Metabolomic phenotyping in medical systems biology

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Systems Biology and the Omics Cascade
Stockholm, 13.6.2008

OMICS revolution over the past decade

- Increased ability to measure large number of ”parts” of the biological systems and their activities
  - Genes and their expression
  - Proteins and their modifications
  - Small molecules (metabolites) and their reactions
  - Imaging technologies, incl. in vivo
  - Microbial populations
  - ”single cell” measurements
  - etc etc

Systems and levels

Hierarchical Mappings

Compartmental Processes

System-wide

System regulation, Disease staging, treatment monitoring, pharmacodynamics

Interstitial

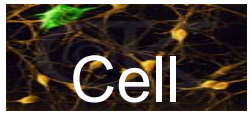
Organ system homeostasis and regulation, metabolite inputs and outputs, endocrine targeting

Need for models linking the phenotype with the genetic & environmental factors

Tissue

Cell Communication, Cytokines, antigen presentation, tissue-type specificity

Intercellular



Gene and Protein Expression and Regulation, Intercellular biochemical activities

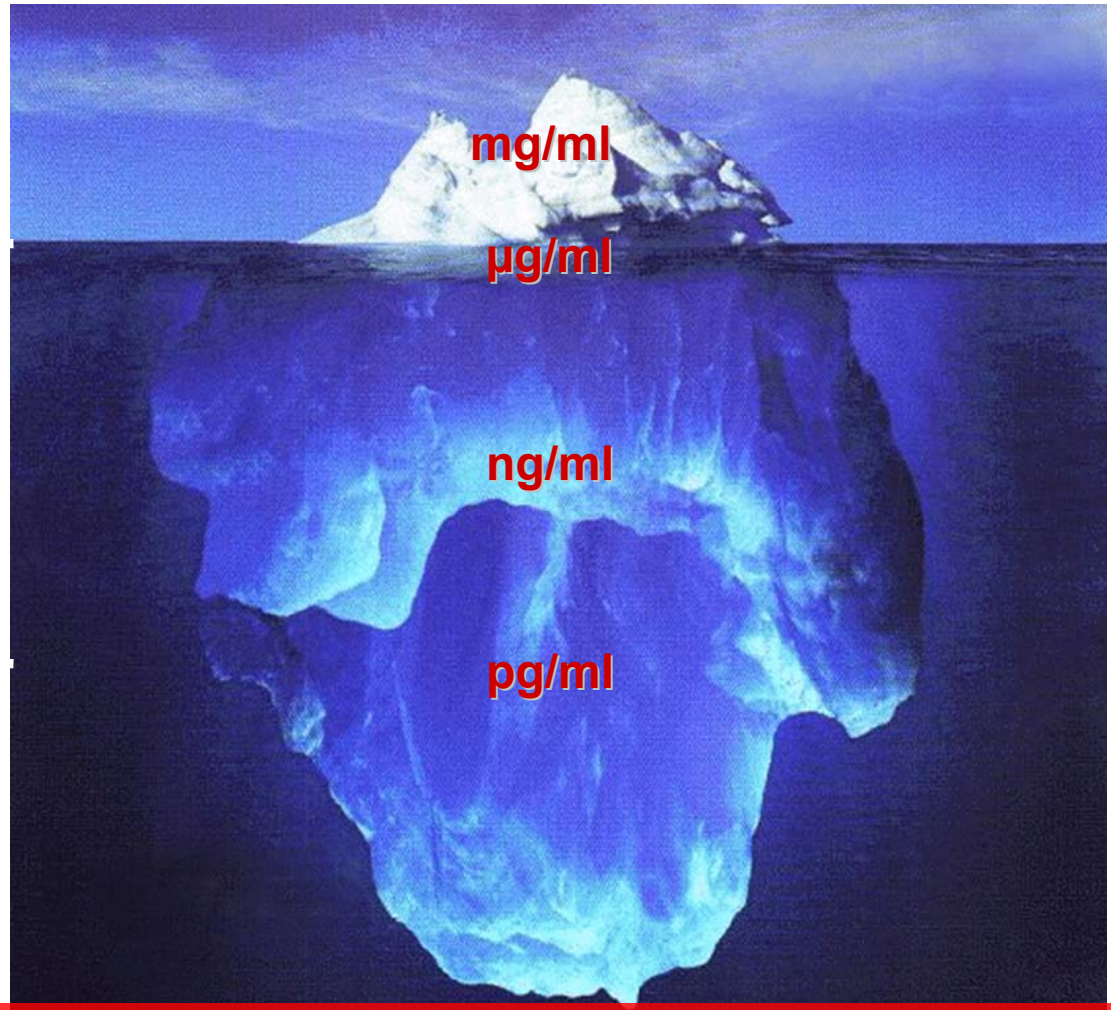
Systems Biology

Metabolome

NMR

Mass Spectrometry

CUSTOM



Homeostasis 'Housekeeping'

Organic Acids

Lipids

Amino Acids

Nucleotides

Steroids

Eicosanoids

Neurotransmitters

Peptides

Trace elements

Biofluid metabolic profile = Phenotype

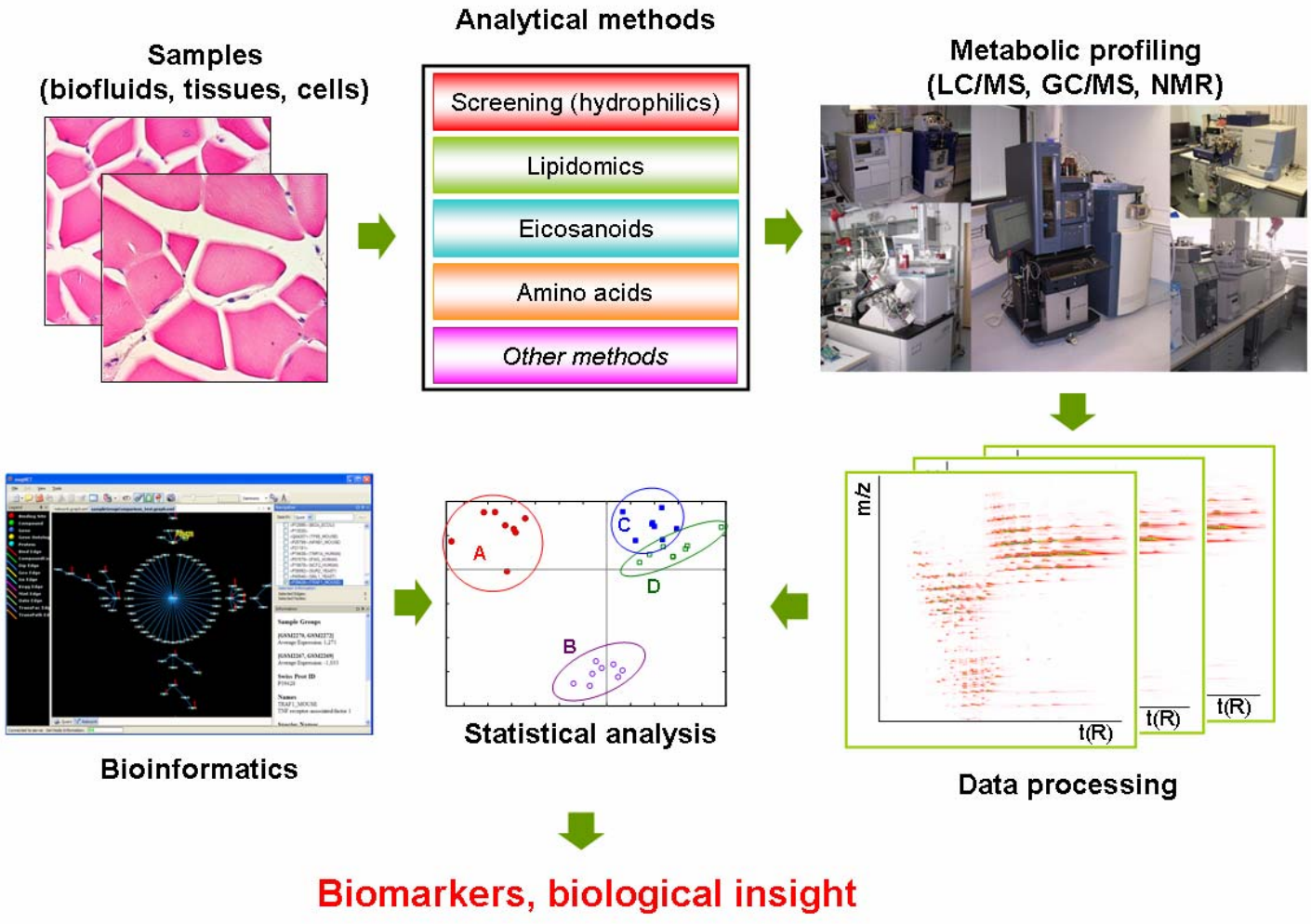
Metabolomics as a platform for systems biology

SENSITIVITY

As proven via the formalism of Metabolic Control Analysis; small changes in activities of individual enzymes lead to small changes in metabolic fluxes, but can lead to ***large changes in metabolite concentrations***

Metabolomics platform

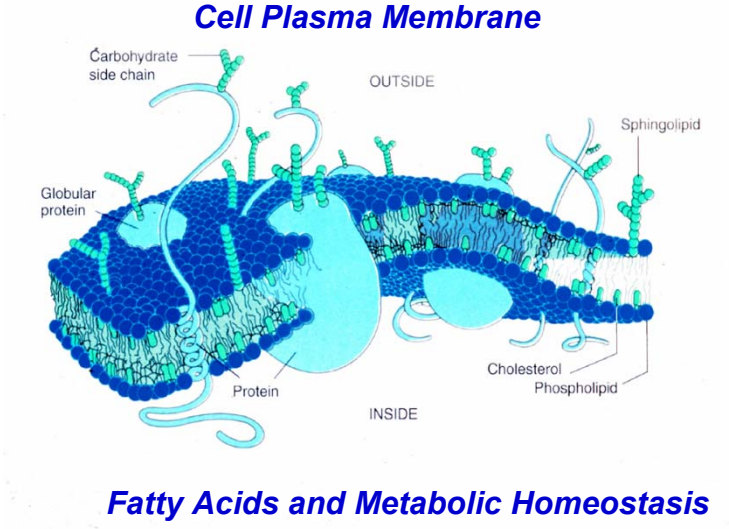
Experiment design + Analytical chemistry + Chemometrics + Bioinformatics



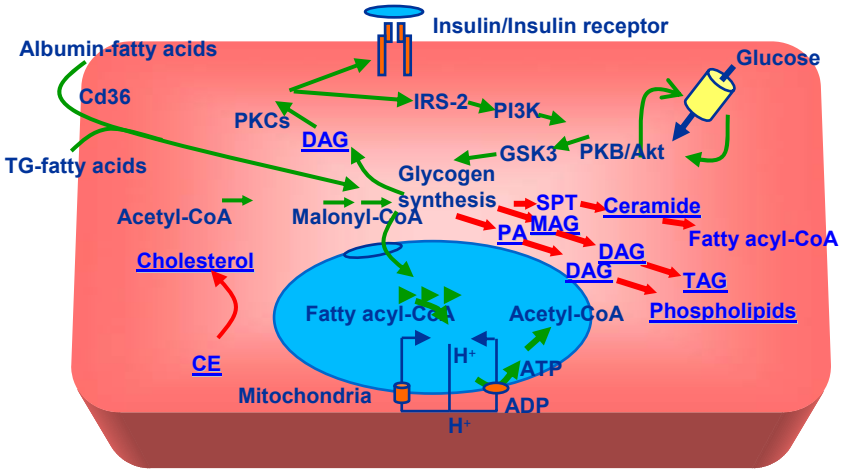
Why measure lipids?

Membrane Structure & Function; Signaling; Energy; Storage

Cell Plasma Membrane



Fatty Acids and Metabolic Homeostasis

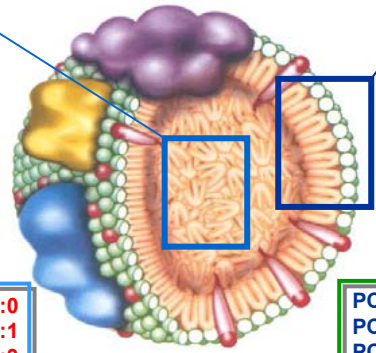


High Density Lipoprotein (HDL)

Storage Lipids

Glycerolipids
Cholesteryl esters

TAG 14:0/16:0/16:0	CE 16:0
TAG 16:0/18:0/18:0	CE 18:1
TAG 18:1/18:1/20:4	CE 20:3
….	CE 20:4
DAG 16:0/16:0	….
DAG 16:0/18:0	
DAG 18:1/18:1	
….	



