Metabolomics approaches applied to study CDV and T2D in PREDIMED

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BROAD INSTITUTE

Broad Institute Metabolomics Platform

A center of collaboration with a focus on development and application of technologies for the systematic analysis of metabolites in biological specimens

Team:

Amy Deik
Kerry Pierce
Kevin Bullock
Justin Scott
Courtney Dennis
Sarah Jeanfavre
Julian Avila, Ph.D.
Daniel Hitchcock, Ph.D.
+ affiliated students & fellows

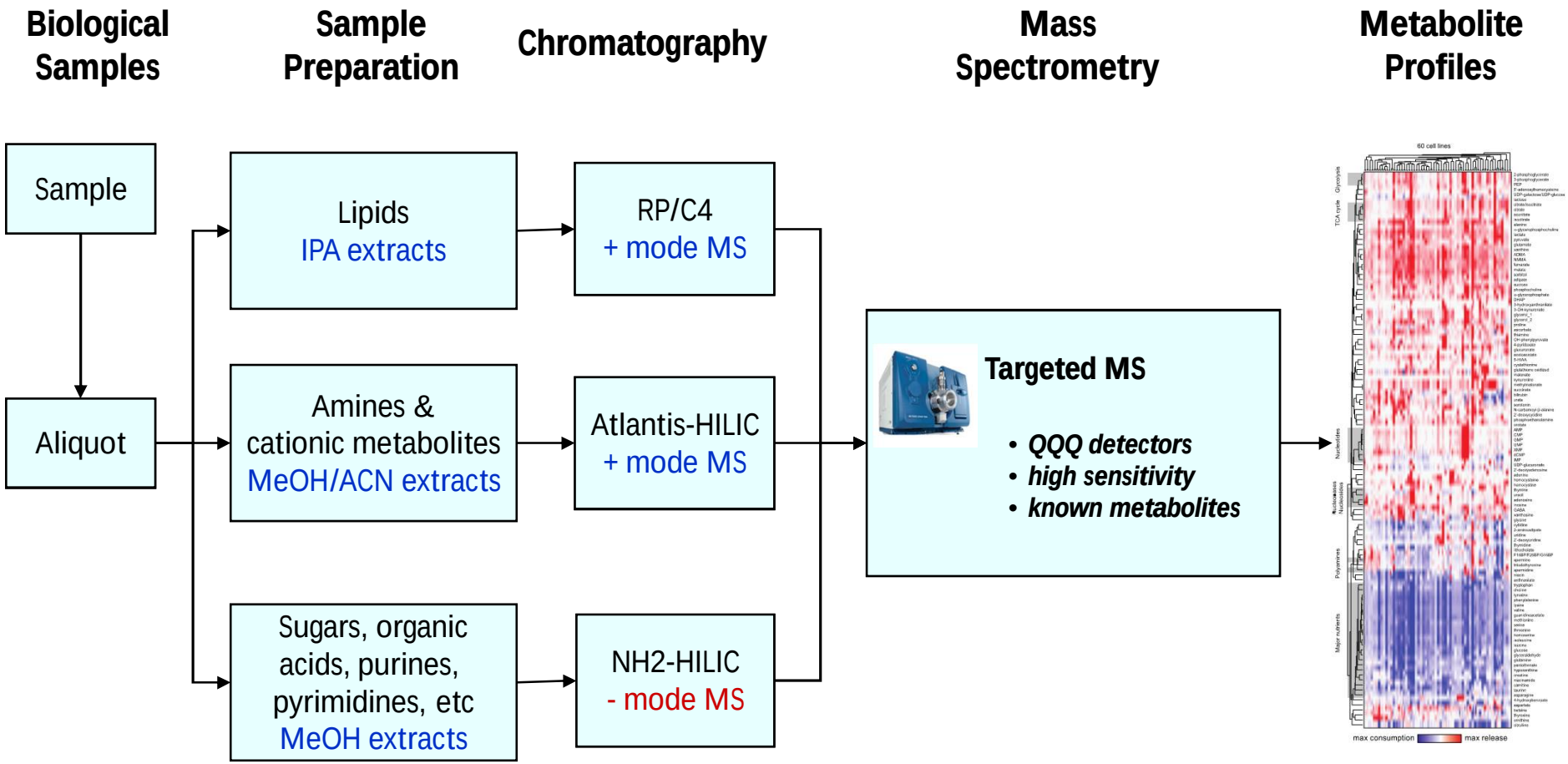
Our areas of focus:

- Characterization of metabolic phenotypes and dependencies
- Drug activity and efficacy
- Discovery of novel metabolites and biological mediators
- Host-microbe interactions
- Signatures of disease/biomarkers

Metabolomics is a significant analytical challenge

- The number of endogenous metabolites is estimated to be less than 10,000 molecules (not including lipids or metabolites from food or the environment…)
- Physical properties differ widely among endogenous metabolites
  - Polarity: range from very polar to very nonpolar
  - Chemical stability: labile to very stable
- Abundance: cellular concentrations range from a few molecules to mM
  - Most techniques have a linear dynamic ranges < 4 orders of magnitude
  - Abundant metabolites may interfere with the analyses of less abundant metabolites
- Multiple analytical methods are needed to obtain full coverage of the metabolome
- Available sample quantity, time, and funding put practical limits on the scope of what can be measured

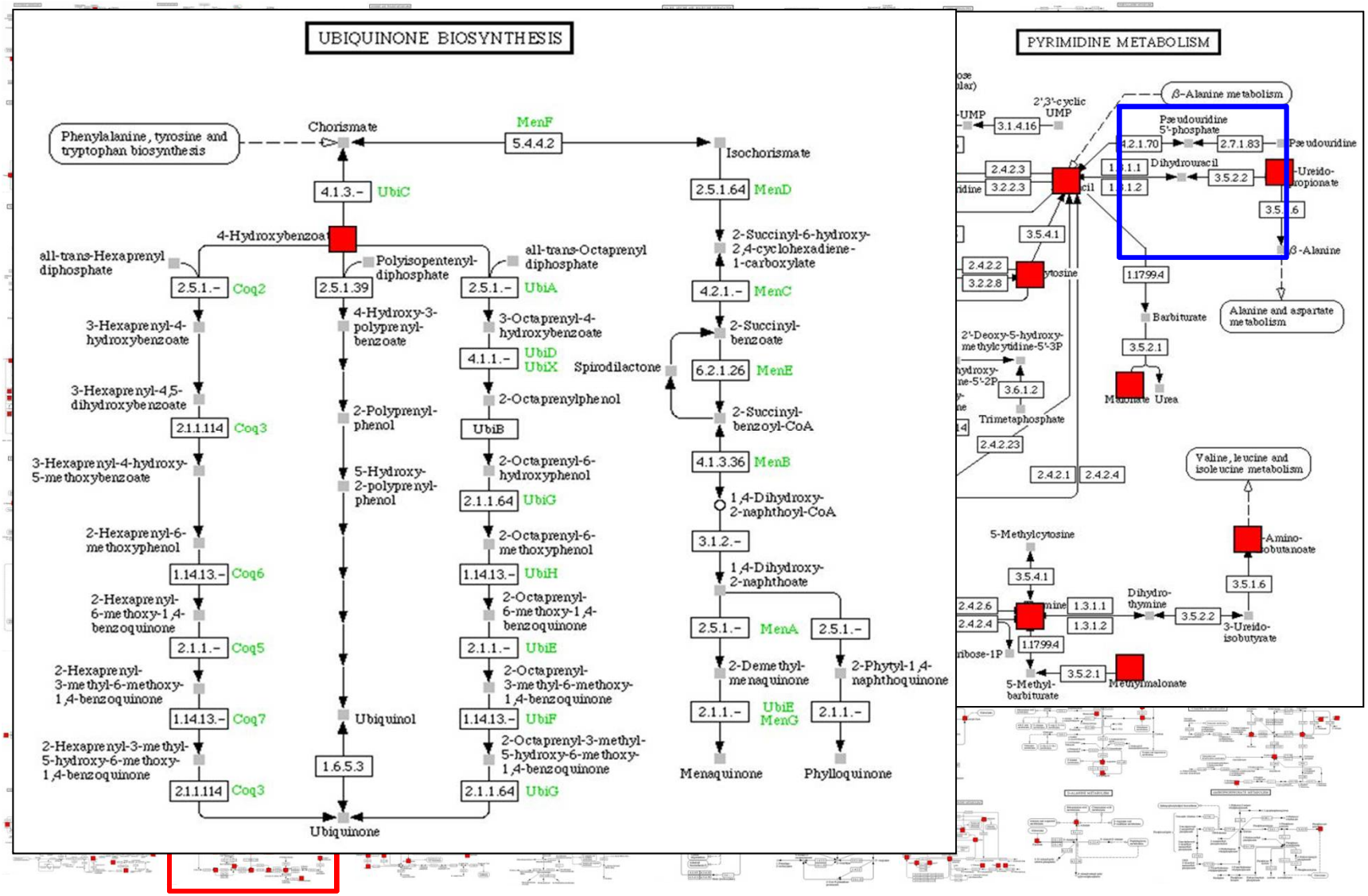
Initial QQQ MS-based metabolomics workflow (ca. 2009)



Sample prep & separation
- Multiple methods
- Methods matched to metabolite physical properties
- Capacity to process and analyze 1000's of samples

Mass spectrometry
- Targeted: Sensitive analyses of metabolites of known ID
- Plasma: 250-300 metabolites of known ID

Targeted metabolic pathway coverage (excluding lipids)

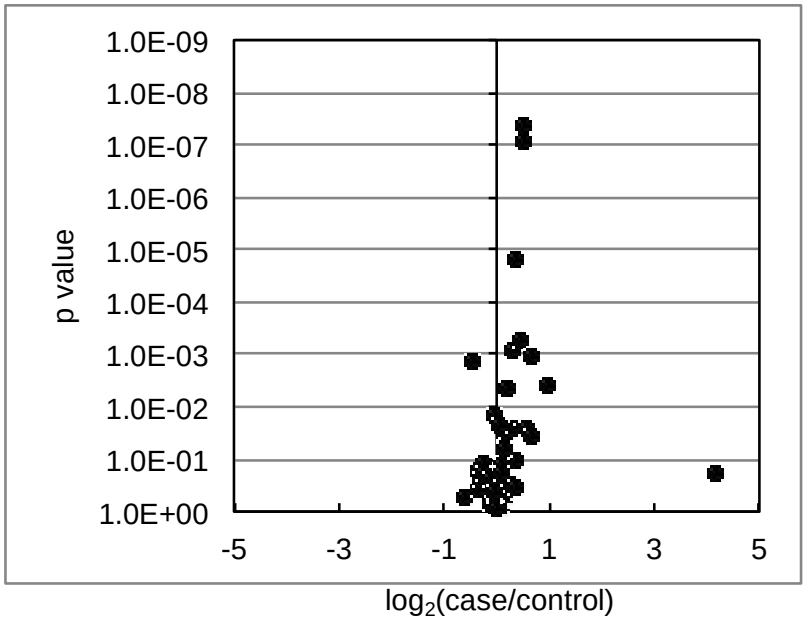


Kyoto Encyclopedia of Genes and Genomes (KEGG), Human Metabolic Pathways

Expanded metabolite coverage using HRAM profiling

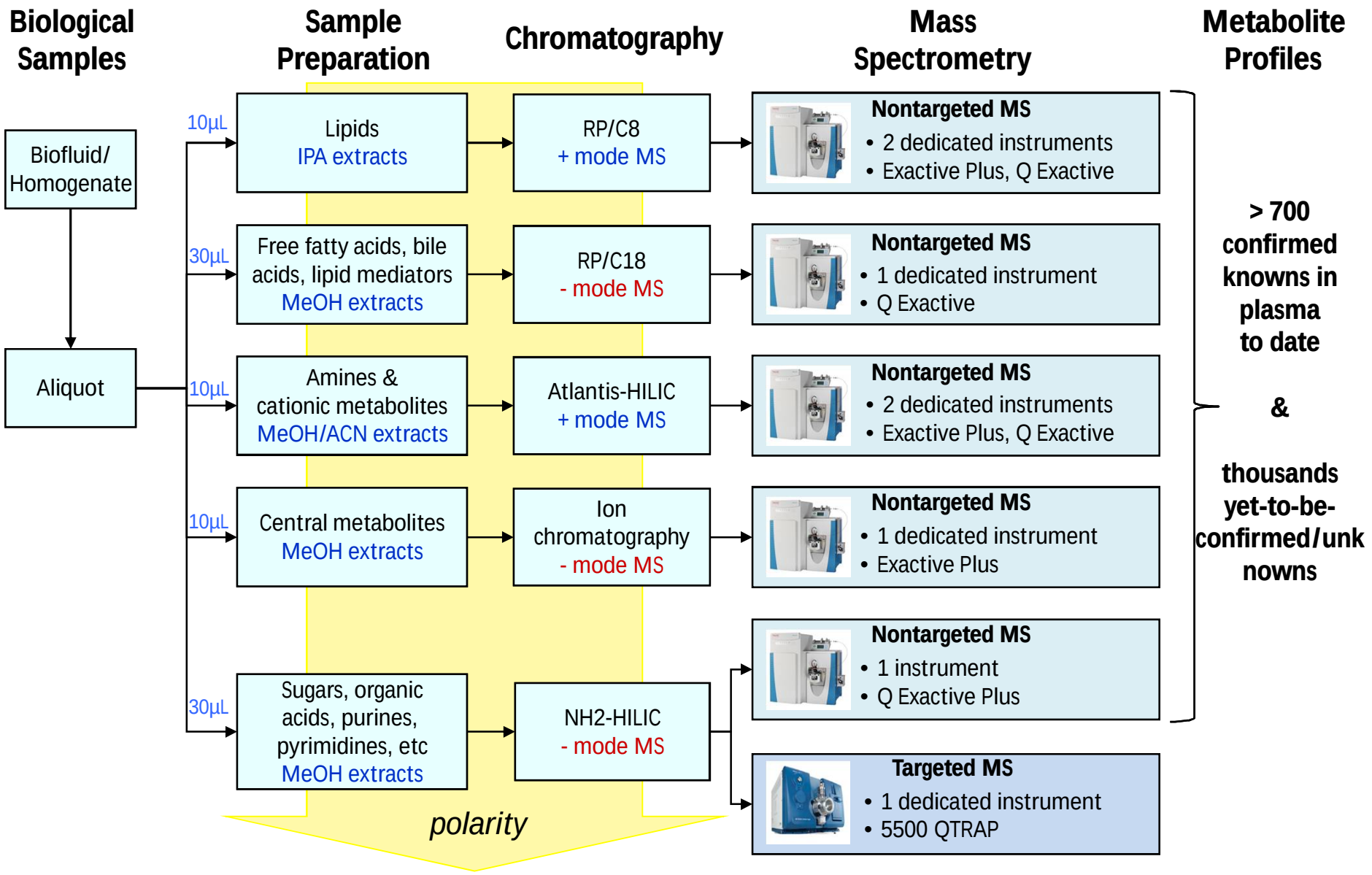
- Example: Analyses of plasma samples of 25 future diabetic cases and 25 age/gender-matched controls
- Single method: HILIC, positive ion mode analysis
- Targeted vs nontargeted processing

