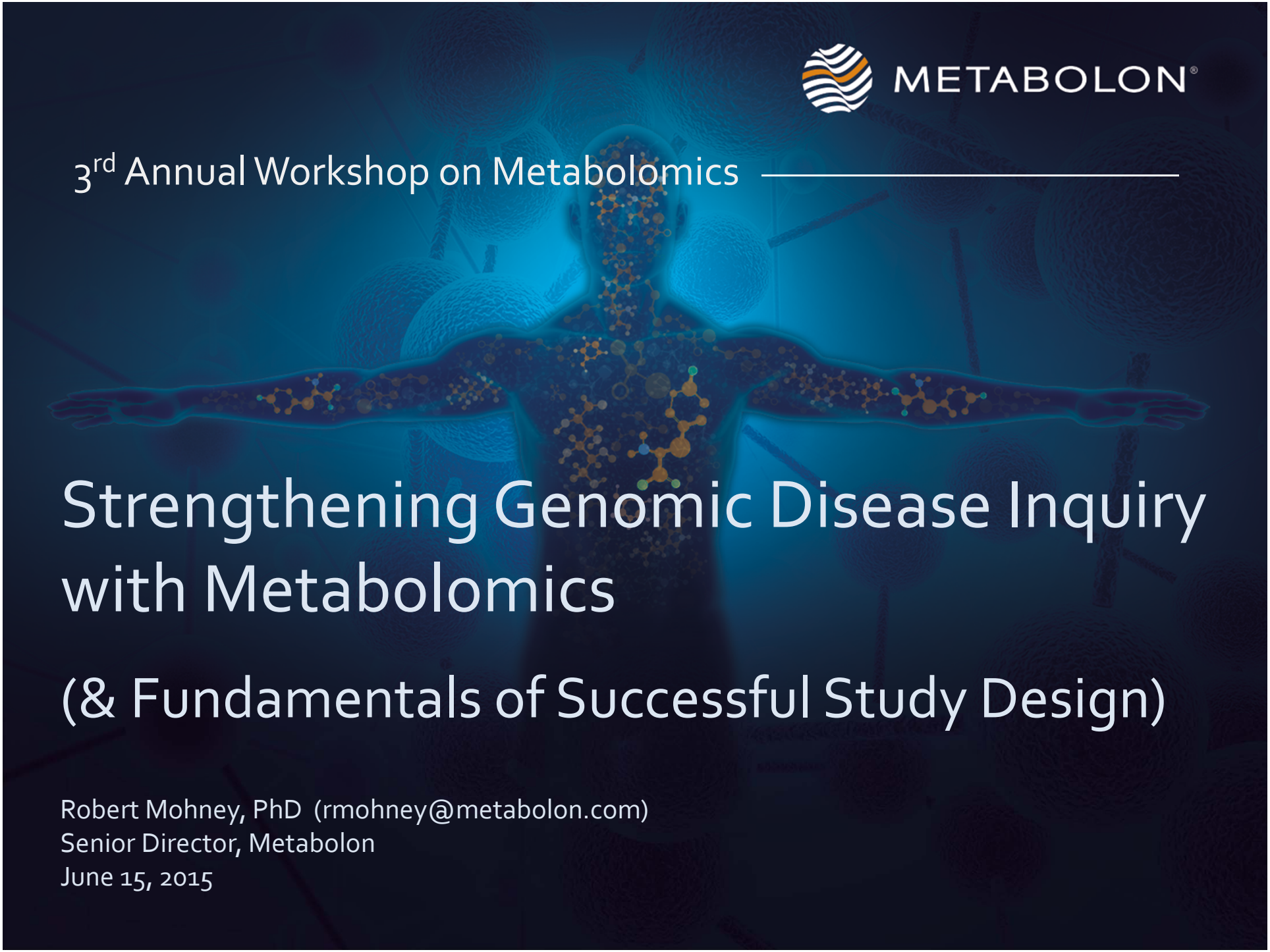


METABOLON®

3rd Annual Workshop on Metabolomics

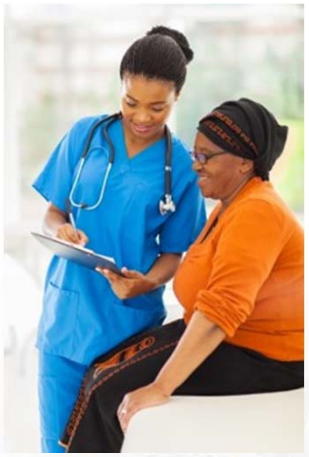
Strengthening Genomic Disease Inquiry with Metabolomics

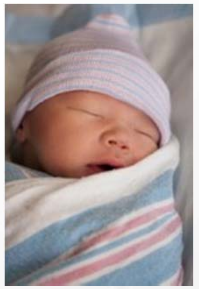
(& Fundamentals of Successful Study Design)

Robert Mohney, PhD (rmohney@metabolon.com)
Senior Director, Metabolon
June 15, 2015

Metabolon Is the Global Leader in Metabolomics

Our technology is advancing life sciences research & improving health

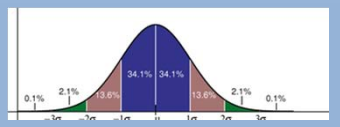




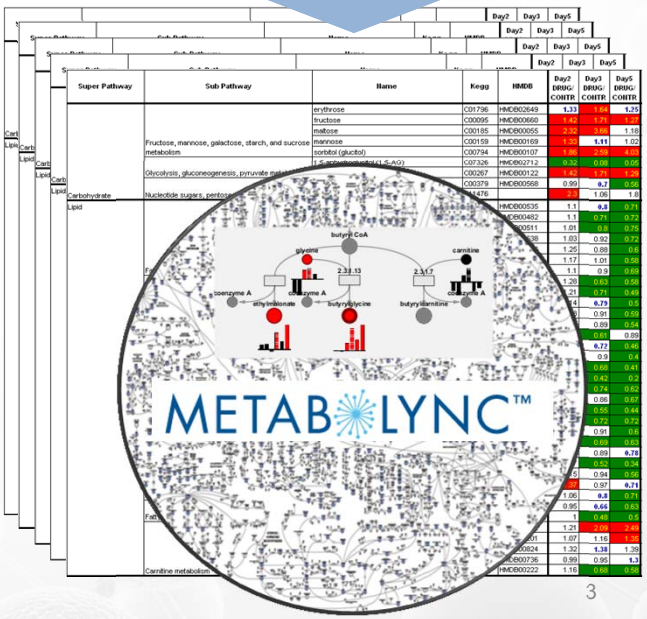
- Founded in 2000
- 150 employees with expertise in biochemistry, mass spectrometry and software development
- 54,000 sq. ft. facility in Research Triangle Park, NC & Sacramento, CA
- CLIA-certified/CAP-accredited lab
- >3,500 studies, >425 publications

METABOLON®

Discovery HD4™ platform launched in April 2014



Statistical Analysis



Interpretation

- >3,500 studies
- Institutional knowledge
- Expert biochemists
- >425 publications

Biomarkers
Mechanistic Understanding
Cellular Characteristics
Drug MoA

Strengthening Genomic Disease Inquiry with Metabolomics

nature genetics

Nature Genetics (2014) 46:543-550.