Membrane Lipids

Phospholipids
Sphingolipids
Sterols

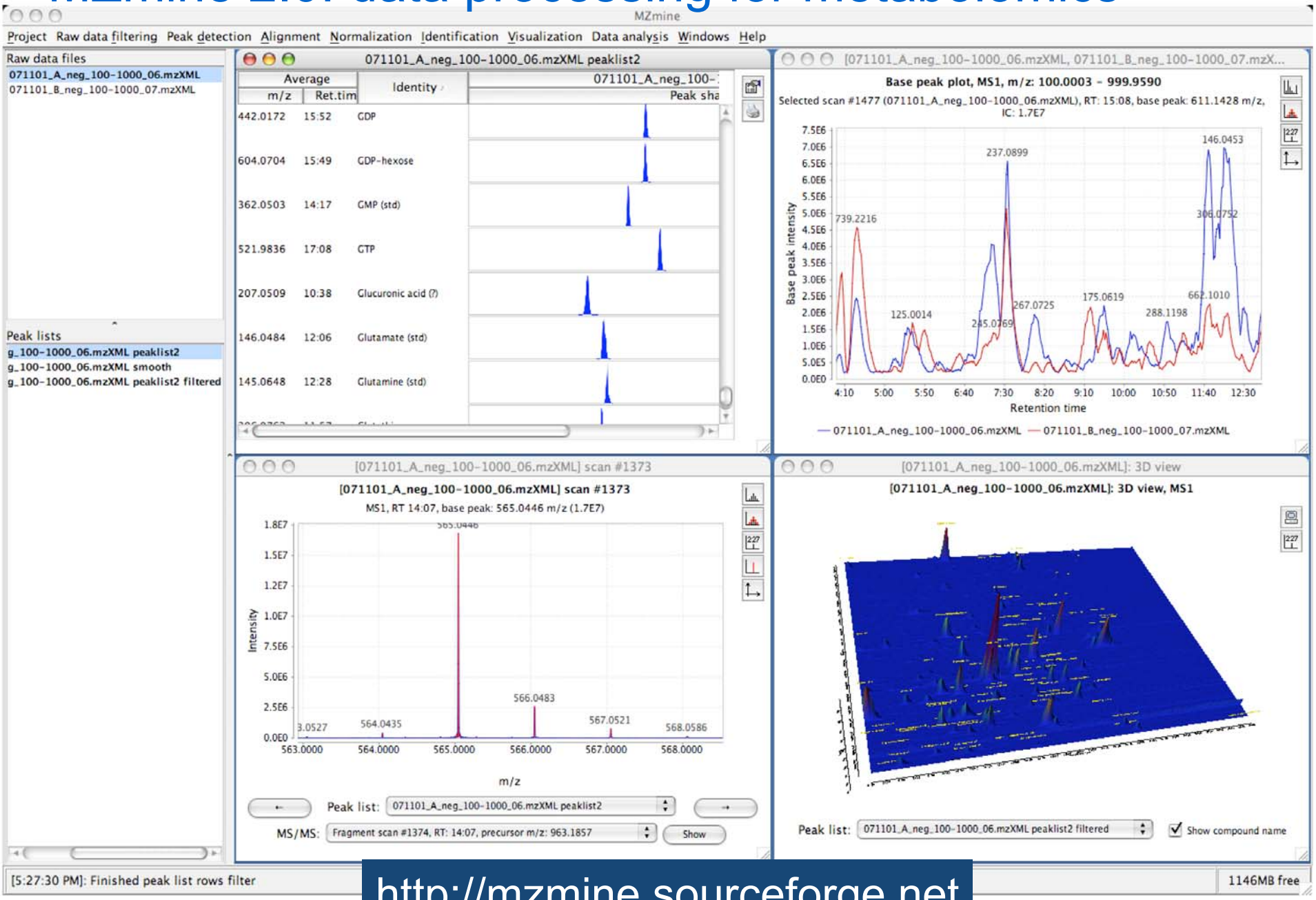
PC 16:0/16:0	Ceramide 18:0
PC 18:1/18:2	Ceramide 18:2
PC 18:0/20:4	Ceramide 24:1
PC 16:0/22:6	….
PC 18:1/20:3	Cholesterol
….	
PE 16:0/22:6	
PE 18:1/20:3	
PE 17:0/18:1	
….	
LPC 16:0	
LPC 18:1	
….	
SM 18:0	
SM 24:1	
….	

Platforms

UPLC-TOF/MS lipidomics
(major phospholipids, sphingolipids, acylglycerols)
10-15μl serum sample used

GCxGC-TOF/MS
(global metabolome)
20μl serum sample used

MZmine 2.0: data processing for metabolomics



http://mzmine.sourceforge.net

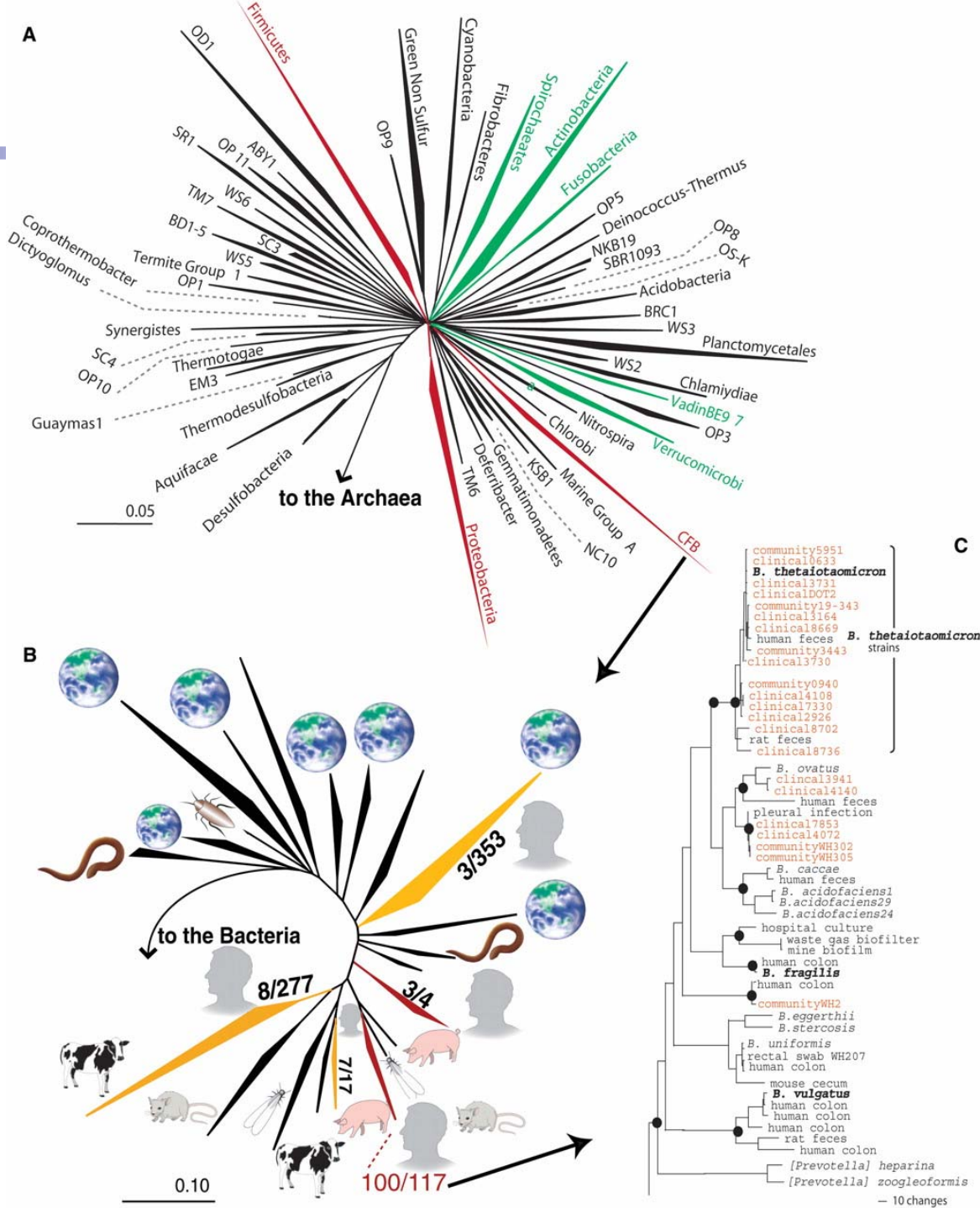
Outline

- 1 genome?
  - **Genetic factors** affecting the metabolic phenotype
- Metabolic states & development
  - Changes of metabolic phenotypes with **age**
- Beneficial autoimmunity?
  - Metabolic phenotypes & **immune response**
- Drug response phenotyping
  - **Tissue-specific drug effect** on metabolic phenotypes

1 genome?

Gut microbes

We carry 10 times more microbial cells as the host mammalian cells (~100 trillion bacteria).

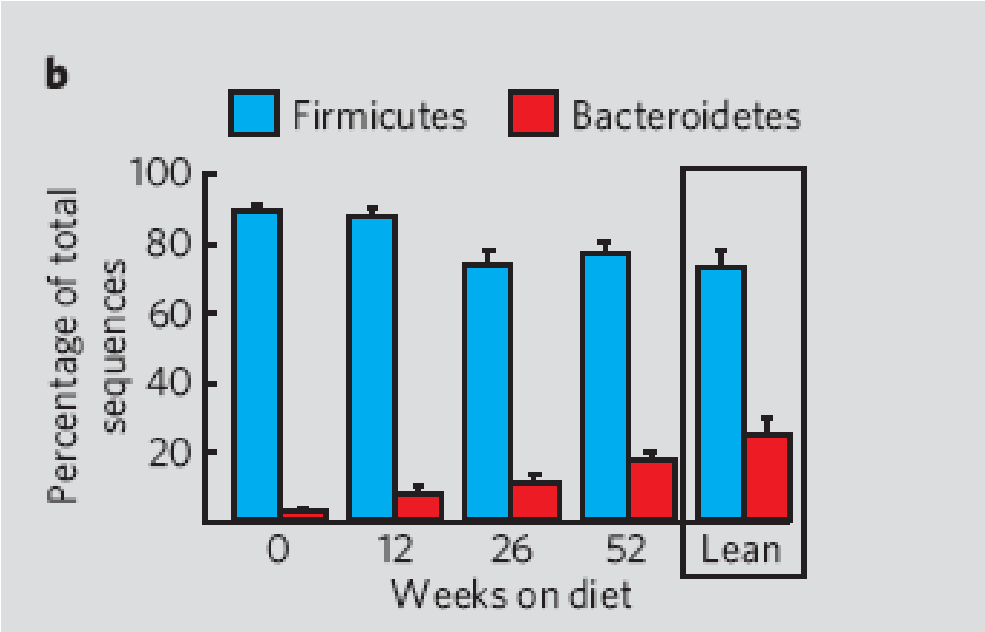


Bäckhed et al, Science (2005)

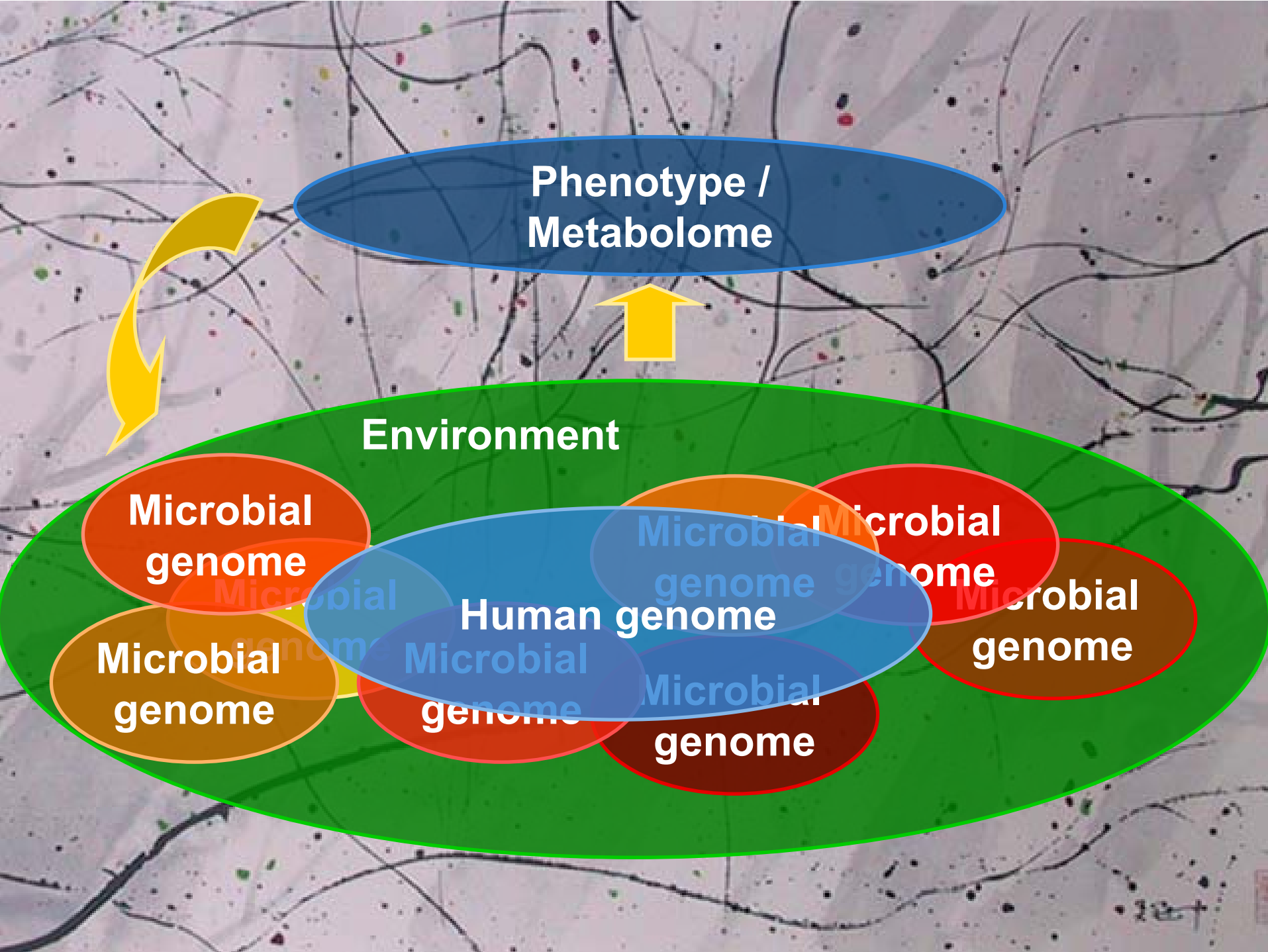
Human gut microbes are associated with obesity and lipid metabolism

