V Congreso Argentino de Espectrometría de Masas
23 al 25 de octubre de 2024
Buenos Aires, Argentina
V CAEM

Microbiome derived bile acids during early life: Insights into the progression to islet autoimmunity

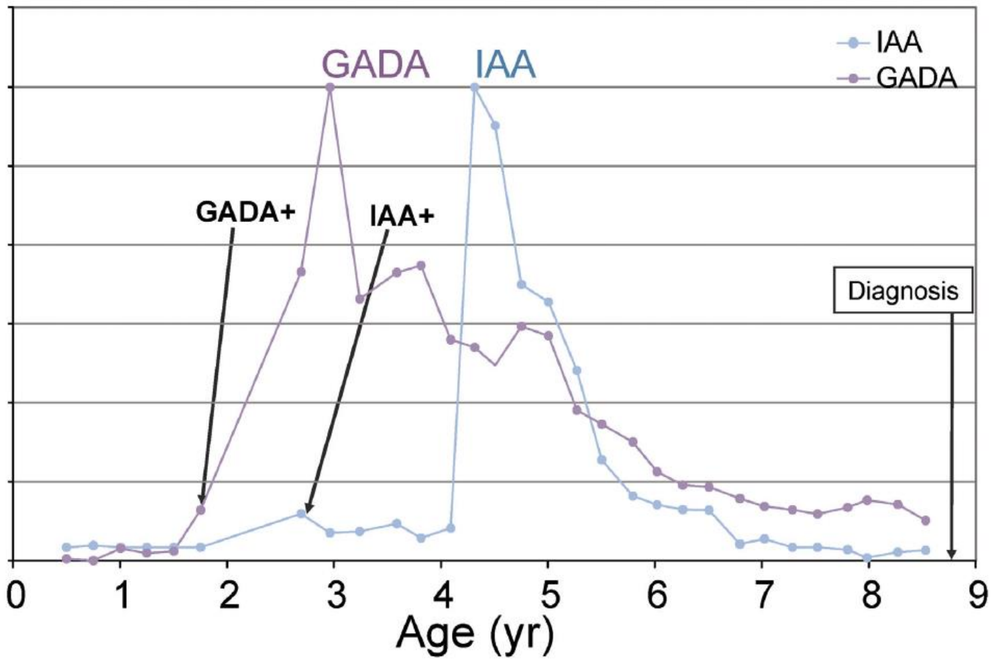
Matej Orešič

Buenos Aires, October 25, 2024

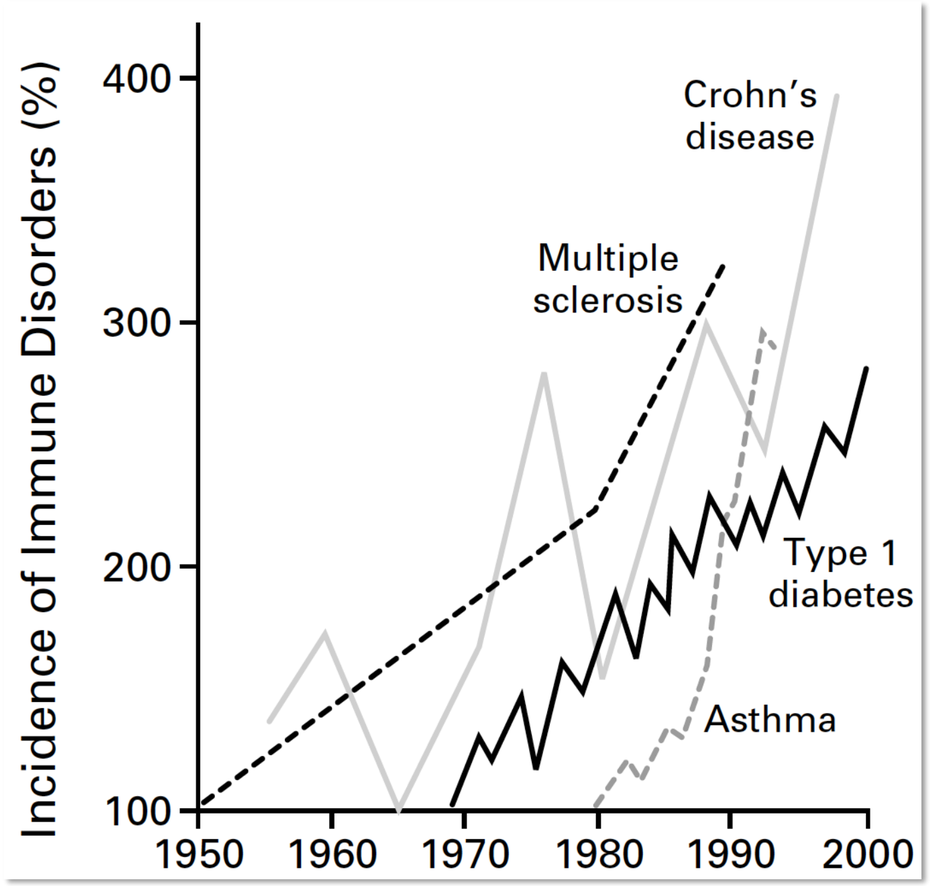
ÖREBRO UNIVERSITY
UNIVERSITY OF TURKU

Type 1 diabetes

- T1D is caused by progressive loss of insulin-secreting capacity due to the selective death of beta cells in the islets of Langerhans
- Although approximately 80% of subjects with T1D carry defined risk-associated genotypes at the human leukocyte antigen (HLA) locus, only 3-7% of the carriers of such genetic risk markers develop the disease
- Seroconversion to islet autoantibody positivity is the first detectable signal implicating initiation of autoimmunity and risk of progression towards diabetes



Environmental causes of immune disorders



Bach JF. N Engl J Med. 2002;347(12):911-920.

