HelmholtzZentrum münchen
German Research Center for Environmental Health

# From Omics to Systems Biology – An Approach to Individuallized Medicine


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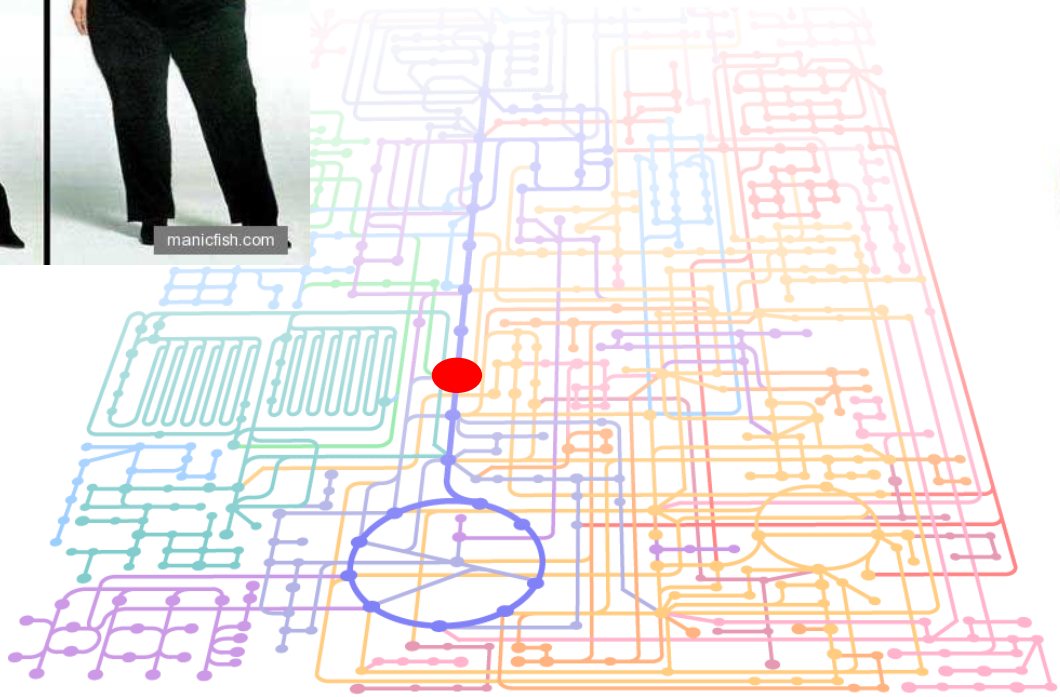
HELMHOLTZ ASSOCIATION

# Combining GWAs and Metabolomics in human serum

- We published several manuscripts combining GWAs and Metabolomics in human serum (**Gieger et al., 2008, Plos Genetics; Illig et al., 2010 Nat Genet; Suhre et al., 2011, Nature; Mittelstrass et al. 2011 Plos Genetics)**
- We found links to complex diseases and pharmacogenomics
- We postulate to treat population groups differentially according to their metabolomic profiles

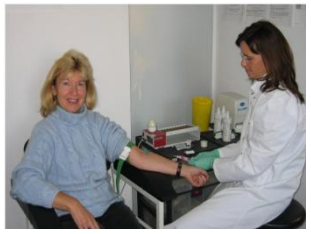
# What makes us different?


# What makes us different?

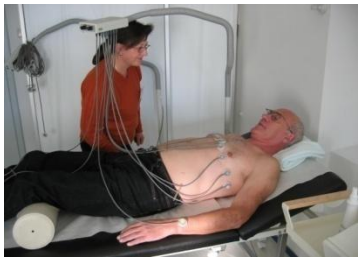



# KORA
## Cooperative Health Research in the Region of Augsburg

**Ressource**



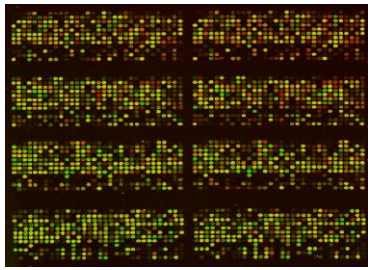
- Cohort study (**18,000 participants**, recruitment age 25 – 74 y) Recruitment 1985, 90, 95, 2000 **(S1-S4)**;
- Follow-up questionnaires 1995, 2000 (all participants)
- Follow-up study centre 2005 (**KORA F3**), 2008 (**KORA F4**)
- Interview, questionnaire, physical measurements, blood, urine, serum, plasma, DNA

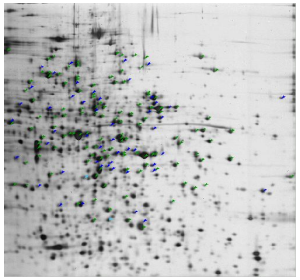


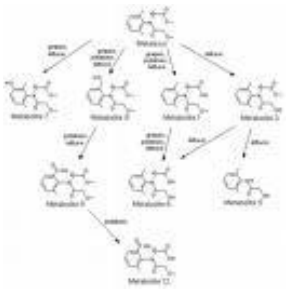
# Challenges in Molecular Epidemiology
# The -omics era - Integration of data

**Challenge:**
**Sequencing**
**of exons and genomes**

**DNA Variation**







**diabetes**

**Many scientists**
**DDZ and HMGU**

**RNA expression** → Proteomics → **Metabolomics**

Epigenetics

- Better understanding of pathophysiology
- Pathway refinement
- Looking for early markers of disease
- Diagnostics
- Individualized Medicine
- New drugs

allergy

# Performed -omics projects in KORA:

Genomics:

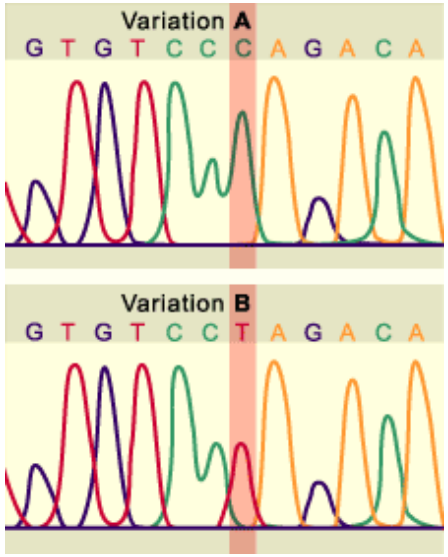
- 4000 GWAs (500 -1000 k) S3/F3, S4/F4
- 11 000 metabochip (200 k), S1, S2, S3/F3, S4/F4
- 4000 cardiochip ( 50 k), S1-S3/F3
- 2000 immunochip (50 k), S4/F4

Transcriptomics: 2500 Illumina 28 k, S4 + F4

Metabolomics: 4000 (163 -300 Metabolites), F3, F4

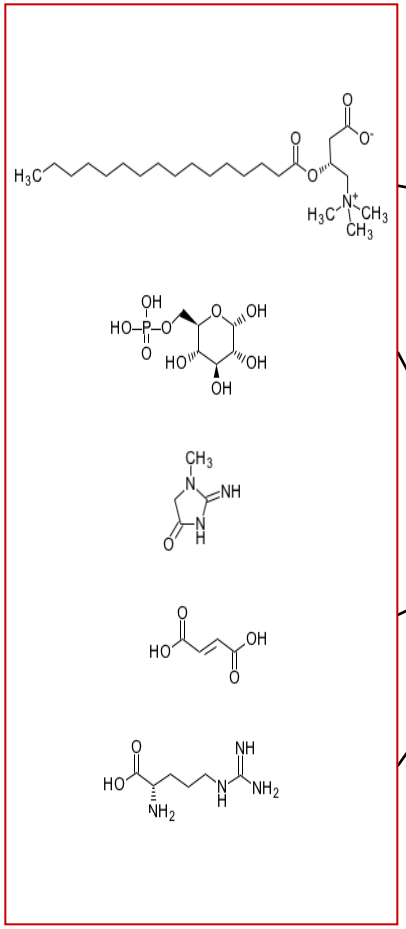
# Combining metabolomics and genomics in KORA

**Genomics (SNPs)** **Metabolomics** **Phenotypes**




GWA of the
KORA F3 + 4 Population
(Affymetrix 500k + 1000k)

500 000 - 1 000.000 SNPs

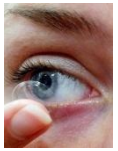


Disease related phenotypes

cardiovascular diseases

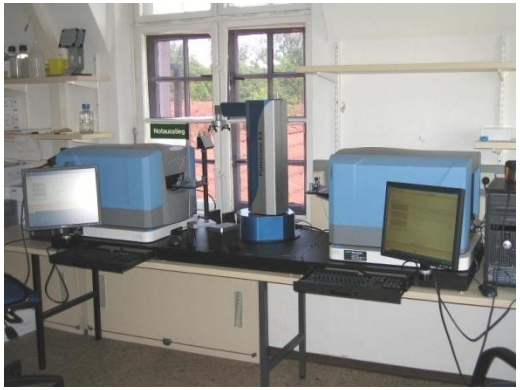
diabetes, obesity

allergy



other ...

# Genotyping Equipment in the Genome Analysis Center



*Illumina*

*SNP Arrays*

Illumina, Affymetrix, Sequenom

*Affymetrix*


*Sequenom*

*Taqman*

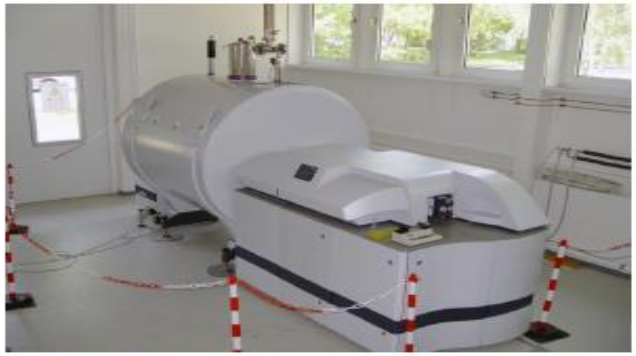
# HMGU metabolomics initiative

metaP

**Partnership of 4 HMGU Institutes: GAC + IBIS + EPI + IOEC**

Hamilton robotics

API 4000 Q Trap



Bruker Apex 12 Tesla

BIOCRATES
LIFE SCIENCES

Products | AbsoluteIDQ™ Kit

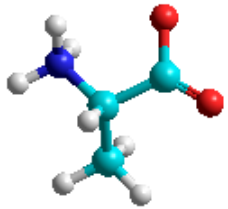
# AbsoluteIDQ™ Kit


## AbsoluteIDQ™ Kit

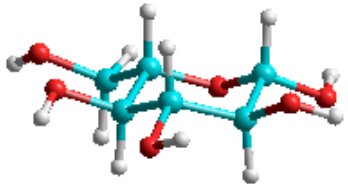
- Keeps your proprietary data in-house.
- Accurately identifies and quantifies over 160 metabolites in over 4 compound classes in just a few minutes per sample.
- Requires very small sample volume (10 µL).

