Metabolomics and other omics of diabetes

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VANDERBILT HEART

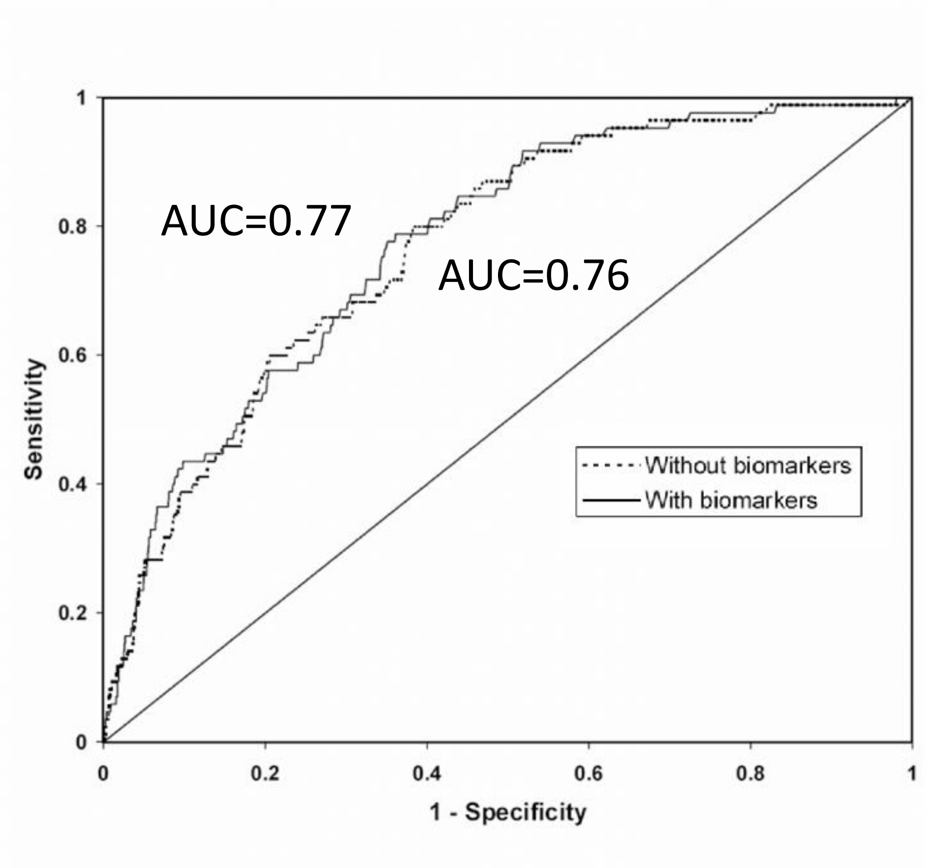
Overview

- Rationale
- Targeted mass spec analyses and DM
- Non-targeted approaches

Modest benefit of conventional biomarkers for identifying who will develop cardiovascular disease

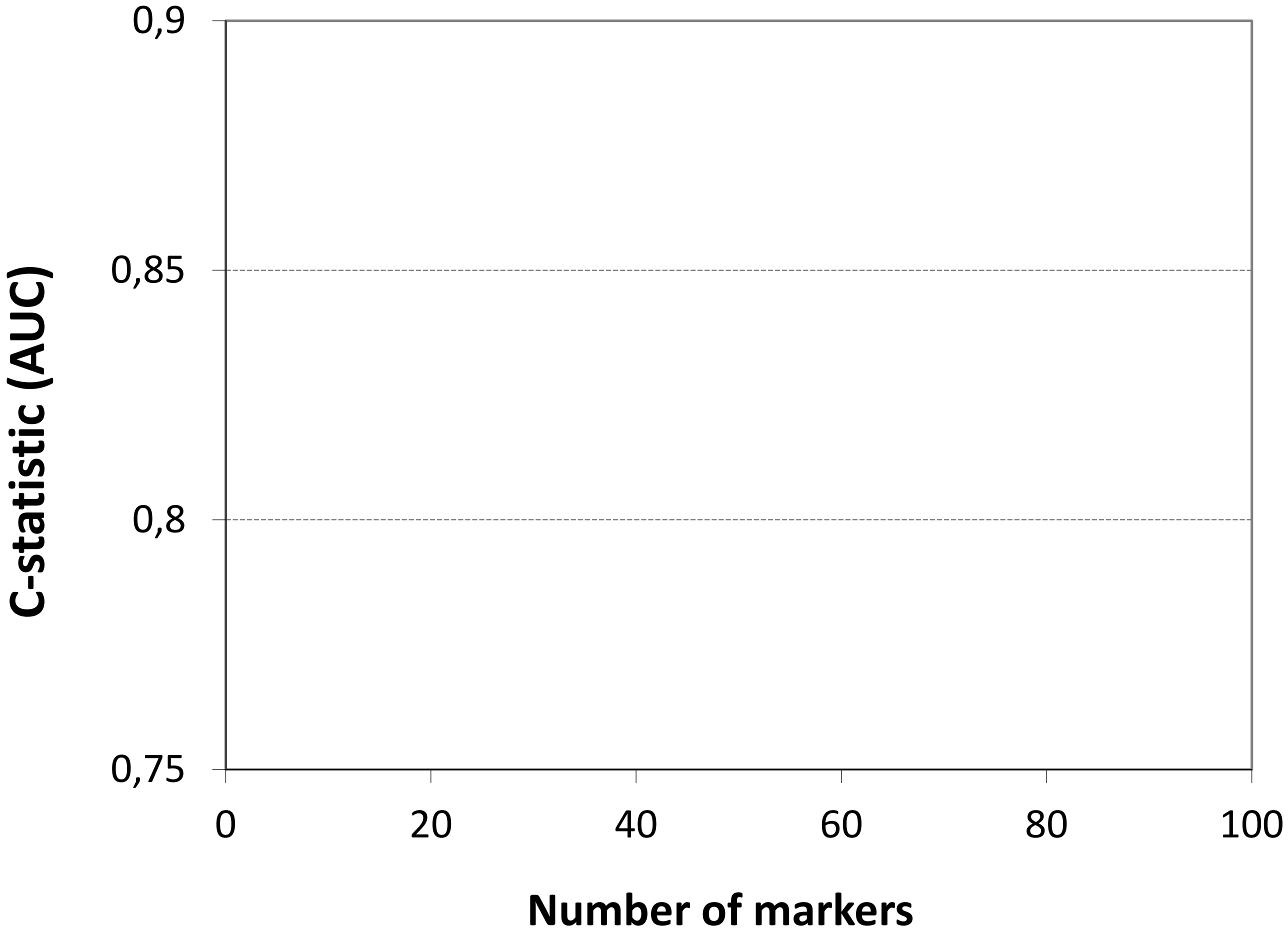
<u>Biomarkers of CV risk</u>

CRP
Fibrinogen
D-dimer
PAI-1
Homocysteine
BNP
ANP
Renin
Aldosterone
Albumin excretion
Total and HDL cholesterol



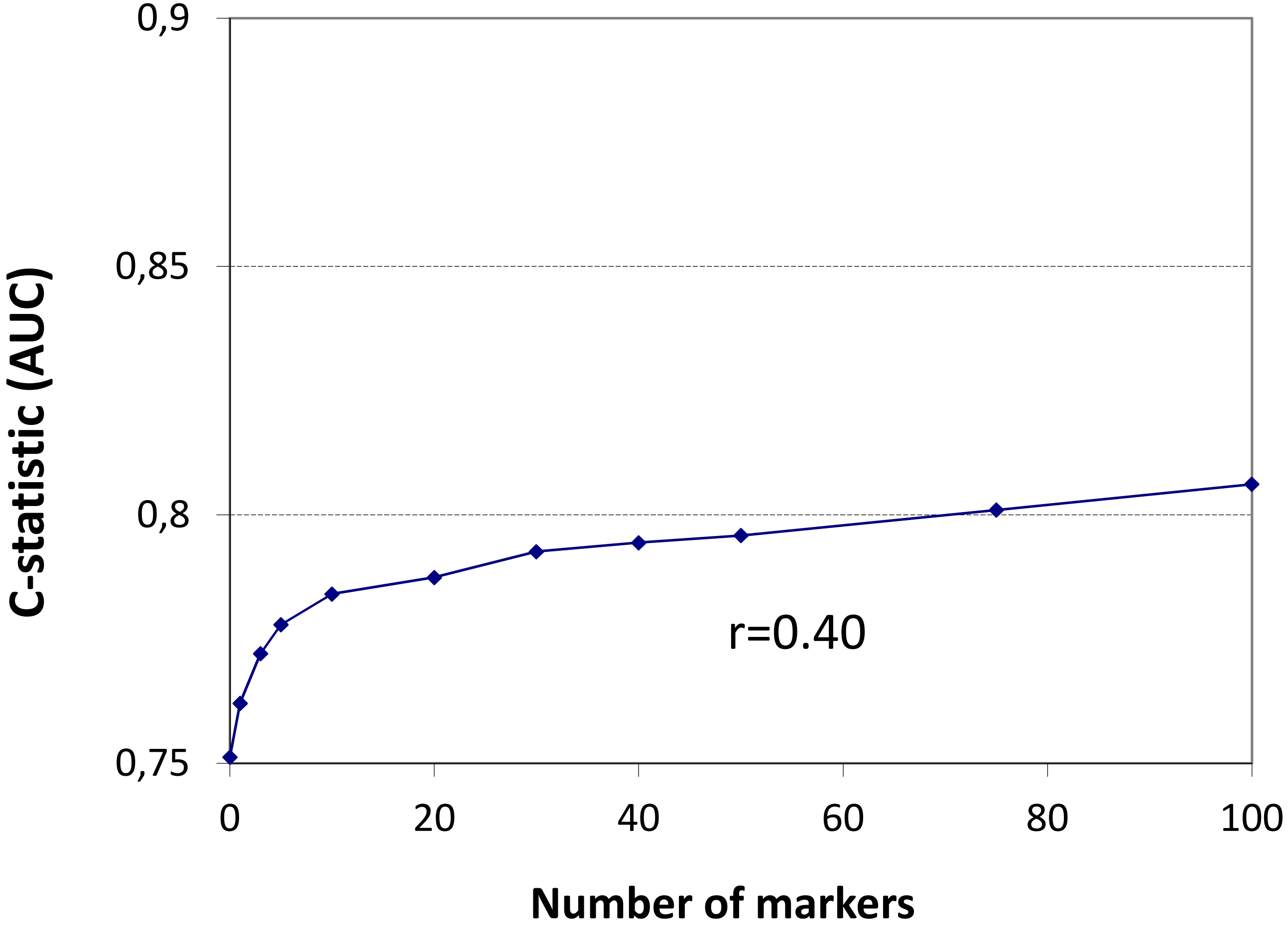
Wang et al, NEJM 2006

Can prognostic value be improved?



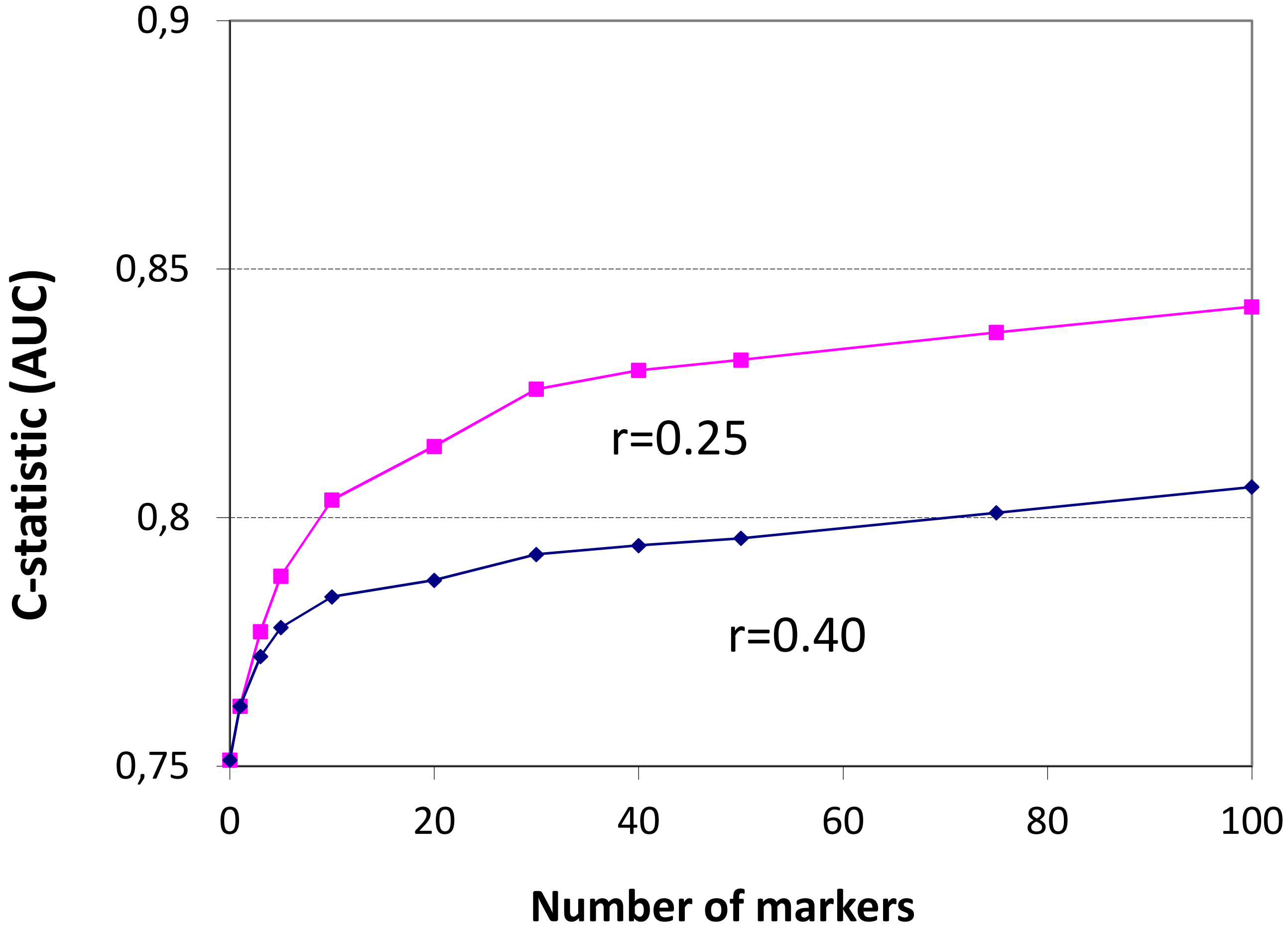
Wang, Circulation 2011
Figure, courtesy of M Pencina

Can prognostic value be improved?



