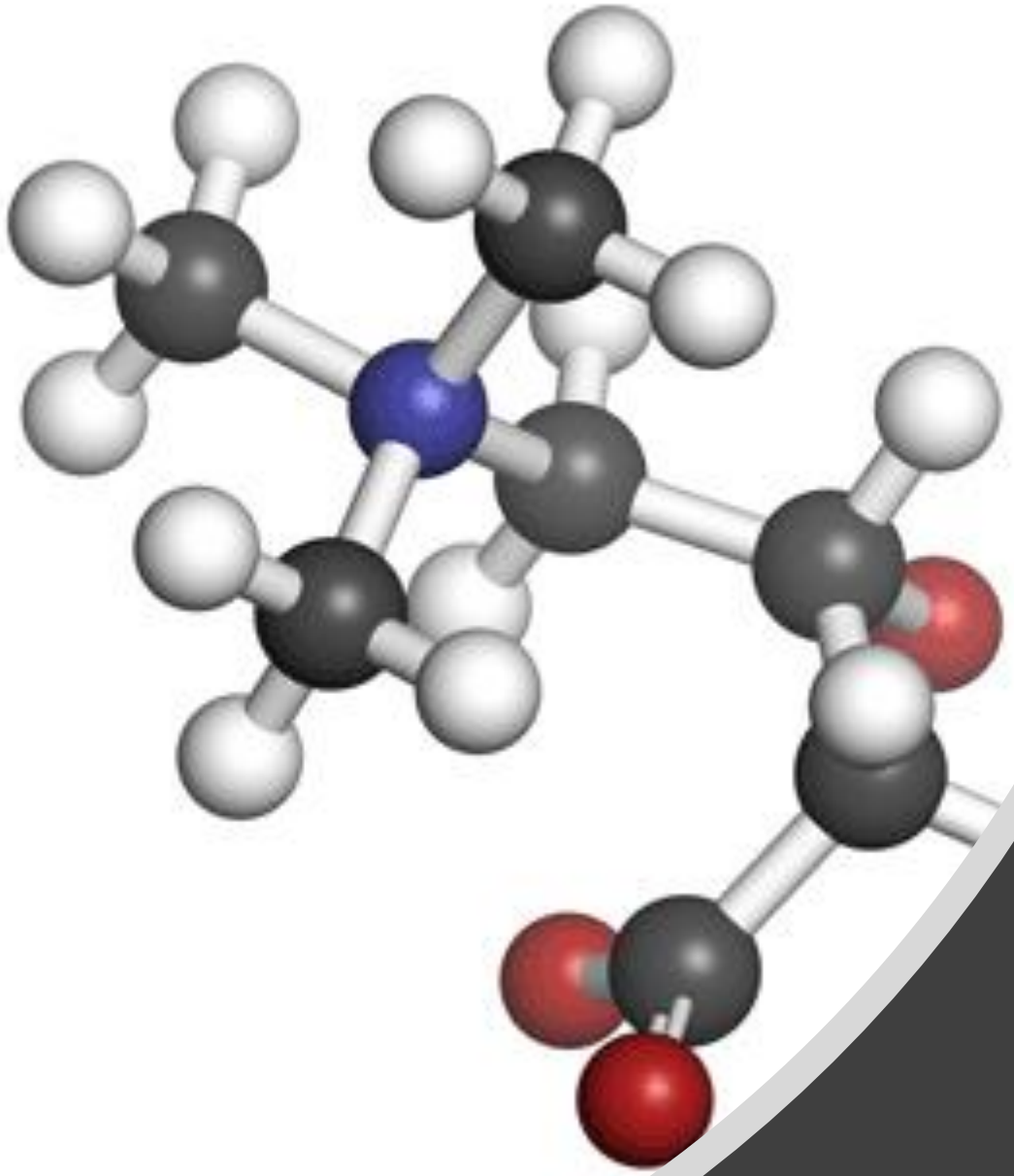


CHANNING DIVISION OF NETWORK MEDICINE

Metabolomics in the multi-omic era: *A key component for clinical translation in asthma*

2019 Breath Biopsy Conference

Jessica Lasky-Su, ScD

November 13, 2019

DNA sequencing timeline

2003

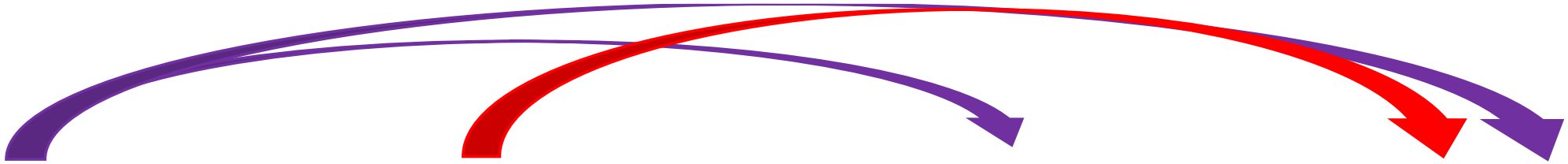
- Human Genome Project, 10 years, $3 billion

June 1, 2007

- James Watson's genome is sequenced
- $2 million dollars
- *"Scientists ultimately hope to bring the cost down to less than $10,000, a target price that many believe will be the turning point in genomic medicine. At that price, many people could afford to have their genomes sequenced, and doctors could then use that data to give their patients more-personalized medical advice." Technology Review, 2007*

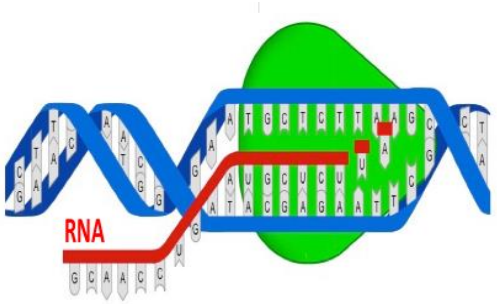
Today: $1000's and a little spit

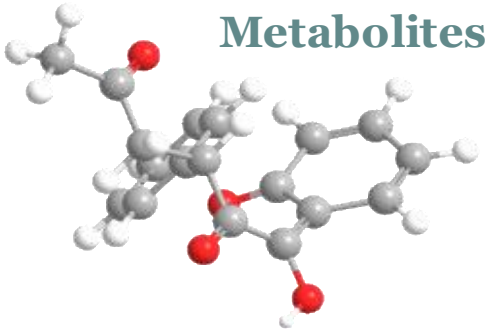
The Central Biological Dogma



GENOME **TRANSCRIPTOME** **PROTEOME** **METABOLOME**

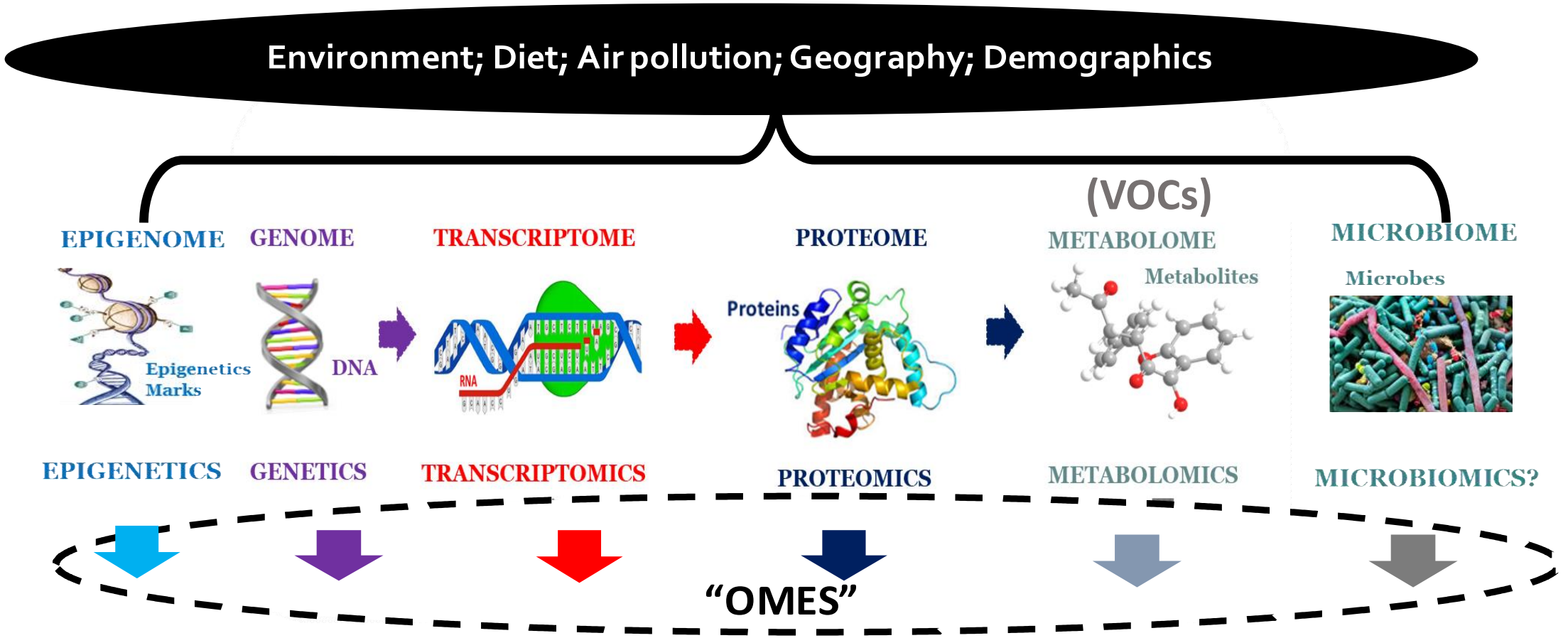
DNA

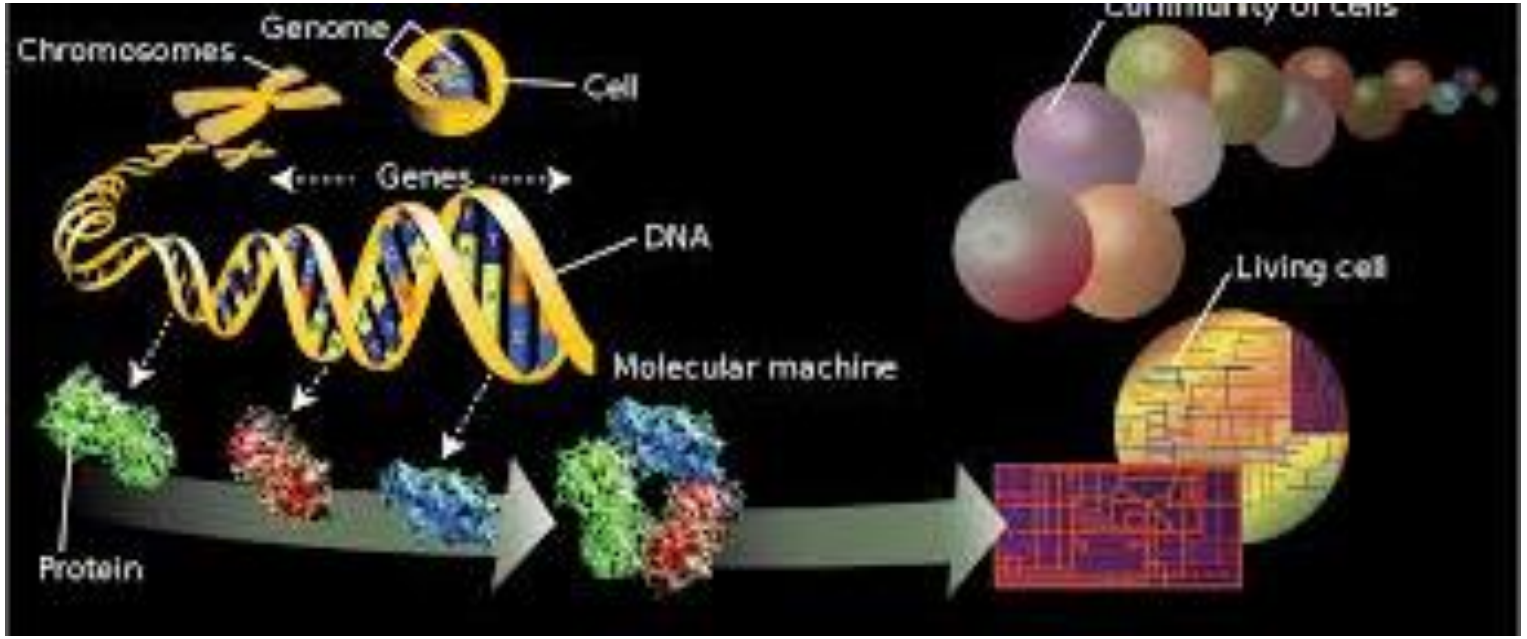


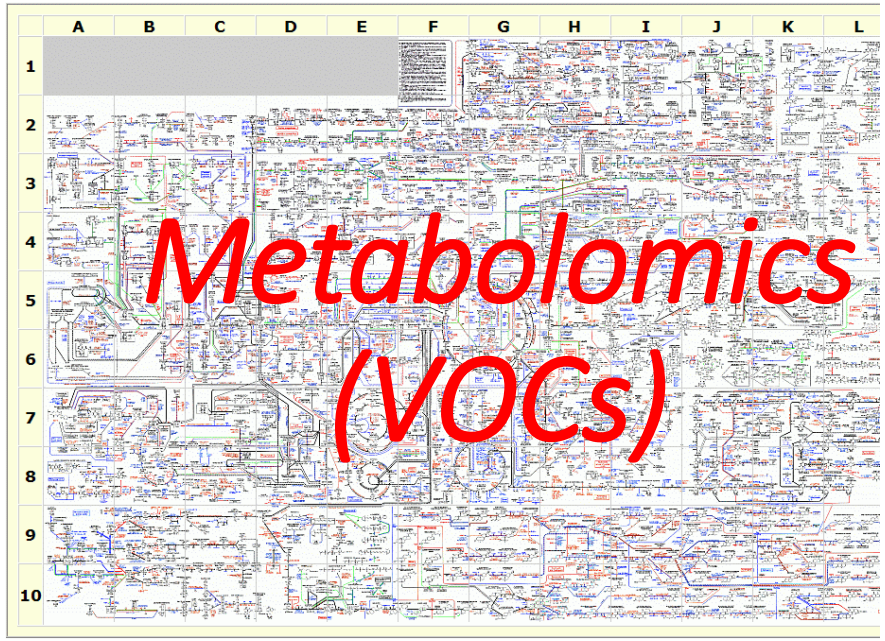


GENETICS **TRANSCRIPTOMICS** **PROTEOMICS** **METABOLOMICS**

The New Biological Dogma







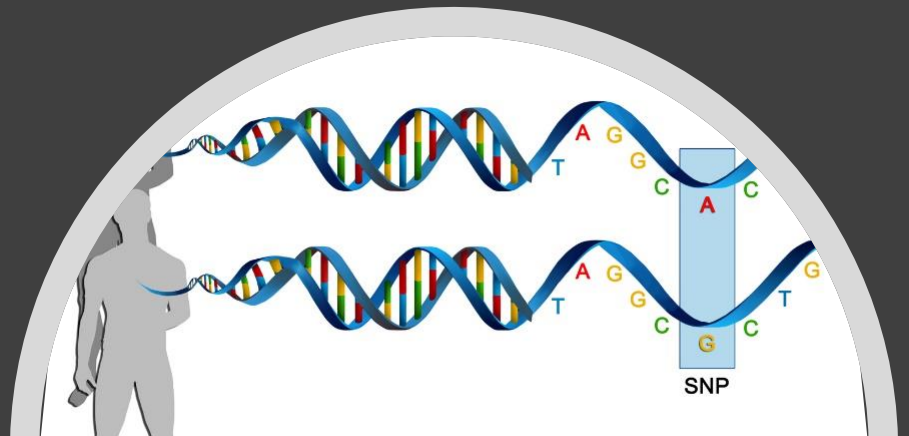
The Omics Revolution

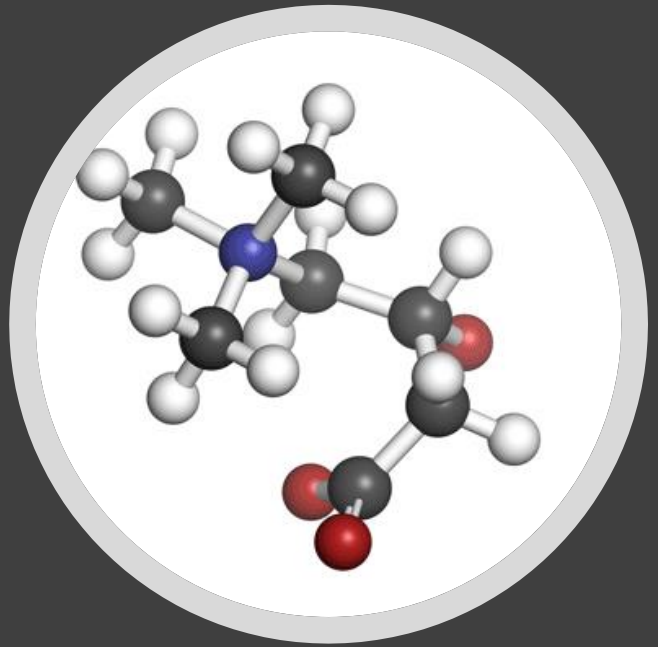
Omics Standard

- Large Sample Size
- Replication
- Multiple Comparison Correction
- Further Biological Interrogation

Outline

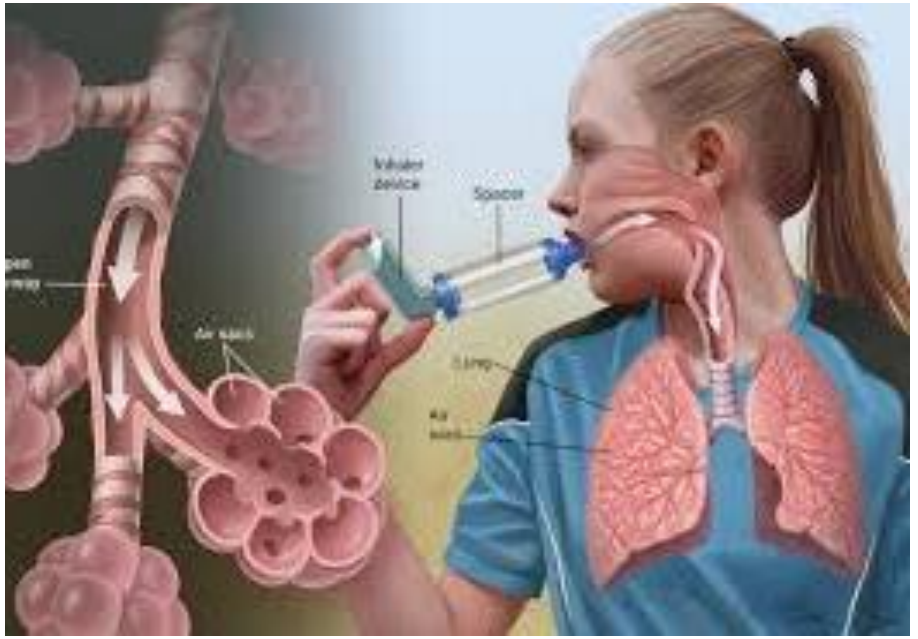
- Longitudinal Birth Cohorts
  - Longitudinal *Metabolomes*
  - Use of *Genetics, Microbiome, Exposures*
- Large Scale Metabolomics
  - Multiple *Metabolomes,* Multiple *Cohorts*





Large Scale Population-based cohorts & *The Omics Revolution*

- Study design
- Multi-omic data collection
- Multiple cohorts
- Large biospecimen collections
- Multiple visits



A *V*ITAMIN *D* *A*NTENATAL *A*STHMA *R*EDUCTION *T*RIAL : ASTHMA PATHOGENESIS

Genetics, Environmental Exposures, Multiomics

Vitamin D & Asthma

Vitamin D plays a critical role in immune response that may reduce inflammation in the airways and the likelihood of developing an infection

The Sydney Morning Herald National

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Lack of vitamin D for children increases the risk of asthma, study finds

Cochrane

Trusted evidence.
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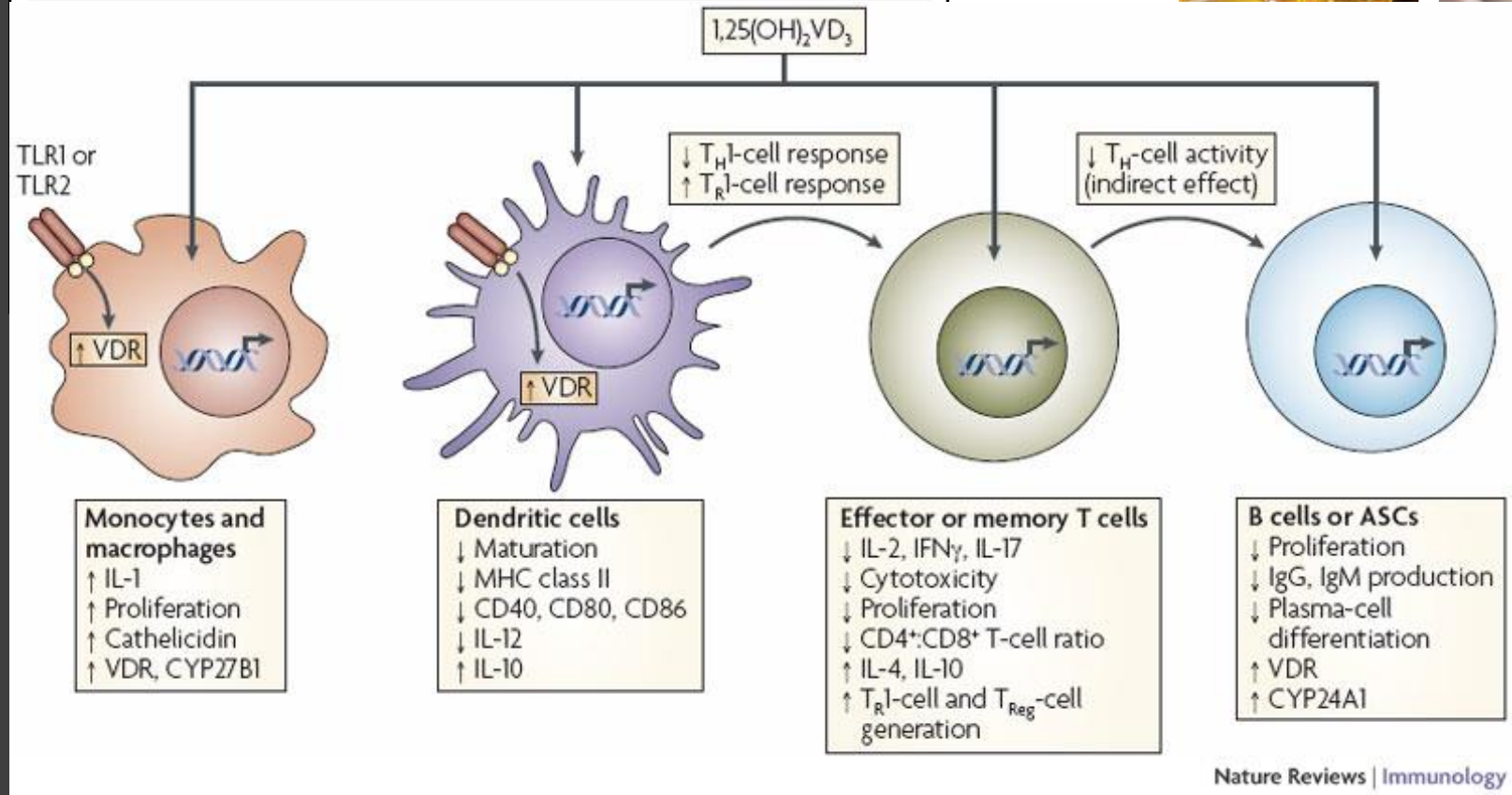
Our evidence About us Get involved News and events