MICROBIAL ECOLOGY

Human gut microbes associated with obesity



Ley et al (Nature, 2006)


Phenotype /
Metabolome
Environment
Microbial
genome
Microbial
genome
Human genome
Microbial
genome
Microbial
genome
Microbial
genome
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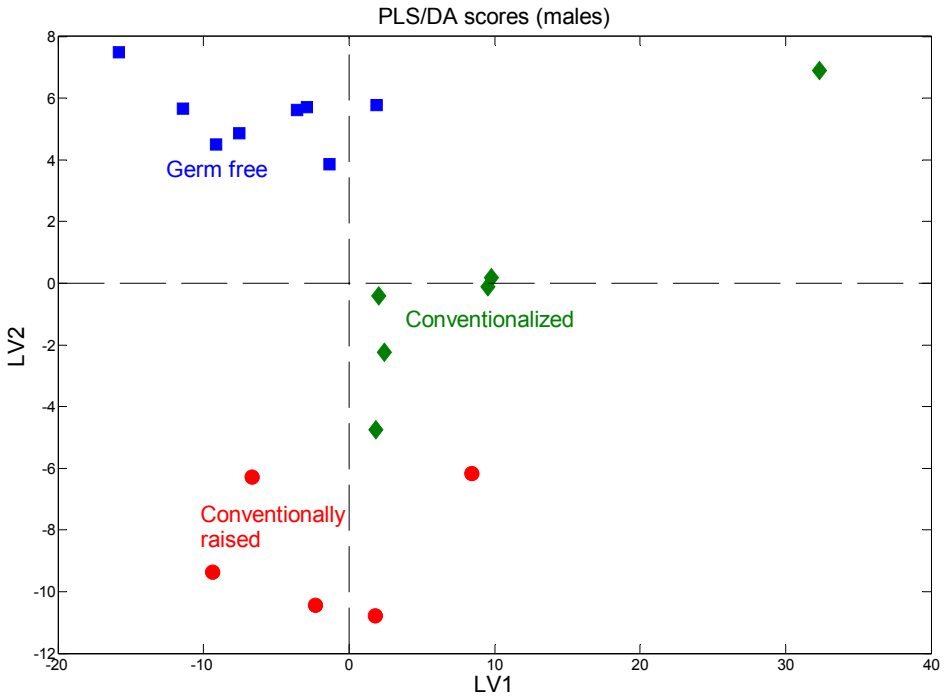
genome

Serum lipidome is affected by gut microbial composition



Comparison of serum lipidomic profiles of three groups of male Swiss Webster mice of different gut microbial composition:

1. **Germ free**
2. **Conventionalized**: GF mice colonized for two weeks at adulthood
3. **Conventionally raised**, i.e., with normal microbiota

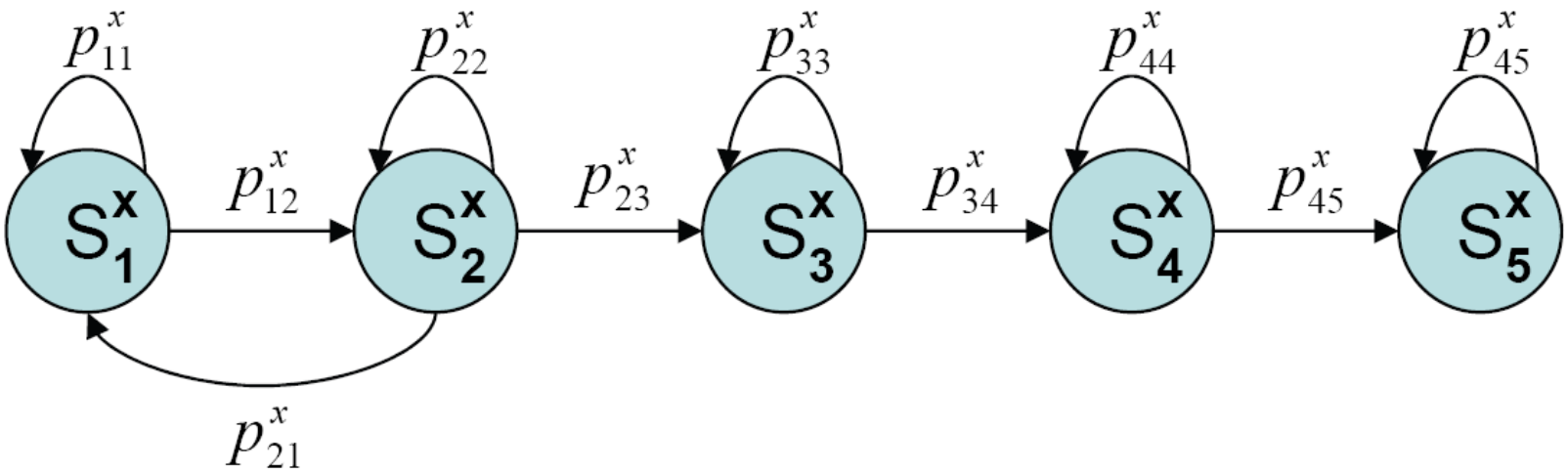
Metabolic states & development

Sample series

- 59 children between 3 months and 4 years of age
  - 27 boys
  - 32 girls
- Serum sample collection every 2-7 months
- Children remained healthy (no chronic disease) throughout the follow-up
- 11 samples per child on average
- Samples from the Type 1 Diabetes Prediction & Prevention study (DIPP)

”Normal” metabolome changes with age

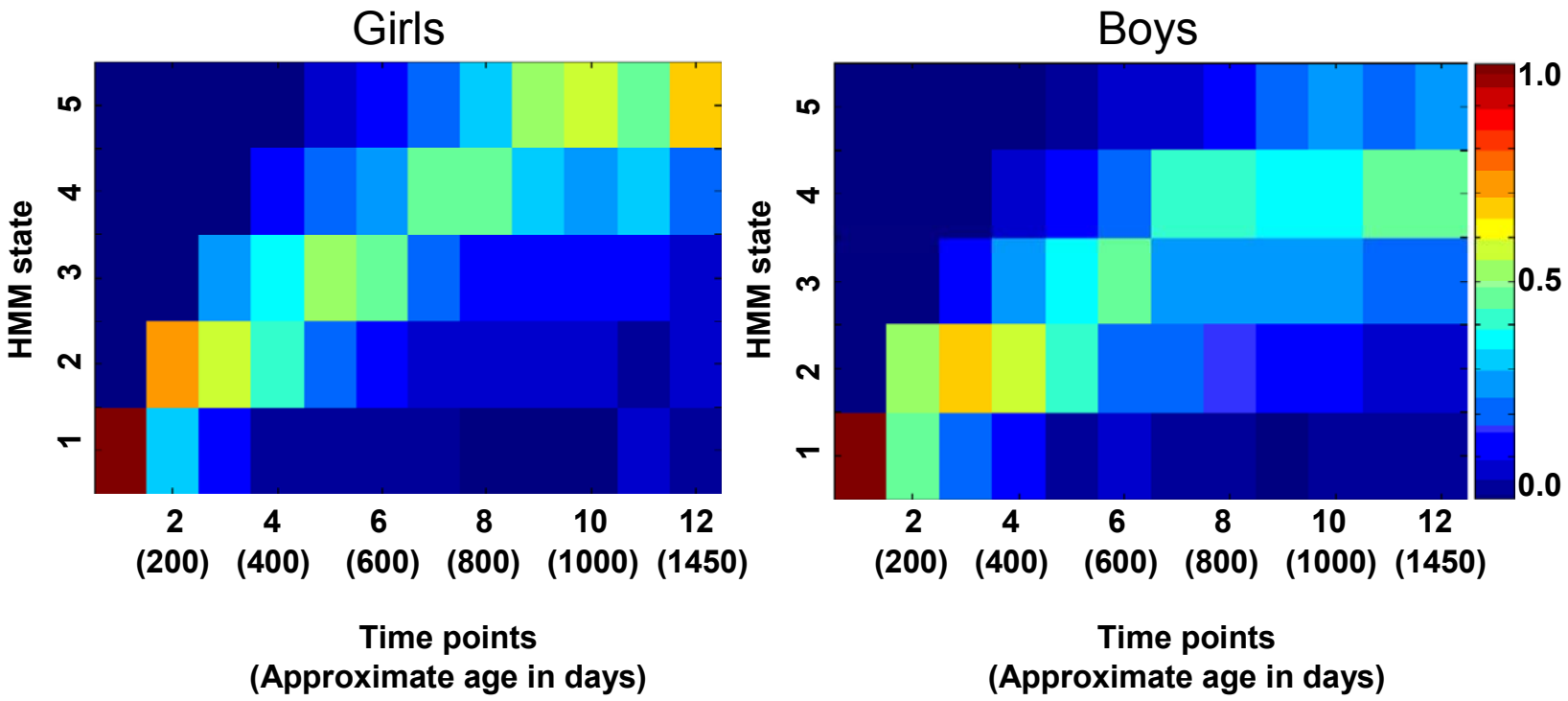
- Describe as progression of metabolic states
- Apply Hidden Markov Model methodology to describe the states and their progression



x - Boys/Girls

Nikkilä et al, Mol. Syst. Biol. (2008)

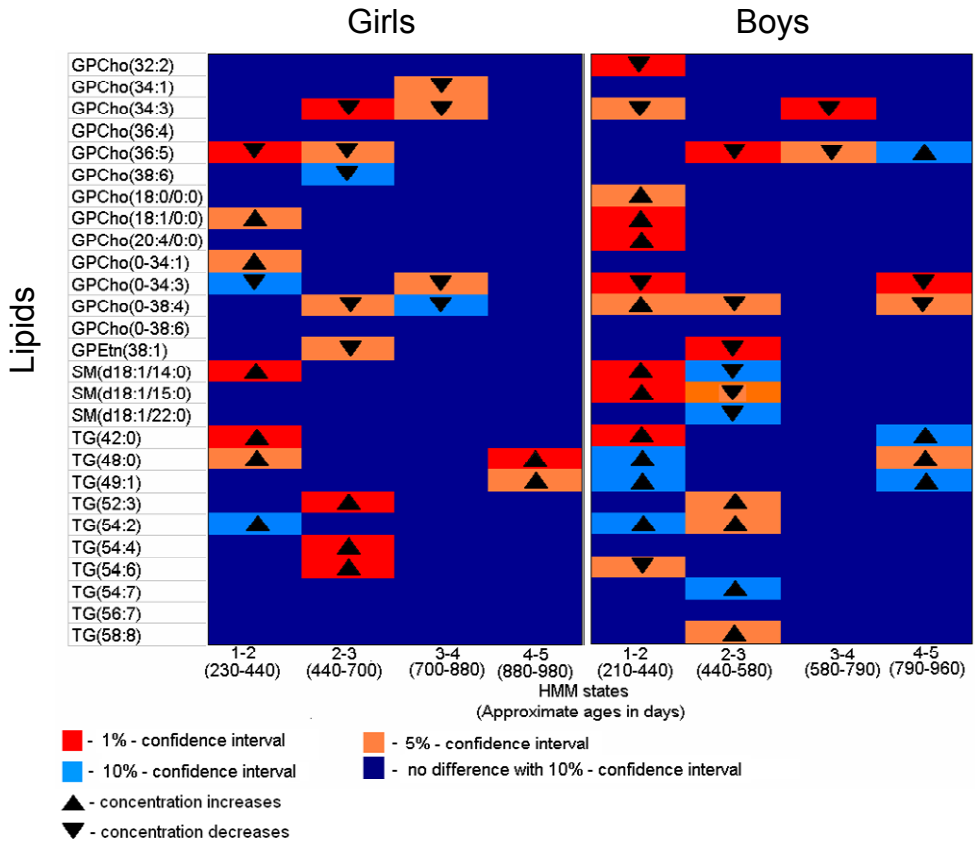
First five years: progression not the same for each child



Nikkilä et al, Mol. Syst. Biol. (2008)

