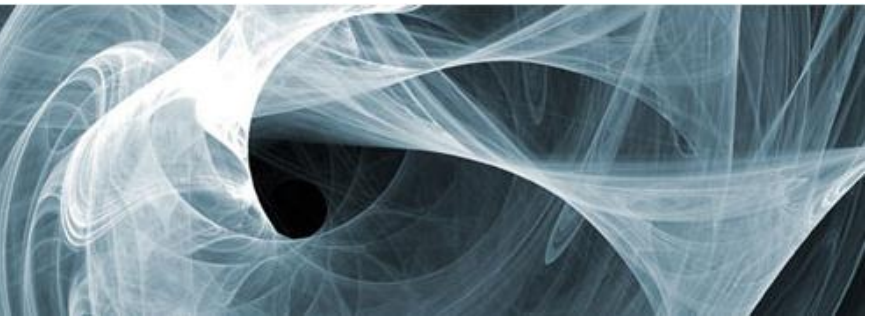


ATTUALITA' DALLA RICERCA: LA METABOLOMICA

Eugenio Baraldi, MD

Women's and Children's Health Department

University of Padova

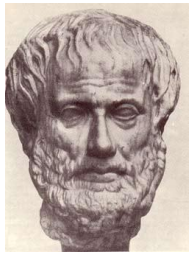
REDUCTIONIST approach

based on dividing biological systems on their constituent parts (genes, RNAs, proteins) and then studying these parts in isolation to finally infer the properties of the system as a whole (e.g. MB).

SYSTEMS BIOLOGY approach
An "holistic" strategy

biological information is not obtained from the detailed properties of each component but from the analysis of the dynamic interaction between several components of a biological system.

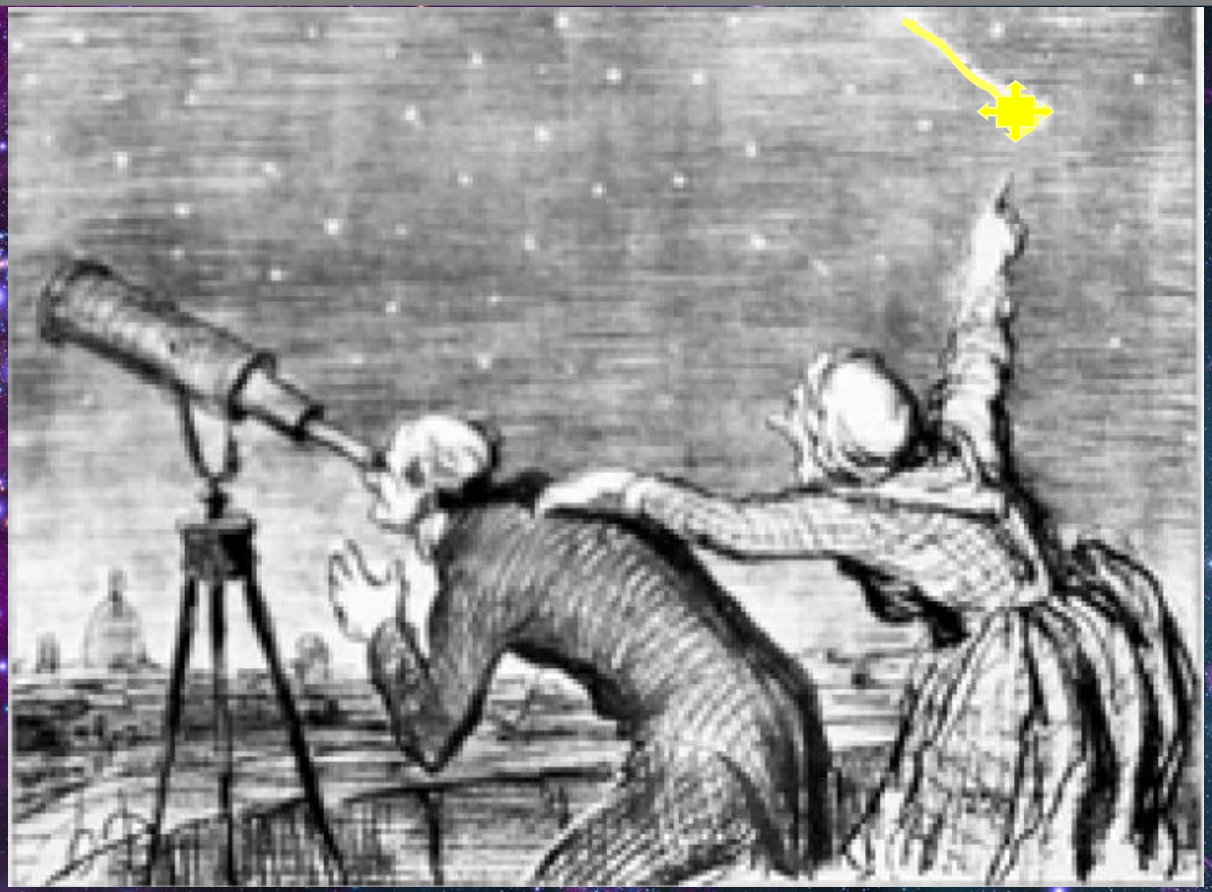
Galileo Galilei
1564-1642



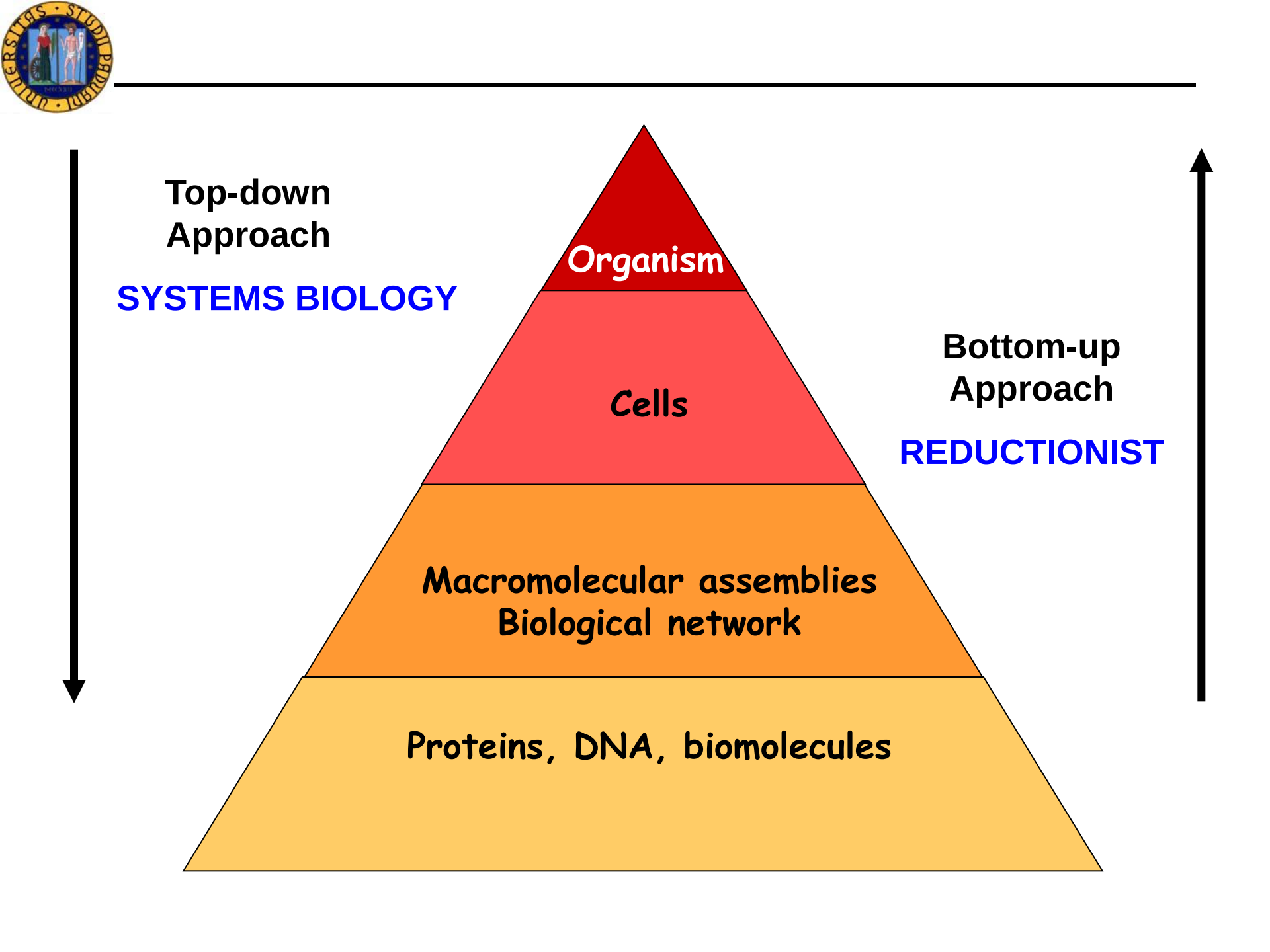
Aristotele

"The whole is more than the sum of its parts"

REDUCTIONISM *versus* SYSTEMS BIOLOGY



INTEGRATION

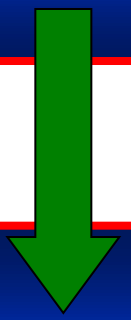

Top-down
Approach
SYSTEMS BIOLOGY
Organism
Cells
Macromolecular assemblies
Biological network
Proteins, DNA, biomolecules
Bottom-up
Approach
REDUCTIONIST

MOVING TOWARD "SYSTEMS MEDICINE"

Traditional strategy

REACTIVE Medicine

"doctors diagnose and treat established disease"



P4 Medicine

PROSPECTIVE Medicine

"centered in preserving health"

Auffray Gen Med 2009

MOVING TOWARD "SYSTEMS MEDICINE" - ASTHMA

Traditional strategy

REACTIVE Medicine

Symptoms and spirometry measure the end result of an established inflammatory process