Our first study in T1D

Published December 15, 2008

JEM

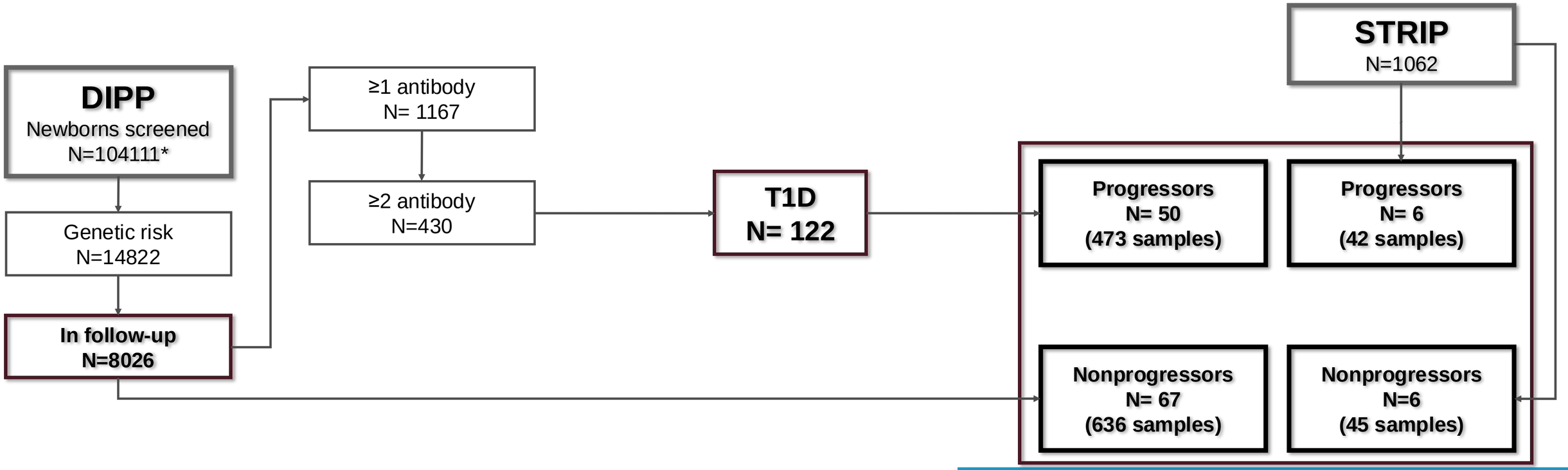
ARTICLE

Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes

Matej Orešič,[1] Satu Simell,[2] Marko Sysi-Aho,[1] Kirsti Näntö-Salonen,[2] Tuulikki Seppänen-Laakso,[1] Vilhelmiina Parikka,[2] Mikko Katajamaa,[1] Anne Hekkala,[4] Ismo Mattila,[1] Päivi Keskinen,[5] Laxman Yetukuri,[1] Arja Reinikainen,[6] Jyrki Lähde,[5] Tapani Suortti,[1] Jari Hakalax,[2] Tuula Simell,[2] Heikki Hyöty,[7,8] Riitta Veijola,[4] Jorma Ilonen,[3,9] Riitta Lahesmaa,[6] Mikael Knip,[5,10] and Olli Simell[2]

J. Exp. Med. Vol. 205 No. 13 2975–2984
www.jem.org/cgi/doi/10.1084/jem.20081800

Finnish Type 1 Diabetes Prediction and Prevention Study (DIPP)

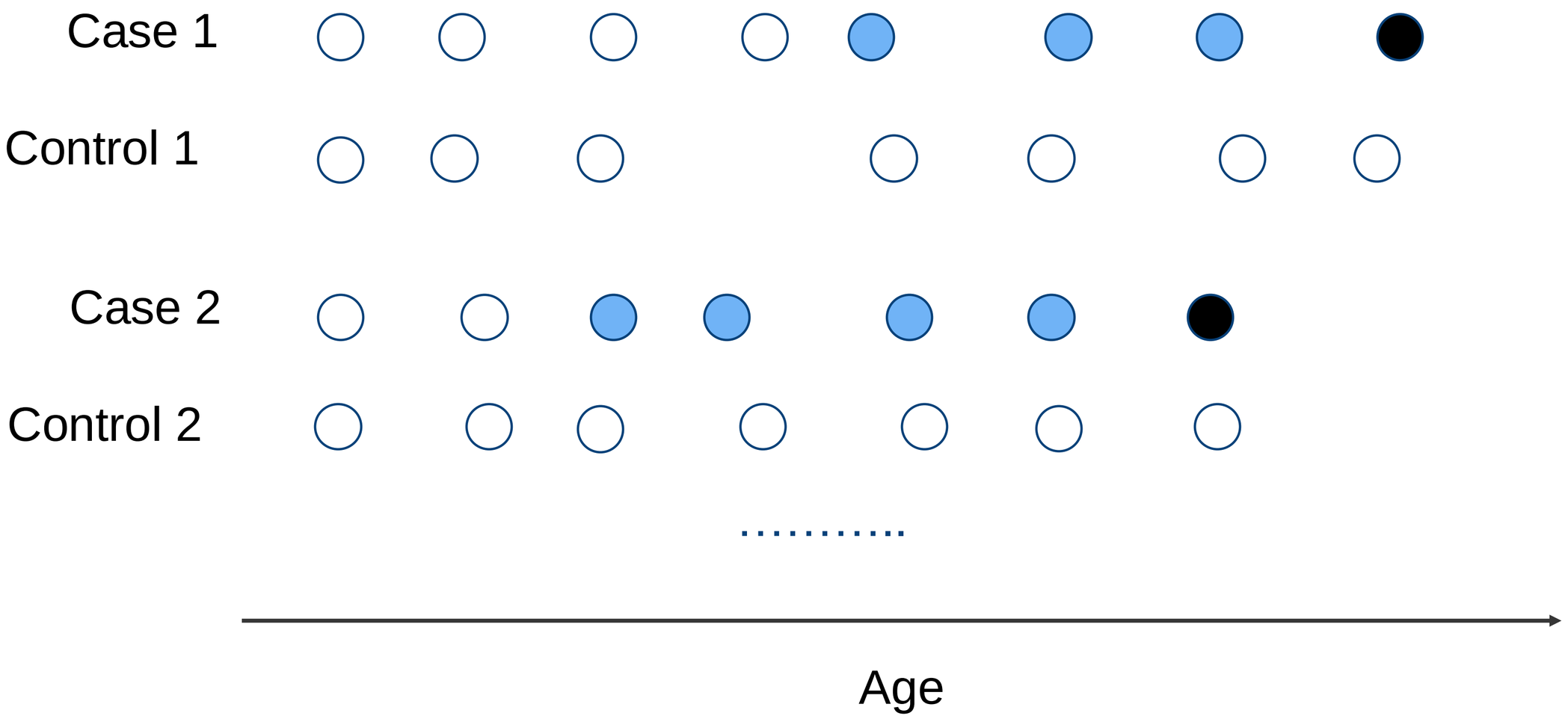


Cases and controls matched by gender, HLA genotype, city and period of birth.