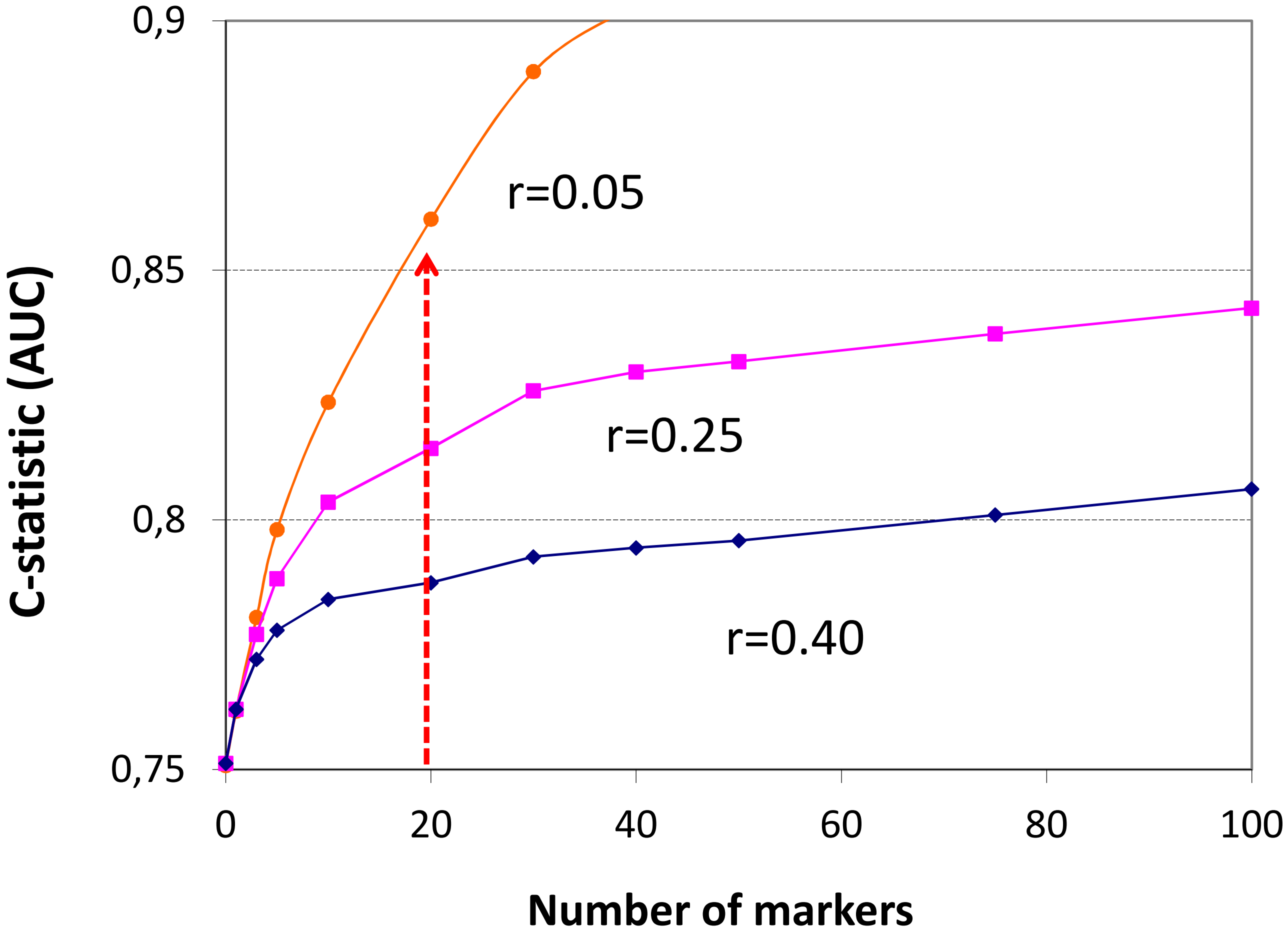
Wang, Circulation 2011
Figure, courtesy of M Pencina

Can prognostic value be improved?



Wang, Circulation 2011
Figure, courtesy of M Pencina

Can prognostic value be improved?



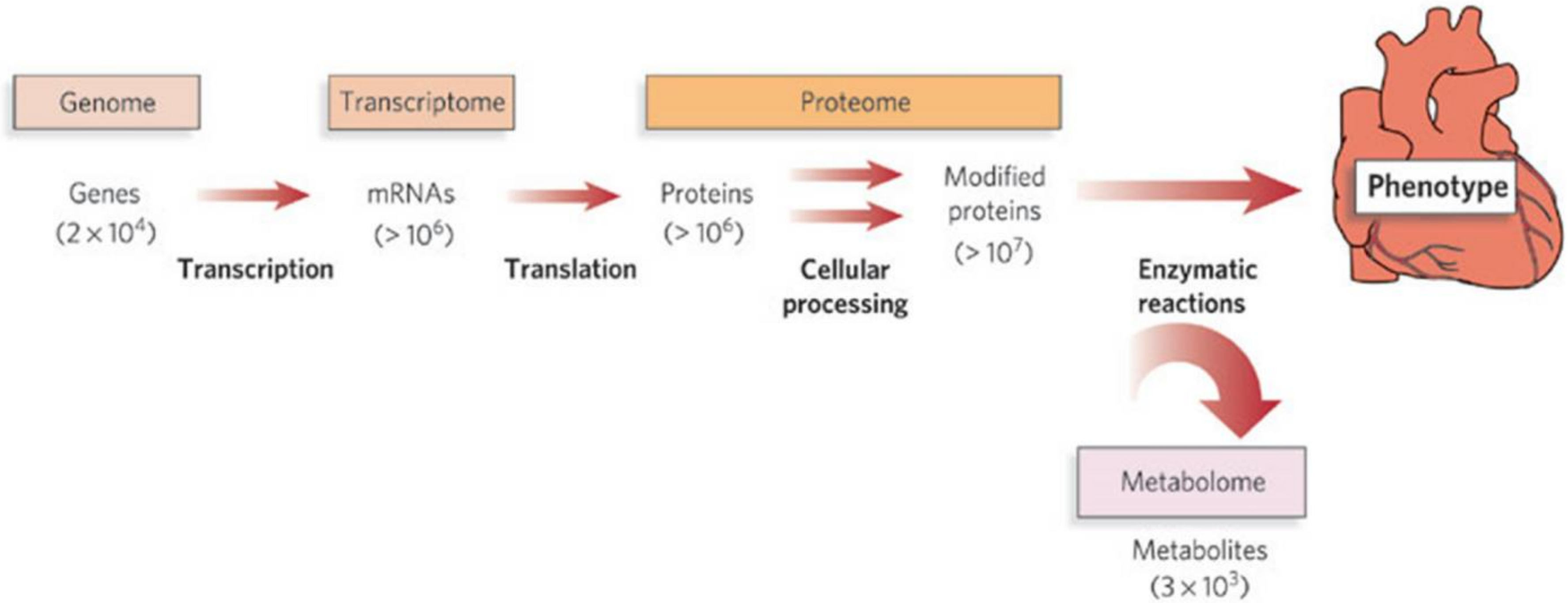
Wang, Circulation 2011
Figure, courtesy of M Pencina

Biomarkers are often correlated with each other

- Examples
  - CRP and fibrinogen (r=0.5), CRP and D-dimer (r=0.3), CRP and PAI-1 (r=0.3)

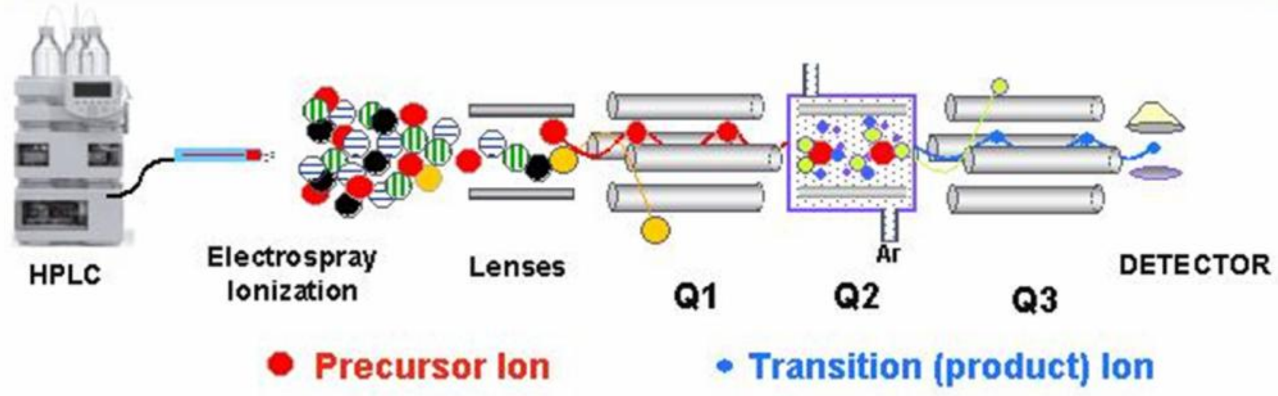
- Little clinical value in measuring multiple biomarkers that capture the same information -- for instance, from the same biological pathway

Using novel technologies to find "uncorrelated" biomarkers



From Gerszten and Wang, Nature 2008

Targeted LC/MS/MS

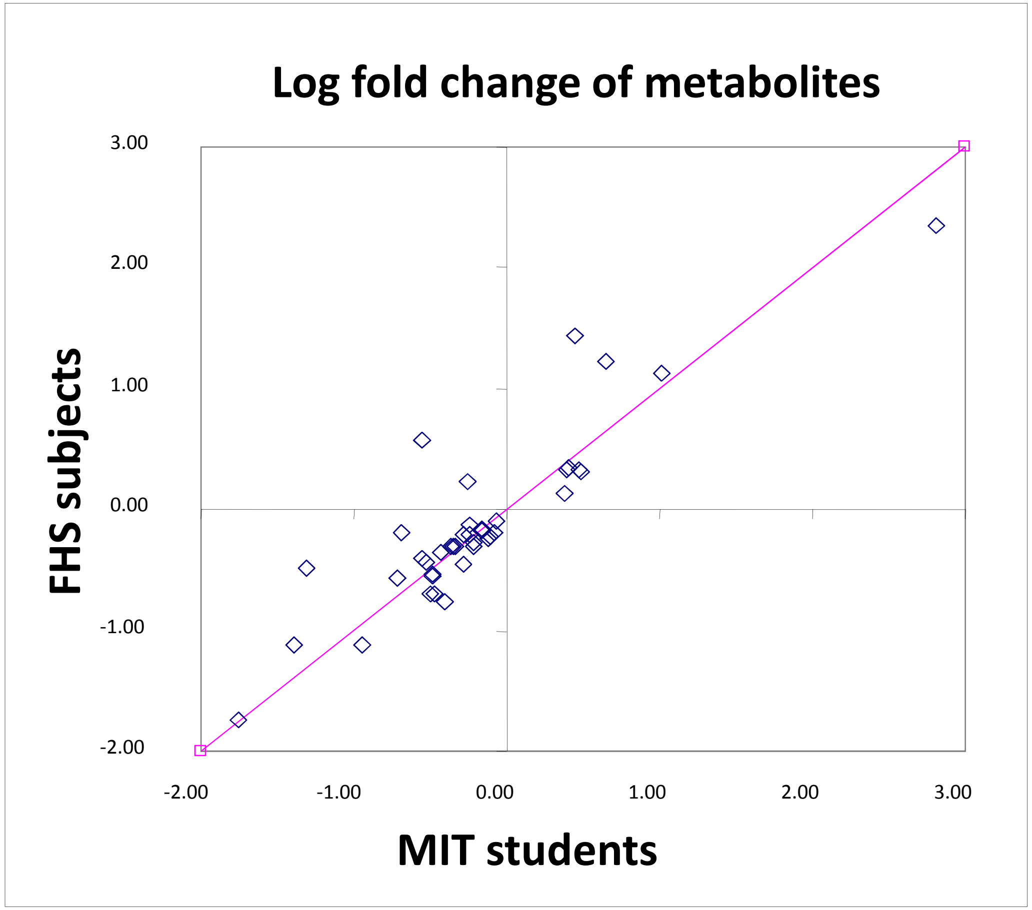


- **Q1 selects and passes only precursor of interest**
- **Q2 selected precursor is fragmented**
- **Q3: monitor specific fragment ions**