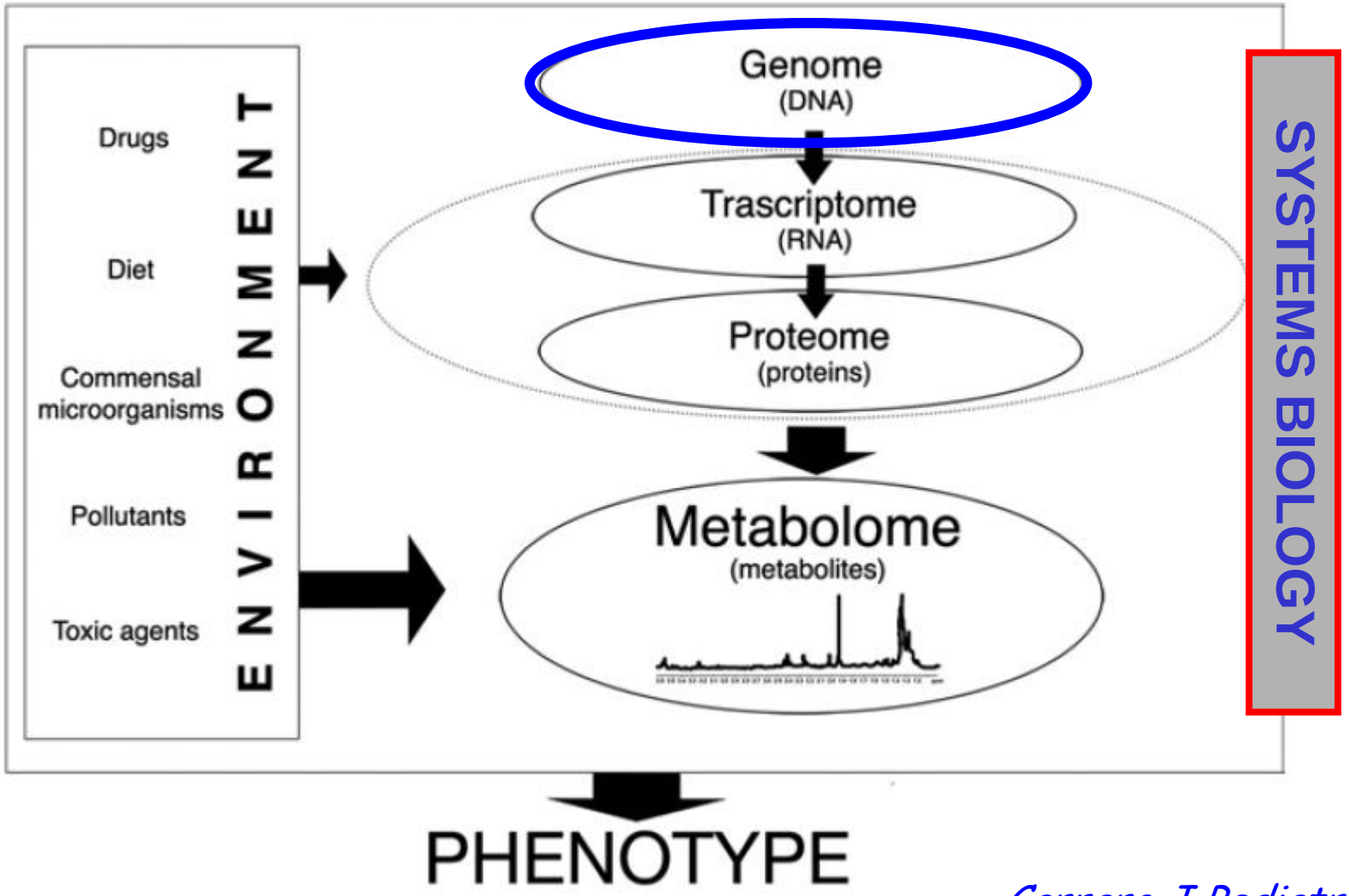
P4 Medicine

PROSPECTIVE Medicine

Biomarkers for early diagnosis before damage has occurred

Auffray Gen Med 2009

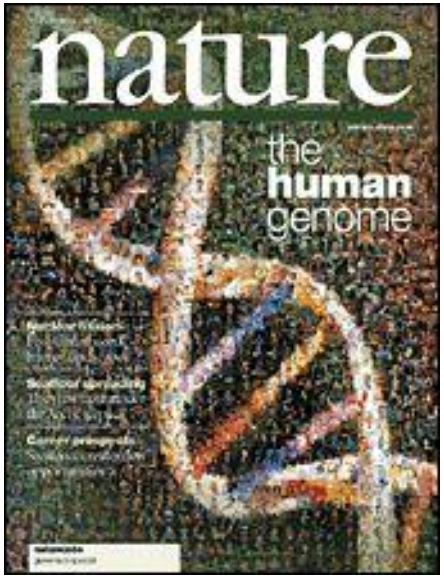
'-OMIC' SCIENCES



Carraro J Pediatr 2009

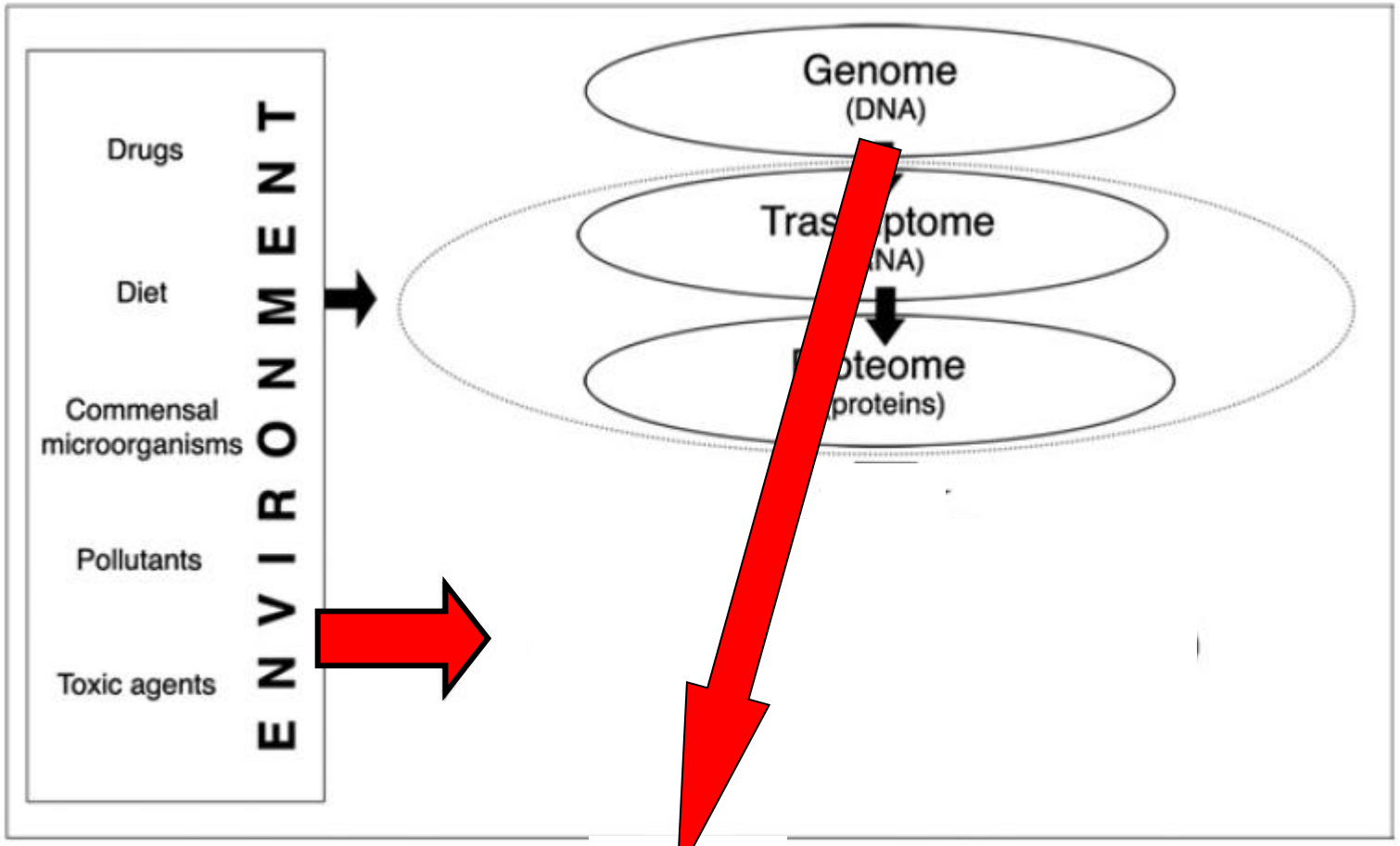
Progetto Genoma Umano

Clinton 26 Giugno 2000
Conferenza alla Casa Bianca



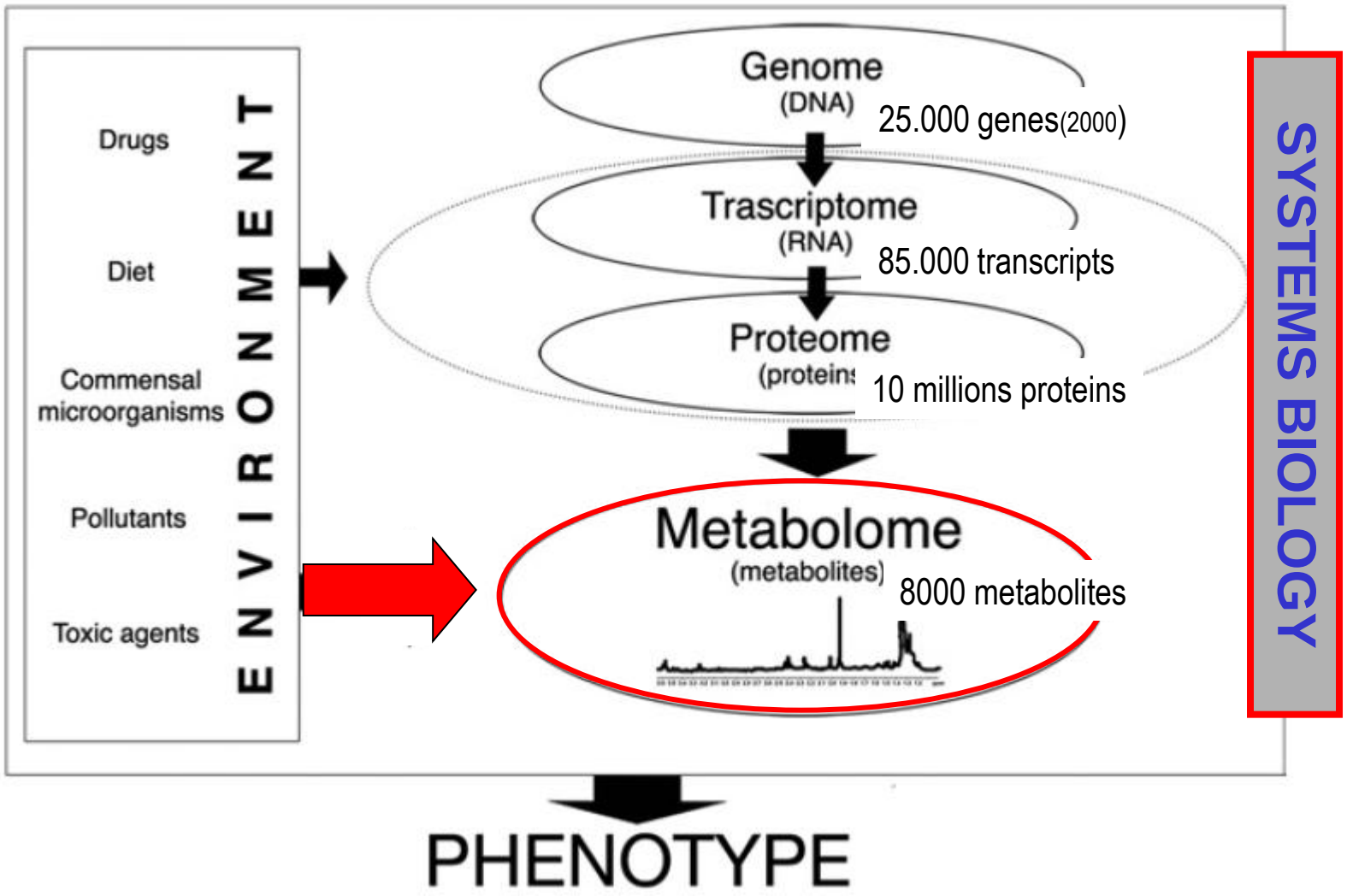
Il Prof Dulbecco, scienziato italiano impegnato nella ricerca sul Genoma Umano. Ha vinto il premo Nobel.

