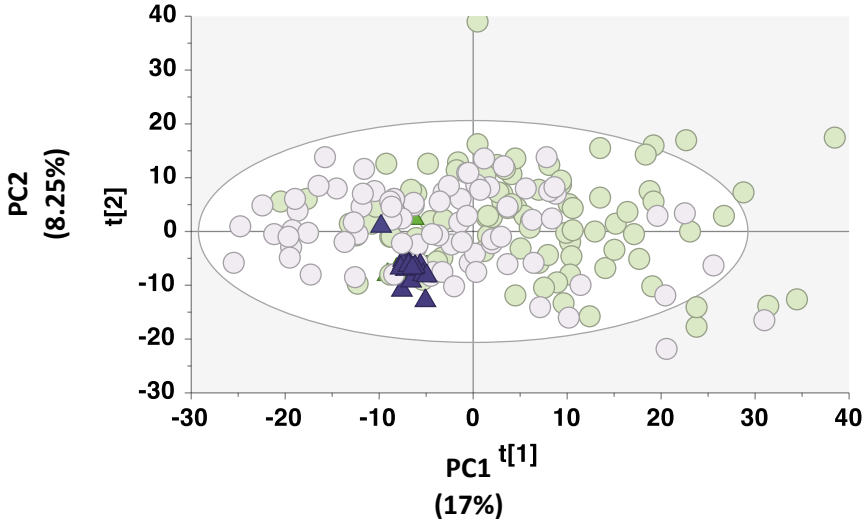
N=165

Raw data



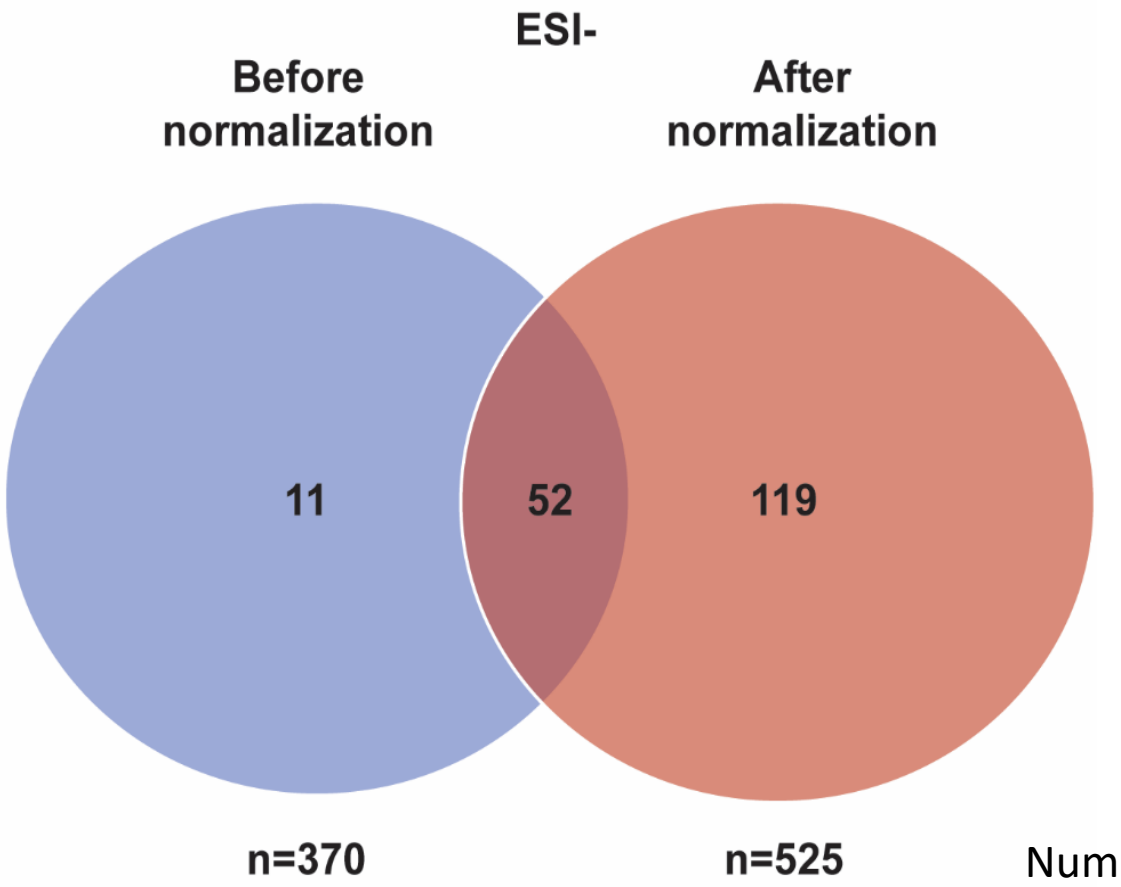
After inter- and intra-batch normalization

Normalized data



Rodriguez-Coira, J., *Metabolites* (2019)

Effect of normalization in the number of significant features



Number of statistically significant
features after comparing control and disease groups

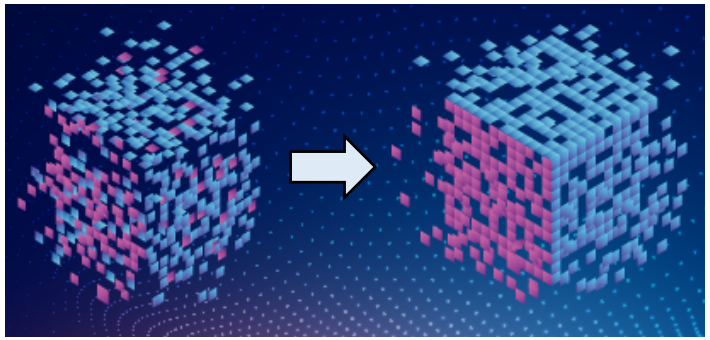
Number of total features that passed quality assurance

Rodriguez-Coira, J; et al. *Metabolites* (2019)

Summary of untargeted metabolomics analysis

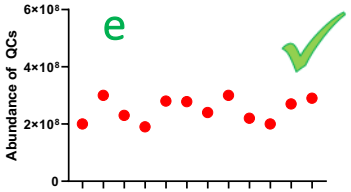
1.Measurement

MassHunter Profinder

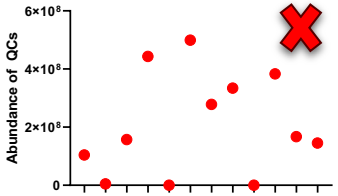


2. Data transformation

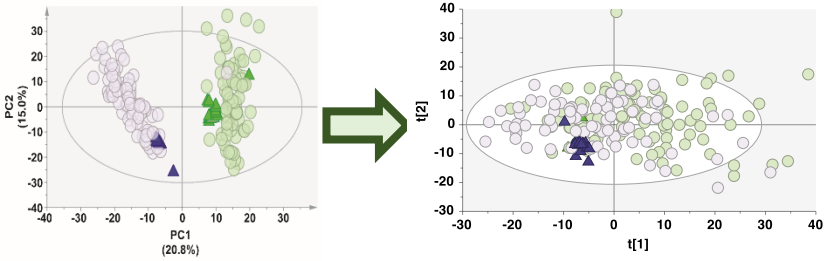
Reliable metabolite



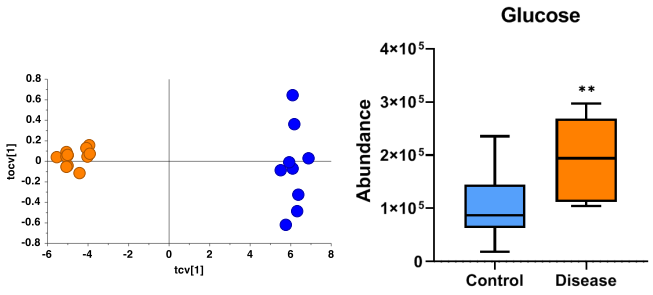
Unreliable Metabolite



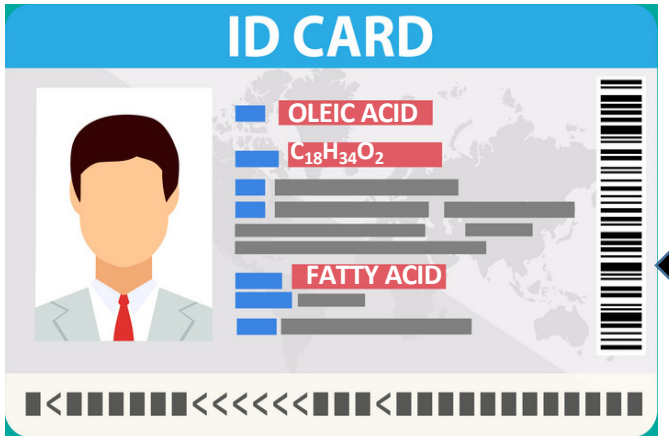
3. Data quality assessment



4.Normalization



5.Statistical analysis



6.Identification

ORAL MUCOSA: A MODEL OF EPITHELIA REMODELLING AND INFLAMMATION

-SEVERE PROFILIN FOOD ALLERGY
-SEVERE RESPIRATORY ALLERGY IN THE ABSENCE OF FOOD ALLERGY

Hypothesis: Oral mucosa integrity is compromised in respiratory allergic patients.

ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

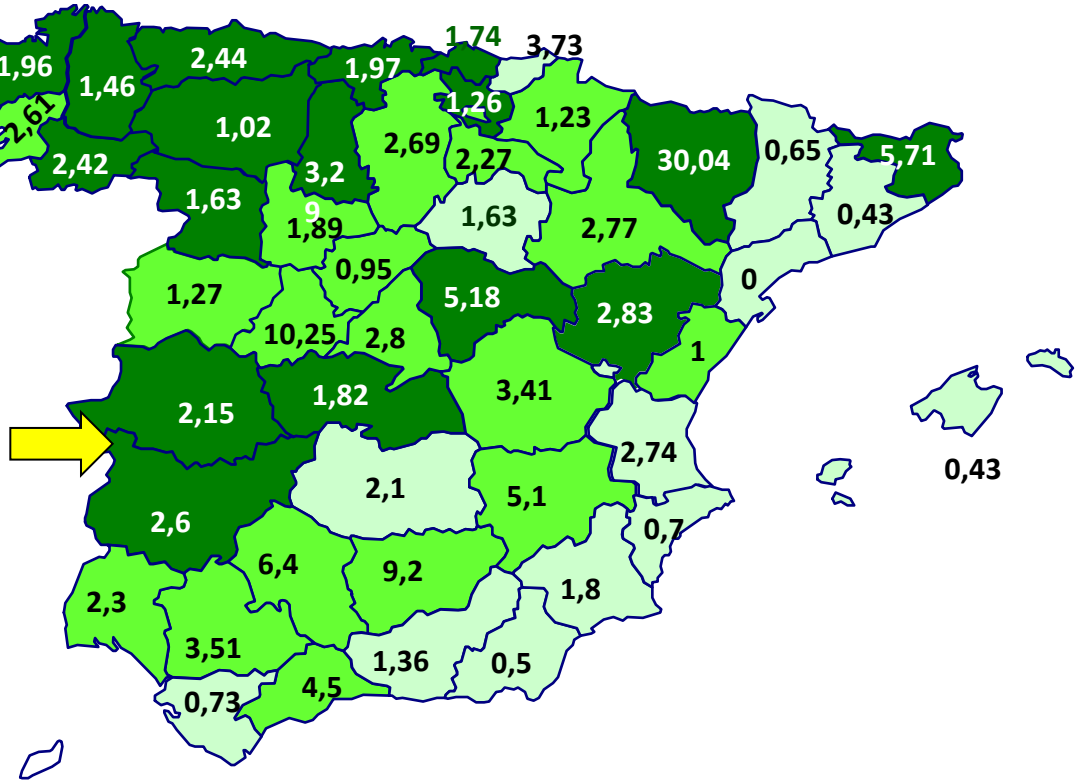
Profilin as a severe food allergen in allergic patients overexposed to grass pollen

M. I. Alvarado[1], L. Jimeno[2], F. De La Torre[2], P. Boissy[2], B. Rivas[1], M. J. Lázaro[1] & D. Barber[3]

[1]Servicio de Alergia, Hospital Ciudad de Coria, Coria; [2]Departamento de I+D y Medical Advisor, ALK-Abelló S.A.; [3]Institute for Applied Molecular Medicine (IMMA), School of Medicine, University San Pablo-CEU, Madrid, Spain

To cite this article: Alvarado MI, Jimeno L, De La Torre F, Boissy P, Rivas B, Lázaro MJ, Barber D. Profilin as a severe food allergen in allergic patients overexposed to grass pollen. *Allergy* 2014; **69**: 1610–1616.

Coria (Caceres):
-60% of prevalence to profilin

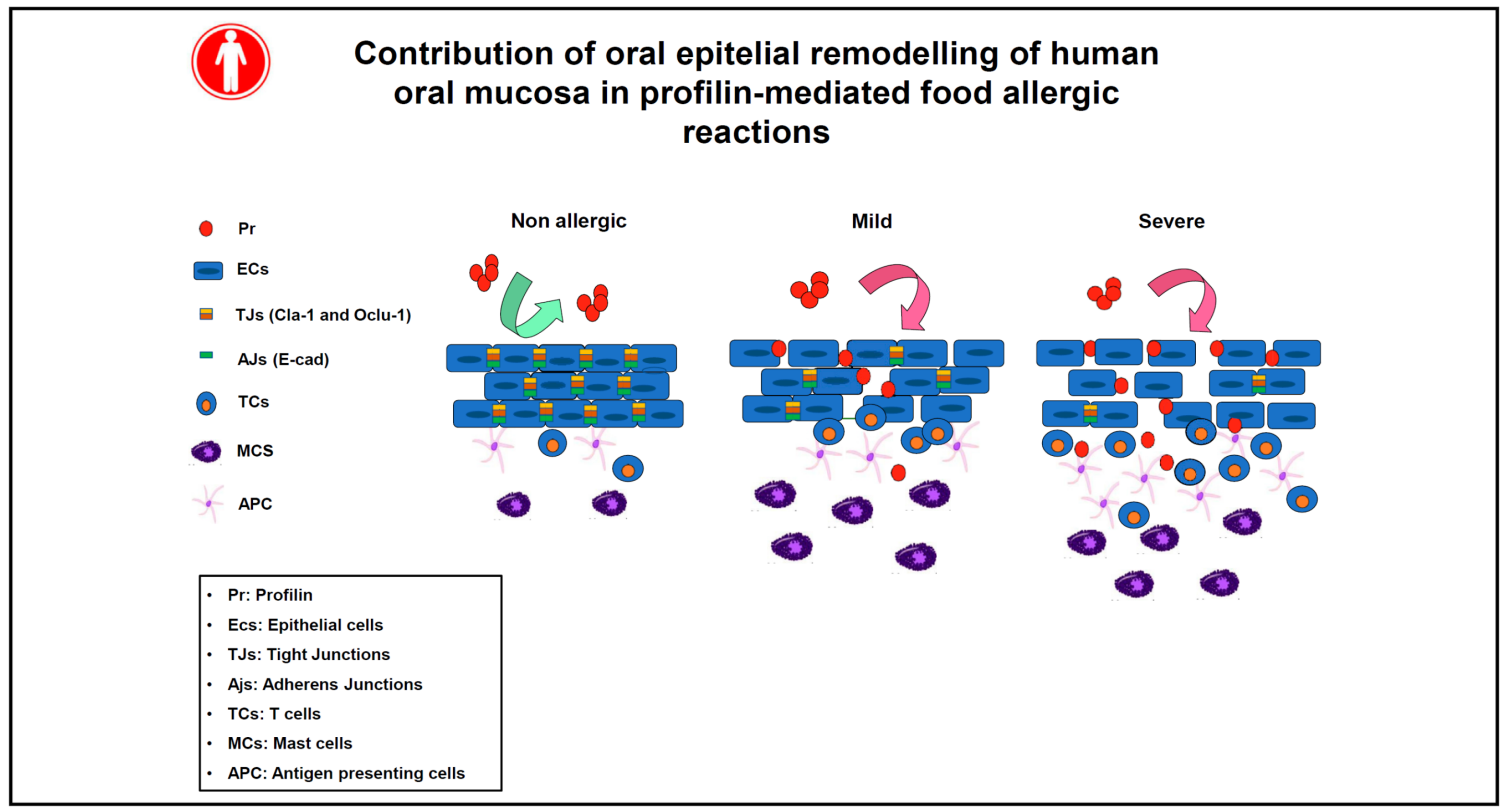


Profilin-mediated food-induced allergic reactions are associated with oral epithelia remodeling

J ALLERGY CLIN IMMUNOL
FEBRUARY 2019

Domenico Rosace, MSc,[a] Cristina Gomez-Casado, PhD,[a] Paloma Fernandez, PhD,[a] Marina Perez-Gordo, PhD,[b] María del Carmen Dominguez, MD,[c] Angel Vega, MD,[c] María Teresa Belver, MD,[d] Tania Ramos, MD,[d] Francisco Vega, MD,[d] Guadalupe Marco, MD, PhD,[e] Manuel de Pedro, MD,[e] Leticia Sanchez, MD,[e] María de las Mercedes Arnas, MD, PhD,[f] Marcela Santaolalla, MD, PhD,[f] Miguel Ángel Saez, MD,[g] Sara Benedé, PhD,[h] Montserrat Fernandez-Rivas, MD, PhD,[e] Carlos Blanco, MD, PhD,[d] Maria Isabel Alvarado, MD, PhD,[c] María M. Escribese, PhD,[a,b] and Domingo Barber, PhD[a] *Madrid and Cáceres, Spain*

GRAPHICAL ABSTRACT



DOI: 10.1111/all.13860

LETTER TO THE EDITOR

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI WILEY

Respiratory allergies with no associated food allergy disrupt oral mucosa integrity

Javier Sanchez-Solares[1]
Maria I. Delgado-Dolset[1]
Leticia Mera-Berriatua[1]
Gonzalo Hormias-Martin[2]
Jose A. Cumplido[3]
Vanesa Saiz[4]
Teresa Carrillo[3]
Carmen Moreno-Aguilar[4]
Maria M. Escribese[1]
Cristina Gomez-Casado[1]
Domingo Barber[1]

[1]*Department of Basic Medical Sciences, Faculty of Medicine, Institute of Applied Molecular Medicine, San Pablo CEU University, Madrid, Spain*

- Highly exposed Olive pollen allergic patients (Cordoba)
- Highly exposed mite allergic patients (Canary Islands)

Understanding severity:

Why, patients that had been tolerating profilin for years on a daily basis, get sensitized and develop severe reactions?

- In the three severity models: **Bad response to intervention (side reactions, lack of effect)**

-Metabolomics of grass pollen severe allergy phenotypes.