52 targeted, known metabolites



9 metabolites p <0.01

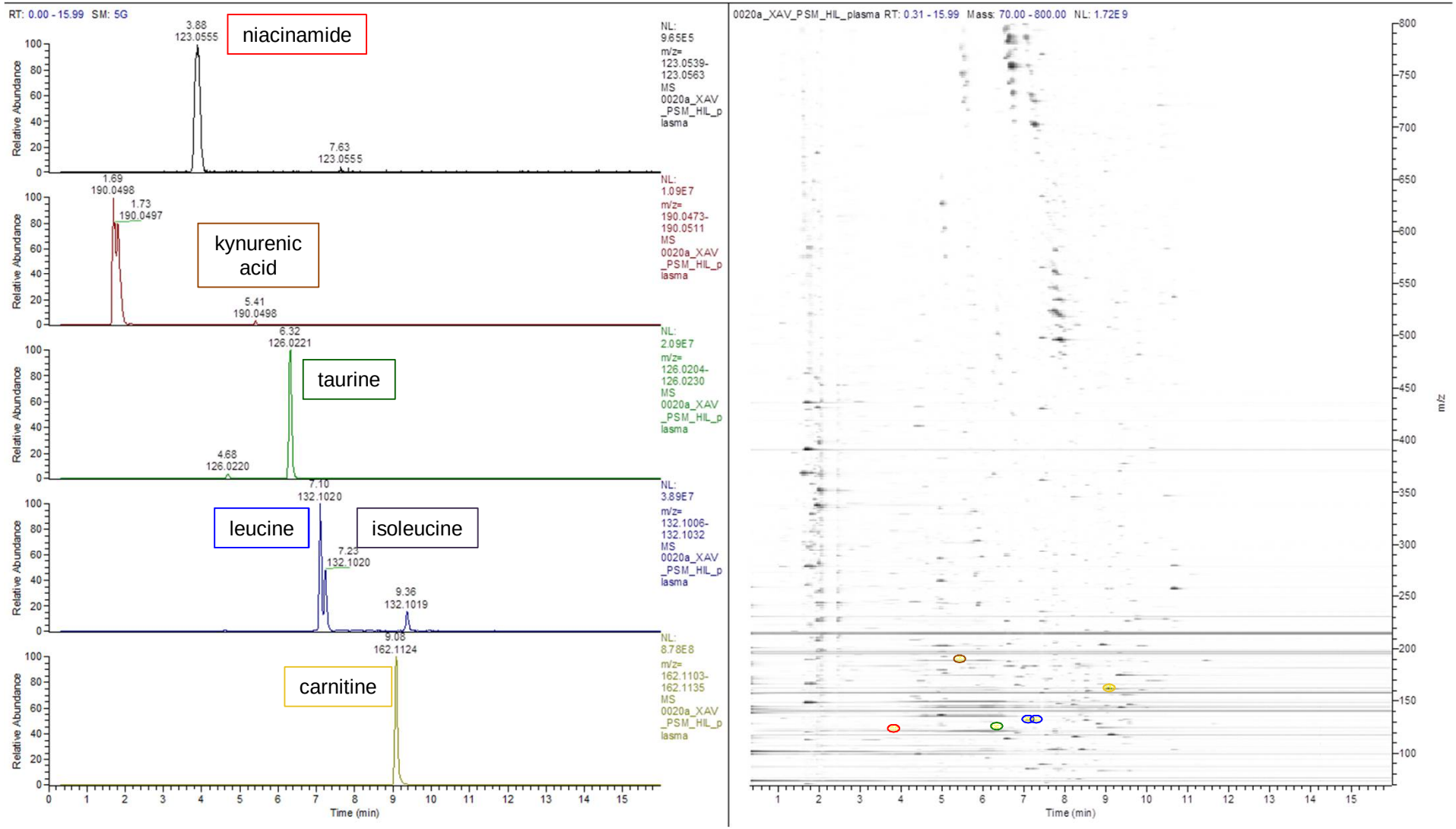
Current LC-MS-based platform



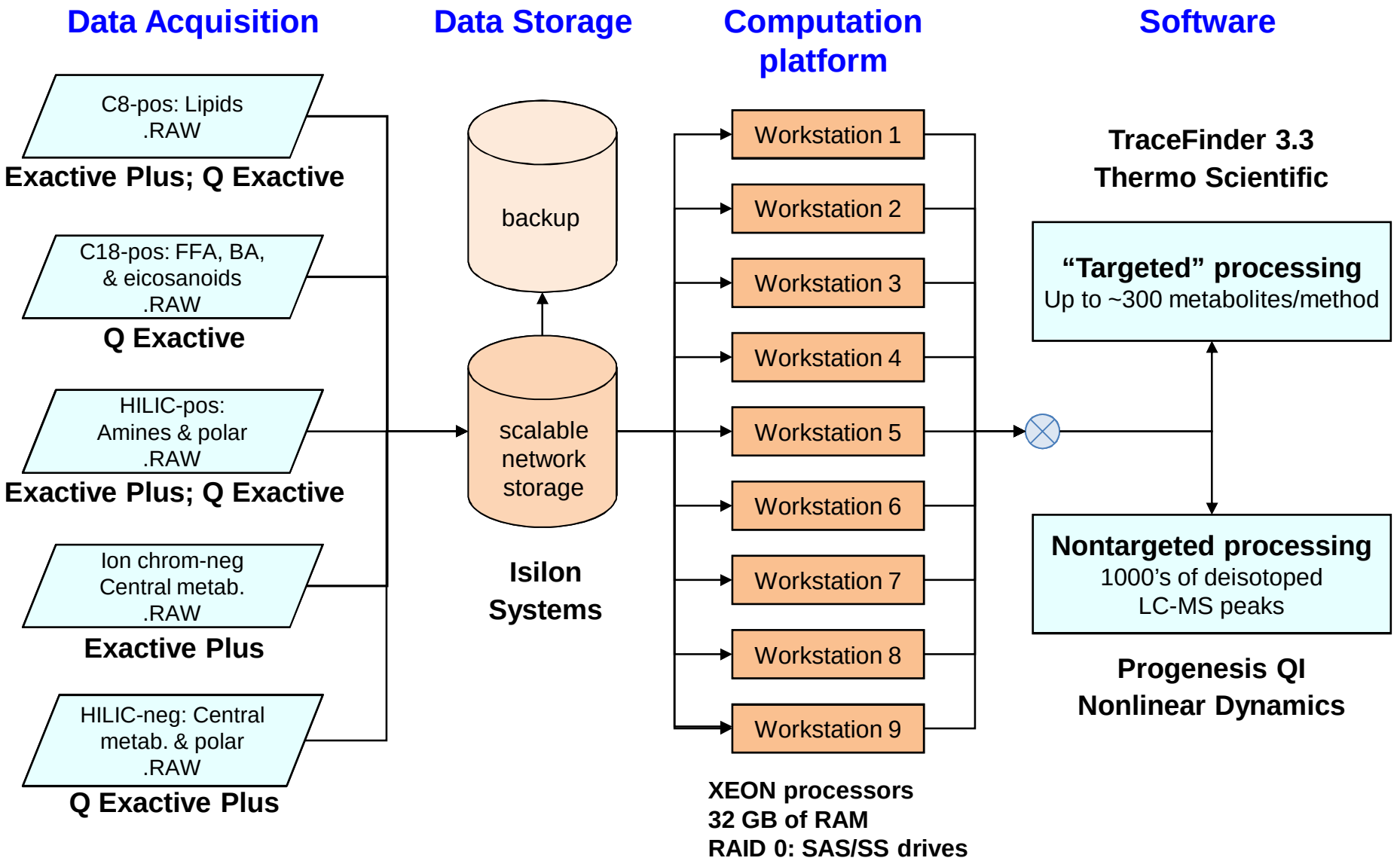
Example of an LC-MS dataset: polar, plasma metabolites

Ion chromatograms (“targeted” peaks)

Full scan dataset (nontargeted)



Data processing workflow for Orbitrap MS data



Output from nontargeted analyses

samples →

Subset of up to ~300 known metabolites

1000's yet to be identified LC-MS peaks

Compound	m/z	RT (min)	Metabolite	PRISMPool01	8582-9	8582-10	8582-11	8582-12	8585-7
TF36	126.1365	7.57	valine-d8	13792180	13722344	12250876	13836339	13143924	13010787
TF37	174.1365	6.84	phenylalanine-d8	16567937	16630155	15375298	15144365	15304933	18320765
9196	126.1026	9.72	1-methylhistamine	2005	523	378	625	658	14602
TF19	137.0709	8.37	1-methylnicotinamide	6871537	867313	7124207	912560	4690913	4313618
4760	252.1089	5.32	2-deoxyadenosine	539		38	146	41	159
TF18	228.0979	6.10	2-deoxycytidine	12711		10591			32844
1319	154.0497	1.90	3-hydroxyanthranilic acid	4577	2635	894	3409	1963	6092
1725	192.0654	2.04	5-HIAA	47233	55167	46529	130324	176744	9493
TF8	221.0921	6.74	5-hydroxytryptophan	350844	152998	152955	49676	6125	368406
TF14	146.1176	9.02	acetylcholine	4046199279	1751909927	572289902	211966268	572596317	3396084940
4624	268.1037	5.12	adenosine	167402	47856	44715	53345	96333	32342
TF10	203.1503	9.92	ADMA	31581647	5486910	1941718	2527949	4237030	8070123
TF2	90.0550	7.98	alanine	75753588	17439592	26593486	14433265	36605999	52221190
9439	258.1095	10.65	alpha-glycerophosphocholine	50645	23001	43408	48539	14480	74458
⋮	⋮	⋮							
7198	82.0659	7.54		1471	2109	1376	1903	1873	2202
8488	82.5378	8.60		1004	4674	28	3891	2747	3040
8355	83.0498	8.44		5531	1612	2660	748	2597	2565
7212	83.0498	7.55		3142	170	622	157	200	4207
6494	83.0611	6.93		20825	16199	16834	16719	19621	21729
791	83.0861	1.75		27521	21335	33249	13261	23248	19603
8187	84.0450	8.24		10873	3617	3896	4705	7725	5530
7714	84.0451	7.85		52797	37063	57828	31601	71652	48960
7016	84.0814	7.41		376	456	351	248	302	686

- Peaks described by m/z and RT
- Subset identified; confirmed by matching mass and RT to standards
- "Known" metabolites missed buy Progenesis QI manually extracted using TraceFinder software
- Reported as LC-MS peak areas

Why so many peaks in the nontargeted data sets?

- Where do the peaks come from?
  - **Metabolites (!)**
    - Multiple ion adducts and bond cleavage products may be formed from a single metabolite during electrospary ionization in MS
    - For polar positive ion mode methods, dominant ion is typically $[M+H]^+$, but $[M+Na]^+$, $[M+K]^+$, and $[M+NH_4]^+$ are often present at measurable levels
    - Redundant peaks share the same retention time as the dominant ion and are highly correlated
  - Contaminants in pre-analytical consumables (e.g. phthalates in plastic consumables)
  - Contaminants in solvents
  - Instrument noise

- How may the data be filtered?
  - Remove peaks with CV above a specific threshold
  - Remove peaks with excessive missing values in pooled plasma samples
  - Filter based on evidence of batch effects

QA/QC for cohort studies

Reference mixtures analyzed before and after to assure system performance

Internal standard added in first step of sample extraction

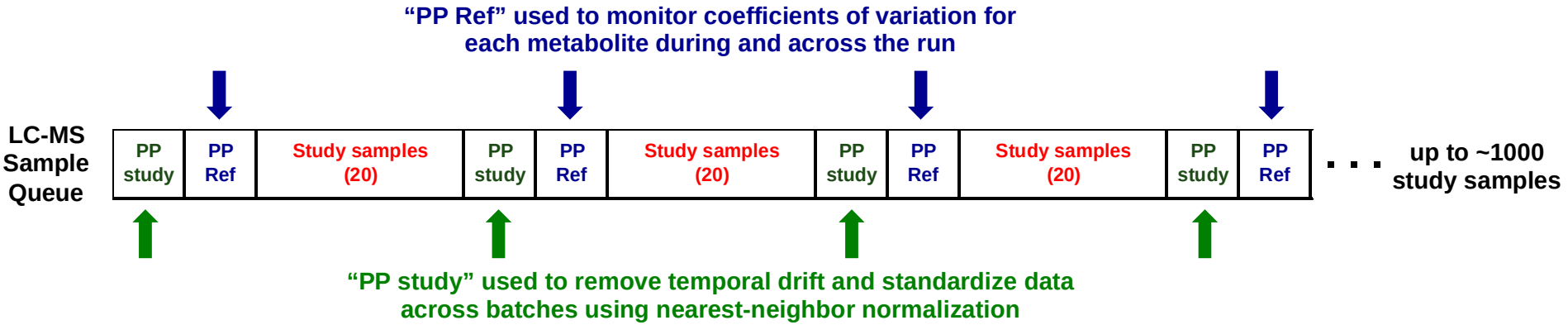
- monitored during analyses
- may be used to standardize data

Pooled study samples analyzed every 20 study samples

- used to standardize data across datasets

Second pooled plasma reference sample, analyzed every 20 study samples

- used to assess: overall reproducibility & impact of standardization procedures



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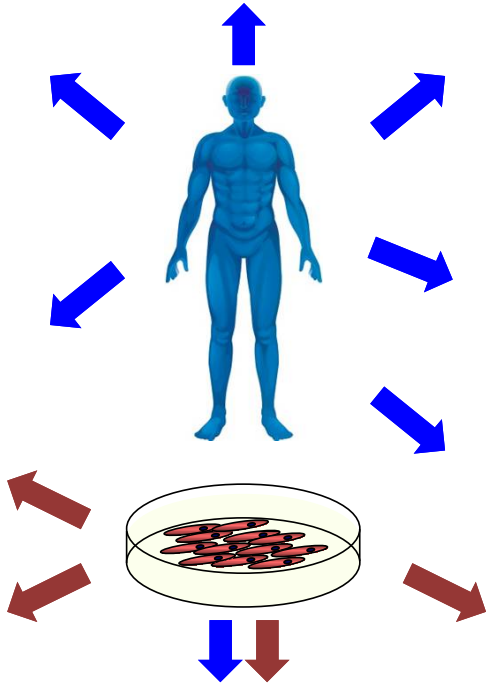