High quality evidence suggests Vitamin D can reduce asthma a

ublished in the Cochrane
ce from randomized trials
n D supplement in addition
ication is likely to reduce

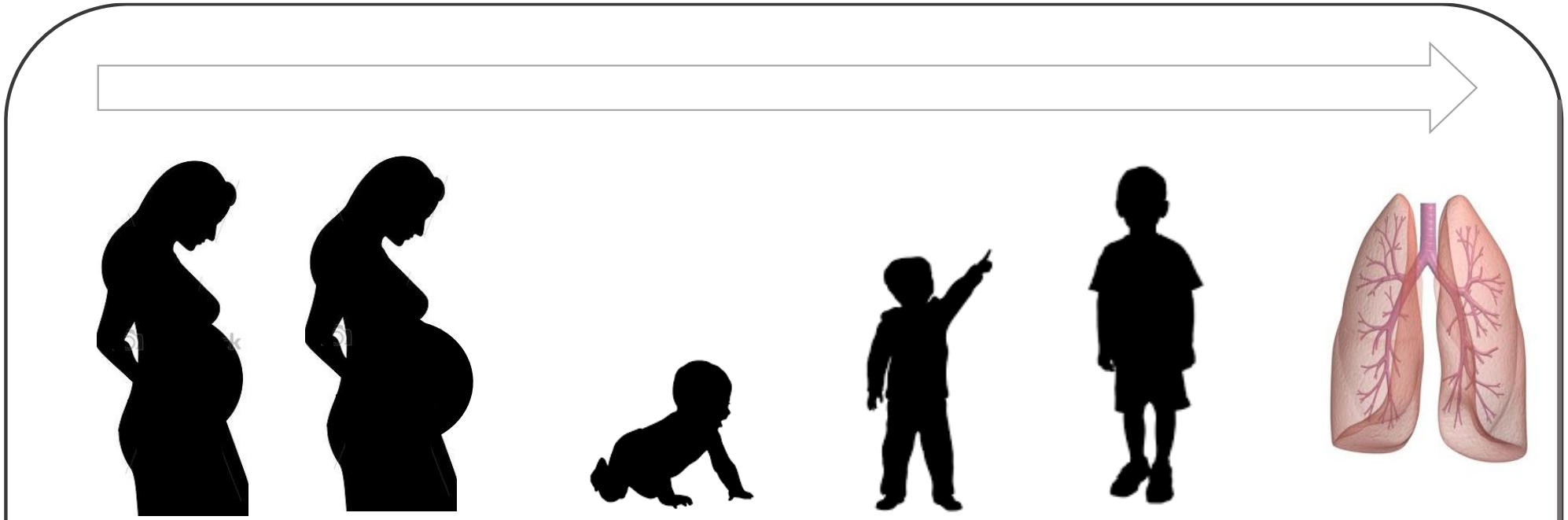
The New York Times

Vitamin D May Reduce Asthma Attacks



VDAART Study Design Overview

(*V*itamin *D* *A*ntenatal *A*sthma *R*eduction *T*rial)



ClinicalTrials.gov identifier: NCT00920621

Maternal Vitamin D reduces the risk of Asthma/Wheeze

Original Investigation

Effect of Prenatal Supplementation With Vitamin D on Asthma or Recurrent Wheezing in Offspring by Age 3 Years

The VDAART Randomized Clinical Trial

Augusto A. Litonjua, MD, MPH; Vincent J. Carey, PhD; Nancy Laranjo, BA; Benjamin J. Harshfield, BA; Thomas F. McElrath, MD, PhD; George T. O'Connor, MD, MS; Megan Sandel, MD, MPH; Ronald E. Iverson Jr, MD, MPH; Aviva Lee-Paritz, MD; Robert C. Strunk, MD, PhD; Leonard B. Bacharier, MD; George A. Macones, MD, MSCE; Robert S. Zeiger, MD, PhD; Michael Schatz, MD, MS; Bruce W. Hollis, PhD; Eve Hornsby, PhD; Catherine Hawrylowicz, PhD; Ann Chen Wu, MD, MPH; Scott T. Weiss, MD, MS

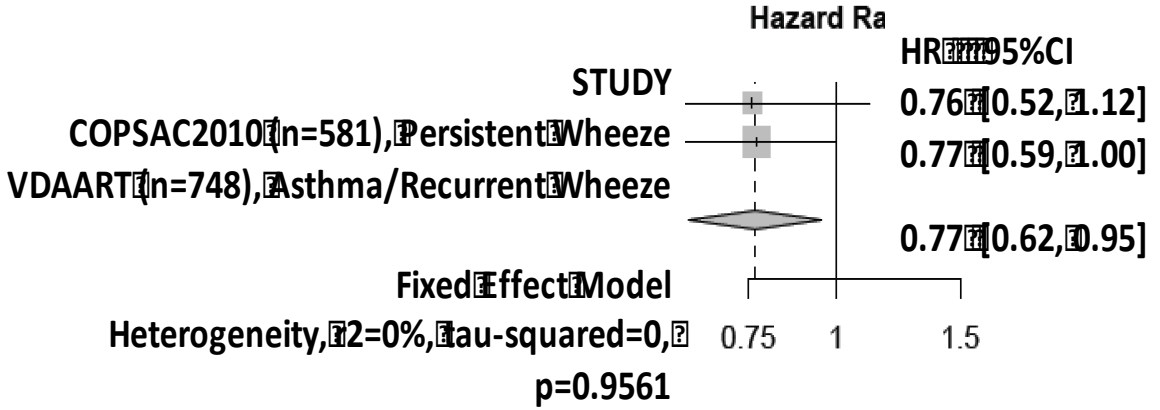
Original Investigation

Effect of Vitamin D_3 Supplementation During Pregnancy on Risk of Persistent Wheeze in the Offspring

A Randomized Clinical Trial

Bo L. Chawes, MD, PhD; Klaus Bønnelykke, MD, PhD; Jakob Stokholm, MD, PhD; Nadja H. Vissing, MD, PhD; Elín Bjarnadóttir, MD; Ann-Marie M. Schoos, MD, PhD; Helene M. Wolsk, MD; Tine Marie Pedersen, MD; Rebecca K. Vinding, MD; Sunna Thorsteinsdóttir, MD; Lambang Arianto, MD; Henrik W. Hallas, MD; Lene Heickendorff, MD, DMSc; Susanne Brix, MSc, PhD; Morten A. Rasmussen, MSc, PhD; Hans Bisgaard, MD, DMSc

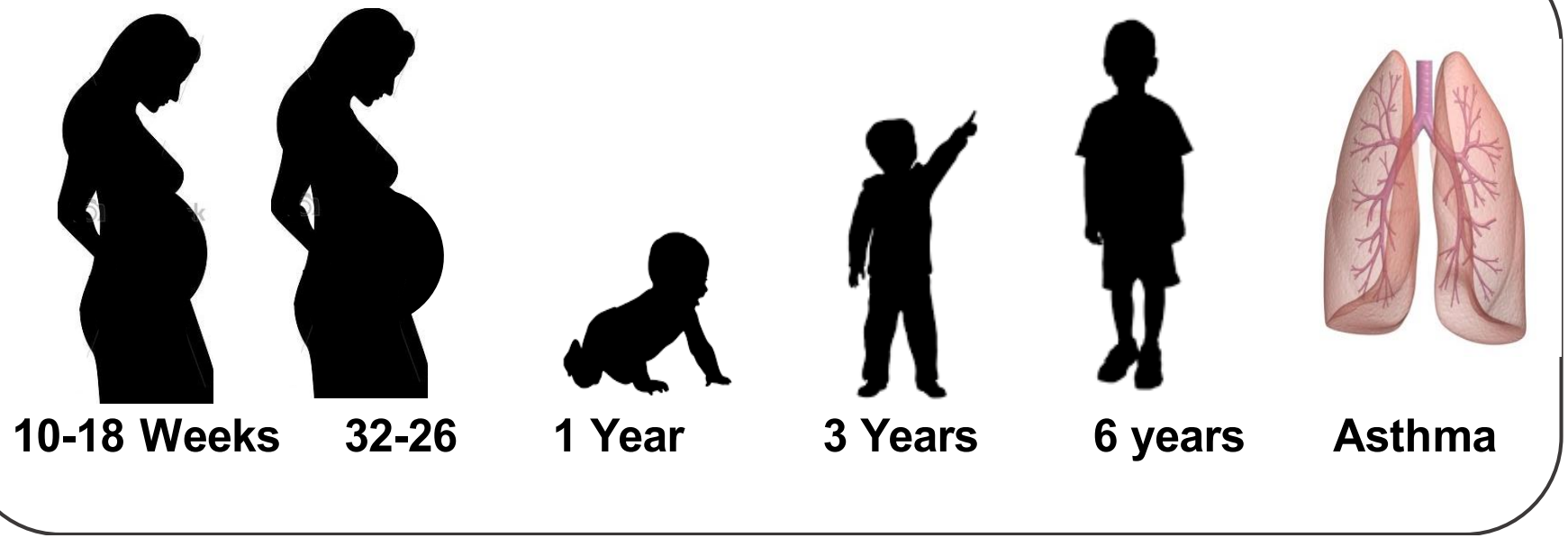
Meta-analysis of the VDAART and $COPSAC_{2010}$ primary findings, overall p-value Vitamin D = 0.01



VDAART Study Design Overview

(*V*itamin *D* *A*ntenatal *A*sthma *R*eduction *T*rial)

MULTI-OMICS

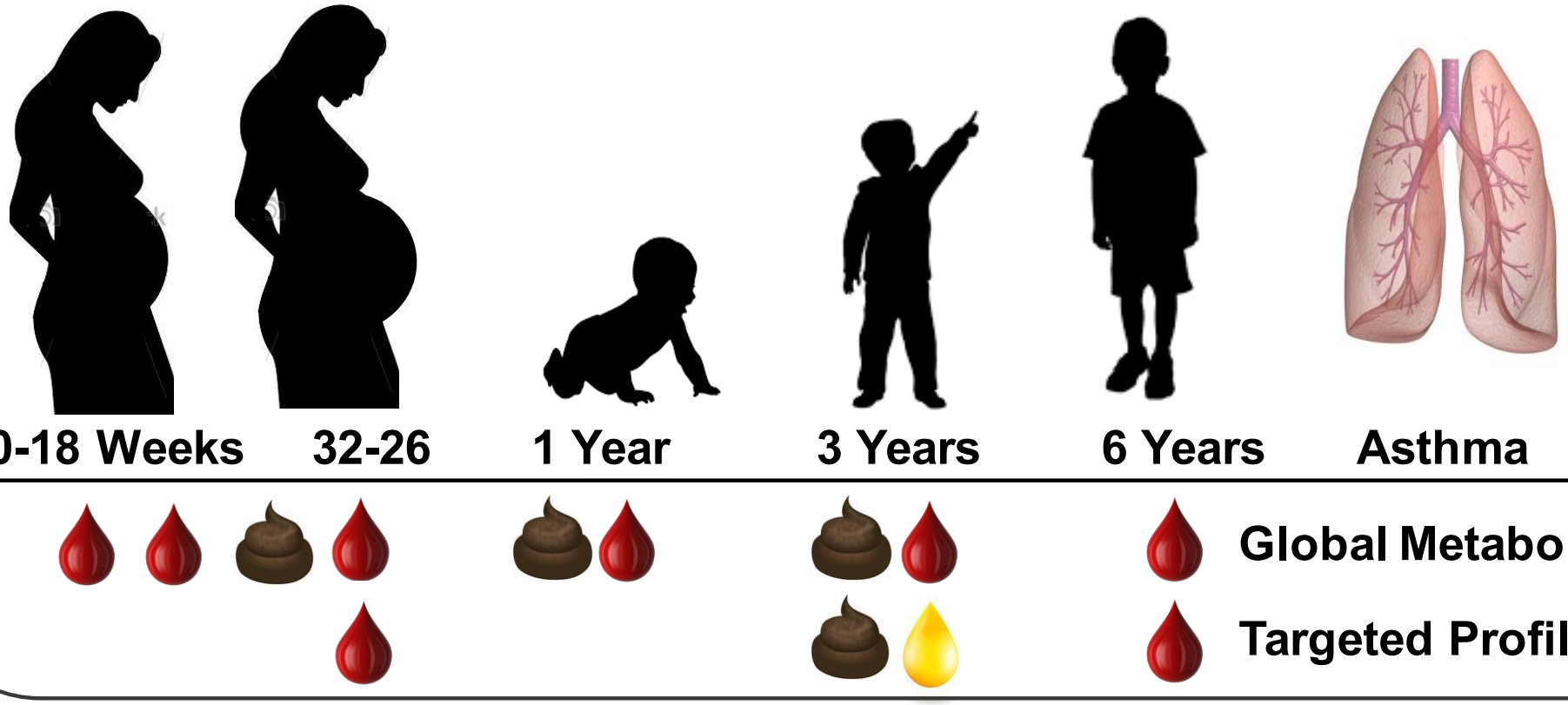


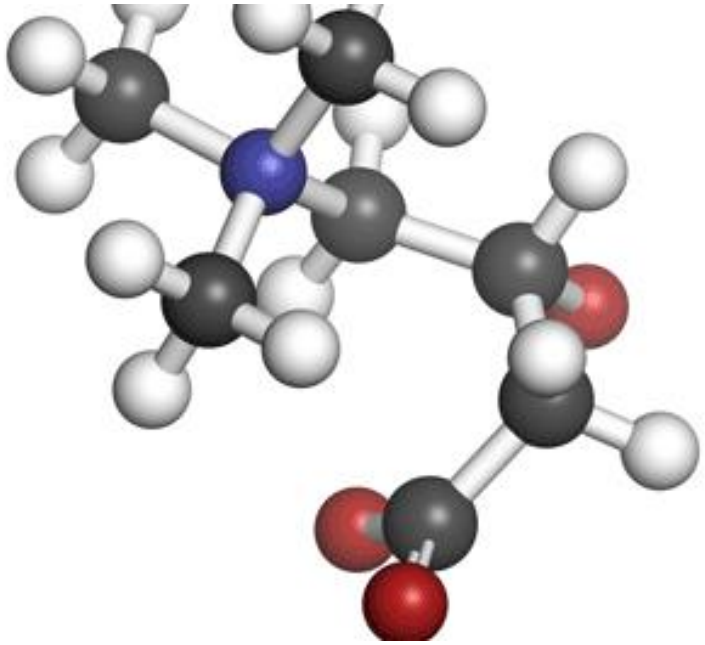
- GWAS
- Gene Expression (3X)
- Microbiome (5X)
- Stool Metabolome (3X)
- Plasma Metabolome (6X)
- Methylation

VDAART Study Design Overview

(*V*itamin *D* *A*ntenatal *A*sthma *R*eduction *T*rial)

METABOLOMICS



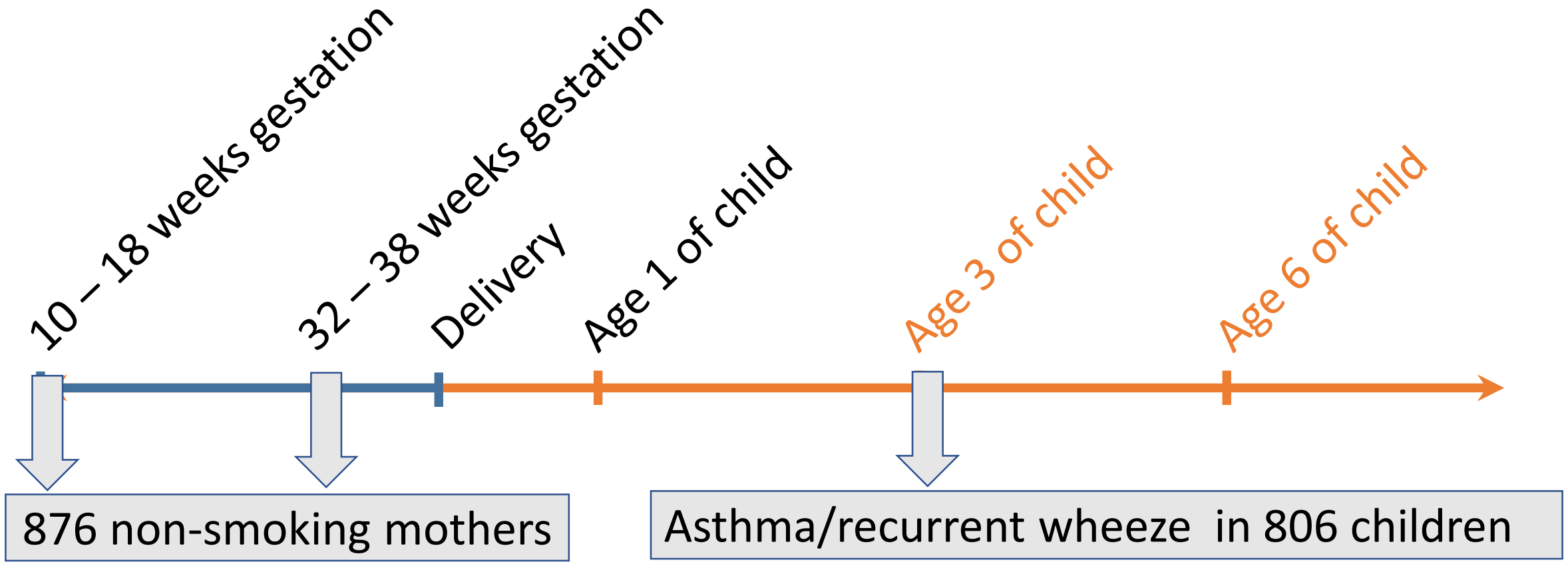


Multiple Metabolomes

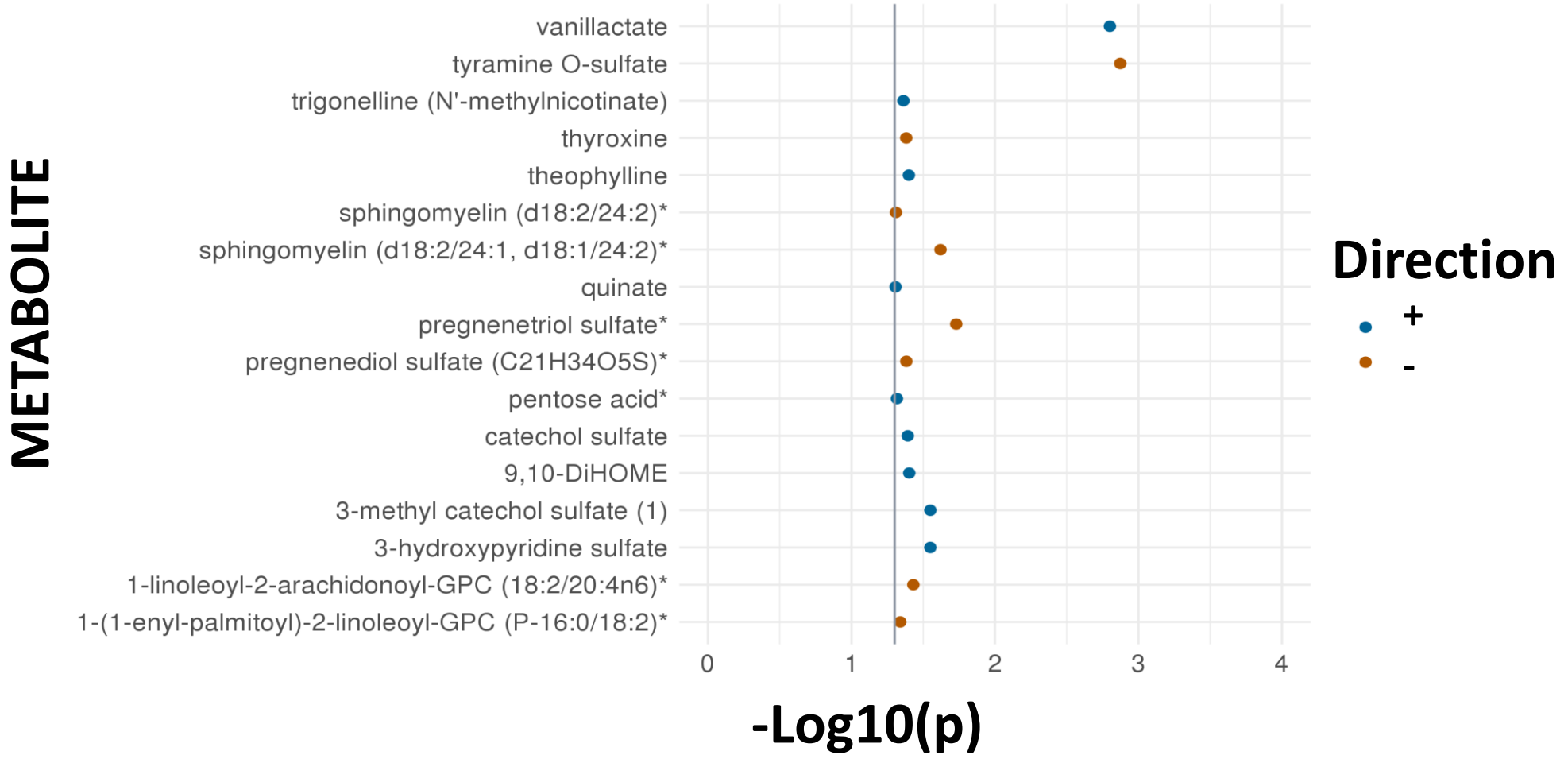
Maternal Metabolomes During Pregnancy & Childhood Asthma