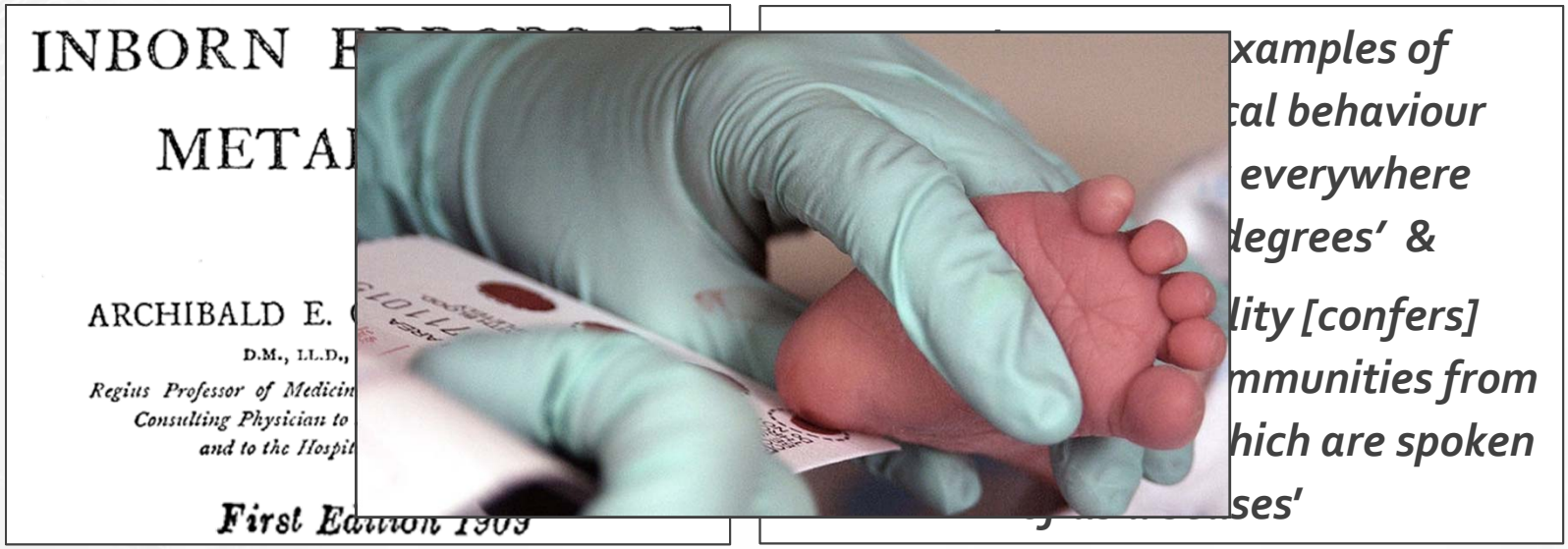
An atlas of genetic influences on human blood metabolites

So-Youn Shin[1,21,23], Eric B Fauman[2,23], Ann-Kristin Petersen[3,23], Jan Krumsiek[4,23], Rita Santos[5], Jie Huang[1], Matthias Arnold[6], Idil Erte[7], Vincenzo Forgetta[8], Tsun-Po Yang[1], Klaudia Walter[1], Cristina Menni[7], Lu Chen[1,9], Louella Vasquez[1], Ana M Valdes[7,10], Craig L Hyde[11], Vicky Wang[2], Daniel Ziemek[2], Phoebe Roberts[2,22], Li Xi[2], Elin Grundberg[8,12], The Multiple Tissue Human Expression Resource (MuTHER) Consortium[13], Melanie Waldenberger[14], J Brent Richards[7,8,15], Robert P Mohney[16], Michael V Milburn[16], Sally L John[17], Jeff Trimmer[18,21], Fabian J Theis[4,19], John P Overington[5], Karsten Suhre[6,20,24], M Julia Brosnan[11,24], Christian Gieger[3,24], Gabi Kastenmüller[6,24], Tim D Spector[7,24] & Nicole Soranzo[1,9,24]

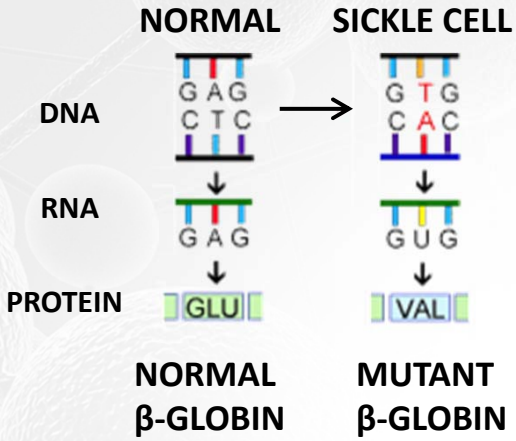
Genome-wide association scans with high-throughput metabolic profiling provide unprecedented insights into how genetic variation influences metabolism and complex disease. Here we report the most comprehensive exploration of genetic loci influencing human metabolism thus far, comprising 7,824 adult individuals from 2 European population studies. We report genome-wide significant associations at 145 metabolic loci and their biochemical connectivity with more than 400 metabolites in human blood. We extensively characterize the resulting *in vivo* blueprint of metabolism in human blood by integrating it with information on gene expression, heritability and overlap with known loci for complex disorders, inborn errors of metabolism and pharmacological targets. We further developed a database and web-based resources for data mining and results visualization. Our findings provide new insights into the role of inherited variation in blood metabolic diversity and identify potential new opportunities for drug development and for understanding disease.

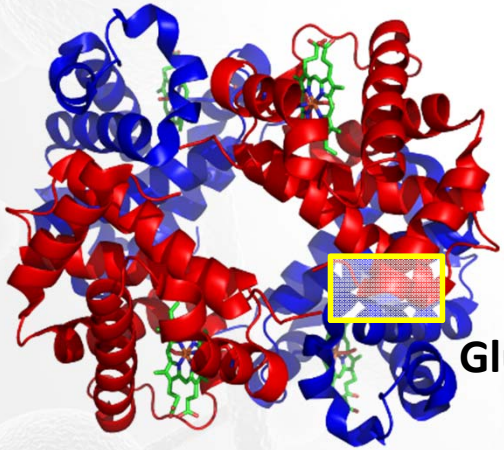
Link Between Biochemistry and Disease

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



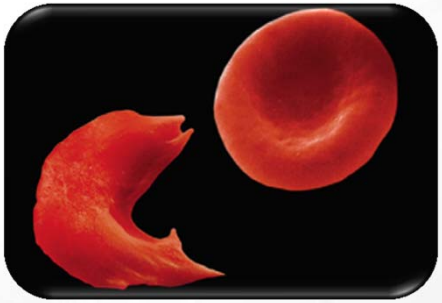
Genetics Emerges & Biochemistry Is Forgotten





$Glu_6 \rightarrow Val_6$

Polymerization



Mutant Gene Product

Disease Phenotype

Paoli et al., (1996) J. Mol. Biol. 256:775.