Major differences between the states

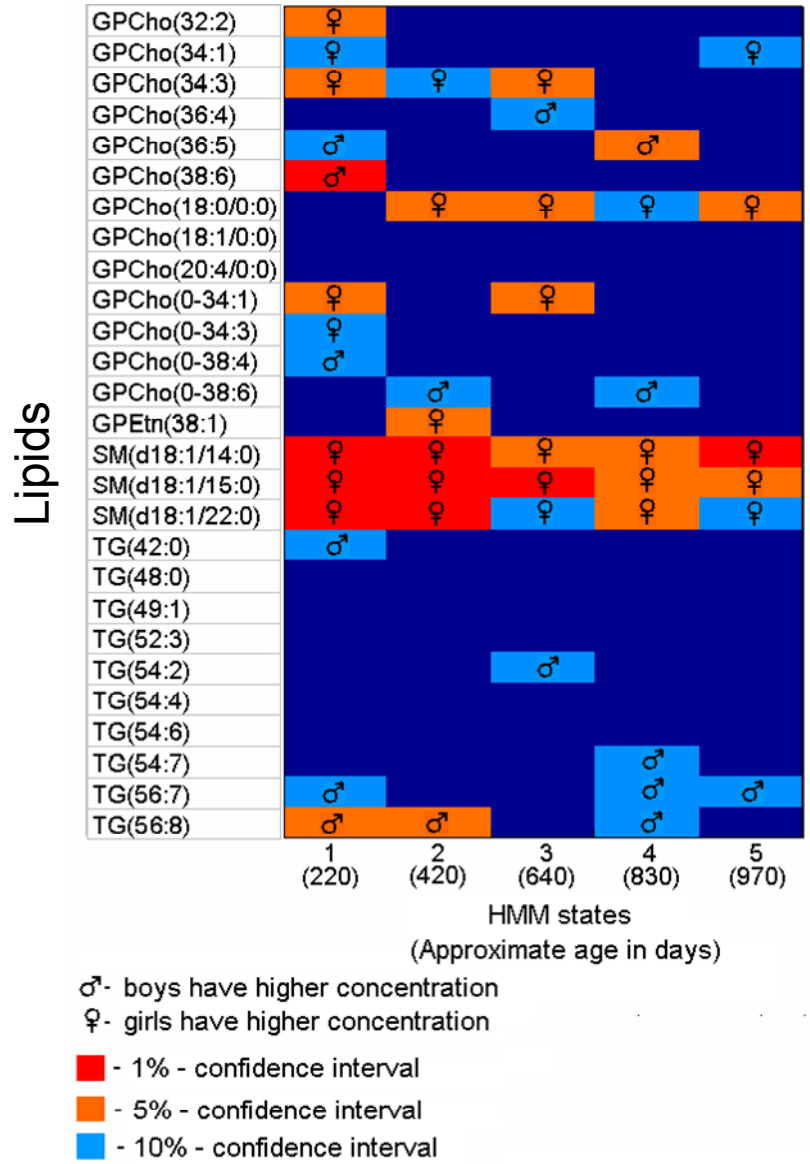
- Upregulation of proinflammatory lysophospatidylcholines and short chain saturated triacylglycerols near 1 year
- Dietary triacylglycerols upregulated near 3 years of age



Nikkilä et al, Mol. Syst. Biol. (2008)

Developmental metabolic differences between girls and boys

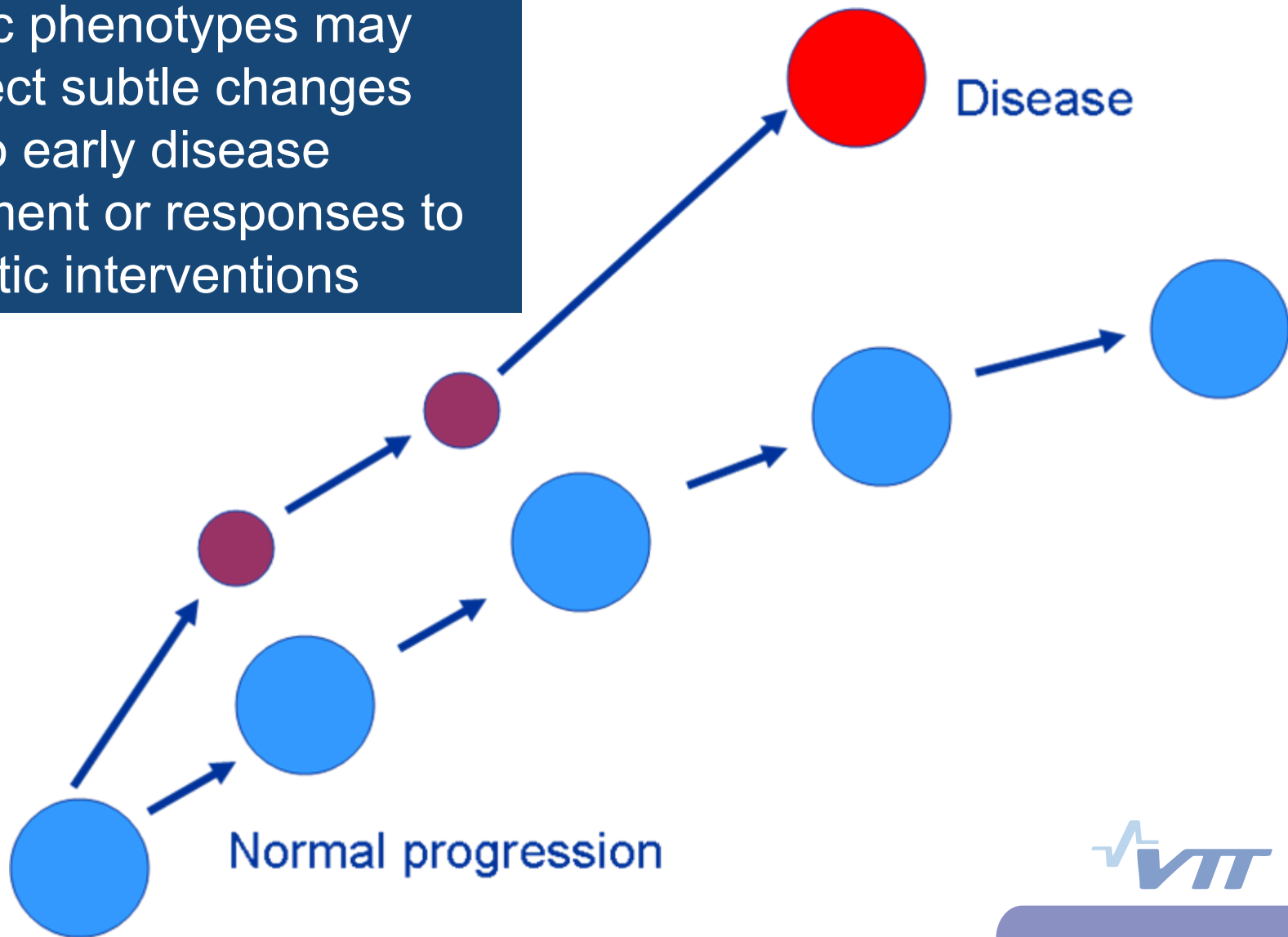
Sphingomyelins consistently elevated in girls



Nikkilä et al, Mol. Syst. Biol. (2008)

Concept: metabolic states and disease

Metabolic phenotypes may help detect subtle changes related to early disease development or responses to therapeutic interventions



Beneficial autoimmunity?

Type 1 diabetes

- T1D is a **chronic autoimmune disease** caused by destruction of the insulin-producing beta cells in the pancreatic islets of Langerhans.
- In most Western countries, the **incidence has increased by 3% per year** during the past 50 years.
- The disease is **multifactorial and polygenic** showing tight linkage with certain HLA-DQ and DR alleles.
- **As only a fraction of those at genetic risk develop T1D**, the impact of environment on disease pathogenesis is obvious.
- A symptom-free prediabetic period is characterized by **T lymphocyte accumulation** to the islets.

Persisting unknowns

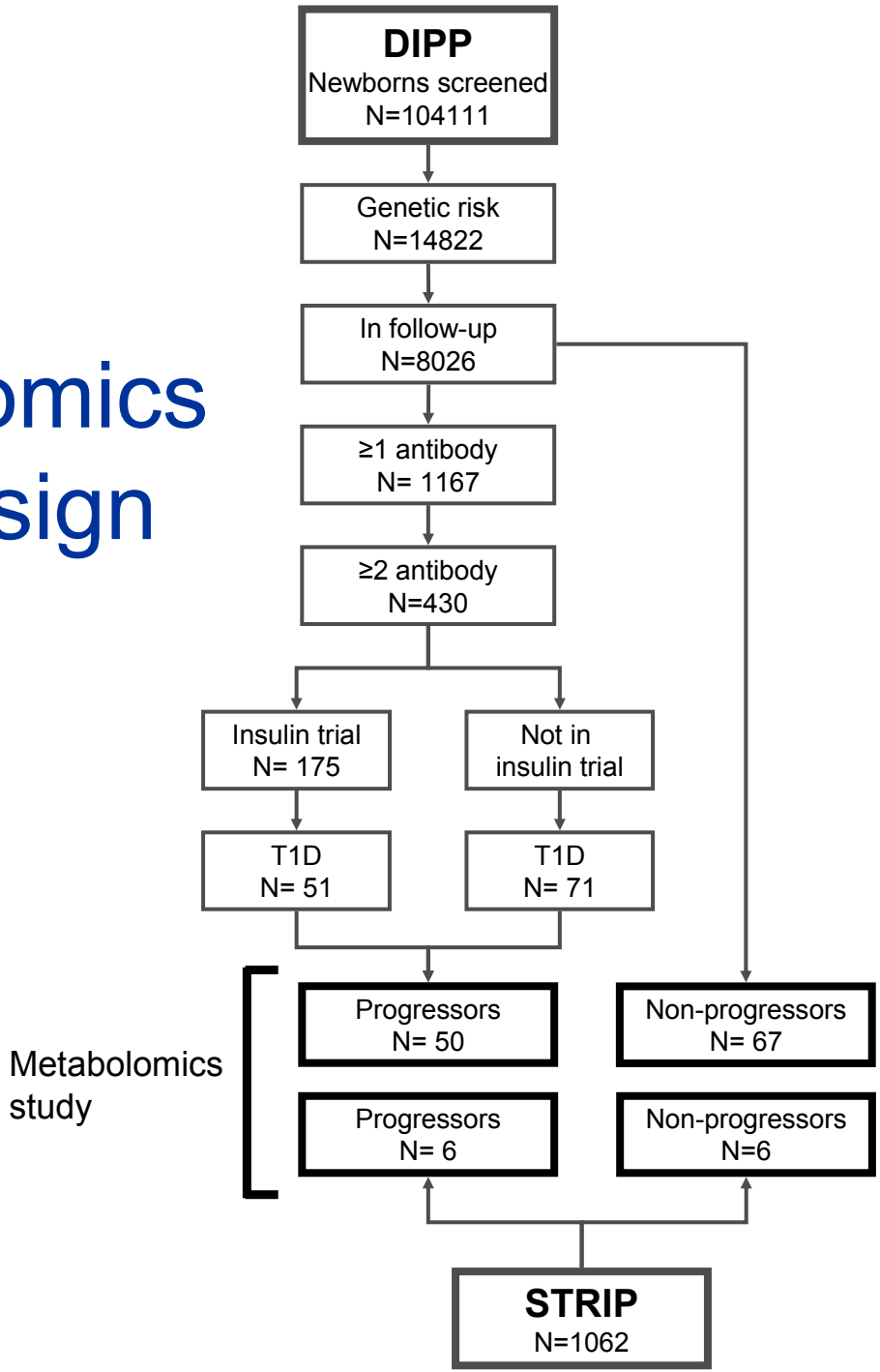
- Disease risk and time of onset?
- Triggers of the disease process(es)?
- Mechanisms regulating progression towards T1D?
- Prevention?

JDRF Center for Prevention of Type 1 Diabetes in Finland

- Type 1 Diabetes Prediction and Prevention Project (DIPP) launched Nov 7, 1994 in Turku
- Oulu joined 1 yr and Tampere 3 yrs later
- 20% of newborns screened in Finland

- SYSDIPP – Systems Biology Approach to Biomarker Discovery in Type 1 Diabetes started in 2005 (Tekes FinnWell Program)

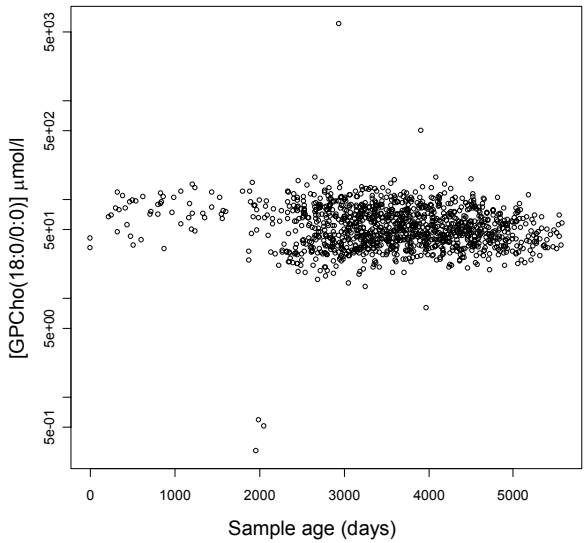
Metabolomics study design



Cases and controls matched by gender, HLA genotype, city and period of birth.