Received: 25 April 2018 | Accepted: 4 July 2018

DOI: 10.1111/all.13563

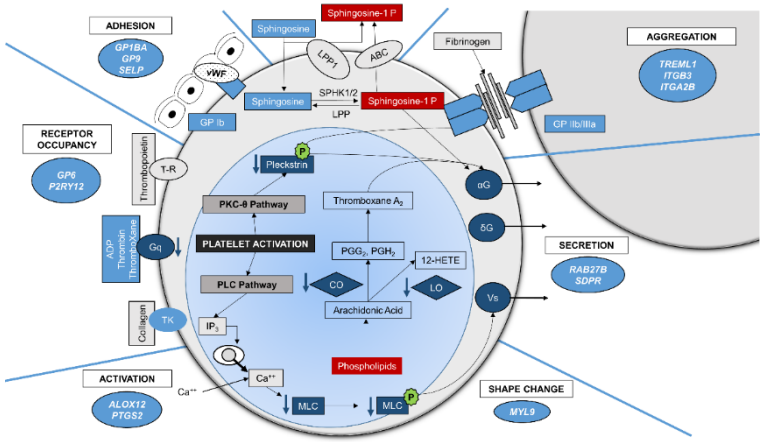
ORIGINAL ARTICLE

Experimental Allergy and Immunology

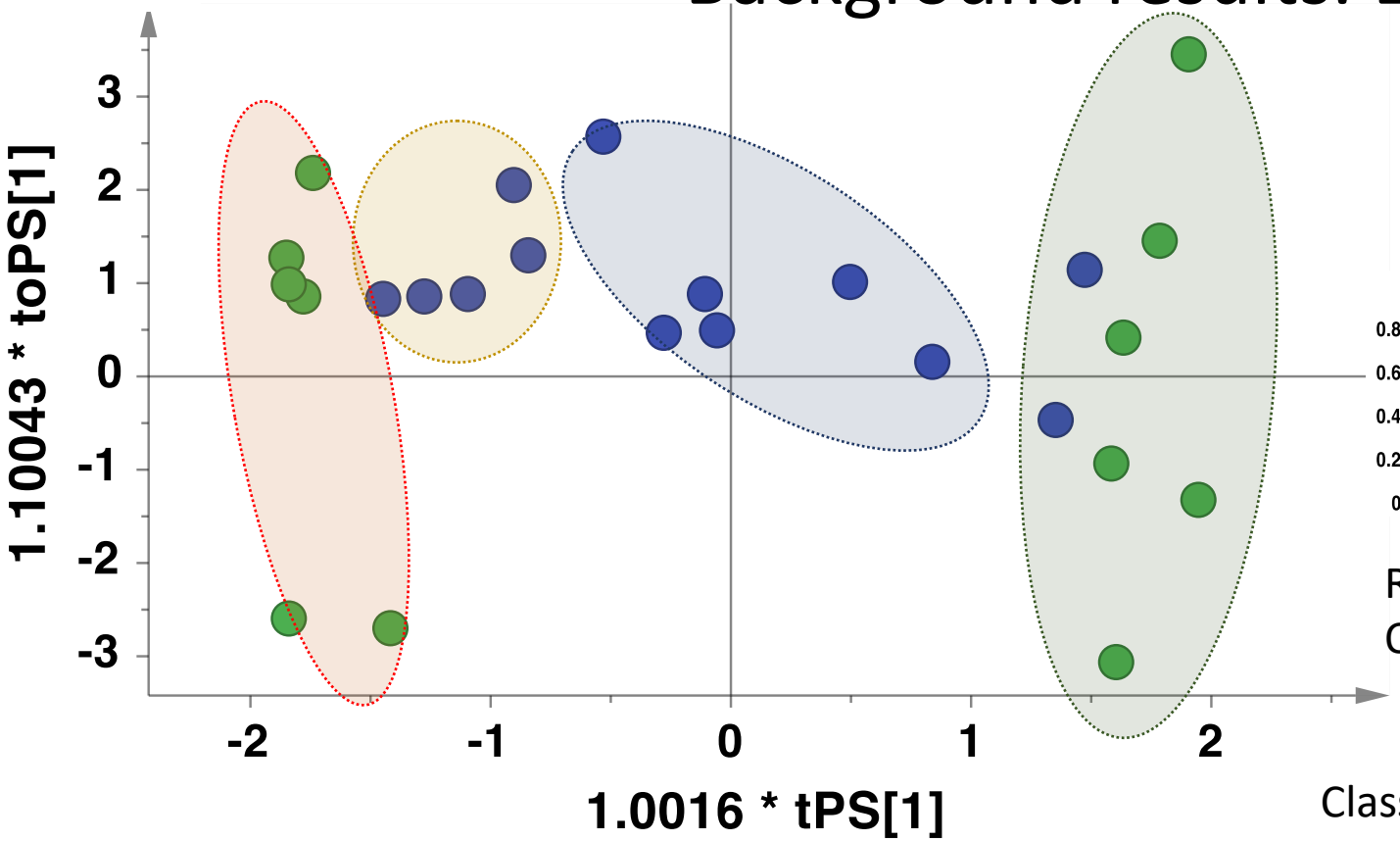
WILEY Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

Multi-omics analysis points to altered platelet functions in severe food-associated respiratory allergy

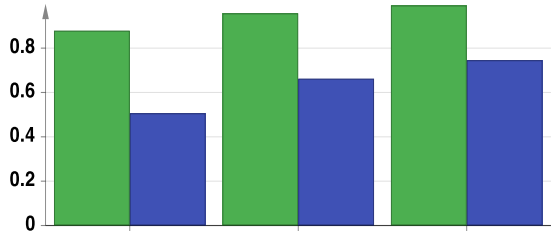
David Obeso[1,2] | Leticia Mera-Berriatua[1] | Juan Rodríguez-Coira[1,2] | Domenico Rosace[1] | Paloma Fernández[1] | Isabel Adoración Martín-Antoniano[1,3] | Marcela Santaolalla[4] | Guadalupe Marco Martín[5] | Tomás Chivato[1,3] | Montserrat Fernández-Rivas[5] | Tania Ramos[6] | Carlos Blanco[6] | María I. Alvarado[7] | Carmen Domínguez[7] | Santiago Angulo[8] | Coral Barbas[2] | Domingo Barber[1] | Alma Villaseñor[1] | María M. Escribese[1,9]



Background results. LC-MS (ESI +)