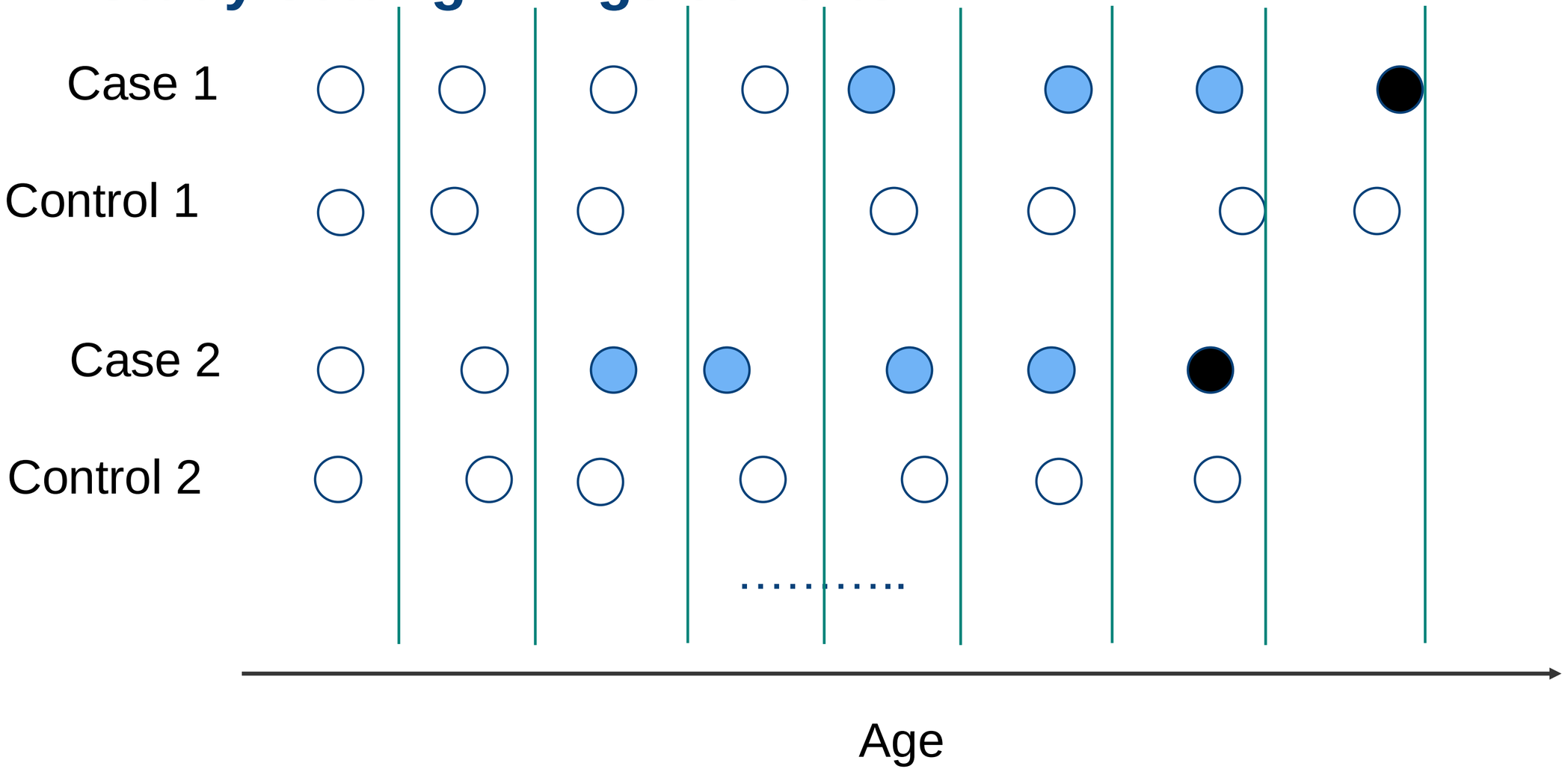
*as of June 2006

M. Orešič et al., *J Exp Med* (2008)

Study setting – how to compare cases and controls?



Study setting → Age cohorts



Lipidomics on complete 1196 sample series

Progressors *vs.* Nonprogressors

- Consistent decrease of multiple phospholipids throughout the follow-up
- Low triglycerides at early age

Commentary | December 15 2008

Causes of early-onset type 1 diabetes: toward data-driven environmental approaches

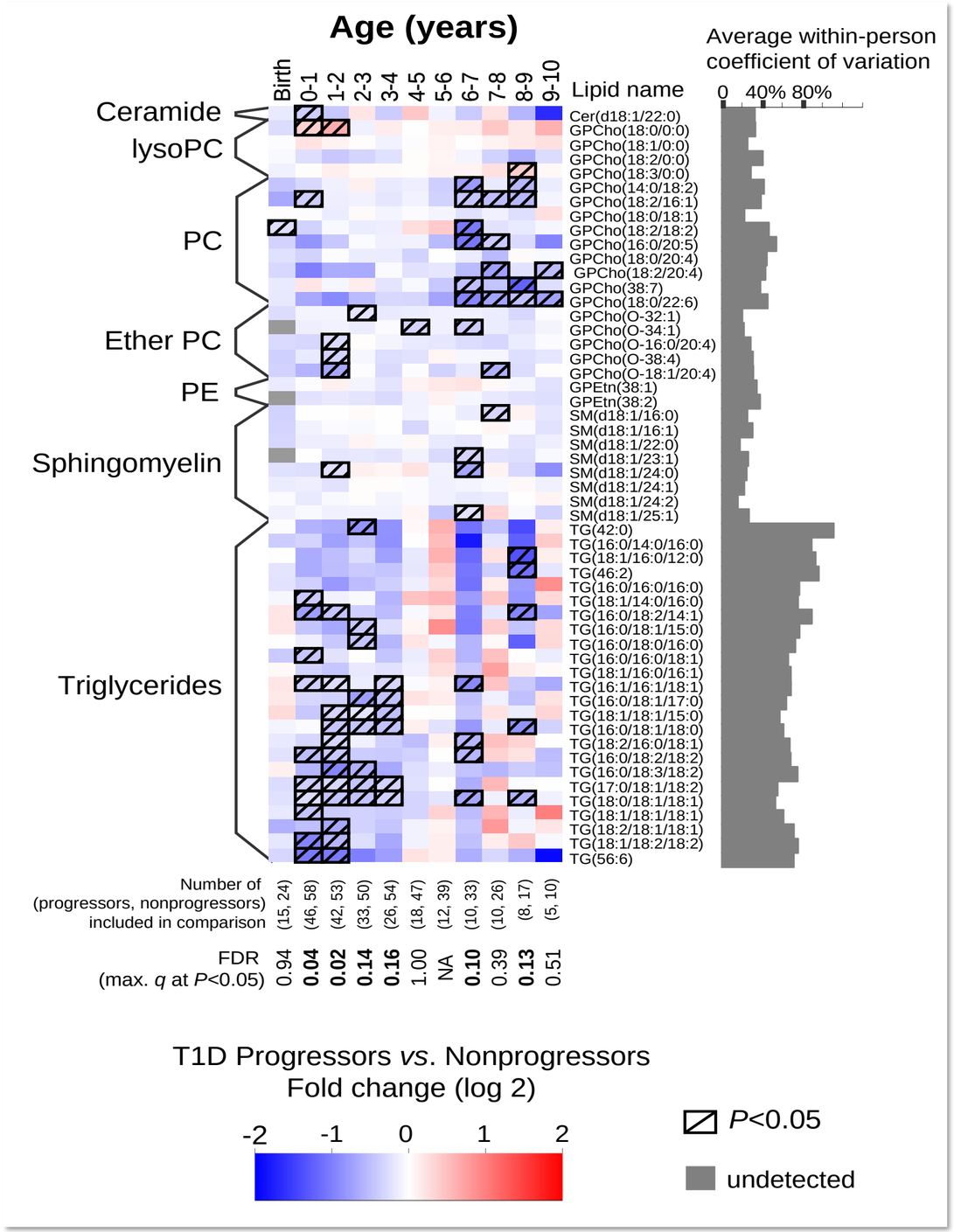
Pierre Bougnères, Alain-Jacques Valleron