# High throughput targeted metabolomics
# Measured metabolites

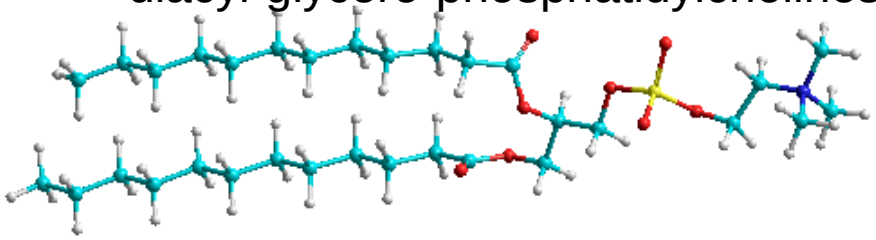
amino acids



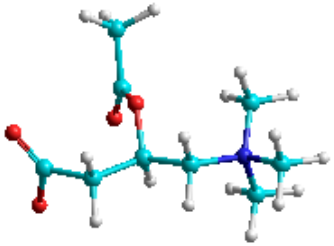
hexose



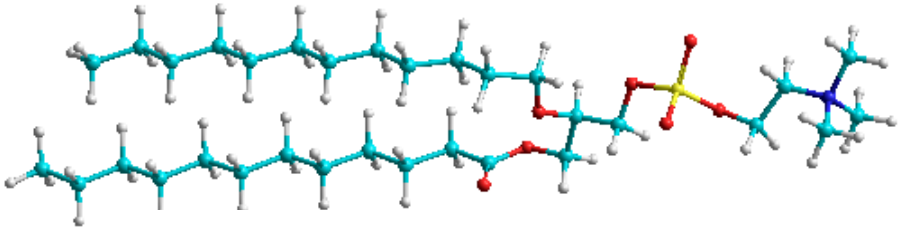
diacyl-glycero-phosphatidylcholines



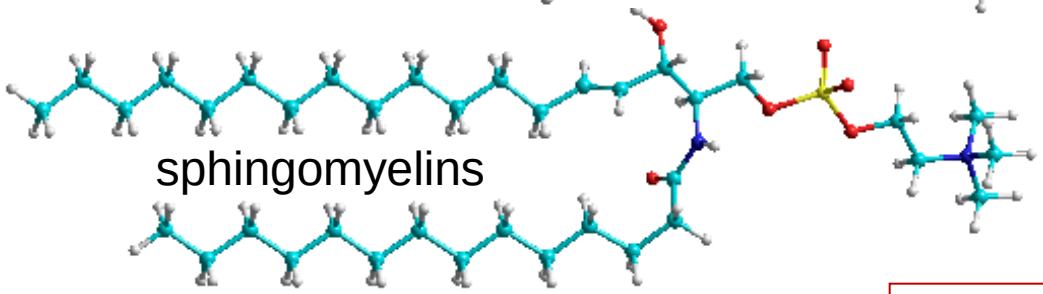
acylcarnitines



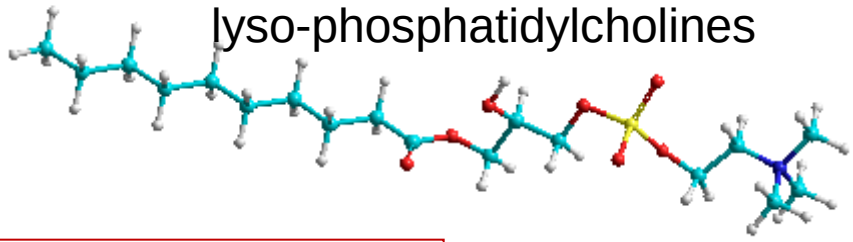
acyl-alkyl-glycero-phosphatidylcholines



sphingomyelins

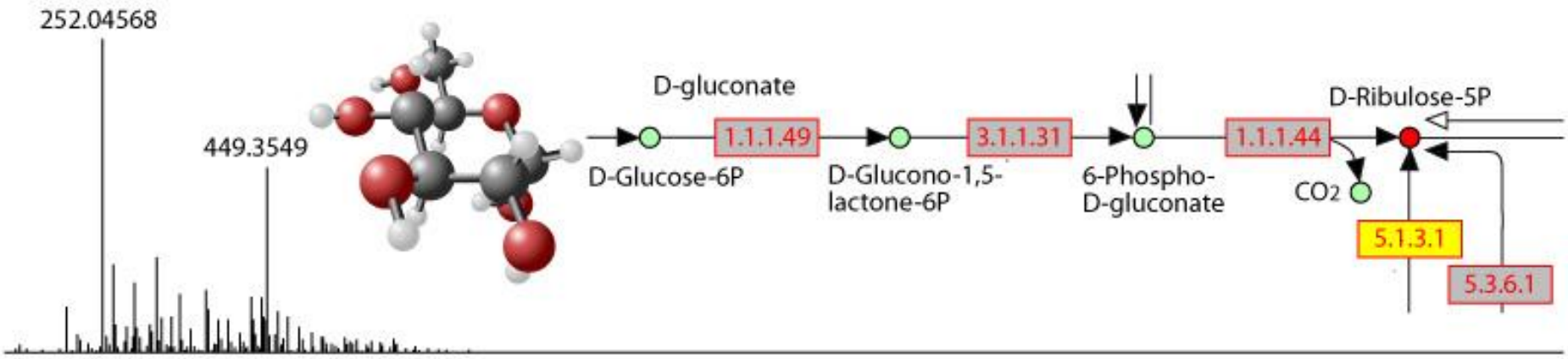


lyso-phosphatidylcholines



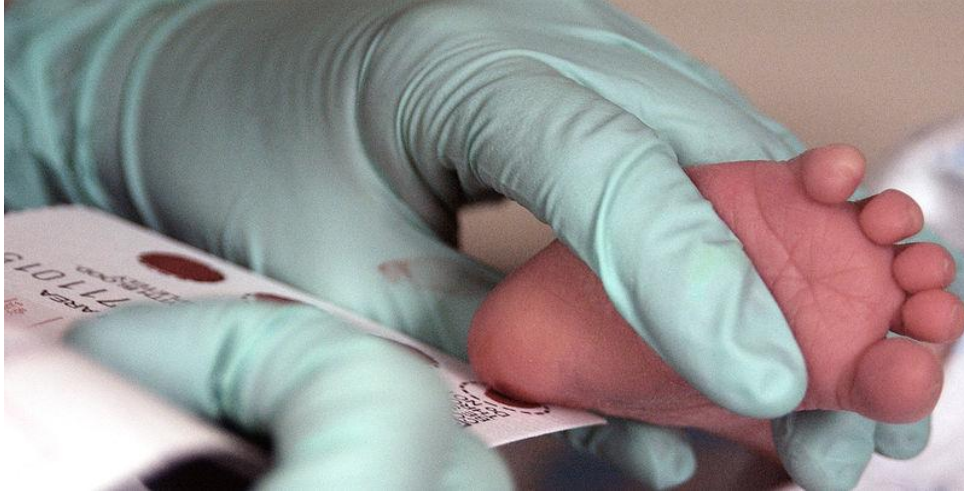
**163 metabolites / sample**

# Metabolomics …




… measuring the true end points of biological processes !

## Start of metabolite research



INBORN ERRORS OF METABOLISM

By

ARCHIBALD E. GARROD, K.C.M.G.

D.M., LL.D., F.R.S., F.R.C.P.

*Regius Professor of Medicine in the University of Oxford*
*Consulting Physician to St. Bartholomew's Hospital*
*and to the Hospital for Sick Children*

*First Edition 1909*

More than 100 years ago, Archibald Garrod already suggested a link between ***chemical individuality*** and ***predisposition to disease***



**Mootha & Hirschhorn, Nat Genet 2010**

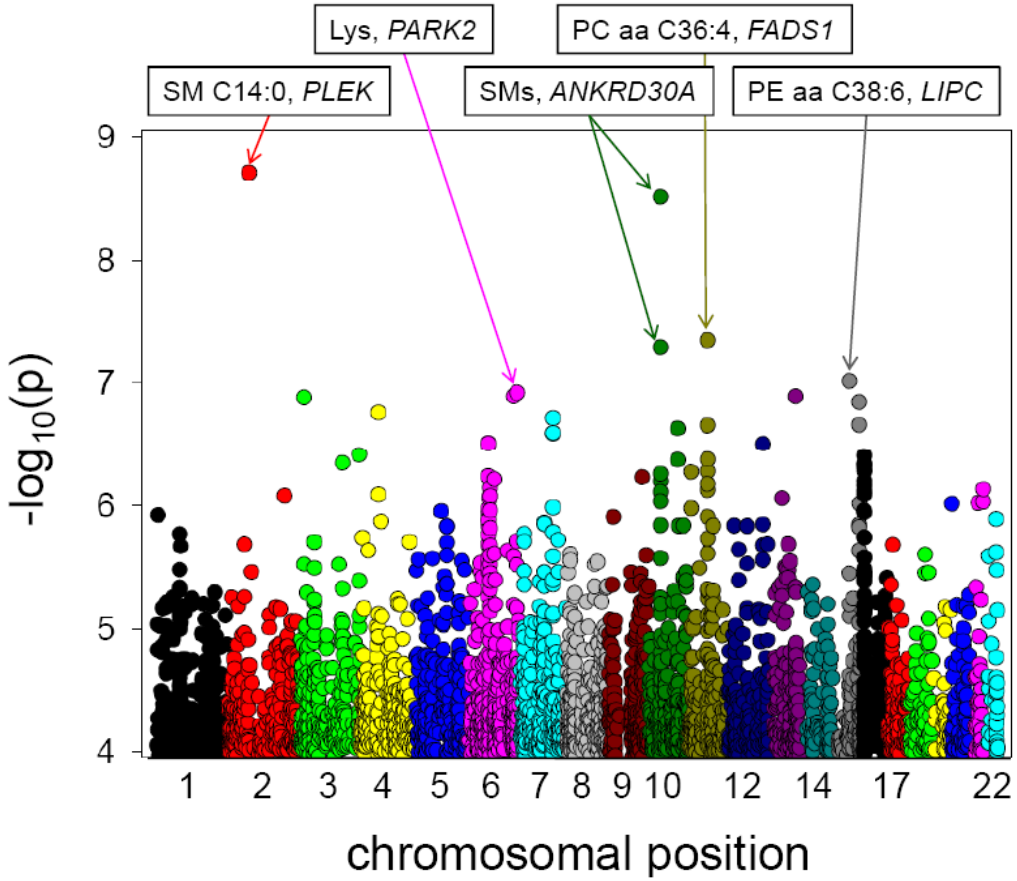
[Inborn errors of metabolism]
… are merely
***extreme examples of variations of chemical behaviour***
which are probably everywhere present in minor degrees