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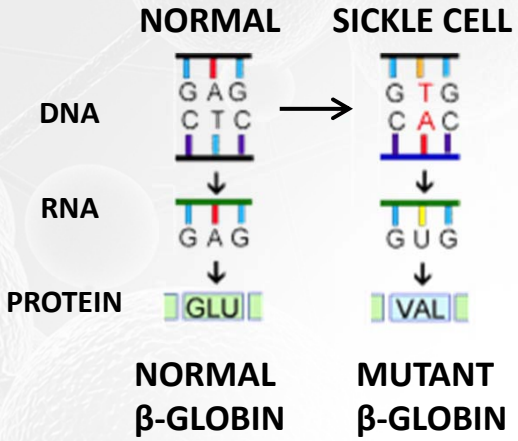
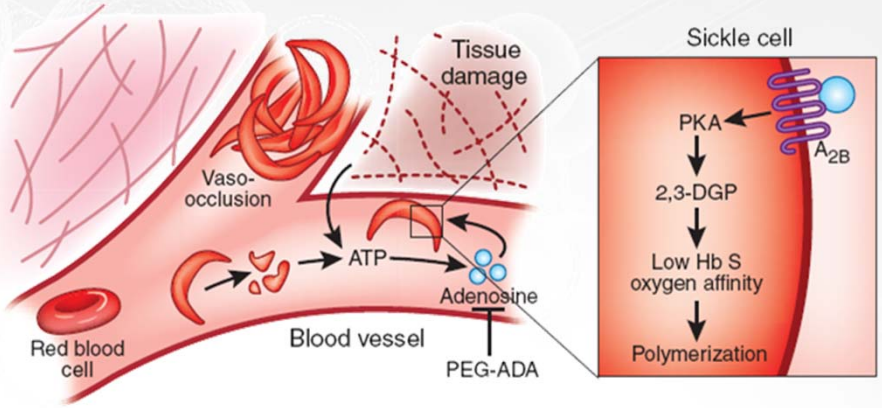
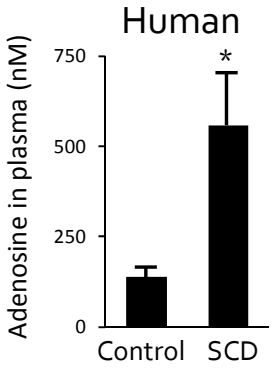
Global Metabolomics Re-Awakens Biochemistry

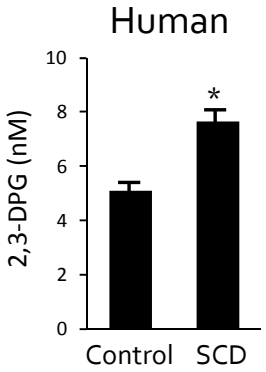


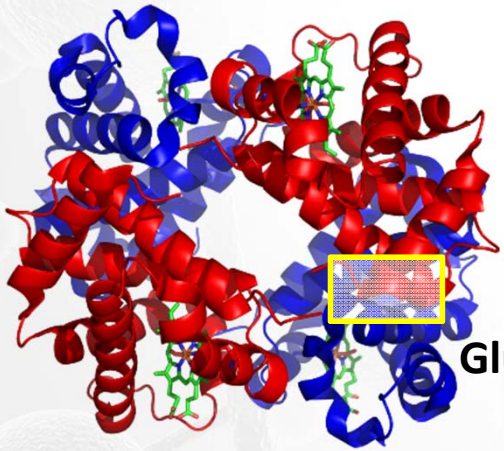


Adenosine



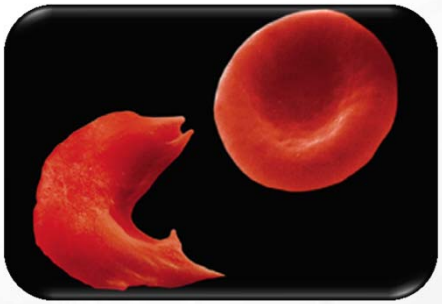
2,3-DPG





$Glu_6 \rightarrow Val_6$

Polymerization



Mutant Gene Product

Disease Phenotype

Paoli et al., (1996) J. Mol. Biol. 256:775.
Zhang et al., (2011) Nature Medicine 17(1):79-86.

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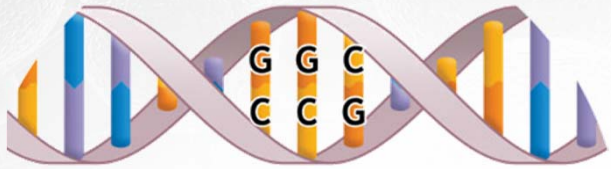
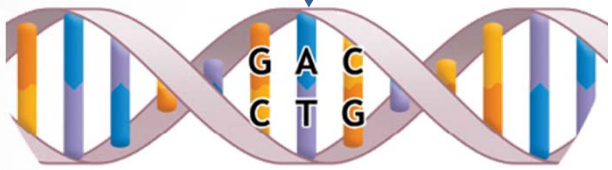
Searching for the Basis of Phenotype with Genomics

CONTROL
(HEALTHY)



SNP

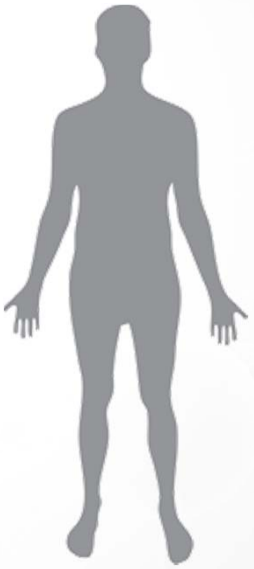


CASE
(DISEASE)

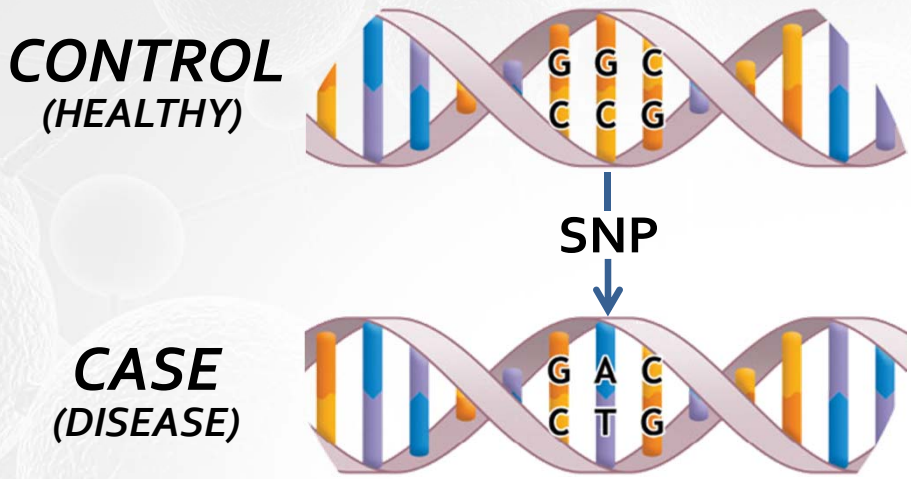
GWAS

Functional info linking genes to phenotype is often weak or absent

Disease Phenotype

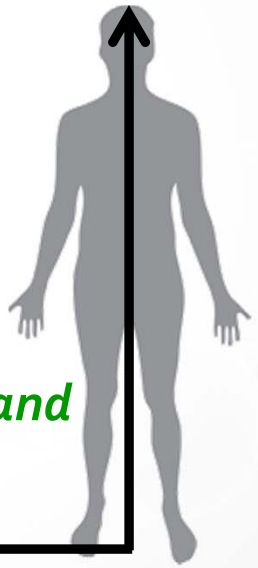


Can Metabolomics Bolster Findings in Genomics Studies?