+ Author and Article Information

Check for updates

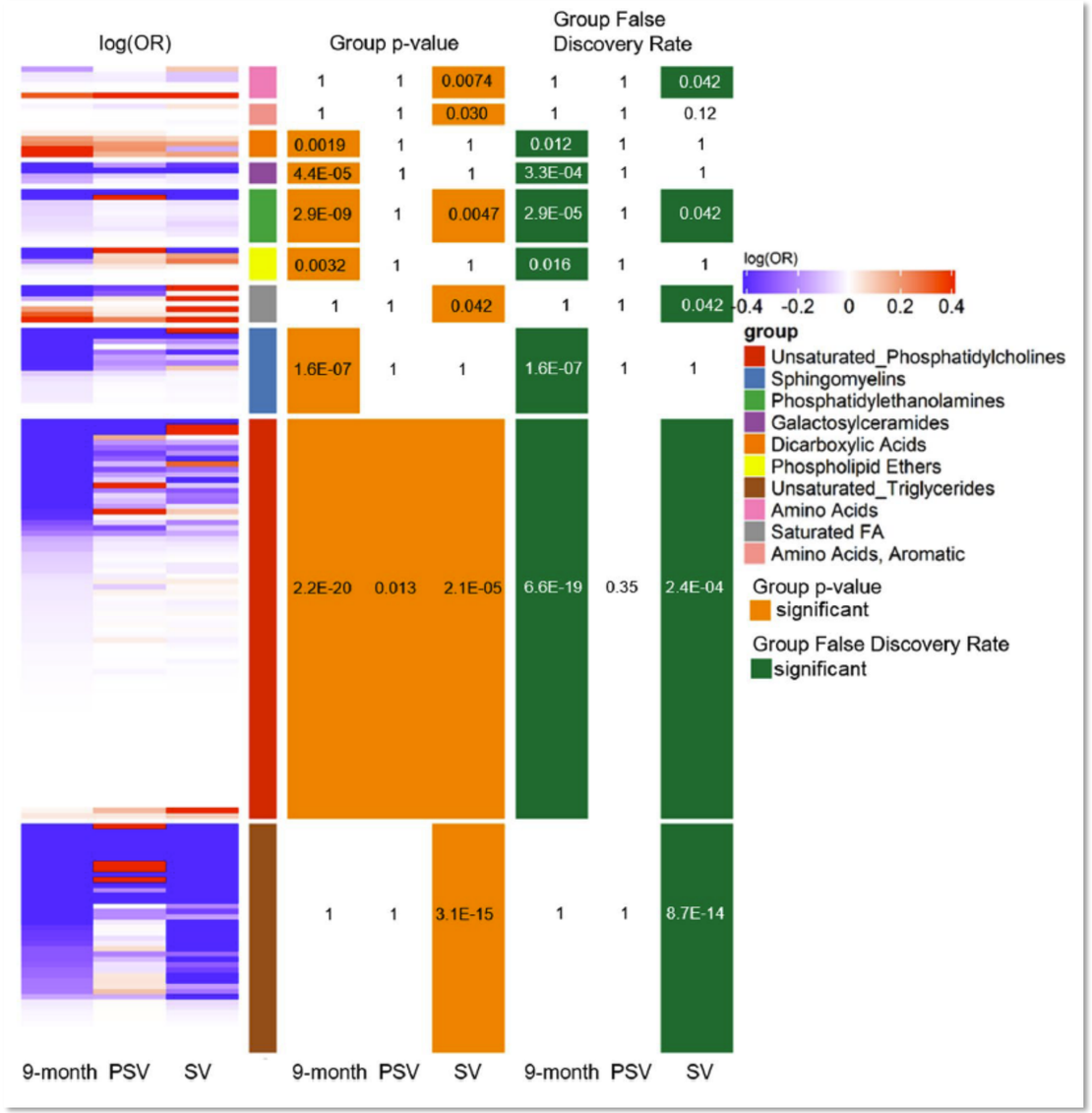
J Exp Med (2008) 205 (13): 2953–2957. https://doi.org/10.1084/jem.20082622

This sort of metabolomic approach to T1D natural history may be a pioneering example of environmental data-driven approaches.



M. Orešič et al., J Exp Med (2008)

Replication of our findings in multi-national TEDDY cohort

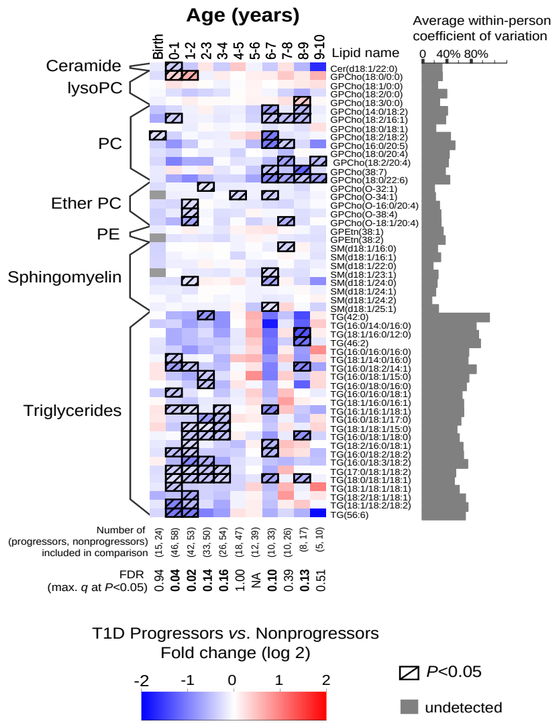


OPEN

Metabolite-related dietary patterns and the development of islet autoimmunity

Received: 16 April 2019
Accepted: 24 September 2019
Published online: 15 October 2019