A.E. Garrod, Lancet, 1902

# Genetics of metabolomics in the population (KORA)

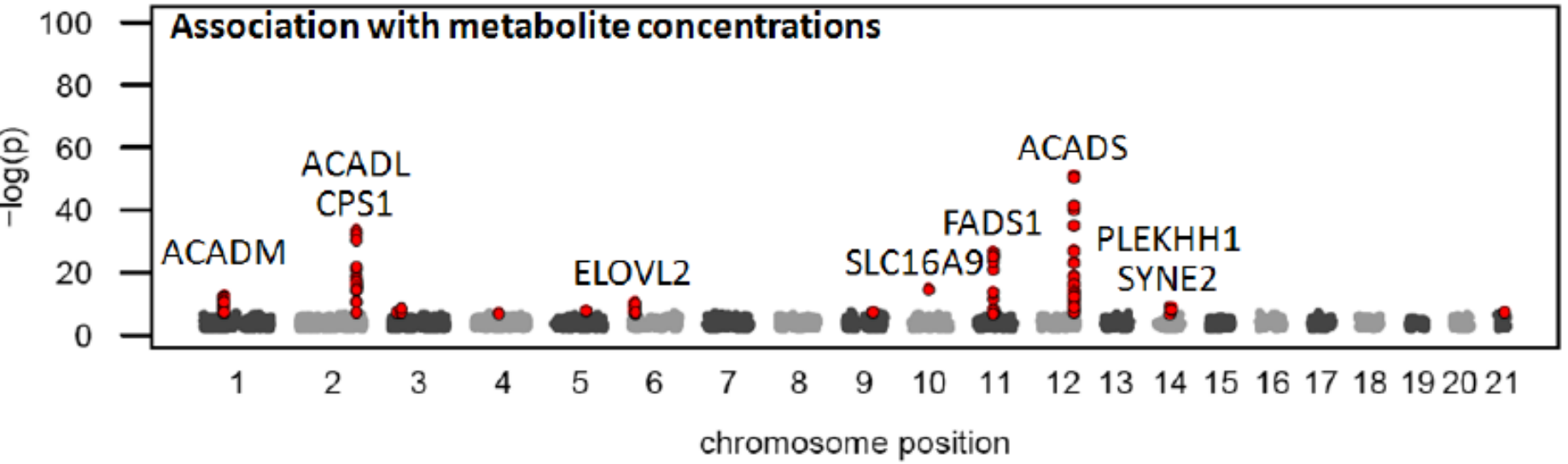
First studies

# Resuts of KORA F3 (288 samples)



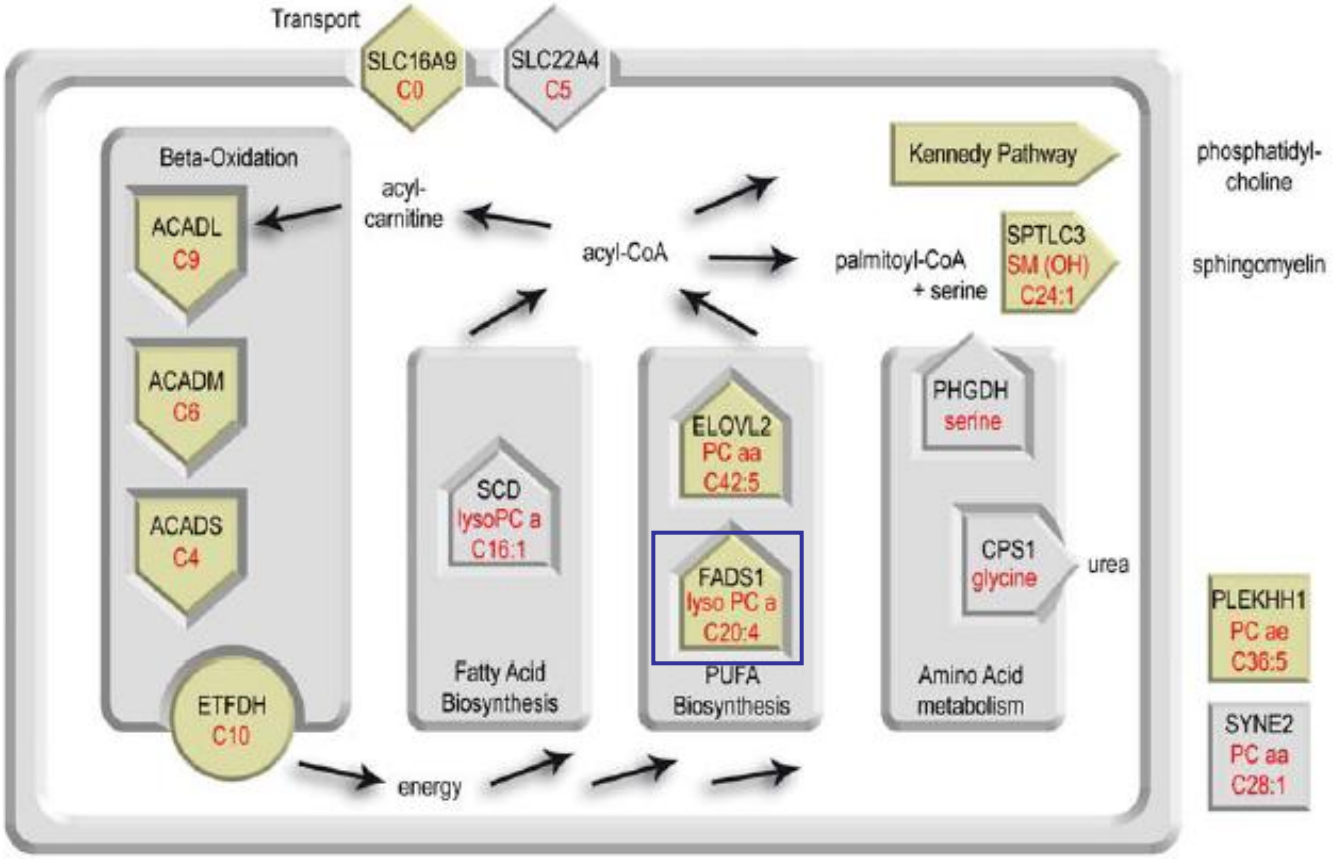

Genome-wide level of significance: $1.33 \times 10^{-9}$

# GWAs in KORA F4 (1800 samples)
# Replication in Twins UK (400 samples)




GWAs significance border $10^{-10}$

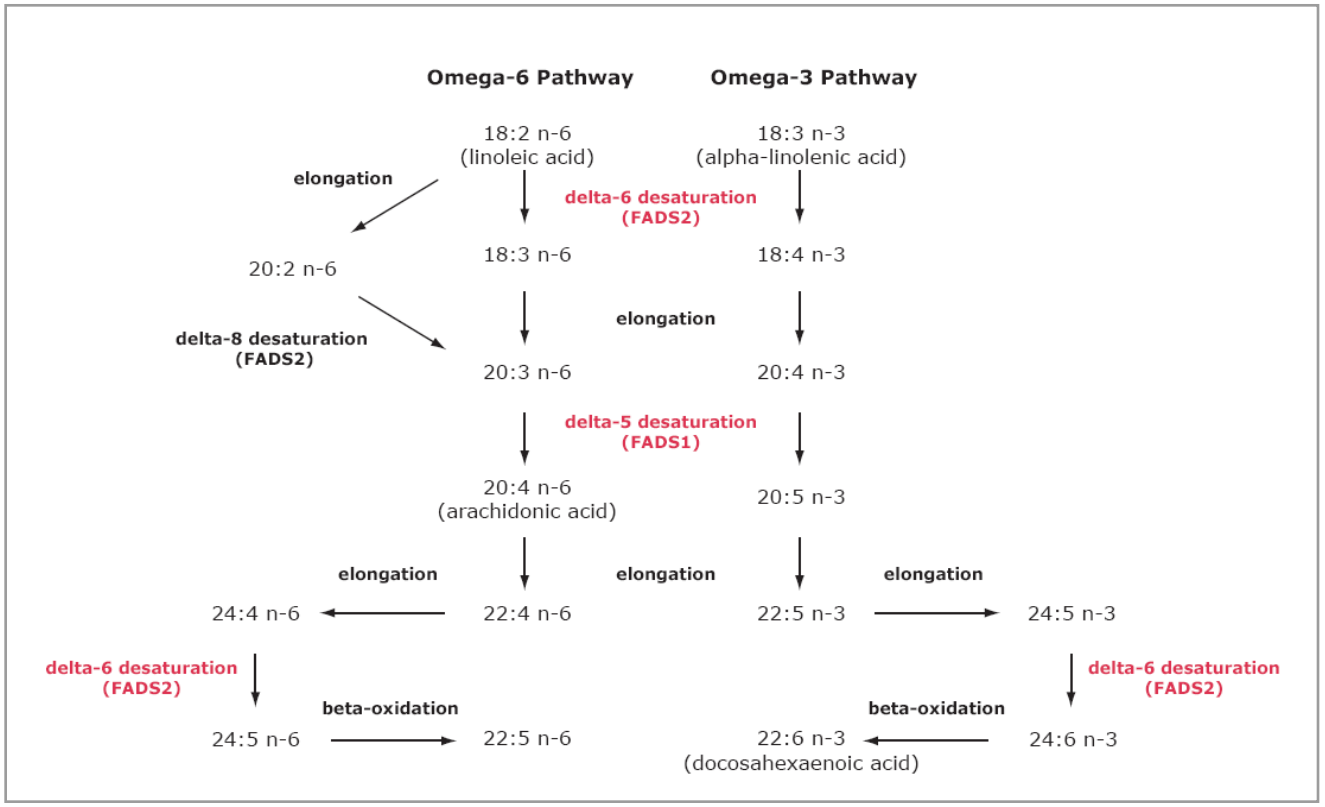
# Summary of detected hits






Illig, et al., Nat Genet, 2010

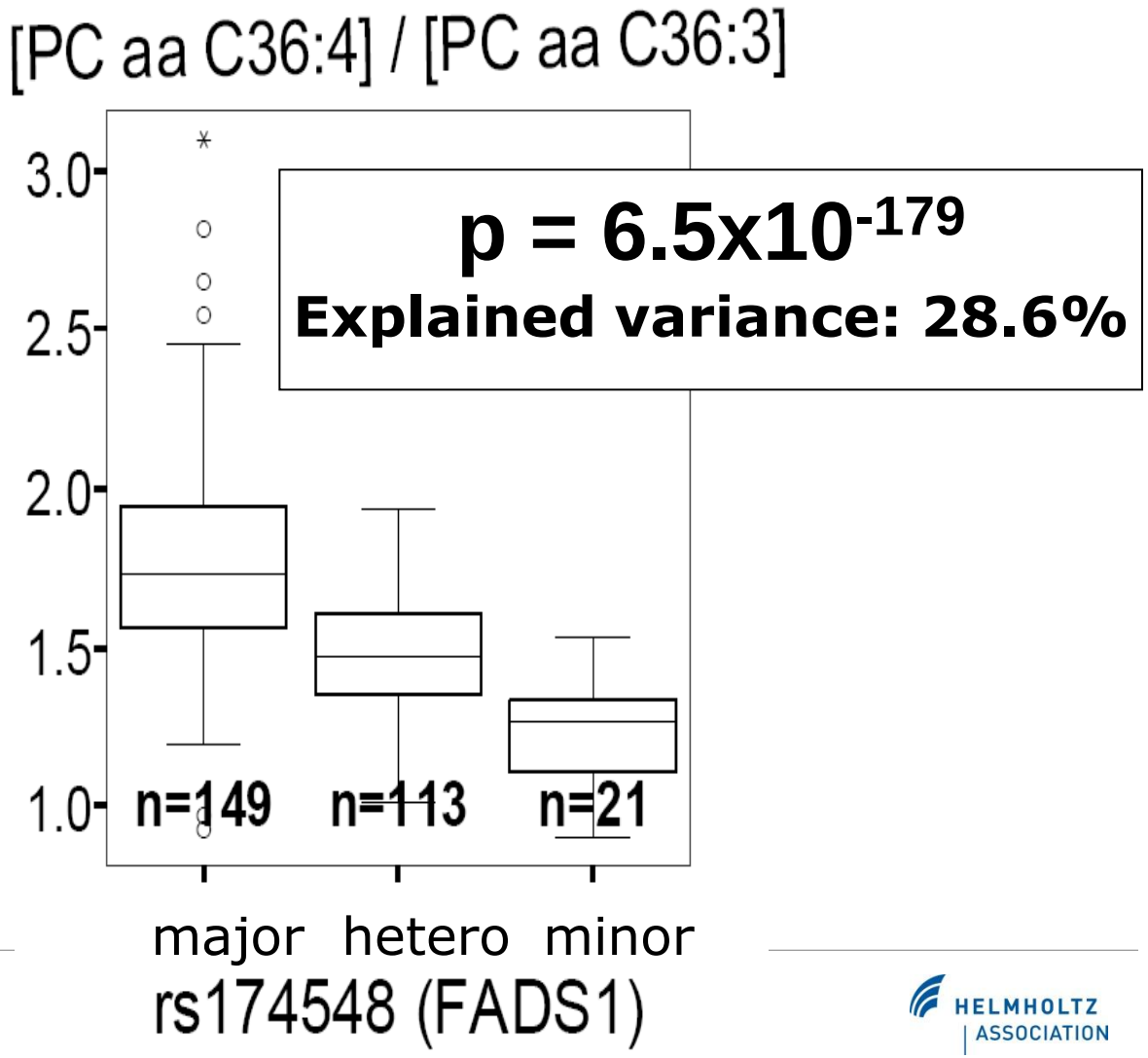
# Function of the delta-5 and delta-6 desaturase (*FADS1* and *FADS2*)