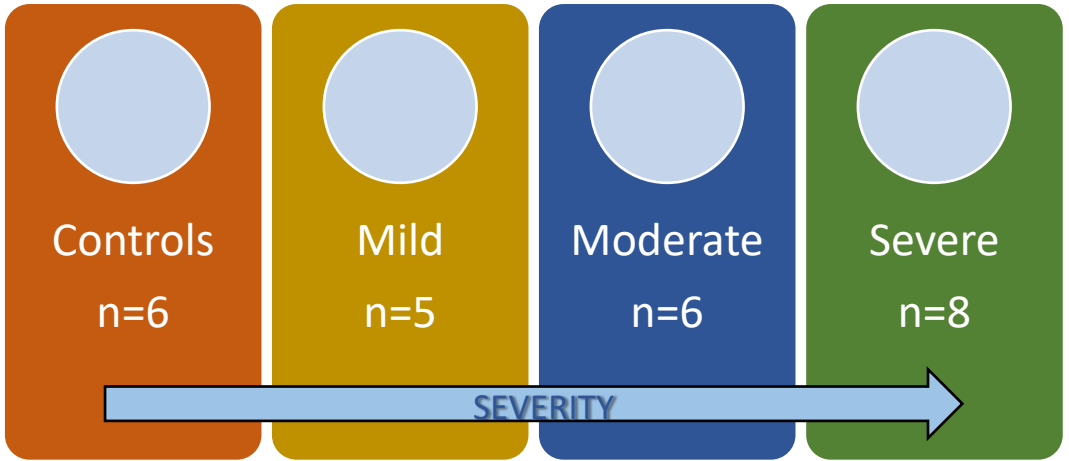


OPLS-DA predicted model



R^2 = 0.99

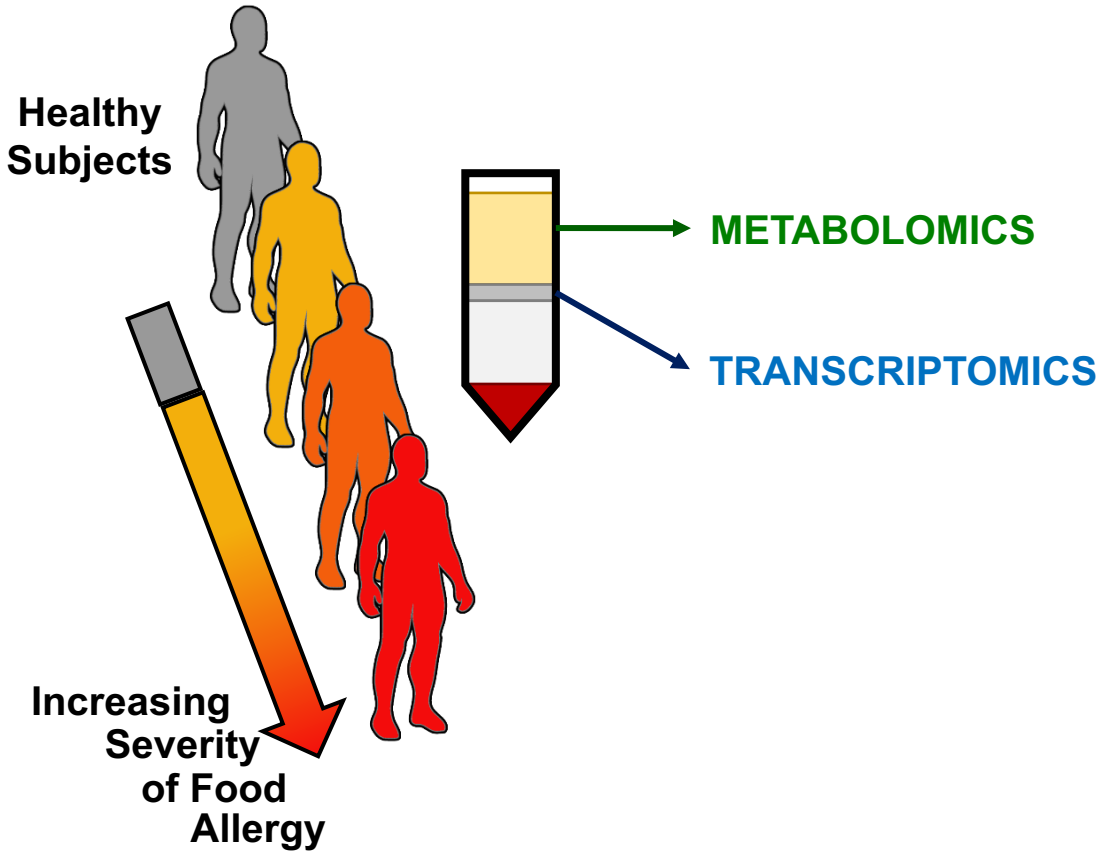
Q^2= 0.75



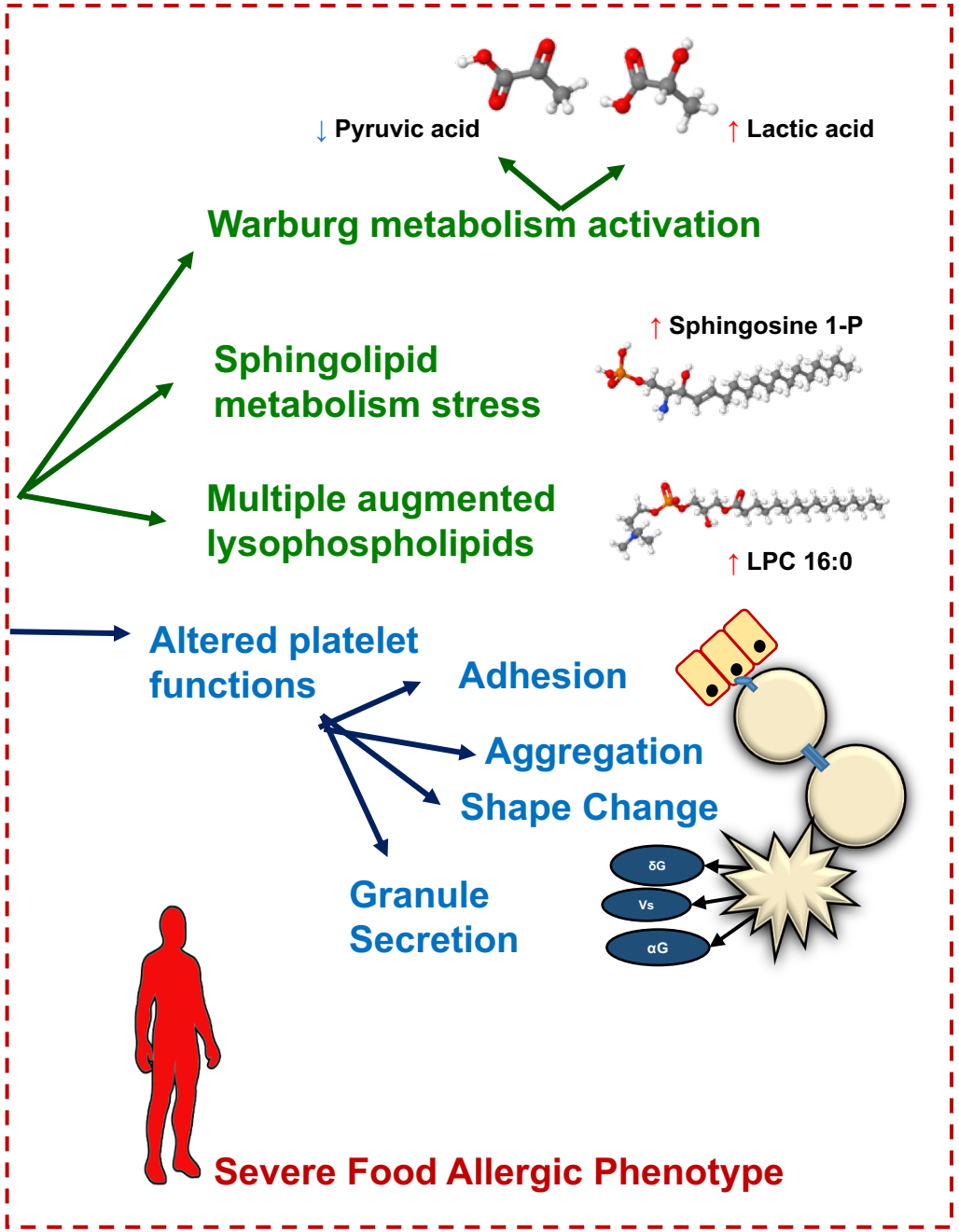
Classes were assigned based on the prediction ecuation:

M29.YVarPS($M29.DA(1))	M29.YPredPS[1]($M29.DA(1))	M29.YVarPS($M29.DA(4))	M29.YPredPS[1]($M29.DA(4))
1	1.00646	0	-0.00645721
1	1.02588	0	-0.0258775
1	1.02419	0	-0.0241894
1	0.904015	0	0.0959849
1	1.0238	0	-0.0238008
1	0.994343	0	0.00565687
--	0.740211	--	0.259789
--	0.260185	--	0.739815
--	0.531348	--	0.468652

SIGNATURES LINKED TO SEVERE PHENOTYPE

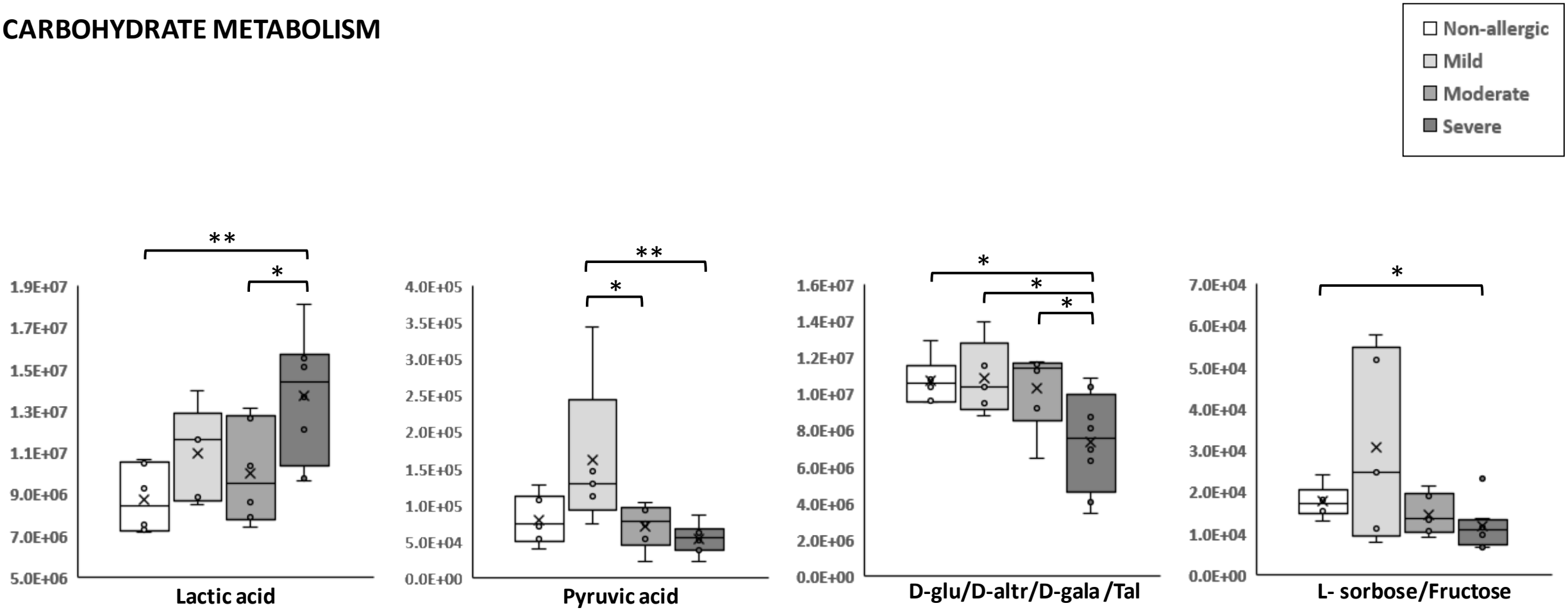


Obeso et al Allergy. 2018



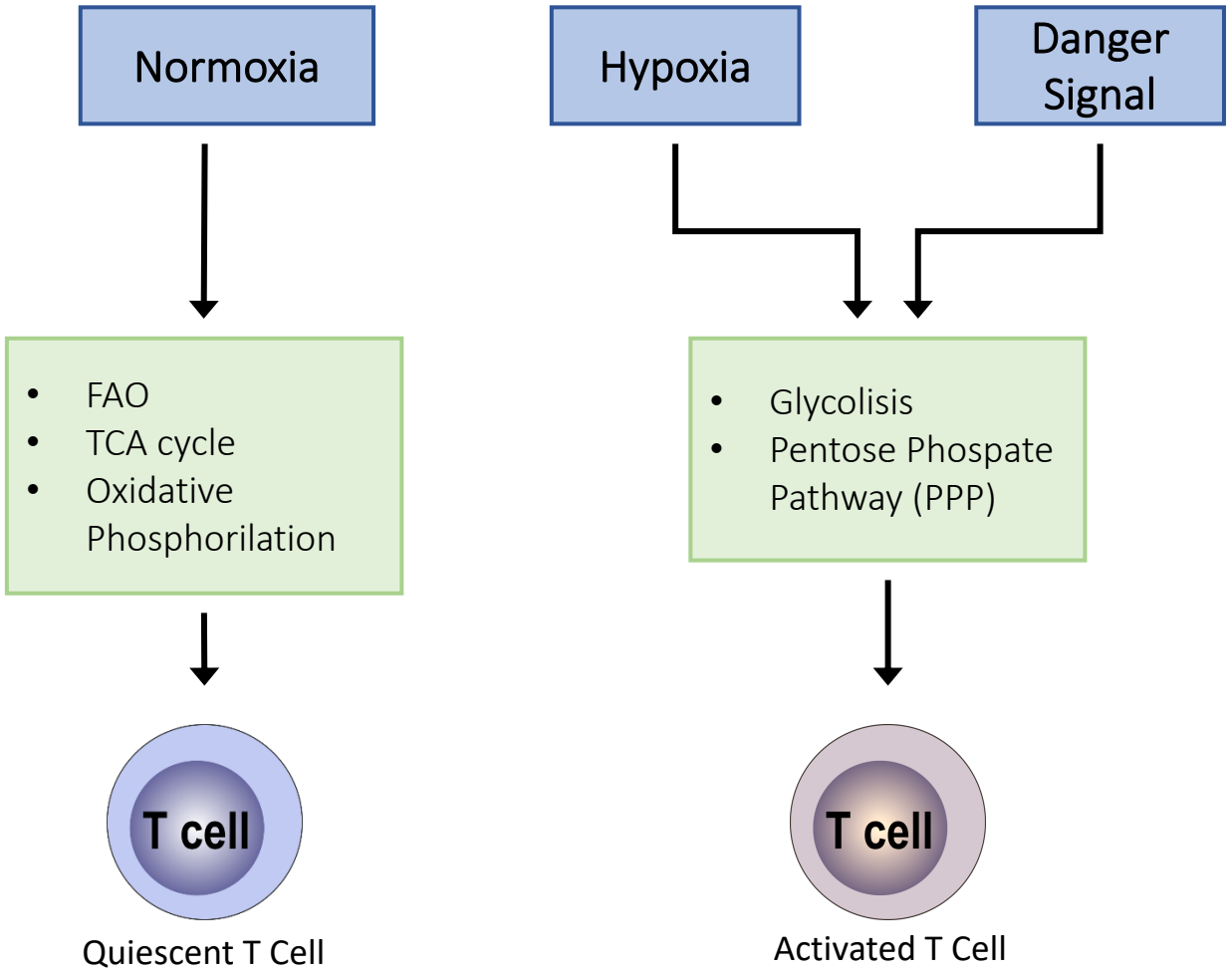
Aerobic Glycolisis (Warburg) metabolism activation