Targeted approach using LC-MS/MS
Metabolite “address”: elution time, MS characterization

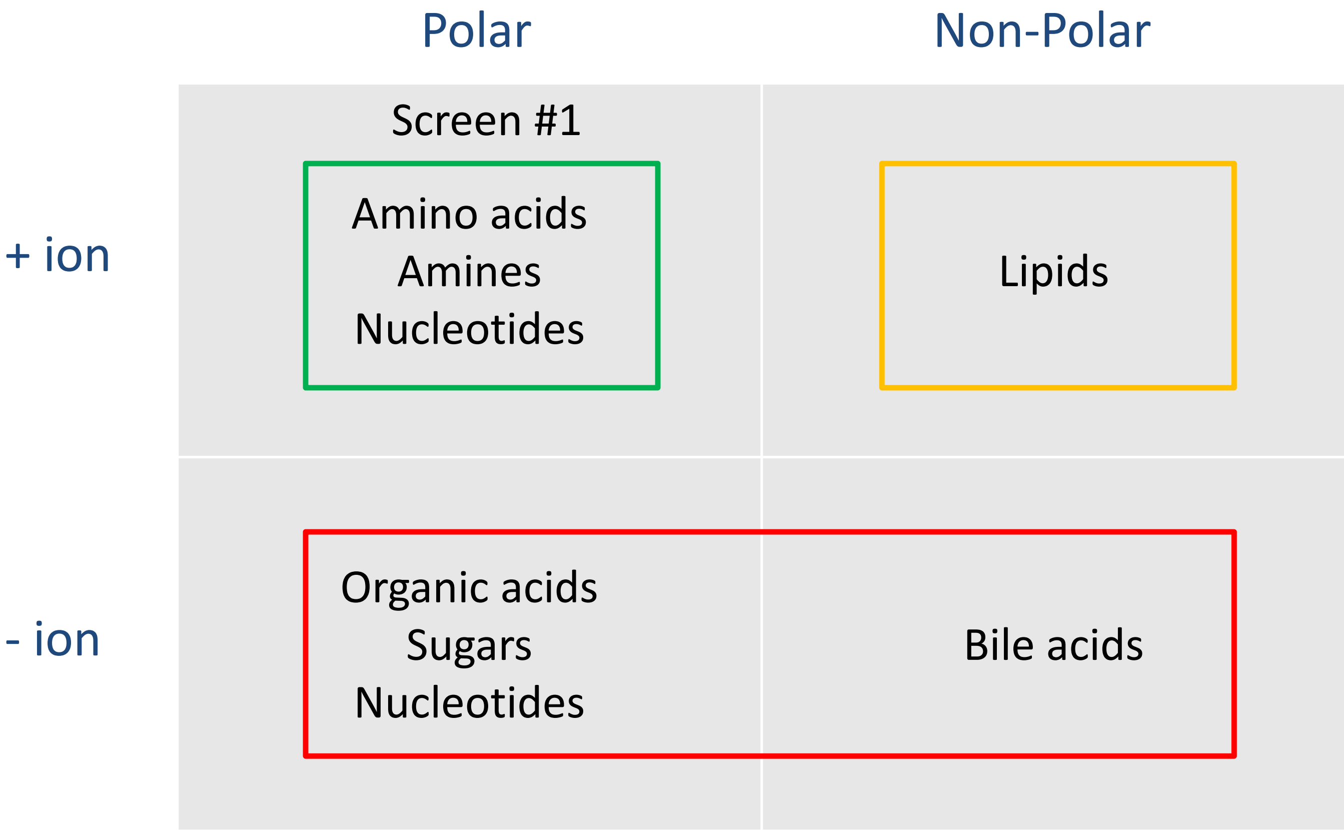
From R Gerszten

Comparison of results from stored FHS samples and fresh MIT samples (OGTT), in healthy normals



Shaham et al, Mol Syst Biol 2008

Targeted mass spec: 3 screens



From E. Rhee

Screen #1 identifies 5 metabolites associated with incident DM

Metabolite	P-value	Odds ratio for individuals in the top quartile*
isoleucine	<0.0001	3.14 (CI, 1.51-6.55)
phenylalanine	<0.0001	2.28 (CI, 1.00-5.20)
tyrosine	<0.0001	2.82 (CI, 1.25-6.34)
leucine	0.0005	3.66 (CI, 1.61-8.29)
valine	0.001	3.14 (CI, 1.43-6.86)

*adjusted for age, sex, BMI, glucose

Similar results even when restricting to individuals who took 12 years to develop diabetes

Wang et al, Nature Medicine 2011

Baseline amino acid levels predict above and beyond insulin measures, OGTT

Model	Isoleucine	Leucine	Valine	Tyrosine	Phenylalanine
	Adjusted odds ratios, per SD increment in metabolite (95% confidence interval)				
Basic model	1.68 (1.26-2.23)	1.54 (1.17-2.03)	1.39 (1.11-1.74)	1.56 (1.18-2.06)	1.70 (1.28-2.25)
+ fasting insulin	1.63 (1.22-2.17)	1.51 (1.14-2.00)	1.36 (1.08-1.71)	1.52 (1.14-2.02)	1.69 (1.27-2.24)
+ HOMA-IR	1.63 (1.22-2.17)	1.51 (1.15-2.00)	1.36 (1.08-1.71)	1.52 (1.15-2.02)	1.69 (1.27-2.24)
+ HOMA-B	1.63 (1.22-2.17)	1.51 (1.14-2.00)	1.35 (1.08-1.71)	1.52 (1.14-2.01)	1.69 (1.27-2.24)
+ OGTT (2-hr glucose)	1.58 (1.18-2.12)	1.46 (1.10-1.93)	1.33 (1.06-1.68)	1.49 (1.13-1.98)	1.68 (1.27-2.24)

Amino acid score and risk of future diabetes

Model	Isoleucine, Phenylalanine, Tyrosine
	Discovery (FHS) 12 year follow-up N=378
1st quartile	1.0 (referent)
2nd quartile	3.48 (1.68 – 7.23)
3rd quartile	2.82 (1.25 - 6.34)
4th quartile	5.99 (2.34 – 15.34)
P for trend	0.0009

Adjusted for age, sex, BMI, fasting glucose

Wang et al, Nature Medicine 2011

Amino acid score and risk of future diabetes

Model	Isoleucine, Phenylalanine, Tyrosine	
	Discovery (FHS) 12 year follow-up N=378	Replication (Malmo) 13 year follow-up N=326
1^{st} quartile	1.0 (referent)	1.0 (referent)
2^{nd} quartile	3.48 (1.68 – 7.23)	2.08 (0.97-4.46)
3^{rd} quartile	2.82 (1.25 - 6.34)	2.59 (1.09-6.15)
4^{th} quartile	5.99 (2.34 – 15.34)	3.93 (1.54-10.04)
P for trend	0.0009	0.006

Adjusted for age, sex, BMI, fasting glucose

Wang et al, Nature Medicine 2011

How helpful is the clinical information provided by metabolites?

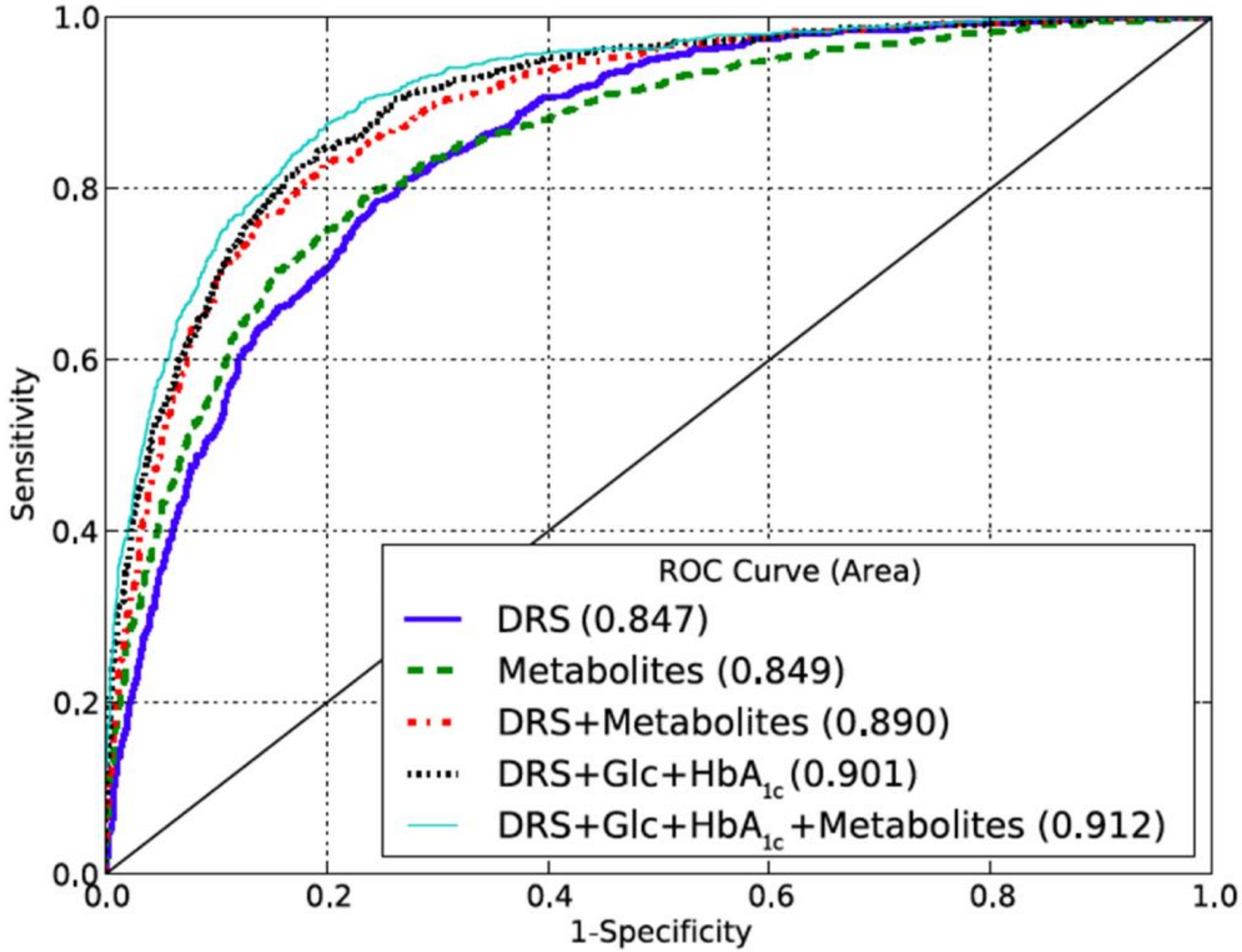
	C-statistic (AUC)
Genotype score	0.641
Metabolite score	0.803
Clinical risk factors	0.856
Clinical + metabolites + genotype	0.880*

p=0.002 vs. clinical risk factors alone

Framingham Offspring Study (n=1,622, 13-year follow up)

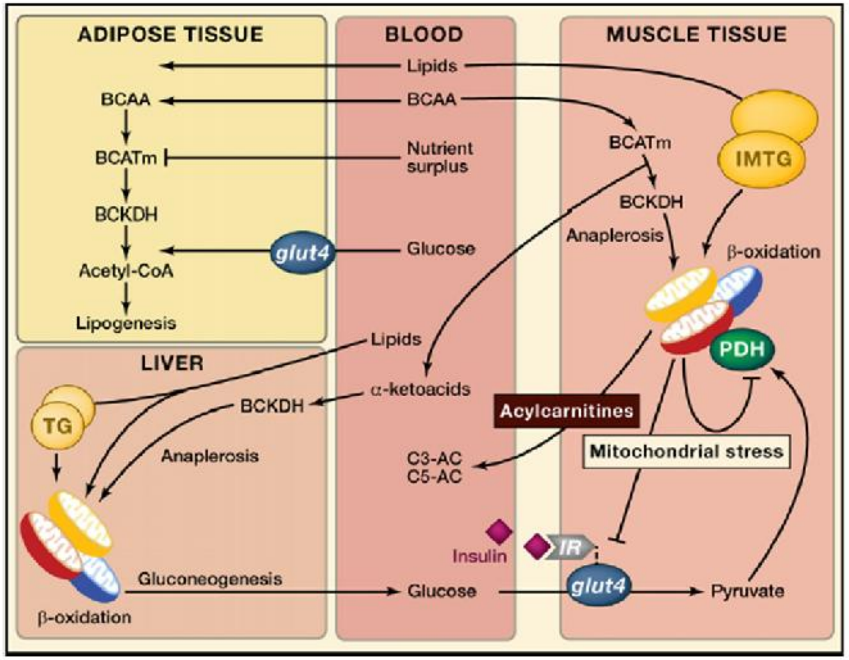
Walford et al, Diabetes Care 2014

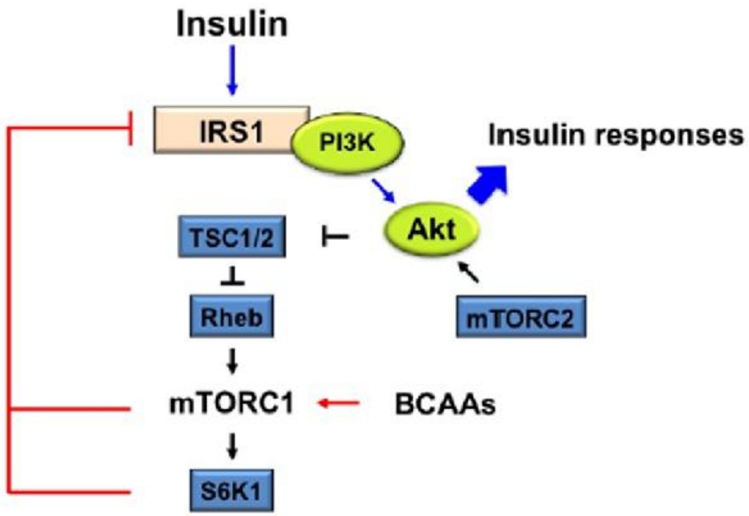
Contribution of metabolites to DM prediction (EPIC-Potsdam study)



Floegel et al, Diabetes 2013

Are the BCAAs playing a causal role?