chr. 11

# Strong effects for certain metabolites and metabolite concentrations

FADS gene cluster and phosphatidylcholines



# FADS is associated with other complex phenotypes

**Lipids: Aulchenko et al., 2009, Nat Genet**

**CVD: Martinelli et al., 2008, Am J Clin Nutr**

**Glucose: Dupuis et al., 2010, Nat Genet**

**Intelligence: Caspi et al., 2007, Proc Natl Acad Sci**

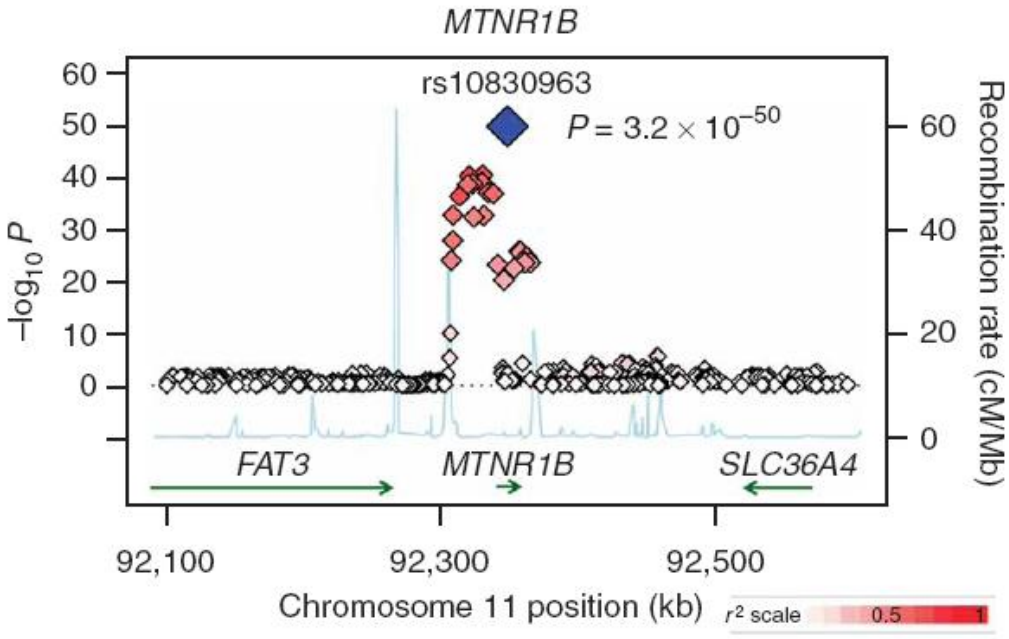
**Attention deficit hyperactivity syndrome: Brookes et al., 2006, Biol Psychiatry**

**Allergic diseases: Lattka et al., 2009, Nutrigenet Nutrigenomics**

Metabolomics as one of the missing links

# Connection of gene – metabolite - association for type 2 diabetes Melatonin receptor 1 B (MTNR1B)

- **MTNR1B expressed in human islets**
- **circadian rhythmicity in melatonin release**
- **circadian patterns in insulin release**
- **MTNR1B mediates inhibitory effect of melatonin on insulin secretion**
- **increased expression of MTNR1B in T2D subjects**






Prokopenko et al. Nat Genet, 2009

# How can gene - metabolite - associations help us in better understanding type 2 diabetes?

| Gene association from international GWAs | Association in our screen |
|---|---|
| **Melatonin-receptor (MTNR1B) associates with fasting glucose and type 2 diabetes (Prokopenko 2009)** | **The same SNP associates in this study with tryptophan and phenylalanine. Tryptophan is a precursor of melatonin (Illig et al., 2010)** |

***Further selected examples:***

***APO*-cluster: apolipoprotein**
Known: blood triglyceride levels ($p<10^{-60}$)
New: PC aa C36:2/PC aa C38:1 ($p=1.8x10^{-11}$)

***GCKR :* glucokinase (hexokinase 4) regulator**
Known: fasting glucose ($p=8x10^{-13}$) and triglyceride ($p=1x10^{-4}$)
New: PC ae C34:2/PC aa C32:2 ($p=3.2x10^{-8}$)

# From Lipidomics to Metabolomics

- About 300 markers from differnt pathways (Metabolon marker set)
- Amino acids
- Carbohydrates
- Cofactors and vitamins
- Metabolites of energy metabolism
- Lipids
- Nucleotides
- Xenobiotics

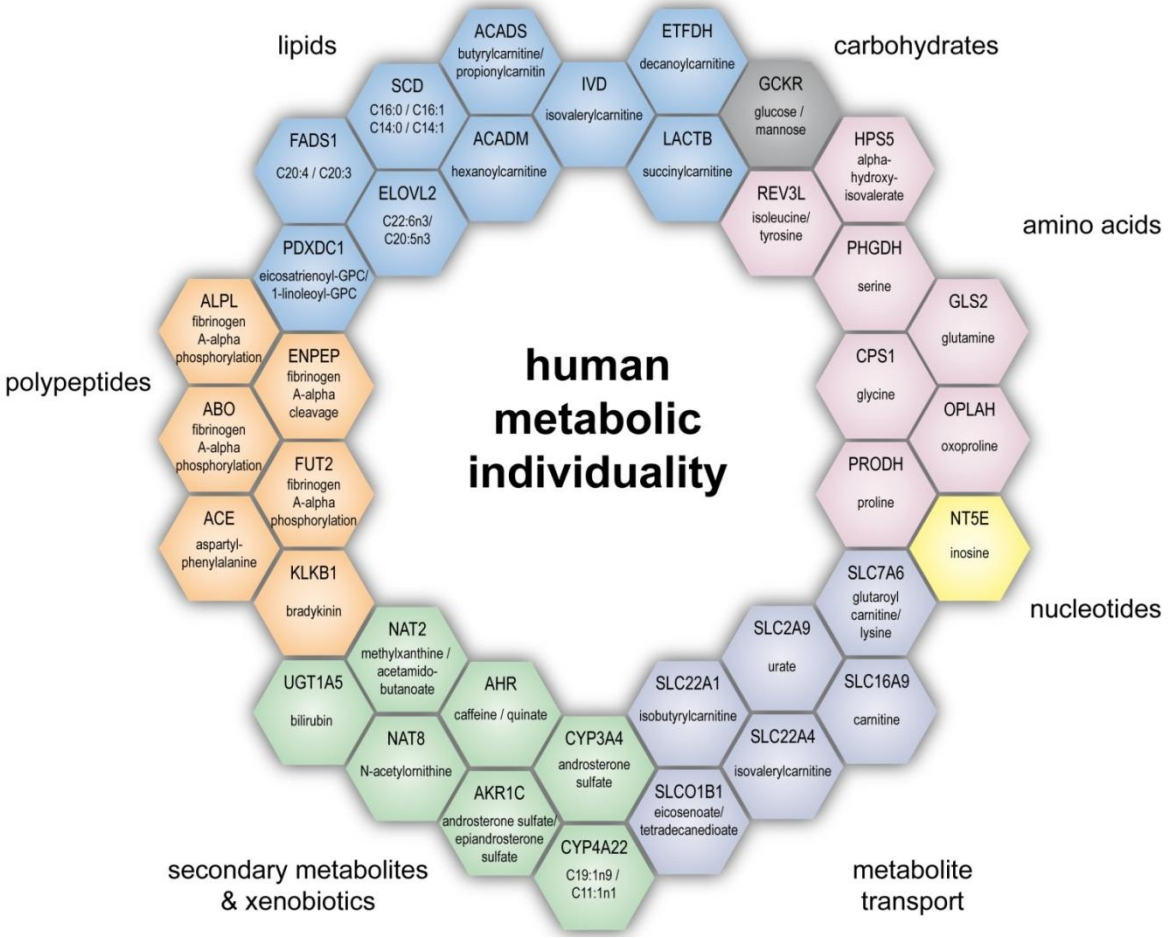
# What did we find?