'-OMIC' SCIENCES

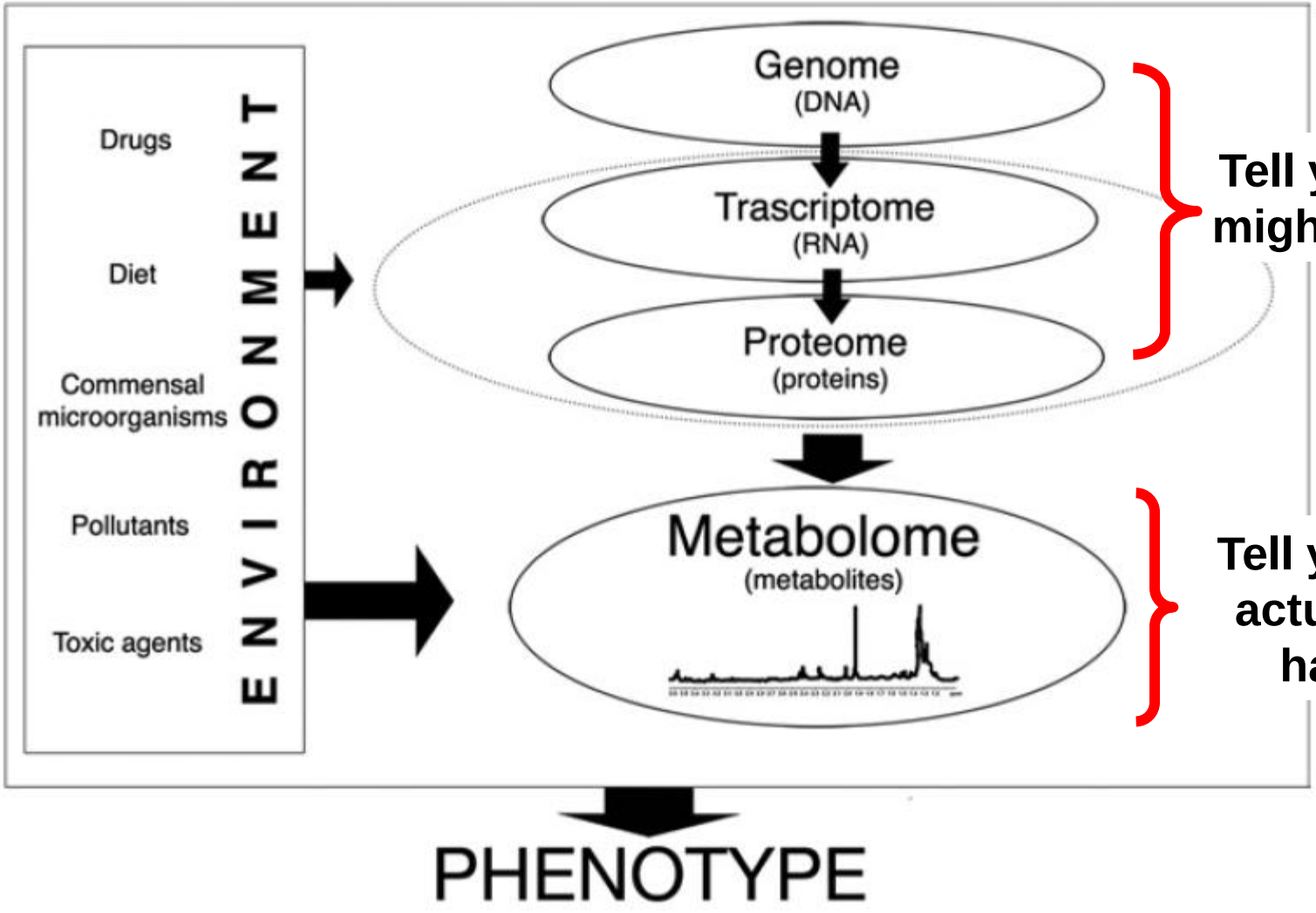


Carraro J Pediatr 2009

'-OMIC' SCIENCES



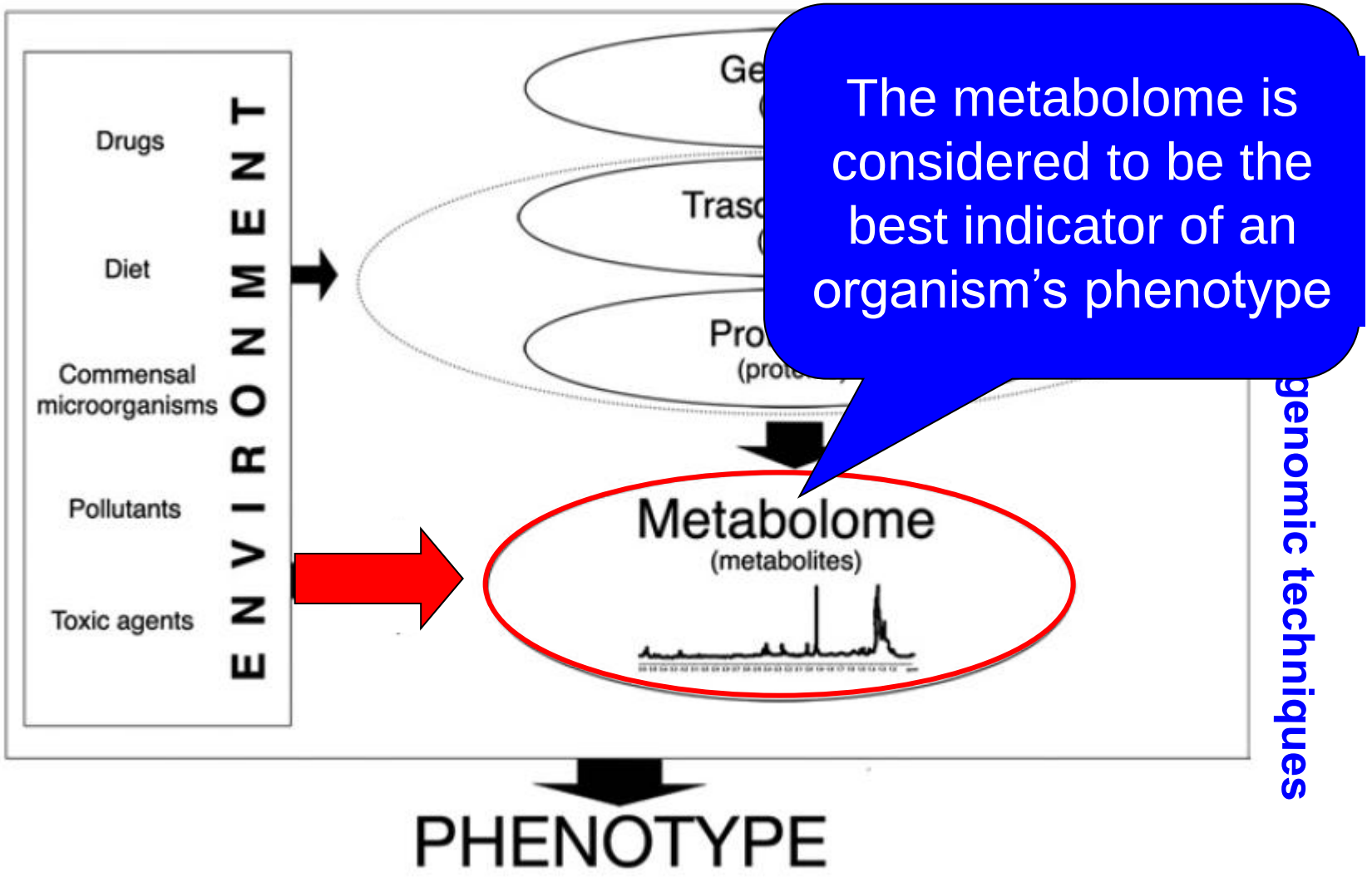
'-OMIC' SCIENCES



Tell you what might happen

Tell you what actually did happen

'-OMIC' SCIENCES

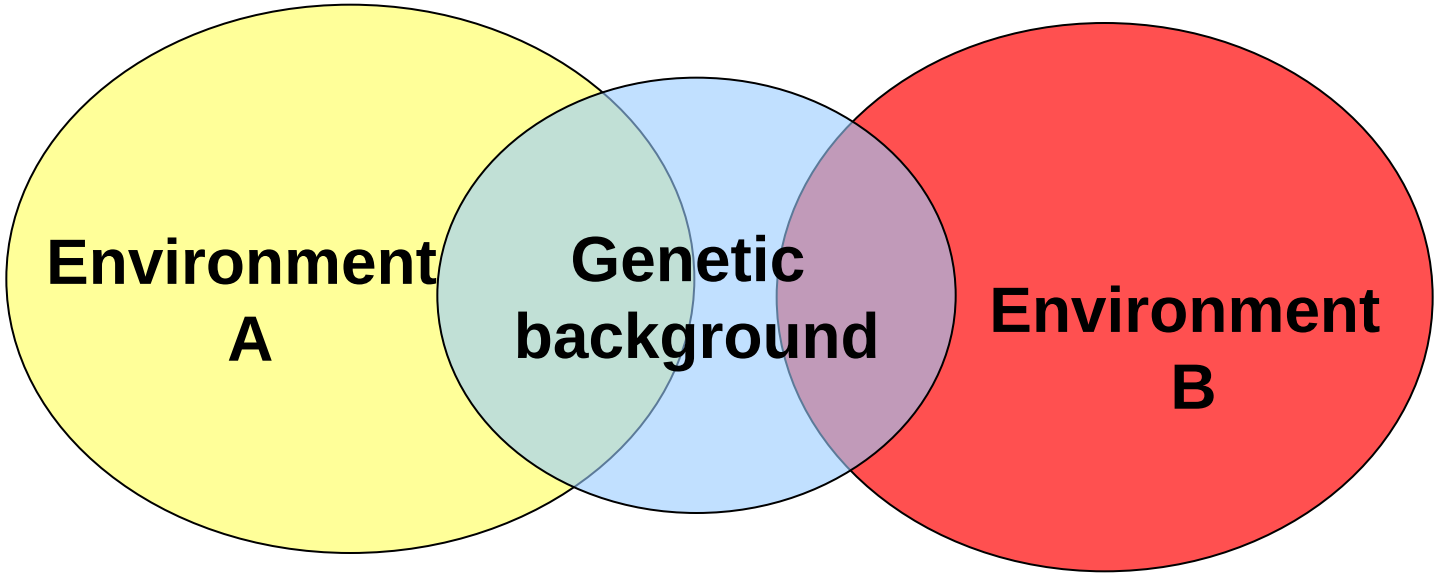


The metabolome is considered to be the best indicator of an organism's phenotype

RELATIONSHIPS BETWEEN GENETIC VARIATIONS AND ENVIRONMENTAL TRIGGERS

Common variants of the fat mass and obesity-associated (FTO) gene* are associated with:

- **higher level of obesity and type 2 diabetes in Western populations**
- **but not in Chinese population !**



*rs9939609, re9930506, re8050136

Li et al Diabetes 2008; 57: 264-268

METABOLOMICS - small (<1kDA) non-peptide metabolites

Metabolomics is the nontargeted measurement of all of the small metabolites that appear in a particular biofluid in a living organism in response to:

- pathological stimuli

- genetic modifications

- enviromental exposures

By measuring and mathematically modelling changes in the levels of products of metabolism found in biological fluids and tissues, matabolomics offers insight into the effect of diet, drugs and disease.

Nicholson & Lindon, Nature 2008
Imperial College-London

The JOURNAL of PEDIATRICS

Metabolomics: A New Frontier for Research in Pediatrics

Silvia Carraro, MD, Giuseppe Giordano, PhD, Fabiano Reniero, PhD, Giorgio Perilongo, MD, and Eugenio Baraldi, MD

SYSTEMS BIOLOGY AND 'OMIC' SCIENCES

METABOLITE FINGERPRINTING

Metabolomics allows the characterization of the metabolite fingerprint of a biological sample without any "a priori" hypothesis allowing hypothesis free profiling of biomarkers, rather than a traditional hypothesis-driven approach.

UNTARGED APPROACH

J Pediatr 2009;154:638-44

Metabolomic analysis

The metabolomic approach can be applied in the analysis of several biofluids:

- ✓ **Urine**
- ✓ **Blood/serum**
- ✓ **Cerebrospinal fluid**
- ✓ **Amniotic fluid**
- ✓ **Bronchoalveolar lavage**
- ✓ **Exhaled breath condensate**

Analytical platforms for metabolomic analysis

NUCLEAR MAGNETIC RESONANCE (NMR) – SPECTROSCOPY

→ detects all hydrogen atoms in metabolites

→Advantages: fast, non destructive

→ Limits: low sensitivity (*micro-molar*)

MASS SPECTROMETRY

→ separation on the basis of mass-to-charge ratio

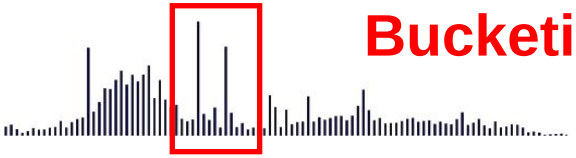
→ combined with separation techniques (LC or GC)

→ higher sensitivity (*nano-molar*)

Integration of NMR and MS

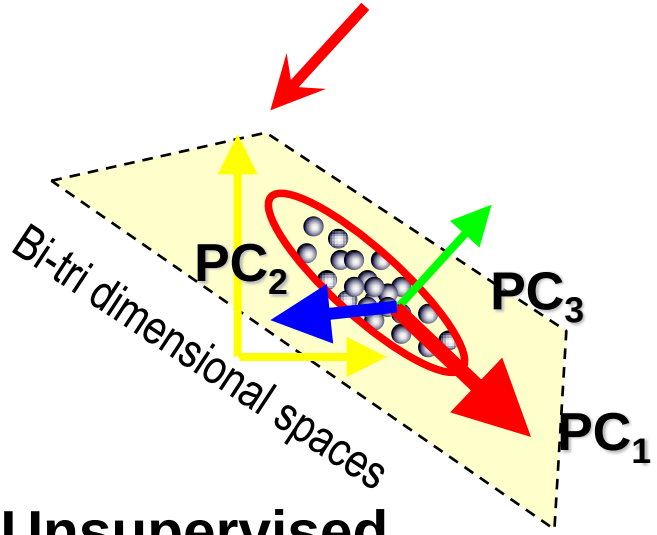
High definition MS

Steps of Metabolomic analysis

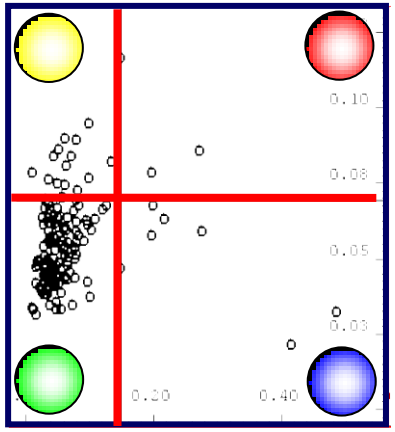


Bucketing

pattern recognition methods **(multivariate statistical analysis)**



Classification



Unsupervised
(PCA: Principal Component a.)
No a priori knowledge of classes

Supervised
(PLS-DA: Partial least squares -discriminant analysis)
Class information is provided