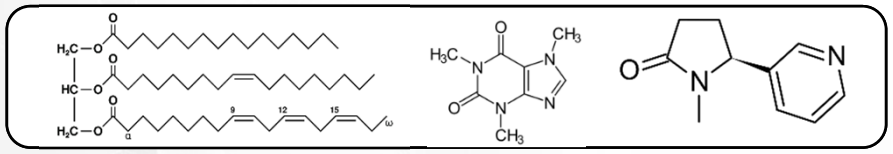
GWAS

Disease Phenotype



Functional link and biomarkers

GWAS + Metabolomics



METABOLON®

Study Design & Data Analysis Workflow

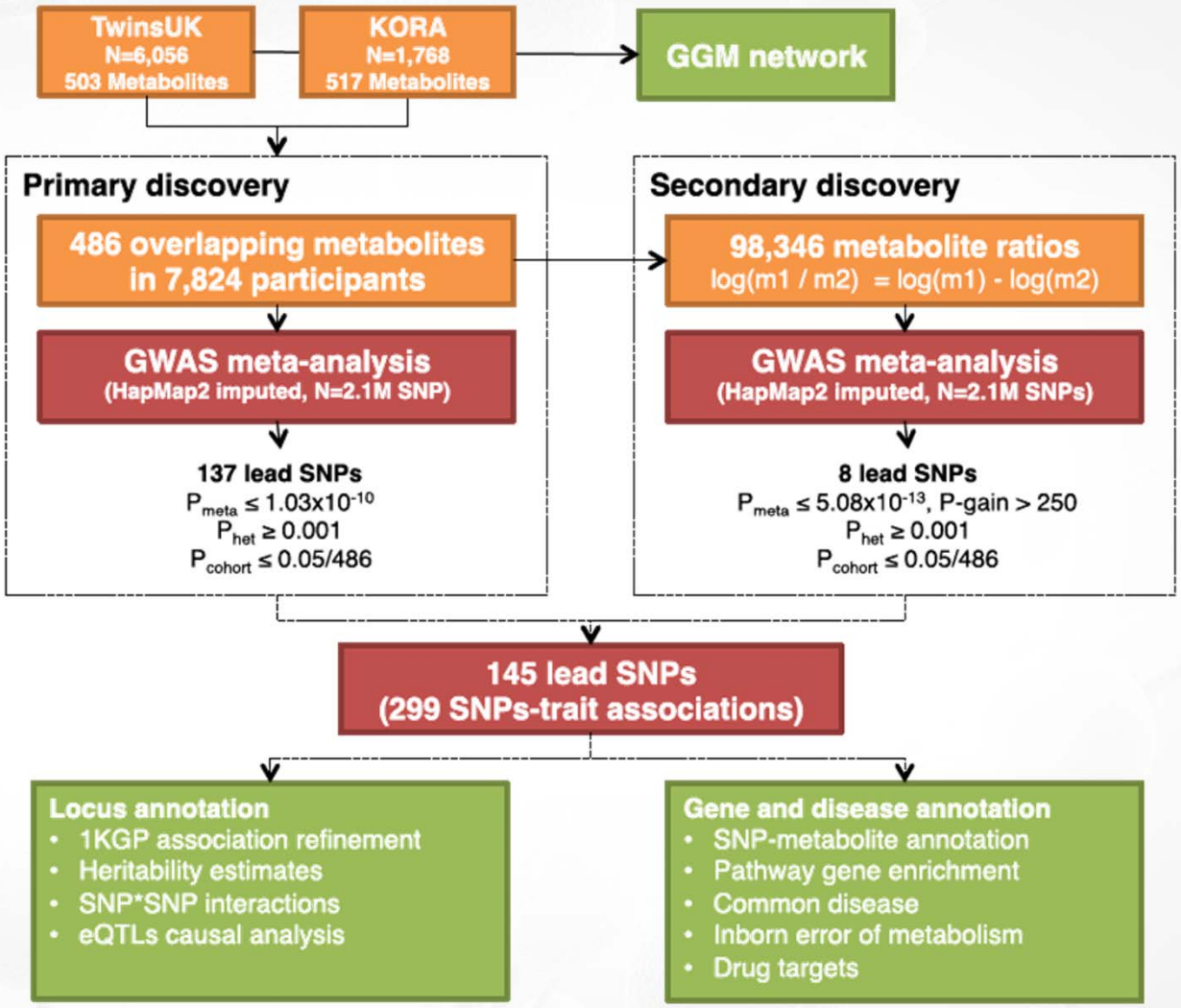
~8,000 subjects

~500 biochemicals,
~100k traits (conc and ratios)

~2.1M genotyped SNPs

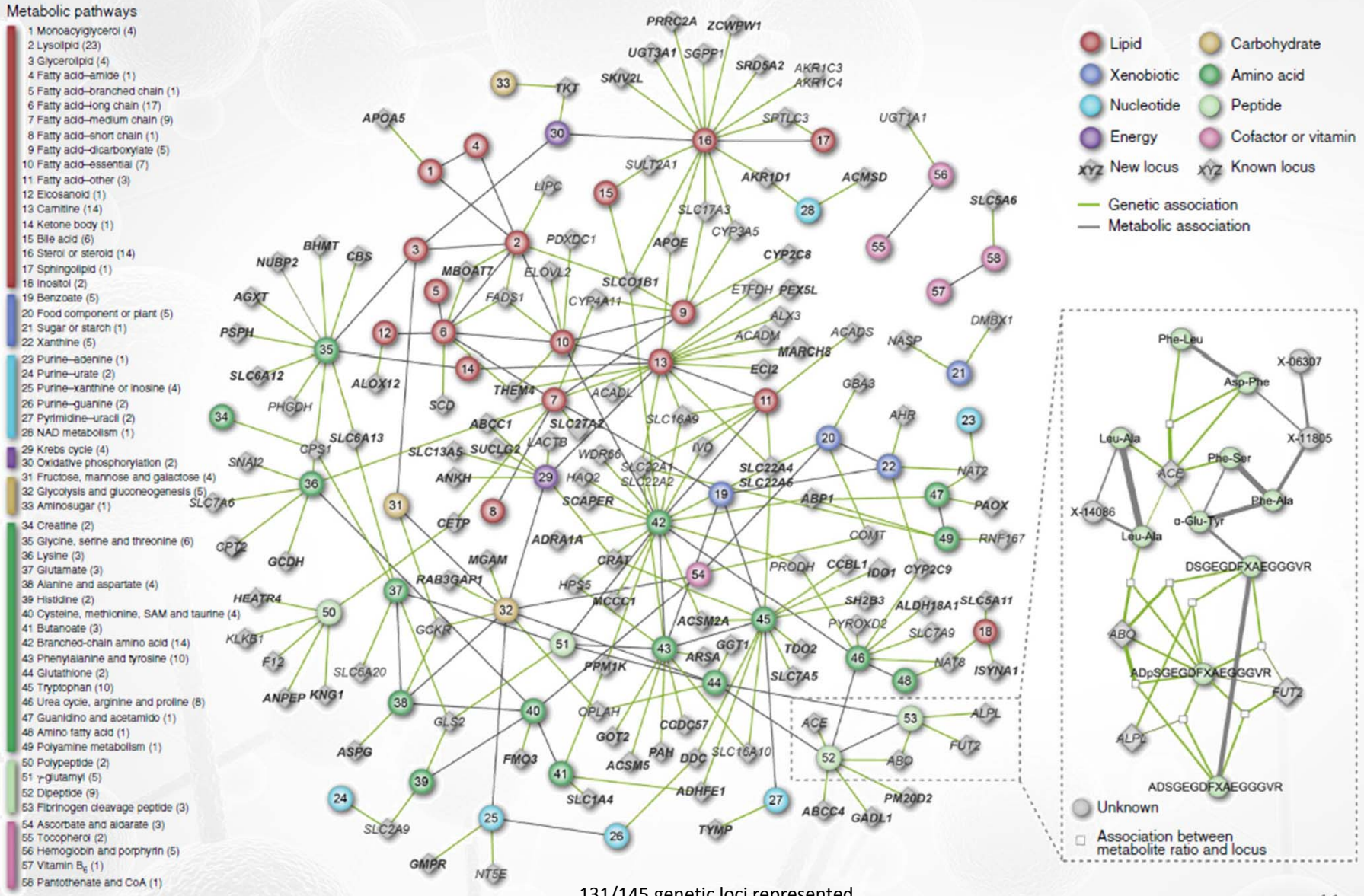
145 metabolic loci associations
(84 novel)