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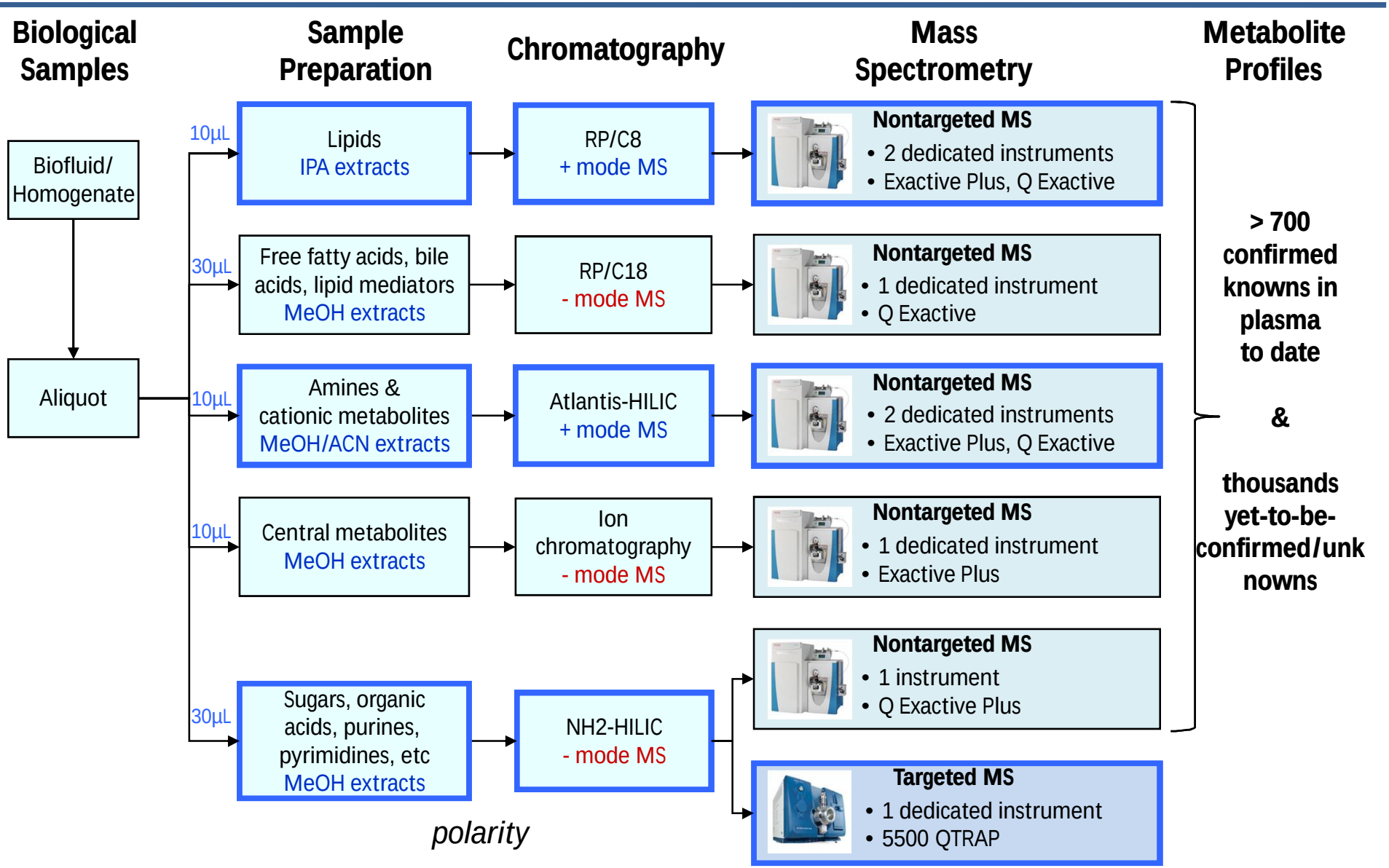
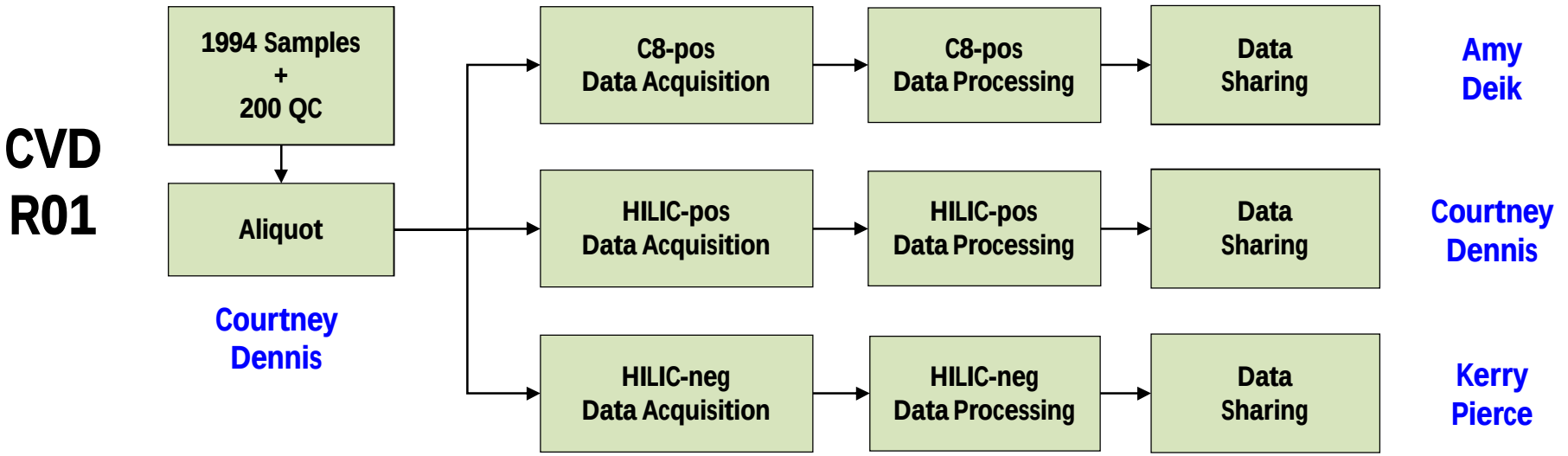
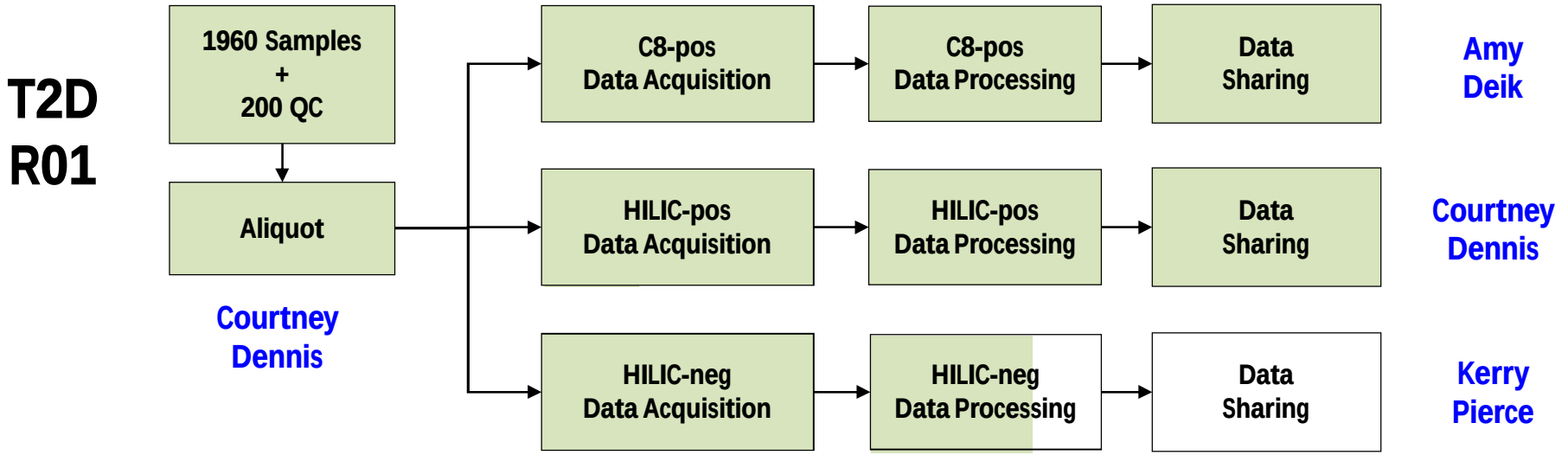
Broad metabolomics platform



Analysis outline and status





Metabolic associations with CVD and T2D in PREDIMED

10:50–11:20	**BCAA and T2D in the PREDIMED Study** Miguel Ruiz-Canela, PhD, Universidad de Navarra, CIBERobn, Pamplona, Spain
11:20–11:40	**Lipidomics of CVD and T2D** Estefanía Toledo, MD, PhD, Universidad de Navarra, CIBERobn, Pamplona, Spain
11.40–12:00	**Lipidomics (PCA analysis and scores)** Cristina Razquin, PhD, Universidad of Navarra, CIBERobn, Pamplona, Spain
1:00–1:30	**Application of network/pathway analysis in the PREDIMED metabolomics study** Daniel Wang, MD, PhD, Harvard T.H. Chan School of Public Health, Boston, MA
1:30–1:50	**Untargeted metabolomics in the PREDIMED** Yan Zheng, MD, PhD, Harvard T.H. Chan School of Public Health, Boston, MA
1:50–2:20	**Acylcarnitines and gut-microbiota related metabolites on T2D and CVD** Marta Guasch-Ferré, PhD, Harvard T.H. Chan School of Public Health, Boston, MA
2:20–2:40	**Tryptophan and urea cycle metabolites** Edward Yu, Harvard T.H. Chan School of Public Health, Boston, MA
3:00–3:30	**Methods to analyze metabolomics data and novel statistical approaches. Metabolomic footprints of the 14-point PREDIMED MedDiet score** Liming Liang, PhD, Harvard T.H. Chan School of Public Health, Boston, MA
3:30–3:50	**Metabolomic footprints of the Mediterranean diet: the effect of the randomized PREDIMED interventions** Cristina Razquin, PhD, Universidad of Navarra, CIBERobn
3:50–4:10	**Plasma trimethylamine-N-oxide and related metabolites involvement with the risk for type 2 diabetes in the PREDIMED trial** Christopher Papandreou, Msc, PhD, Rovira i Virgili University

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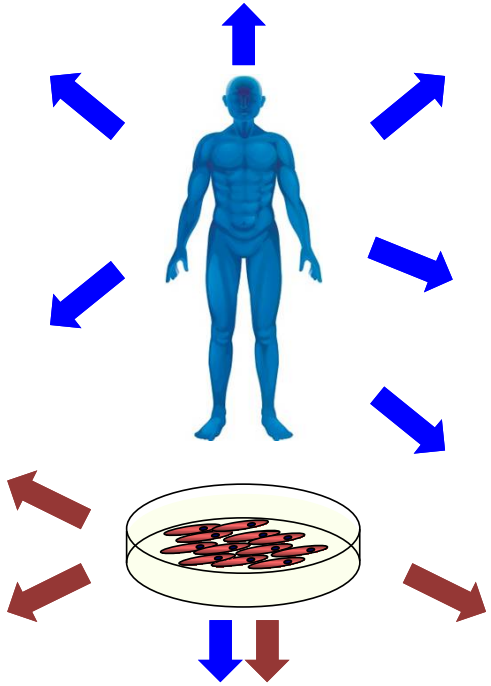

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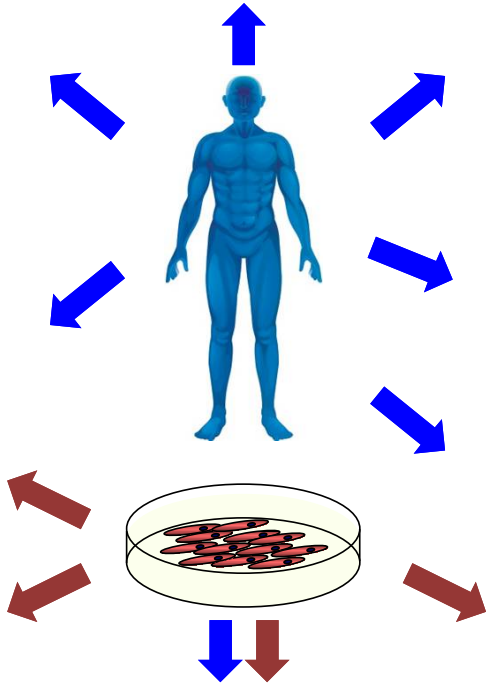

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Metabolomics of the Women's Health Initiative (WHI)

Background

- Launched in 1991 and consisted of a set of clinical trials and an observational study
- Included two postmenopausal hormone therapy trials:
  - estrogen-plus-progestin study of women with a uterus
  - estrogen-alone study of women without a uterus
- Enrolled 161,808 generally healthy postmenopausal women