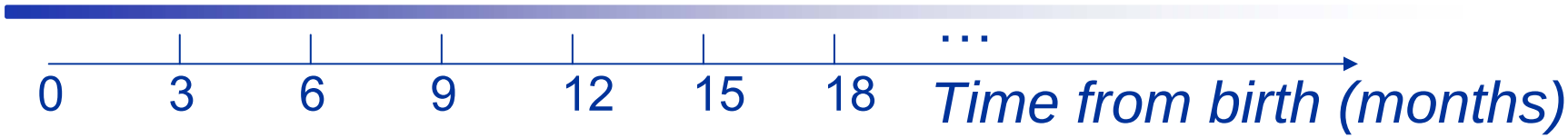
Sample series

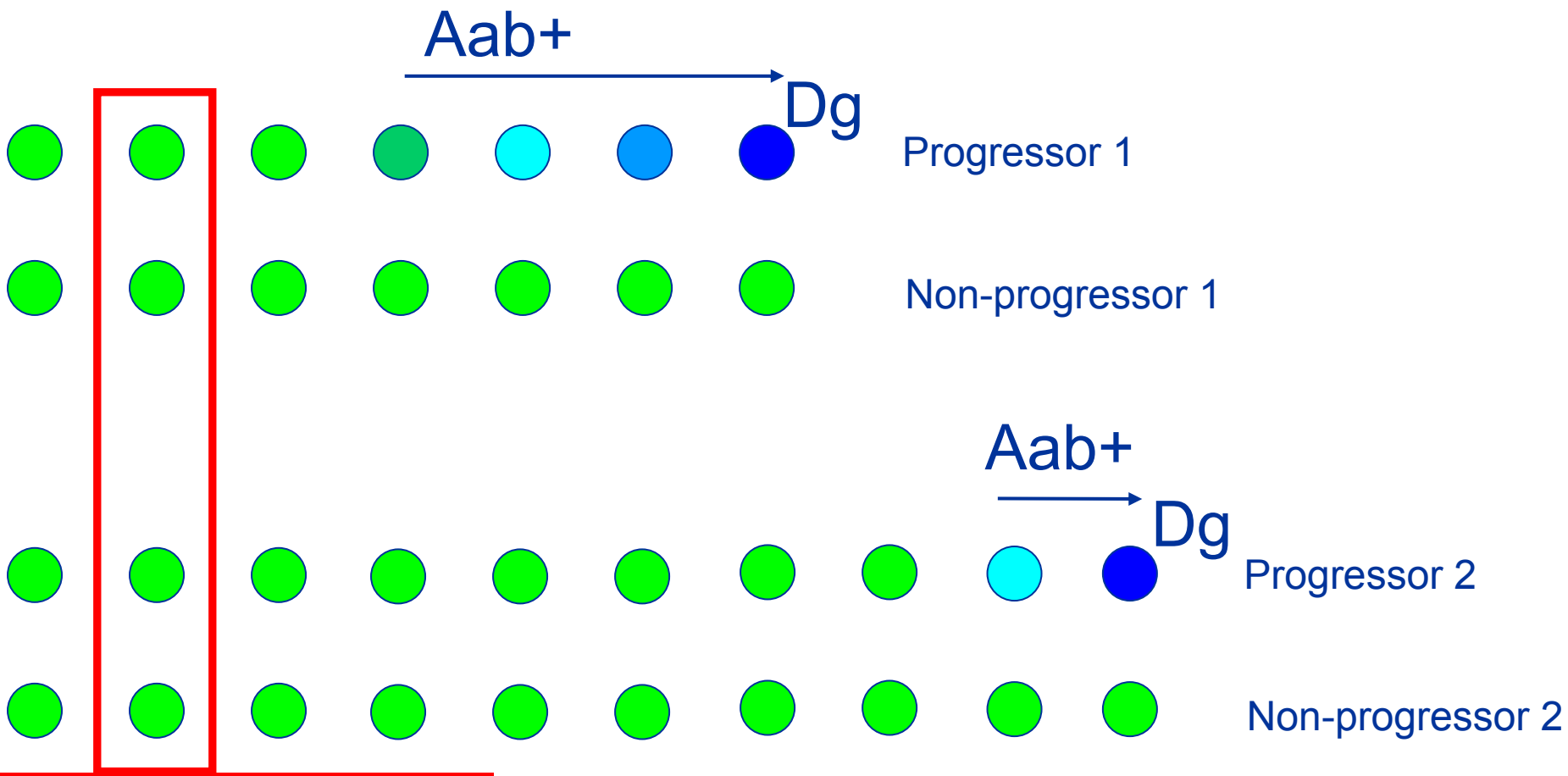
Batch	City of birth	Study	Year of birth	Age at diagnosis	Number of progressors	Number of non-progressors	Number of samples
1	Turku	DIPP	1994-2001	1-11y	13	26	441
2	Turku	DIPP	1996-1999	1-6y	10	13	185
3	Oulu	DIPP	1996-2001	1-8y	27	28	483
4	Turku	STRIP	1990	3-13y	6	6	87
				TOTAL	**56**	**73**	**1196**



No effect of sample age (r=-0.05, P=0.94)

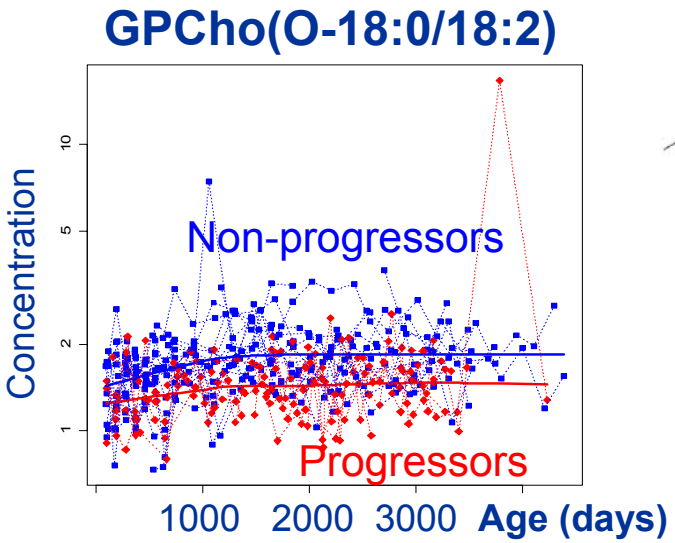
Age-based comparison

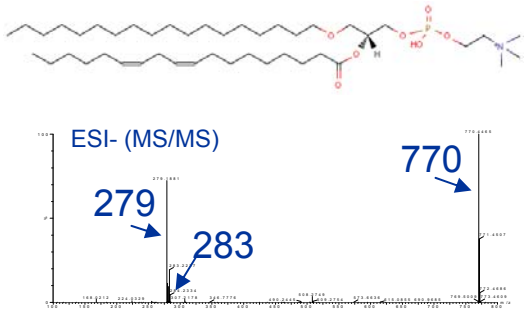




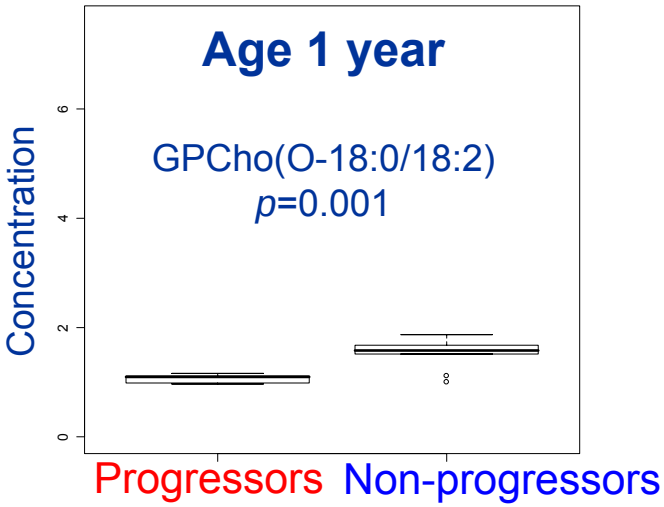
Age based comparision of molecular profile changes between cases and controls

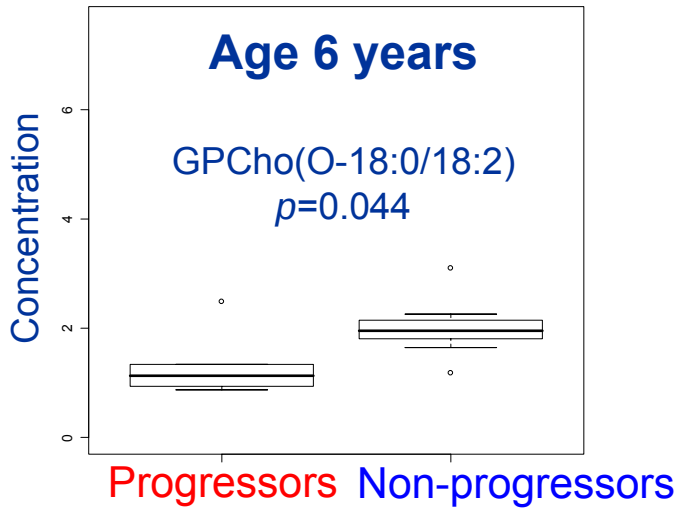
Ether-linked phosphocholines decreased in individuals who later developed autoimmunity and Type 1 Diabetes



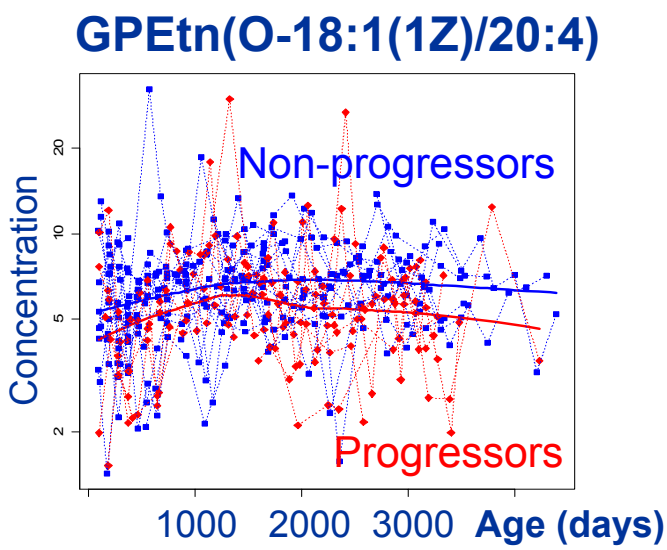


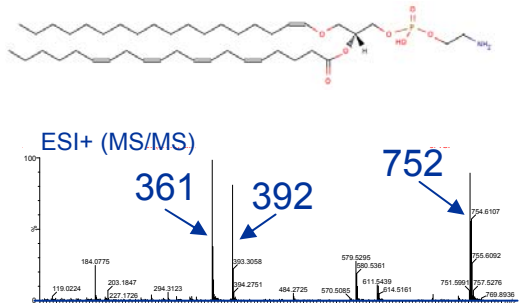
Plasmalogens are most abundant class of ether linked phospholipids, known as endogenous antioxidants.