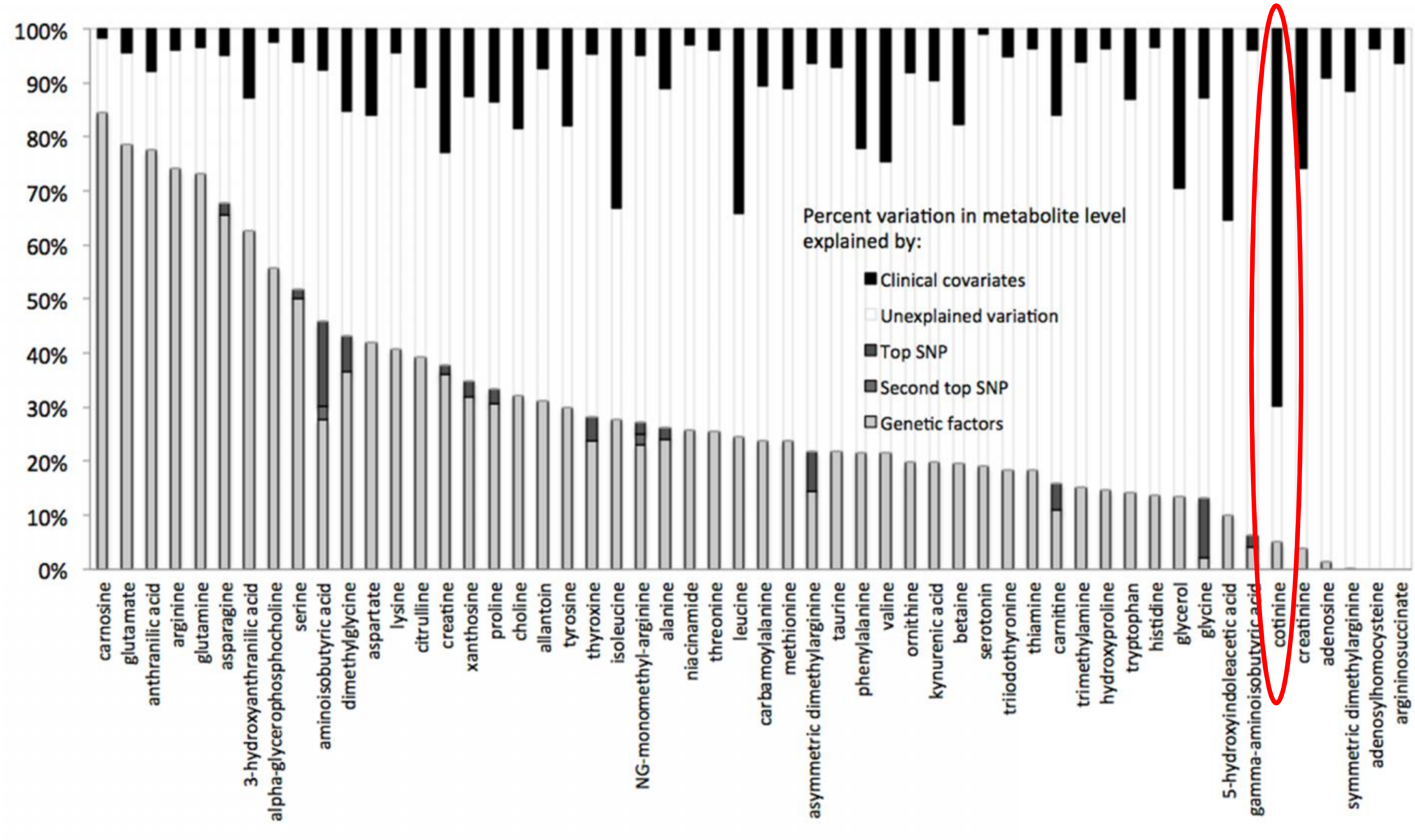


Newgard, Cell Metab 2012
Yoon, Nutrients 2016

Physical activity, diet, and amino acids (Framingham)

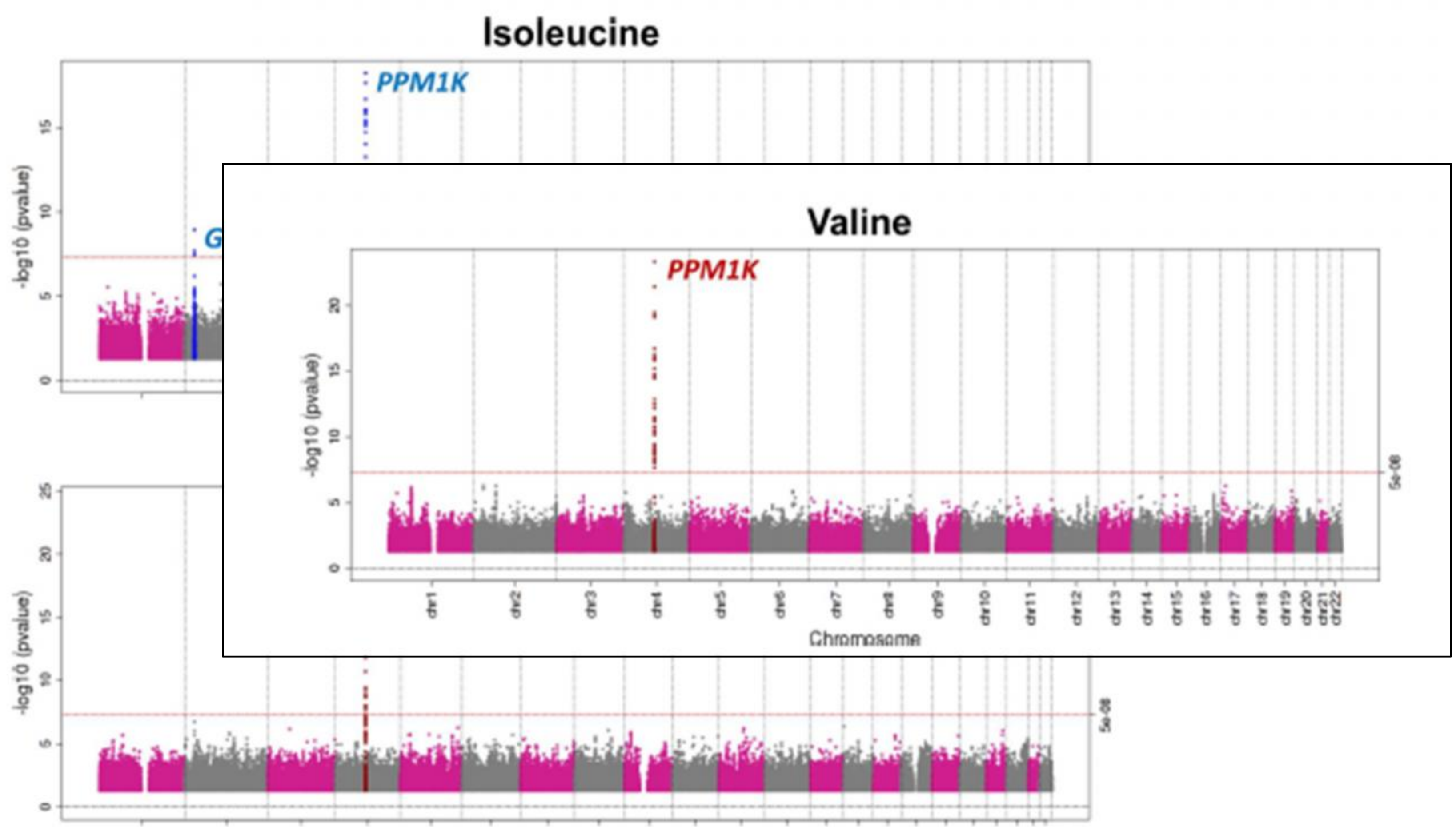
	Cases ($n = 189$)	Matched controls ($n = 189$)
Physical activity index	35 ± 6.2	35 ± 7.3
Total caloric intake, kcal	1,982 ± 660	1,866 ± 600
Total protein intake, g	82 ± 28	78 ± 28
Phenylalanine intake, g	3.6 ± 1.2	3.4 ± 1.3
Tyrosine intake, g	3.0 ± 1.0	2.8 ± 1.1
Leucine intake, g	6.5 ± 2.2	6.1 ± 2.3
Isoleucine intake, g	3.9 ± 1.3	3.7 ± 1.4
Valine intake, g	4.3 ± 1.5	4.1 ± 1.5

No significant correlations of amino acids with FFQ variables or physical activity index