VDAART: Metabolomics

Mother during pregnancy

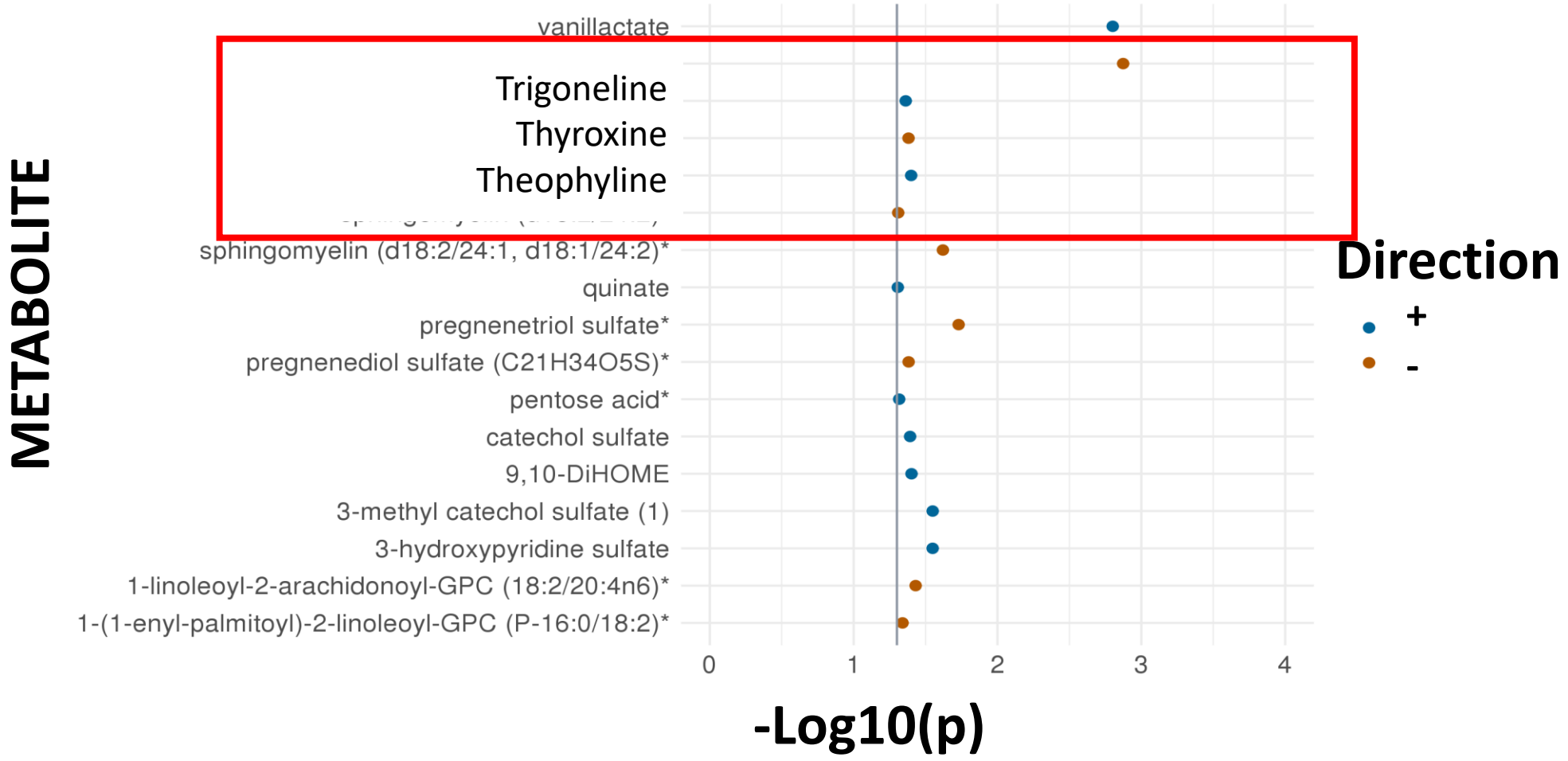


VDAART: Metabolomics: *early pregnancy & child asthma/wheeze by 3*



Model adjusted for: baseline maternal age, gestational age, vitamin D level, asthma history, race, study site, treatment group, SES proxy (education level, income category)

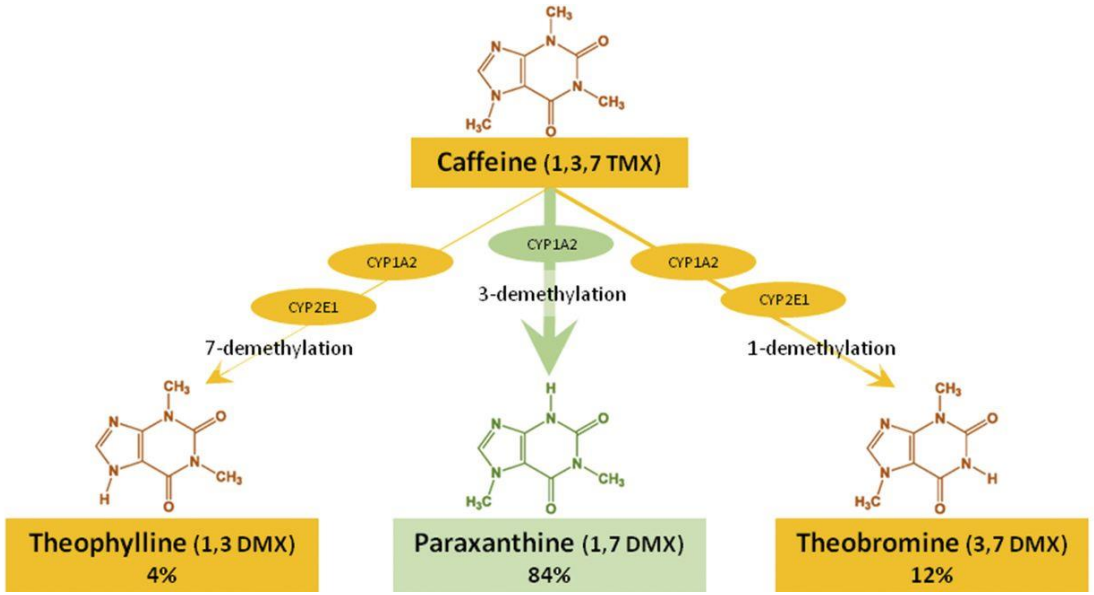
VDAART: Metabolomics: *early pregnancy & child asthma/wheeze by 3*



Model adjusted for: baseline maternal age, gestational age, vitamin D level, asthma history, race, study site, treatment group, SES proxy (education level, income category)

Theophylline

- Known asthma bronchodilator
- Anti-inflammatory effects
- Not commonly used today



Theophylline

Caffeine Metabolite

Caffeine missing in 18.6% samples

Caffeine dichotomized (detectable vs. below LoD)

Presence of caffeine associated with lower odds of age 3 asthma

- Beta = -0.45
- P-value = 0.038

Additional linear models

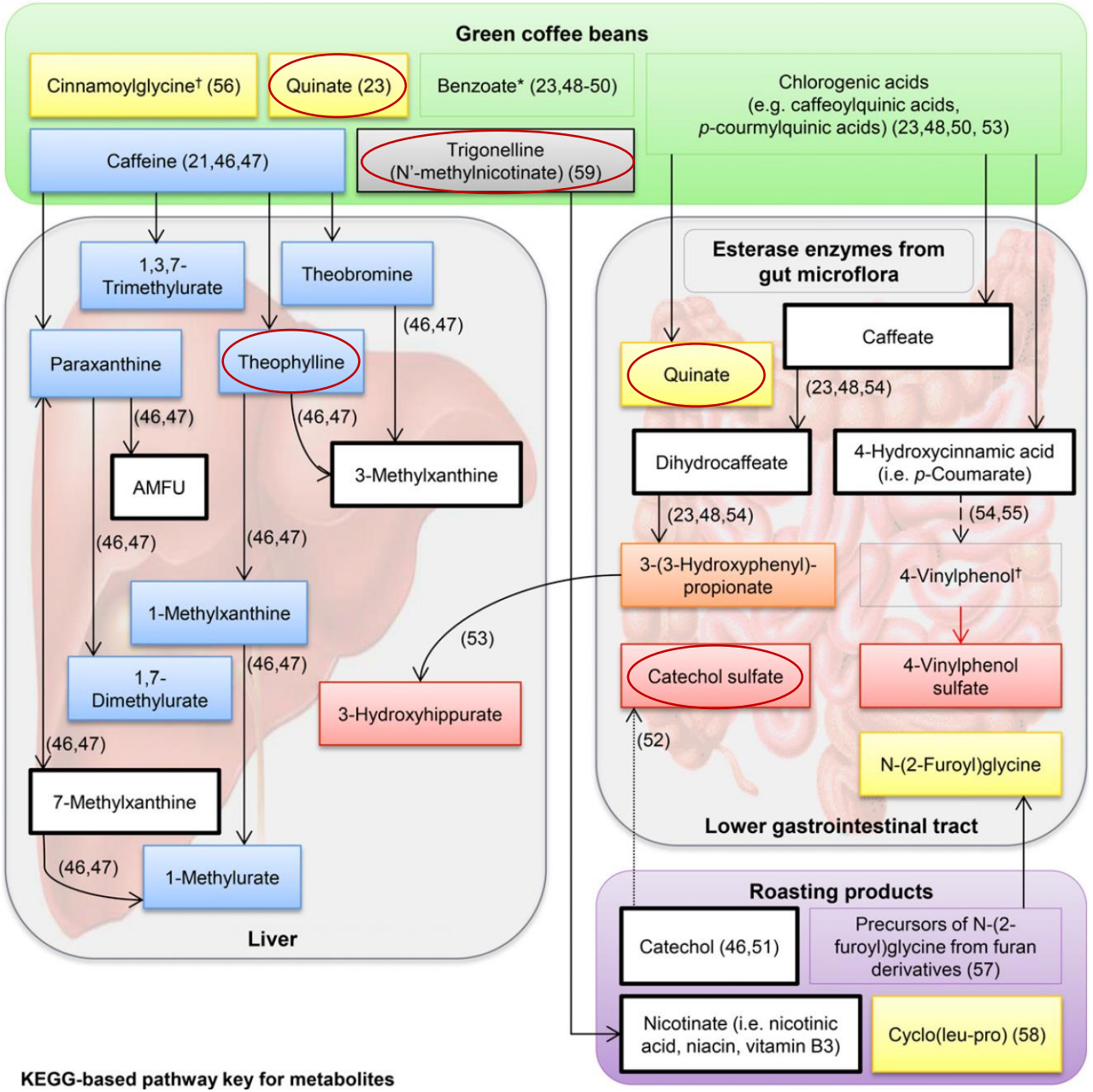
Replication Currently underway in independent cohort (COPSAC)

Metabolomic profile of habitual *coffee* intake

❖Guertin et al 2015 AJCN (serum)

- Red circle: significant in VDAART ($p<0.05$)

Metabolite	Total coffee
Trigonelline	X
Theophylline	X
Quinate	X
Catechol sulfate	X
3-methyl catechol sulfate	X
3-hydroxypyridine sulfate	X
Multiple testing procedure	*Bonferroni (7.61×10^{-5})*



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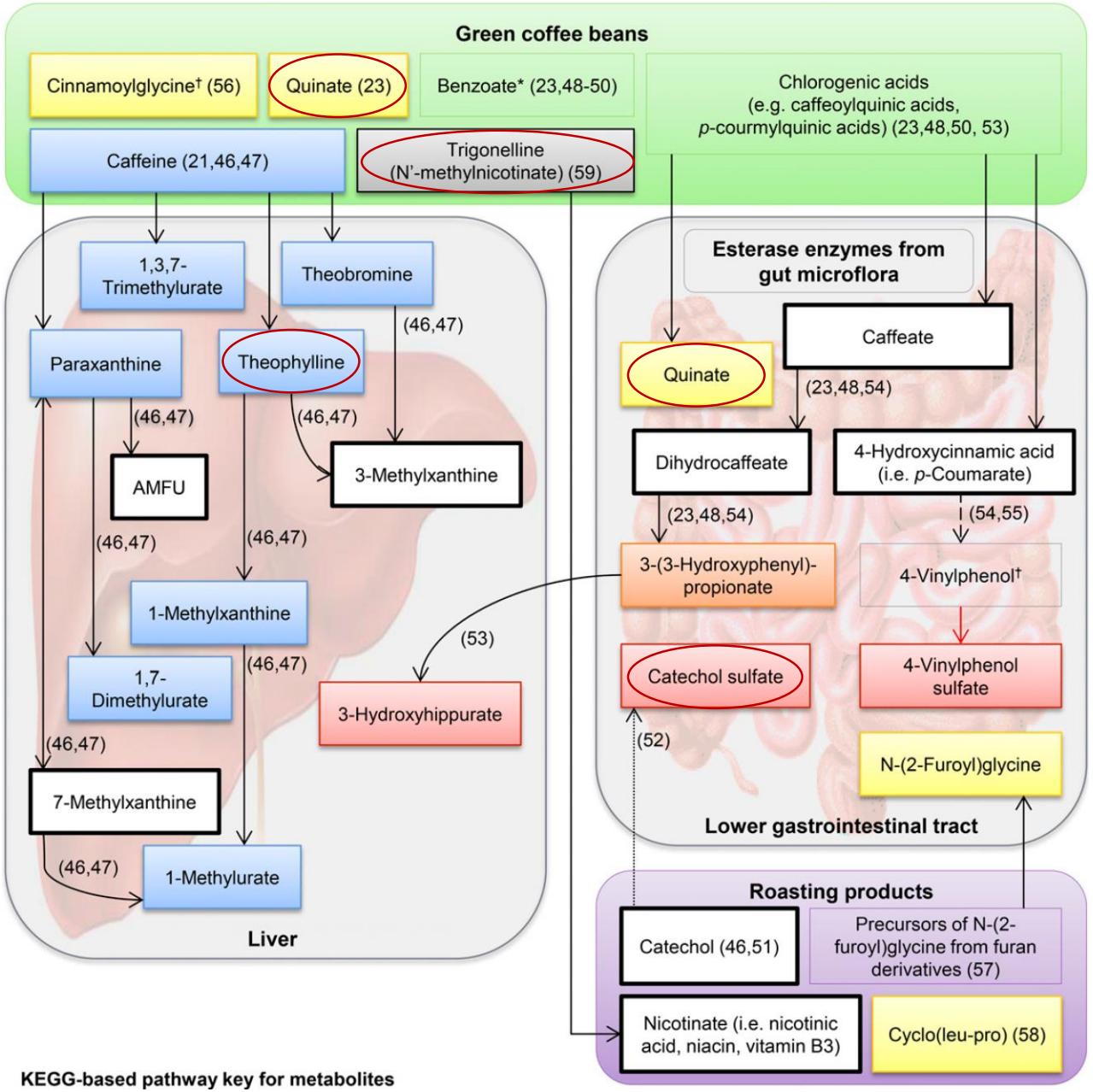
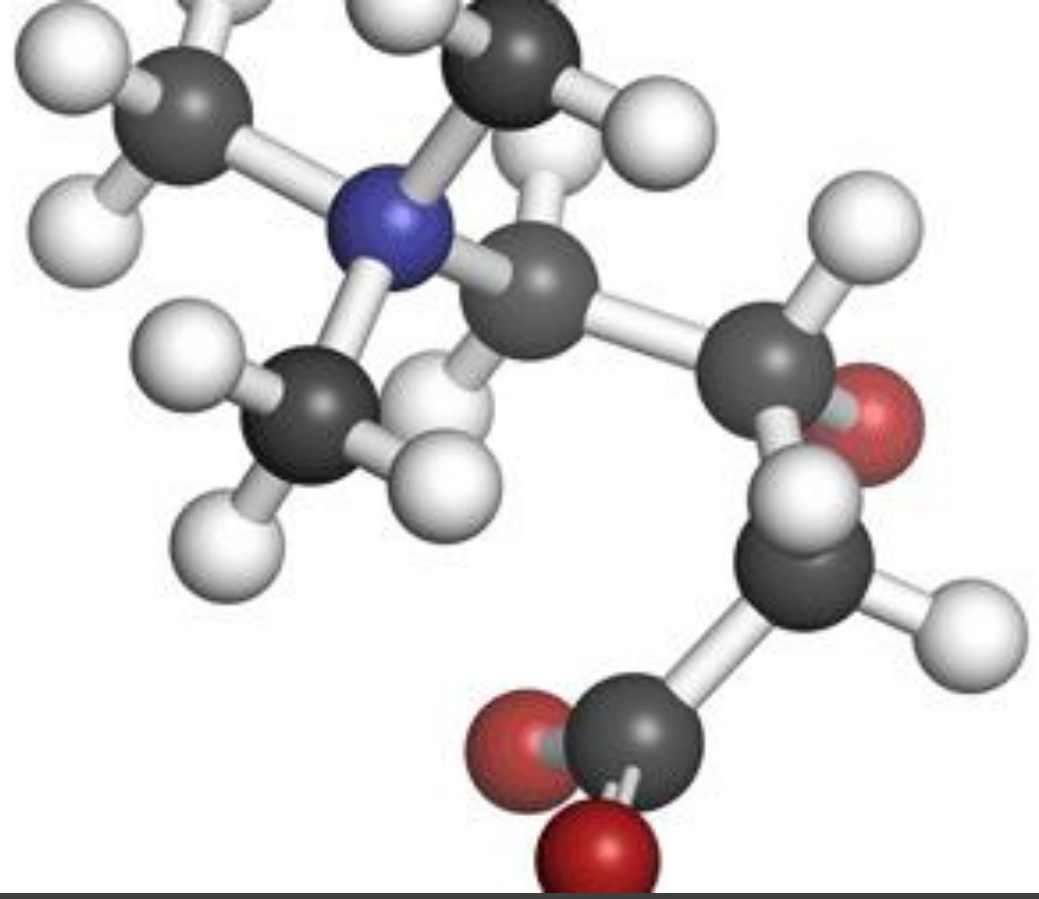




Multiple Metabolomes:

Child Metabolomes (age 1&3) and Vitamin D

Child Serum Vitamin D & the Plasma Metabolomes (Age 1, n=451 & Age 3, n=407)

Age 1

Metabolite	Super-pathway	Beta	P-value
docosadienoate (22:2n6)	Lipid	-2.69	4.44E-08
ergothioneine	Xenobiotics	-2.53	1.91E-06
docosapentaenoate (n3 DPA; 22:5n3)	Lipid	-2.43	2.01E-06
docosatrienoate (22:3n3)	Lipid	-2.37	2.40E-06
1,5-anhydroglucitol (1,5-AG)	Carbohydrate	-2.36	2.69E-06
sphingomyelin (d18:2/24:2)*	Lipid	-2.22	6.98E-06
docosapentaenoate (n6 DPA; 22:5n6)	Lipid	-2.29	7.73E-06
dihomo-linoleate (20:2n6)	Lipid	-2.17	1.17E-05
erucate (22:1n9)	Lipid	-2.13	1.37E-05
4-hydroxychlorothalonil	Xenobiotics	-2.40	2.78E-05

Age 3

Metabolite	Super-pathway	Beta	P-value
gamma-glutamylglycine	Peptide	-2.12	1.72E-07
gamma-glutamylisoleucine*	Peptide	-1.82	5.49E-06
alpha-hydroxycaproate	Lipid	-1.79	8.00E-06
gamma-glutamylglutamate	Peptide	-1.79	8.54E-06
pantothenate	Cofactors Vitamins	1.85	1.21E-05
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	Lipid	-1.72	1.96E-05
gamma-glutamylhistidine	Peptide	-1.71	2.17E-05
erythronate*	Carbohydrate	-1.69	2.28E-05
glycerophosphoethanolamine	Lipid	-1.68	2.32E-05
campesterol	Lipid	-1.74	2.57E-05

Overlap of Age 1 & Age 3: n-6 PUFA metabolites

- Results for n-6 PUFA metabolites in our data set

Metabolite	Age 1 result		Age 3 result	
	Estimated effect	P-value	Estimated effect	P-value
linoleate (18:2n6)	-1.67	6.60×10^{-4}	-1.66	4.86×10^{-5}
arachidonate (20:4n6)	-1.79	2.91×10^{-4}	-1.42	4.36×10^{-4}
docosapentaenoate (n6 DPA; 22:5n6)	-2.29	7.73×10^{-6}	-1.47	3.38×10^{-4}
linolenate (α or γ; 18:3n3 or n6)	-1.35	6.15×10^{-3}	-1.55	1.48×10^{-4}
dihomo-linolenate (20:3n3 or n6)	-2.12	4.35×10^{-5}	-1.30	1.54×10^{-3}

ENT80 cutoff: 6.93×10^{-4} in age 1 analysis and 7.89×10^{-4} in age 3 analysis

Replication in independent asthma cohort: CAMP (n=561)

n-6 PUFAs

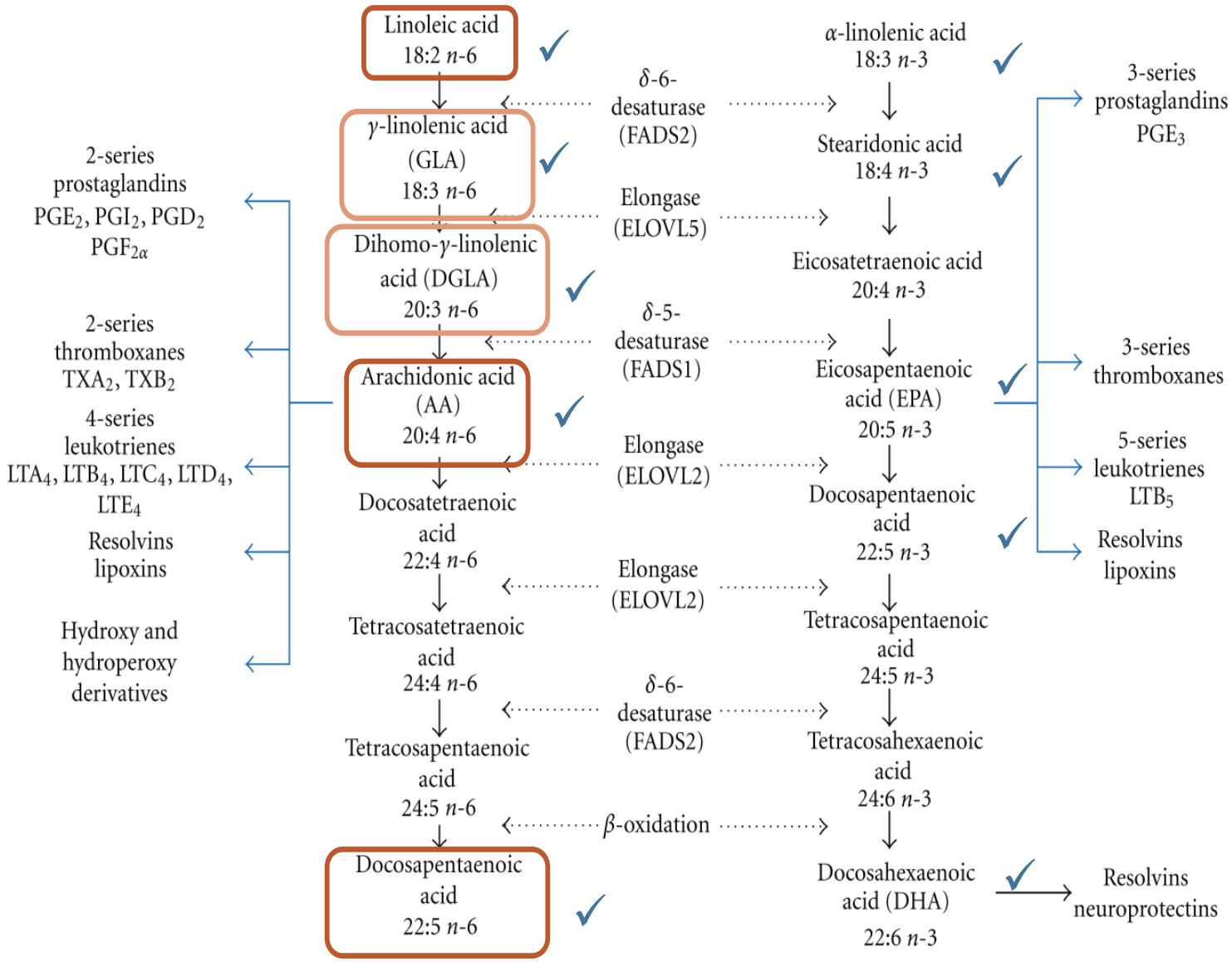
End of trial results	Metabolite	Estimated effect	P-value
n-6 PUFAs	linoleate	-2.45	3.82×10^{-5}
	γ-linolenate	-2.37	9.51×10^{-5}
	docosapentaenoate (DPA)	-2.11	4.60×10^{-4}
	arachidonate	-2.01	1.24×10^{-3}