Ethanolamine plasmalogen also decreased





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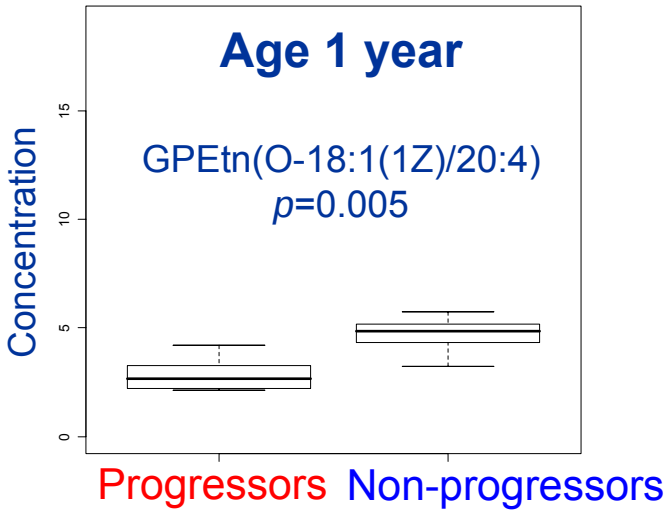
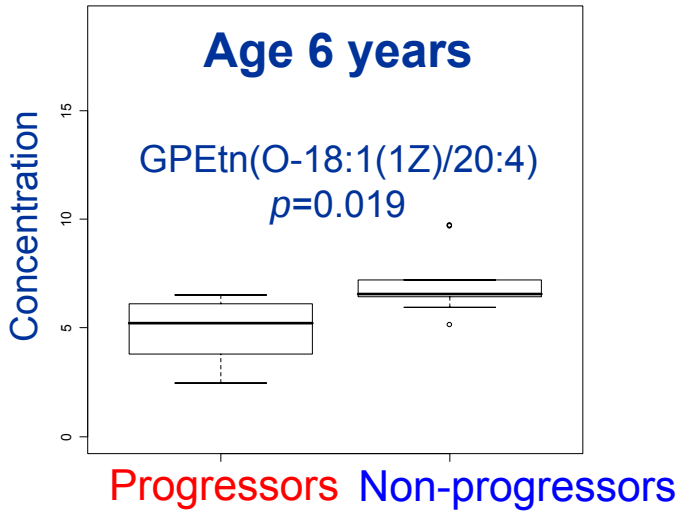


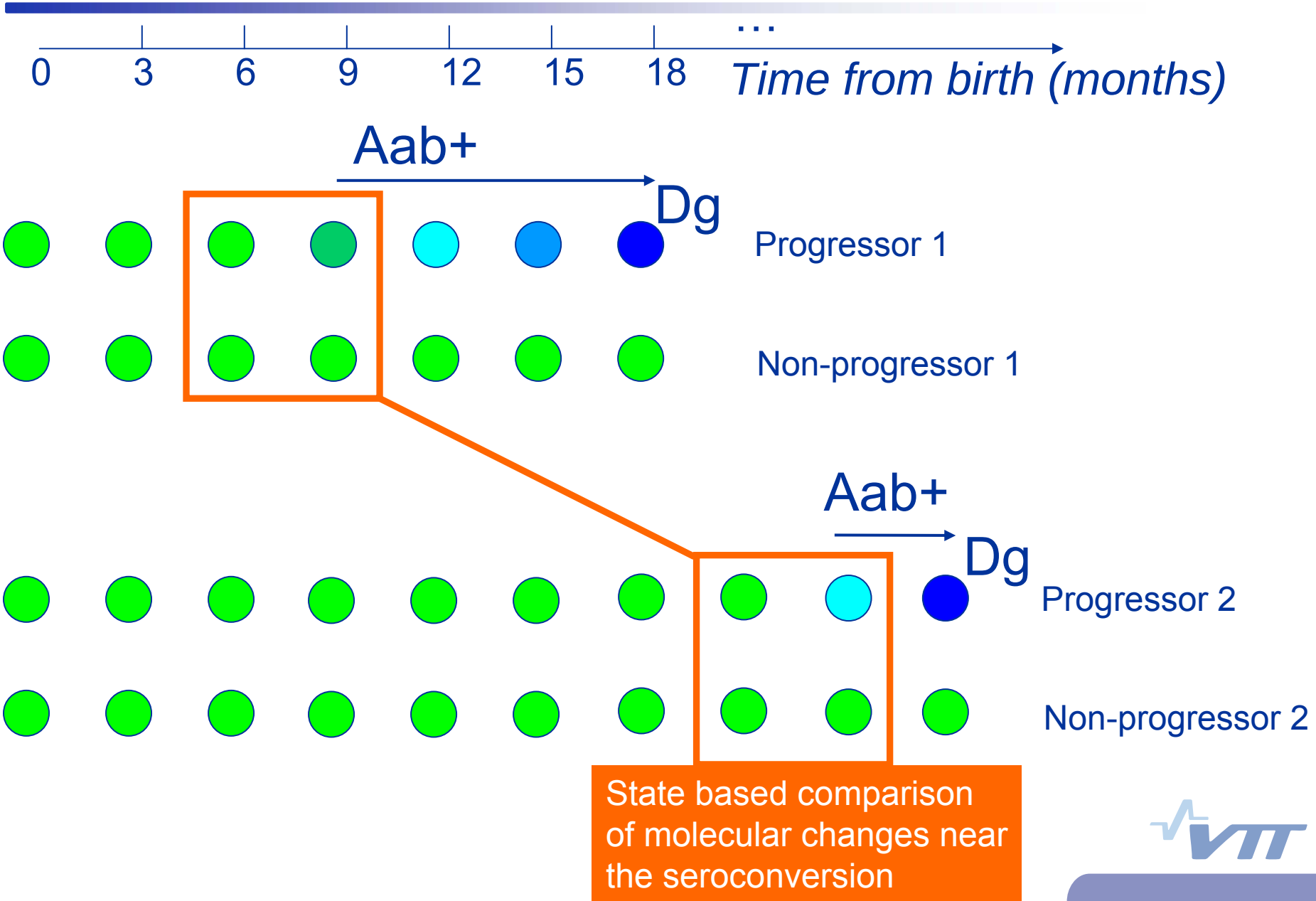


Can differences be explained by genetic risk?

High risk: DR3-DQ2/DR4-DQ8
Medium risk: DR4-DQ8/x
(x = any haplotype except DR2-DQ6, DR5-DQ7 or DR3-DQ2)

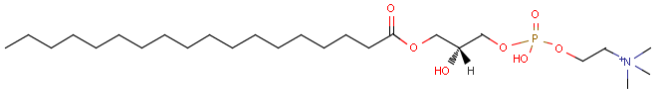
No significant association of HLA-associated genetic risk and the lipid profiles

Seroconversion for islet autoimmunity

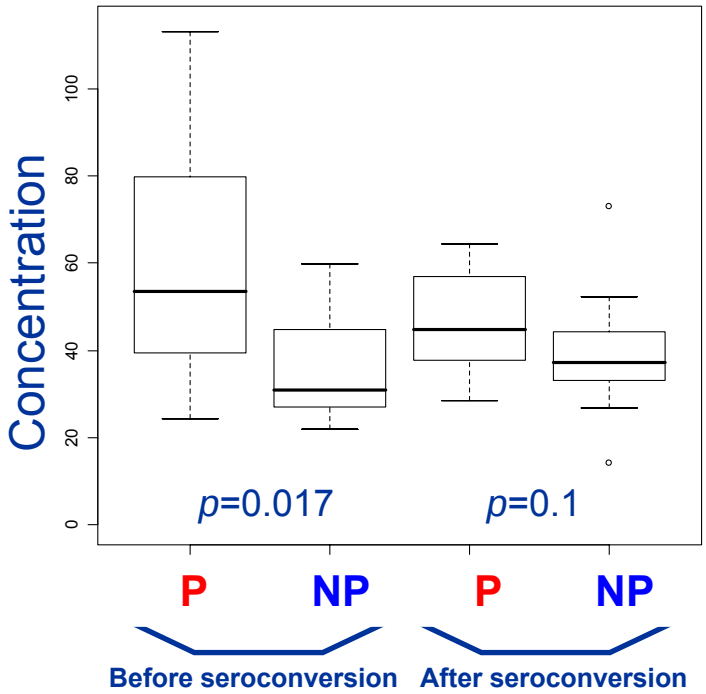


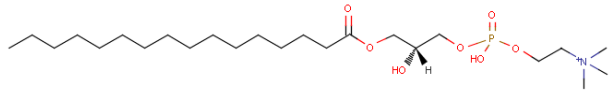
Lipidomic profiles near seroconversion for islet autoimmunity

Elevated lysophosphatidylcholines

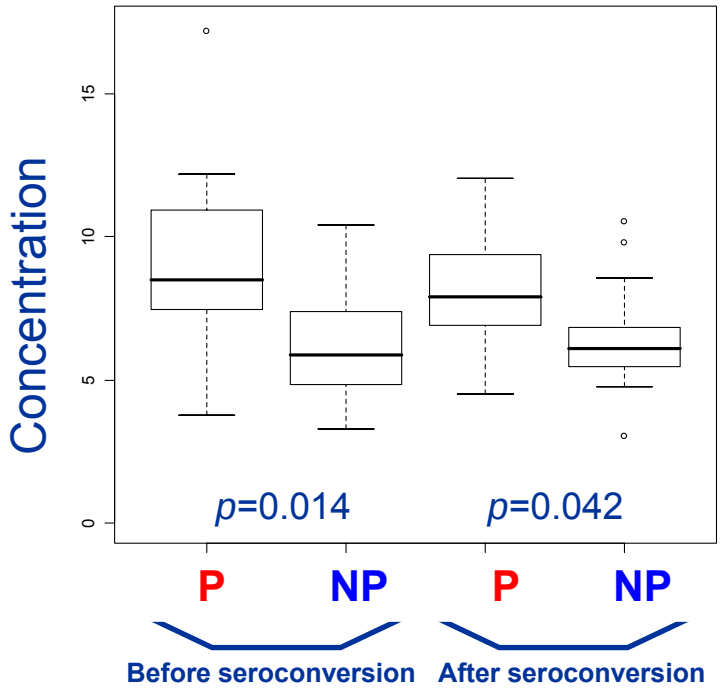


GPCho(18:0/0:0)





GPCho(16:0/0:0)



Progressors 3-6 months prior to seroconversion (Ser-) and 3-6 months after seroconversion (Ser+), with matched non-progressors.

Implications

- Our findings clearly favor early immunomodulation, rather than immunosuppression, as a preventive therapy, with the aim to boost the beneficial component of autoimmunity.

- The factors leading to metabolic stress and autoimmune responses clearly need to be investigated in further studies in the context of autoimmune diseases in general.

Drug response phenotyping

Statins

- Lipid lowering drugs
- Reduction in atherosclerotic complications
- Higher doses of statins are being recommended today for lowering of cholesterol

→Increased risk of myopathy (muscle toxicity)

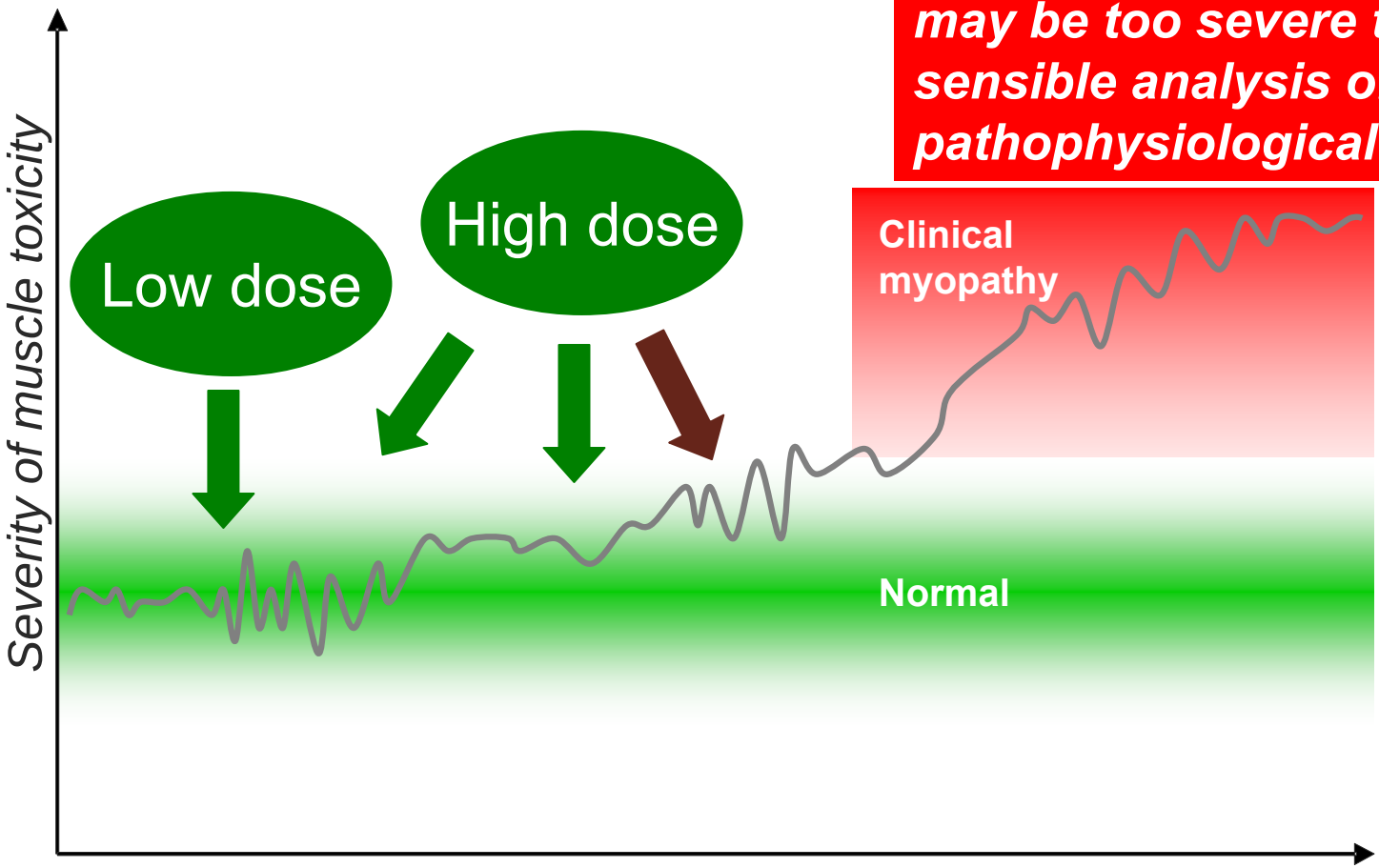
- Mechanisms or biomarkers of myopathy not known