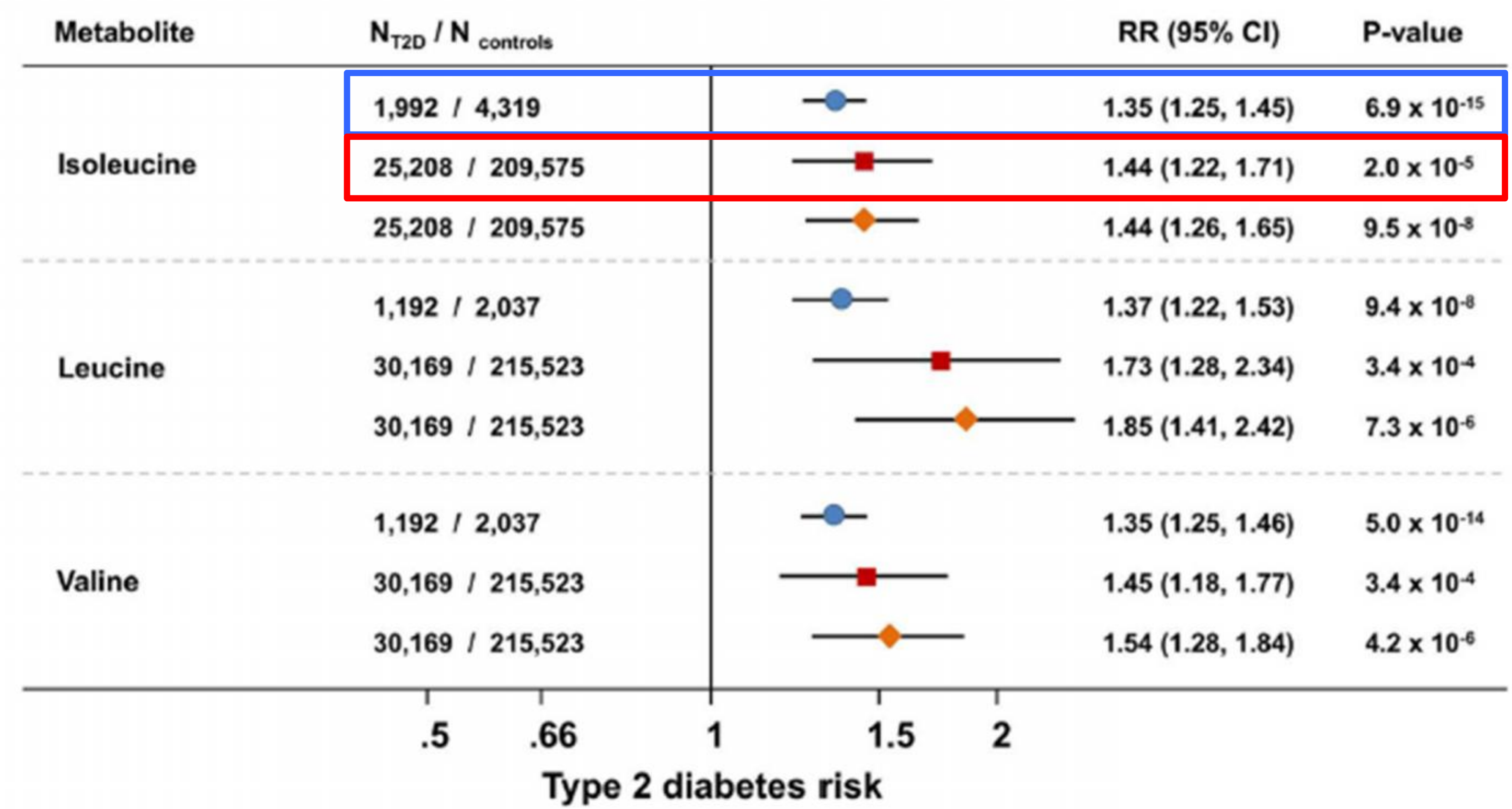
Rhee et al, Cell Metabolism 2013

BCAAs: genetic determinants



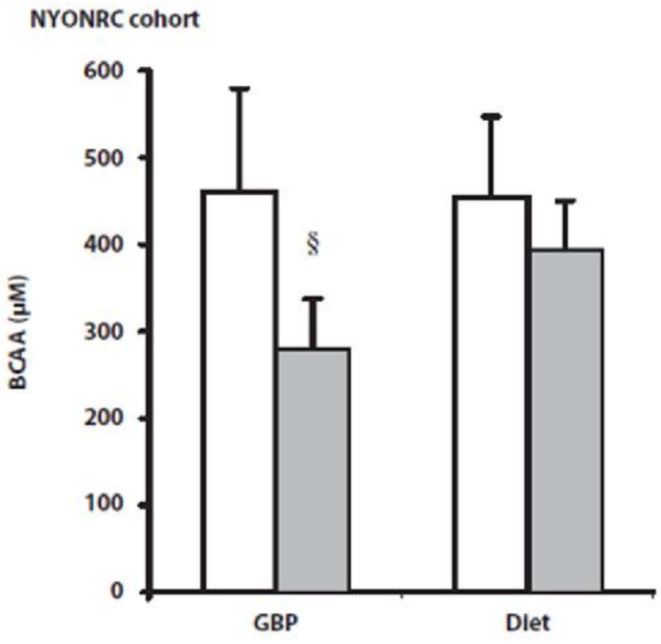
Lotta et al PLoS Med 2016

BCAA and DM: Mendelian randomization analyses



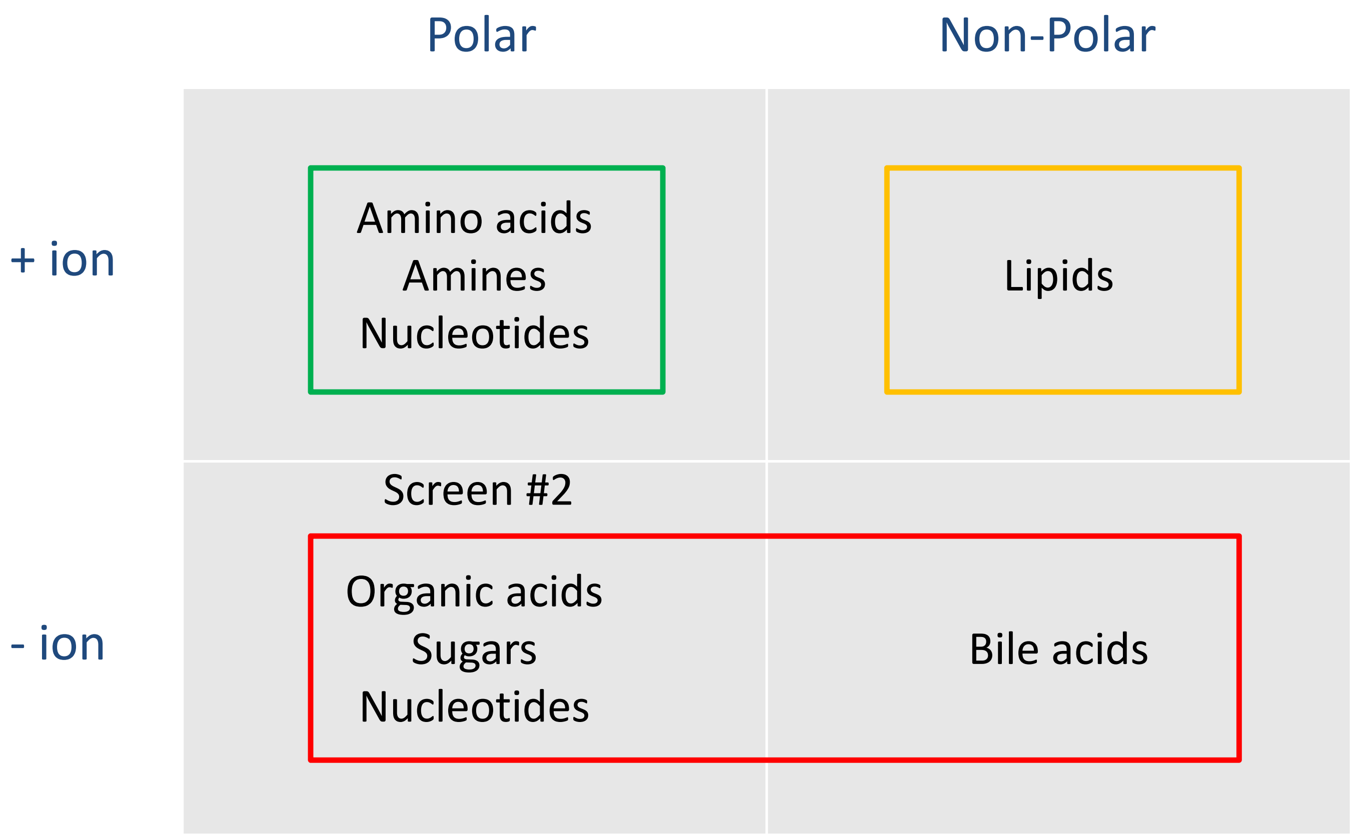
Lotta et al PLoS Med 2016

Impact of surgical and medical weight loss on BCAAs



Laferrere et al, Sci Transl Med 2011

Targeted mass spec: 3 screens



From E. Rhee

Screen #2 identifies 2-aminoadipate as a predictor of diabetes risk

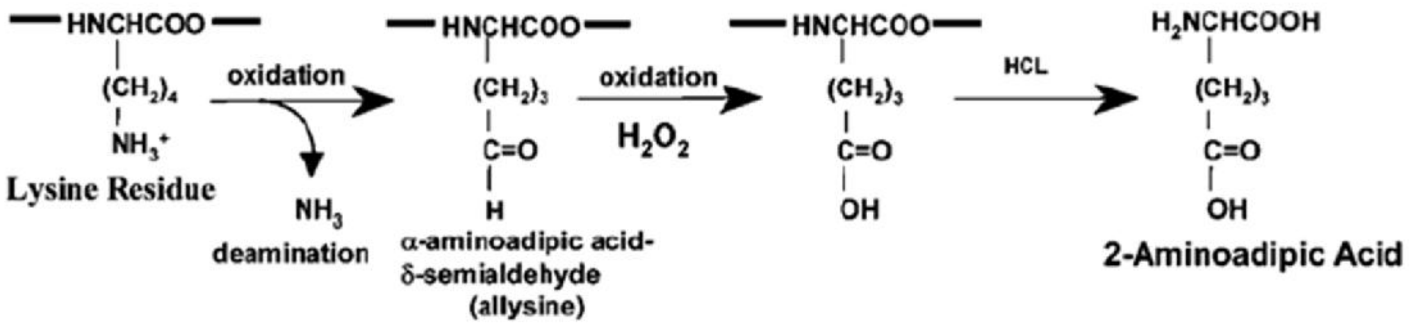
Metabolite	Paired T-statistic	P-value
2-aminoadipate	3.39	0.0009
quinolinate	2.53	0.0121
PEP	2.49	0.0138
UDP-galactose/UDP-glucose	2.42	0.0164
hippurate	-2.19	0.0294
F1P/F6P/G1P/G6P	2.24	0.0265
beta-hydroxybutyrate	-1.95	0.0529
UDP	1.91	0.0583
3-methyladipate	-1.85	0.0657
salicylurate	1.77	0.0780
isocitrate	1.61	0.11
alpha-glycerophosphate	1.58	0.12

Wang et al, JCI 2013

2-aminoadipic acid and risk of future DM

Model	2-aminoadipic acid Framingham Heart Study (188 cases, 188 controls) 12-year follow-up	Malmö Diet and Cancer (162 cases, 162 controls) 13-year follow-up	Combined sample (350 cases, 350 controls)
As continuous variable			
Per SD increment	1.60 (1.19-2.16)	1.57 (1.15-2.14)	1.59 (1.28-1.97)
P	0.002	0.004	<0.0001
As categorical variable			
1^{st} quartile	1.00 (Referent)	1.00 (Referent)	1.00 (Referent)
2^{nd} quartile	1.34 (0.72-2.49)	2.19 (1.07-4.48)	1.66 (1.05-2.63)
3^{rd} quartile	1.71 (0.82-3.54)	1.45 (0.68-3.07)	1.56 (0.93-2.61)
4^{th} quartile	4.49 (1.86-10.89)	3.96 (1.63-9.59)	4.12 (2.22-7.65)
***P* for trend**	0.001	0.01	<0.0001

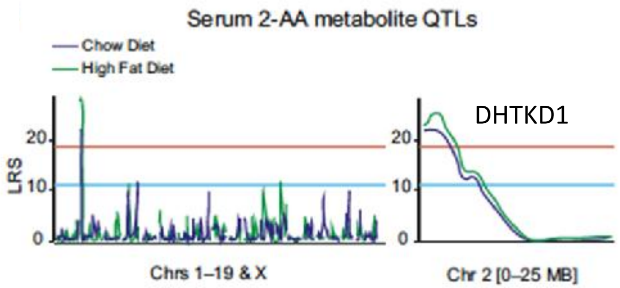
2-aminoadipic acid is a product of lysine degradation

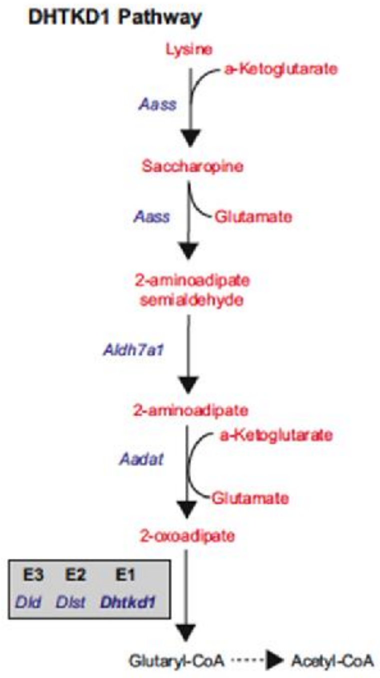


J Biochem 2007

- In FHS, moderately correlated with lysine (r = 0.38), insulin (r = 0.25), and HOMA-IR (r=0.24), but not lysine intake
- Only modest correlation with BCAA or aromatic amino acids (r<0.2)