* Bonferroni correction cutoff: $0.05/501 = 9.98 \times 10^{-5}$

† ENT80 cutoff: 1.42×10^{-3} in baseline analysis and 1.19×10^{-3} in end of trial analysis

Results Primary model: n-6 PUFAs

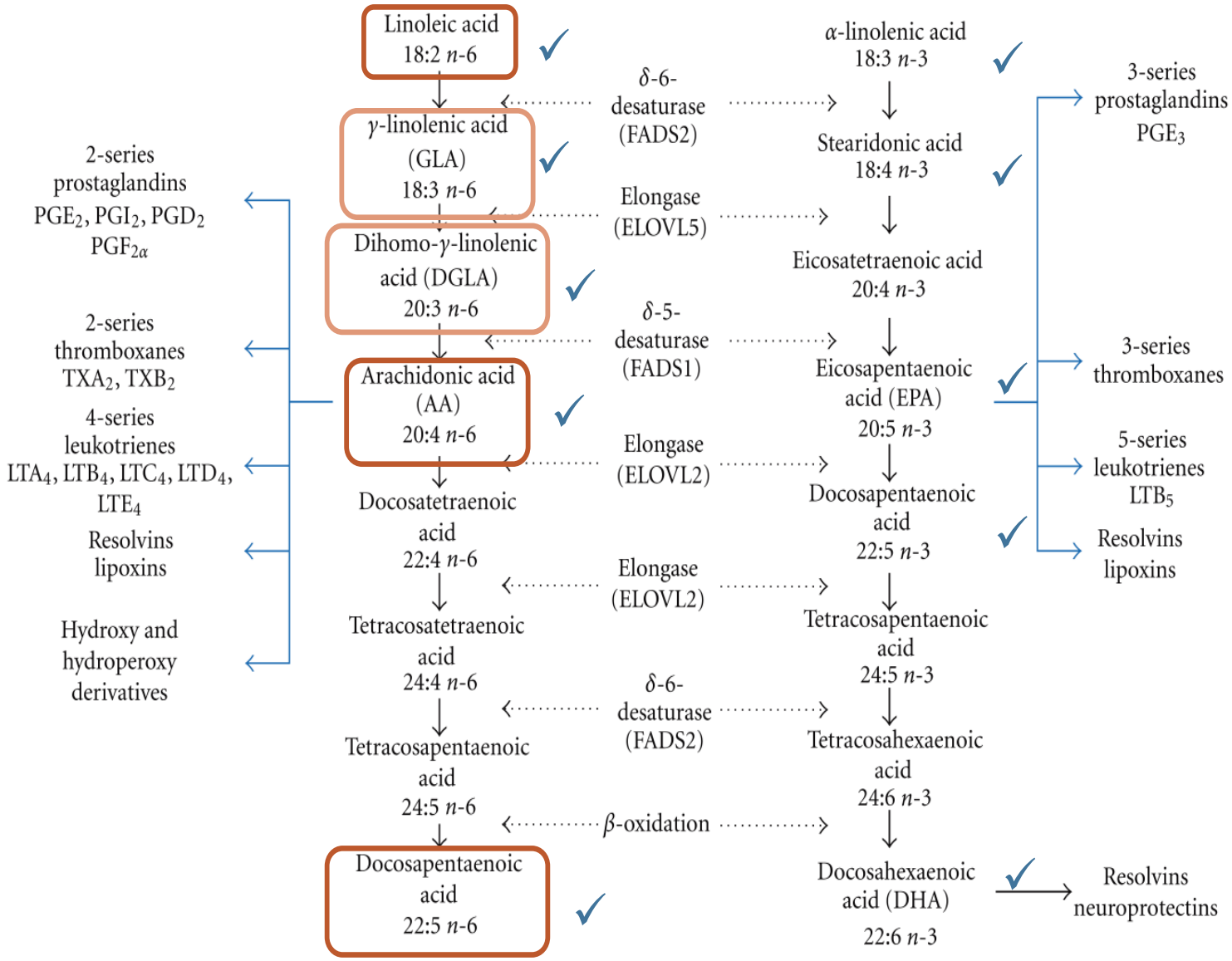
n-6 PUFA-derived eicosanoids have pro-inflammatory and bronchoconstriction effects



Schmitz, Ecker 2007 Progress in Lipid Research; Patterson et al. 2012 Journal of Nutrition and Metabolism

Results Primary model: n-6 PUFAs

Western Lifestyle: Indoors, Sunscreen Dietary Patterns



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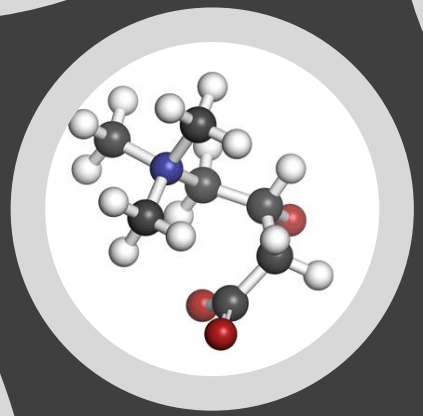


Metabolome vs. FFQ, Exposure:

ASTHMA & ALLEGRY

Metabolome and Nutrition ancillary VDAART analyses

J Allergy Clin Immunol Pract. 2018 Aug 23. pii: S2213-2198(18)30515-4. doi: 10.1016/j.jaip.2018.07.039. [Epub ahead of print]

Dietary and plasma polyunsaturated fatty acids are inversely associated with asthma and atopy in early childhood.

Lee-Sarwar K[1], Kelly RS[2], Lasky-Su J[2], Kachroo P[2], Zeiger RS[3], O'Connor GT[4], Sandel MT[5], Bacharier LB[6], Beigelman A[6], Laranjo N[2], Gold DR[7], Weiss ST[2], Litonjua AA[8].

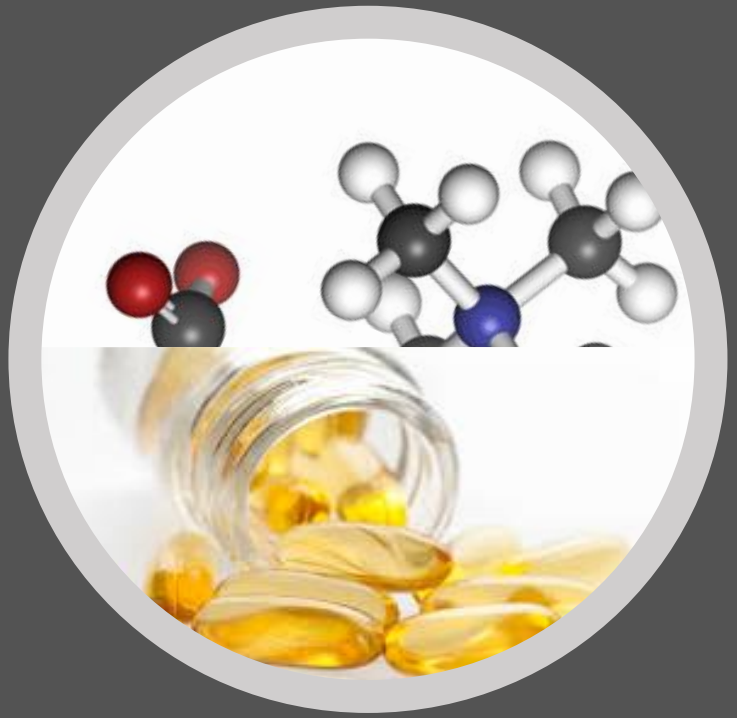
Plasma metabolomics

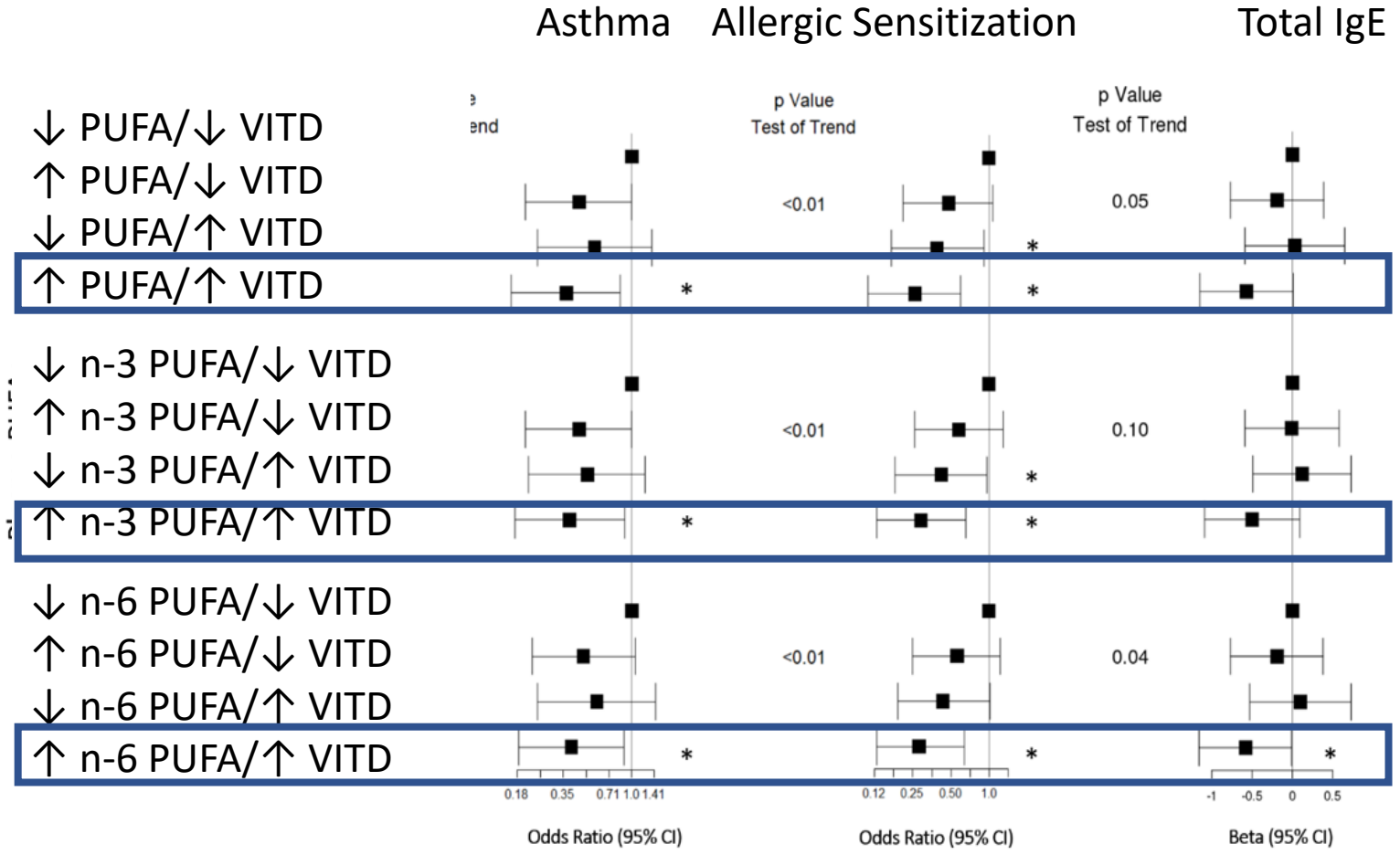
Dietary PUFA intake (FFQ)

Associations between PUFA and outcomes, including asthma and/or recurrent wheeze, allergic sensitization, and total IgE at age 3 years

Also evaluated the combined effects of antenatal vitamin D and early childhood PUFA on outcomes.

Joint Associations Plasma PUFA & Vitamin D





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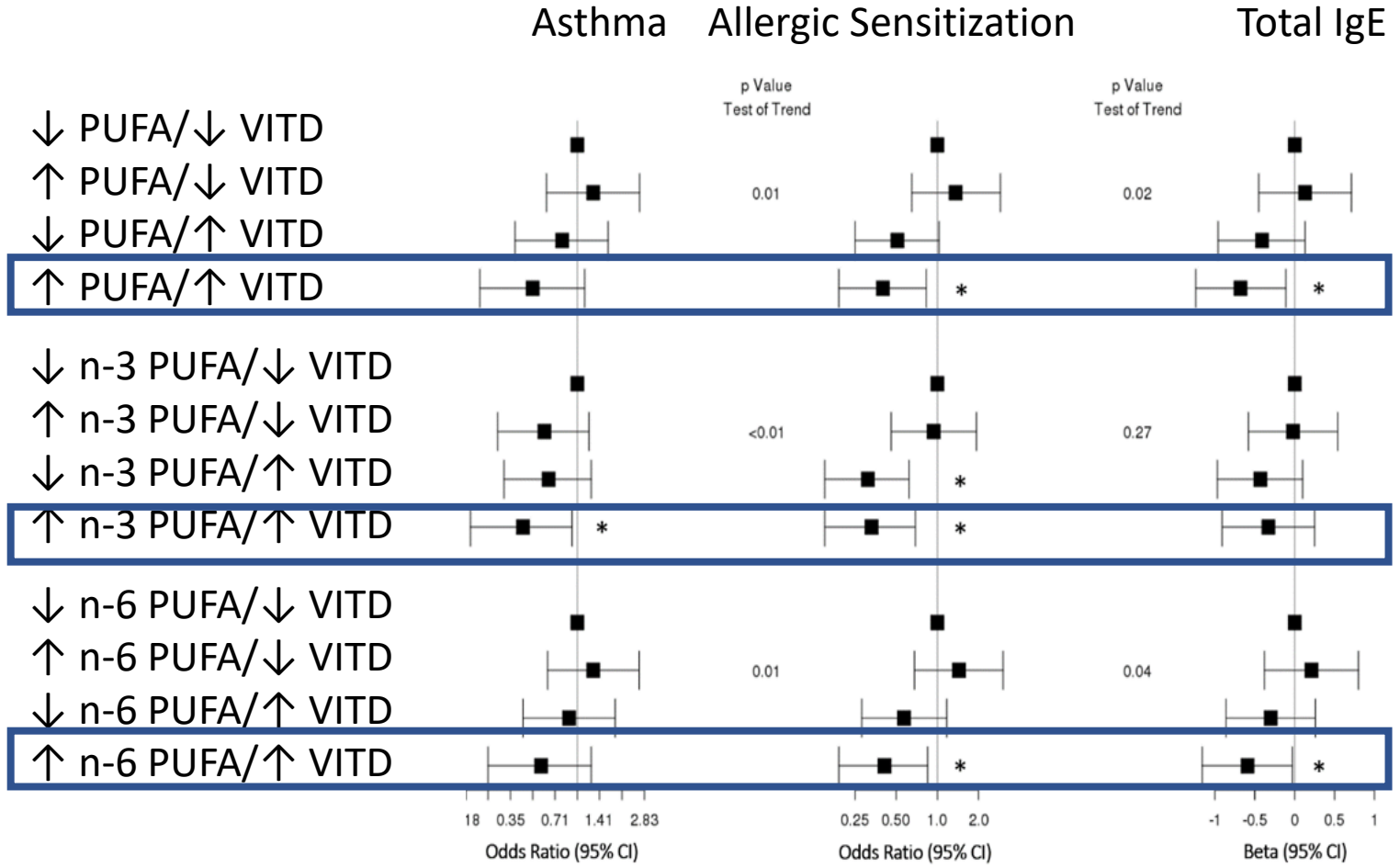
Joint Associations Dietary PUFA & Vitamin D



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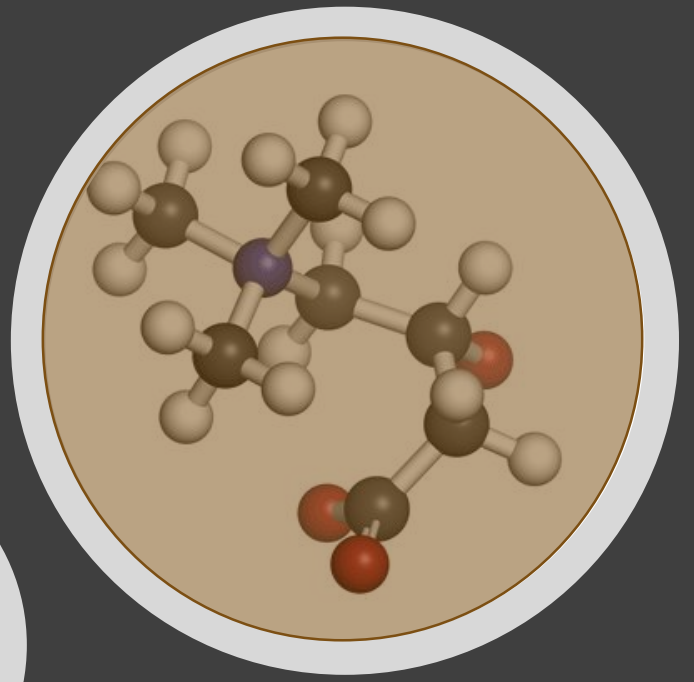
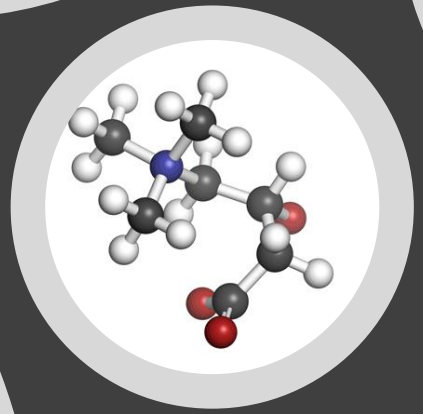
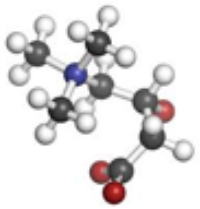
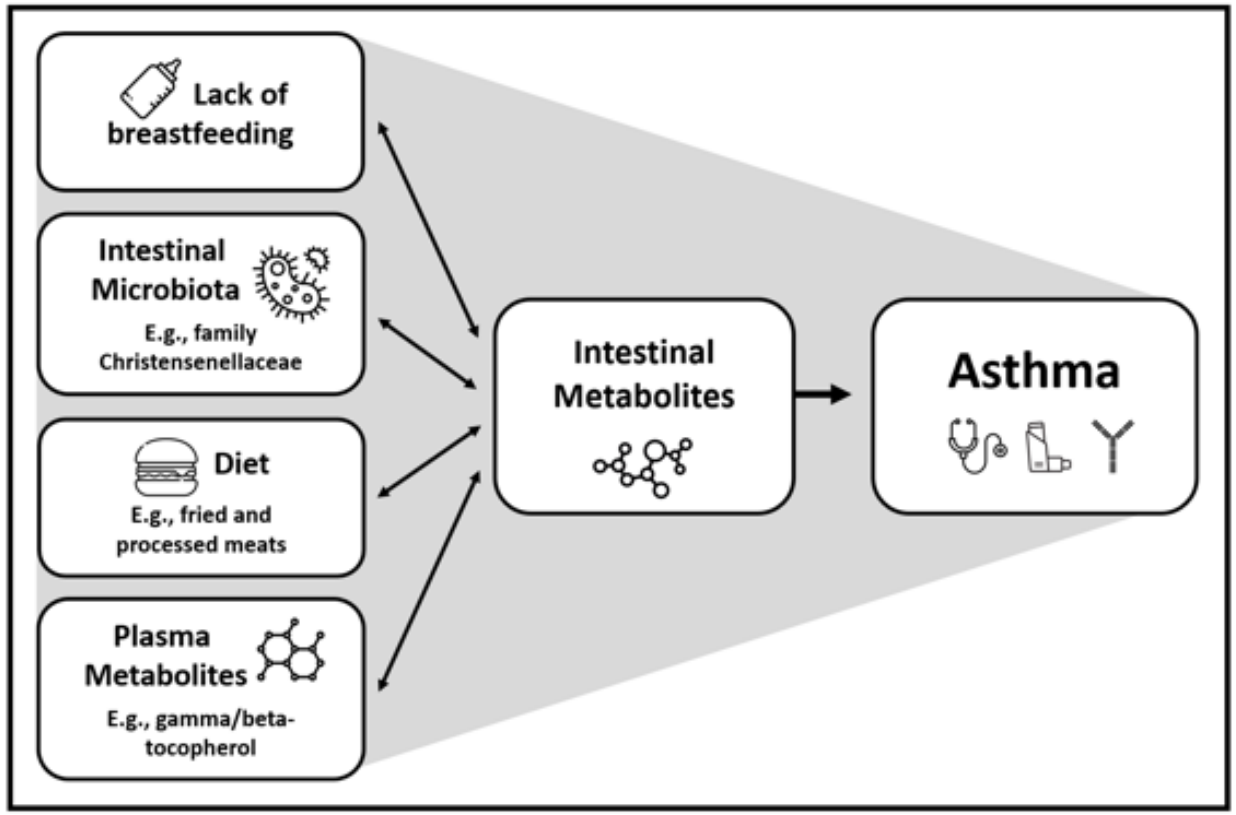
Lee-Sarwar K[1], Kelly RS[2], Lasky-Su J[2], Kachroo P[2], Zeiger RS[3], O'Connor GT[4], Sandel MT[5], Bacharier LB[6], Beigelman A[6], Laranjo N[2], Gold DR[7], Weiss ST[2], Litonjua AA[8].