Statin induced muscle toxicity

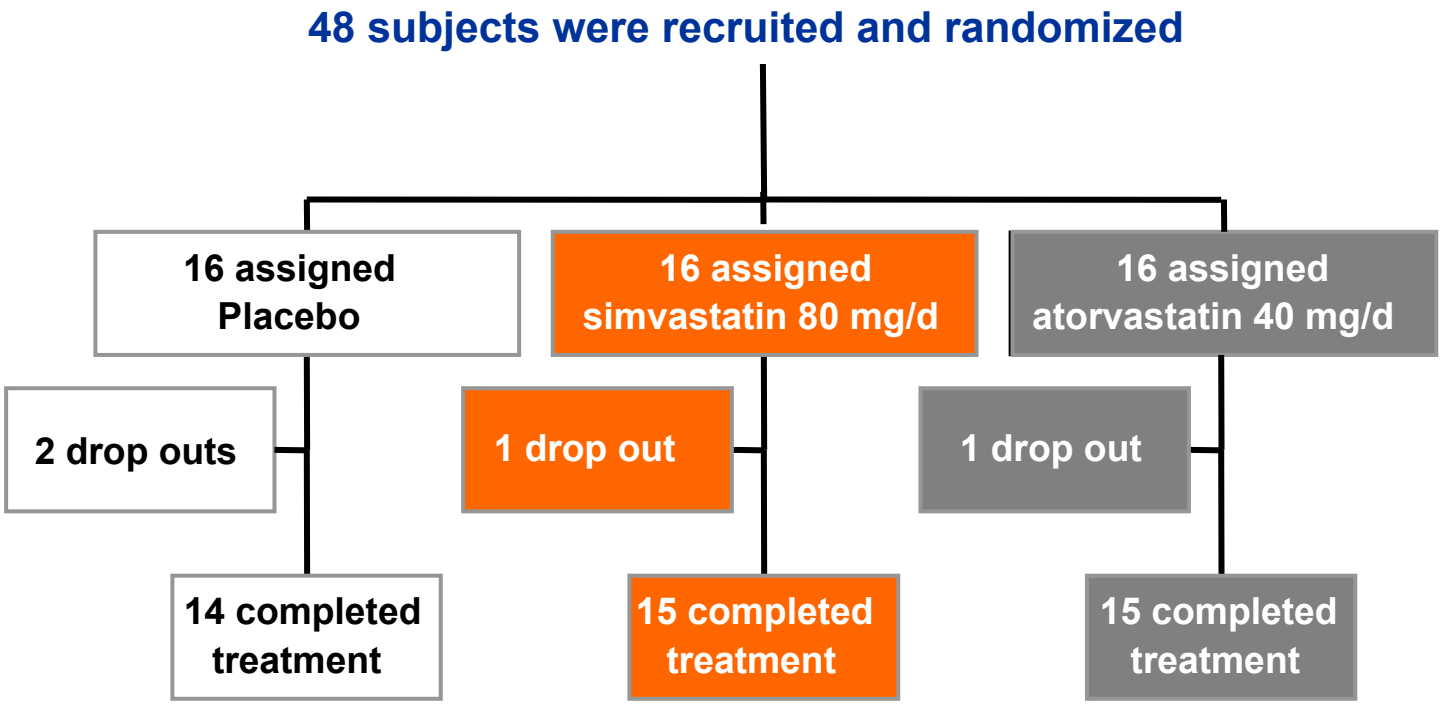
- Muscle complaints without creatine kinase elevations occur in 5-10% of patients in clinical trials
  - However, the complaints occure with similar frequency in statin and placebo groups, thus it is commonly believed they are not drug related
- In recent PRIMO study (N=7924, high dose statins) 10.5% patients complained of muscle pain, highest rate of 18% associated with simvastatin treatment

High dose statin trial – strategy to elucidate early mechanisms of statin induced myopathy

Myopathy cases: Muscle damage may be too severe to permit sensible analysis of specific pathophysiological phenomena



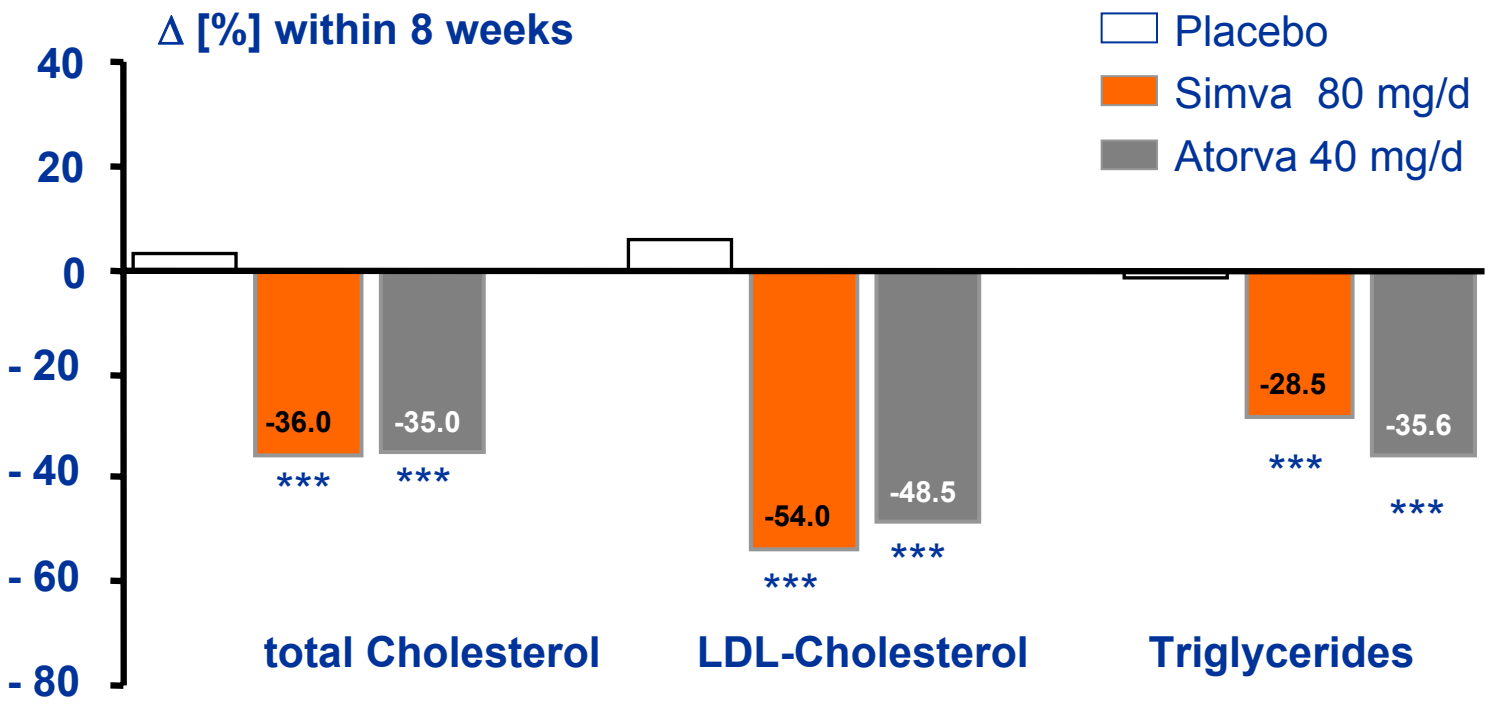
Study design



Muscle biopsies were obtained at baseline and at the end of 8 weeks follow-up

H. Päivä et al, Clin Pharmacol Ther (2005)

Plasma lipids

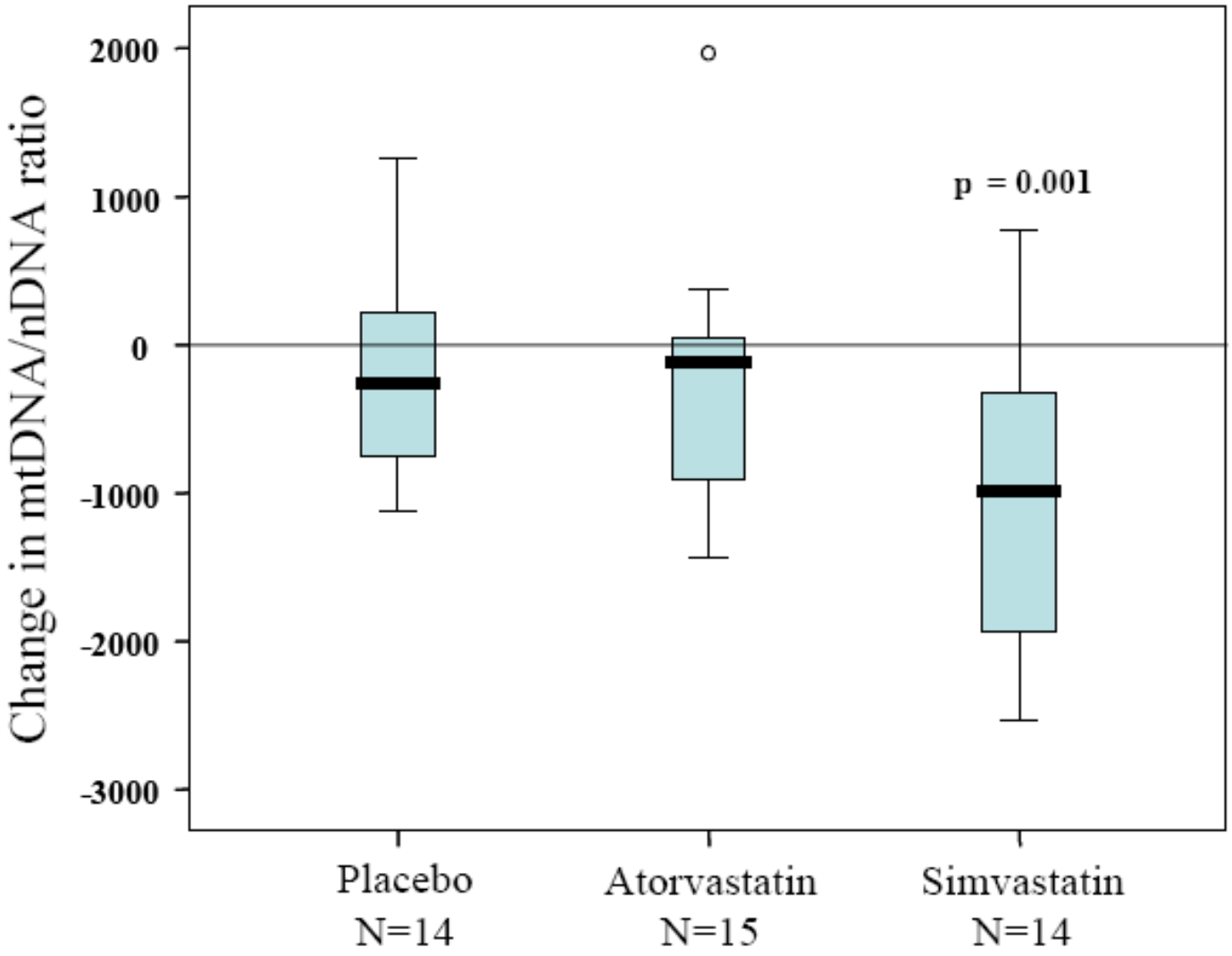


* $P < 0.05$ Paired t-test -
** $P < 0.01$ baseline vs. endpoint
*** $P < 0.001$ within groups

H. Päivä et al, Clin Pharmacol Ther (2005)

Mitochondrial DNA in skeletal muscle

Figure 1. Changes in mtDNA/nDNA ratio after 8 weeks of high-dose statin treatment.