CHD study

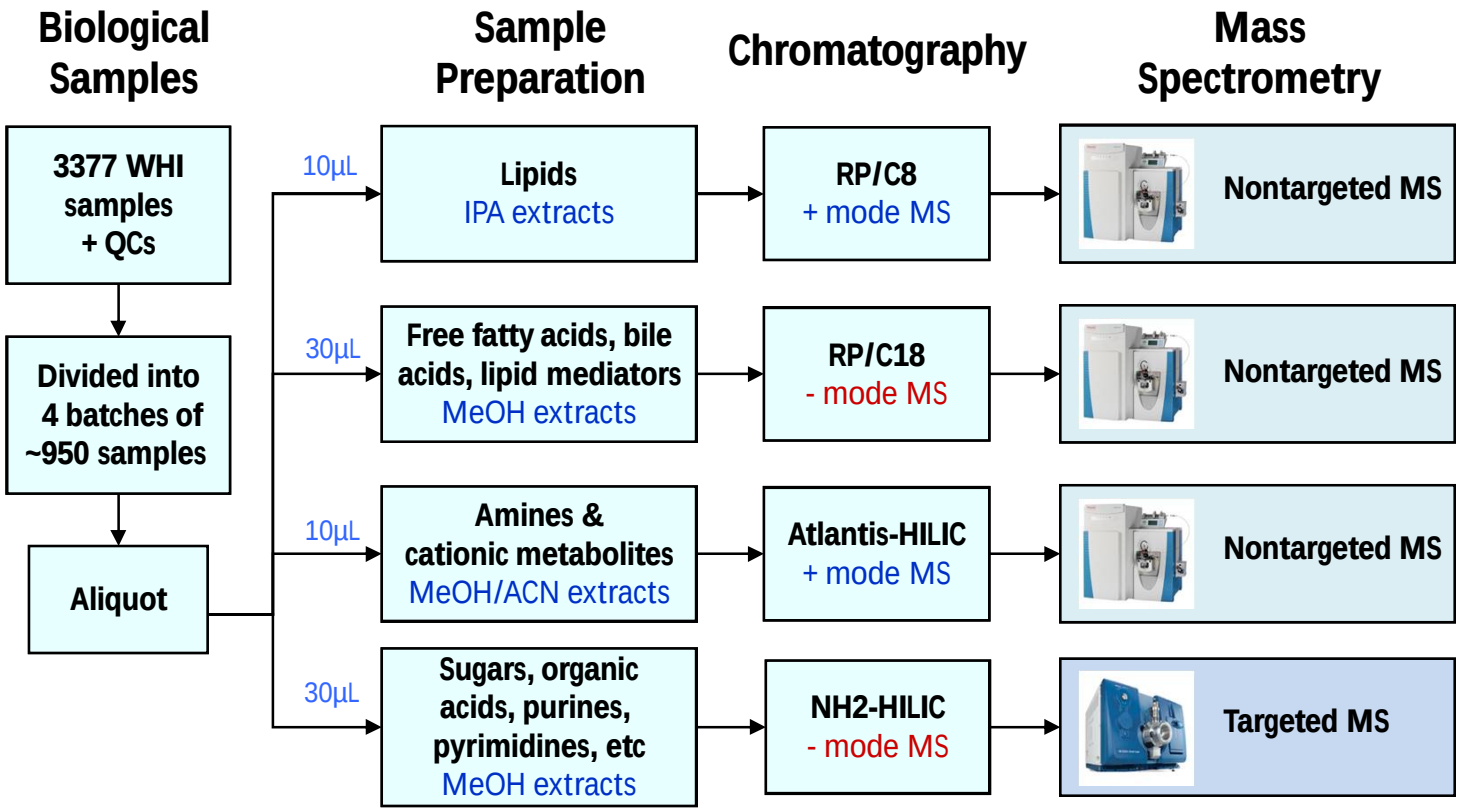
- **Goal:** Identify metabolites associated with nonfatal and silent MI and CHD death
- Samples were from the observational study (WHI-OS; 93,726 participants) and the placebo arms of the hormone therapy trials (WHI-HT; 27,347 participants)
- Nested case-control study with participants matched based on 5-year age, race/ethnicity, hysterectomy status, and 2-year enrollment window
- Median time to CHD event among cases was 5.8 years in the discovery cohort and 4.2 years in the validation cohort

Kathy Rexrode

Baseline Characteristics

N (%) or mean (SD)	WHI-OS Discovery (n=944)	WHI-HT Validation (n=624)
Cases	472 (50%)	312 (50%)
Age, years	67 (7)	66 (7)
Race		
White	696 (73%)	510 (81%)
Black	138 (15%)	75 (12%)
Other	110 (12%)	22 (7%)
Systolic blood pressure, mmHg	133 (19)	134 (19)
Diabetes, reported	103 (11%)	96 (15%)
BMI, m^2/kg	28 (6)	29 (6)
Total cholesterol, mg/dL	232 (47)	235 (41)
HDL cholesterol, mg/dL	54 (16)	50 (14)
Smoking status, reported		
Current	76 (8%)	90 (14%)
Former	416 (44%)	226 (36%)
Never	452 (48%)	311 (50%)
Aspirin use, reported	214 (23%)	171 (27%)
Statin use, reported	81 (9%)	96 (15%)
Anti-hypertensive use, reported	235 (25%)	186 (30%)
Anti-diabetic use, reported	60 (6%)	70 (11%)

WHI metabolomics



371 metabolites Initially annotated (~700 currently)

>5000 unknowns measured consistently across four batches

60 annotated metabolites sig. dysregulated in WHI-OS discovery cohort

Validation In WHI-HT

Metabolites associated with risk of future CHD

Metabolite	WHI-HT Adjusted for matching[1]		WHI-HT Adjusted for risk factors[2]	
	Odds Ratio	p value	Odds Ratio	p value
Succinate	1.42 (1.20, 1.69)	2.9E-05	1.56 (1.30, 1.87)	8.2E-07
Glutamate	1.64 (1.38, 1.95)	5.5E-09	1.50 (1.25, 1.80)	8.3E-06
Glutamine	0.61 (0.50, 0.73)	1.4E-08	0.67 (0.55, 0.82)	2.5E-05
PGE2	1.38 (1.17, 1.62)	1.0E-04	1.34 (1.12, 1.59)	9.0E-04
2-Hydroxyglutarate	1.45 (1.22, 1.72)	1.0E-05	1.35 (1.13, 1.62)	9.3E-04
CMP	1.38 (1.17, 1.63)	1.1E-04	1.33 (1.11, 1.59)	1.4E-03
IMP	0.75 (0.64, 0.89)	4.9E-04	0.77 (0.65, 0.92)	3.3E-03
Sucrose	1.40 (1.18, 1.65)	7.2E-05	1.29 (1.08, 1.54)	4.8E-03

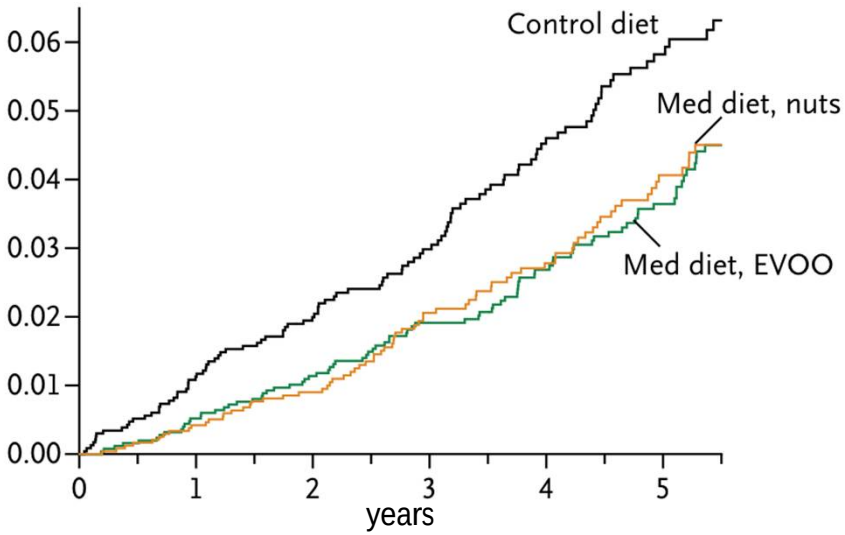
[1] Adjusted for baseline age, race/ethnicity, hysterectomy status, and enrollment window

[2] Adjusted for baseline age, race/ethnicity, hysterectomy status, and enrollment window, aspirin use, statin use, anti-hypertensive use, smoking, systolic blood pressure, diabetes, total and HDL cholesterol

Preliminary validation in PREDIMED

- PREvención con DIeta MEDiterránea (PREDIMED) was a primary prevention trial designed to determine if adherence to the Mediterranean Diet could prevent CVD in a high-risk population

Incidence of the primary end point (acute MI, stroke, and CVD death)



N Engl J Med 2013; 368:1279-90

- Case-cohort metabolomics study of 980 PREDIMED participants to determine associations between metabolites and diet and CVD risk
  - median 4.8 years of follow up
  - 229 cases of CVD (nonfatal stroke, nonfatal MI, or CVD death
  - 79 cases of CHD

Miguel Martinez-Gonzalez, Frank Hu

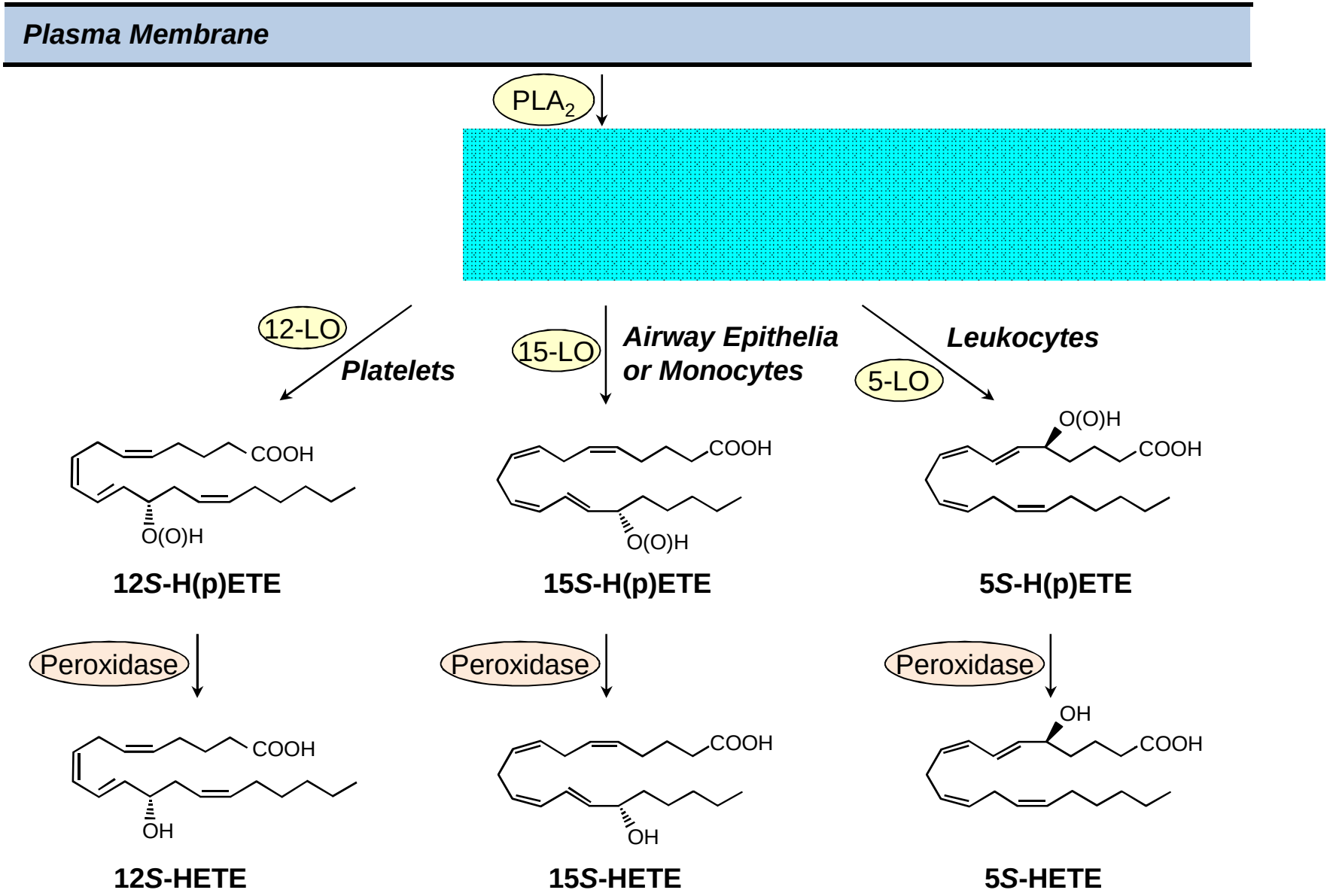
Hydroxy-PCs are associated with CVD and CHD in PREDIMED

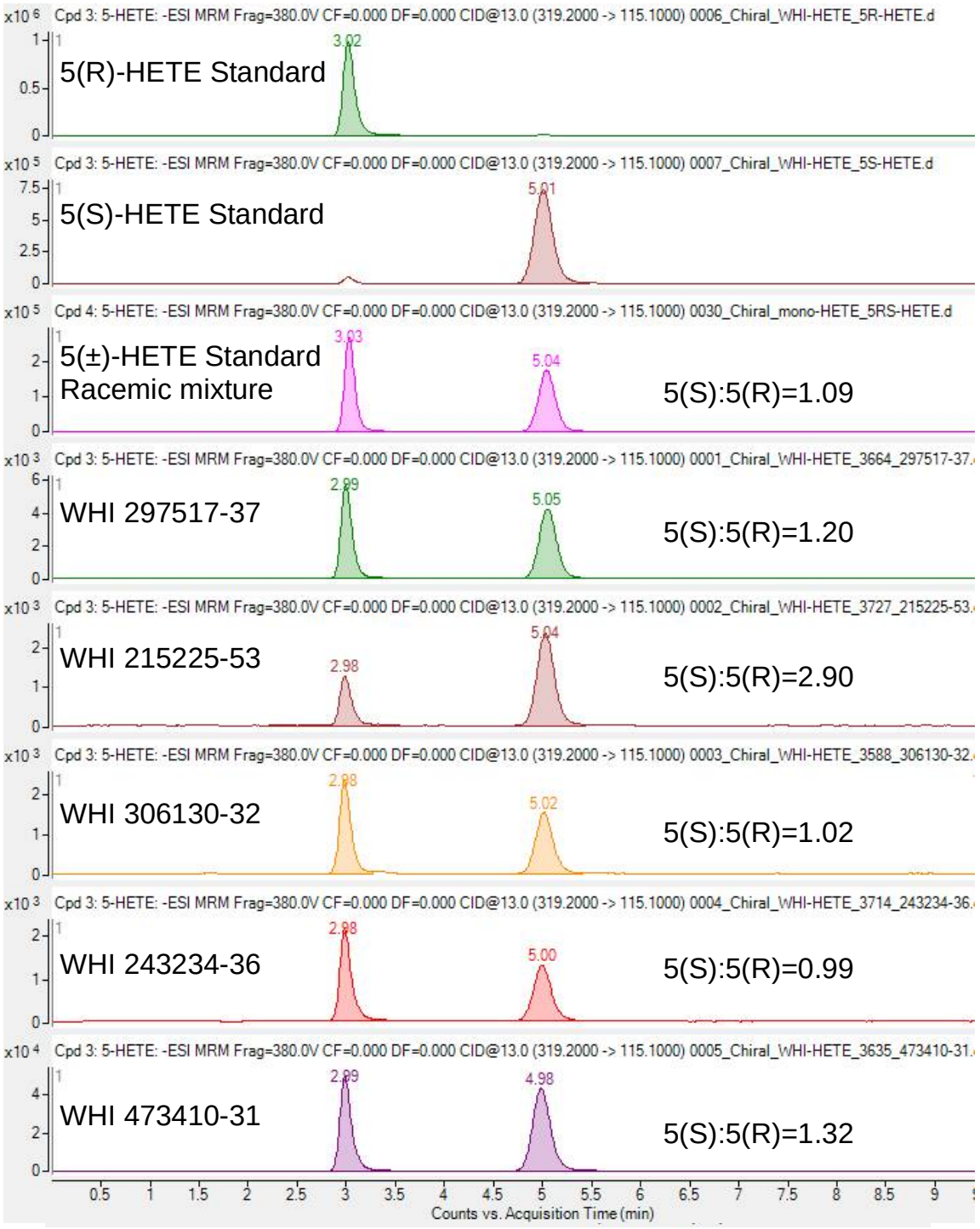
Metabolite	PREDIMED* CVD (n=229 cases)		PREDIMED* CHD (n=79 cases; both genders)	
	Odds Ratio	p value	Odds Ratio	p value
C34:2 hydroxy-PC	1.40 (1.15, 1.70)	7.6E-04	1.56 (1.09, 2.24)	2.0E-02
C36:4 hydroxy-PC	1.36 (1.12, 1.66)	2.0E-04	1.59 (1.10, 2.30)	1.0E-02