Randi K. Johnson[1], Lauren Vanderlinden[2], Brian C. DeFelice[3], Katerina Kechris[2], Ulla Uusitalo[4], Oliver Fiehn[3,5], Marci Sontag[1], Tessa Crume[1], Andreas Beyerlein[6,7], Åke Lernmark[8], Jorma Toppari[9,10], Anette-G. Ziegler[7], Jin-Xiong She[11], William Hagopian[12], Marian Rewers[13], Beena Akolkar[14], Jeffrey Krischer[4], Suvi M. Virtanen[15,16,17], Jill M. Norris[1] & The TEDDY Study Group*

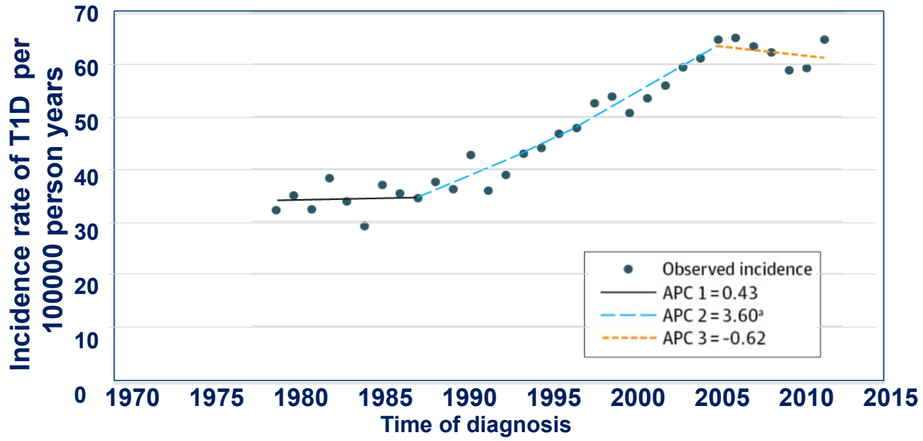


M. Orešič et al., J Exp Med (2008)

Hypothesis: Potential role of environmental stressors

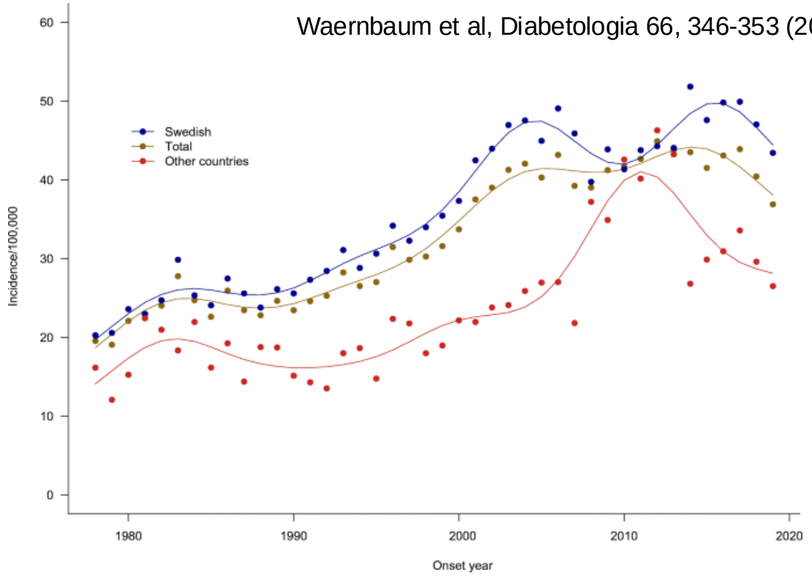
- Our earlier findings indicate that children who later progress to T1D acquire a risk metabolic phenotype already *in utero*.
- Altered phospholipid (incl. sphingolipid) metabolism may play a direct role in T1D pathogenesis *via* impact on immune system (*not discussed here*)
- What could be the cause of altered phospholipid levels in newborns at risk of T1D?

T1D incidence in Finland and Sweden has stabilized or decreased



JAMA. 2013;310(4):427-428. doi:10.1001/jama.2013.8399

Waernbaum et al, Diabetologia 66, 346-353 (2023)



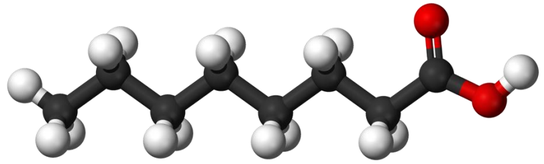
Incidence of childhood-onset (0−14 years) type 1 diabetes

Increased immigration from countries with lower reported incidence does not explain the levelling off of trend

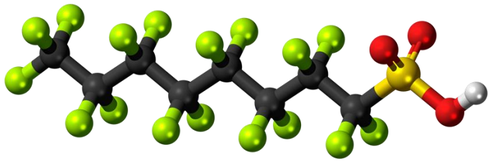
Explanation for change in incidence rate among the risk factors?

- Obesity? No (no change in trend over past decade)
- Enterovirus infections? No (infections actually increased)
- Diet/gut microbiome? No (weak association with incidence)
- Environmental contaminants?

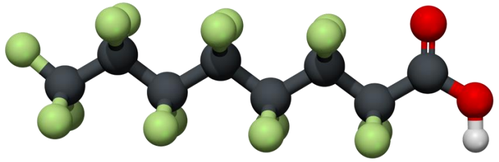
- **Per- and poly-fluorinated alkyl substances (PFAS)** are a group of man-made chemicals.
- Widely used since the 1950s in household and industrial products that resist heat, oil, stains, grease and water, *e.g.*, in food packaging, non-stick cookware, outdoor clothing, carpet protection, cleaners, fire fighting foams etc.
- EU introduced tighter regulation of PFOS in 2009 (phased out by industry already in 2002); currently complete ban on PFAS is considered in EU.



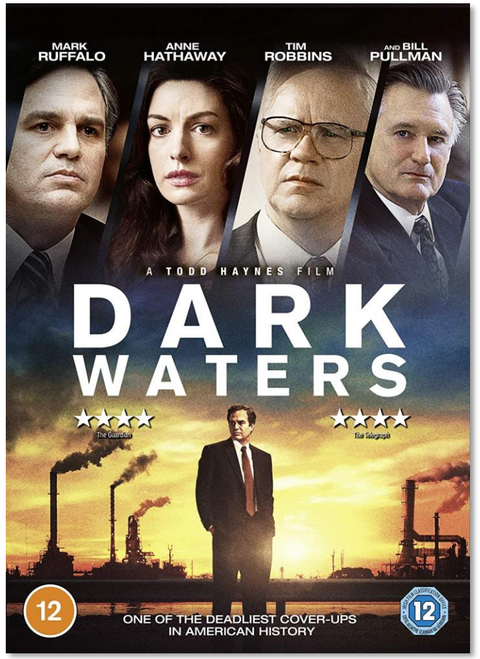
Octanoic acid



Perfluorooctanesulfonic acid
PFOS



Perfluorooctanoic acid
PFOA



- PFOS and PFOA are well absorbed orally and are very slowly eliminated from the body in humans with a half life of approximately nine and four years, respectively.
- PFAS have been shown to alter eukaryotic cell membrane properties and increase membrane permeability.