CARBOHYDRATE METABOLISM



Obeso et al Allergy. 2018

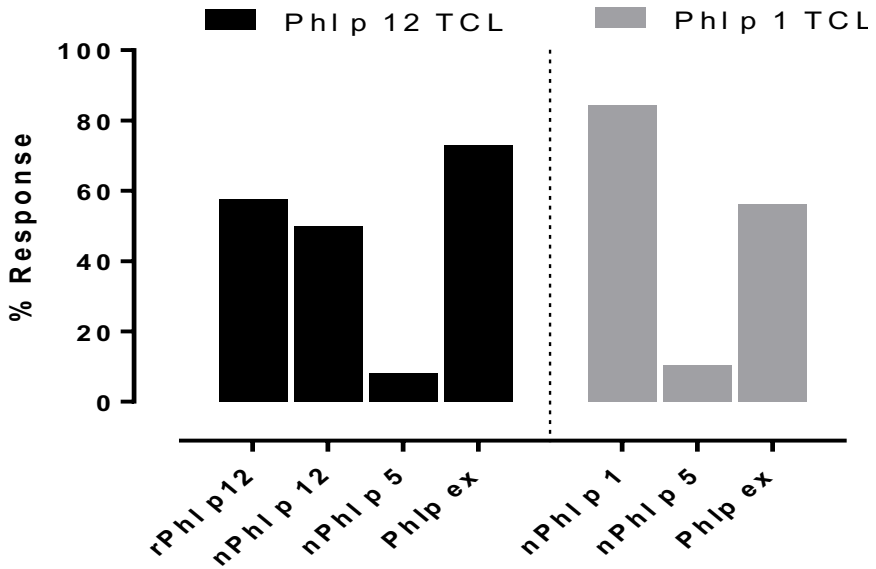
T cell metabolism



Ghesquière, B., *Nature Immunology* (2014)

CEU
Universidad
San Pablo

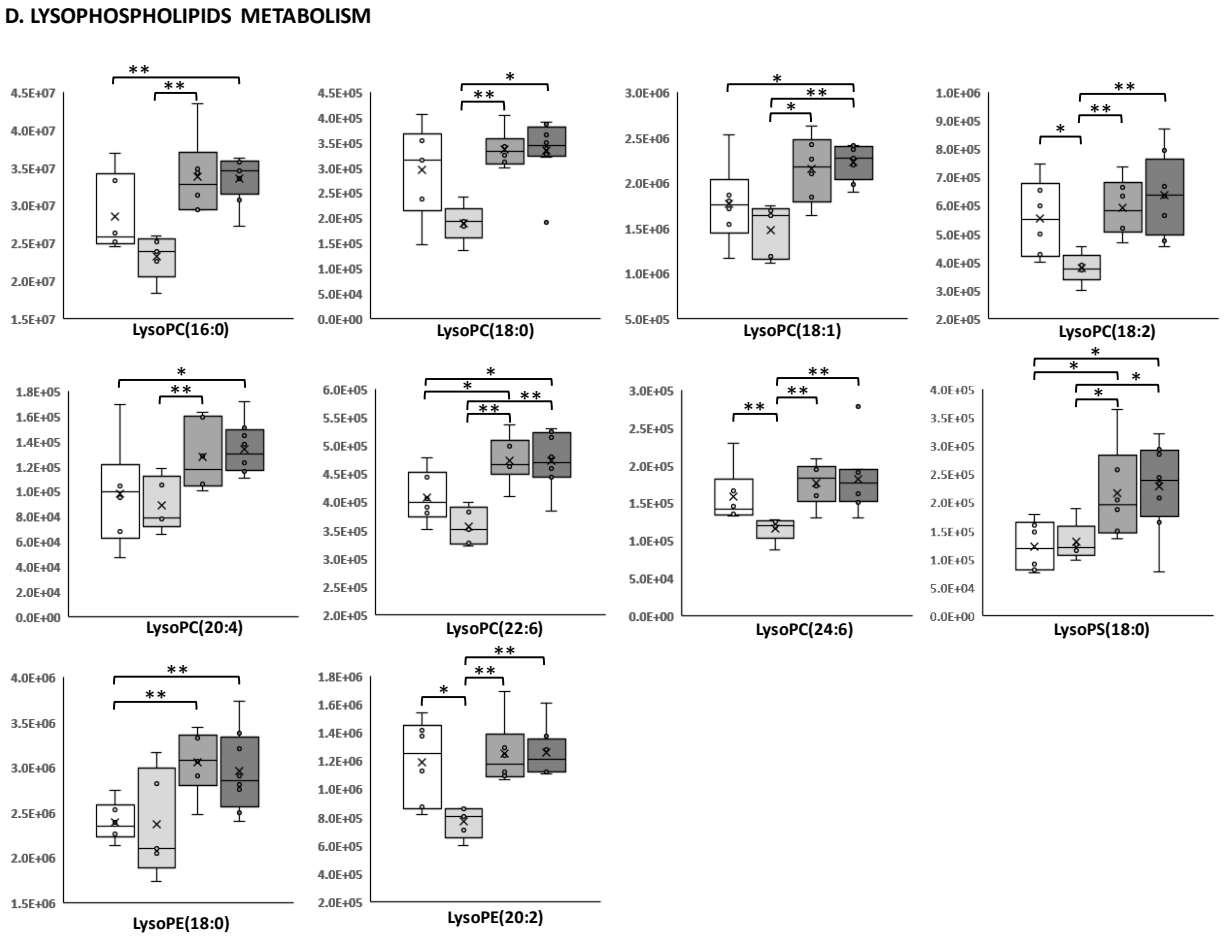
Profilin induces strong T cell proliferation in Spanish sensitized patients compared to Nordic ones.



found in association with PFS. In contrast to the Danish cohort, T cells from the Spanish patients responded vigorously to profilin and were comparable to the major allergen Phl p 1, in terms of both response prevalence and strength. Thus, the T-cell response to Phl

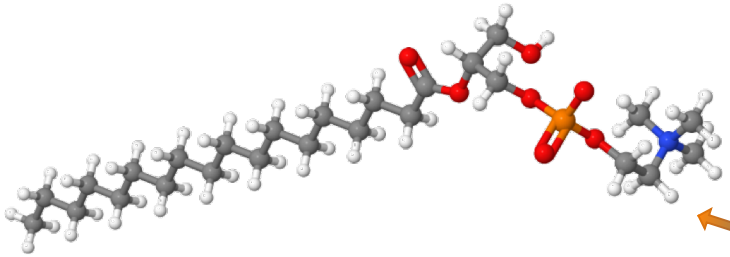
Lund G et al. Allergy 2018

LYSOPHOSPHOLIPIDS SIGNALLING



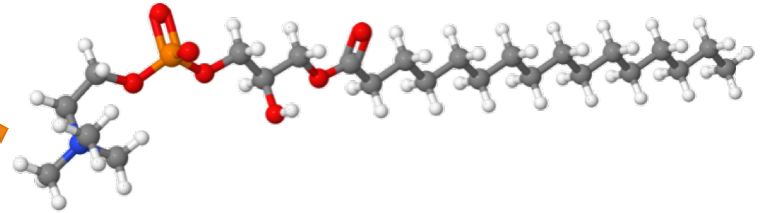
Obeso et al Allergy. 2018

Alteration of LYSOPHOSPHOLIPID metabolism: Lysophosphocholine 16:0 and 18:0 (LPC 16:0 & LPC 18:0)



Products of PLA_2 enzyme

LPC 18:0 | LPC 16:0



Ether-linked phospholipids
PAF synthesis

Eicosanoids source
Arachidonic acid and derivates

Palmitic acid source
Synthesis of Sphingosine

Allergy. 2013;68(5):629-36. doi: 10.1111/all.12110. Epub 2013 Mar 1.

Integrative genetic and metabolite profiling analysis suggests altered phosphatidylcholine metabolism in asthma.

Ried JS[1], Baurecht H, Stückler F, Krumsiek J, Gieger C, Heinrich J, Kabesch M, Prehn C, Peters A, Rodriguez E, Schulz H, Strauch K, Suhre K, Wang-Sattler R, Wichmann HE, Theis FJ, Illig T, Adamski J, Weidinger S.