Bioinformatic tools

Structural identification → Human Metabolome Database

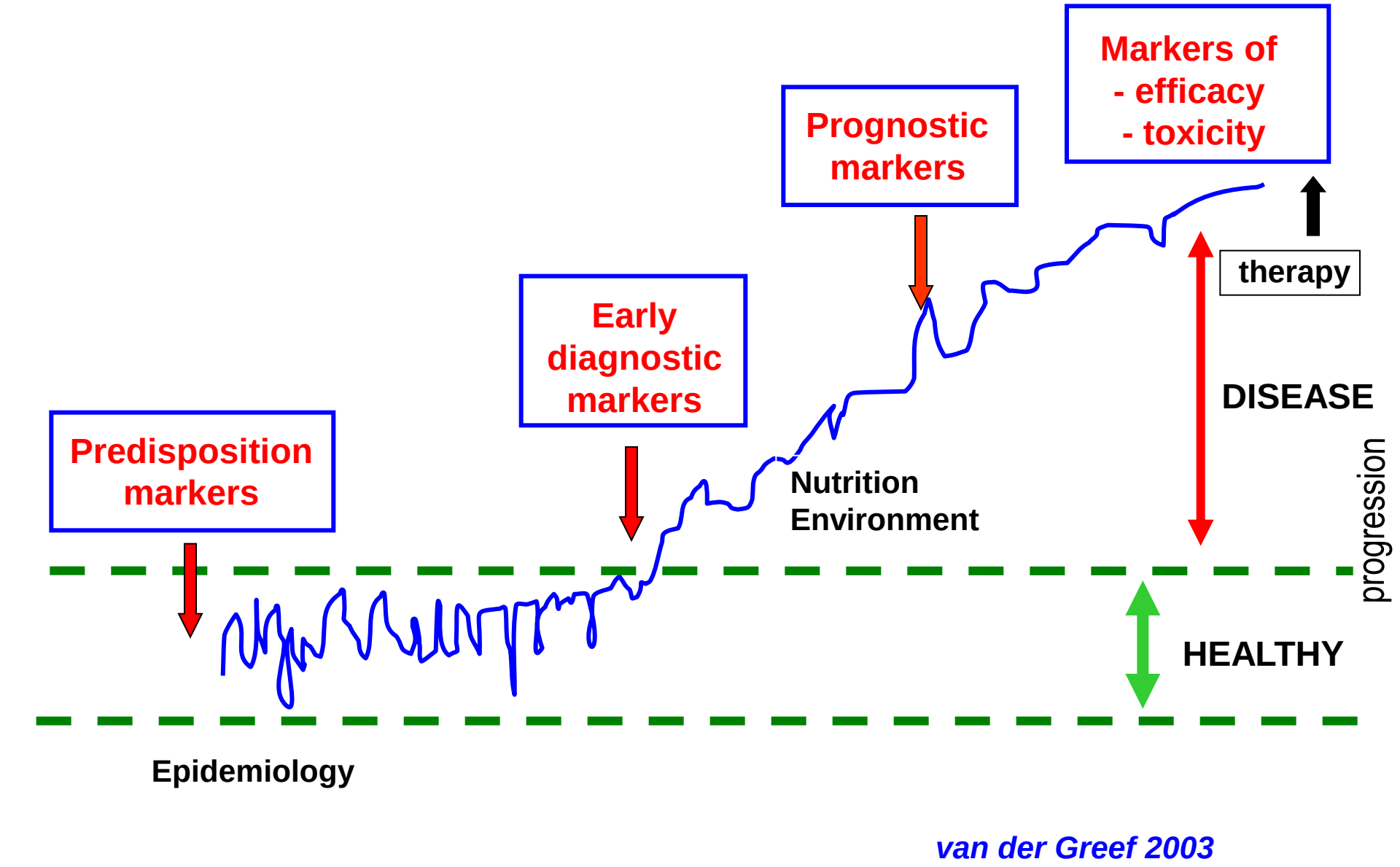
"2020 visions: Metabolomics"

By 2020, personalized health could involve doctors monitoring the metabolic activities of a patient…….

The use of mathematical models to interpret metabolic data obtained using NMR and MS will help us to understand the changing patterns of human disease, and generate new targets for drug or nutritional interventions.

Nicholson, Nature 2010

POTENTIAL ROLE OF BIOMARKERS



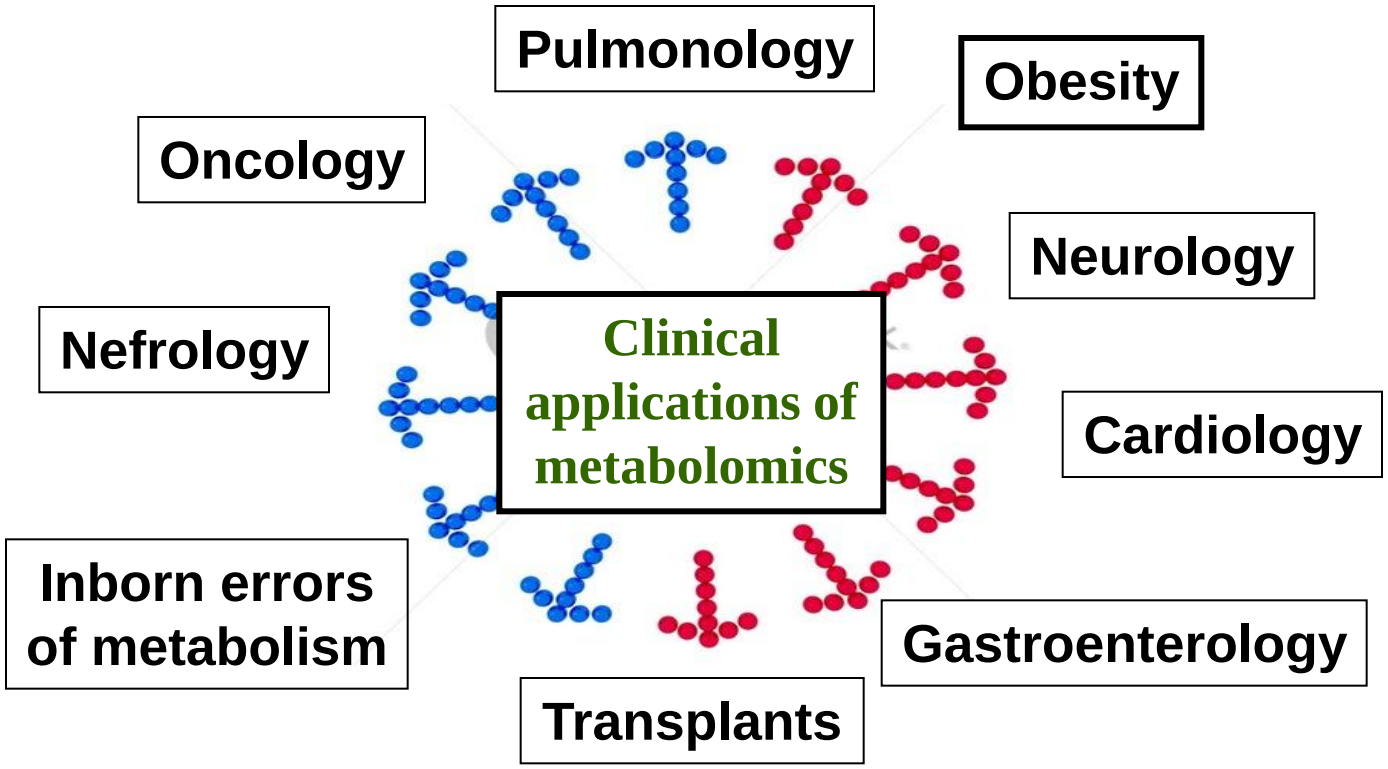
van der Greef 2003

The JOURNAL of PEDIATRICS

Metabolomics: A New Frontier for Research in Pediatrics

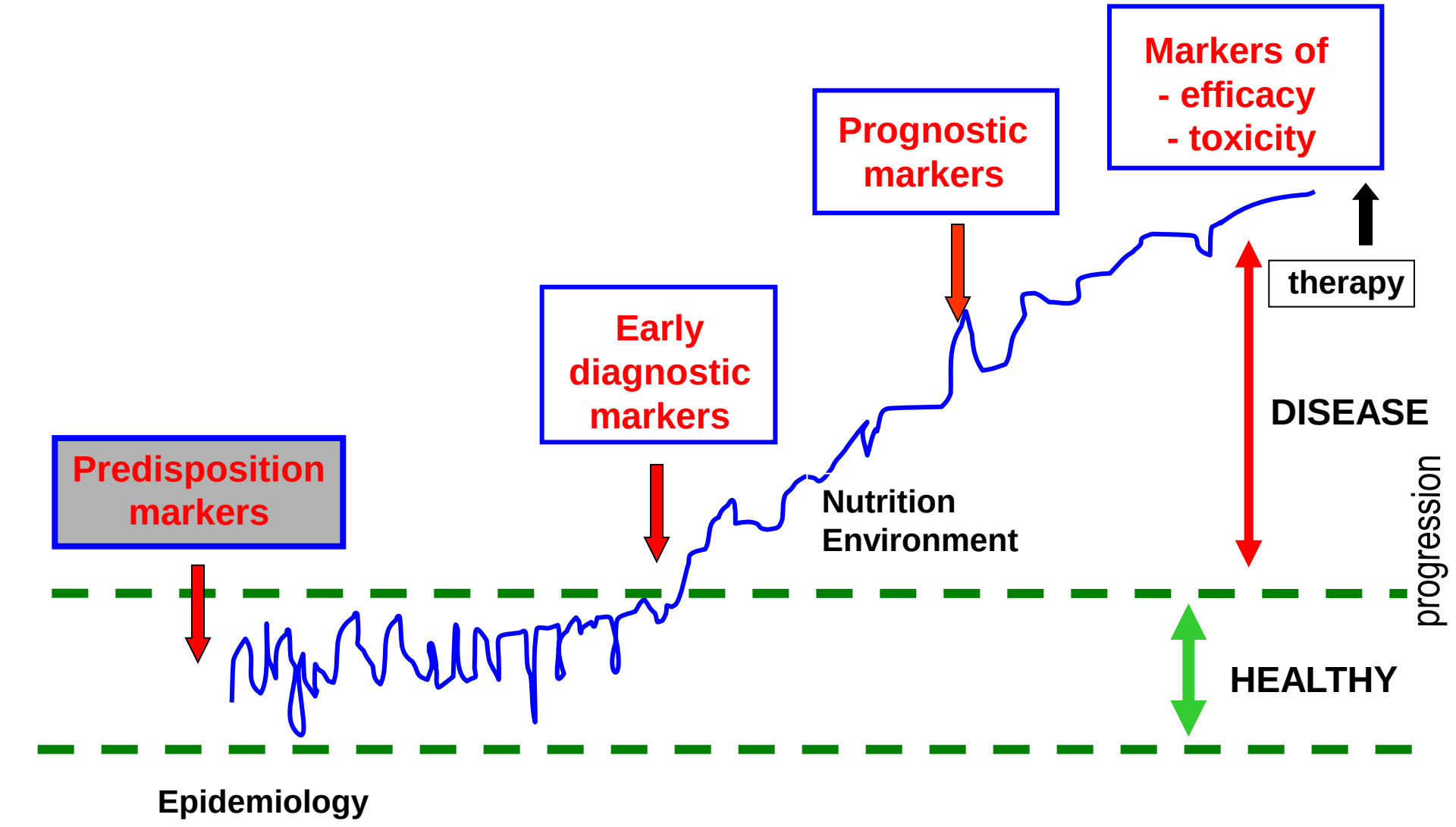
SILVIA CARRARO, MD, GIUSEPPE GIORDANO, PHD, FABIANO RENIERO, PHD, GIORGIO PERILONGO, MD, AND EUGENIO BARALDI, MD