Metabolomes, Gut Microbiome, Dietary intake: ASTHMA & ALLERGY



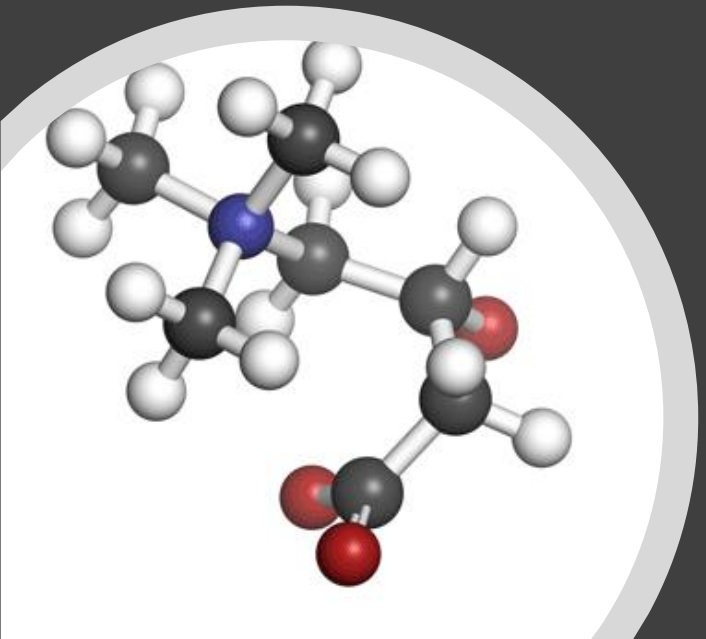


Integrative Analysis of the Intestinal Metabolome of Childhood Asthma

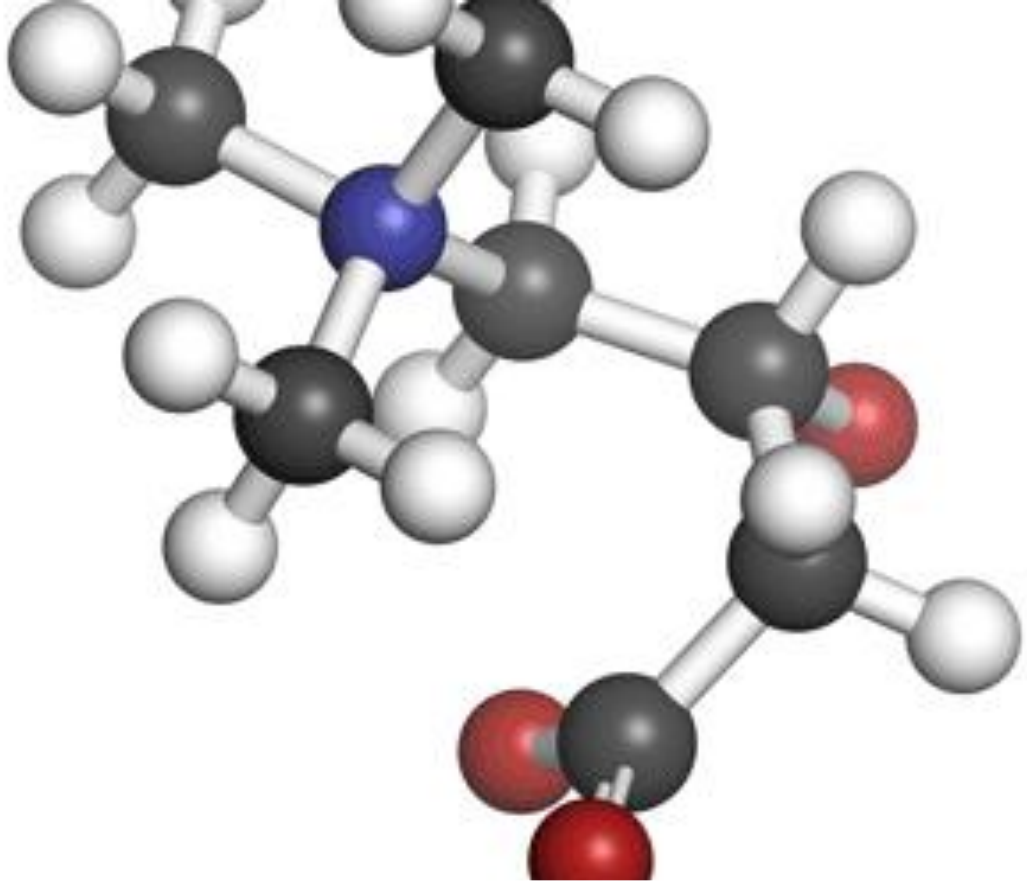
Integrative analyses revealed significant interrelationships between the intestinal metabolome and the *intestinal microbiome, plasma metabolome,* and *diet* in association with childhood asthma.

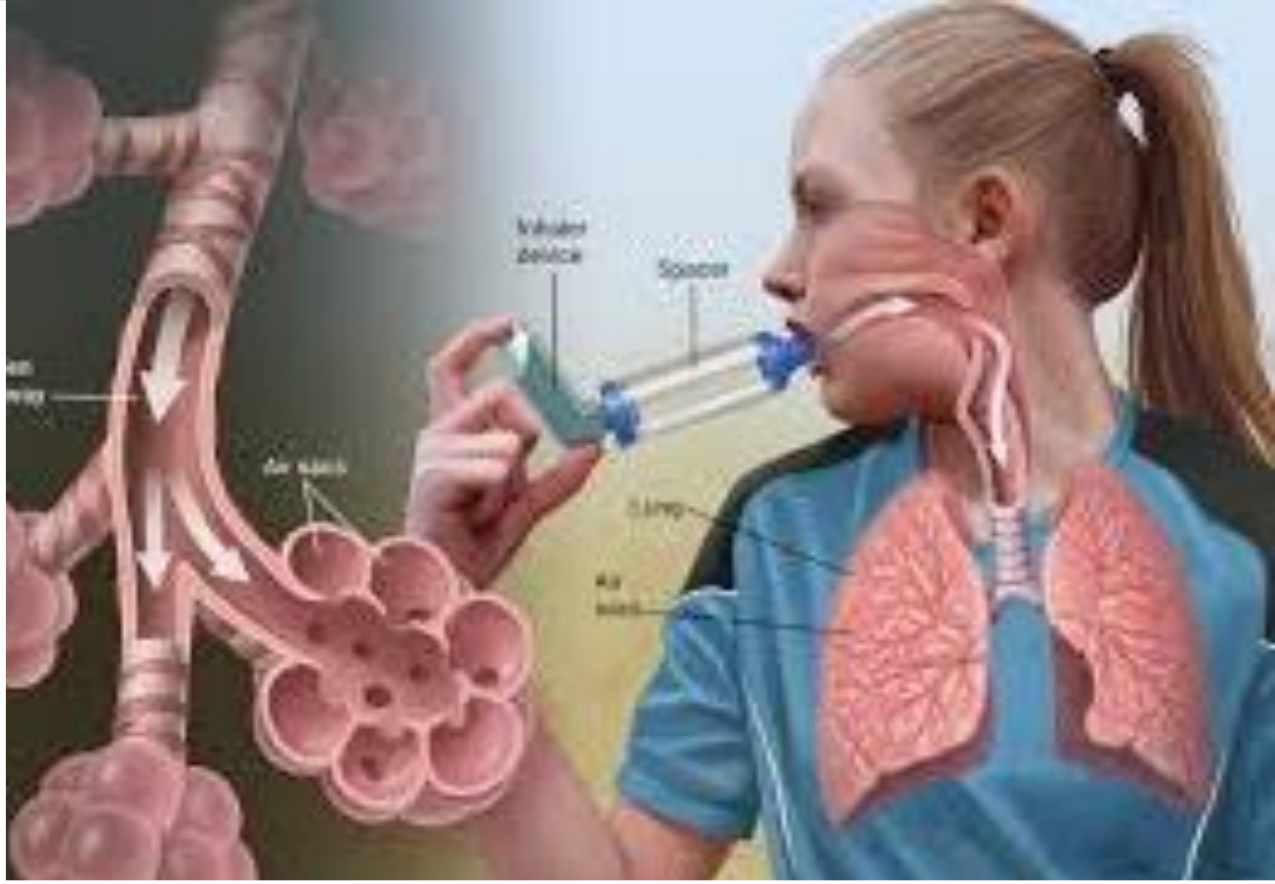
Copenhagen Prospective Studies on Asthma in Childhood (COPSAC)

- Analogous pre-birth cohort to VDAART
- Randomized mothers to Vitamin D
- Denmark, all Caucasian
- 517 Children
- Plasma metabolomics at 6 months

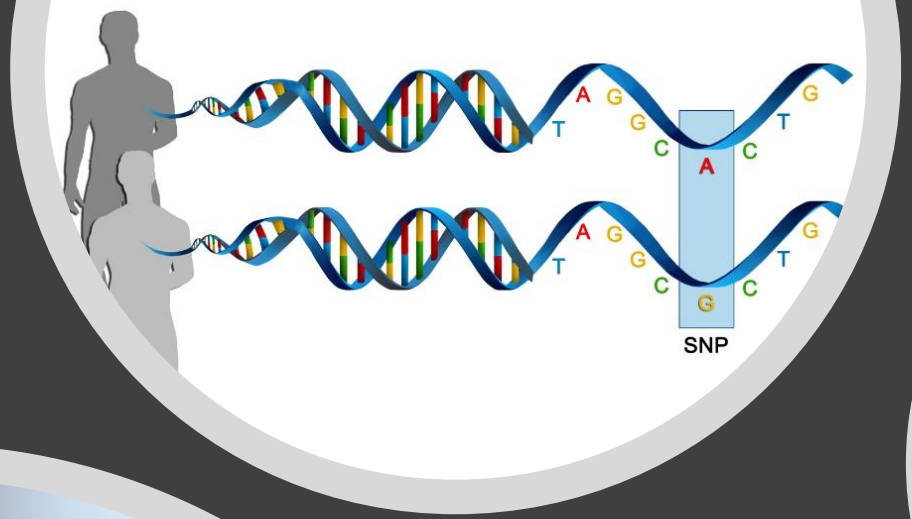


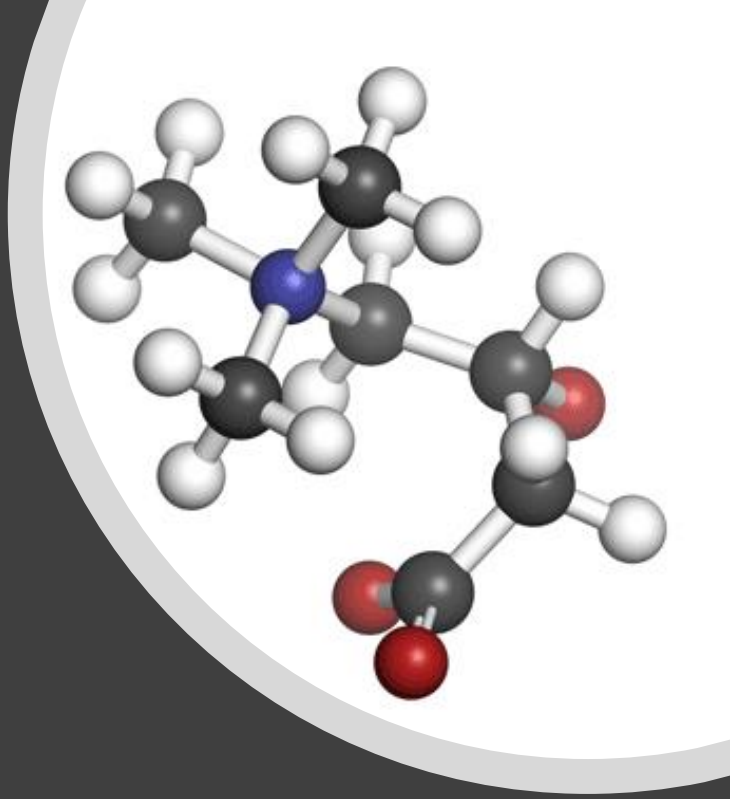
COPSAC





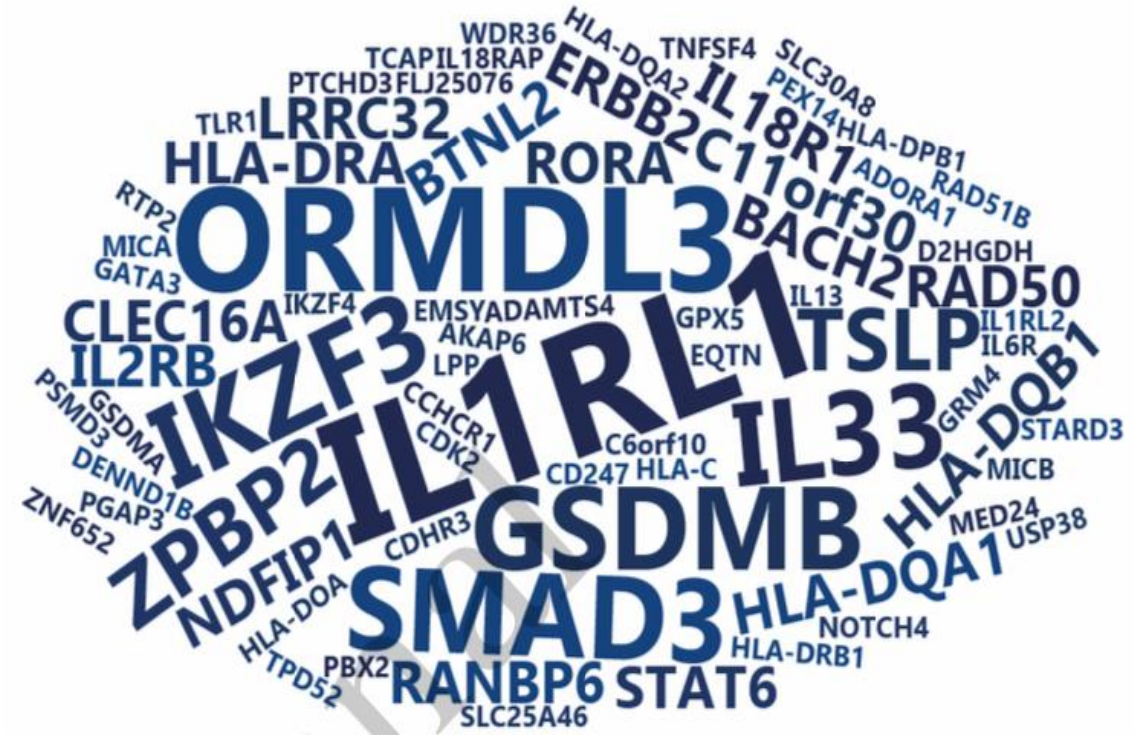
The Plasma Metabolome and Asthma Development by Age 3



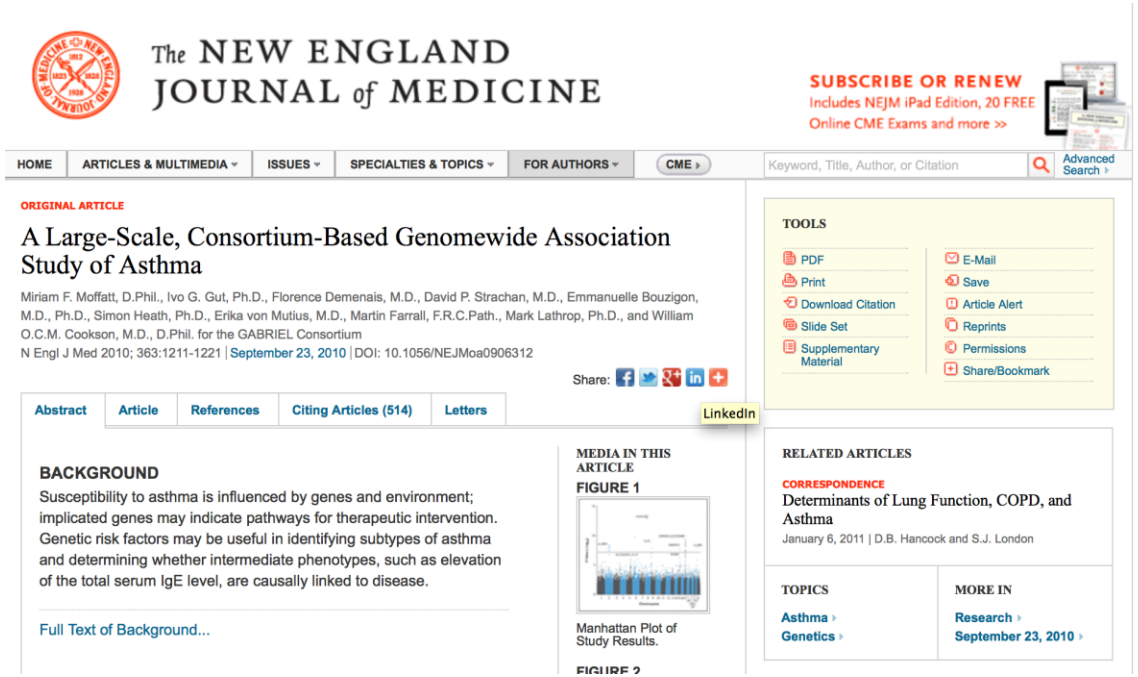


Do *Genetics* and the Metabolome play a role in the relationship between vitamin D and asthma?

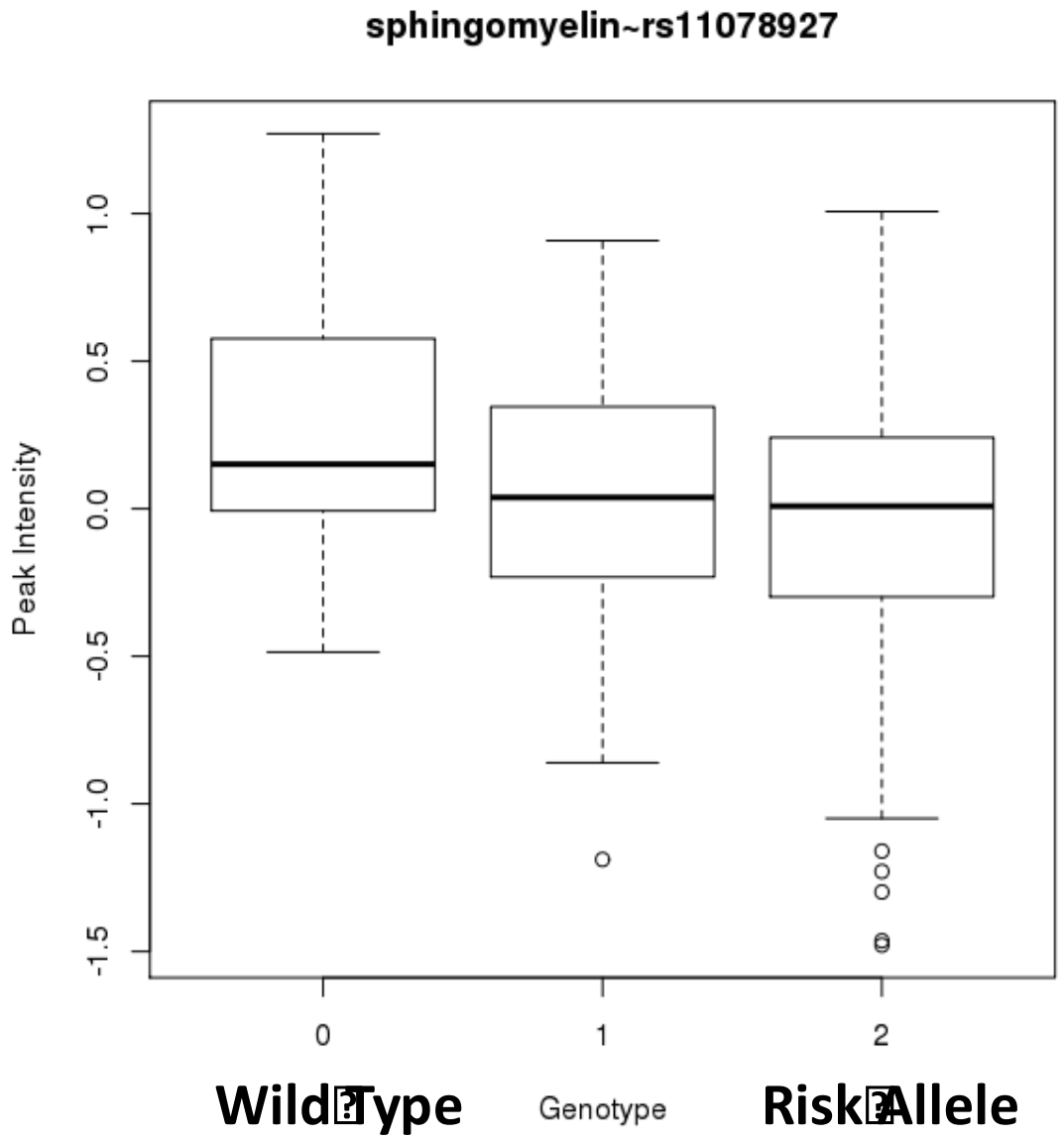
ORMDL sphingolipid biosynthesis regulator 3 (ORMDL3)
Located in the Sphingolipid Pathway



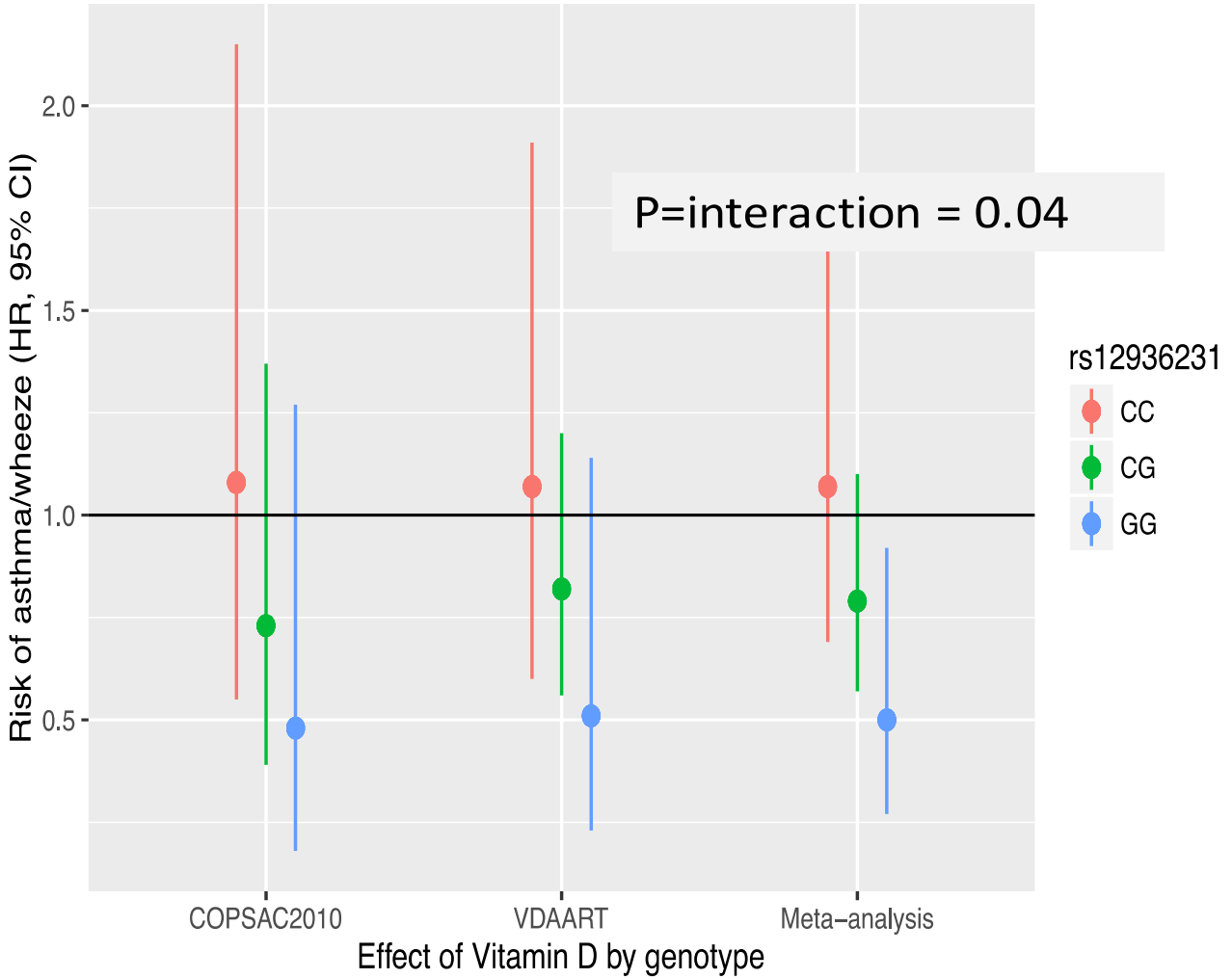
Kim & Ober Allergy Asthma Immunol Res. (2019)