* Adjusted for age, gender, intervention group, statin use, smoking, systolic blood pressure, diabetes, total and HDL cholesterol

- mean age 68 years
- 46% male

Hydroxyeicosatetraenoic acid (HETE) synthesis




Cpd 3: 5-HETE: -ESI MRM Frag=380.0V CF=0.000 DF=0.000 CID@13.0 (319.2000 -> 115.1000) 0006_Chiral_WHI-HETE_5R-HETE.d
5(R)-HETE Standard
3.02
Cpd 3: 5-HETE: -ESI MRM Frag=380.0V CF=0.000 DF=0.000 CID@13.0 (319.2000 -> 115.1000) 0007_Chiral_WHI-HETE_5S-HETE.d
5(S)-HETE Standard
5.01
Cpd 4: 5-HETE: -ESI MRM Frag=380.0V CF=0.000 DF=0.000 CID@13.0 (319.2000 -> 115.1000) 0030_Chiral_mono-HETE_5RS-HETE.d
5(±)-HETE Standard
Racemic mixture
3.03
5.04
5(S):5(R)=1.09
Cpd 3: 5-HETE: -ESI MRM Frag=380.0V CF=0.000 DF=0.000 CID@13.0 (319.2000 -> 115.1000) 0001_Chiral_WHI-HETE_3664_297517-37.
WHI 297517-37
2.99
5.05
5(S):5(R)=1.20
Cpd 3: 5-HETE: -ESI MRM Frag=380.0V CF=0.000 DF=0.000 CID@13.0 (319.2000 -> 115.1000) 0002_Chiral_WHI-HETE_3727_215225-53.
WHI 215225-53
2.98
5.04
5(S):5(R)=2.90
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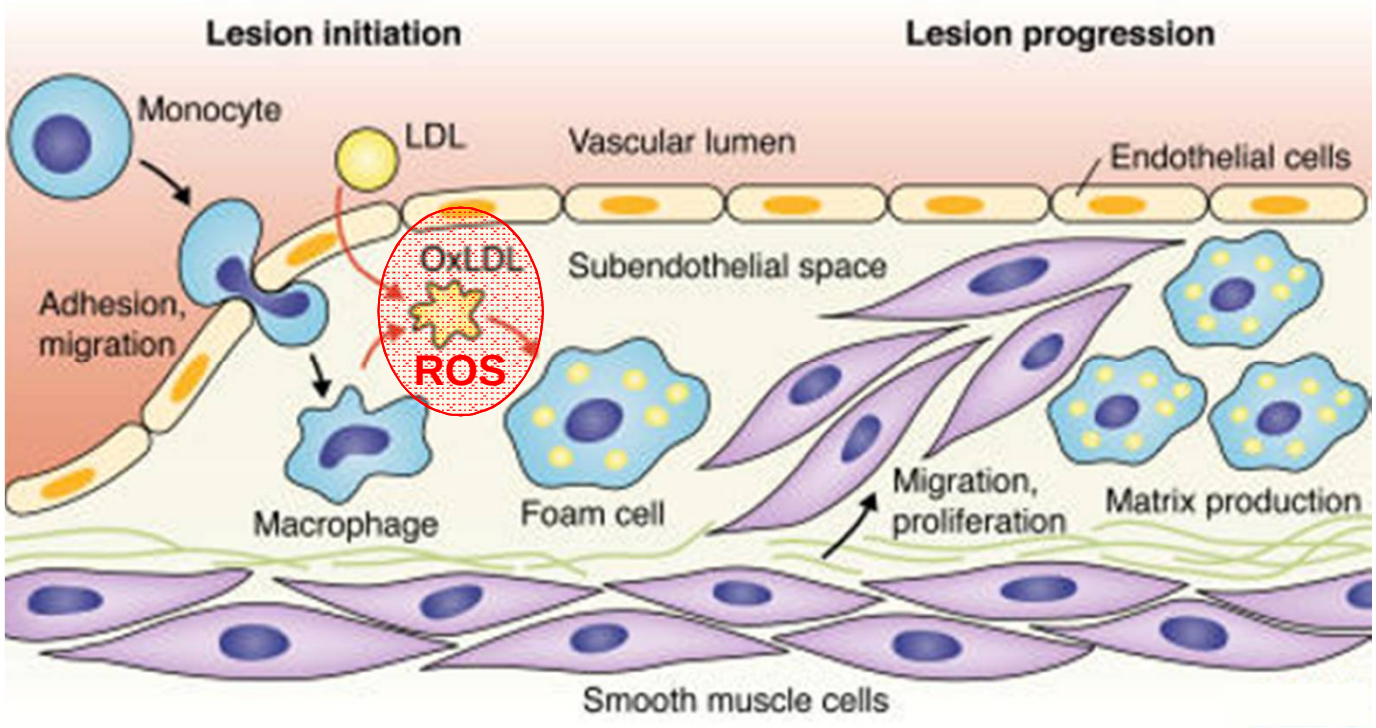
WHI 306130-32
2.98
5.02
5(S):5(R)=1.02
Cpd 3: 5-HETE: -ESI MRM Frag=380.0V CF=0.000 DF=0.000 CID@13.0 (319.2000 -> 115.1000) 0004_Chiral_WHI-HETE_3714_243234-36.
WHI 243234-36
2.98
5.00
5(S):5(R)=0.99
Cpd 3: 5-HETE: -ESI MRM Frag=380.0V CF=0.000 DF=0.000 CID@13.0 (319.2000 -> 115.1000) 0005_Chiral_WHI-HETE_3635_473410-31.
WHI 473410-31
2.99
4.98
5(S):5(R)=1.32
Counts vs. Acquisition Time (min)

Linkage between oxidized lipids and atherosclerosis



Is there a common driver for OxLDL, hydroxy-PC, and mono-HETE generation?

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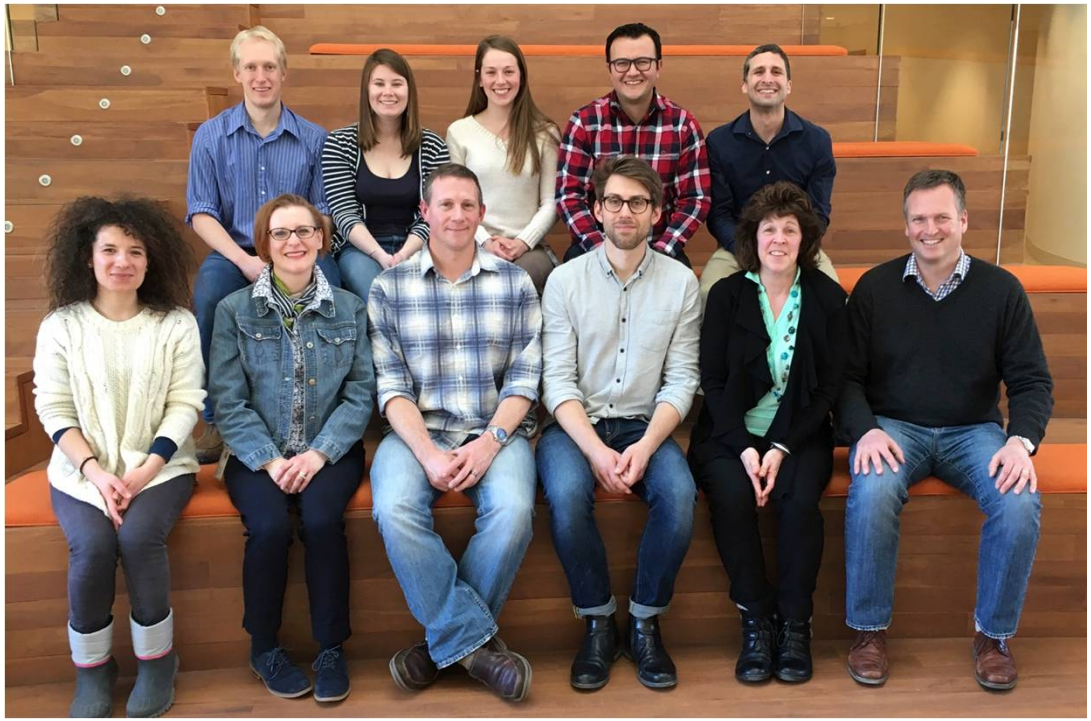
R01-HL118264
R01-DK102896
HHSN268201300008C



Type 2 diabetes

- Metabolic predictors of future T2D in FHS, MDC, MCDS, NHS, DPP
- Metabolic phenotyping of SLC16A11 haplotypes associated with T2D
- Intervention response in DPP
- Influence of diet on metabolic profiles and T2D in PREDIMED

Wang ***Nat Med*** 2011; 17:448-53
Rhee ***J Clin Invest*** 2011; 121:1402–1411
Wang ***J Clin Invest*** 2013;123:4309-4317
SIGMA Consortium. ***Nature*** 2014; 506:97-101
Magnusson ***Diabetes*** 2015; 64:3010-6
Walford ***Diabetes*** 2016; 65:1424-33

Renal disease

- CKD progression
- Prediction of CV mortality
- Novel markers of uremia

Kalim S et al. ***J Am Heart Assoc*** 2013; 2(6)
Rhee E et al. ***J Am Soc Nephrol*** 2010; 21:1041
Rhee E et al. ***Am J Nephrol*** 2016; 43:366-74
Tran MT et al. ***Nature*** 2016; 531:528-32

Cancer metabolism

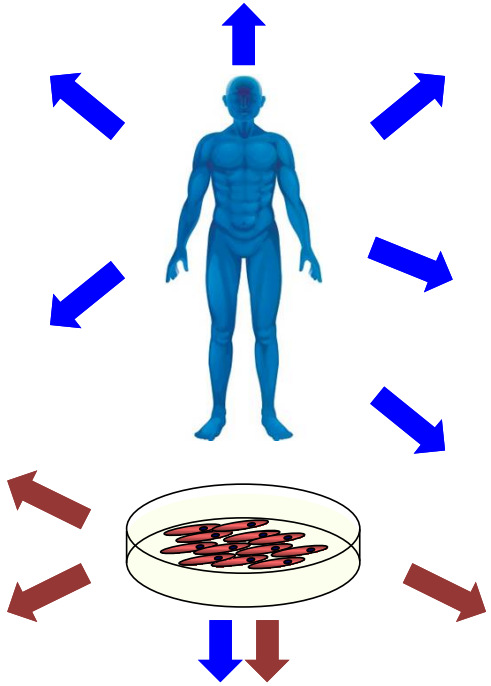
- Metabolic dependencies of cancer cells

Birsoy ***Nature*** 2014; 508:108-12
Israelsen ***Cell*** 2013;155:397-409
Vazquez F ***Cancer Cell*** 2013; 23:287-301
Jain M ***Science*** 2012; 336:1040-4
Wang ***Cell*** 2014;158:1309-23
Davidson ***Cell Metab*** 2016; 23:517-28
Kryukov ***Science*** 2016; 351:1214-8

Cardiovascular disease

- Predictors of CHD in DPP
- Predictors of CHD in WHI
- Influence of diet on metabolic profiles and CVD in PREDIMED

Guasch-Ferré ***Am J Clin Nutr*** 2016; 103:1408-16
Ruiz-Canela ***Clin Chem*** 2016; 62:582-92
Lewis GD ***J Am Coll Cardiol*** 2016; 67:174-89.



Mitochondrial disorders

- Markers of mitochondrial disease
- Mitochondrial dysfunction

Shaham ***PNAS*** 2010; 107:1571-5
Leoni ***Mol Genet Metab*** 2012; 105:463-71
Chen ***Cell Rep*** 2014; 7:27-34
Gohil ***J Biol Chem*** 2013; 288:35387-95
Bau ***Elife*** 2016; 5. pii: e10575.

Cancer

- Early plasma indicators of pancreatic cancer
- Effect of metformin and lifestyle on risk in the DPP
- Risk factors for breast cancer in younger women
- Dietary and hormonal determinants of cancer
- Predictors of prostate cancer

Mayers ***Nat Med*** 2014; 20:1193-8

Microbiome & disease

- Microbiome in IBD
- Gut microbiome and T1D
- Bile acid profiles associated with *C. difficile* infection

Meelu ***Inflamm Bowel Dis*** 2014; 20:1139-46
Kostic ***Cell Host Microbe*** 2015; 17:260-73
Allegretti ***Aliment Pharmacol Ther*** 2016; 43:1142-53

Infection & immunity

- Metabolic signaling and metabolism in immune cells
- Influence of infection on metabolism and vise versa

Tannahill ***Nature*** 2013; 496:238-42
Mascanfroni ***Nat Med*** 2015; 21:638-46
Wang ***Cell*** 2015; 163:1413-27
Matheson ***Cell Host Microbe*** 2015; 18:409-23
Graham ***Nat Commun*** 2015; 6:7838
Palsson-McDermott ***Cell Metab*** 2015; 21:65-80
Rothhammer ***Nat Med*** 2016; 22:586-97

Metabolic predictors of T2D in the FHS: elevated at baseline, 4-12 years before diagnosis

Odds ratio for future diabetes: Plasma isoleucine, phenylalanine, and tyrosine

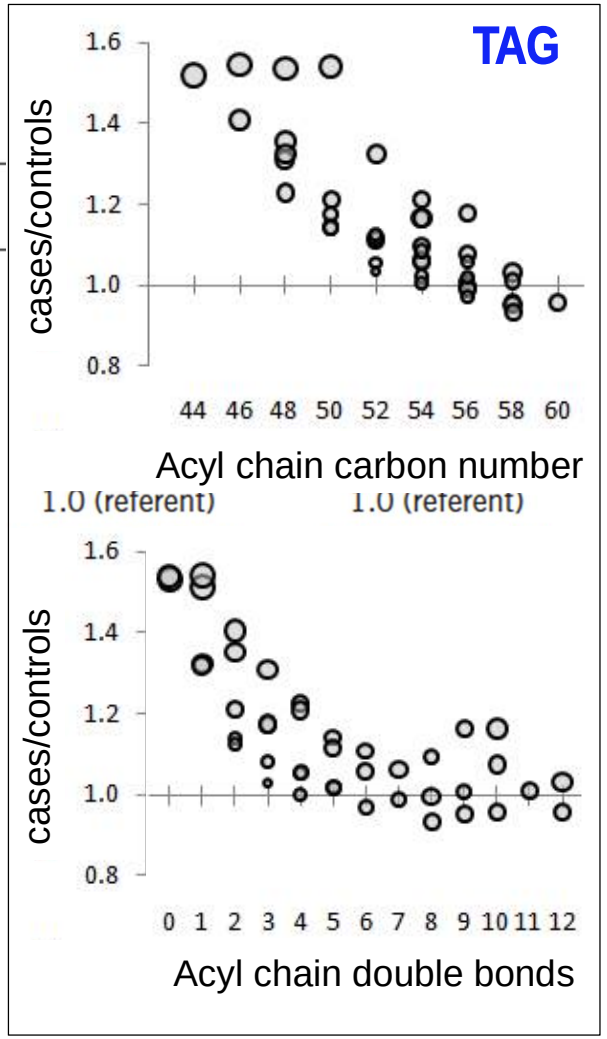
BCAA/AA

	Discovery (FHS) 12 year follow-up (n=378)	Validation (Malmö) 13 year follow-up (n=326)
1st quartile	1.0 (referent)	1.0 (referent)
2nd quartile	3.48 (1.68-7.23	2.08 (0.97-4.46)
3rd quartile	2.82 (1.25-6.34)	2.59 (1.09-6.15)
4th quartile	5.99 (2.34-15.34)	3.93 (1.54-10.04)
P for trend	0.0009	0.006