http://gwas.eu/si



Shin et al., (2014) Nature Genetics 46(6):543-550.

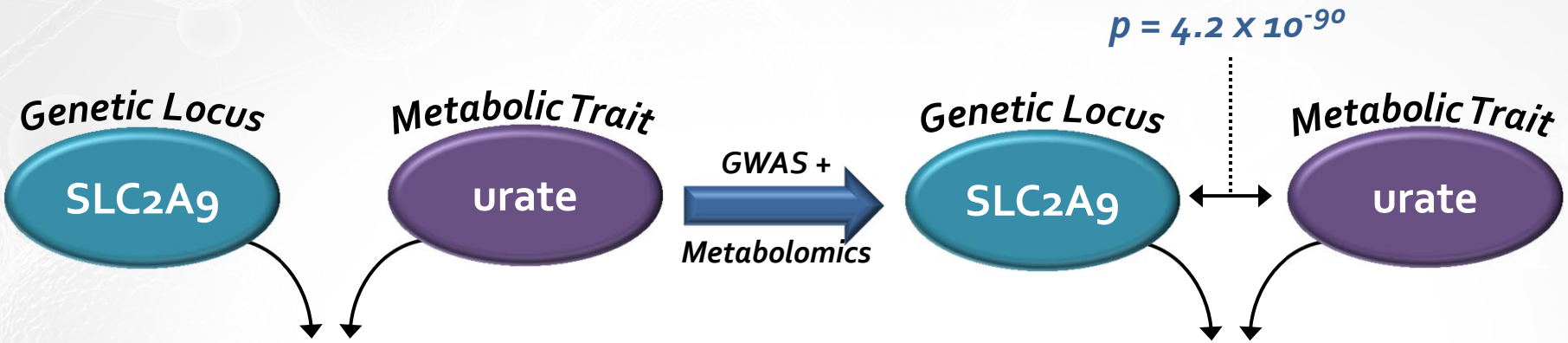
Network View of Genetic & Metabolic Associations



131/145 genetic loci represented

Shin et al., (2014) Nature Genetics 46(6):543-550.

Metabolomics Facilitates Validation of Known Associations



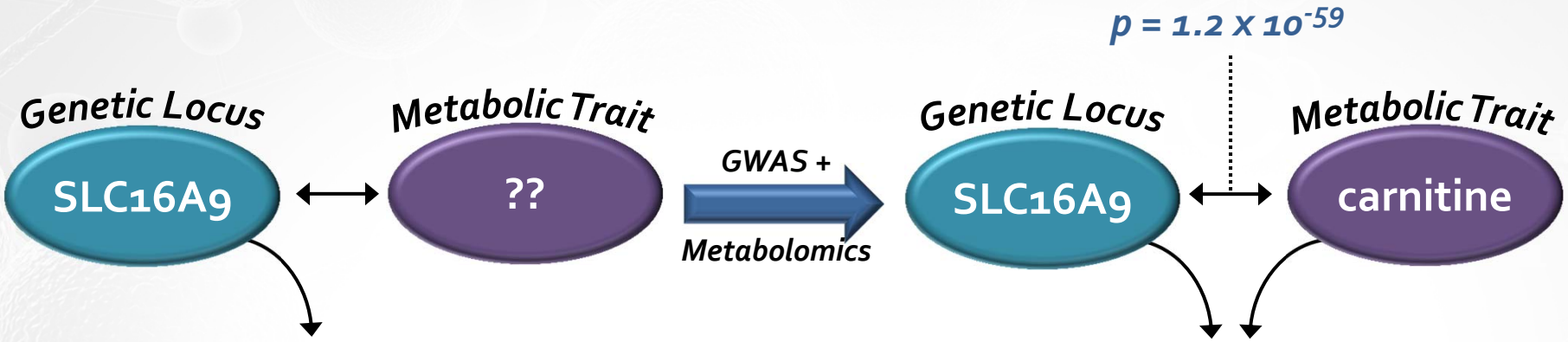
- Glucose transporter family
- Variants associated with gout (a disease of high urate)

(Archibald Garrod's father was the first to associate high levels of uric acid with gout.)

- Strong association reaffirms link
- Emphasizes importance to disease

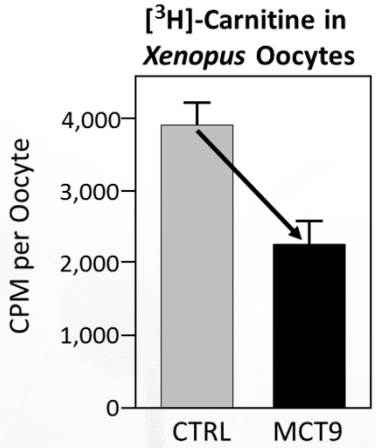
Suhre et al., (2011) Nature 477(7362):54-60.

…and Identification of Novel Associations



MCT9: Function?