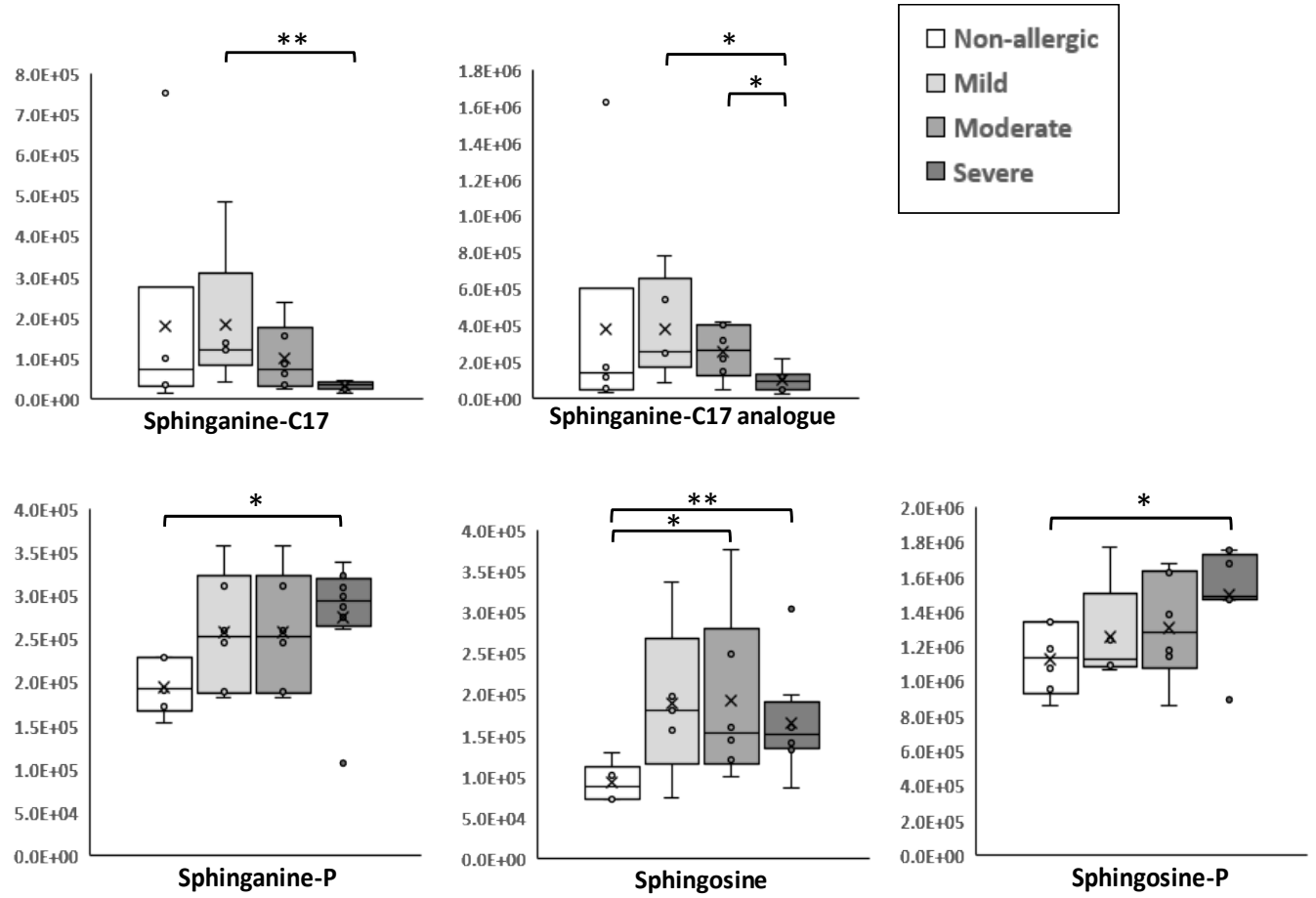
Allergy Asthma Immunol Res. 2014 Jan; 6(1): 61–65. PMCID: PMC3881403
Published online 2013 Dec 5. doi: 10.4168/aair.2014.6.1.61 PMID: 24404395

Bioactive Lysophosphatidylcholine 16:0 and 18:0 Are Elevated in Lungs of Asthmatic Subjects

Mark Yoder,[1] Yan Zhuge,[2] Yang Yuan,[4] Oksana Holian,[2] Sherry Kuo,[1] Richard van Breemen,[4] Larry L. Thomas,[3] and Hazel Lum[1][2]

Author information ► Article notes ► Copyright and License information ► Disclaimer

Sphingolipid metabolism

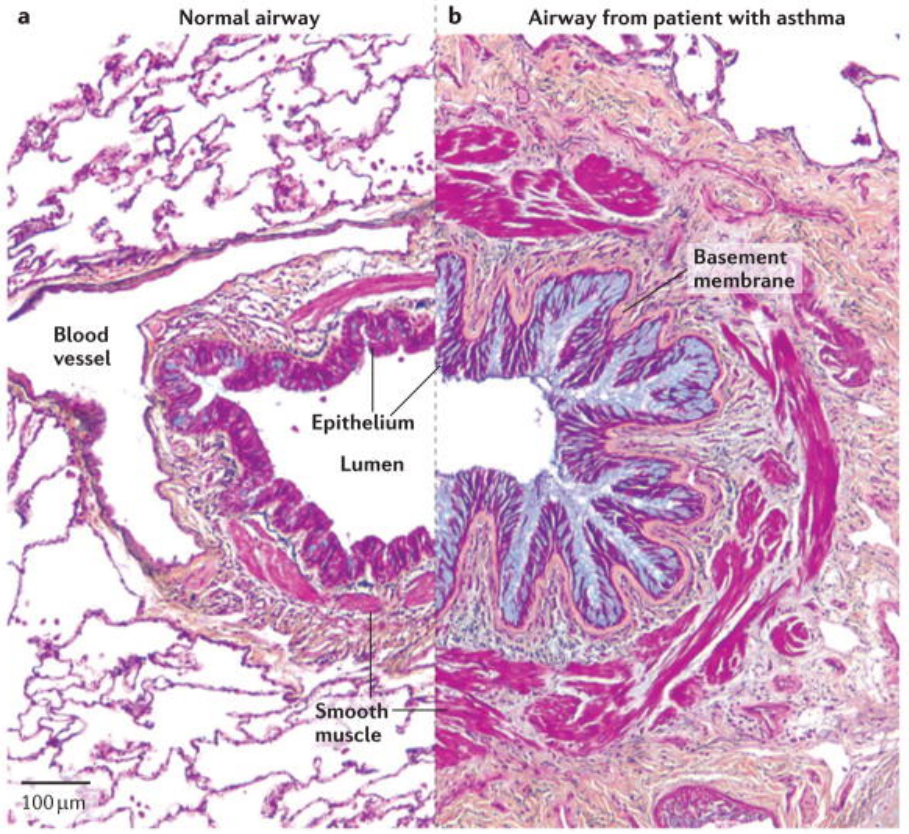


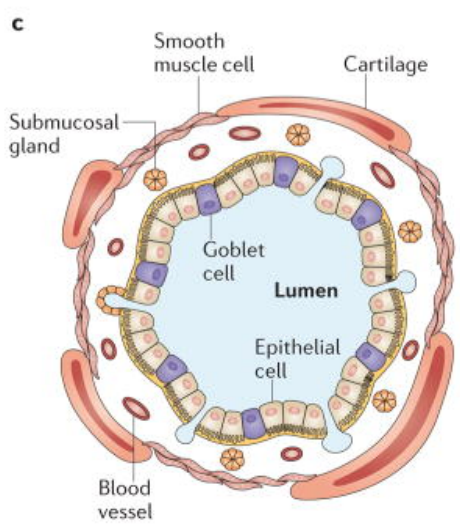
Obeso et al Allergy. 2018

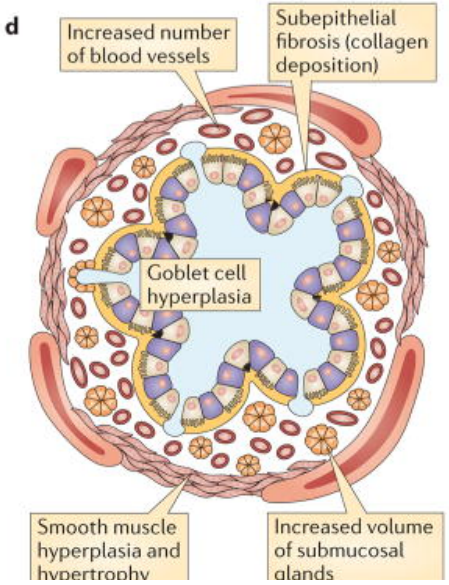
Asthma

- Asthma is a multifactorial, chronic syndrome, which varies over time and involves genetic and environmental interactions.