Model	Isoleucine	Leucine	Valine
Models adjusting for age, sex, BMI and fasting glucose (*n* = 378)			
Metabolite as continuous variable			
Per s.d.	1.72 (1.22–2.42)	1.62 (1.15–2.28)	1.57 (1.17–2.09)
P	0.0024	0.005	0.002
Metabolite as categorical variable			
First quartile	1.0 (referent)	1.0 (referent)	1.0 (referent)

2-aminoadipate

	FHS (188 cases, 188 controls) 12-year follow-up	MDC (162 cases, 162 controls) 13-year follow-up
As continuous variable		
Per SD increment	1.60 (1.19–2.16)	1.57 (1.15–2.14)
P value	0.002	0.004
As categorical variable		
First quartile	1.00 (Referent)	1.00 (Referent)
Second quartile	1.34 (0.72–2.49)	2.19 (1.07–4.48)
Third quartile	1.71 (0.82–3.54)	1.45 (0.68–3.07)
Fourth quartile	4.49 (1.86–10.89)	3.96 (1.63–9.59)
P value for trend	0.001	0.01



Wang TJ *et al*. *Nat Med* 2011; 17:448-453

Rhee EP et al. *J Clin Invest* 2011; 121:1402-11

Wang TJ et al. *J Clin Invest* 2013; 123:4309-17

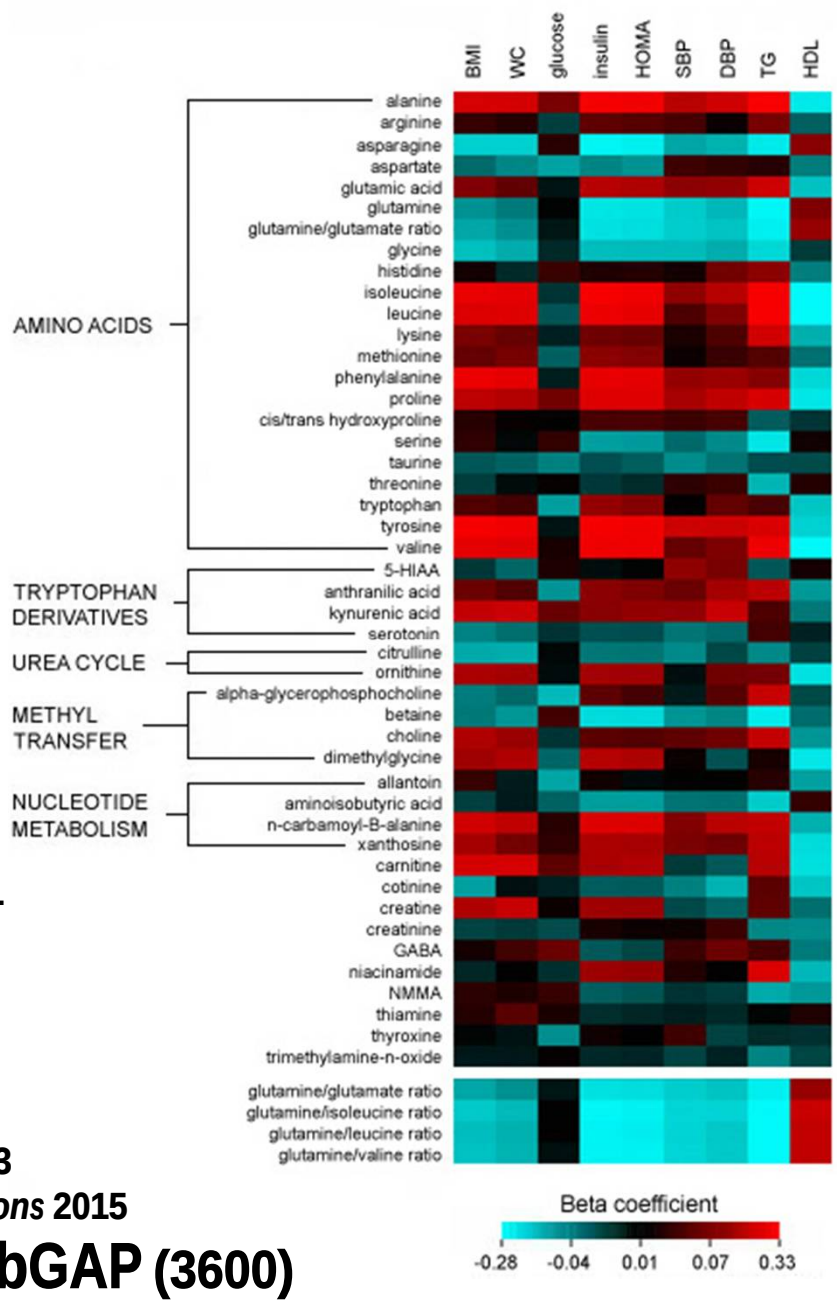
Nontargeted profiling of FHS: associations with HOMA-IR

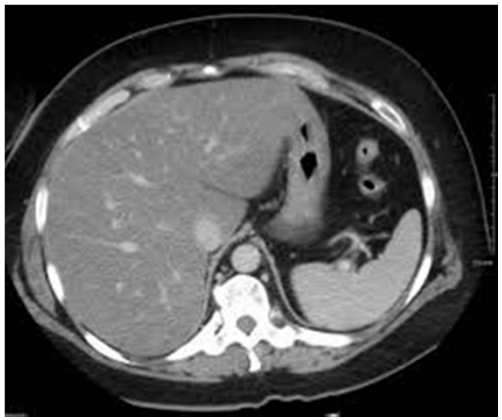
- 1000 FHS Gen 3 participants
- Nontargeted HILIC-pos method (knowns + unknown peaks)
- ~5000 peaks were observed in >80% of individuals
- ~500 peaks associated with key metabolic traits

Table 2: Targeted analysis on new platform		
Metabolites associated with HOMA-IR		
Compound	**β estimate**	**P value**
glutamate	0.0292	1.53E-40
valine	0.0198	7.32E-18
tyrosine	0.0204	2.35E-17
C5 carnitine	0.0185	3.04E-16
isoleucine	0.0180	5.77E-16
alanine	0.0195	2.84E-15
leucine	0.0164	1.73E-13
C3 carnitine	0.0152	1.50E-11
glycine	-0.0166	3.85E-11
3-hydroxyanthranilic acid	0.0153	3.86E-09
C6 carnitine	0.0148	5.16E-09
acetylglycine	-0.0137	8.21E-08
phenylalanine	0.0128	2.14E-07
sarcosine	0.0123	2.86E-07
dimethylglycine	0.0117	4.86E-07

Table 3: Non-targeted analysis on new platform			
Metabolites associated with HOMA-IR			
m/z	**Retention time**	**β estimate**	**P value**
783.6359	7.17	0.0258	5.63E-32
313.2733	1.63	0.0277	1.72E-30
612.5556	1.63	0.0275	7.52E-30
202.1185	7.79	0.0254	7.68E-25
575.5028	1.61	0.0247	1.16E-23
116.1073	7.87	0.0231	1.31E-23
606.6179	1.66	0.0227	5.97E-23

Metabolite correlations with phenotypes in the FHS





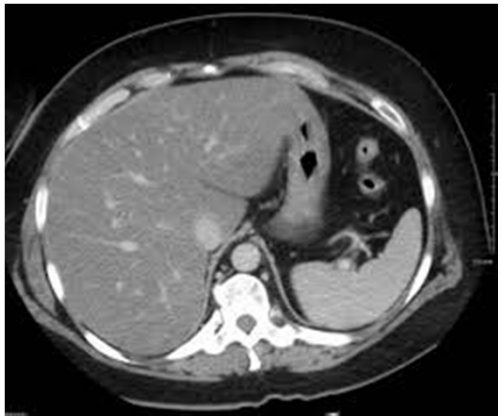
~200 peaks associated with hepatic fat (age and sex adjusted)

Wang *Nature Medicine* 2011
Rhee *JCI* 2011
Cheng *Circulation* 2012
Ho *Diabetes* 2011
Rhee *Cell Metabolism* 2013
Wang *JCI* 2013
Robert *Cell Metabolism* 2013
Cheng *Nature Communications* 2015

Data available on dbGAP (3600)

Robert Gerszten
John O'Sullivan
Jordan Morningstar

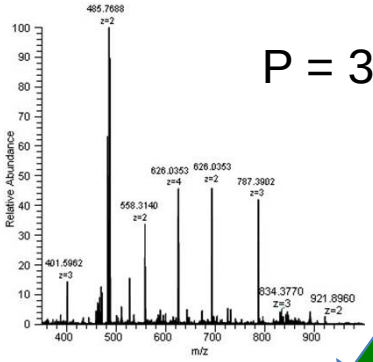
Cmpd #5836 (m/z 202.1185) is associated with hepatic fat in FHS



~200 peaks associated with hepatic fat (age and sex adjusted)

Phenotype	covariates	n	beta	p value
LPR	AGE1:sex	464	-0.197	2.28E-24
LPR	AGE1:sex:bmi1	464	-0.175	4.81E-16
LPR	AGE1:sex:smoke1:alc1	463	-0.201	6.22E-25
LPR	AGE1:sex:smoke1:alc1:HDL1:log(tg1):gluc1:diab:HTN1	457	-0.186	1.49E-16
LPR	AGE1:sex:smoke1:alc1:HDL1:log(tg1):gluc1:diab:HTN1:bmi1	457	-0.174	1.71E-13

Gene:
AGXT2



P = 3.65E-9

(alanine-glyoxylate aminotransferase 2)

Unknown Metabolite:

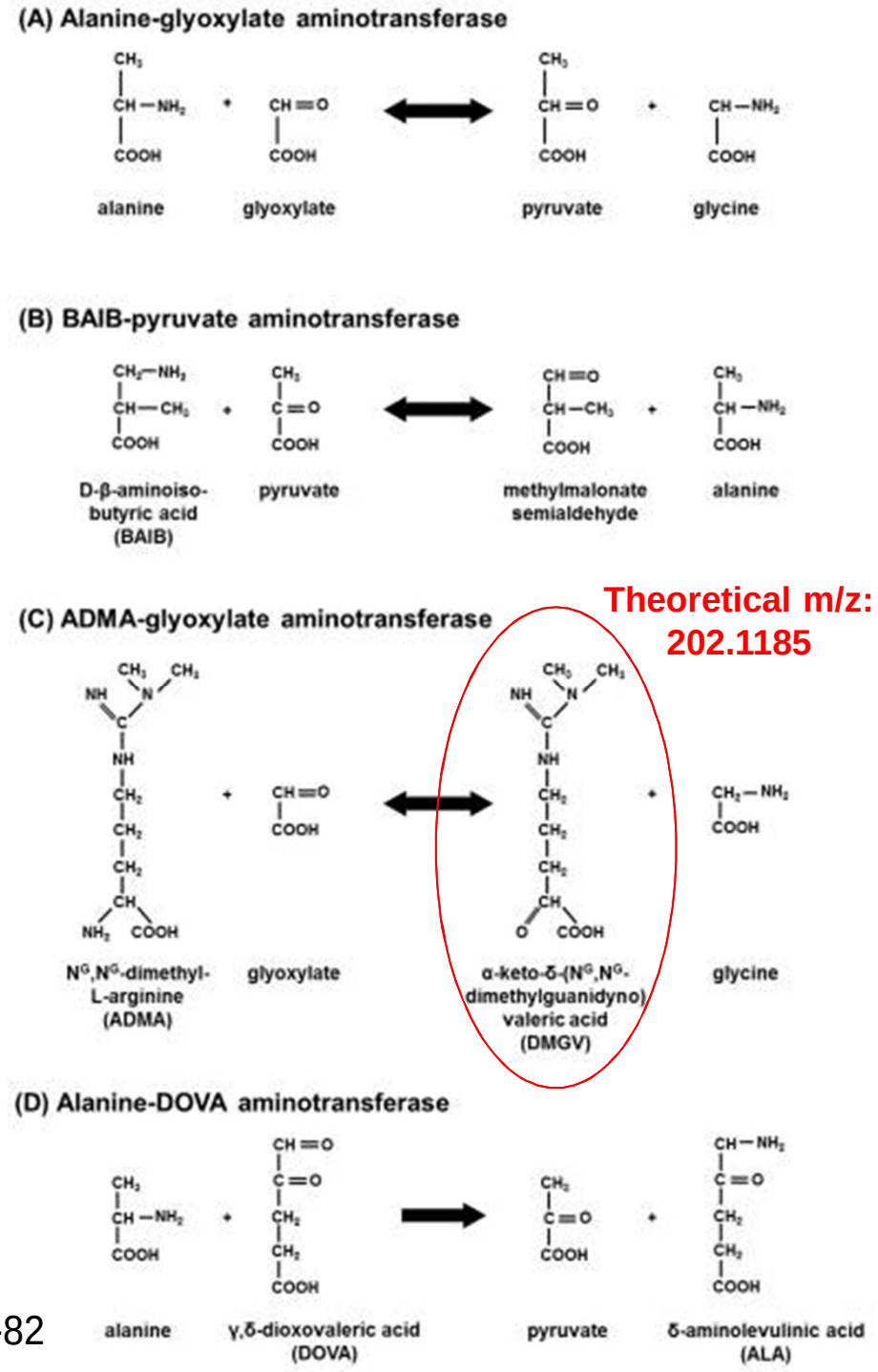
m/z 202.1185

P = 6.22E-24

Phenotype:
Liver Fat
(CT Scan)