Genetic regulation of 2-AAA metabolism: animal and human data



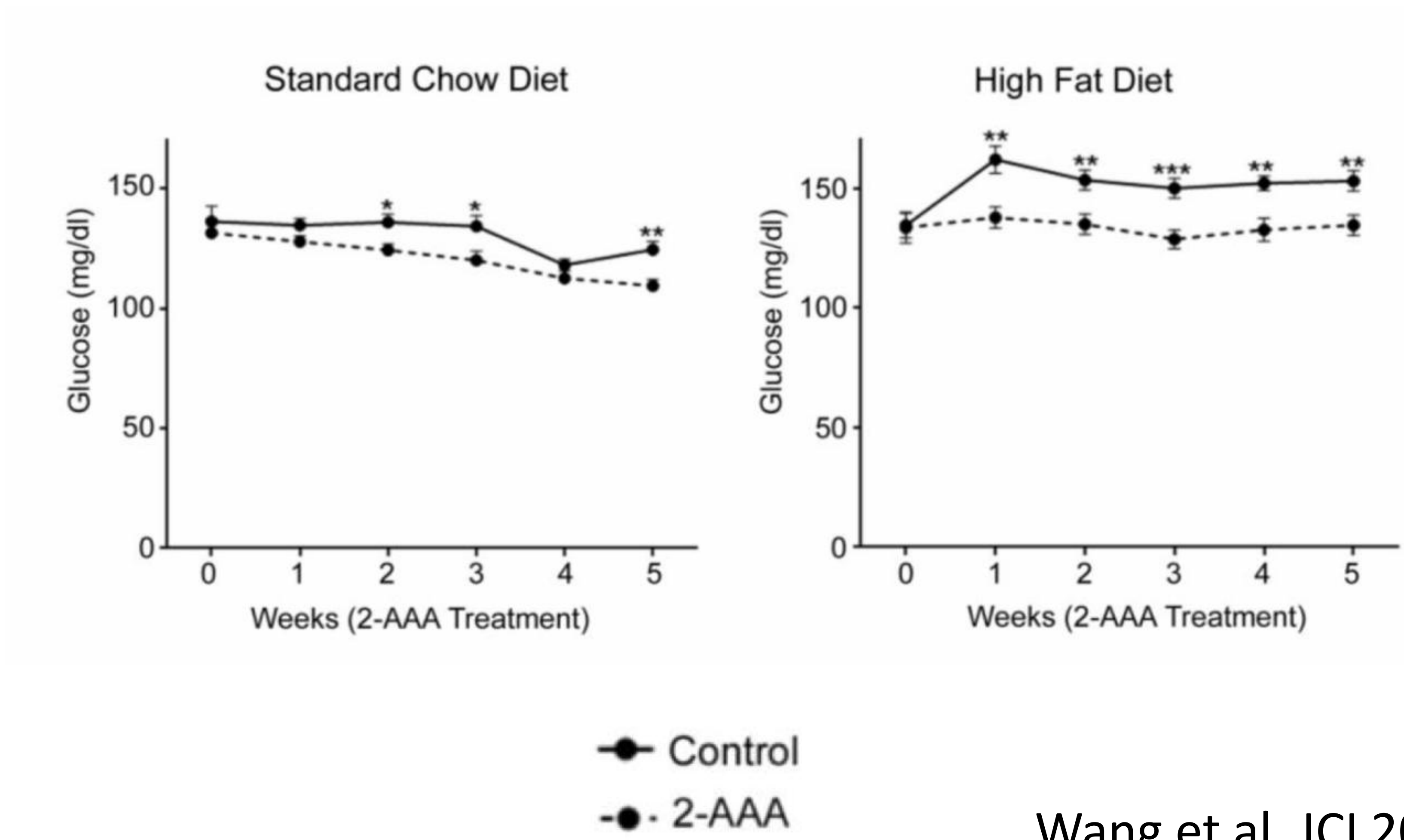


DHTKD1 variants and plasma 2-AAA (Framingham): p=0.04-0.05

DHTKD1 variants and type 2 DM (DIAGRAM consortium): p=0.007

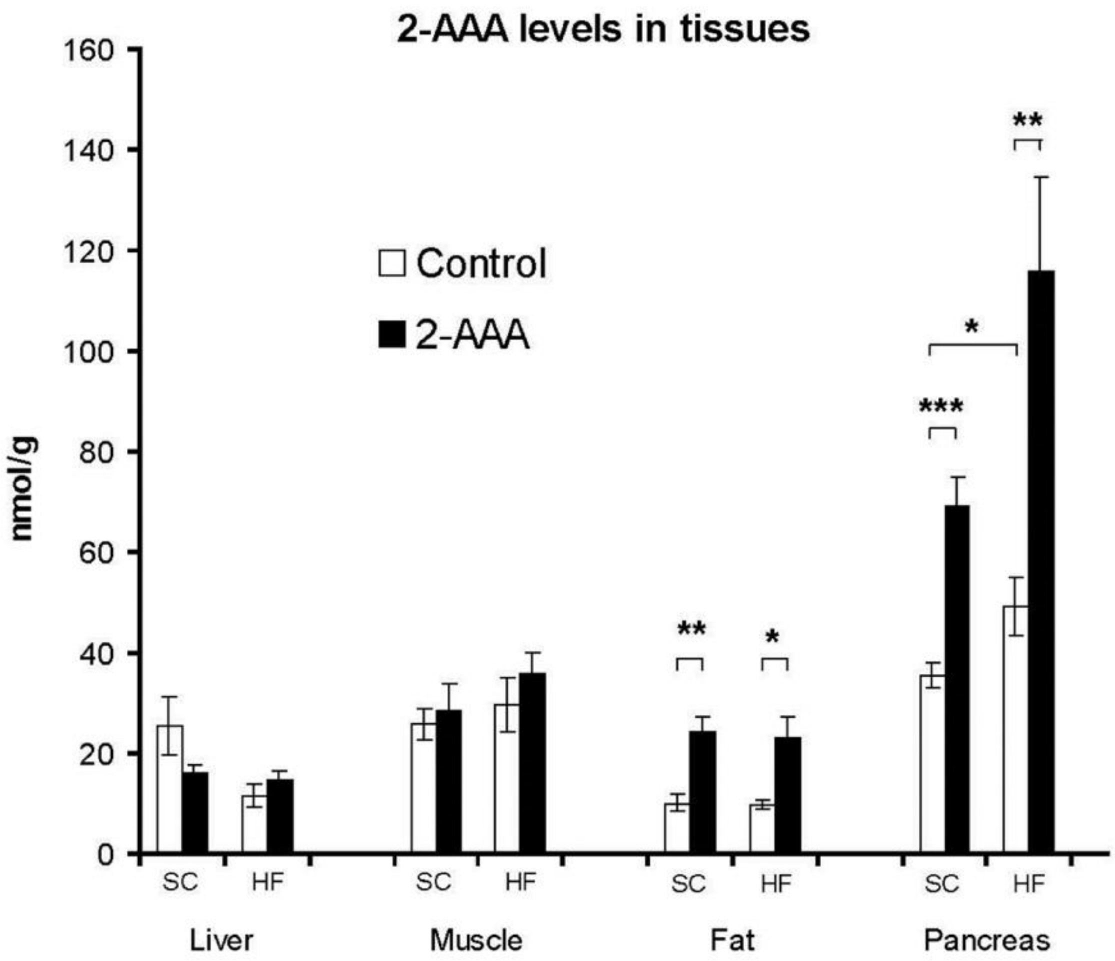
Wu et al, Cell 2014

2-AAA feeding modulates fasting glucose

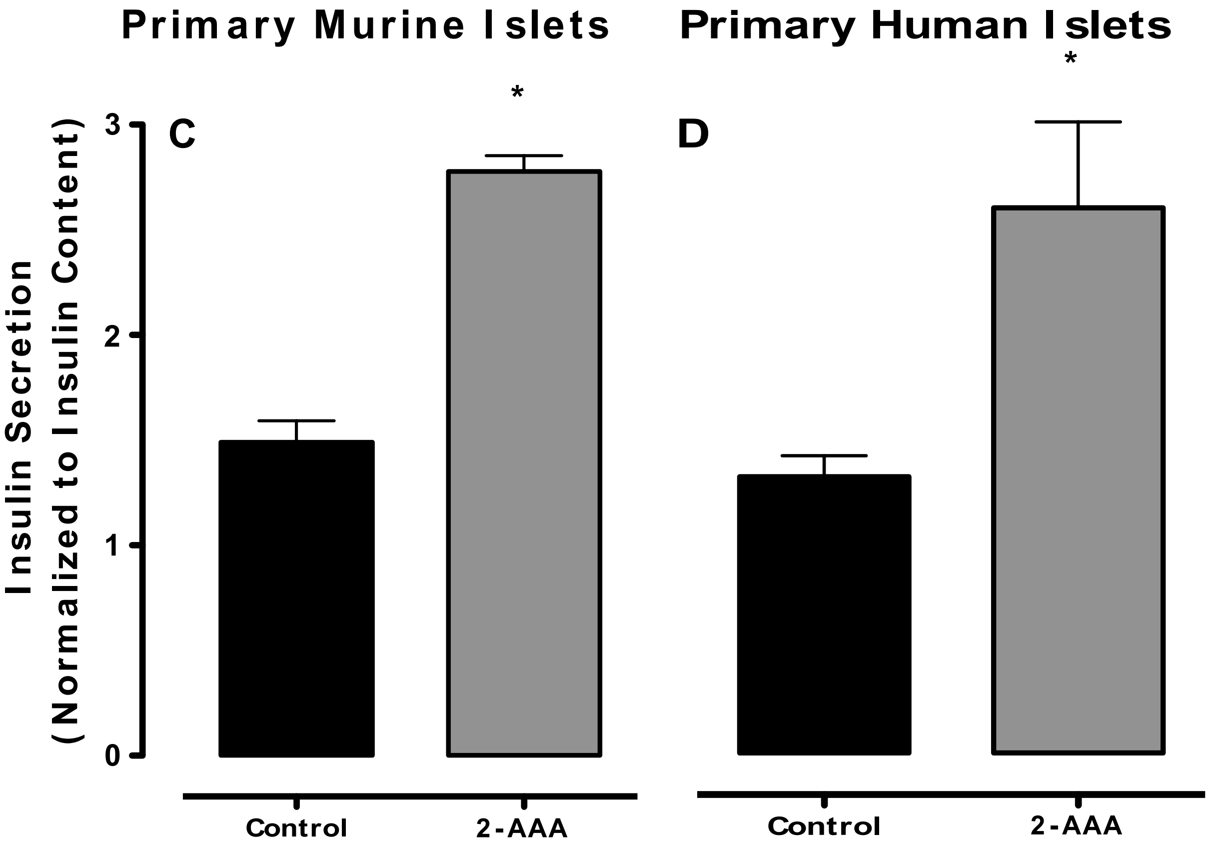


Wang et al, JCI 2013

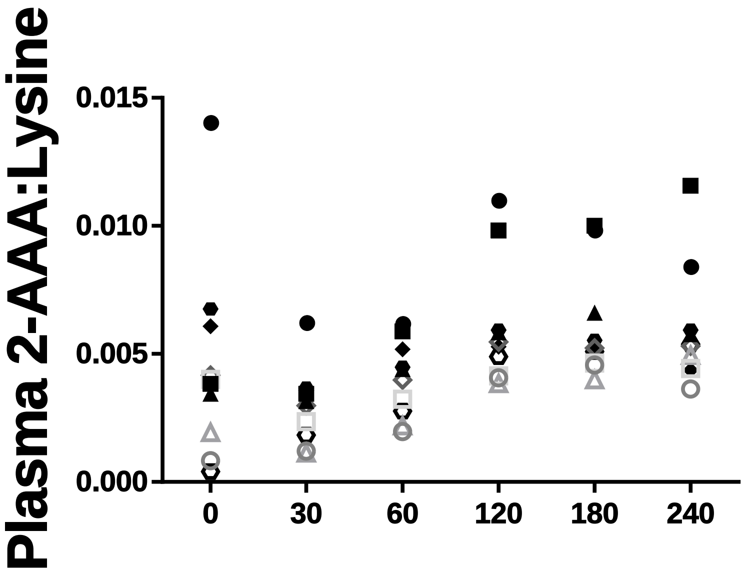
2-AAA levels are enriched in the pancreas

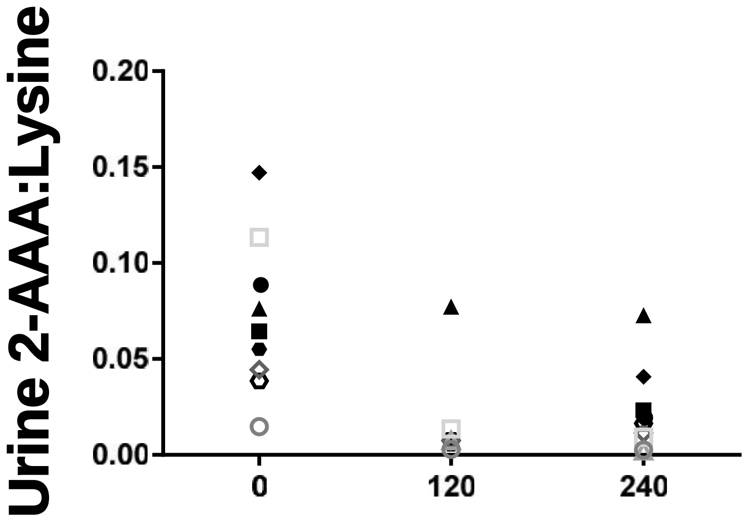


2-AAA enhances insulin secretion



Inter-individual variability in 2-AAA:Lysine at baseline and preserved over time



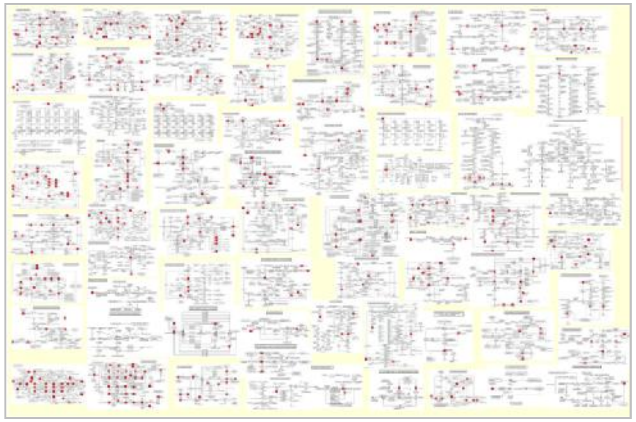


Jane Ferguson

Overview

- Rationale
- Targeted mass spec analyses and DM
- Non-targeted approaches

Human Metabolome

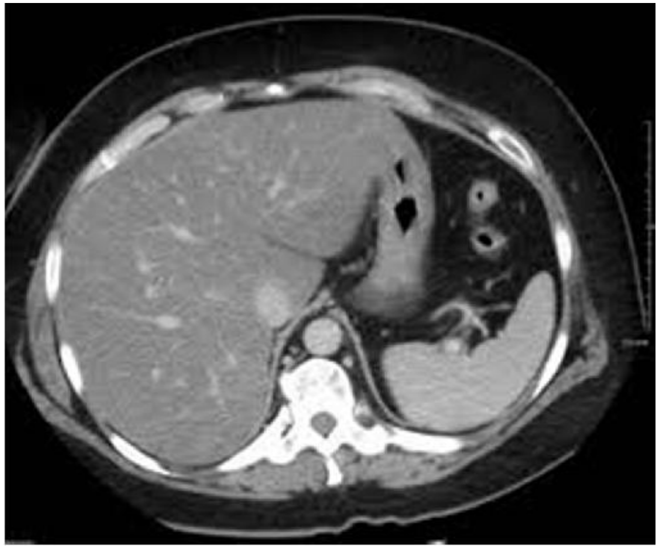


Initial experience with non-targeted approach in Framingham

- 1,000 Framingham Gen 3 participants
- HILIC chromatography/ positive ion mode MS on QExactive (hybrid method)
- ~7,000 total peaks
- ~5,000 peaks were observed in >80% of individuals
- 987 "factors"