SYSTEMS BIOLOGY AND 'OMIC' SCIENCES



J Pediatr 2009;154:638-44

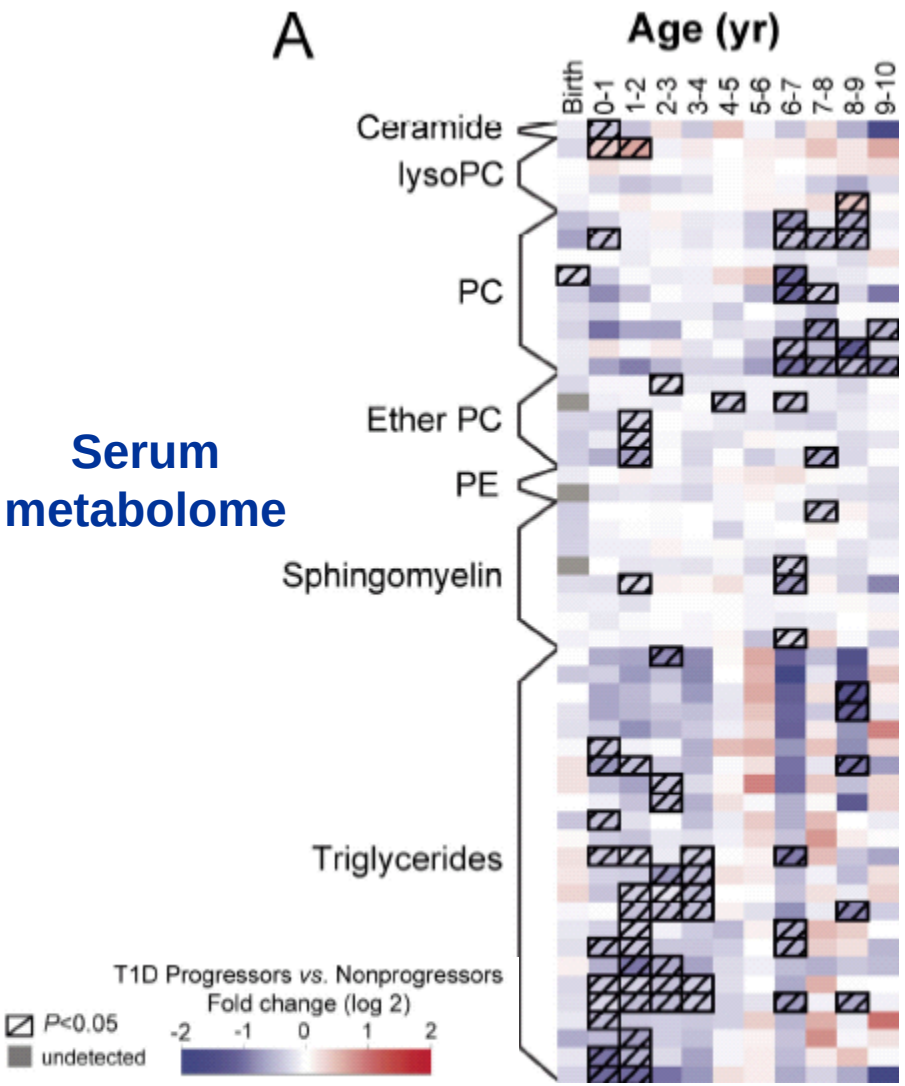
POTENTIAL ROLE OF BIOMARKERS



van der Greef 2003

Metabolomics in early diagnosis of type 1 diabetes

Serum metabolome



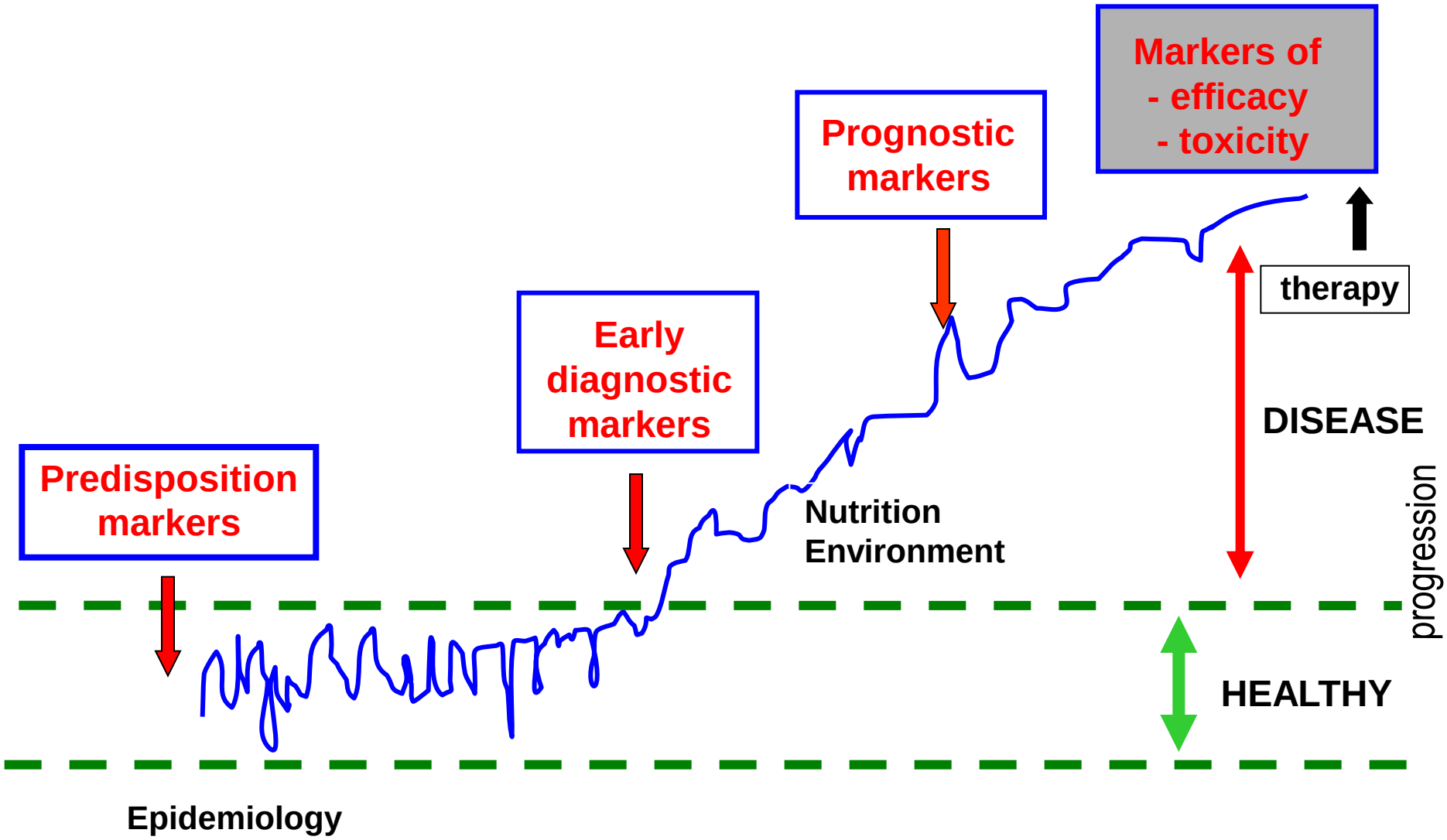
Finland birth cohort study (1994) – 12 yrs newborns with genetic risks (HLA)

Metabolic dysregulation precedes overt autoimmunity (islet autoantibodies) in type 1 diabetes

Immunomodulatory intervention before the onset of the disease?

Oresic, J Exp Med 2009

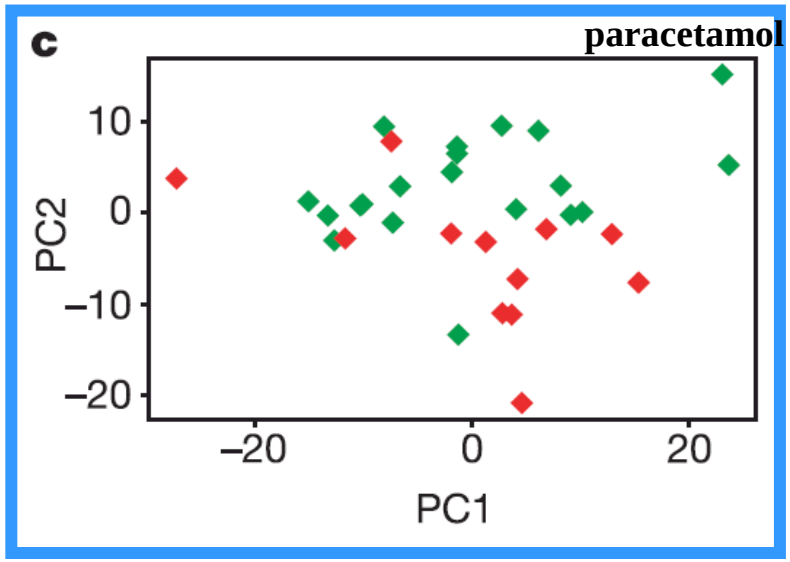
POTENTIAL ROLE OF BIOMARKERS



van der Greef 2003

Metabolomics in prediction of response to therapy

Clayton, Nicholson, Nature 2006



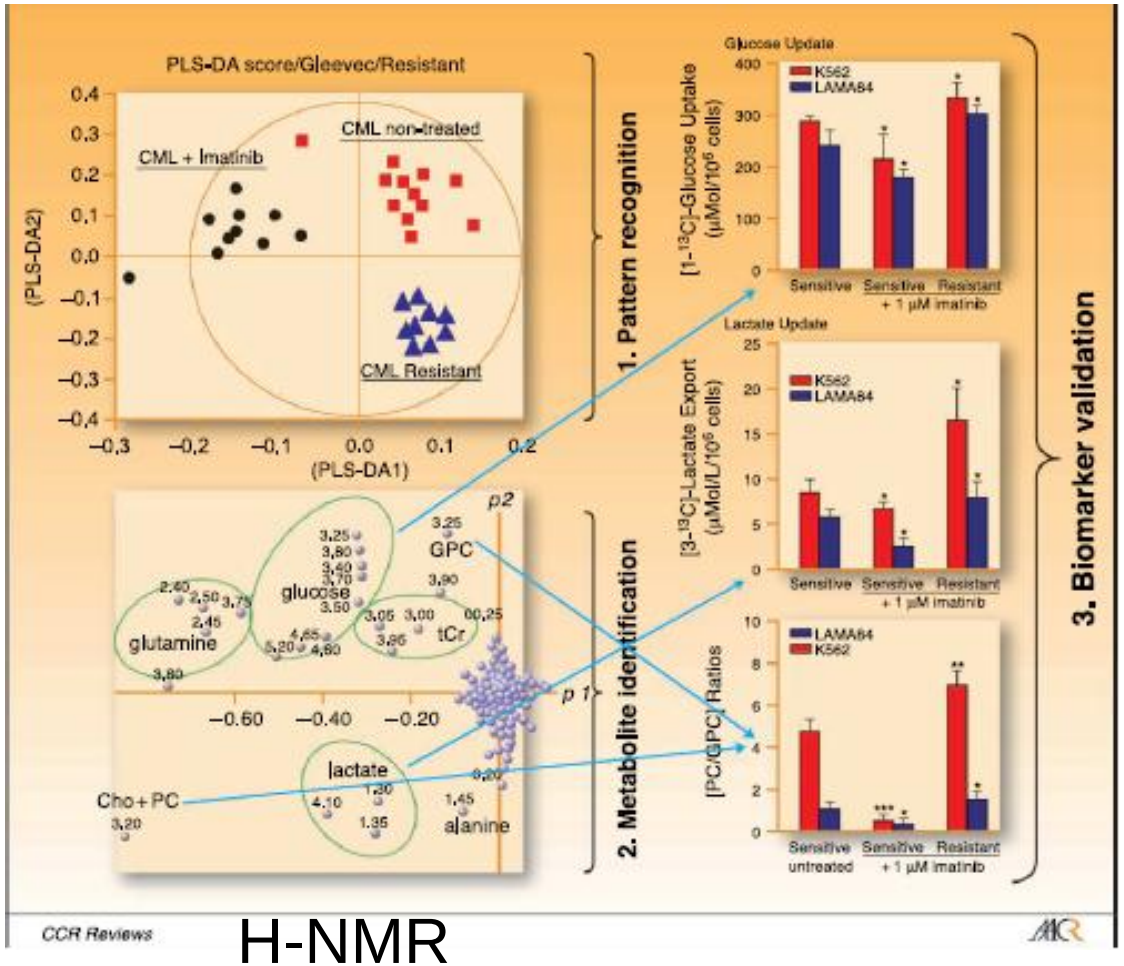
Significant association between <u>pre-dose</u> urinary biochemical profile and post-dose extent of liver damage sustained after paracetamol administration.

pharmacometabolomics

To predict the effectiveness and/or toxicity of a drug on the basis of the individual pretreatment metabolomic characteristics

Clinical Applications of Metabolomics in Oncology: A Review

Jennifer L. Spratlin,[1] Natalie J. Serkova,[2] and S. Gail Eckhardt[1]