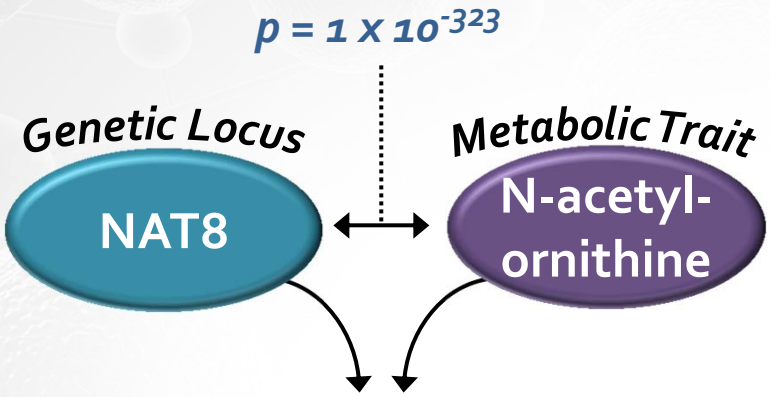
- Metabolite association suggests function as carnitine transporter



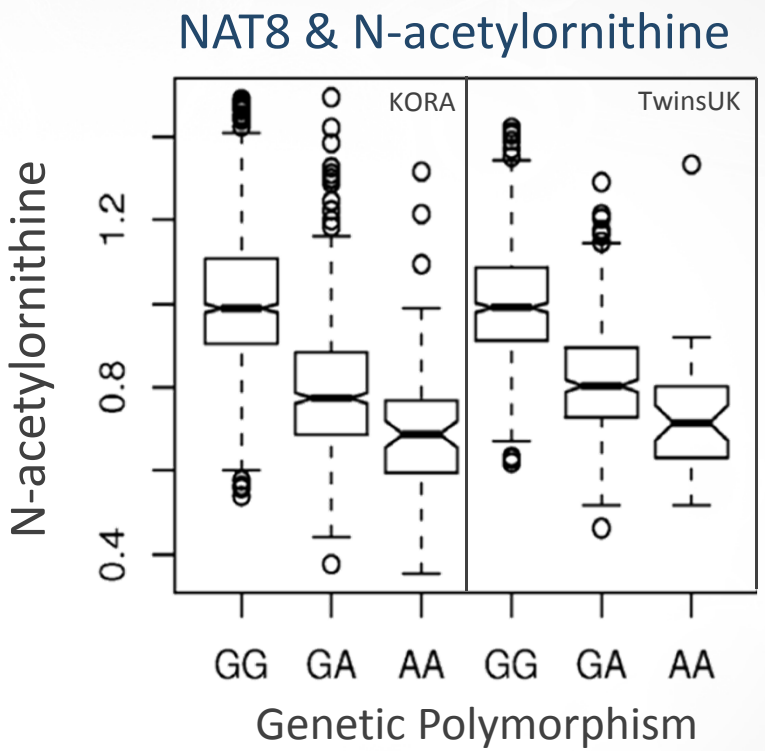
- SLC16A9/MCT9 exports labeled carnitine
- External labeled carnitine not imported (not shown)

Suhre et al., (2011) Nature 477(7362):54-60.

Metabolic Locus Linked to Kidney Disease



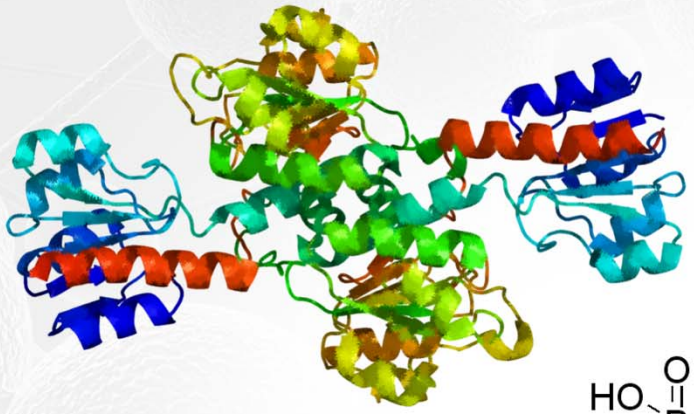
- Gene variants associated with chronic kidney disease
- Here, levels of N-acetylornithine correlated with renal function (eGFR)



Role of N-acetylation in CKD warrants exploration.
Serum N-acetylornithine may represent a biomarker for kidney function.

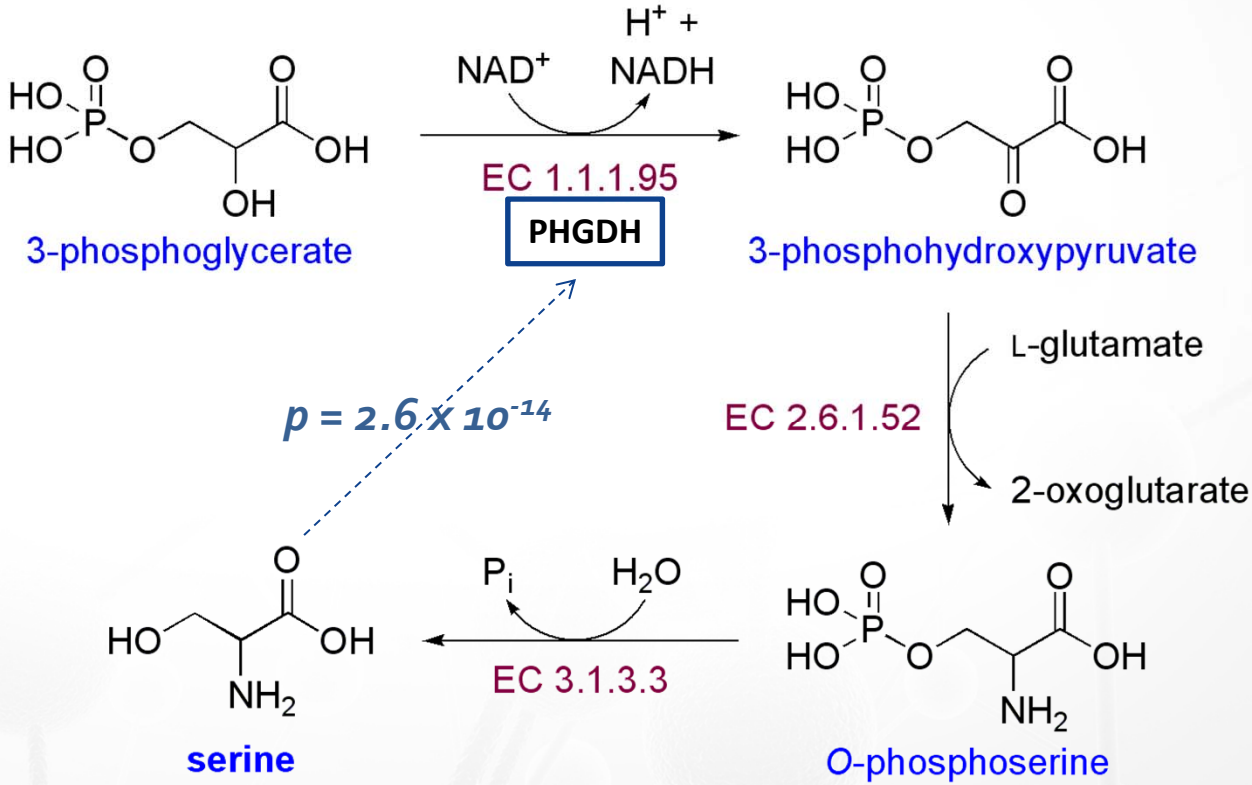
Suhre et al., (2011) Nature 477(7362):54-60.
Sekula et al., in review.