Sphingomyelin levels according to *ORMDL3* genotype

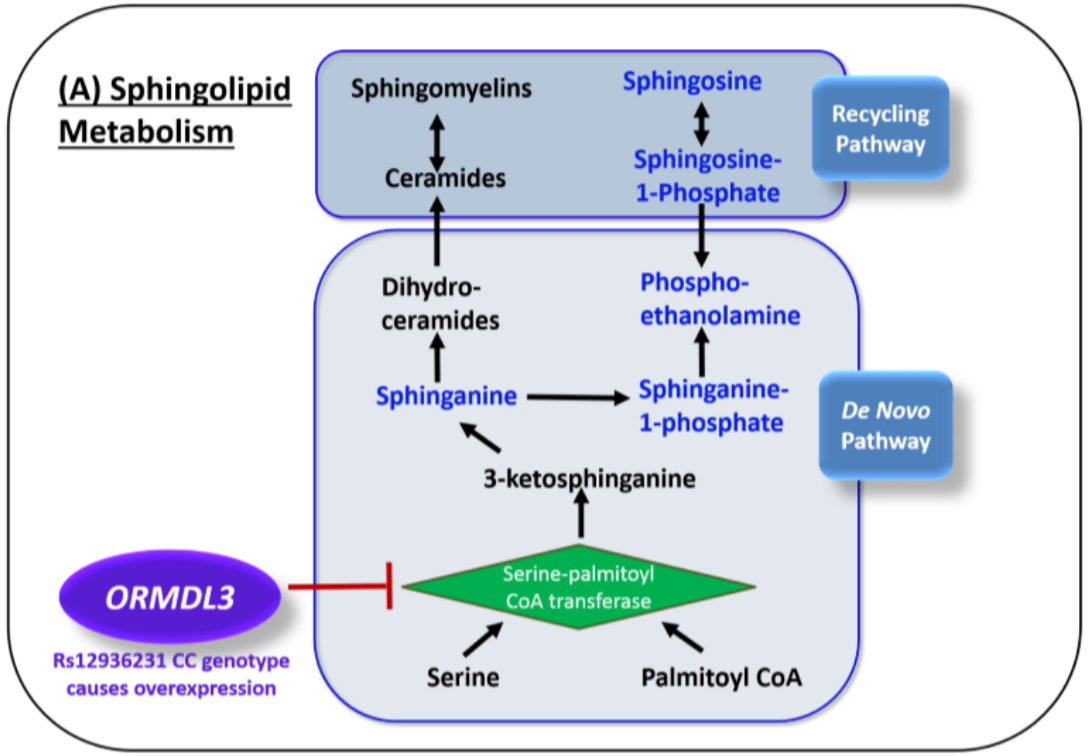


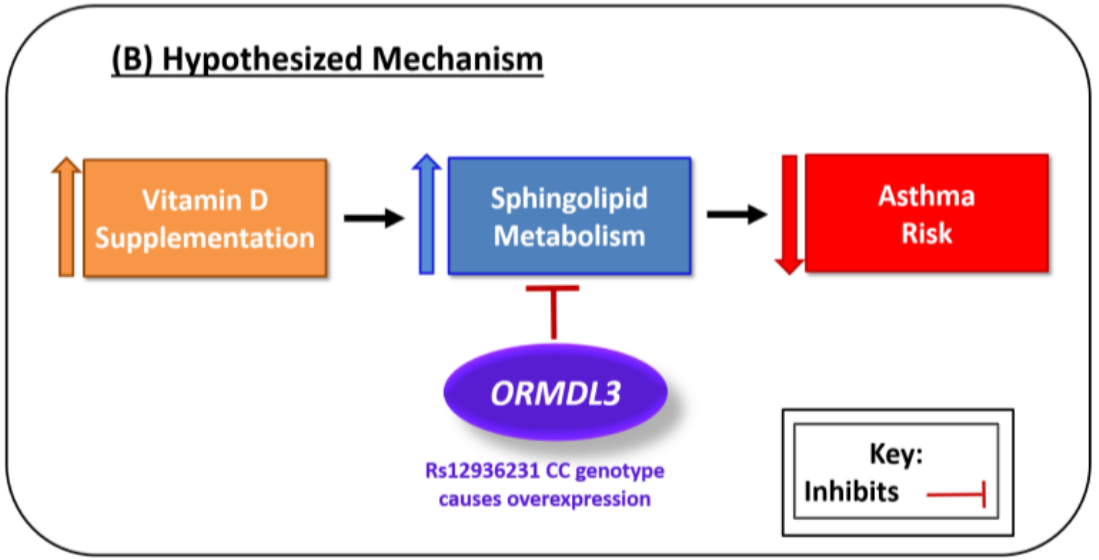
*ORMDL3**vitamin D interaction



ORMDL3 is a key regulator of Sphingolipid biosynthesis

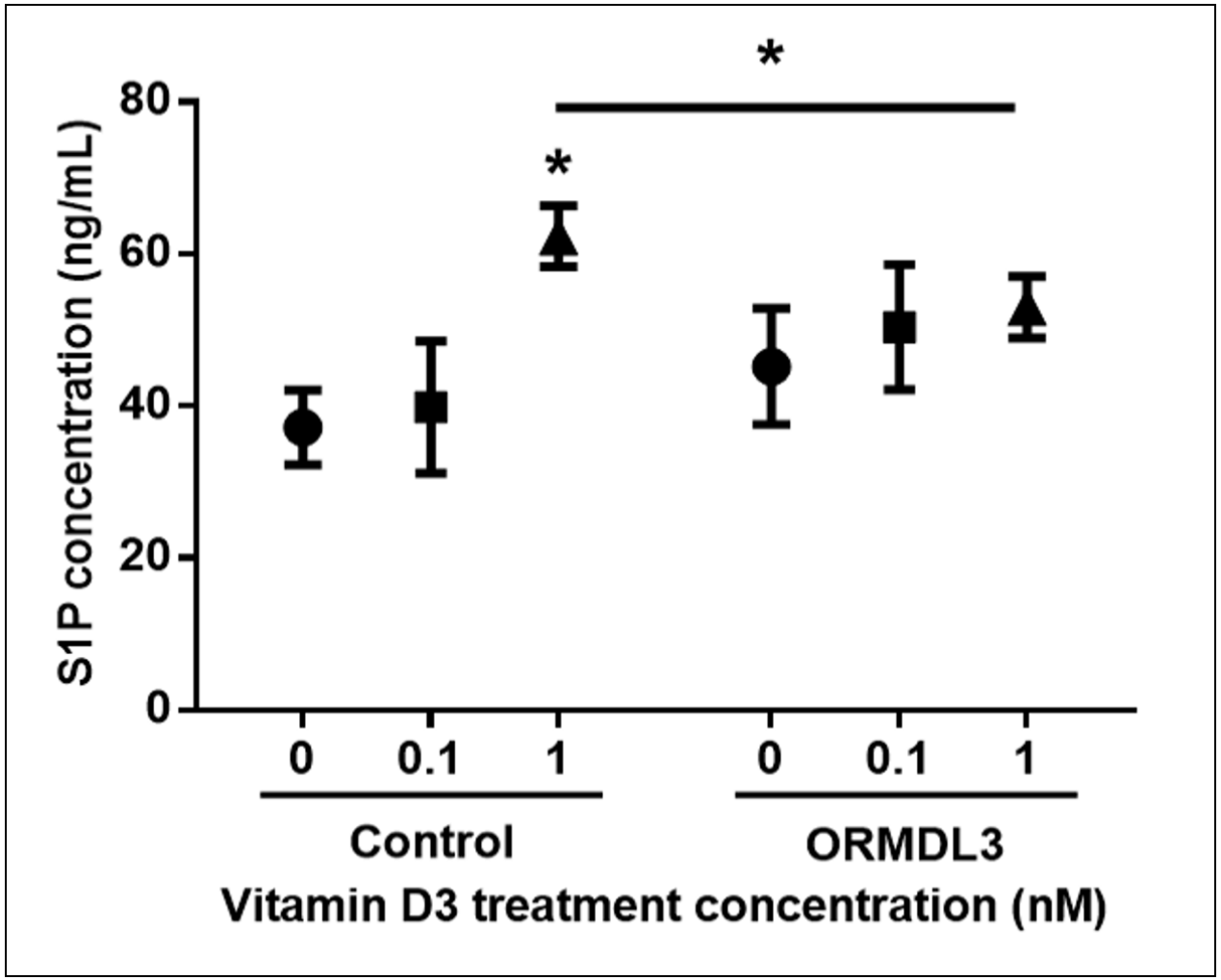
- The asthma associated C-allele increases expression of *ORMLD3*
- *ORMDL3* inhibits a key step in sphingolipid metabolism
- Vitamin D shown to activate sphingolipid metabolism
- Sphingolipid metabolism linked to airway hyper reactivity, inflammation and asthma





Human Airway Epithelial Cells

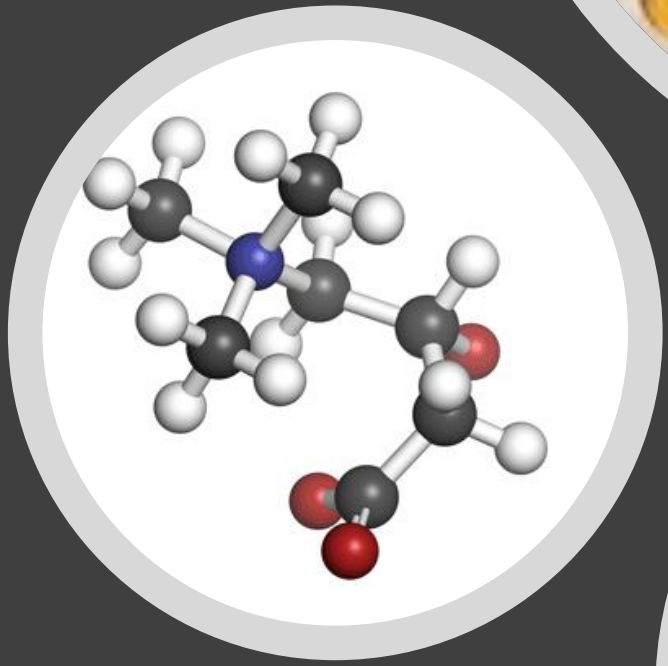
- Vitamin D increases Sphingolipids and this effect is blocked by ORMDL3
- Overexpression of *ORMDL3* showed decreased production of S1P when treated with Vitamin D, relative to vector controls

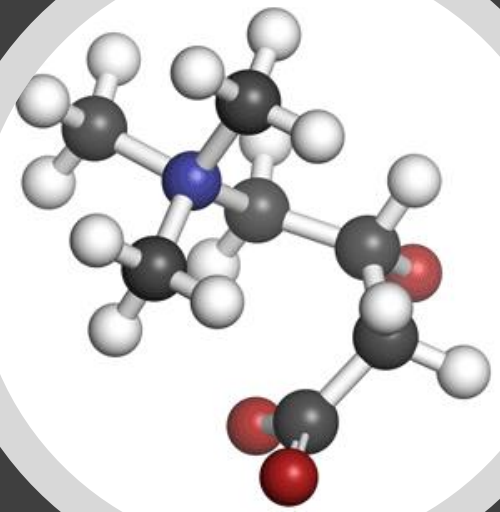


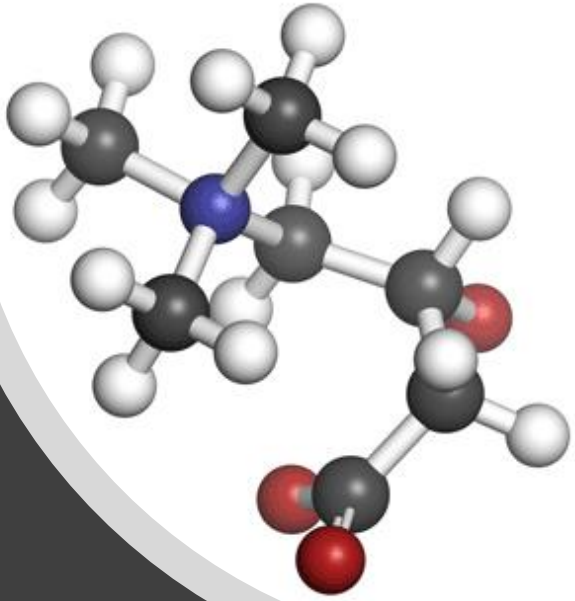
VDAART Metabolomics findings to date:

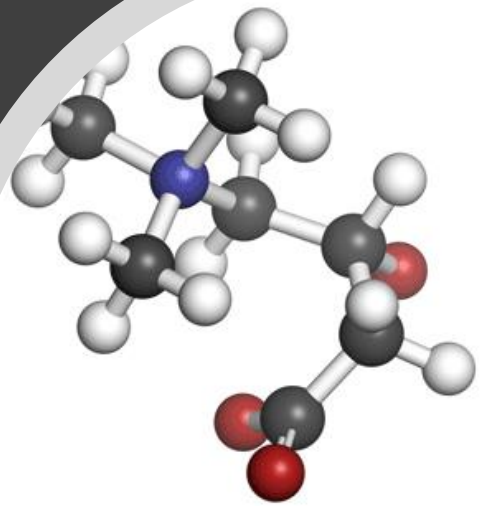
DISEASE PATHOGENSIS

- Key connector of other omics
- Importance of longitudinal measures
- Identify important exposures
- Replication







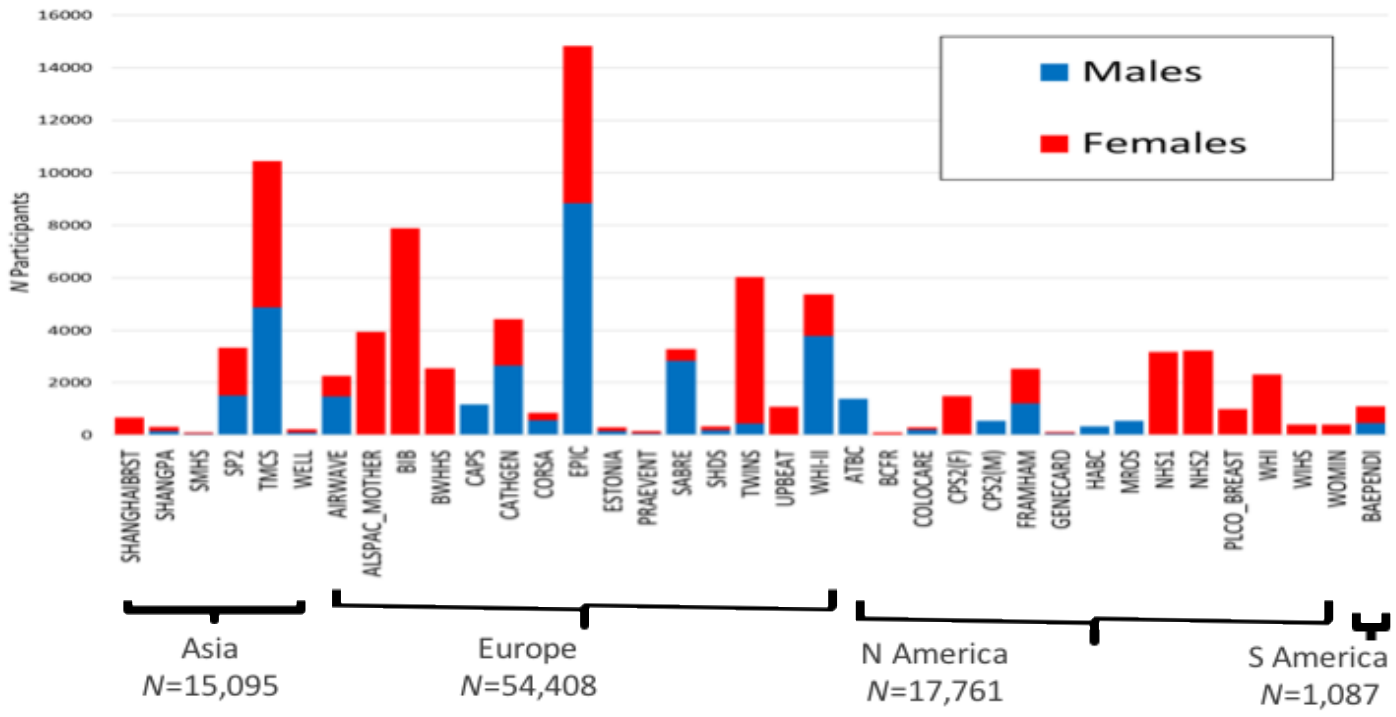


Large-Scale Metabolomics:

Multiple Metabolomes

Meta-Analysis of BMI in >88,000 individuals

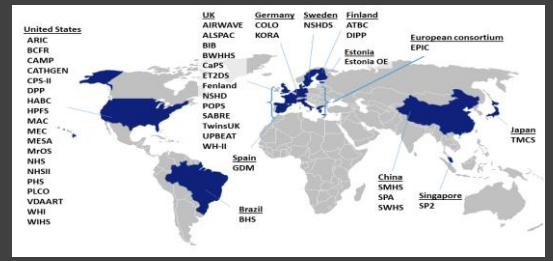
BMI Metabolomics Meta-Analysis



Multiple Metabolomes

COMETS

- Trans-NIH International Metabolomics Consortium
- 50+ prospective cohorts
- ~150,000 participants
- BMI Metabolomics Meta-Analysis using COMETs Analytics
  - 37 cohorts
  - 88,351 individuals



BMI metabolomic meta-analysis

COMETS Analytics Cycle and Features

Meta-Analyses
Centralized analyses of aggregated cohort data

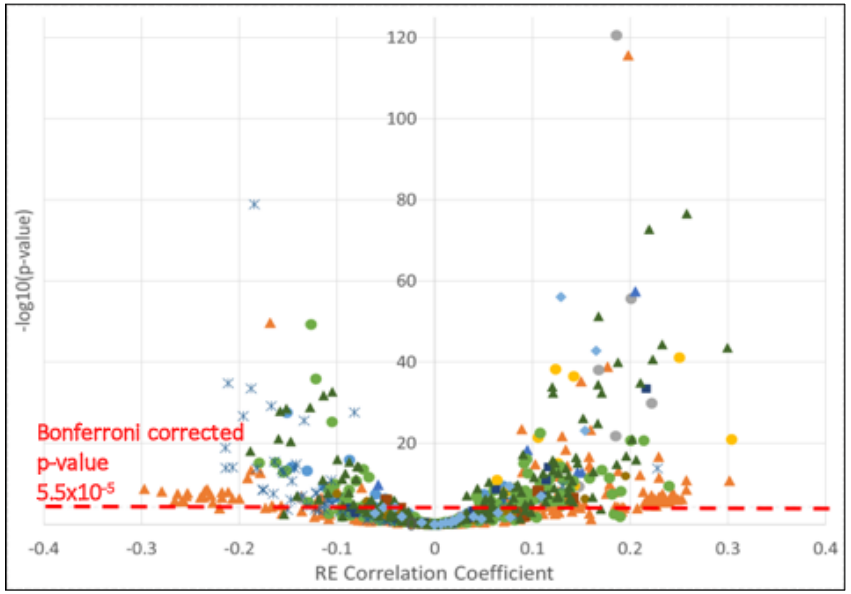
Metabolite Harmonization
Create mapping of metabolites across cohorts and platforms

Cohort-Specific Analyses
Conduct patient level analyses for data exploration, and approved, manuscript proposals

Data Preparation
Facilitate creation of common input file

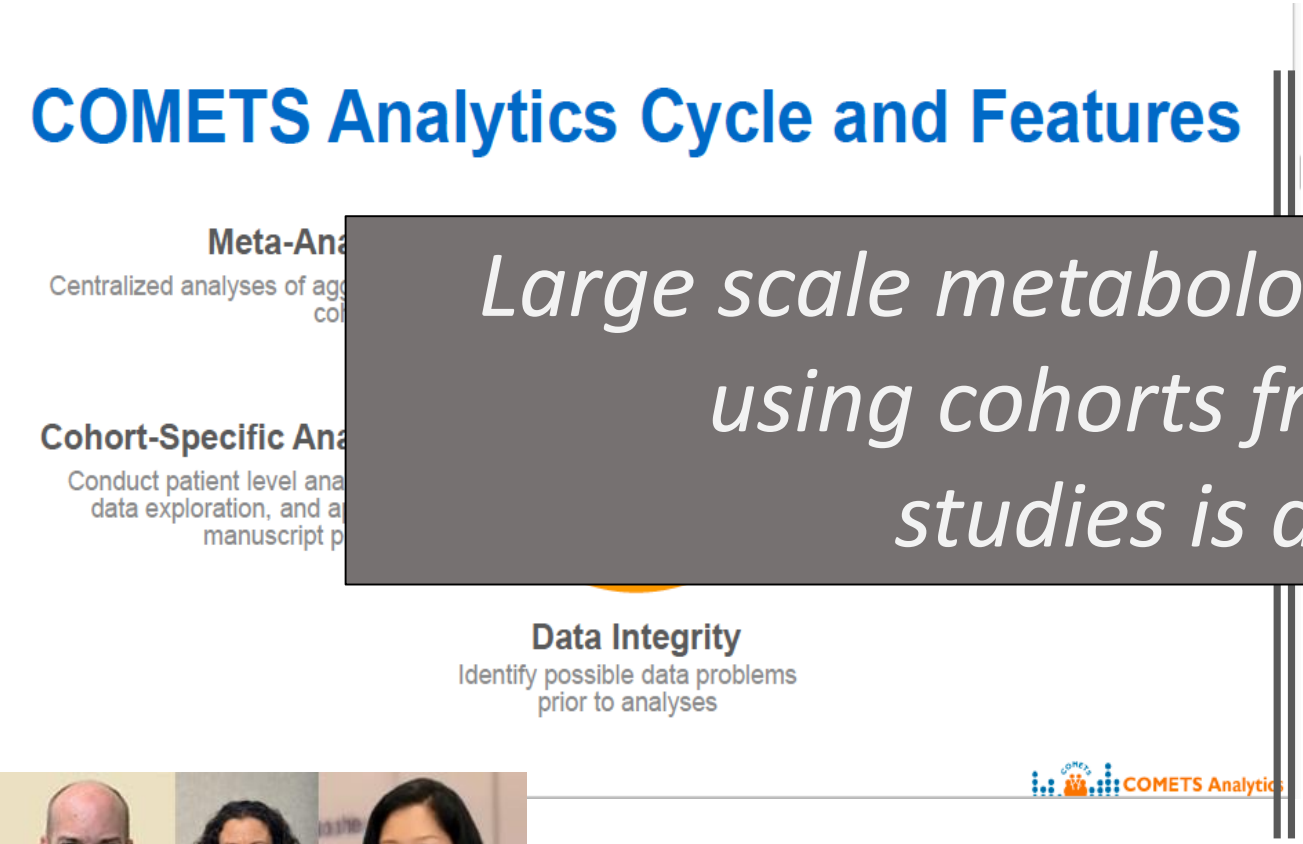
Data Integrity
Identify possible data problems prior to analyses