H-NMR

IMATINIBIB treatment in chronic myeloid leukemia

Markers validation to distinguish metabolic signatures of responsiveness and resistance to Imatinibib

Clin Cancer Res 2009

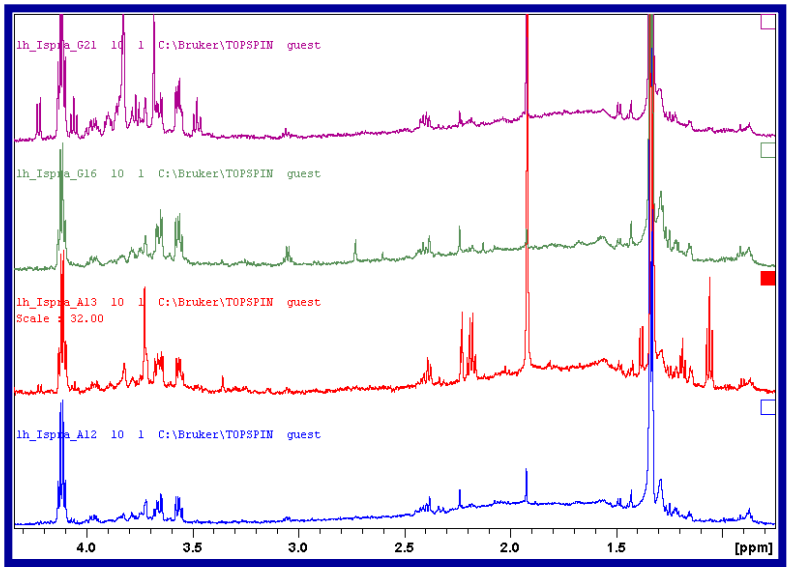
Metabolomic analysis

The metabolomic approach can be applied in the analysis of several biofluids:

- ✓ **Urine**
- ✓ **Blood/serum**
- ✓ **Cerebrospinal fluid**
- ✓ **Bronchoalveolar lavage**
- ✓ **Exhaled breath condensate**

BACKGROUND

"Breathomics": application of the metabolomics approach to exhaled breath condensate



<u>Metabolomics</u>: biofluid analysis based on spectroscopic techniques such as mass spectrometry and NMR based spectroscopy

<u>EBC</u>: a biofluid non invasively collected, which is believed to mirror the composition of airway lining fluid

Metabolomics analysis of EBC: “breathomics”

Study subjects:

- 25 asthmatic children with well-controlled asthma
- 11 healthy controls

Procedures:

- FE_{NO} measurement
- spirometry
- EBC collection and storage at –80 °C

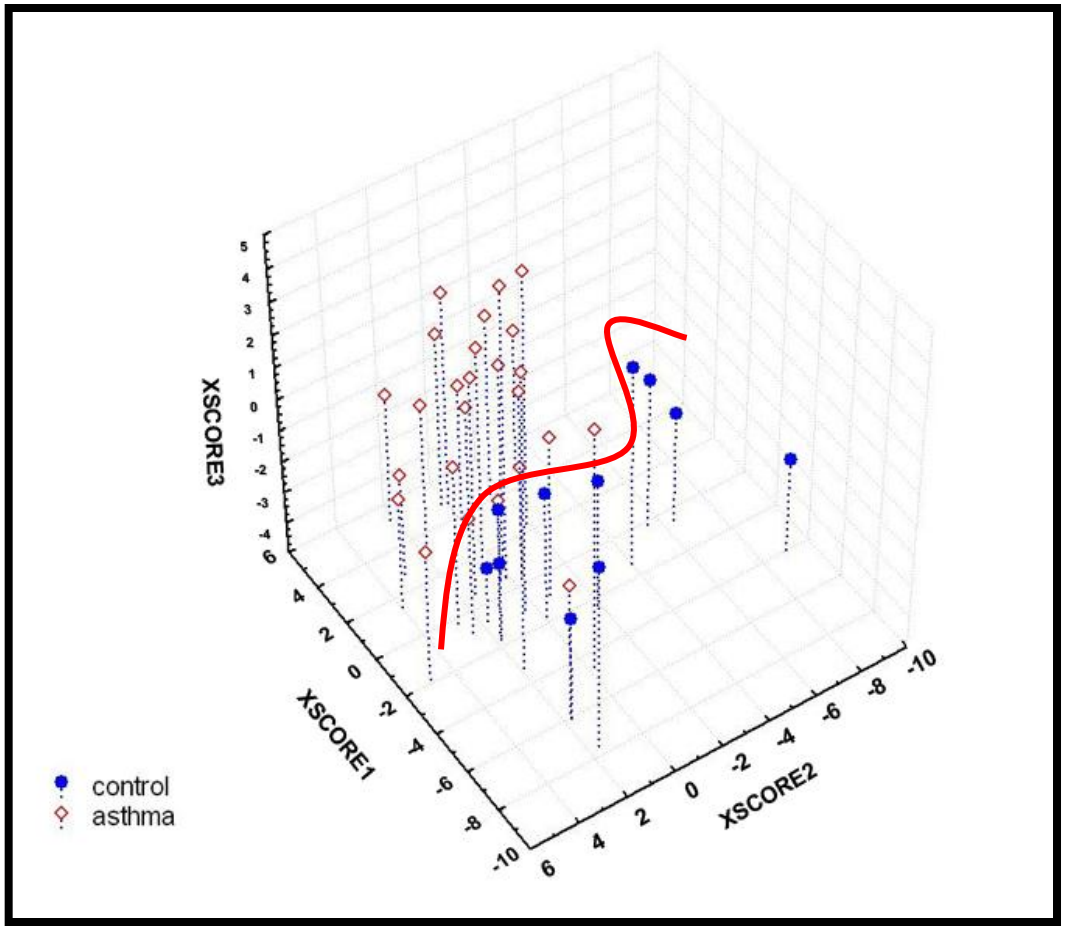
Metabolomic analysis:

- NMR analysis
  - 600 MHz Bruker Biospin spectrometer
  - 3 mm cryogenic probe at 300 °K

Carraro et al AJRCCM 2007

METABOLOMIC analysis of Exhaled Breath Condensate

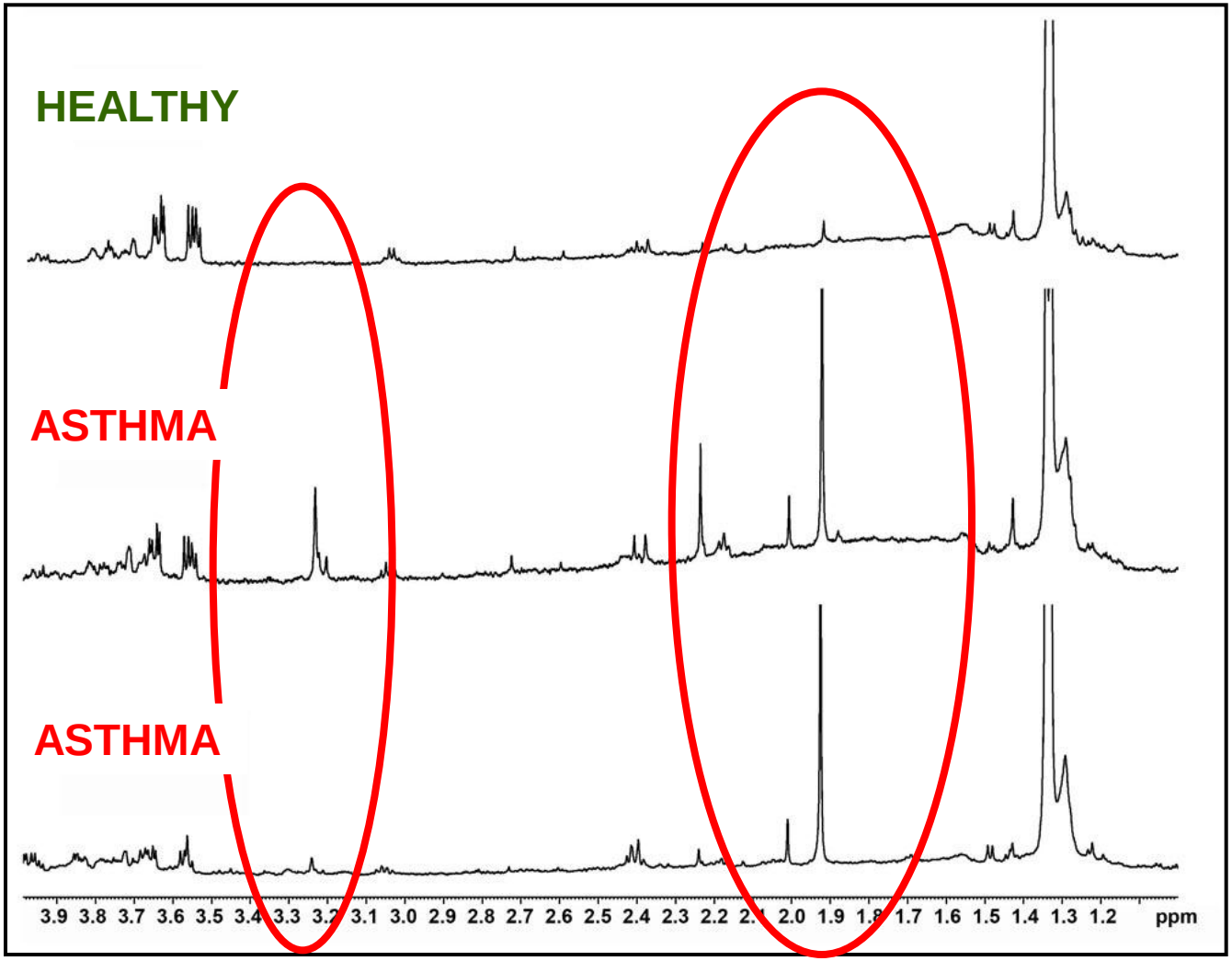
"BREATHOMICS"



Pattern recognition methods (PLS-DA) applied to NMR-metabolomics spectra discriminates healthy and asthmatic children with a 95% success rate

Carraro et al AJRCCM 2007

Metabolomics analysis of EBC: NMR spectra



oxidized compounds

acetylated compounds

Problematic severe asthma in children, not one problem but many: a GA^{2}LEN initiative

G. Hedlin*,¶¶, A. Bush#,¶¶, K. Lødrup Carlsen¶,¶¶, G. Wennergren+,¶¶, F.M. de Benedictis§, E. Melén‡,¶¶, J. Paton**, N. Wilson#,¶¶ and K-H. Carlsen##,¶¶ on behalf of the Problematic Severe Asthma in Childhood Initiative group++

Problematic severe asthma
(poor control before consideration of other issues)

Difficult-to-treat asthma
Additional issues:
- Comorbid conditions
- Medication, adherence and technique
- Smoking
- Allergen exposure
- Environmental exposure
- Psychosocial

Severe therapy-resistant asthma
(despite high-dose therapy)
Consider for treatment beyond guidelines