- GWAs in KORA F4 (1786 samples)
- Replication in Twins UK (1056 samples)
- 37 loci with genome wide significance ($10^{-12}$)
- 24 new loci
- 13 replications
- In all regions good candidates with enzymes linked to the metabolites
- 16 cases of associations with disease or pharmacogenetic effects
- Explained variability for 25 loci between 10 and 60% (very strong effects)

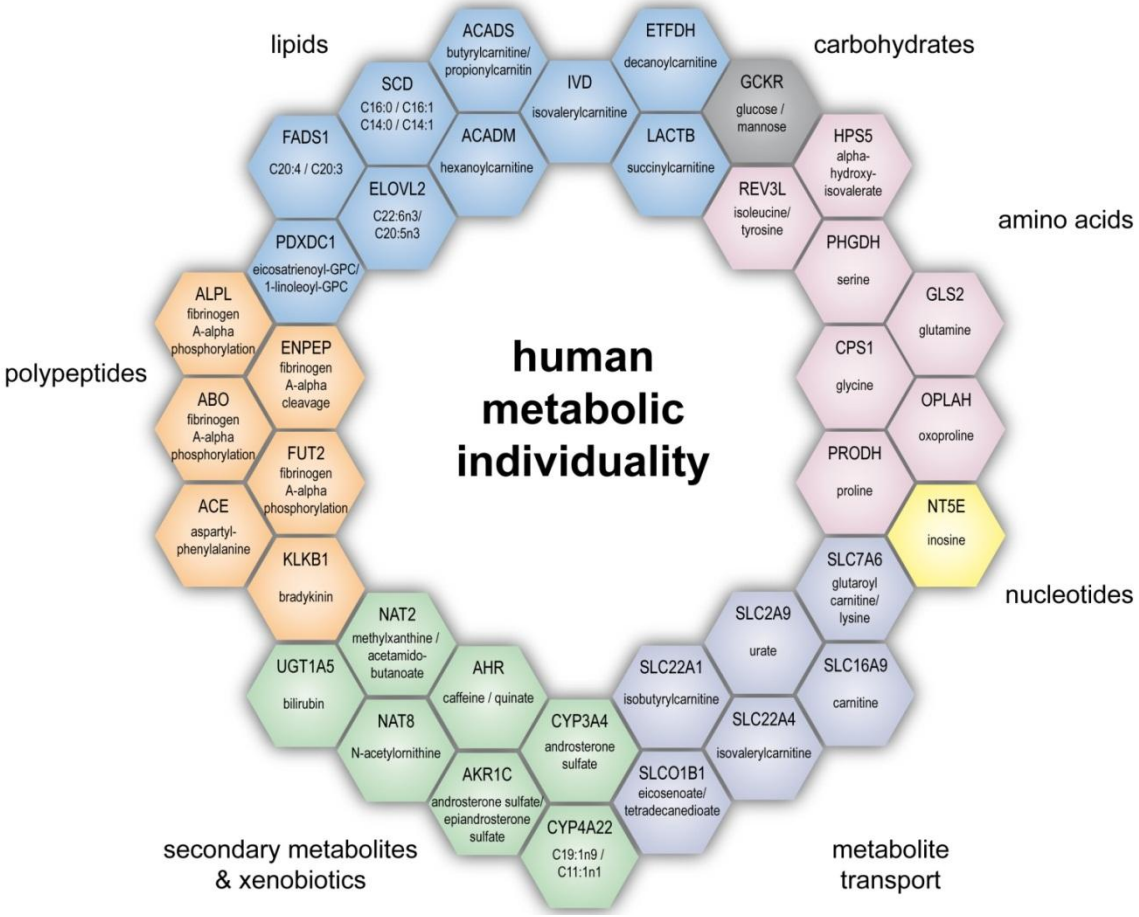


Suhre et al., Nature in resubmission

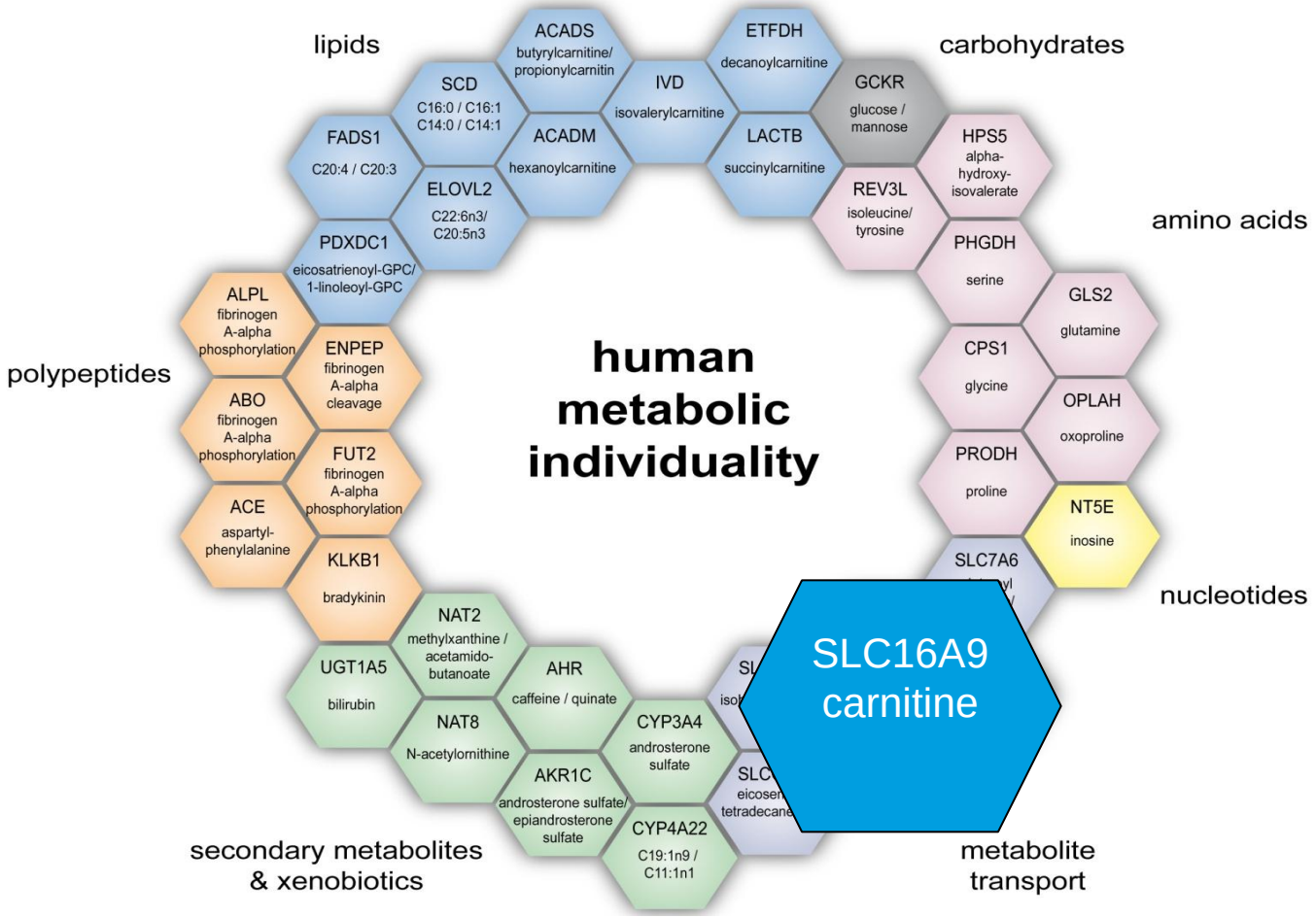
# Detected hits



# Detected hits



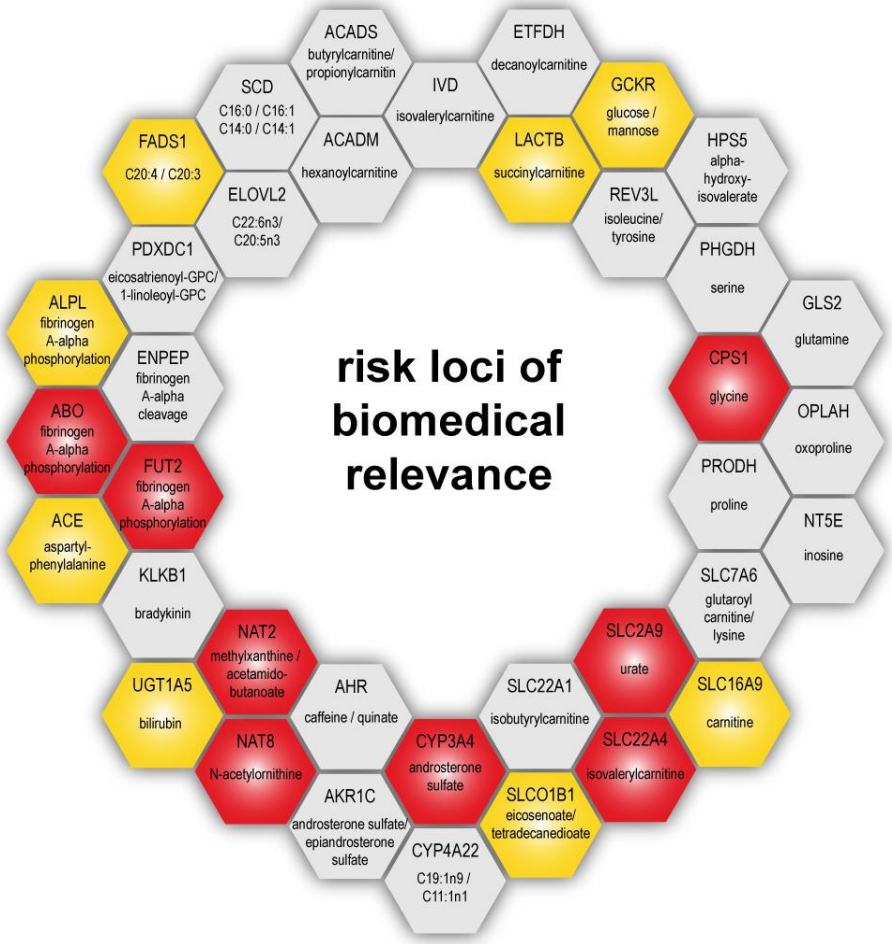
# Main results
# Explaining function of gene products



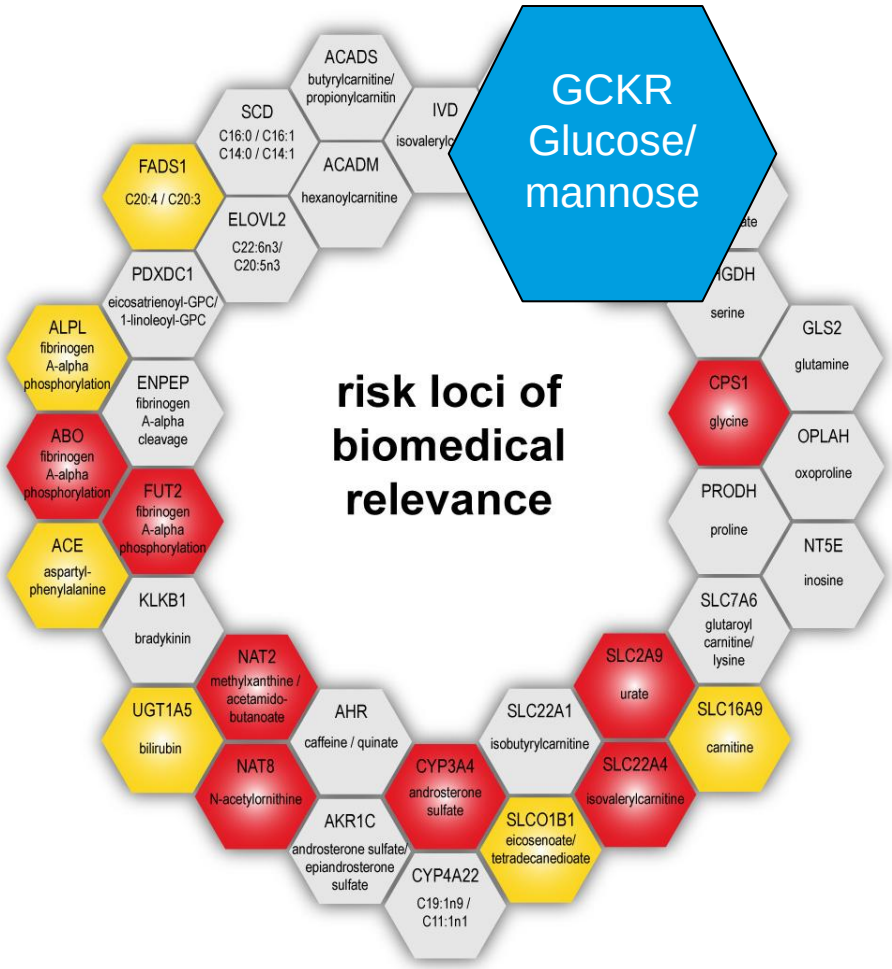
# Explaining function of gene products

- Association of SLC16A9 with carnitine
- Function: monocarboxylic acid transporter
- Functional test in Xenopus oocytes: [$^{3}$H] carnitine uptake by the protein
- Result: SLC16A9 is a sodium and pH-dependent carnitine efflux transporter