COMETS Analytics

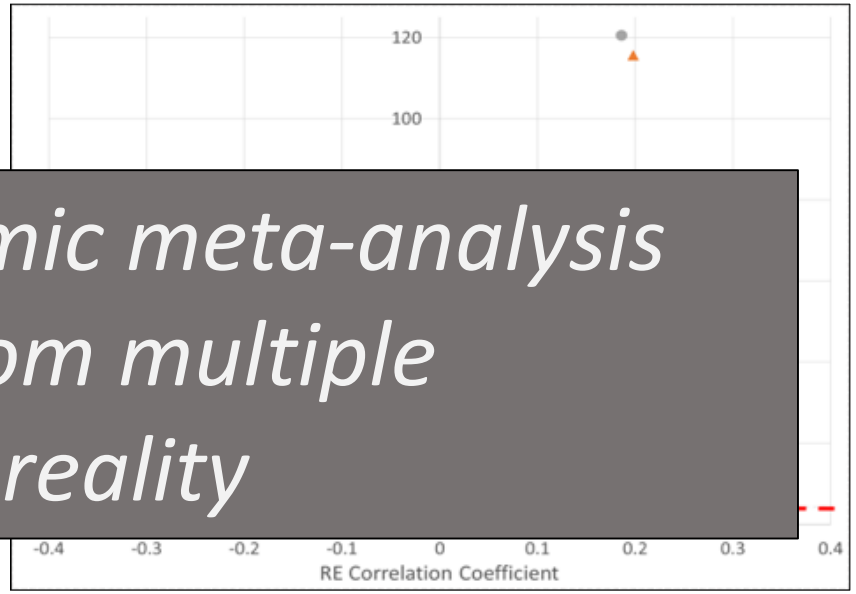


- Strongest most robust finding: Glutamate
- Based on 50,673 participants from 20 cohorts
- $P=2.9x10^{-44}$

BMI metabolomic meta-analysis

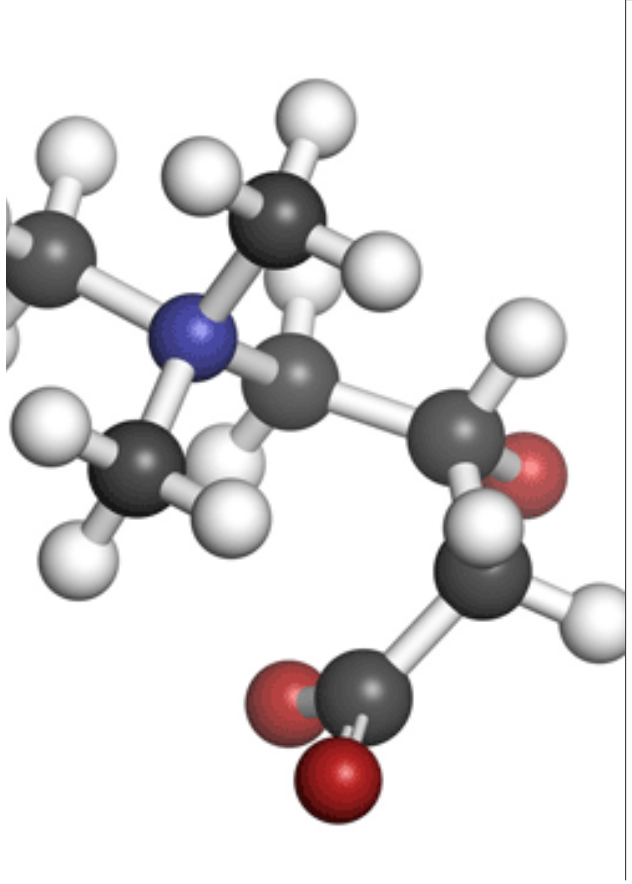


Large scale metabolomic meta-analysis using cohorts from multiple studies is a reality



- Strongest most robust finding: Glutamate
- Based on 50,673 participants from 20 cohorts
- P=2.9x10^{-60}






Metabolomics & Prevalent Asthma

Using 2 large population-based cohorts

Metabolomics & Prevalent Asthma

Using 2 large population-based cohorts, RCTs, further analyses in Partners biobank participants

To identify the metabolomic signatures of asthma in EPIC-Norfolk cohort

Validate/Replicate the findings in Partners HealthCare Biobank

Assess the impact of ICS use on the biological profile of asthma

Further Replication of top hits in a relevant clinical trial of ICS use: Childhood Asthma Management Program (CAMP)

Use the biobank for further assessment using EMRs

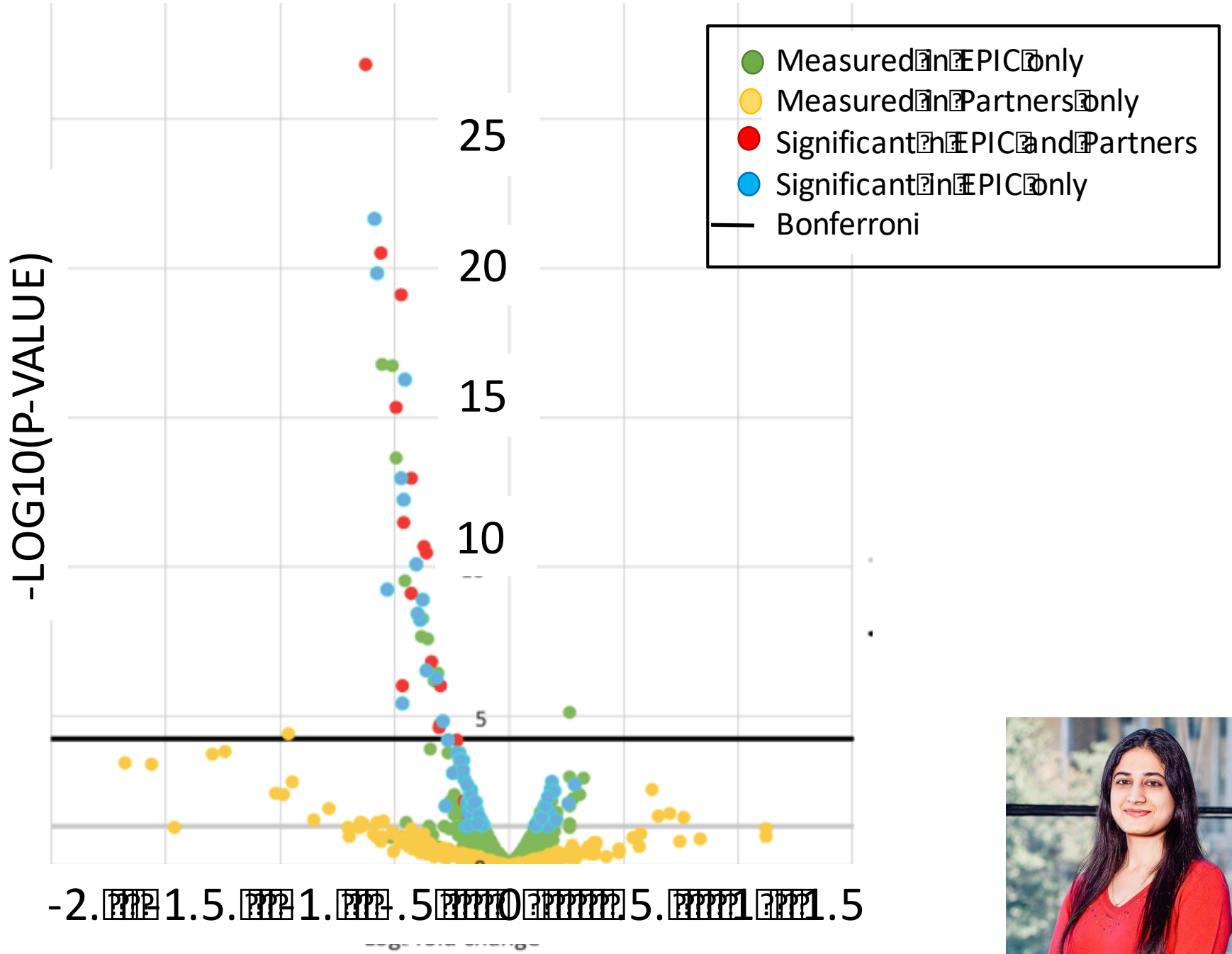
Aims/Objectives

Cohorts:
Asthma &Plasma Metabolomics

Discovery cohort EPIC-Norfolk (Day *et al., Br J Cancer*. 1999)		
Large multi-center, longitudinal cohort study: nutrition, lifestyle	10,754 Subjects 661 Cases 10,093 Controls	Caucasian, 53% smoke, 60 years of age

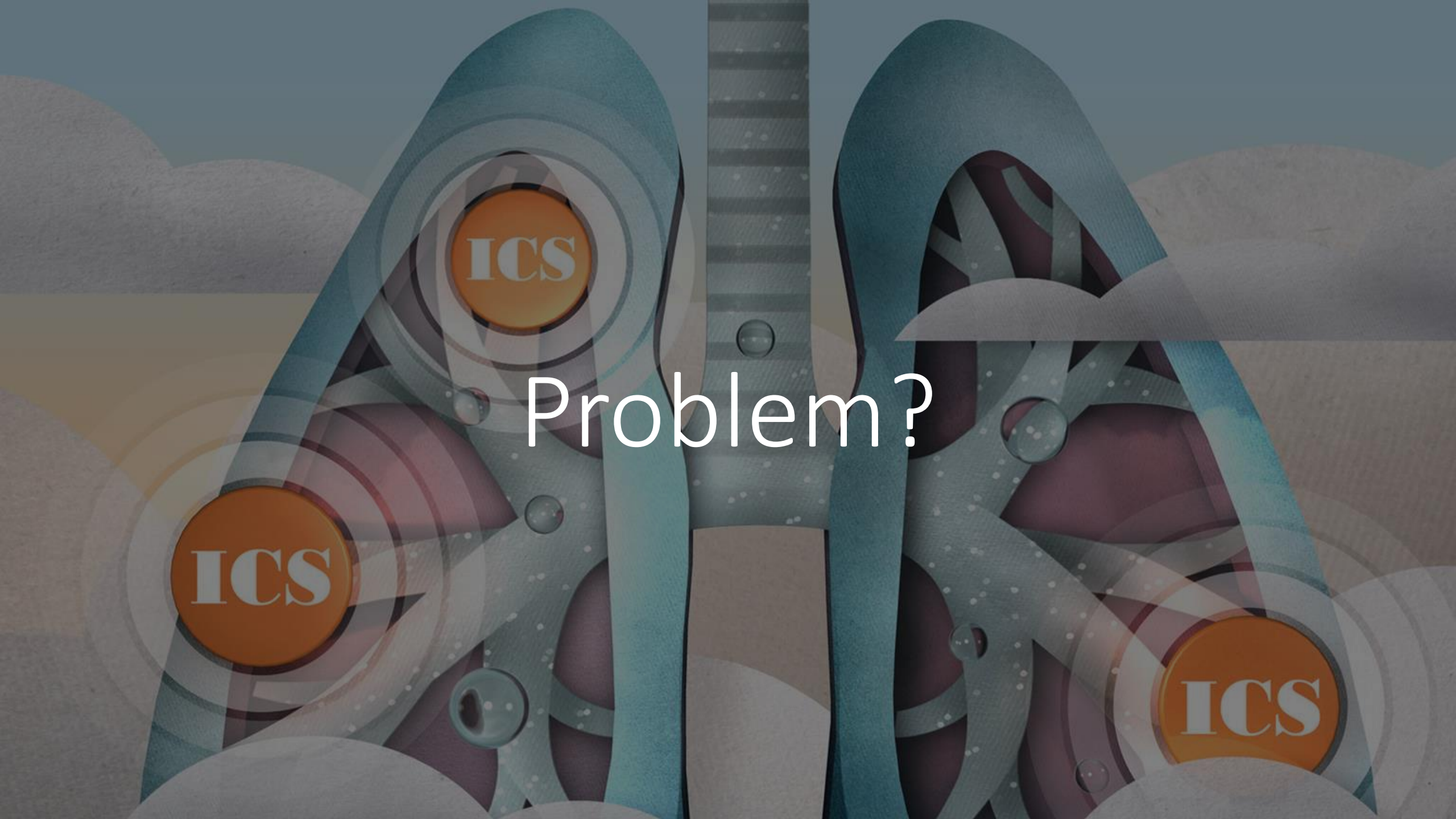
Replication cohort: Partners HealthCare Biobank		
Large bio-repository >100,000 consented patients linked to EMR Available Plasma, Serum, DNA	613 Subjects 295 Cases 318 Controls	77% Caucasian, 60% smoke, 30 years of age

Asthma in EPIC and Partners Biobank

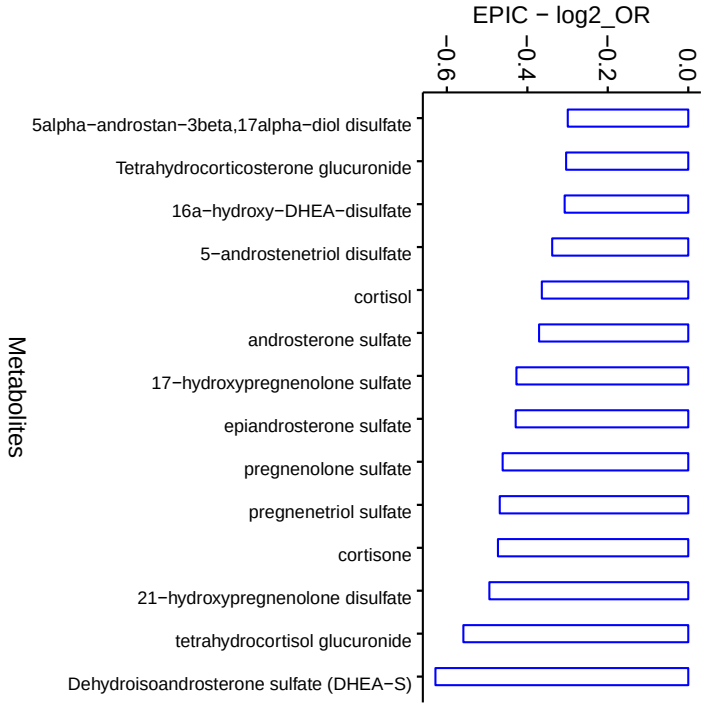


Top Association Findings

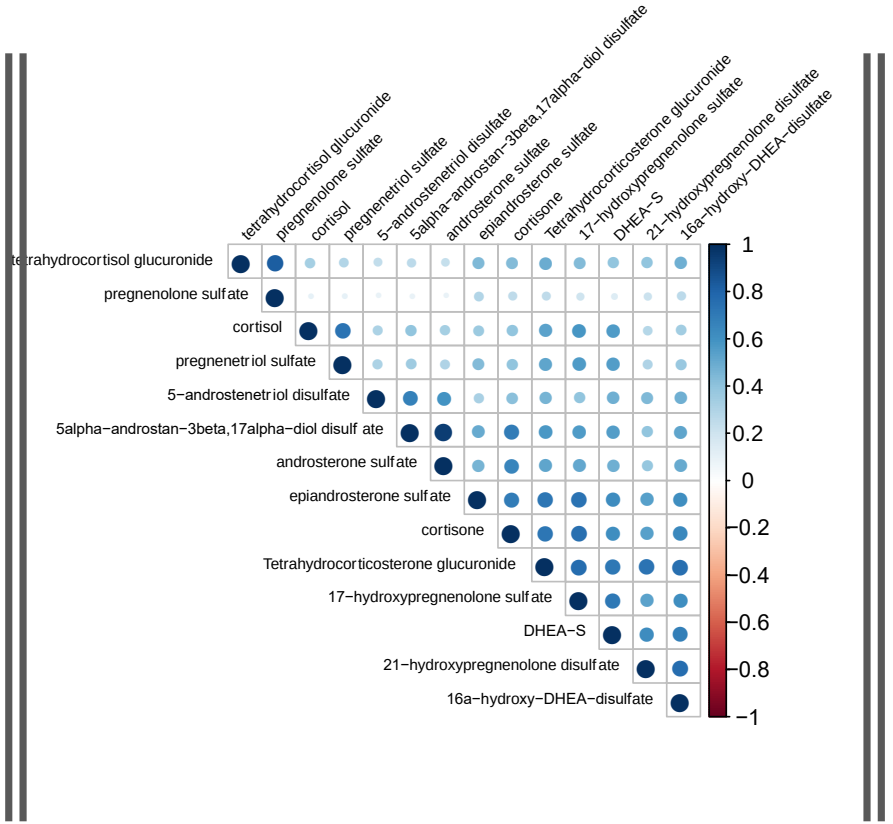
	Metabolite	EPIC-Norfolk (N=10,754)		Partners Healthcare Biobank (PHB) (N=613)	
		OR	*P*-value*	OR	*P*-value**
Androgenic Steroid	Dehydroisoandrosterone sulfate (DHEA-S)	0.65	1.4×10^{-27}	0.36	2.7×10^{-4}
	Epiandrosterone sulfate	0.74	1.1×10^{-13}	0.46	8.3×10^{-4}
	5alpha-androstan-3beta,17alpha-diol disulfate	0.81	1.8×10^{-5}	0.64	6.3×10^{-3}
	16a-hydroxy-DHEA-disulfate	0.81	2.5×10^{-5}	0.66	0.019
Cortico-steroid	Tetrahydrocortisol glucuronide	0.68	2.9×10^{-21}	0.66	0.025
	Cortisone	0.72	7.8×10^{-20}	0.30	3.0×10^{-5}
	Cortisol	0.78	3.3×10^{-11}	0.37	8.3×10^{-5}
	Tetrahydrocorticosterone glucuronide	0.81	9.9×10^{-7}	0.68	0.024
Pregnenolone Steroid	17-hydroxypregnenolone sulfate	0.74	7.4×10^{-10}	0.65	0.028
	21-hydroxypregnenolone disulfate	0.71	4.5×10^{-16}	0.58	9.3×10^{-3}
	Pregnenolone sulfate	0.73	3.2×10^{-12}	0.47	2.5×10^{-4}
	Pregnenetriol sulfate	0.72	1.0×10^{-6}	0.55	0.015
	5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	0.78	3.0×10^{-7}	0.71	0.047
Androstane Steroid	Androsterone sulfate	0.77	2.1×10^{-11}	0.48	2.0×10^{-3}
	5-androstenetriol disulfate	0.79	1.5×10^{-7}	0.66	0.036

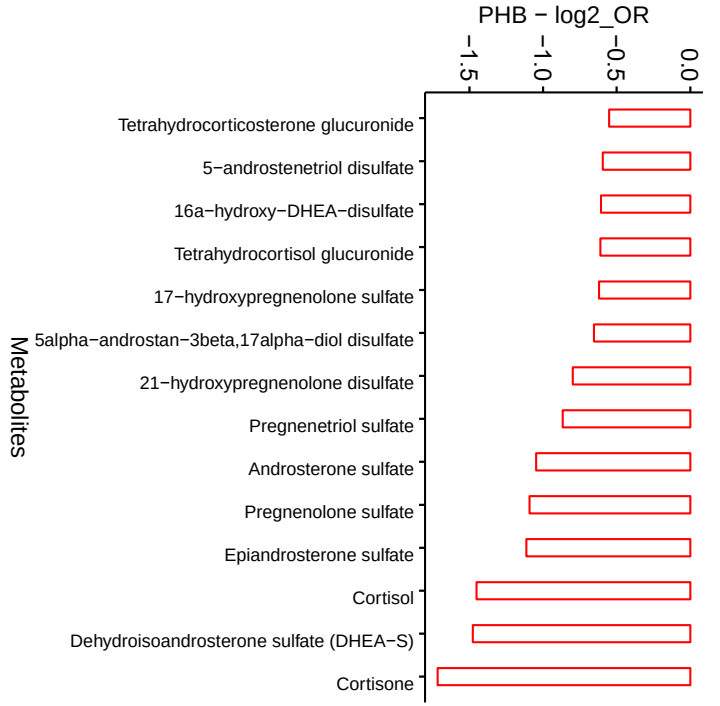


Problem?



EPIC

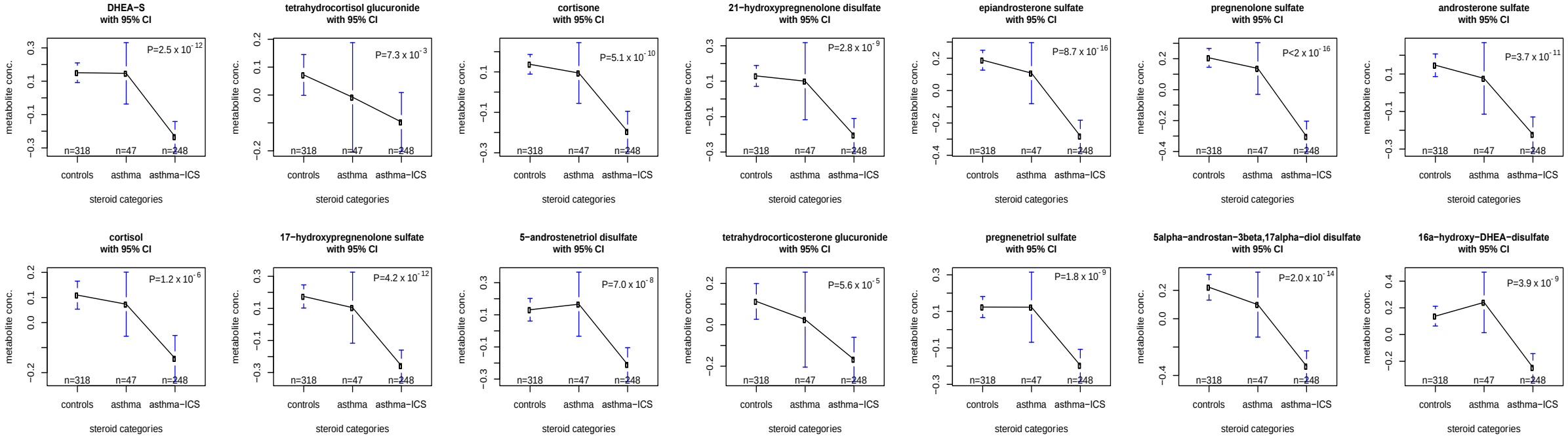




PHB

Direction of Effect?