ERJ 2010

EBC METABOLOMIC PROFILING IN CHILDREN WITH SEVERE ASTHMA

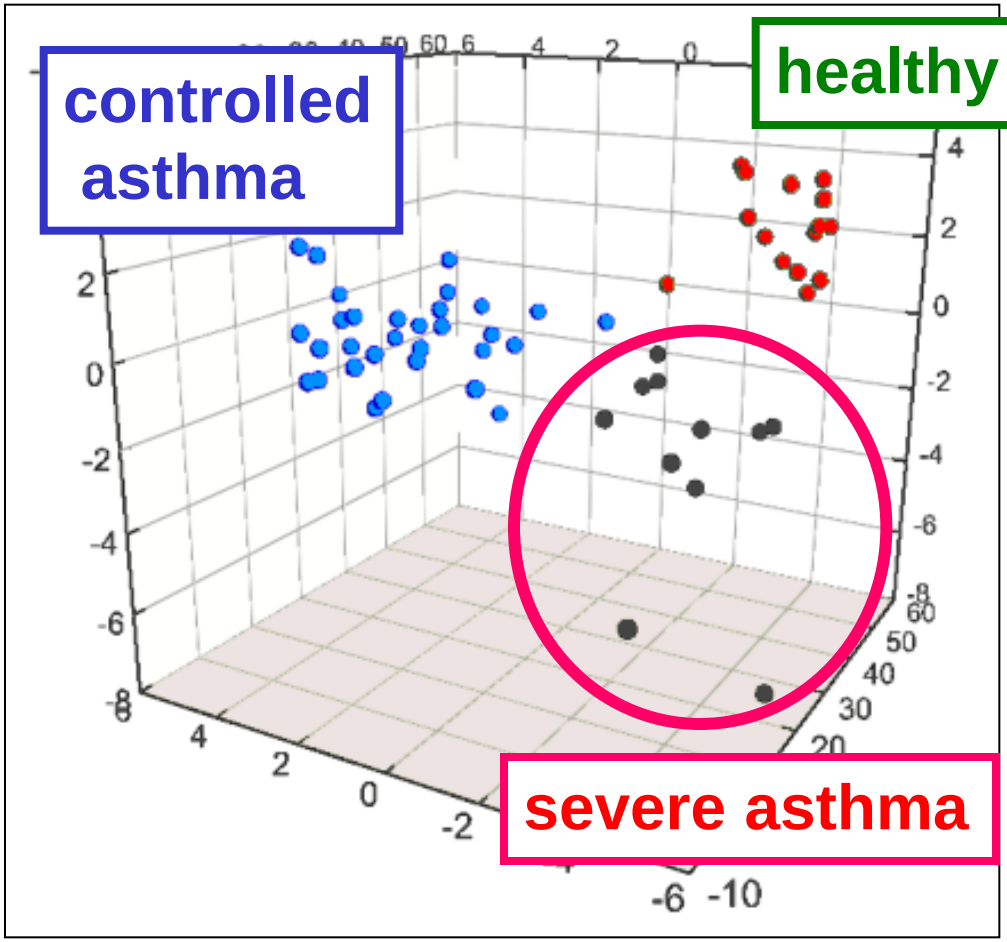
STUDY SUBJECTS

- **Well-controlled asthma: 14 children steroid-naïve, 17 children ICS treated**
- **Severe asthma: 11 children (age 8-16) with poorly controlled asthma although treated with high dose ICS and multiple controller medications (LABA, montelukast..).**
- **Healthy children: 15 children (age 9-17)**

METABOLOMIC ANALYSIS:

HPLC coupled with MS (Orbitrap-Fischer)

METABOLOMIC PROFILING OF CHILDREN WITH SEVERE ASTHMA



Baraldi et al ERS meeting 2010

METABOLOMIC PROFILING OF CHILDREN WITH SEVERE ASTHMA

By searching the Human Metabolome Database (HMDB), we were able to characterize, from a chemical point of view, the putative biomarkers potentially having a role in asthma pathophysiology

SEVERE ASTHMA

Var **225** → compound chemically related to retinoic acid

Var **127** → deoxyadenosine

NONSEVERE ASTHMA

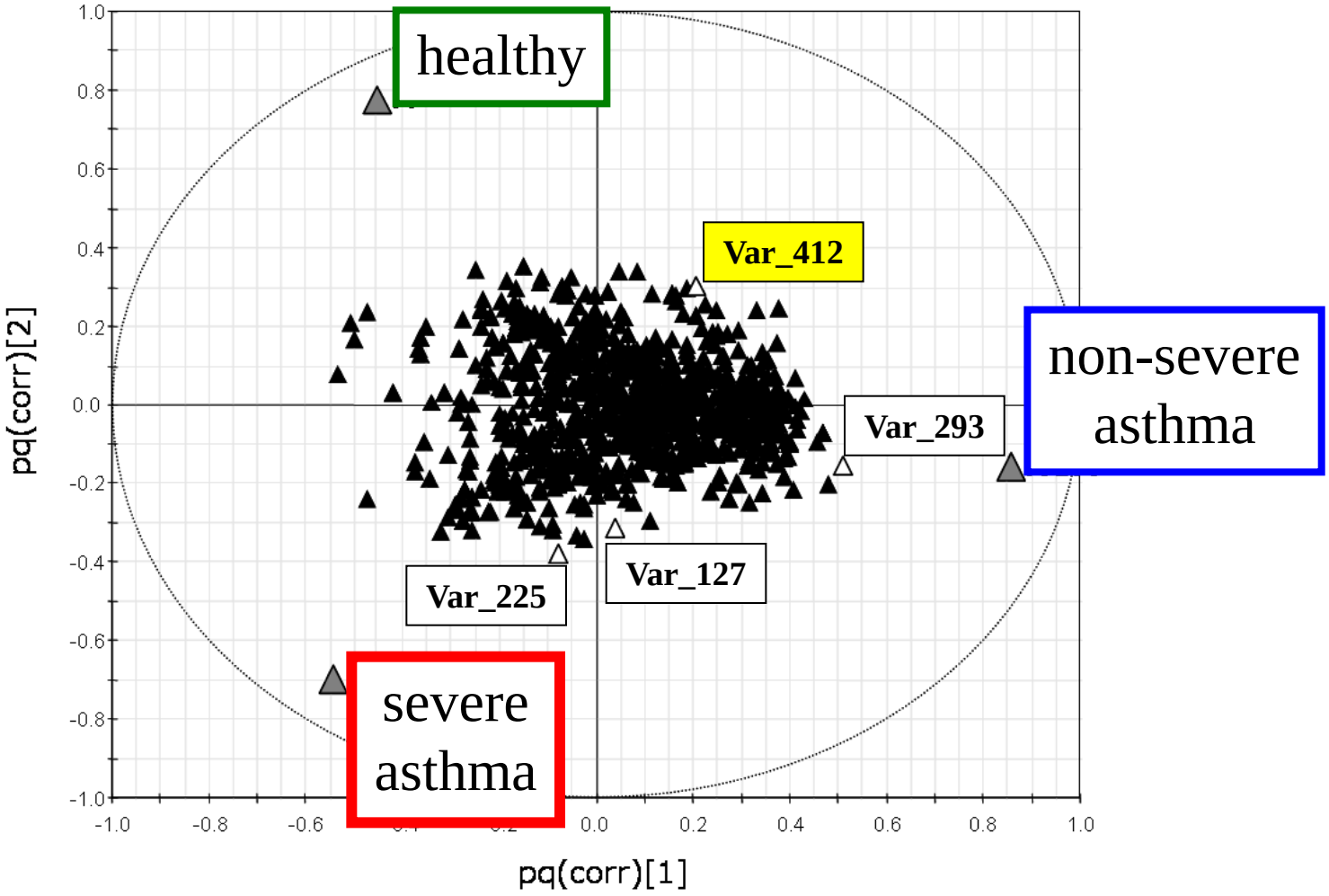
Var **293** → prostanoid (thromboxane B2?)

Var **412** → vit.D metabolite

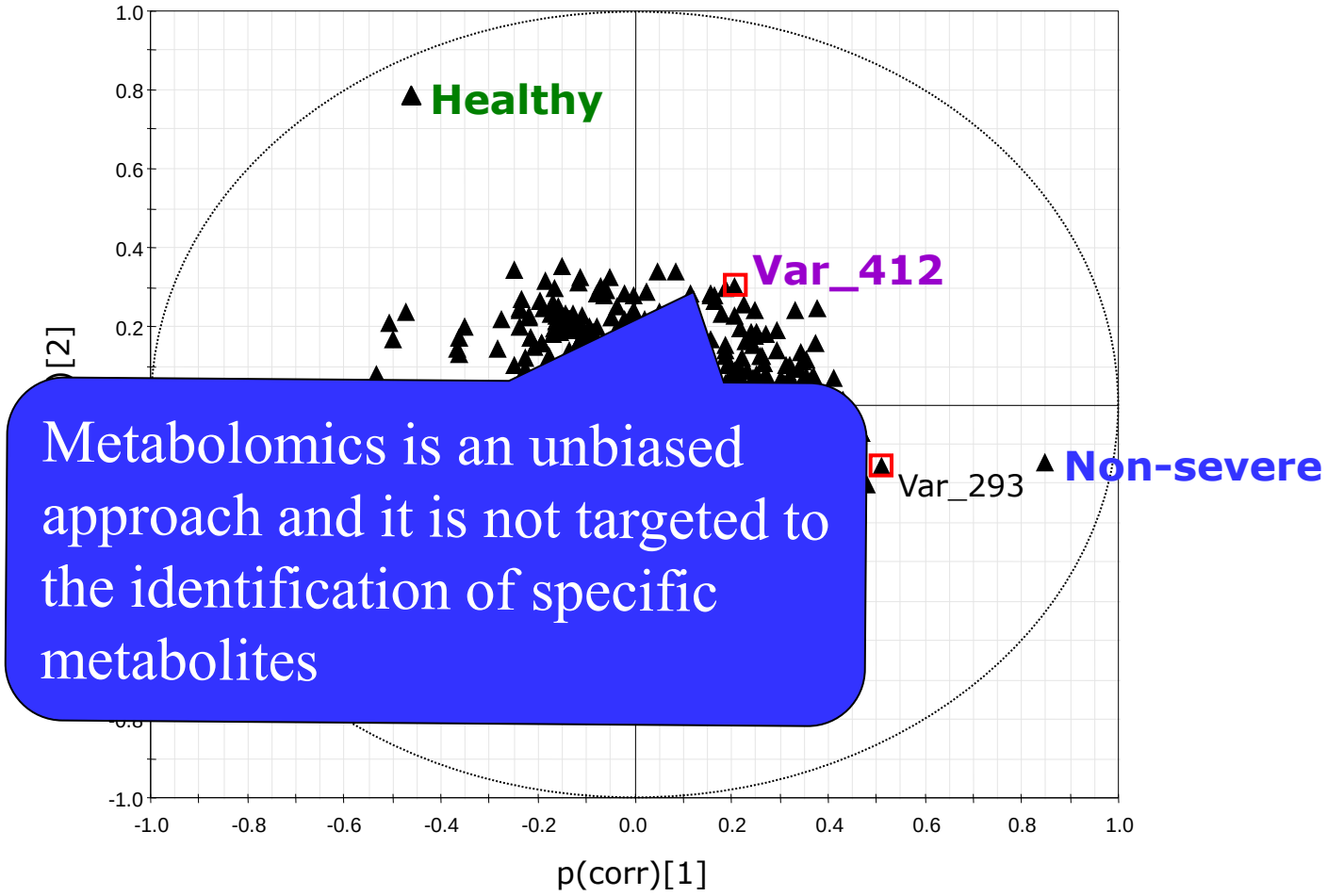
HEALTHY

Var **412** → vit.D metabolite

Breathomics and severe asthma in children



O2PLS-Discriminant Analysis: comparison of the 3 groups

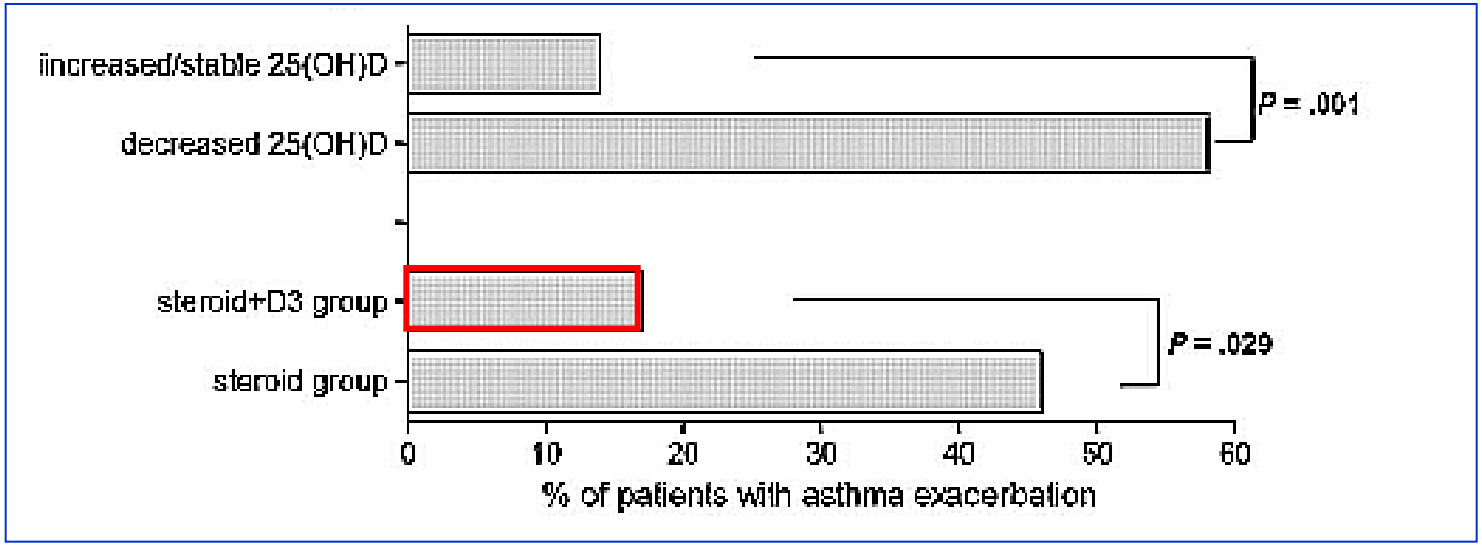


Correlation loading plot (pq(corr) plot)

Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection

Majak JACI 2011

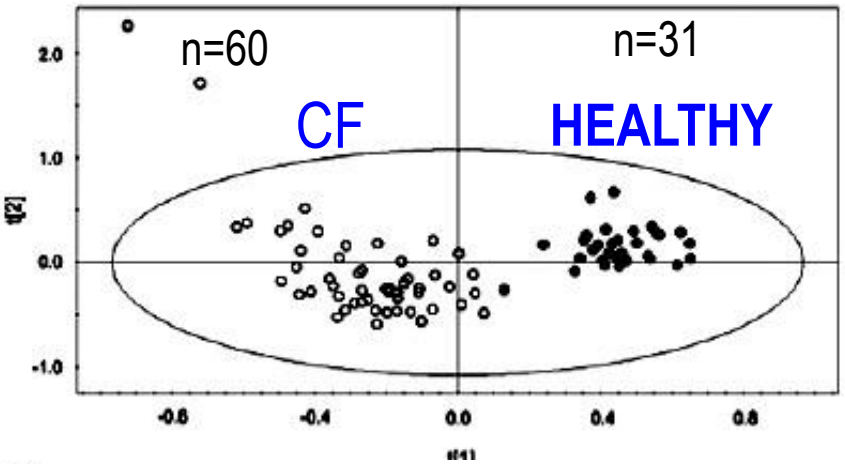
6 months trial, ICS or ICS+ Vit D 500 UI

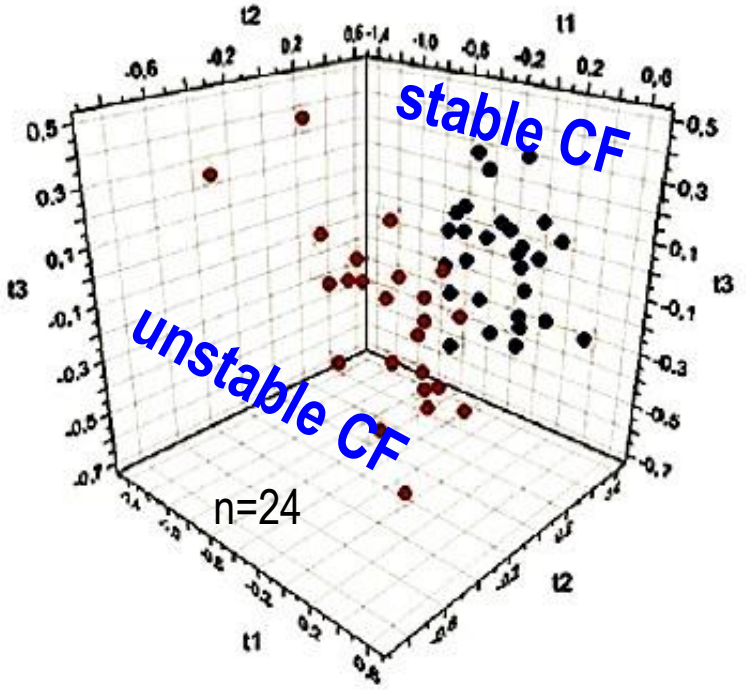


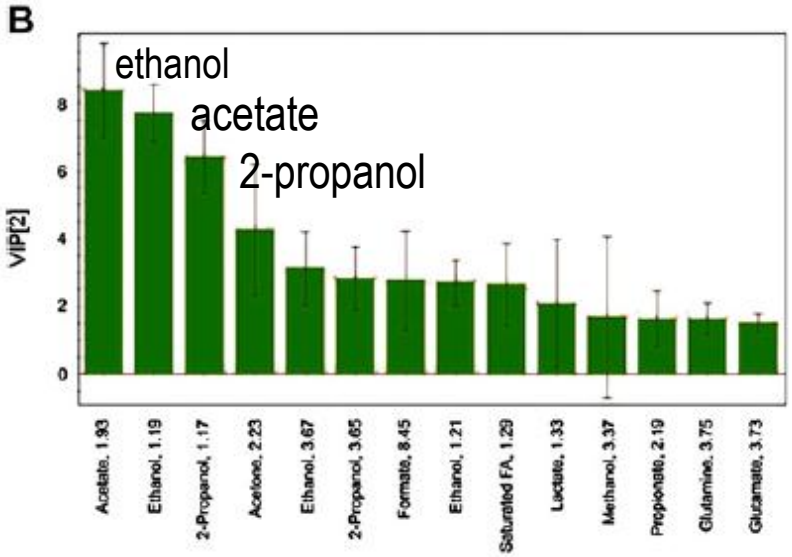
Asthma exacerbations triggered by viral infections were lower in the ICS+ Vit D group

Children with decreased 25(OH)D levels: risk of exacerbation x 8

NMR SPECTROSCOPY METABOLOMIC PROFILING OF EBC IN PATIENTS WITH STABLE AND UNSTABLE CYSTIC FIBROSIS

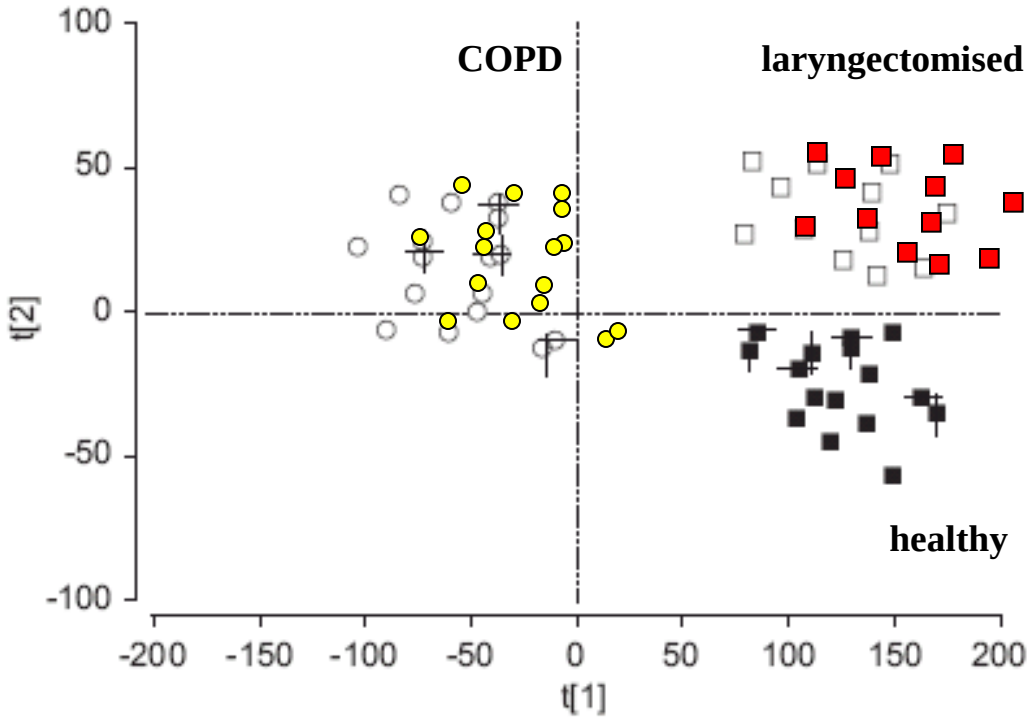






Montuschi Thorax 2012

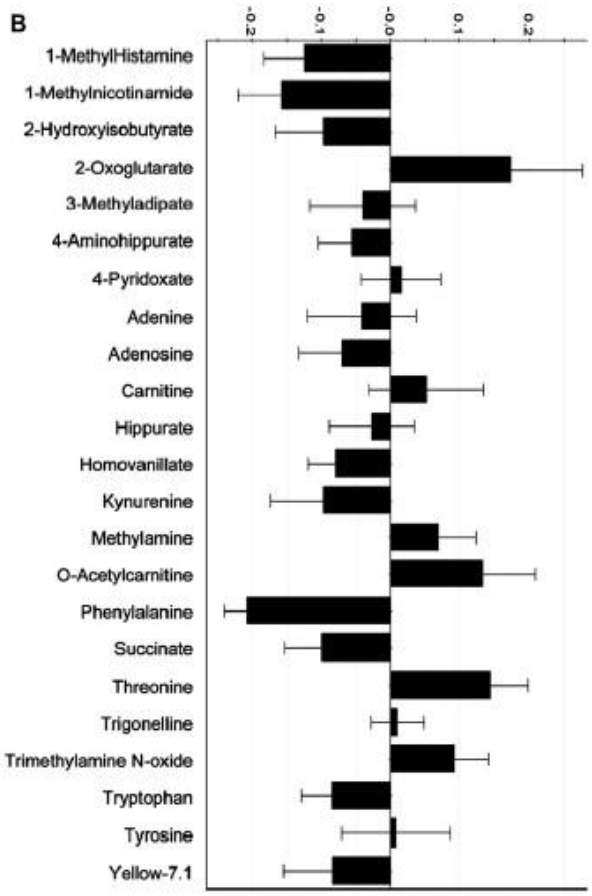
Metabolomics analysis of EBC - COPD

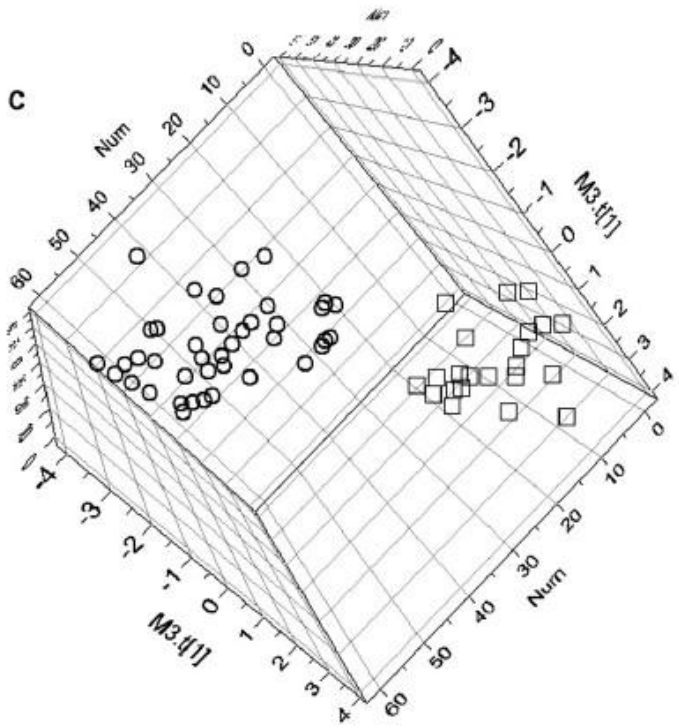


5 NMR-metabolomics signals differentiate between COPD and "non respiratory" subjects

De Laurentiis, ERJ 2008

Metabolomic profiling of asthma: Diagnostic utility of urine nuclear magnetic resonance spectroscopy





Saude JACI 2011;127:757-64

PROFILO METABOLOMICO DI BAMBINI CON INFEZIONI RESPIRATORIE RICORRENTI

Obiettivi dello studio:

1) ricerca di biomarkers che permettano una caratterizzazione biochimico-metabolica dei bambini con IRR
2) valutare se vi sono biomarkers predittivi di risposta alla terapia con Pidotimod

The Journal of Maternal-Fetal and Neonatal Medicine, Month 2009; 22(S3): 1–4

informa
healthcare

REVIEW

Metabolomics: A new tool for the neonatologist

LUIGI ATZORI[1,4], ROBERTO ANTONUCCI[2], LUIGI BARBERINI[3], JULIAN L. GRIFFIN[4], & VASSILIOS FANOS[2]

[1]*Department of Toxicology, University of Cagliari, Italy,* [2]*Section of Neonatal Intensive Care Unit, Department of Paediatrics and Clinical Medicine, University of Cagliari, Italy,* [3]*Department of Neurological Sciences, University of Cagliari, Italy, and* [4]*Department of Biochemistry, University of Cambridge, Cambridge, UK*

Science fiction?

Just remember that only a few years ago none of us had a mobile phone, e-mail or access to Internet

- ✓ Metabolomics … characterizatio… …ological sample without …
- ✓ Metabolomics has th… …ntial for the identification of early biomarkers of disease… …nd novel drug targets.
- ✓ Metabolomics is one of the platforms paving the way to the development of a personalized medicine