Environmental Science & Technology Letters

Letter

Cite This: *Environ. Sci. Technol. Lett.* 2018, 5, 237–242

pubs.acs.org/journal/estlcu

PFOA and PFOS Disrupt the Generation of Human Pancreatic Progenitor Cells

Shuyu Liu,[†,‡] Nuoya Yin,[†,‡] and Francesco Faiola*[,†,‡]

Toxicology Reports 3 (2016) 664–672

Contents lists available at ScienceDirect

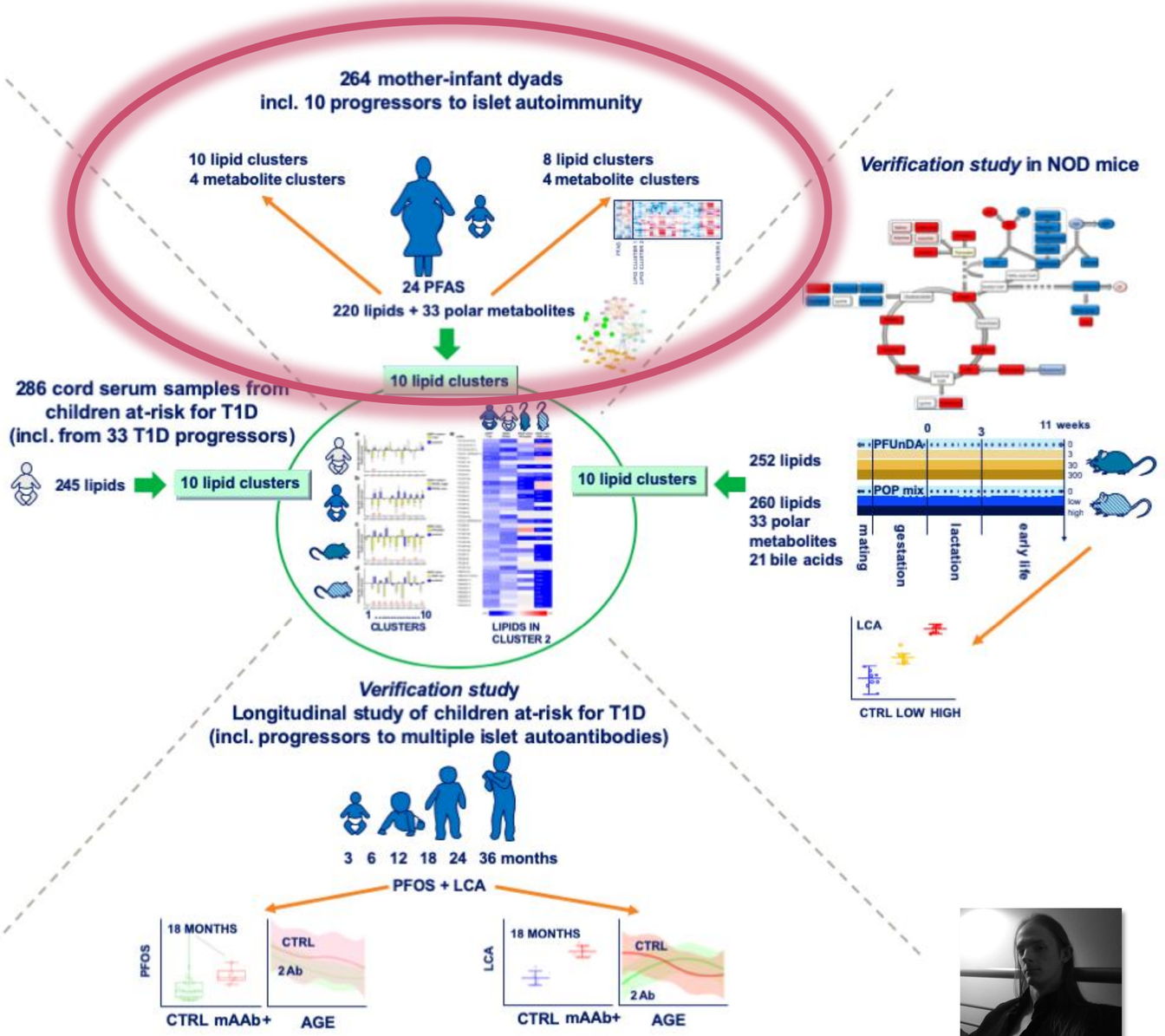
Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Exposure to perfluoroundecanoic acid (PFUnDA) accelerates insulitis development in a mouse model of type 1 diabetes

Johanna Bodin*, Else-Carin Groeng, Monica Andreassen, Hubert Dirven, Unni Cecilie Nygaard