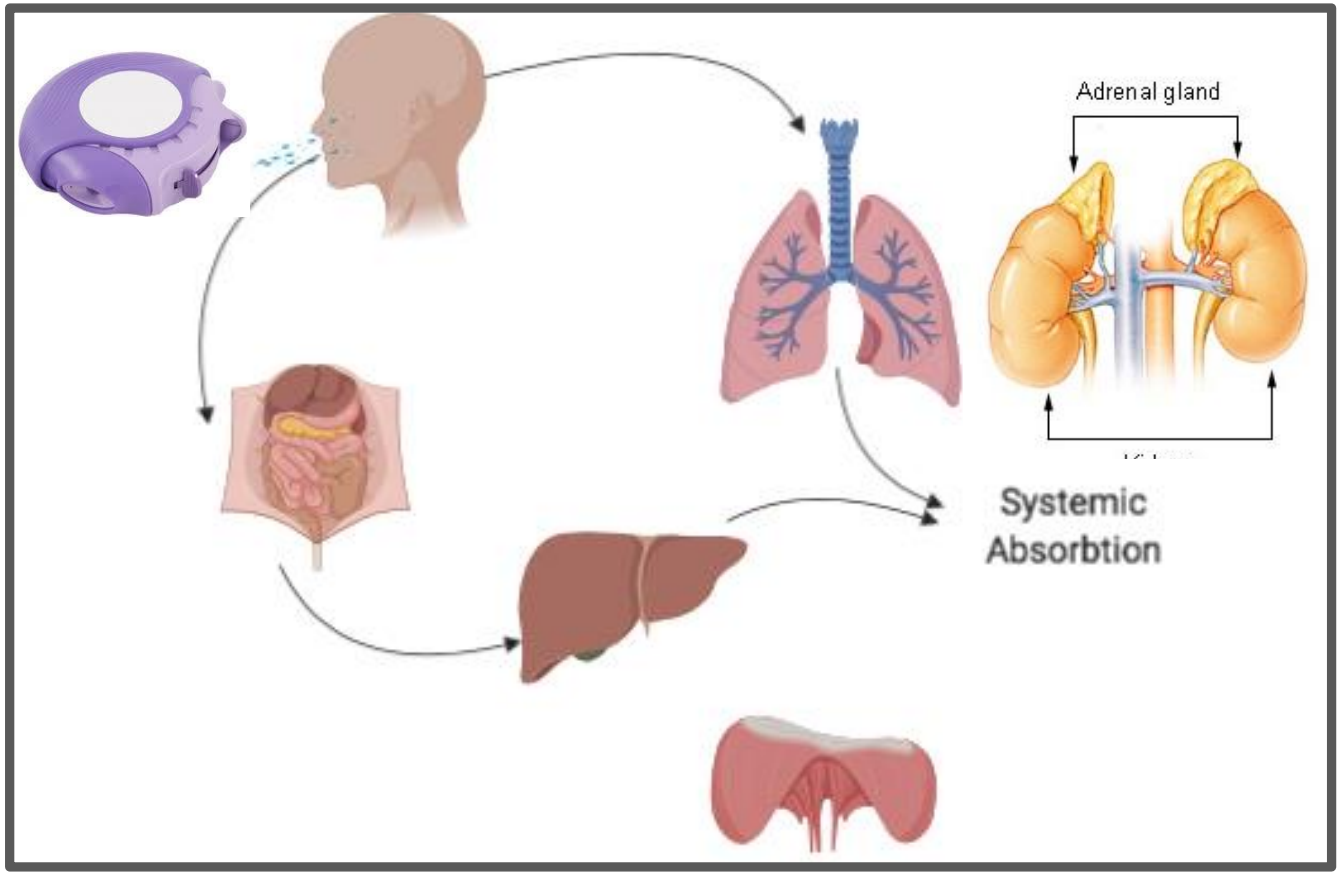
Effect of ICS on Steroid Metabolites among *Controls, Asthma (No ICS), Asthma ICS*



SUB-CLINCIAL ADRENAL SUPRESSION

- Adrenal glands do not produce adequate amounts of steroid hormones, primarily cortisol
- Systemic Absorption from ICS use leads to sub-clinical adrenal insufficiency.
- Subtle Symptoms – underdiagnosed
- Clinical trials to date insufficient

Could this be much more common than we recognize?



Replication in CAMP

RCT of ICS use

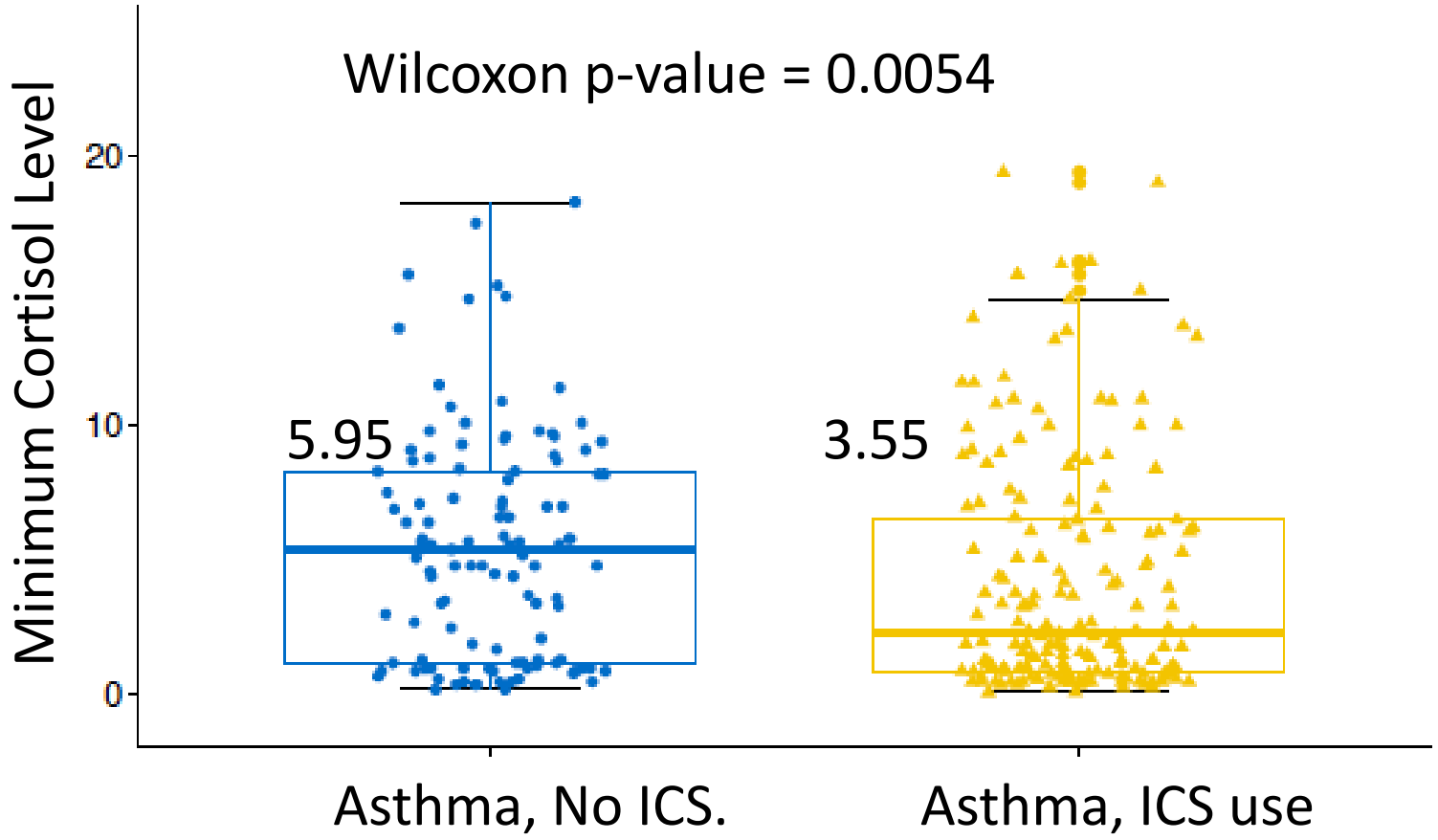
Outcome	Estimate	P*
Cortisol	-0.87	0.007
age	-0.009	0.452
Cortisol*age	0.07	0.008
Cortisone	-0.61	0.044
age	0.03	0.004
Cortisone*age	0.04	0.07

- **Double-blind RCT** of Budesoinide (ICS) vs. inhaler/pacebo
- **Long follow-up period:** 4.8 year post-trial period
- **Metabolomic data** (Broad) available for 560 asthmatics at baseline & end of trial
- Cortisol and Cortisone are the only 2 metabolites available
- Metabolomics is **Qualitative**
- Use ~100,000 EMRs in PBB for **Quantitative** data

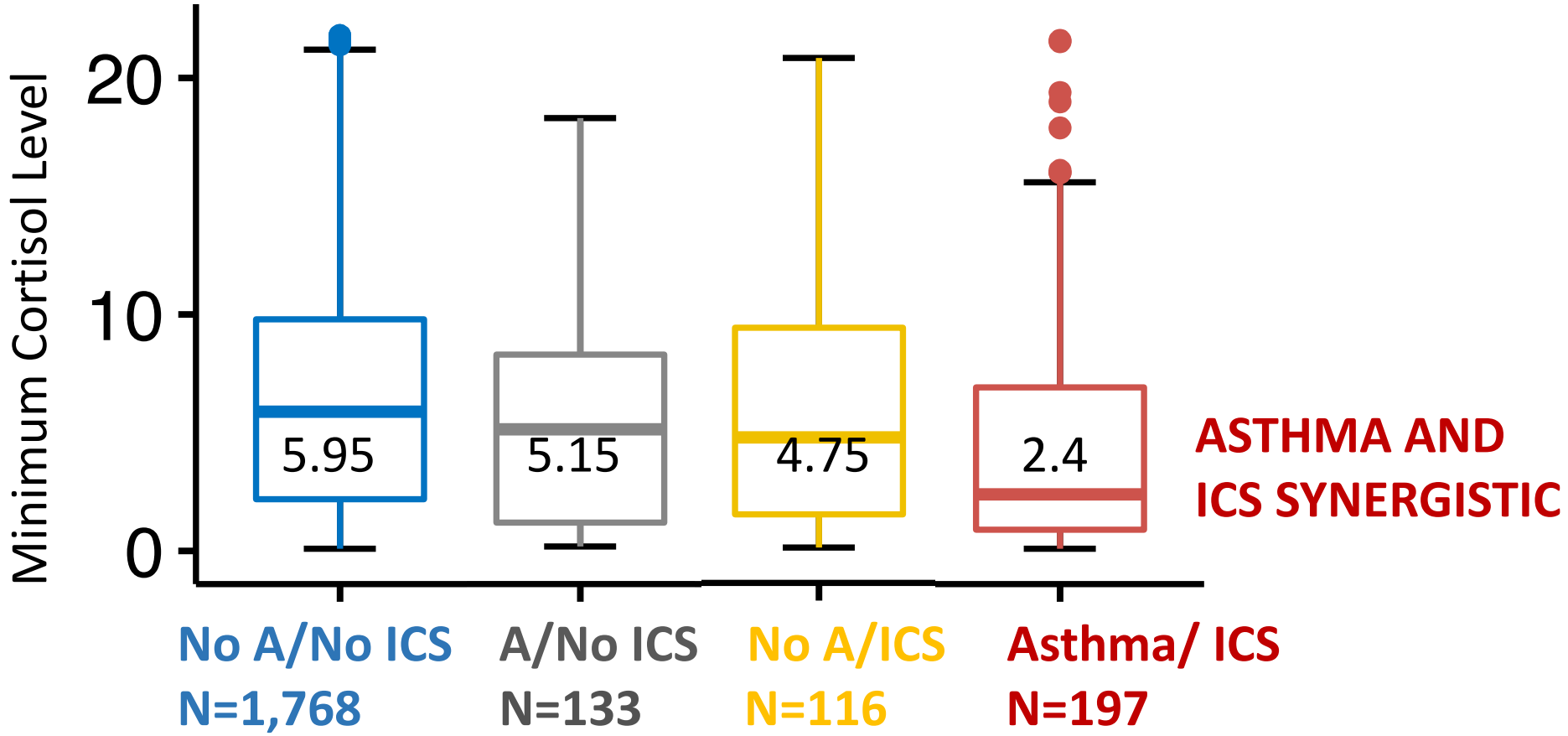
- Predictor: ICS intake
- Outcome is metabolite
- Adjusted for age, sex, race, bmi and ICS*age interaction

PBB: Minimum Cortisol levels in Asthmatics

No ICS vs. ICS



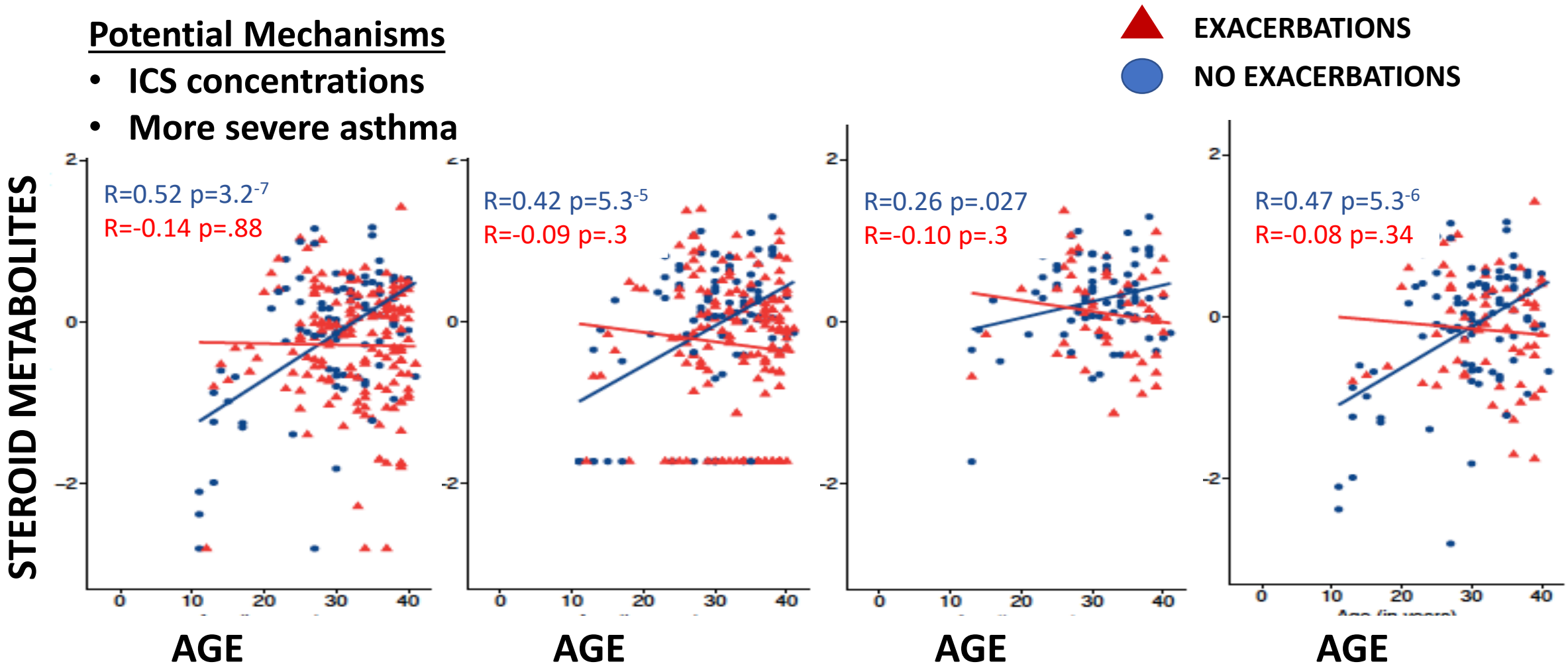
Cortisol levels in all PBB subjects (n=2,214)

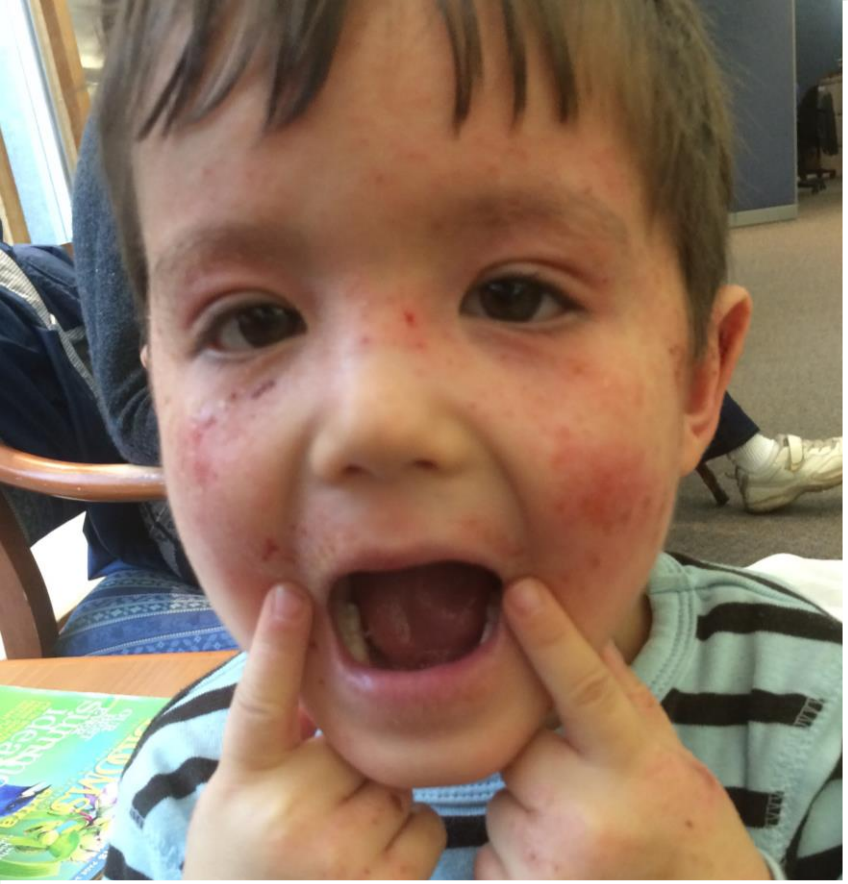


PBB: ICS Asthmatics *with exacerbations* have no change in steroid levels across age compared to those *without exacerbations*

Potential Mechanisms

- **ICS concentrations**
- **More severe asthma**



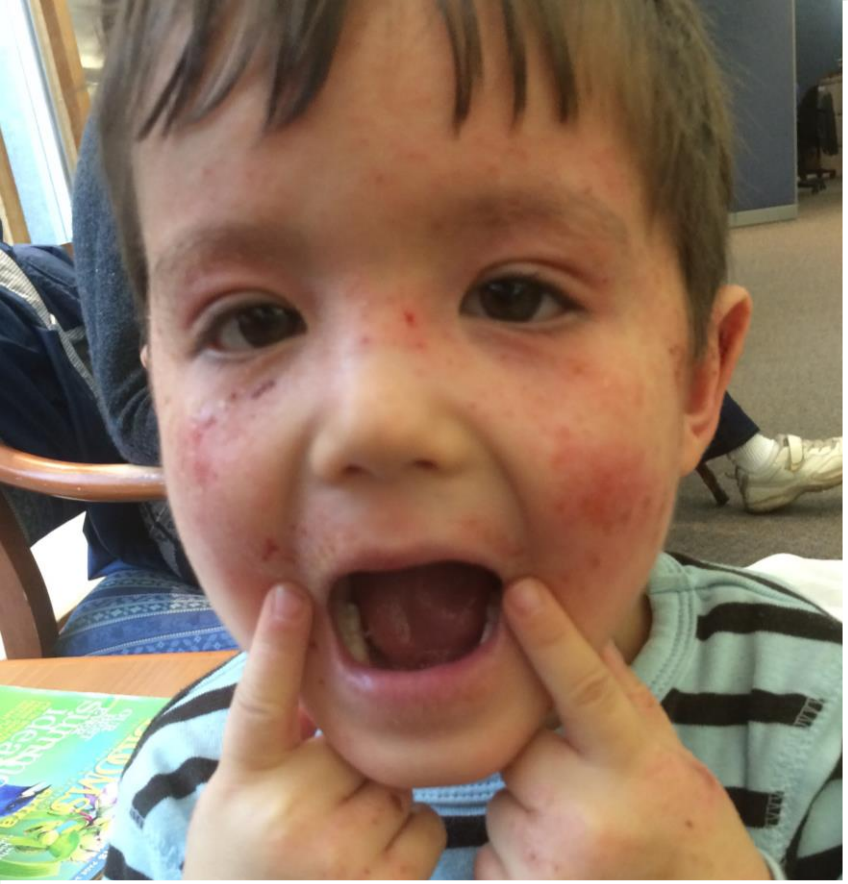


Age 4

Age 6

Age 8

”Natey”



Age 4

Age 6

Age 8

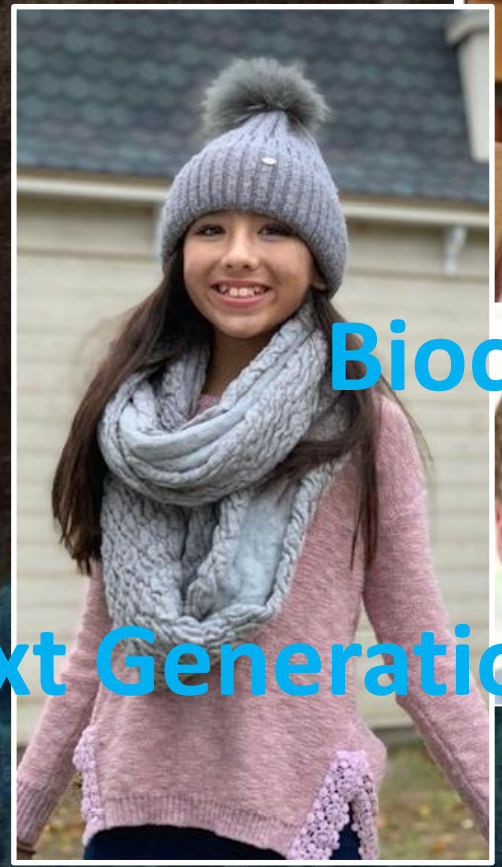
”Natey”

Bringing Metabolomics to the Forefront of *OMICS* in Precision Medicine

Collect	Collect multi-omics continually
Capitalize on	Capitalize on study design
Validate	Validate with other derived measures and cohorts
Apply	Apply novel methodological approaches to link omics together
Form	Collaborations with colleagues
Identify	Robust findings
Develop	Simple tests (VOCs)!
Work	Together

No Free Solo

Statisticians



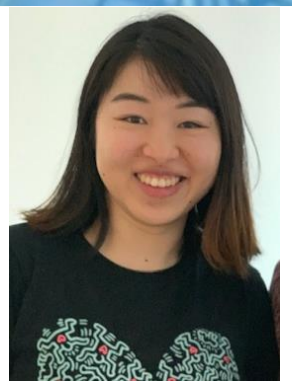
Biochemists

Next Generation

MDs

Post Docs

Acknowledgements



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