# Risk loci of biomedical relevance



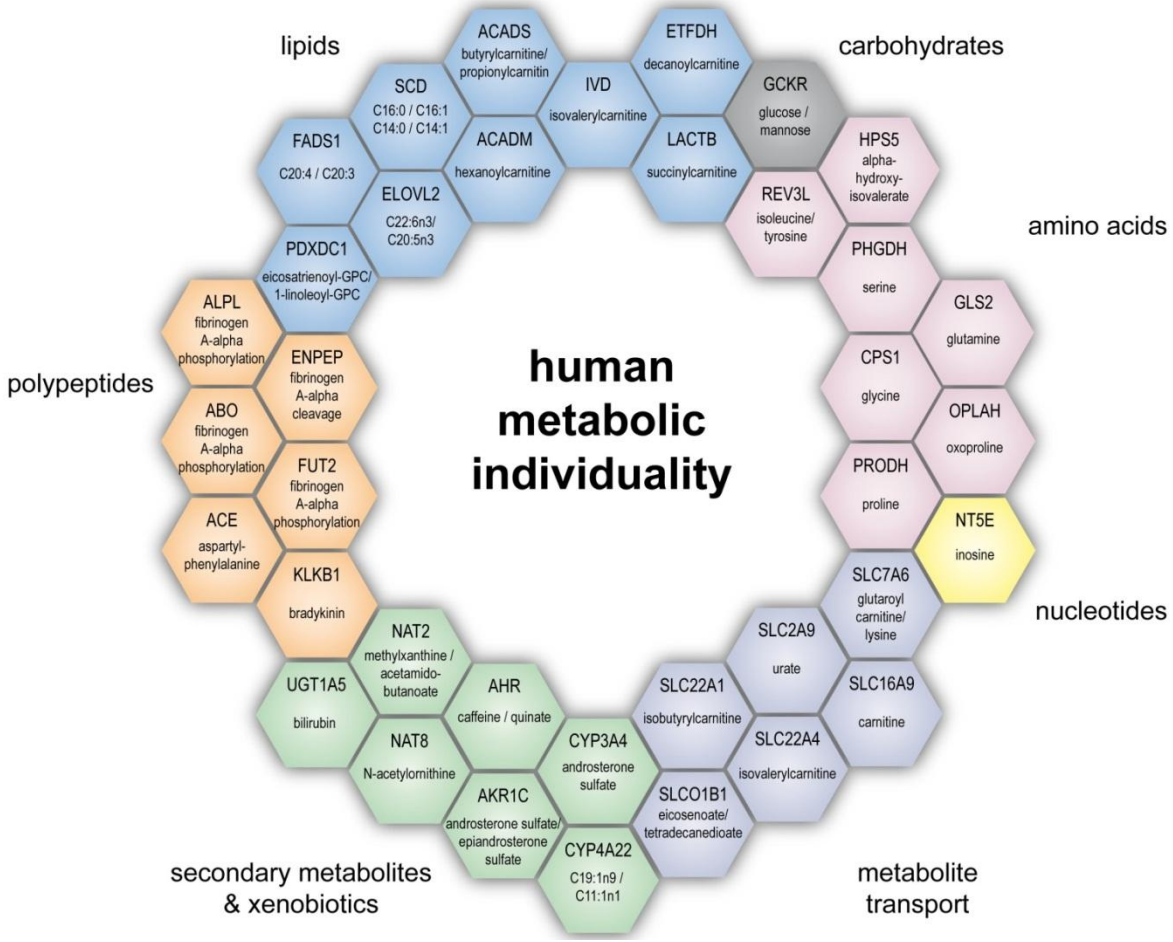
# Diabetes


GCKR
Glucose/
mannose
risk loci of
biomedical
relevance
ACADS
butyrylcarnitine/
propionylcarnitin
SCD
C16:0 / C16:1
C14:0 / C14:1
ACADM
hexanoylcarnitine
IVD
FADS1
C20:4 / C20:3
ELOVL2
C22:6n3/
C20:5n3
PDXDC1
eicosatrienoyl-GPC/
1-linoleoyl-GPC
ALPL
fibrinogen
A-alpha
phosphorylation
ENPEP
fibrinogen
A-alpha
cleavage
ABO
fibrinogen
A-alpha
phosphorylation
FUT2
fibrinogen
A-alpha
phosphorylation
ACE
aspartyl-
phenylalanine
KLKB1
bradykinin
NAT2
methylxanthine /
acetamido-
butanoate
UGT1A5
bilirubin
NAT8
N-acetylornithine
AHR
caffeine / quinate
AKR1C
androsterone sulfate/
epiandrosterone
sulfate
CYP3A4
androsterone
sulfate
CYP4A22
C19:1n9 /
C11:1n1
SLC22A1
isobutyrylcarnitine
SLCO1B1
eicosenoate/
tetradecanedioate
SLC2A9
urate
SLC22A4
isovalerylcarnitine
SLC7A6
glutaroyl
carnitine/
lysine
SLC16A9
carnitine
PRODH
proline
NT5E
inosine
CPS1
glycine
OPLAH
oxoproline
GLS2
glutamine
serine

# Diabetes

- GCKR is a major pleiotropic risk locus for diabetes-related traits, such as fasting, glucose and insulin, triglyceride levels , and CKD
- Strong association of this locus with the mannose to glucose ratio
- Fasting mannose  lower in carriers of the risk allele, as opposed to glucose.
- Physiological role of mannose other than its use in protein glycosylation?
- Mannose as a differential biomarker or even as a point of intervention in diabetes???

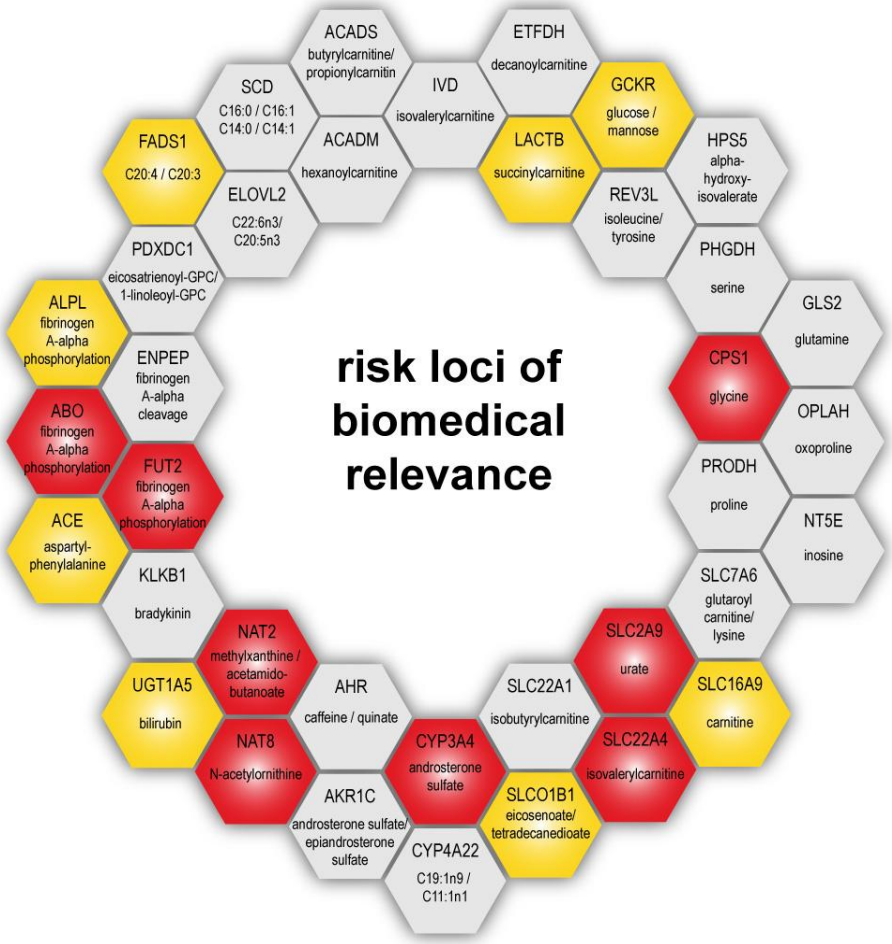
# Lipid disorders



# Lipid disorders and obesity

- *LACTB associated with succinylcarnitine concentrations*
- *LACTB  a HDL cholesterol risk locus*
- Functional link between succinate-related pathways and HDL metabolism
- *LACTB* identified by a systems biology approach as a potential obesity gene
- Transgenic mice with an increase in gene expression of the hepatic succinate metabolism.
- Succinylcarnitine concentrations associated with body mass index
- *LACTB as a target for obesity medication*

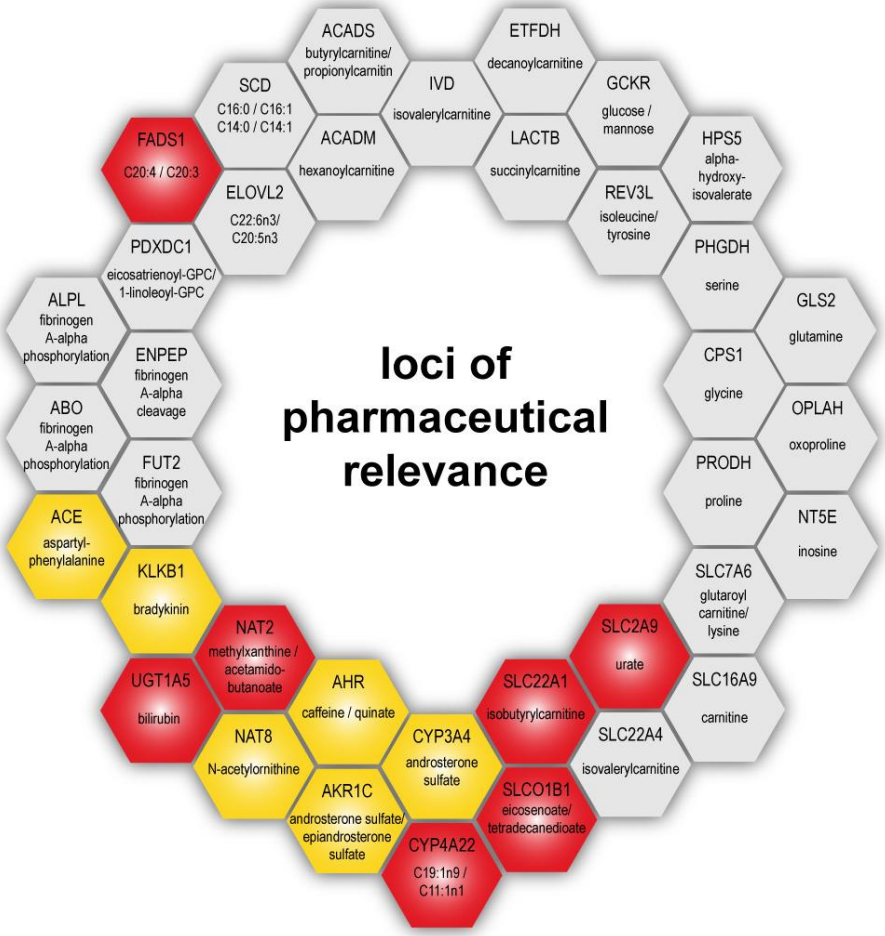
# Coronary artery disease



# Coronary artery disease

- *ABO, CPS1, NAT8, ALPL, KLKB1 associated with CAD*
- *ABO, ALPL associated with FAaP (*involved in blood coagulation properties)
- Basis of the association of *ABO with CAD??*
- FAaP may be a biomarker for acute myocardial infarction
- *CPS1 also associated with CKD as well as with* homocysteine levels (CAD risk factor)
- *NAT8 is linked to CKD via ornithine acetylation b*eing a risk factor for CAD
- *KLKB1 associated with bradykinin* concentrations (*blood pressure)*