Gerszten and Clish (Broad Institute)

Unique phenotyping in Framingham Gen 3



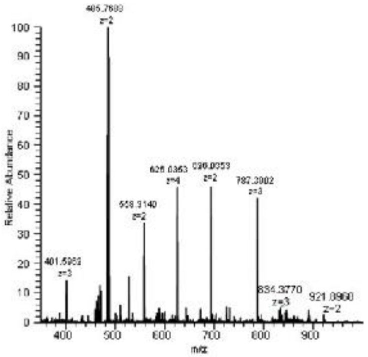
~250 unknown peaks associated
with hepatic fat by CT

With Caroline Fox

Top metabolites associated with liver fat by CT (Framingham)

	m/z	RT	P Value
1	202.1185	7.79	2.28E-24
2	551.5034	1.61	5.49E-22
3	386.2536	1.99	1.53E-18
4	606.6179	1.66	3.17E-17
5	612.5556	1.63	1.88E-16
6	578.5864	1.66	2.50E-16
7	634.6491	1.65	3.80E-16
8	116.1073	7.87	4.16E-16
9	223.9720	7.77	1.20E-14
10	313.2733	1.63	1.55E-14

O’Sullivan et al



Unknown Metabolite
Mass = 202.1185

p = 6.22E-24

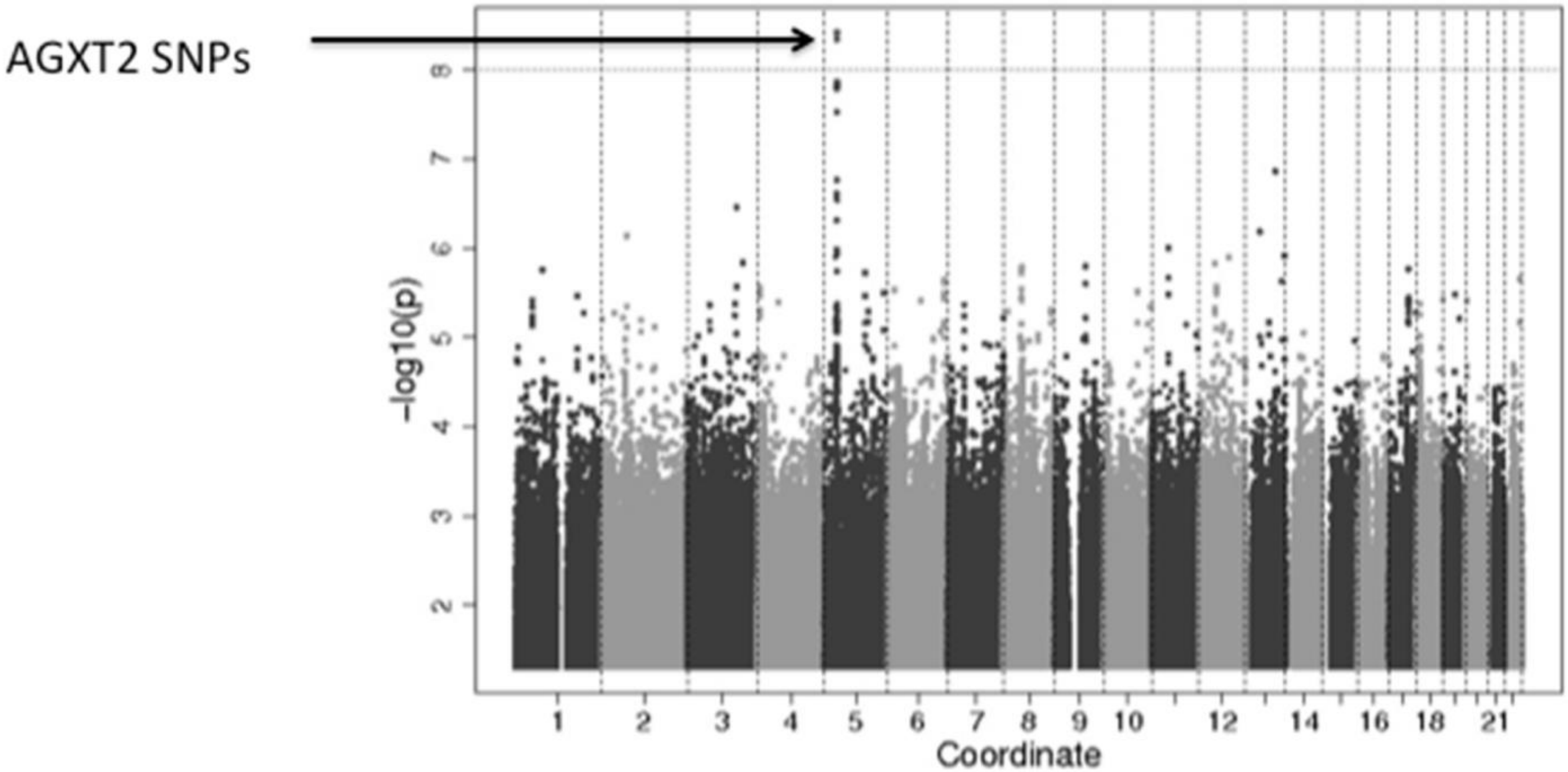
<u>Phenotype:</u>
Liver Fat
(CT Scan)

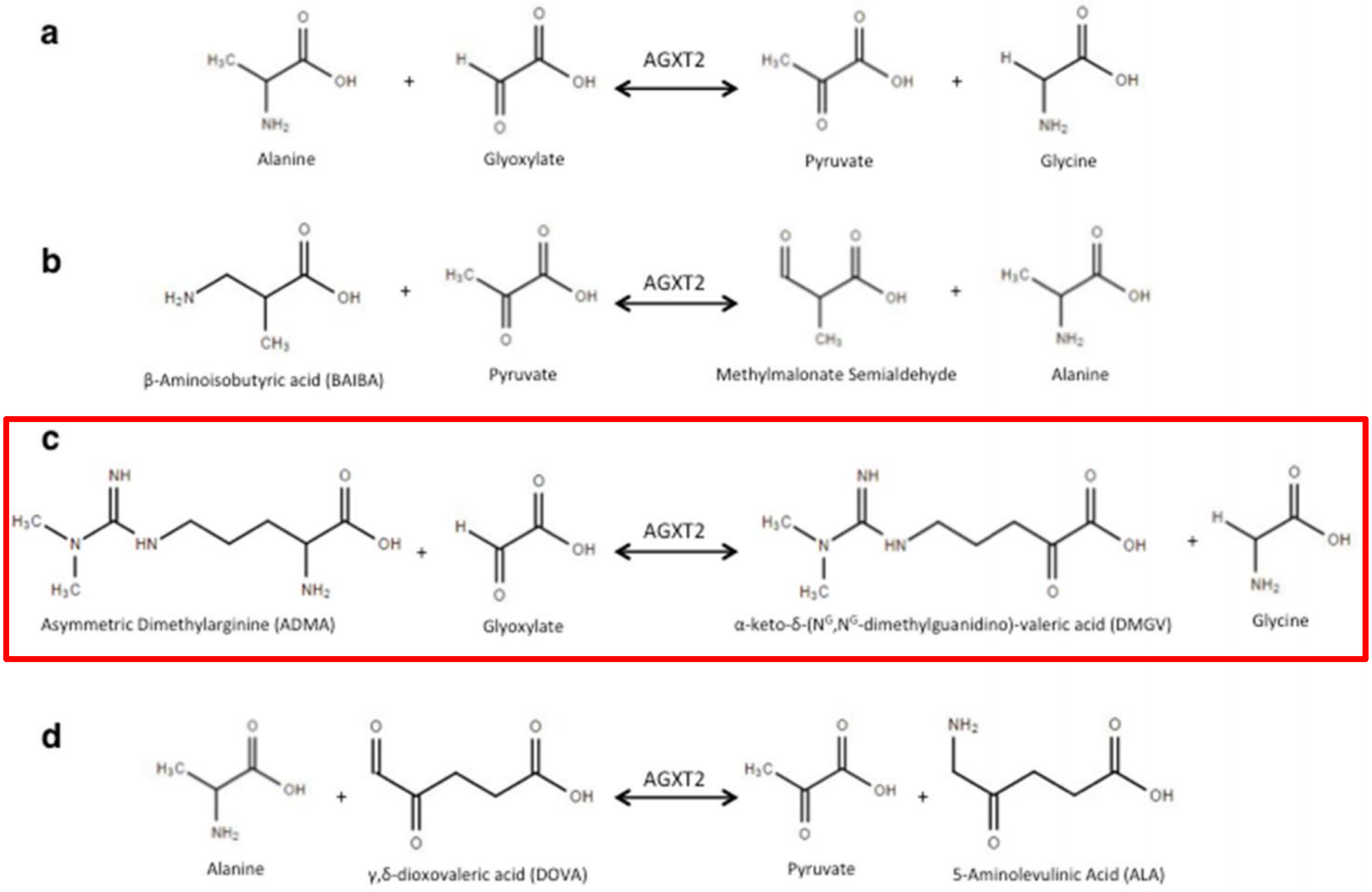
<u>Potential database matches</u>

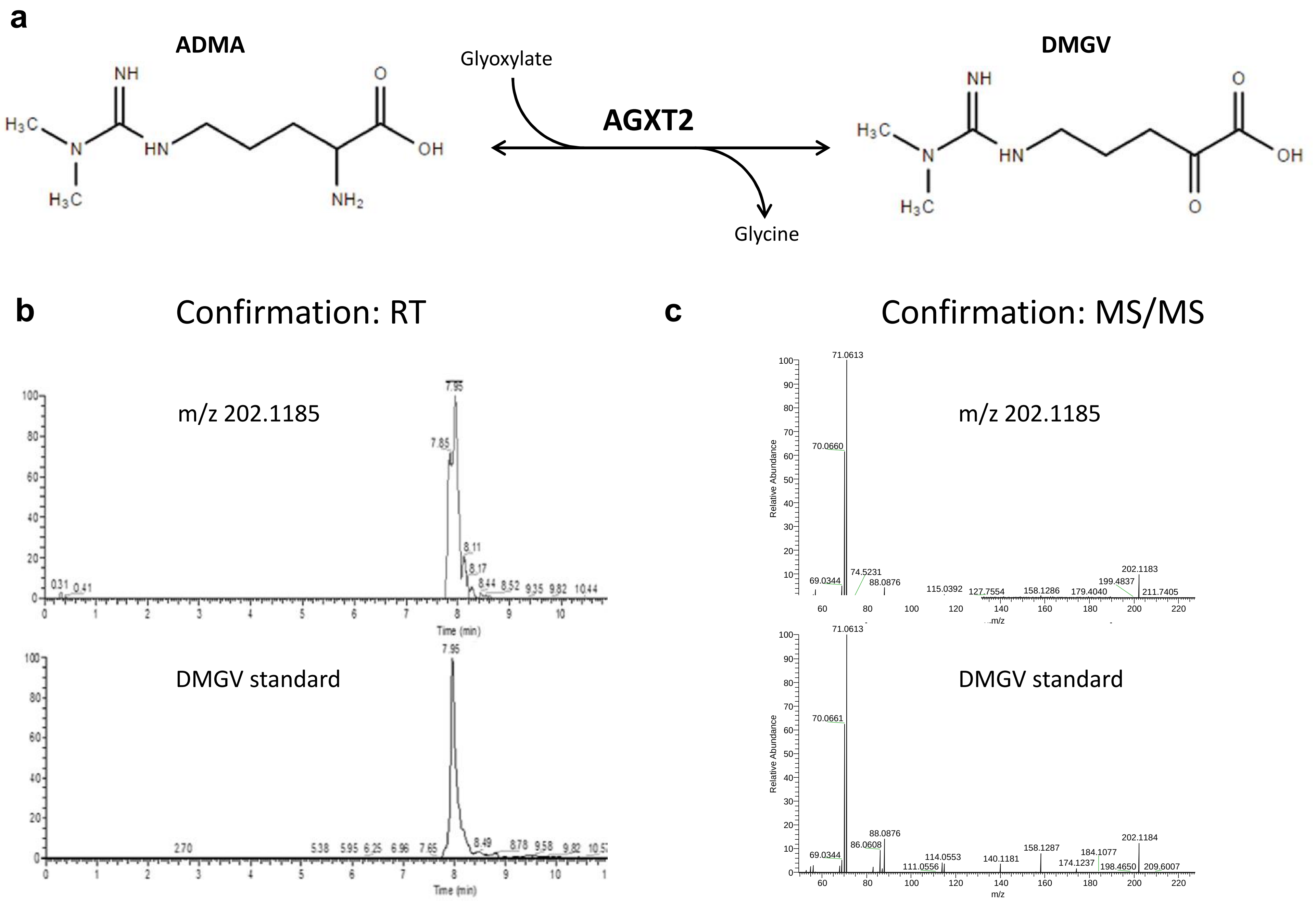
L-Threonine
L-Allothreonine
Hydroxyethyl glycine
4-Amino-3-hydroxybutyrate
L-Homoserine
D-Alanyl-D-alanine
Alanyl-Alanine
4-Acetamido-2-aminobutanoic acid
1-Methylhistidine
3-Methylhistidine
Ethyl lactate
2-Methyl-3-hydroxybutyric acid
3-Hydroxy-2-methyl-[R-(R,S)]-butanoic acid
Erythronilic acid
2-Ethylhydracrylic acid
2-Hydroxy-3-methylbutyric acid
4-Hydroxyisovaleric acid
3-Hydroxyisovaleric acid
3-Hydroxyvaleric acid
4-Hydroxyvaleric acid
2-Hydroxy-2methylbutyric acid
Diethyl carbonate
3-Hydroxy-2-methyl-[S-(R,R)]-butanoic acid
2-Hydroxyvaleric acid