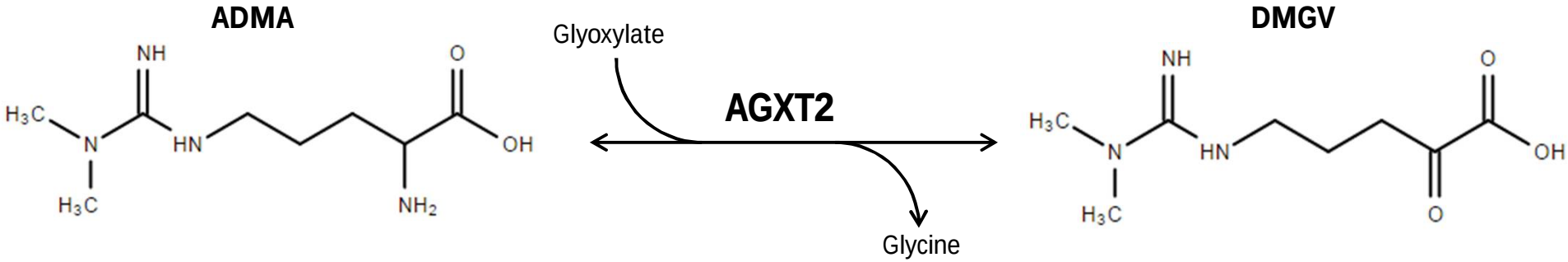
L-Threonine
L-Allothreonine
Hydroxyethyl glycine
4-Amino-3-hydroxybutyrate
L-Homoserine
D-Alanyl-D-alanine
Alanyl-Alanine
4-Acetamido-2-aminobutanoic acid
1-Methylhistidine
3-Methylhistidine
Ethyl lactate
2-Methyl-3-hydroxybutyric acid
3-Hydroxy-2-methyl-[R-(R,S)]-butanoic acid
Erythronilic acid
2-Ethylhydracrylic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

AGXT2:
A Multifunctional enzyme

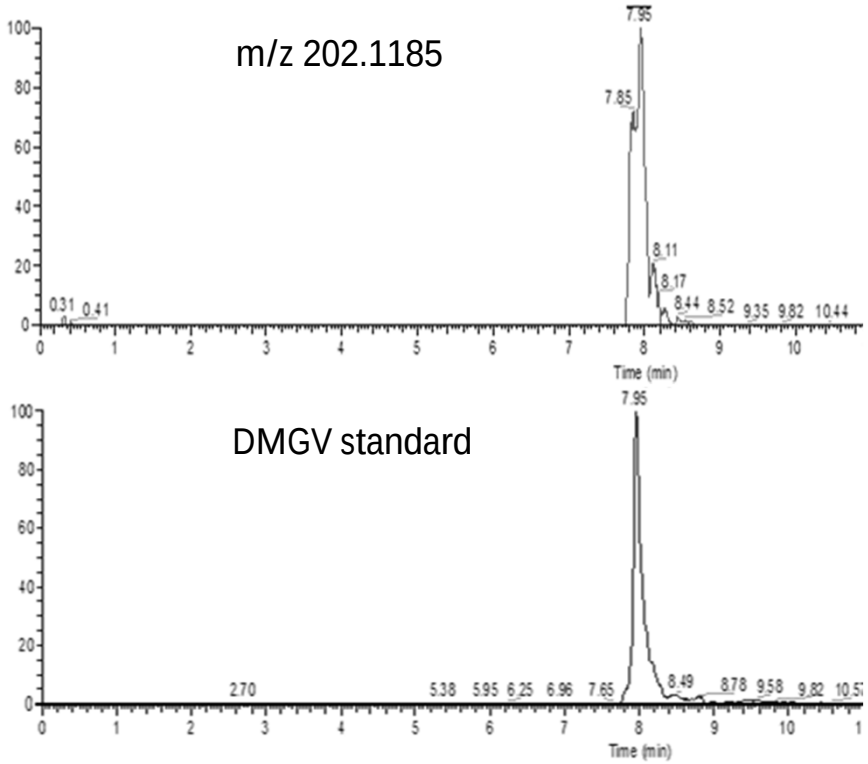


DMGV: α-keto-dimethyl-δ-(N^G,N^G-dimethylguanidynol) valeric acid

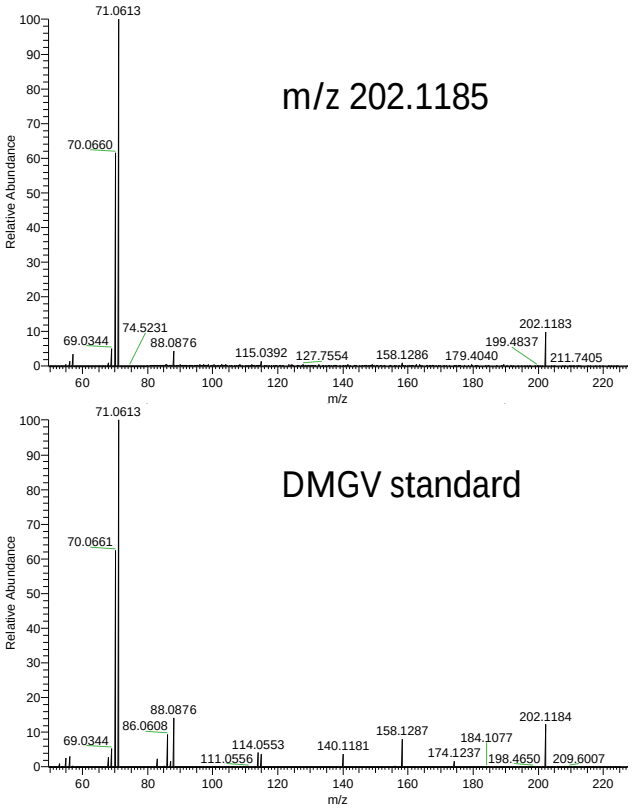
Rodionov RN et al. *Trends Pharmacol Sci* 2014; 35:575-82



Confirmation: RT



Confirmation: MS/MS



DMGV predicts incident DM in FHS Gen 3 and MDC

- FHS Gen 3 (4 yrs f/u)
  - 20 incident cases of DM
  - 1.8-fold increase per SD increment
  - p = 0.00045 (age- and sex-adjusted)

- Malmo Diet and Cancer Study (12.6 yrs f/u)
  - 196 incident cases of DM
  - 1.6-fold increase per SD increment
  - p = 8.6E-4 (adjusted for age, sex, glucose, and BMI)

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NIH:
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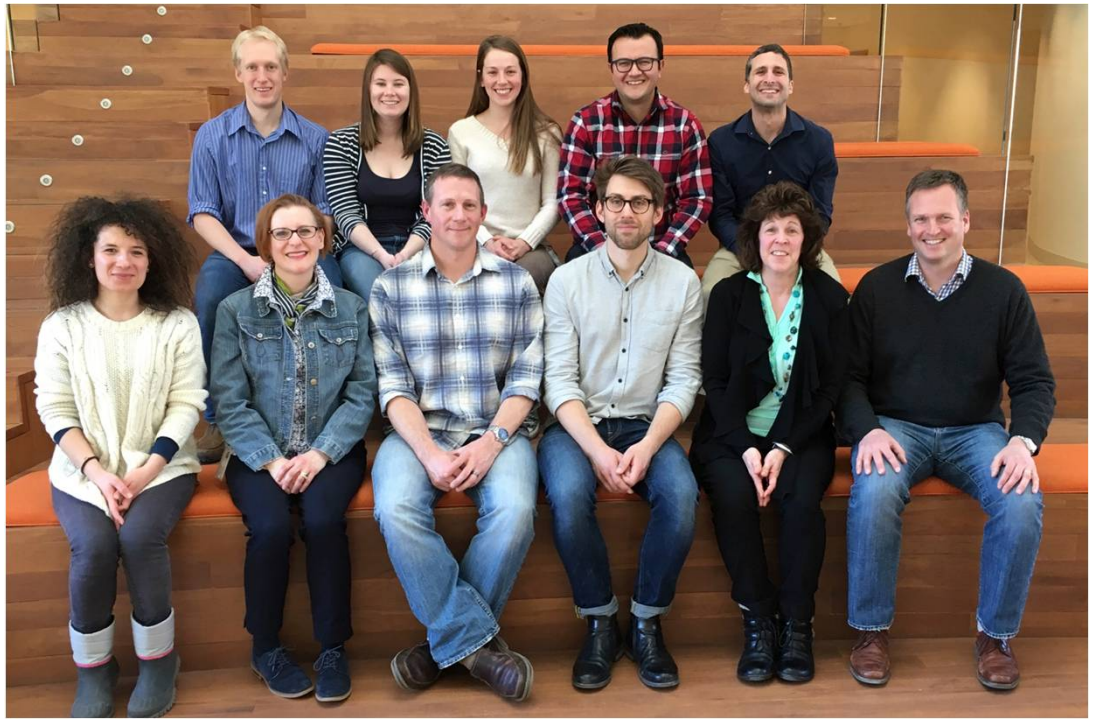
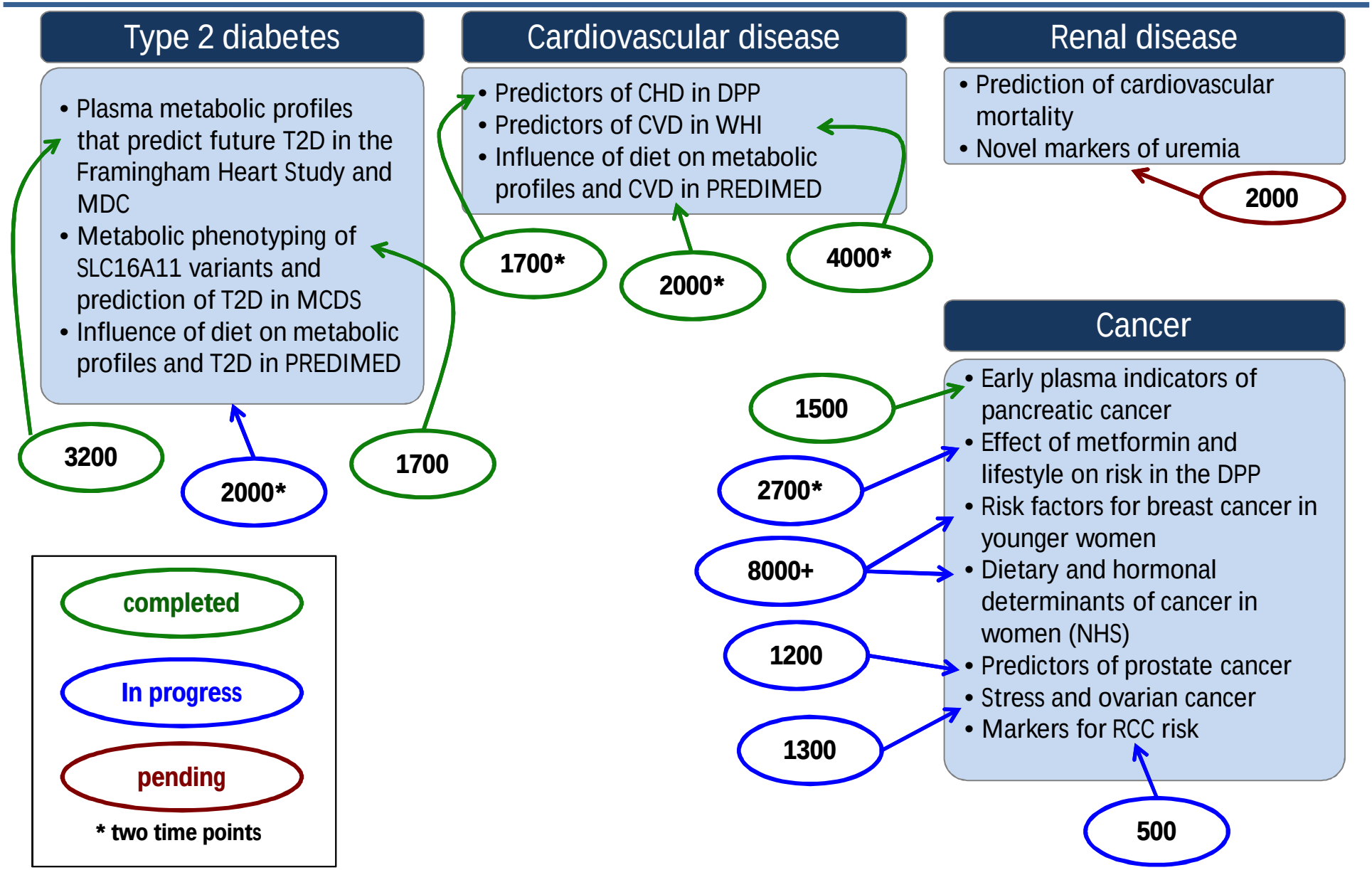
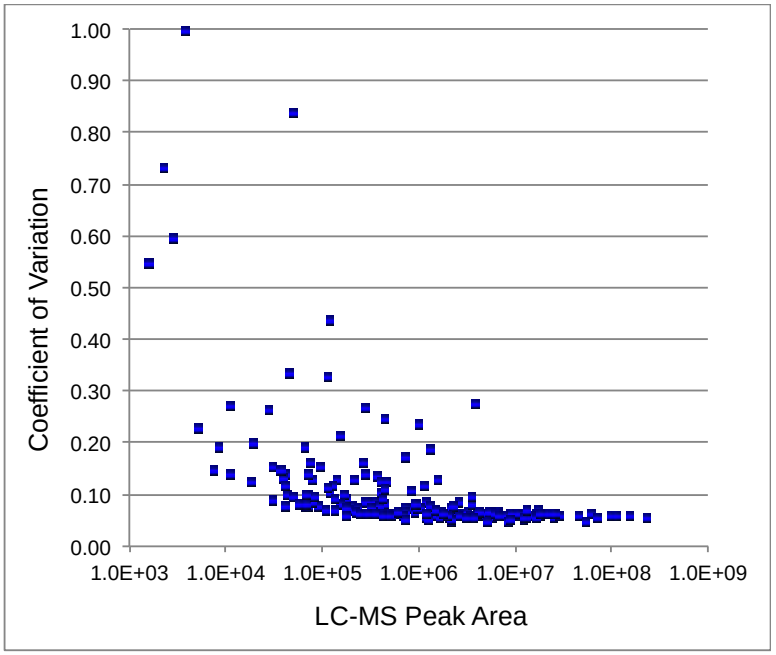
R01-DK081572
R01-DK102896
N01-HC25195
HHSN268201300008C