- <u>It is characterized by:</u>
  - Airway obstruction
  - Mucus hypersecretion
  - Airway hyper-responsiveness
  - Inflammatory cell infiltration
  - Airway epithelial remodeling







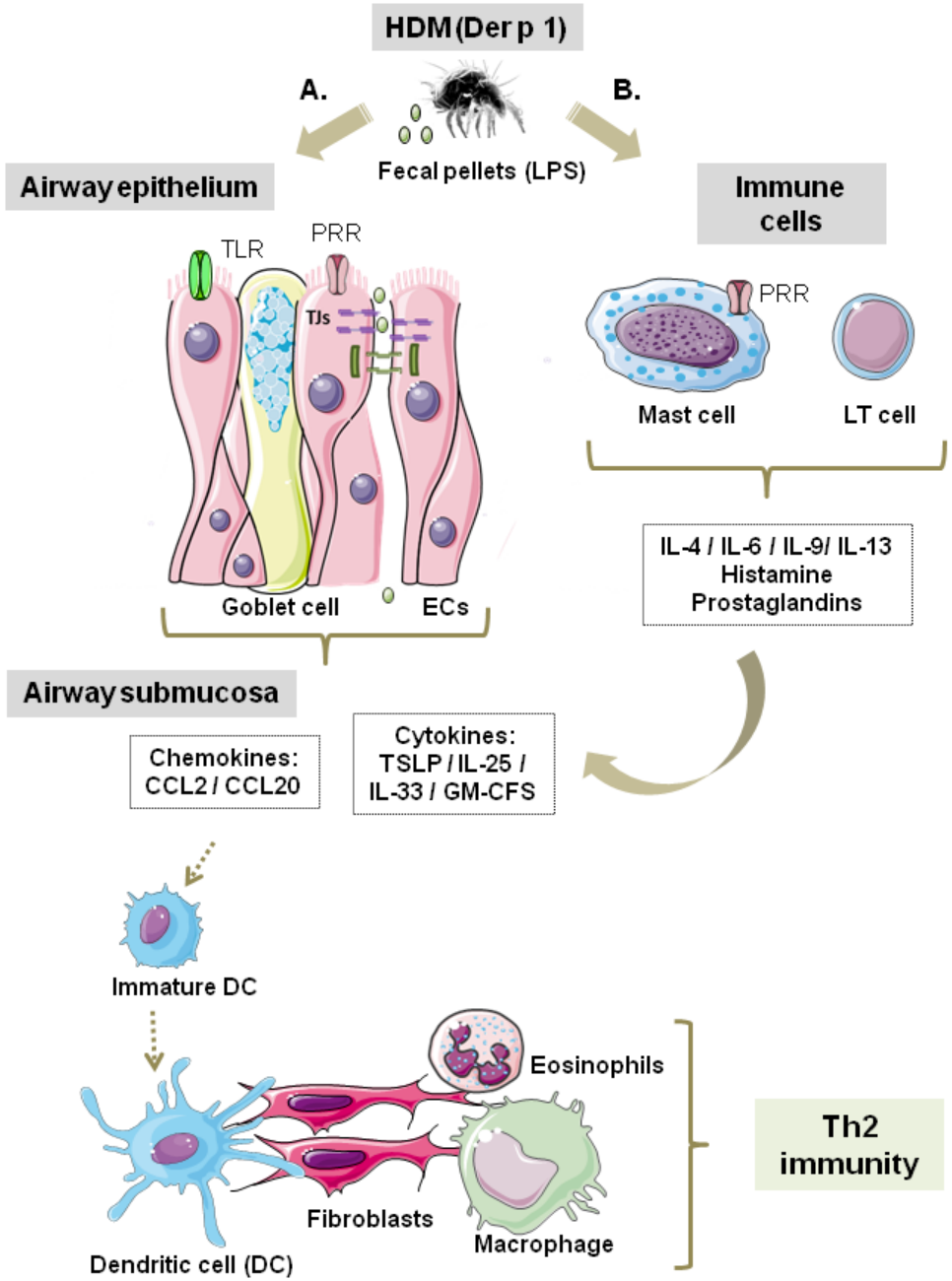
Nat Rev Immunol. 2015 Jan; 15(1): 57–65.

HDM Allergic Asthma

- House dust mites (HDM) are the most prevalent sensitizers associated with allergic asthma.

- The major mite allergens Der p 1 and Der p 2 have the ability to induce asthmatic status through different mechanisms:

  - Der p 1 is a cysteine protease capable of inducing tissue damage and inflammation by cleaving tight-junction
  - Der p 2 displays an allergenic role by mimicking the function of MD-2 in the activation of TLR4.



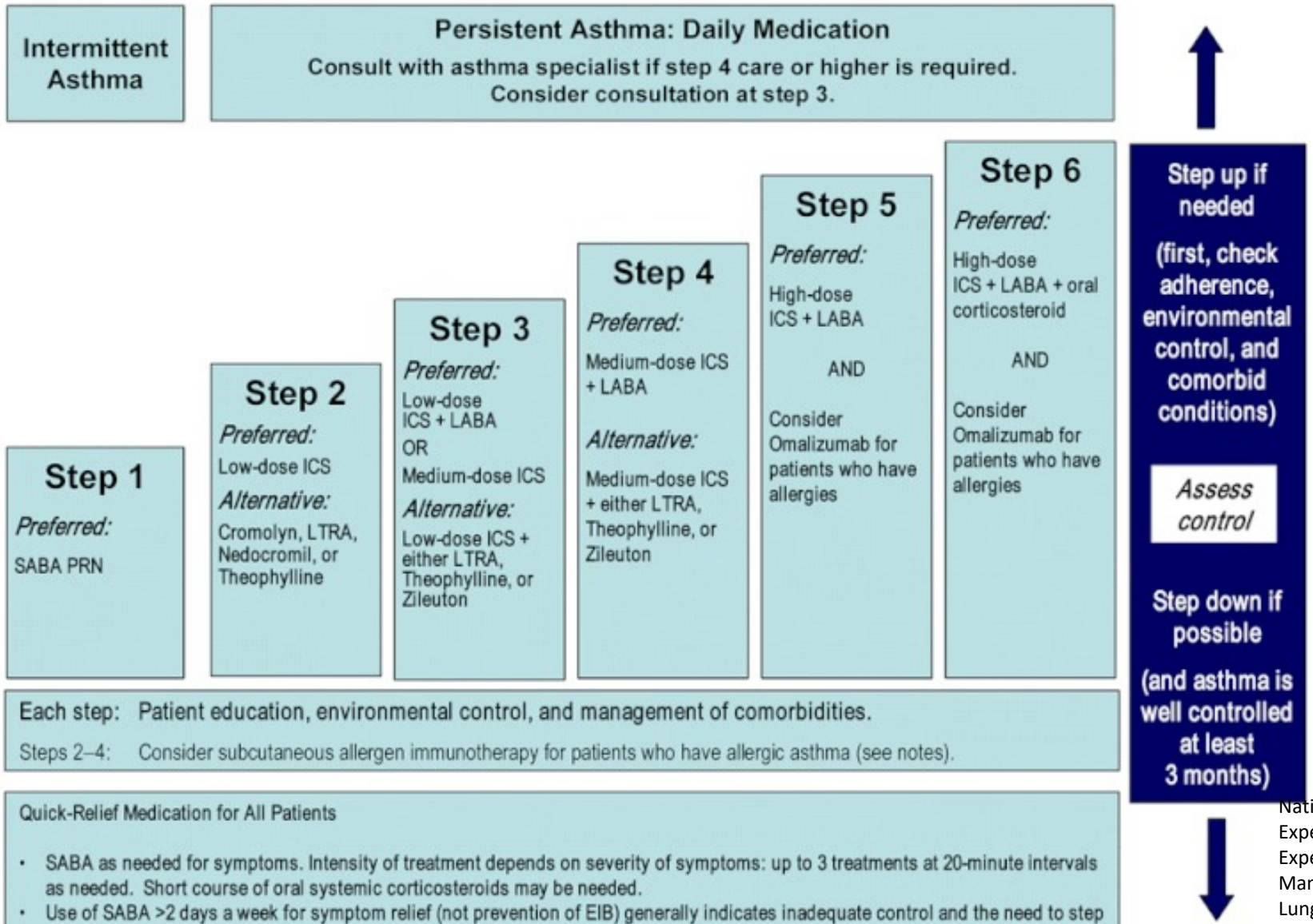
Clin Exp Allergy. 2017 Apr;47(4):442-456.

HDM High exposure area

- Canary Islands present the highest levels of HDM exposure in Spain.
- Tropical temperatures and high humidity.
- Around 80% of allergic patients are sensitized to HDM

Hospital Universitario de
Gran Canaria Doctor Negrín

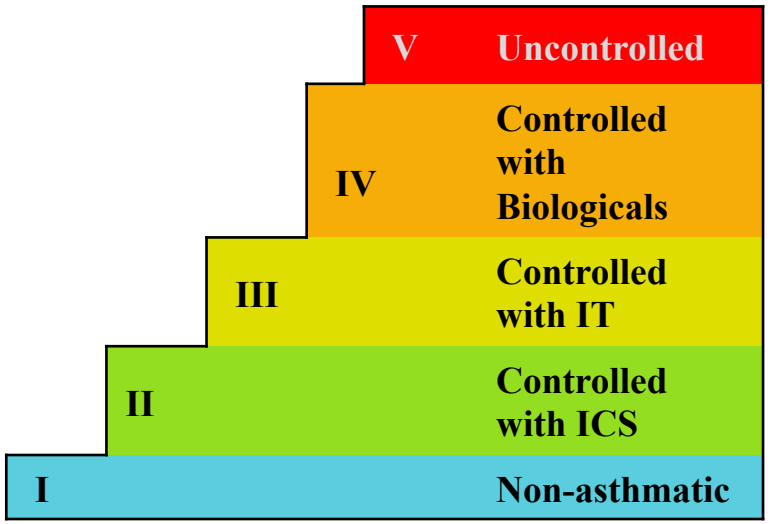
Stepwise approach to managing asthma



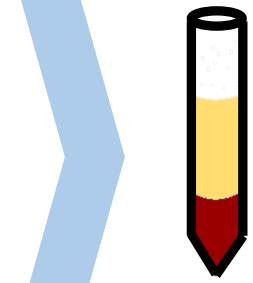
National Asthma Education and Prevention Program, Third Expert Panel on the Diagnosis and Management of Asthma. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. Bethesda (MD): National Heart, Lung, and Blood Institute (US); 2007 Aug. Figure 4-5, [STEPWISE APPROACH FOR MANAGING ASTHMA IN YOUTHS ≥12 YEARS OF AGE AND ADULTS].