Genetic Variant Affects Key Intermediates in Metabolic Pathways (Cancer Link)



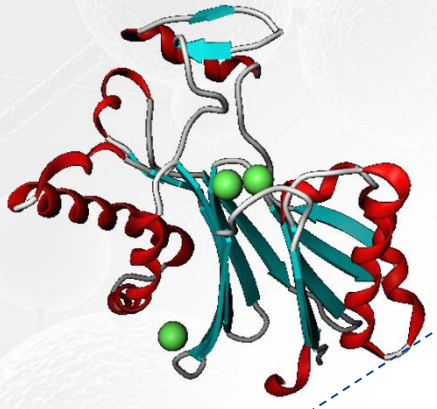
PHGDH

Serine biosynthesis contributes a significant amount of anaplerotic flux into the TCA cycle. Thus, PHGDH is a cancer target.



Ichihara et al., (1955) PNAS 41(9):605-609.
Possemato et al., (2011) Nature 476(7360):346-350.

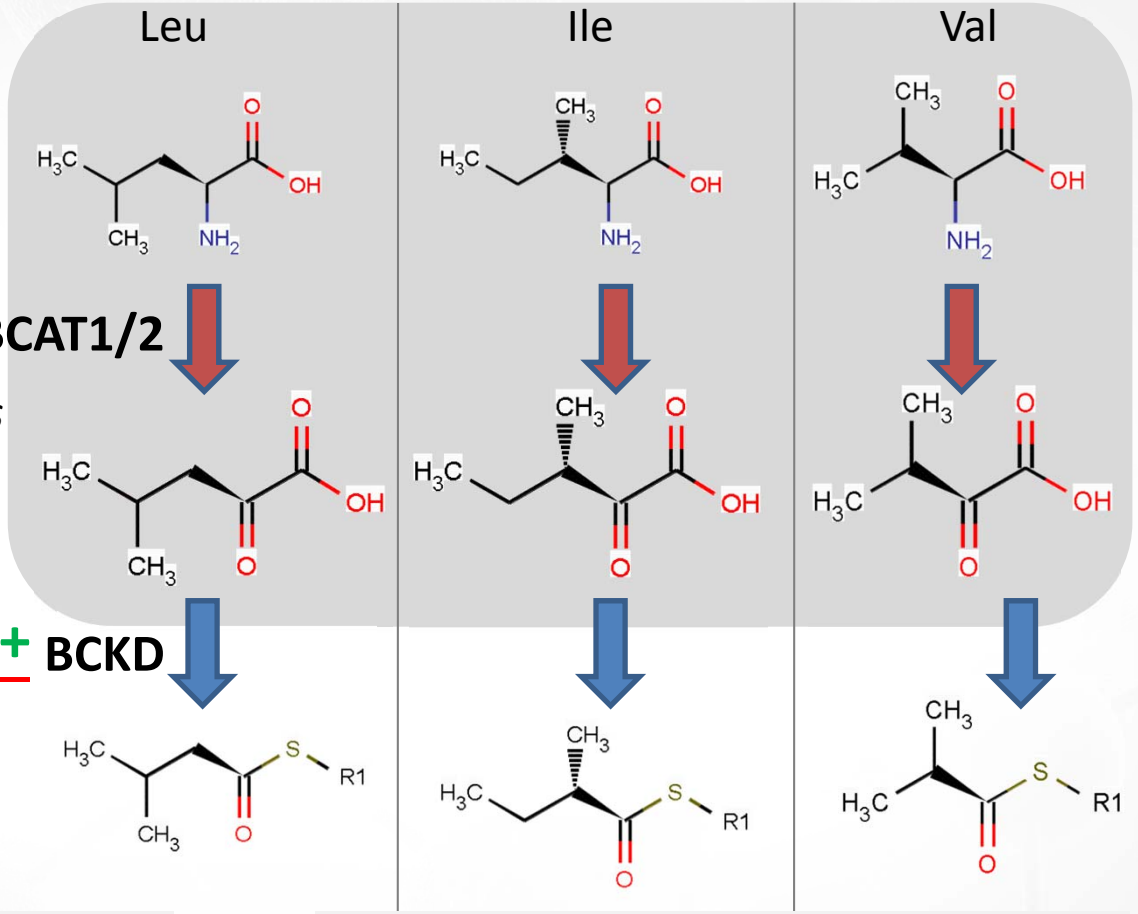
Genetic Variant Affects Regulator of Key Enzymes, Rather than the Enzyme Itself



strong p-values

PPM1K

(Protein Phosphatase, Mg^{2+}/Mn^{2+}-dependent, 1K)

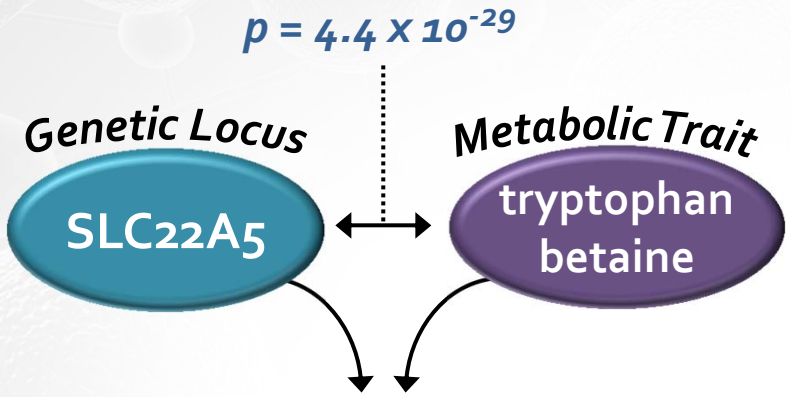


IEM: PPM1K mutations result in Maple Syrup Urine Disease, Mild Variant

Diabetes: Variants in PPM1K also associated with T2D in other GWAS.

Menni et al., (2013) Diabetes 62(12):4270-4276.
Lu et al., (2009) J Clin Invest 119(6):1678-1687.

Metabolic Locus Linked to Crohn's Disease



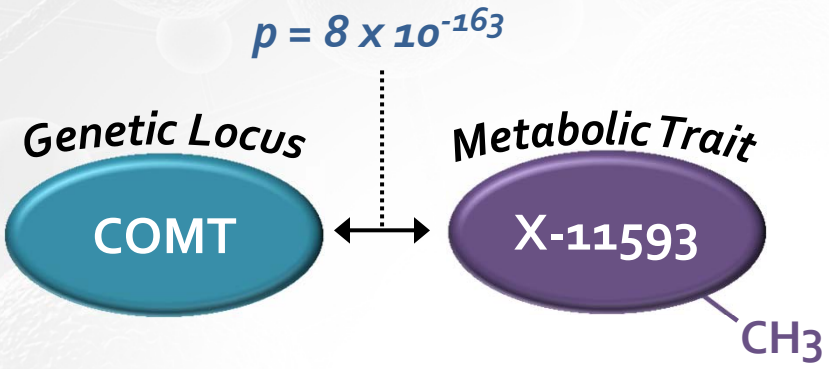
- Encodes OCTN2 (a Na^+-dependent transport of carnitine into cells and removal of cationic drugs from intestine)
- Actively expressed in the intestinal epithelium, macrophages and T cells
- Trp betaine is diet-derived (legumes)