# Loci with pharmaceutical relevance



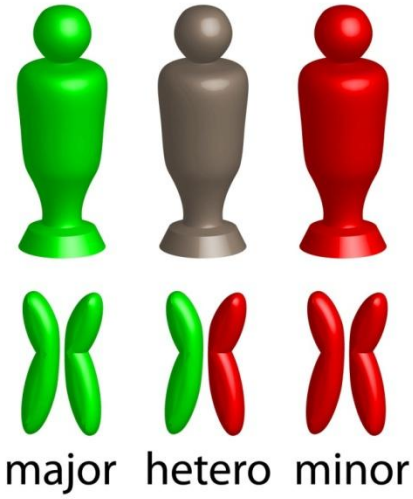
# Pharmacogenomics

- *Pharmacogenomics Knowledge Base: identification of seven of* our loci reported to associate with toxicity or adverse reactions to medication
- *SLC22A1 with metformin pharmacokinetics*
- *FADS1 with* response to statin therapy
- *SLCO1B1 with statin-induced myopathy*
- *NAT2 and in CYP4A loci are associated with toxicities* to docetaxel and thalidomide treatment
- *UGT1A associated with irinotecan toxicity*
- *SLC2A9 with etoposide IC*

# Pharmacogenomics

- Associations with metabolic traits provide a novel biochemical basis for the genotype-dependant reaction to drug treatment
- Redesign of the respective drug molecules to avoid adverse reactions
- Early identification of potentially adverse pharmacogenetic effects??

# The future?
## The *"genetically determined metabotype"* - its possible role in drug testing




MAF = 20%
major = 64%
hetero = 32%
minor = 4%

**Strong-responder**

**Poor-responder**

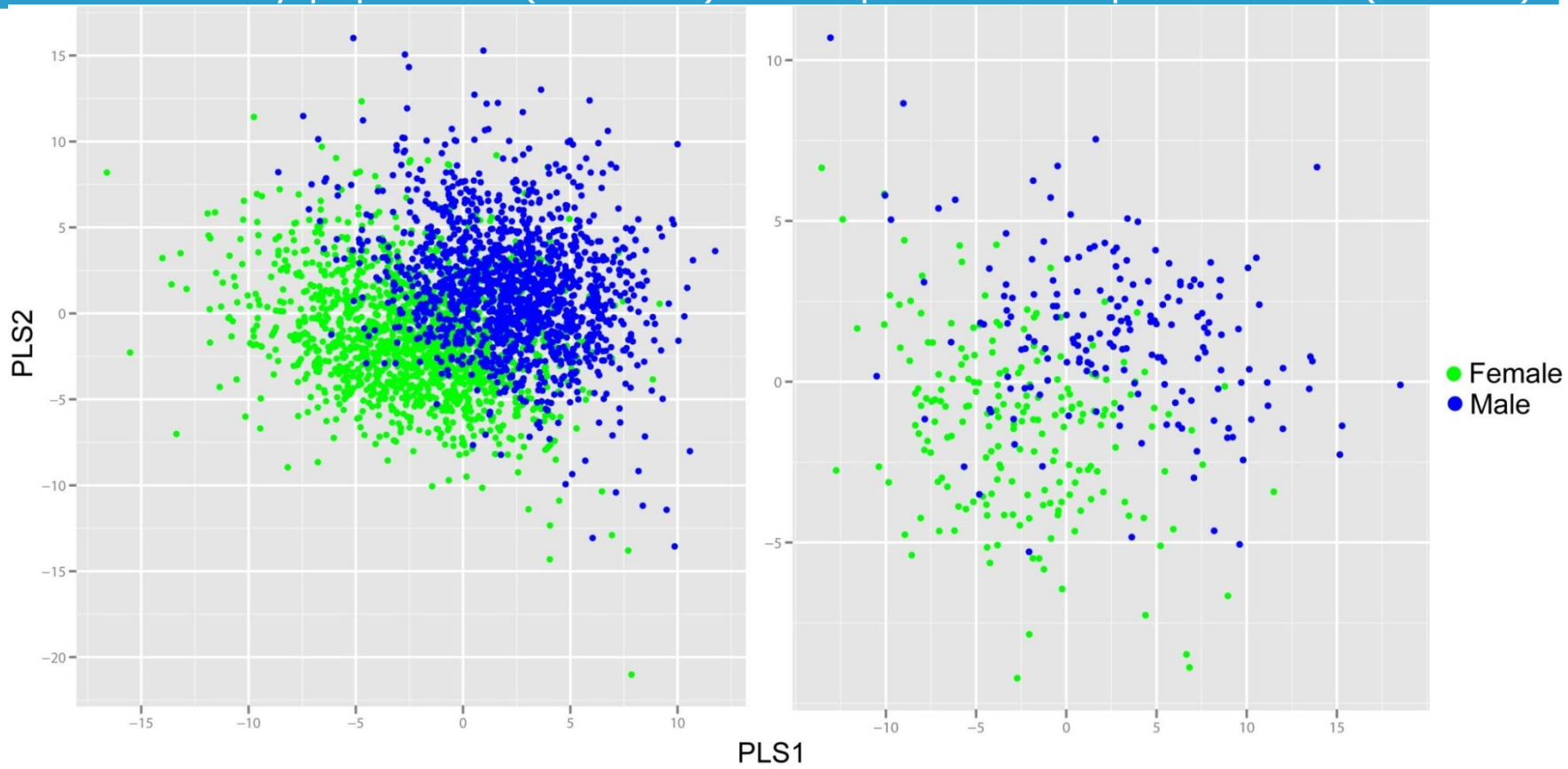
**Non-responder**



**Dihydropyrimidine dehydrogenase (DPYP) gene is associated strongly with fluoropyrimidine-related toxicity in cancer patients, Gross 2008)**

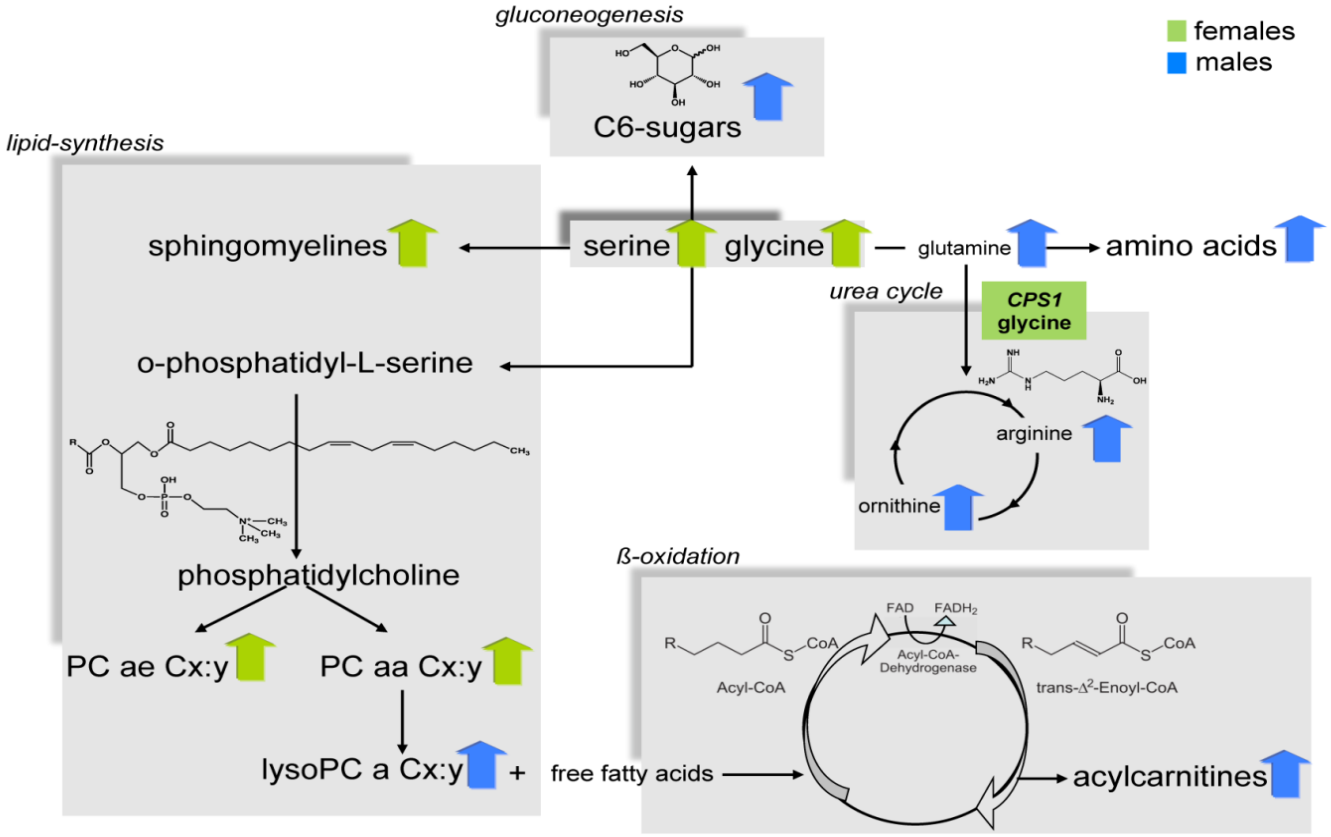
# Gender specific metabolite differences (Biocrates kit)