Impact of chemical exposures on islet autoimmunity



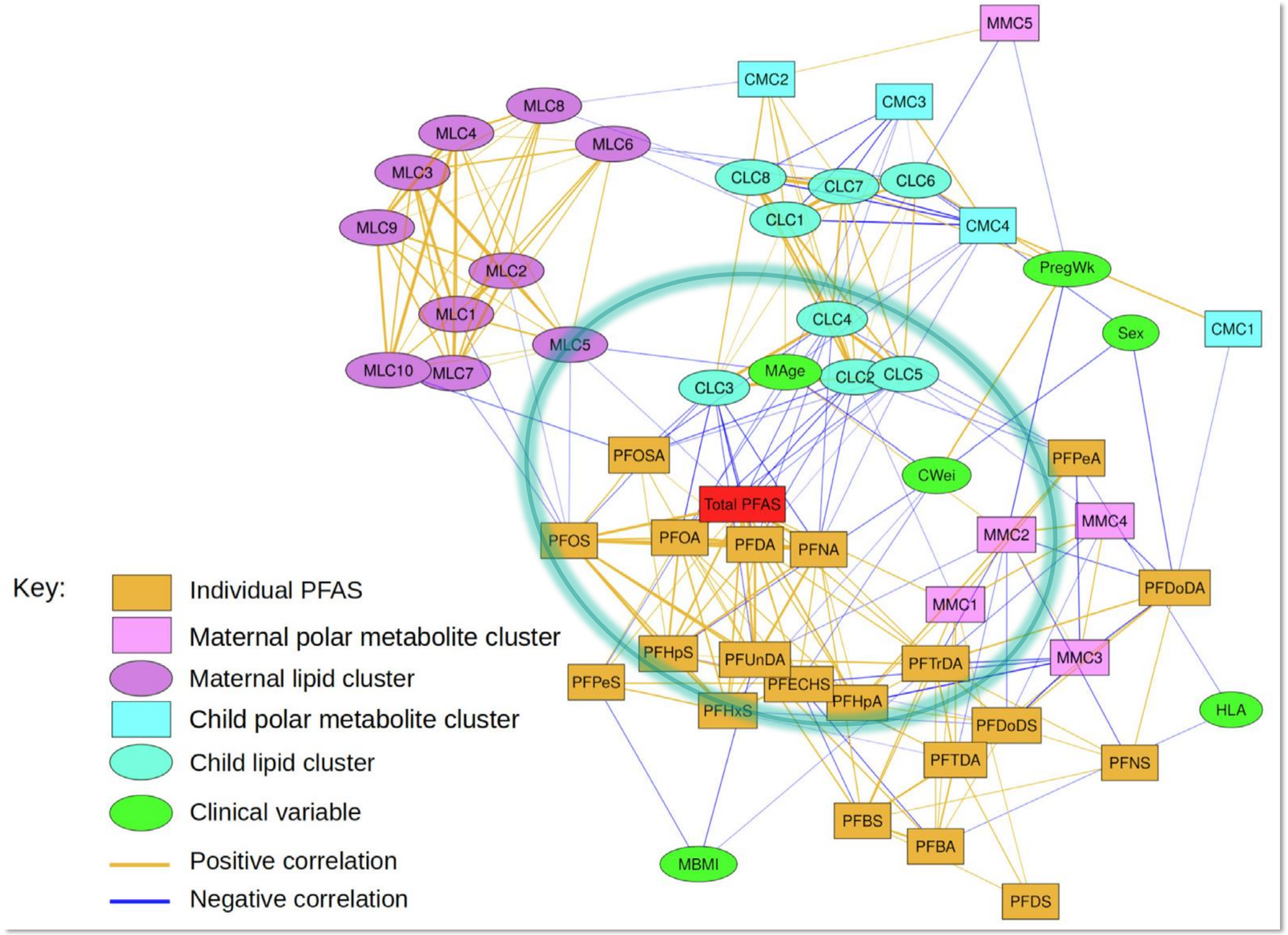
Aidan McGlinchey

Profs. Tuulia Hyötyläinen

Mikael Knip

McGlinchey A, …,, Orešič M, Knip M, Hyötyläinen T. Environ Int. 2020

Partial correlation network analysis



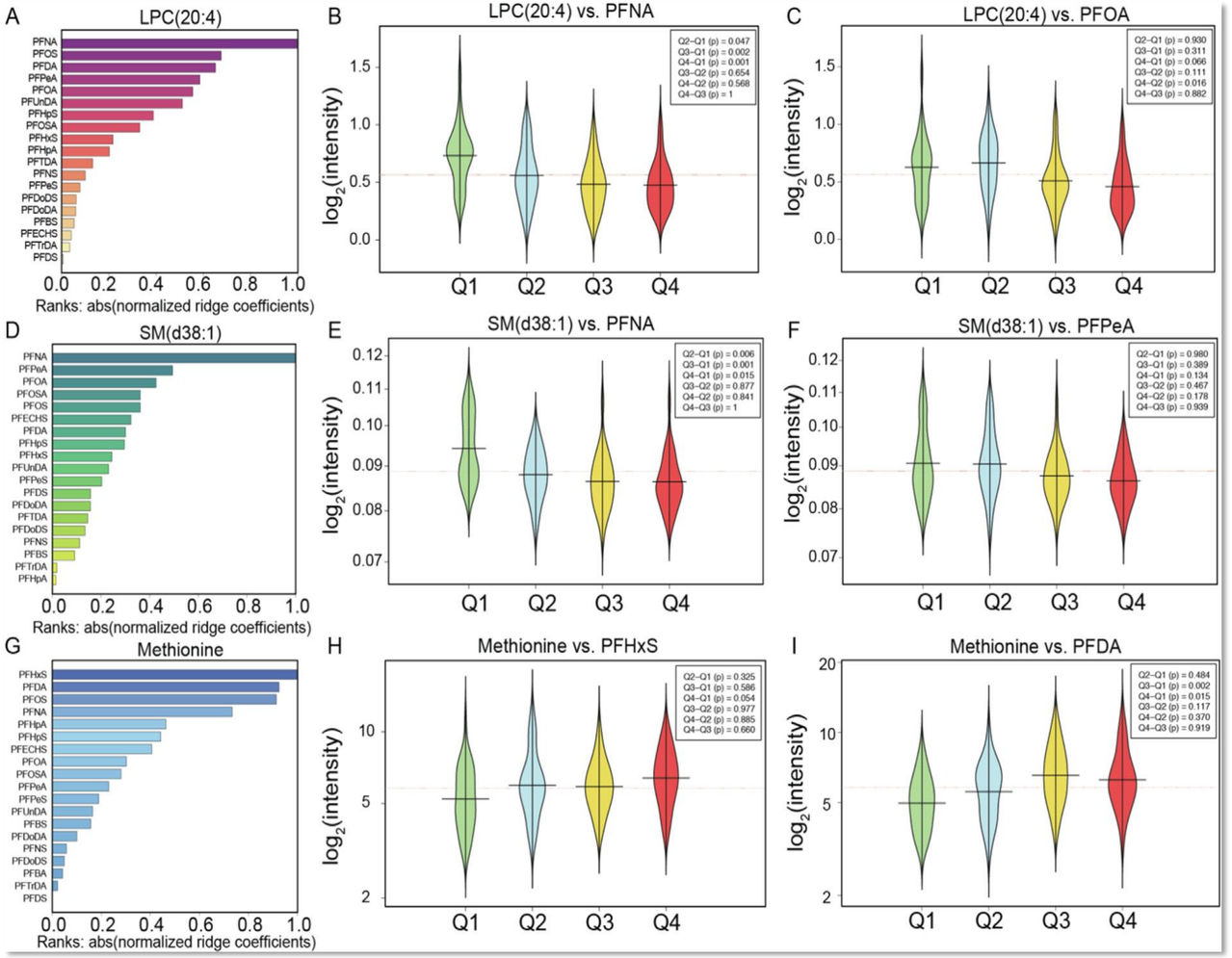
qp-graph, non-rejection rate 0.4

Cord serum clusters

Cluster	General description	Main lipids
CLC1	Cholesterol esters, DGs, TGs	CE(18:1), CE(16:0), CE(20:4), TG(18:2/18:1/18:1), DG(36:2), TG(18:1/18:1/18:1)
CLC2	Phospholipids with PUFAs, SMs	SM(42:1), PC(38:4), PC(38:6), PC(40:6), PC(18:0/18:0)
CLC3	Main TGs	TG(18:2/18:1/16:0), TG(14:0/18:1/18:1), TG(16:0/18:2/18:2)
CLC4	LPCs	LPC(18:0), LPC(18:1),LPC(20:4)
CLC5	Abundant PCs and SMs	SM(42:2), PC(18:0/18:1), SM(42:3), SM(38:1)
CLC6	Odd-chain PCs	PC(35:1), PC(33:1), PC(35:2)
CLC7	Phospholipids with PUFAs	PC(36:3), PC(40:7), PC(O-40:6)
CLC8	Odd-chain TGs	TG(45:0), TG(47:0), TG(47:1), TG(47:2)
CMC1	Amino acids, dicarboxylic and hydroxyl acids	Alanine, Glycine, Glutamic acid, Fumaric acid Malic acid, Citric acid, Lactic acid
CMC2	2- and 3-butyric acids, main free fatty acids	2-Hydroxybutyric acid, 3-Hydroxybutyric acid Palmitic acid, Linoleic acid, Oleic acid, Stearic acid
CMC3	BCAAs, sugar derivatives	Valine, Leucine, Isoleucine, Proline, Succinic acid, Glutamine, Fructose, Indole-3-propionic acid, Cholesterol