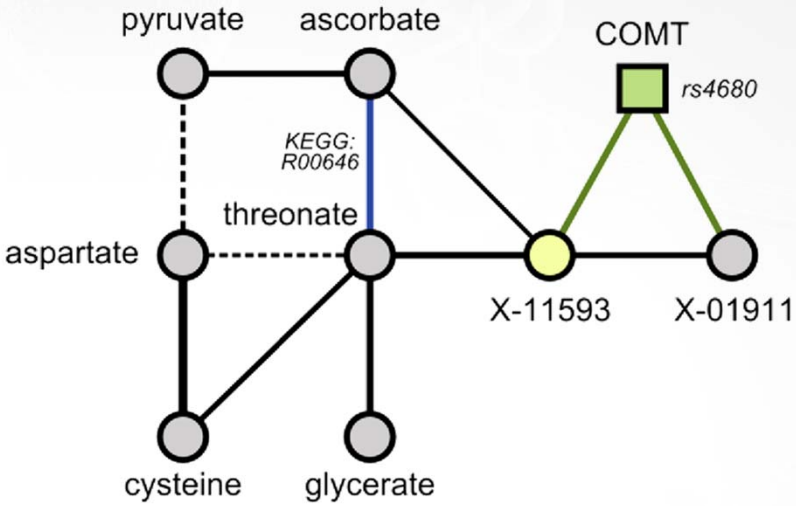
http://healthmaven.blogspot.com

Variants of OCTN2 are linked to inflammatory bowel diseases like CD.

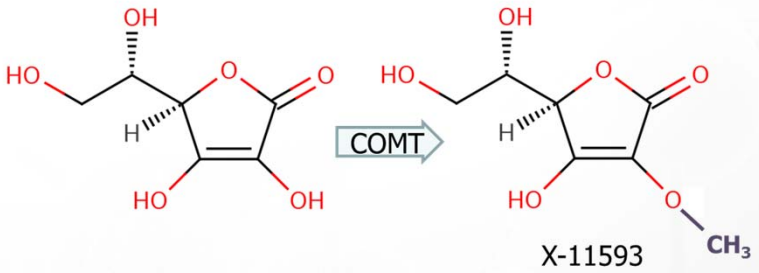
Shin et al., (2014) Nature Genetics 46(6):543-550.
http://gwas.eu/si

Identification of Unknown Biochemicals



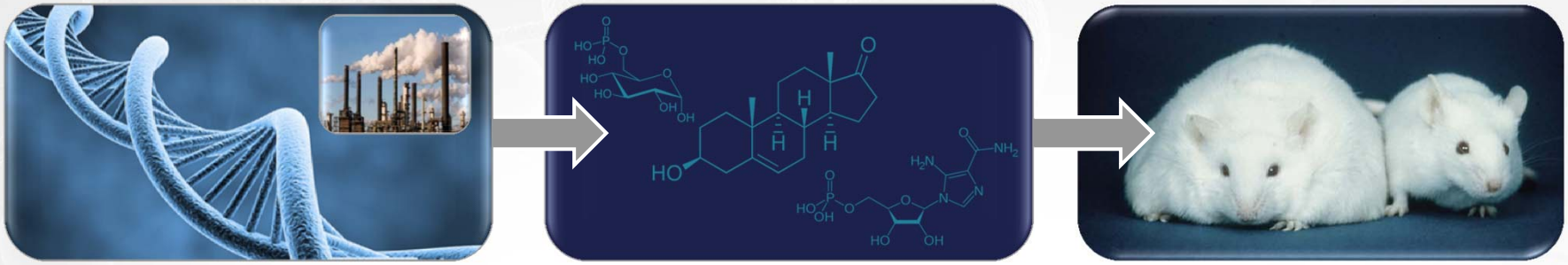


- COMT methylates catecholamine neurotransmitters (DA, Epi, NE), thereby leading to their degradation
- Strong association with a methylated unnamed metabolite



Chen et al., (2004) Am J Human Genet 75:807-821.
Krumsiek et al., (2012) PLoS Genet 8(10):e1003005.

Genomics + Metabolomics Summary



Genes (SNPs) + Environment | Metabotype | Phenotype

Metabolites are sensitive and strong links between genes and disease phenotypes.

Metabolomics facilitates discovery of biomarkers that mechanistically bridge genetics and phenotype.

Combining metabolomics with population genetics can provide new biological insights into human health and disease, therapeutic response, etc.

3rd Annual Workshop on Metabolomics

Fundamentals of Successful Study Design

METABOLON®

Define a Clear Objective

The first and most critical step in any successful scientific study is to clearly define the study's objective.

- **Are you seeking general information to help you form a hypothesis?**
- **Do you have a hypothesis in mind that you wish to validate?**
- **Are you hoping to discover biomarkers for a disease?**
- **Do you want to understand the MOA of a potential drug candidate?**

The Metabolon Advantage:
Metabolon offers every investigator study design assistance from an experienced Ph.D. scientist.

METABOLON®

Utilize Strong Study Design Elements

Strong study design elements are central to uncovering biologically significant results.

- **Select the appropriate sample matrix (or combination of matrices)**
- **Collect adequate exposures (dose and time of collection)**
- **Employ controls for each tested variable – don't skimp!**
- **Take steps to minimize excess variation – maintain consistency!**

A Tip from Metabolon: The more inherent variation you can control for, the fewer samples that are required for the study.

Power Your Metabolomics Study for Success

Strong study design can deliver biologically significant results, but a well-powered study can provide statistically significant results.

	Cell Culture	Small Animals	Human Studies
Optimal	>7	>10	>50
Rigorous	6-7	8-10	40-50
Acceptable	4-5	6-7	25-40

Fewer Required

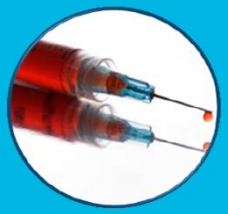
- Strong phenotype or treatment effect (toxicology study)
- Repeated sampling from the same subject
- Multiple time points
- Multiple doses of a drug/inhibitor

More Required

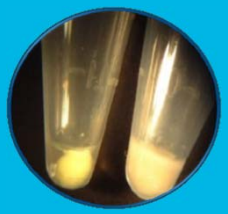
- Subtle phenotype or treatment effect (diet- or exercise-induced changes)
- Mixed populations of subjects (mixed gender, wide-ranging age or BMI)
- Multiple-site collections

Considerations of Sample Type, Amount, & Power

Sample Quantities Recommended for Optimal Results:



Biological Fluids: 100 ul



Cells: 100 ul pellet

Tissues: 100 mg

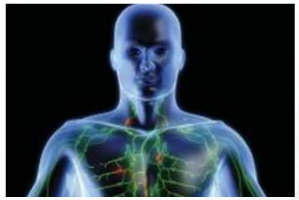
Cell-Based Studies

	t1	t2	t3
Vehicle Control	5	5	5
Drug Dose 1	5	5	5
Drug Dose 2	5	5	5

Small Animal Studies

	Chow	HFD
WT	8	8
KO	8	8
Overexpression	8	8

Human Studies



	♂	♀
Control	30	30
Case	30	30

Robert Mohney, PhD (rmohney@metabolon.com)
Senior Director, Metabolon