**A** KORA F4 study population (n= 3060)  **B** Replication sample KORA F3 (n= 377)




**77% of all analyzed metabolites show significant differences between males and females**

# Systematic view of metabolic variations in the metabolism of males and females

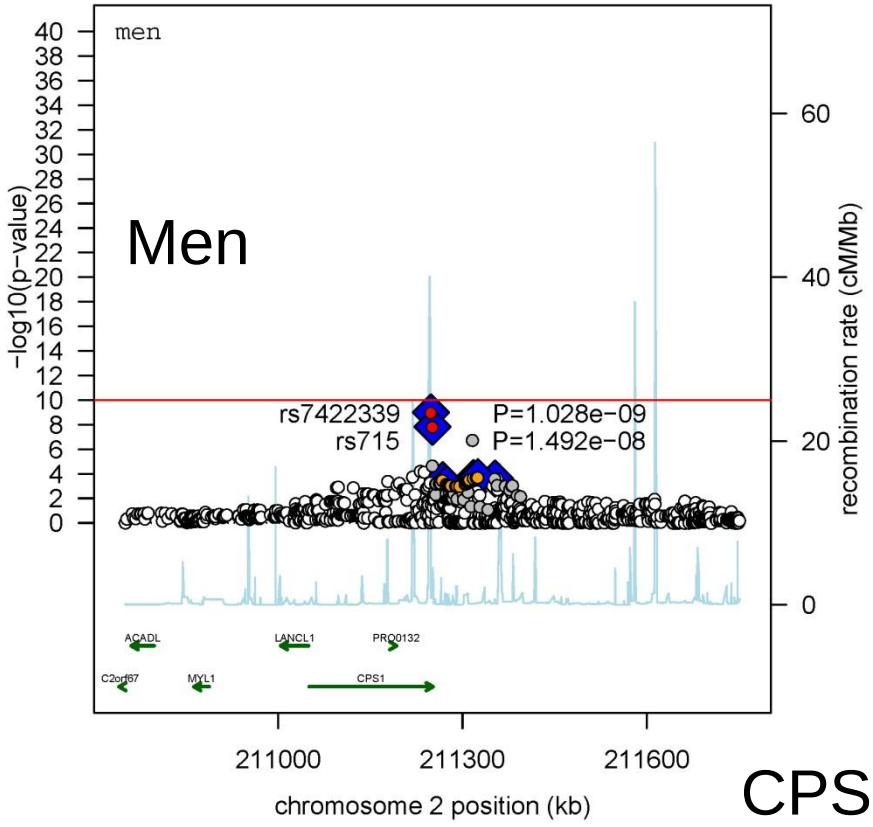


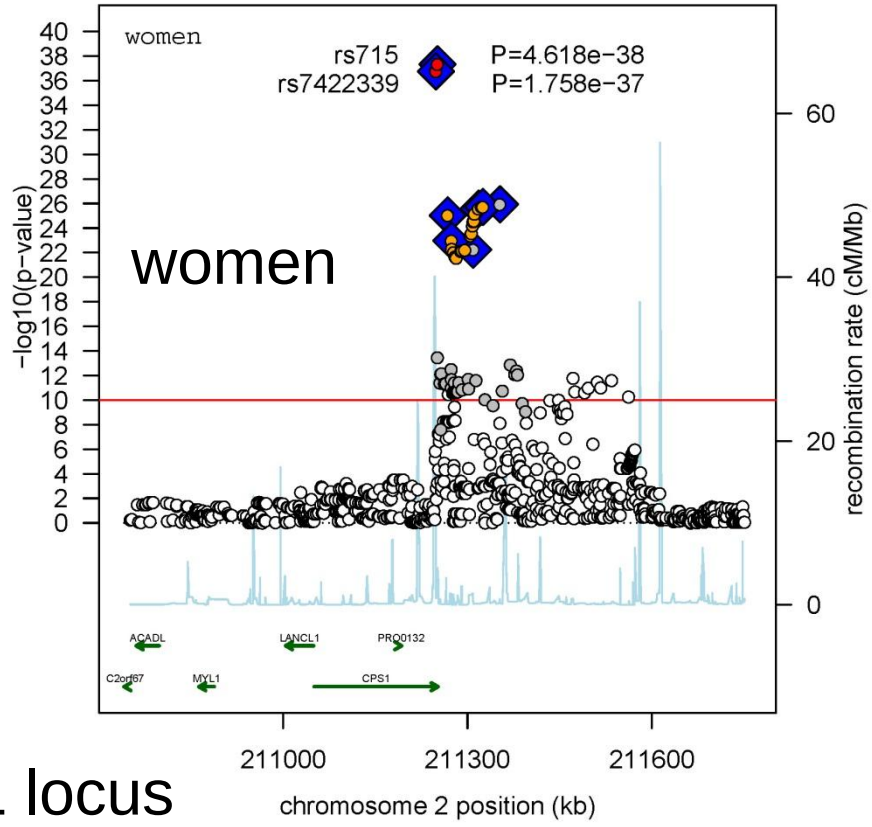

Sex specific medication and prediction?



**Mittelstrass et al, Plos Genetics, 2011, 7(8):e1002215**

# Glycine is regulated genetically different between males and females by the CPS 1 gene

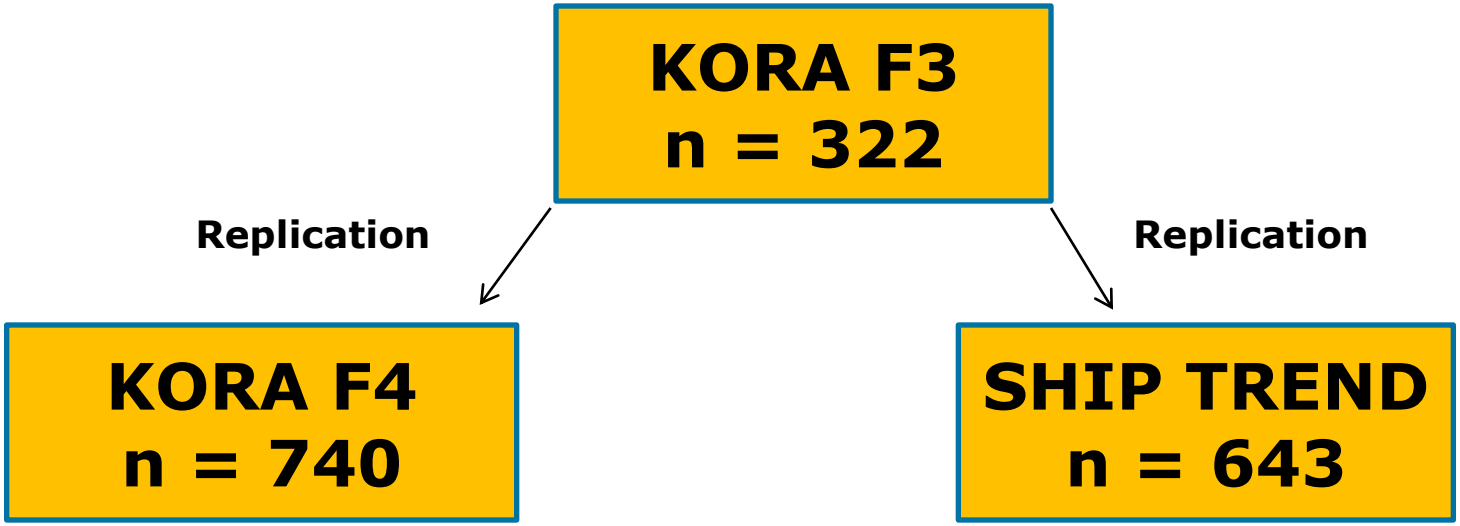



CPS 1 locus

# Genomics and transcriptomics

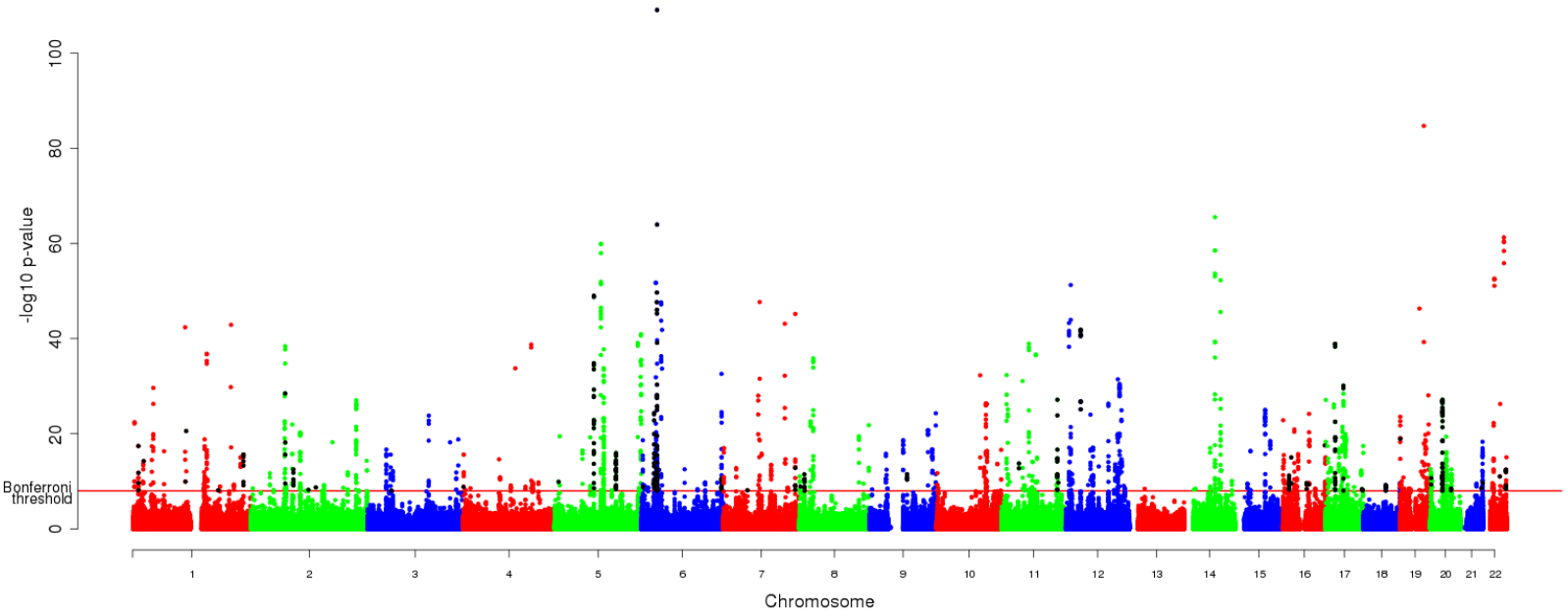
- GWAs data (Affymetrix, Illumina)
- Genome wide expression, Illumina 28 k chips
- **First study in human whole blood**






Metha et al., submitted

# Results in KORA F3




- 363 eQTLs in cis: 98.6% replicated in KORA F4 and SHIP
- 33 eQTLs in trans: 86.7% replicated in KORAF4 and SHIP
- Large effects: mean expression variability explained 19%
- Detection of causal genes for complex diseases in large LD blocks
- 35 eSNPs (11 novel) identified in GWAS of complex diseases



Metha et al., submitted

# What comes next?

- Combining all of the omics techniques to detect common features for complex disases
- Detection of early marker for disease
- Systems Biology approaches
- Validation of new targets for drug development
- Detection of rare sequence mutations by NGS

# Many thanks to…

## Helmholtz Munich

**Christian Gieger**

**Kirstin Mittelstrass**

**Rui Wang Sattler**

**Jerzy Adamski**

**Holger Prokisch**

**Thomas Meitinger**

**Werner Mewes**

**Karsten Suhre**

## King´s College London

**Guangju Zhai**

**Bernet S Kato**

**Tim D Spector**

**Nicole Soranzo**

## Innsbruck University

**Florian Kronenberg**