Metabolomic fingerprint

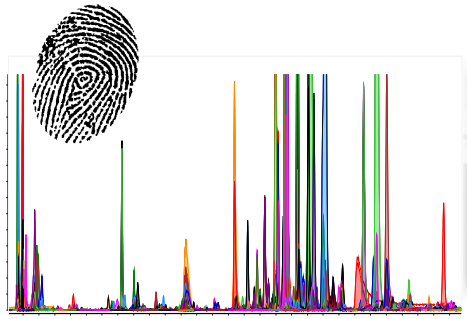
Patient Stratification



Global Metabolomic Profiling

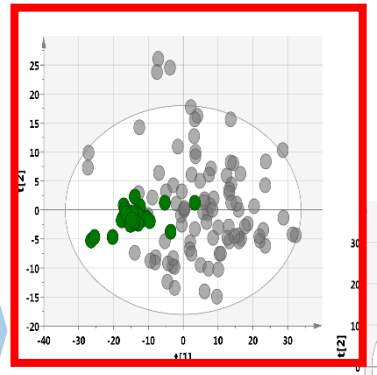


Sample collection & treatment

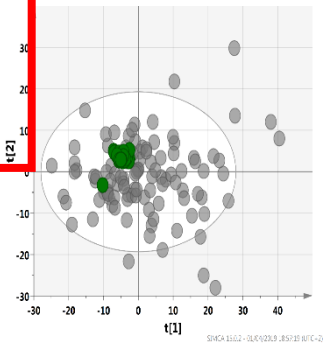


LC-QTOF-MS analysis

Data treatment & Analysis



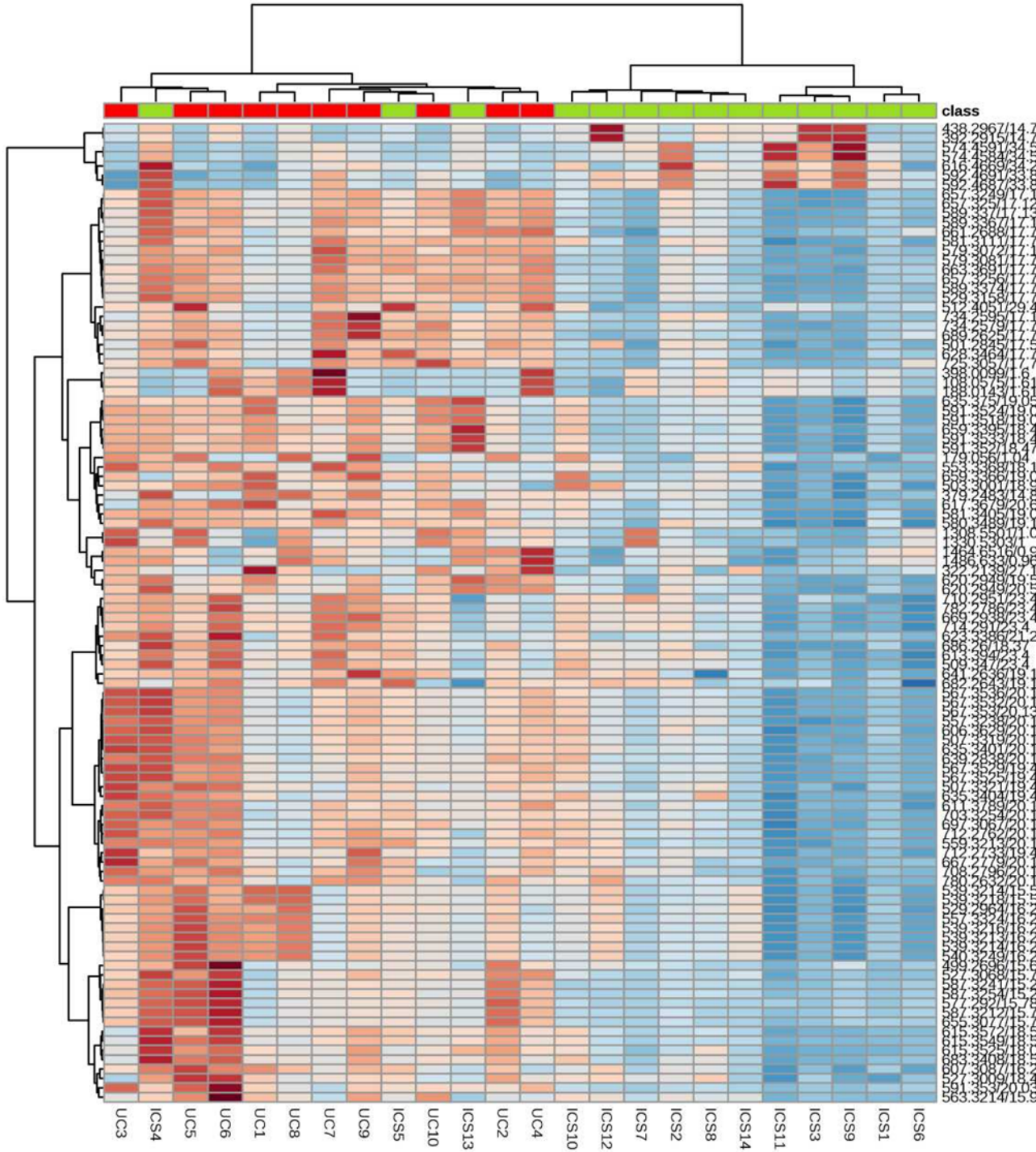
582 signals for LC-MS (ESI -)



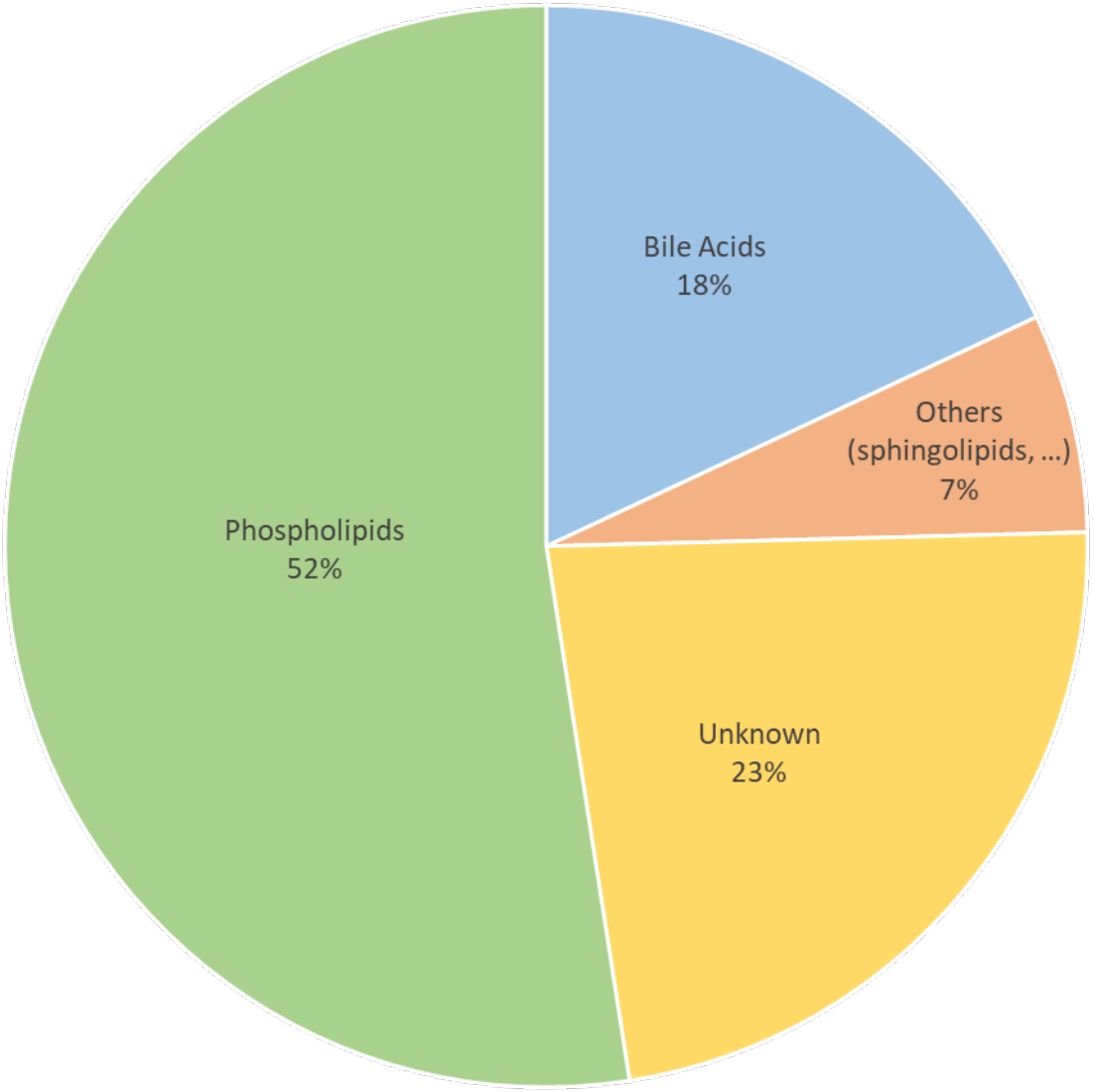
734 signals for LC-MS (ESI +)

Uncontrolled severe allergic asmathic patients show a differential metabolic fingerprint.

			61	IV
		21	84	III
	25	21	171	II
47	41	30	98	I
II	III	IV	V	



Tentative identification of differential metabolites in severe uncontrolled patients



Recommendations

-Working with metabolomics in allergy needs:

-A perfect technical execution (Skilled analytical team)

-Adequate Clinical Models

-Biochemical Interpretation

-Complementary approaches: Other opmics, Immunological data, Immunohistochemoistry…….)

-In summary , a multidisciplinary team

Universidad San Pablo CEU: Facultad de Medicina. IMMA

CEU
Centro de Excelencia en Metabolómica y Bioanálisis CEMBIO
Universidad San Pablo

Maria Escribese, PhD
Coral Barbas, PhD
Tomas Chivato MD,PhD
Juan Rodriguez-Coira MSc
Alma Villaseñor, PhD
David Obeso MSc
Leticia Mera MSc
Elisa Zubeldia MSc
Marina Perez-Gordo, PhD
Cristina Gomez-Casado,PhD
Adoración Martin PhD
Paloma Fernandez, PhD
Javier Moratinos
Ricardo Arroyo
Virginia Garcia
Tomas Barker MSc
Marisa Delgado MSc