O’Sullivan et al

GWAS of the unknown metabolite reveals association with *AGXT2* locus

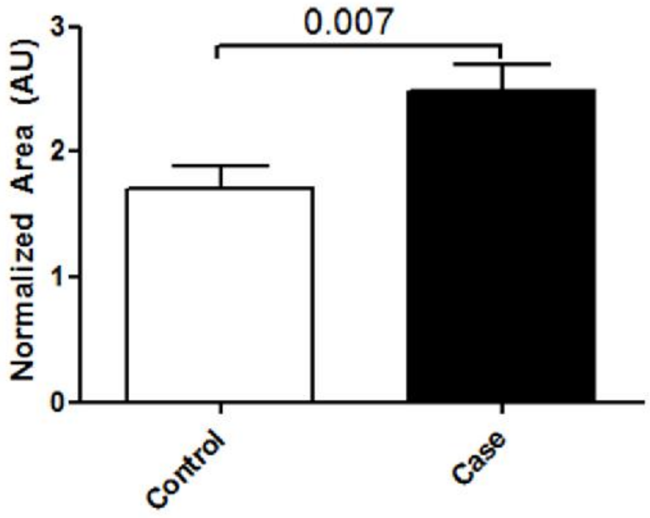



a
Alanine
Glyoxylate
AGXT2
Pyruvate
Glycine
b
β-Aminoisobutyric acid (BAIBA)
Pyruvate
AGXT2
Methylmalonate Semialdehyde
Alanine
c
Asymmetric Dimethylarginine (ADMA)
Glyoxylate
AGXT2
α-keto-δ-(N^G,N^G-dimethylguanidino)-valeric acid (DMGV)
Glycine
d
Alanine
γ,δ-dioxovaleric acid (DOVA)
AGXT2
Pyruvate
5-Aminolevulinic Acid (ALA)


a
ADMA
DMGV
Glyoxylate
AGXT2
Glycine
b
Confirmation: RT
m/z 202.1185
DMGV standard
Time (min)
c
Confirmation: MS/MS
m/z 202.1185
DMGV standard
Relative Abundance
m/z
71.0613
70.0660
70.0661
202.1183
202.1184
88.0876

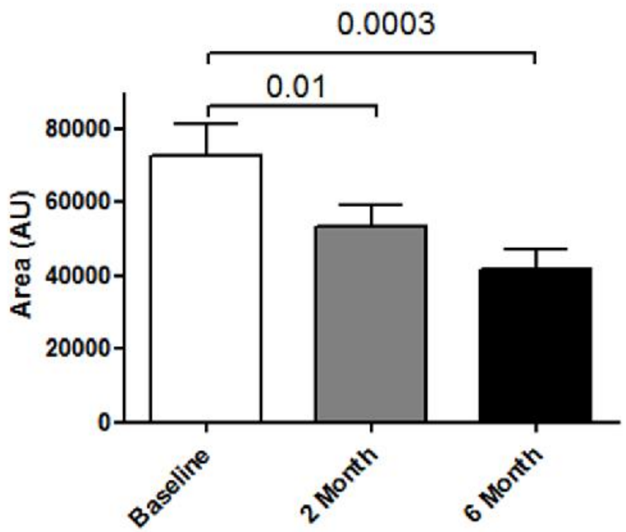
Novel metabolite is associated with NASH, and decreases with surgical weight loss

Biopsy-Proven NASH Cohort



Age-, sex- and BMI-matched

Gastric Bypass Cohort



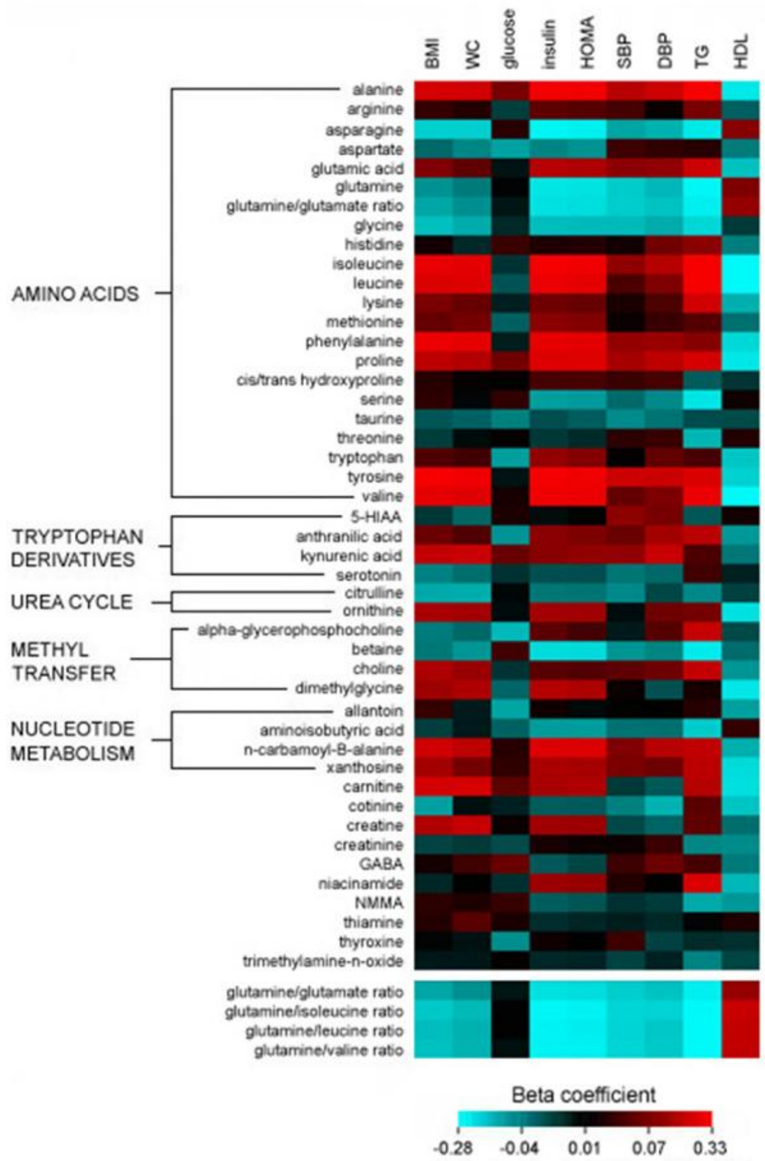
With Kathleen Corey

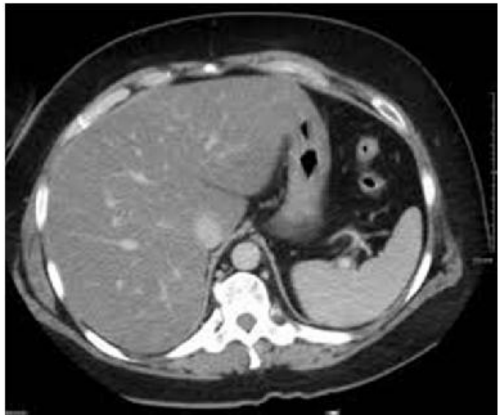
Novel metabolite (DMGV) is associated with future diabetes

- FHS (4 yr follow-up)
  - 20 incident cases of DM
  - 1.8-fold increase per SD, $p = 0.00045$

- MDC (12.6 yr follow-up)
  - 196 incident cases of DM
  - 1.6-fold increase per SD, $p = 0.0008$

Publicly-available metabolomic data from FHS





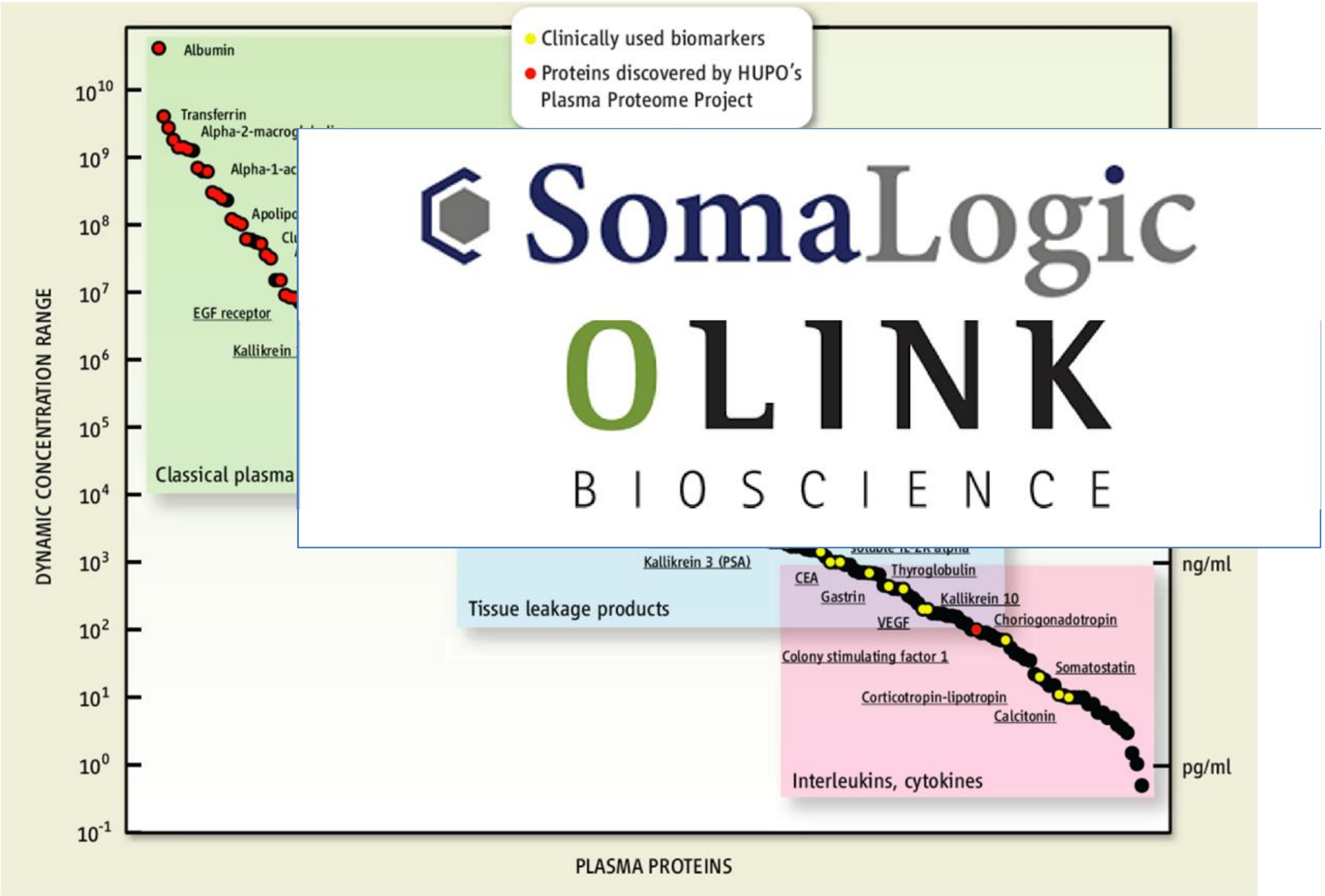
Data available on dbGAP (>2,500)

Nat Medicine 2011
JCI 2011a, 2011b
Circulation 2012
Diabetes 2012
Cell Metabolism 2013a, 2013b

Metabolomics in multi-ethnic cohorts

- Diabetes Prevention Program
  – Walford, Florez, Ma, Temprosa
- Shanghai Women’s and Men’s Health Study
  – Shu, Zheng
- Southern Community Cohort Study
  – Lipworth-Elliot, Blot
- Jackson Heart Study
  – Wilson

Other omics?



Clinically used biomarkers
Proteins discovered by HUPO's Plasma Proteome Project
SomaLogic
OLINK
BIOSCIENCE
DYNAMIC CONCENTRATION RANGE
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
10^{-1}
Albumin
Transferrin
EGF receptor
Classical plasma
Kallikrein 3 (PSA)
Tissue leakage products
CEA
Gastrin
Thyroglobulin
Kallikrein 10
VEGF
Choriogonadotropin
Colony stimulating factor 1
Somatostatin
Corticotropin-lipotropin
Calcitonin
Interleukins, cytokines
ng/ml
pg/ml
PLASMA PROTEINS

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Eugene Rhee
Jennifer Ho
Mark Berenson
Debbie Ngo
Jose Florez
Geoff Walford

Framingham Heart Study

Vasan Ramachandran
Martin Larson
Daniel Levy
Emelia Benjamin

Broad Institute

Clary Clish

Jackson Heart Study

James Wilson

DPP Research Group

Yong Ma
Ella Temprosa

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