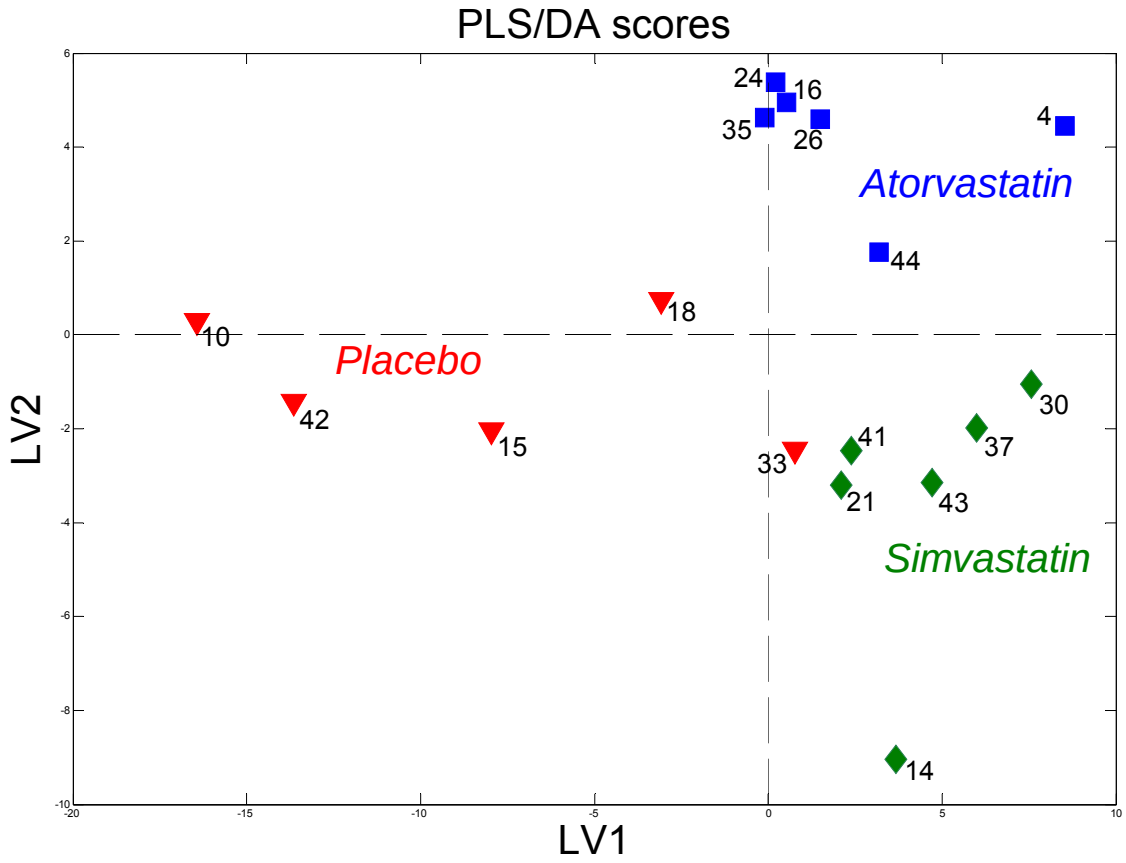
BA Schick et al (Clin Pharmacol Ther. 2007)

Pathway analysis; GSEA (muscle)

- No significant changes in placebo or atorvastatin groups
- Several upregulated pathways in simvastatin group (FDR q<0.1)

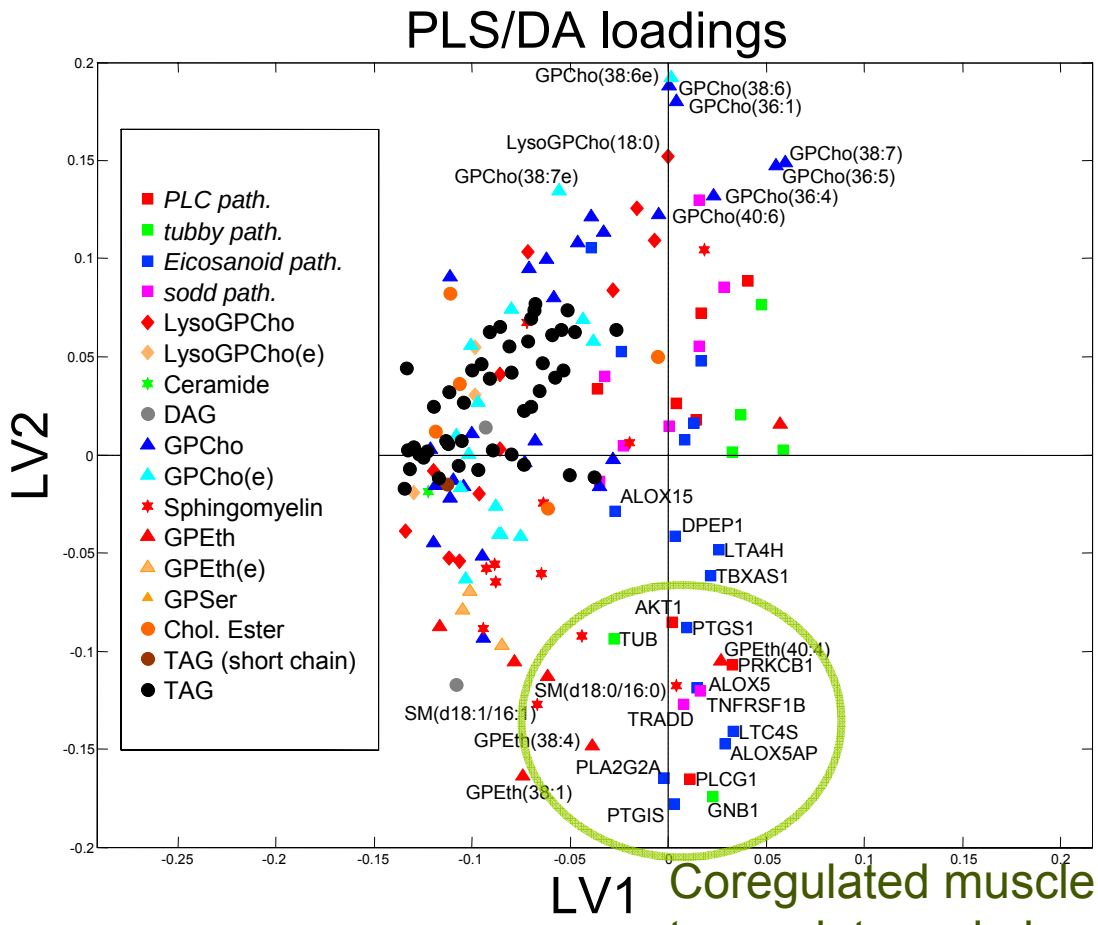
NAME	Source	SIZE	ES	NES	NOM p-val	FDR q-val
ST_T_Cell_Signal_Transduction	Signalling Transduction KE	45	0,58	1,68	0,000	0,055
soddPathway	BioCarta	10	0,83	1,69	0,014	0,060
Eicosanoid_Synthesis	BioCarta	15	0,79	1,66	0,000	0,071
plcPathway	BioCarta	8	0,78	1,69	0,000	0,072
tubbyPathway	BioCarta	7	0,70	1,70	0,000	0,076
caspasePathway	BioCarta	23	0,68	1,65	0,008	0,077
CR_DEATH	Brentani PNAS 2003 (31)	76	0,53	1,64	0,005	0,079
hivnefPathway	BioCarta	58	0,56	1,64	0,000	0,085
ephA4Pathway	BioCarta	10	0,86	1,61	0,004	0,085
deathPathway	BioCarta	33	0,59	1,61	0,005	0,089
MAP00590_Prostaglandin_and_leukotriene_metabolism	GenMAPP	19	0,72	1,62	0,005	0,089
nkcellsPathway	BioCarta	20	0,64	1,60	0,000	0,090
SA_CASPASE_CASCADE	SigmaAldrich	19	0,63	1,59	0,000	0,091
rac1Pathway	BioCarta	22	0,73	1,62	0,004	0,092
ST_Dictyostelium_discoideum_cAMP_Chemotaxis_Pathway	Signalling Transduction KE	33	0,64	1,61	0,007	0,093
nktPathway	BioCarta	29	0,62	1,62	0,018	0,094
eosinophilsPathway	BioCarta	8	0,77	1,58	0,025	0,094
tall1Pathway	BioCarta	15	0,56	1,60	0,003	0,095
il17Pathway	BioCarta	15	0,78	1,57	0,023	0,095
CBF_LEUKEMIA_DOWNING_AML	Manually Curated	75	0,60	1,58	0,023	0,097
ureacyclePathway	BioCarta	7	0,87	1,57	0,008	0,098
cell_motility	GO	116	0,61	1,58	0,014	0,098
MAP00562_Inositol_phosphate_metabolism	GenMAPP	20	0,65	1,57	0,032	0,099

PLS/DA on combined serum lipid and muscle gene expression data (4 pathways: PLC, eicosanoid, sodd, and tubby)



Laaksonen et al, PLoS ONE (2006)

PLS/DA on combined serum lipid and muscle gene expression data (4 pathways: PLC, eicosanoid, sodd, and tubby)

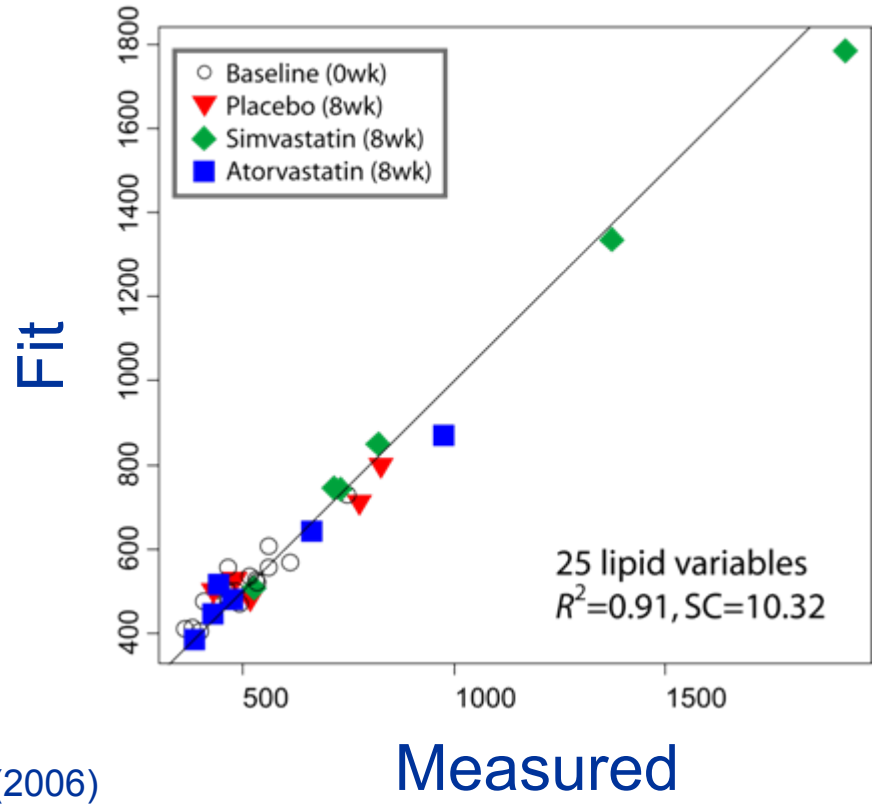


Coregulated muscle transcripts and plasma lipids in simvastatin group

Laaksonen et al, PLoS ONE (2006)

lasso regression of lipid data on ALOX5AP

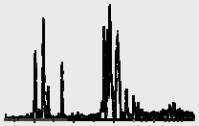
- Shrinkage regression method, which performs continuous variable selection causing some of the regression coefficients to be exactly zero
- Shrinkage reduces the variance of the regression estimates



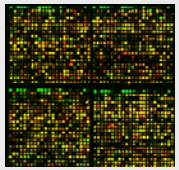
Laaksonen et al, PLoS ONE (2006)

Concept: Drug response phenotyping using systems approach

Controls

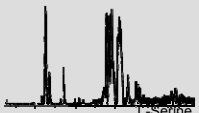


Metabolic profiles

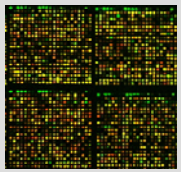


Transcriptional and other data

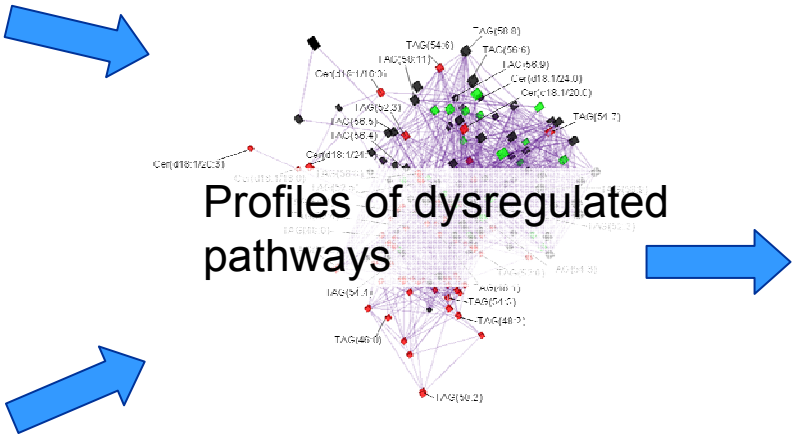
Drug intervention

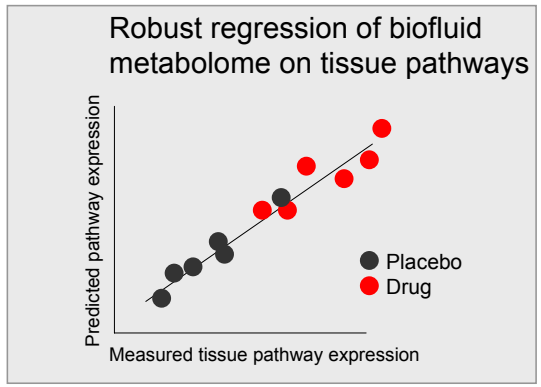


Metabolic profiles



Transcriptional and other data





Extend biofluid metabolomics to monitor tissue sensitive biomarkers in larger populations

Summary

- Medical systems biology aims to elucidate complex networks linking phenotypes with genes and environment
- Biofluid metabolome is a quantitative measure of the phenotype
- Metabolic phenotype depends on genetic factors reflected in host and microbial cells
- Changes of metabolic phenotypes can be described in terms of metabolic states
- Autoimmunity may be physiological and beneficial response to metabolic stress
- Tissue-specific drug responses are reflected in changes in metabolic phenotypes

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- DIPP parents & children

Gut microbiota

- Fredrick Bäckhed

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