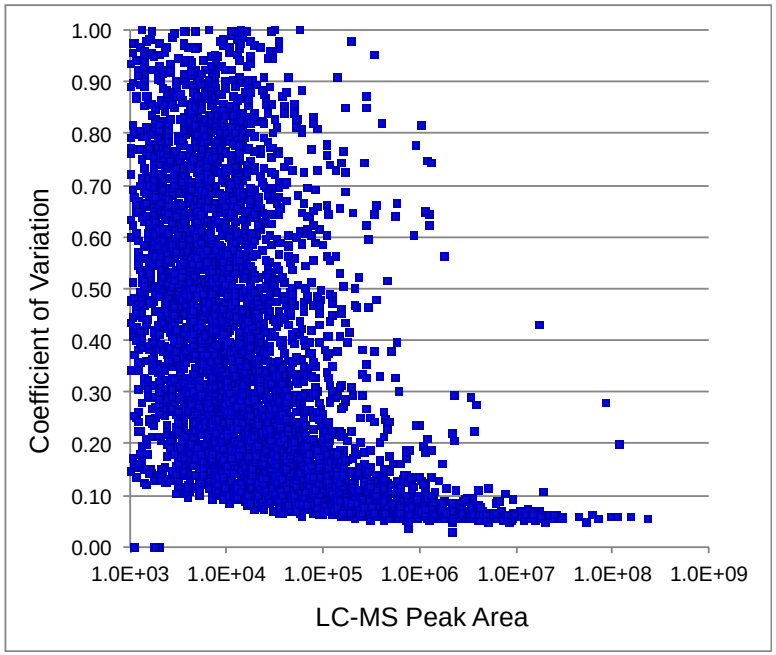


Additional metabolomics studies in longitudinal cohorts



CVs of C8-pos (lipid) peaks in reference pooled plasma



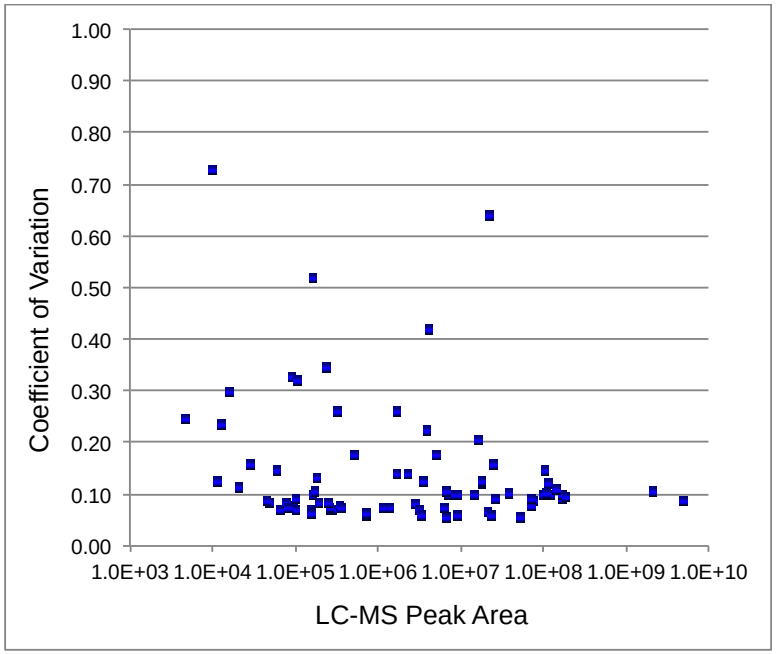


CV	KNOWNS (200)		ALL (6358)	
	# peaks	%	# peaks	%
< 1.00	200	100%	5400	85%
< 0.50	195	98%	3817	60%
< 0.25	188	94%	2471	39%
< 0.20	184	92%	2080	33%
< 0.15	175	88%	1546	24%
< 0.10	150	75%	929	15%

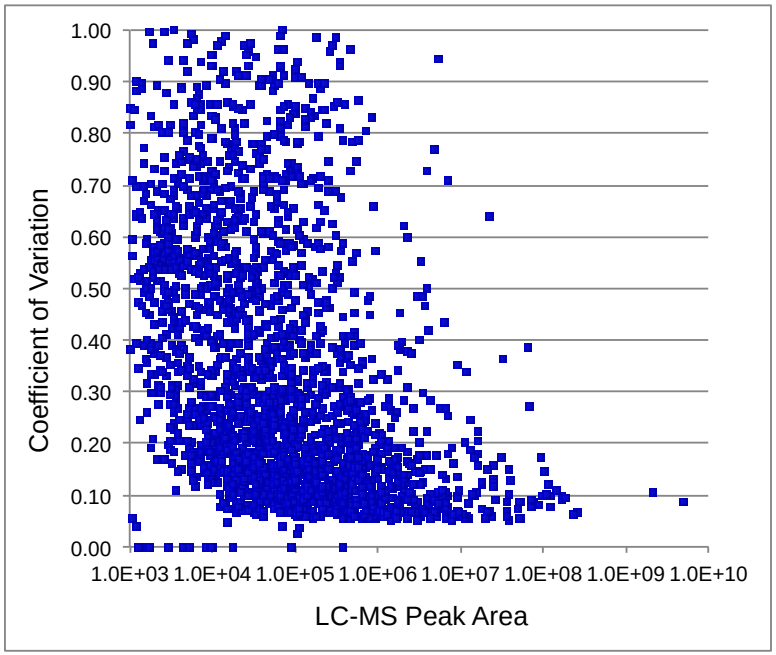
n = 102 PP samples

CVs of HILIC-pos (polar metabolite) peaks in reference pooled plasma

83 Knowns



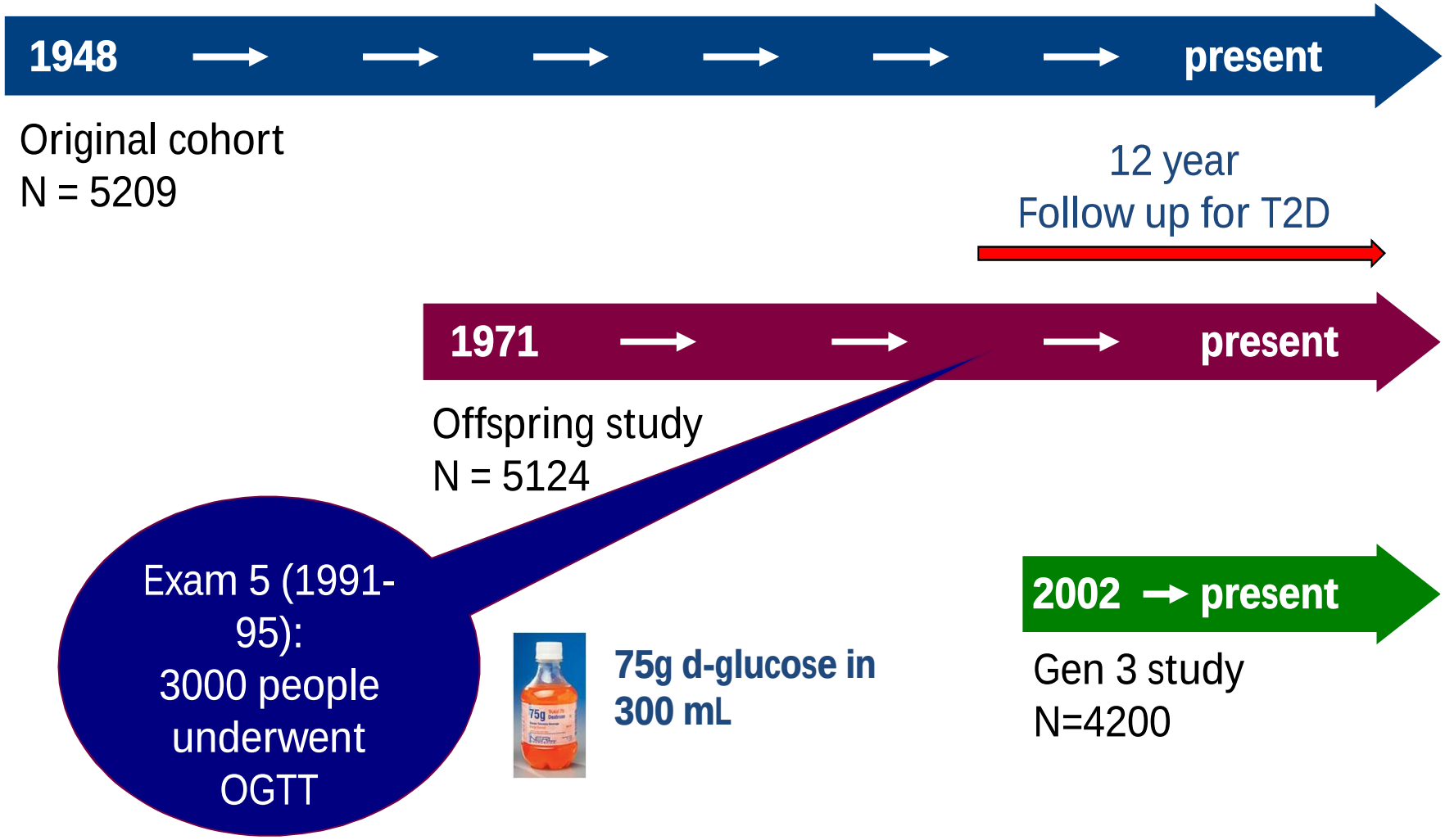
All 2833 peaks



CV	KNOWNS (200)		ALL (2833)	
	# peaks	%	# peaks	%
< 1.00	78	94%	2355	83%
< 0.50	75	90%	1708	60%
< 0.25	68	82%	1130	40%
< 0.20	64	77%	945	33%
< 0.15	60	72%	668	24%
< 0.10	42	51%	334	12%

n = 99 PP samples

Framingham Heart Study: Longitudinal population-based study



Robert Gerszten, Thomas Wang

Study design: Metabolic predictors of T2D

- Initial nested case-control study:
  - 189 future T2D cases and 189 matched controls, pre- & post-OGTT
  - 756 samples in discovery set
- Matching based on fasting glucose, age, sex, BMI, and hypertension status

Table 1 Baseline characteristics (Framingham Offspring Study)

	Cases (n = 189)	Matched controls (n = 189)	Random cohort (n = 400)
Clinical characteristics			
Age, years	56 ± 9	57 ± 8	55 ± 9
Women, %	42	42	58
Body mass index, kg m^{-2}	30.5 ± 5.0	30.0 ± 5.5	26.8 ± 4.6
Waist circumference, cm	102.3 ± 12.1	99.6 ± 13.5	90.8 ± 13.8
Hypertension, %	53	53	27
Parental history of diabetes[a], %	31	18	21
Physical activity index	35 ± 6.2	35 ± 7.3	35 ± 6.0
Total caloric intake, kcal	1,982 ± 660	1,866 ± 600	1854 ± 581
Total protein intake, g	82 ± 28	78 ± 28	76 ± 26
Phenylalanine intake, g	3.6 ± 1.2	3.4 ± 1.3	3.4 ± 1.1
Tyrosine intake, g	3.0 ± 1.0	2.8 ± 1.1	2.8 ± 1.0
Leucine intake, g	6.5 ± 2.2	6.1 ± 2.3	6.0 ± 2.1
Isoleucine intake, g	3.9 ± 1.3	3.7 ± 1.4	3.6 ± 1.3
Valine intake, g	4.3 ± 1.5	4.1 ± 1.5	4.0 ± 1.4
Other laboratory tests			
Fasting glucose, mg dl^{-1}	105 ± 9	105 ± 9	94 ± 9
2-h glucose (OGTT), mg dl^{-1}	126 ± 32	118 ± 30	103 ± 27
Serum triglycerides, mg dl^{-1}	192 ± 114	151 ± 90	138 ± 93
Fasting insulin, μIU ml^{-1}	13.7 ± 9.9	11.9 ± 8.8	8.1 ± 7.2
HOMA-IR	3.5 ± 2.6	3.1 ± 2.3	1.9 ± 1.8
HOMA-B	2.7 ± 2.0	2.4 ± 1.7	1.8 ± 1.5

Values are mean ± s.d. or percentage. [a]Parental history information missing in 57 participants.

Wang TJ *et al*. *Nat Med* 2011; 17:448-453.

Many metabolic changes in response to OGTT:

However, no difference between incident cases and controls observed

Metabolite	DF	t-statistic	P-value	Percent Change post vs. pre
3-OH-anthranilic acid	34	4.82	<.0001	-20.66%
5-HIAA	187	6.39	<.0001	-7.45%
5-hydroxytryptophan	92	5.21	<.0001	-21.20%
ADMA/SDMA	187	12.5	<.0001	-13.41%
alanine	188	6.16	<.0001	-5.17%
aminoisobutyric acid	187	15.42	<.0001	-24.01%
arginine	187	6.24	<.0001	-13.78%
asparagine	187	18.28	<.0001	-23.96%
aspartate	188	12.33	<.0001	-21.99%
betaine	187	-4.16	<.0001	3.12%
carnitine	187	-6.79	<.0001	5.05%
choline	188	-4.23	<.0001	4.67%
cis/trans-hydroxyproline	187	20.97	<.0001	-26.42%
citrulline	187	36.37	<.0001	-39.09%
dimethylglycine	187	5.18	<.0001	-4.27%
glutamic acid	188	9.75	<.0001	-12.21%
glutamine	188	6.48	<.0001	-5.12%
glycerol	138	13.8	<.0001	-42.57%
glycine	187	11.06	<.0001	-11.75%
histidine	187	13.22	<.0001	-14.89%
isoleucine	188	36.6	<.0001	-37.40%
kynurenic acid	187	11.01	<.0001	-29.19%
leucine	188	35.72	<.0001	-36.14%
lysine	188	10.26	<.0001	-15.31%
methionine	188	20.4	<.0001	-28.72%
niacinamide	188	6.59	<.0001	-17.31%
NMMA	187	10.92	<.0001	-12.26%
ornithine	187	13.92	<.0001	-22.26%
phenylalanine	187	24.23	<.0001	-22.36%
proline	187	19.09	<.0001	-12.13%
serine	187	18.44	<.0001	-20.57%
serotonin	181	7.44	<.0001	-39.28%
taurine	188	14.35	<.0001	-11.91%
thiamine	188	4.24	<.0001	10.16%
threonine	187	31.47	<.0001	-22.74%
trimethylamine-n-oxide	188	4.85	<.0001	-5.13%
tryptophan	187	13.55	<.0001	-12.88%
tyrosine	188	28.39	<.0001	-28.53%
valine	188	30.22	<.0001	-21.13%
xanthosine	188	7.5	<.0001	-12.15%
alpha-glycerophosphocholine	188	-3.97	0.0001	22.13%

Summary

- Metabolomics can reveal early metabolic changes in subclinical disease
- Elevated branched chain and aromatic amino acids, 2-aminoadipic acid, and shifts in TAG fatty acid content predict future T2D
- Plasma BCAA are also associated with risk of future pancreatic cancer diagnosis and may be linked to increased protein turnover
- We have identified novel oxidized lipids associated with CHD risk
  - First study showing link between either hydroxy-PCs or mono-HETEs and CHD prospectively in humans

Current directions:

- Extending T2D work in a number of different studies; foci include: other ethnic groups, treatment and lifestyle interventions, and prediction in more youthful participants
- Applying current nontargeted methods to T2D, PDAC, and other cancers; exploring associations with genetics and imaging data