CMC4	Amino acids	Serine, Threonine, Methionine, Aspartic acid Phenylalanine, Asparagine, Glycerol-3-phosphate, Ornithine, Lysine

McGlinchey A, …,, Orešič M, Knip M, Hyötyläinen T. Environ Int. 2020

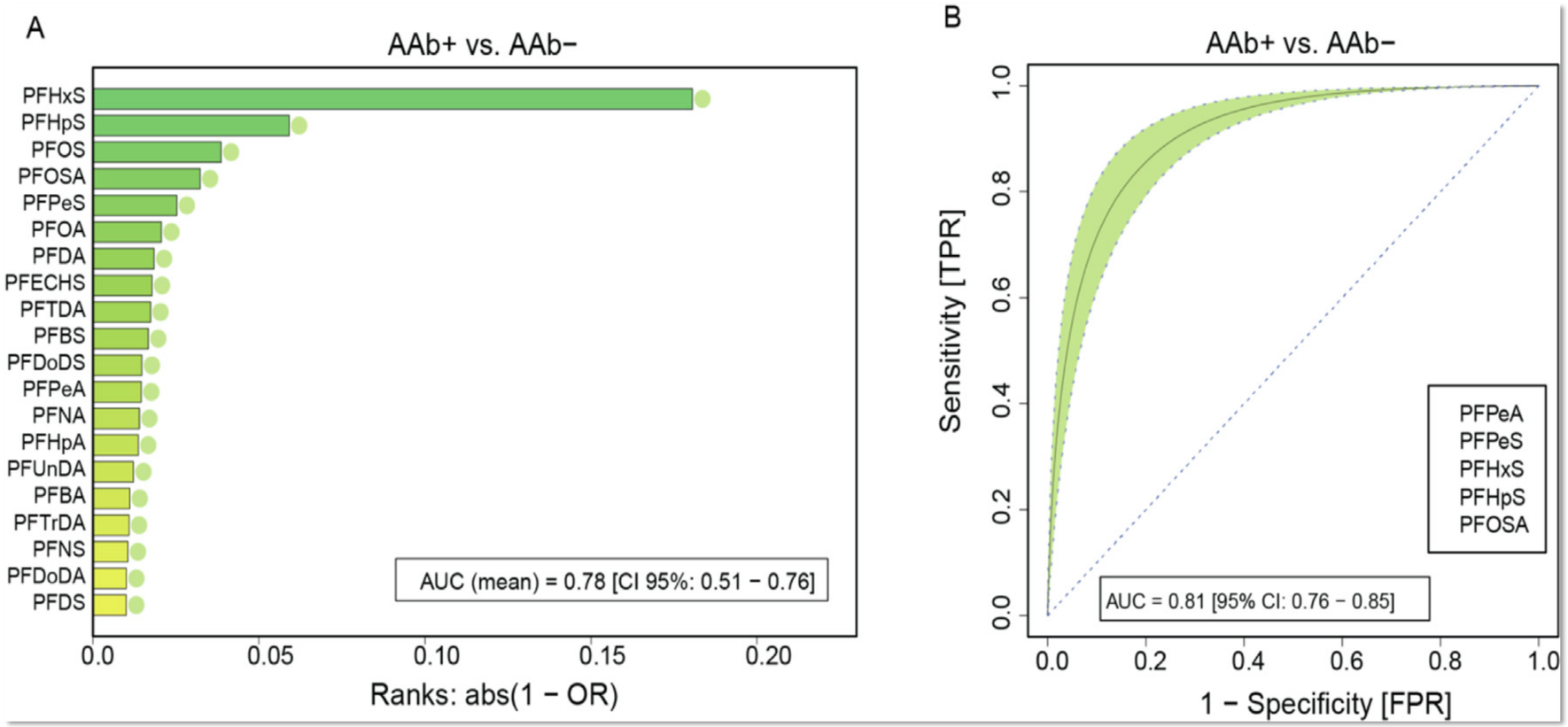
Impact of prenatal exposure to PFAS (quartiles) on specific cord serum lipids & polar metabolites from newborns



ANOVA adj.p < 0.05 for all

McGlinchey A, *et al.* Environ Int. 2020 Oct;143:105935.

Exposures in prediction of progression to islet autoimmunity



Logistic ridge regression (LRR) models

McGlinchey A, …,, Orešič M, Knip M, Hyötyläinen T. Environ Int. 2020

PFAS exposure & genetic risk for T1D

- PFAS exposure alone cannot explain increased risk of T1D. For example, the incidence of T1D in Finland is about 50 cases per 100,000 population, while, *e.g.*, in China the incidence is about 1/100,000.
- Yet, in Chinese urban areas, the levels of PFAS in newborns are markedly higher, about an order of magnitude, than in Finland (Sinisalu et al, Metabolomics, 2021)
- Interaction of HLA risk genotype & exposure?

Impact of prenatal exposure to PFAS on lipid profiles in newborns is excacerbated by increased HLA-associated risk of T1D.

Lipid	Interaction HLA*PFAS
LPC(18:1)	0.067
LPC(18:2)	0.11
LPC(20:3)	0.025
LPC(20:4)	0.041
LPC(22:5)	0.61
LPC(22:6)	0.025
PC(40:8)	0.12
SM(d36:0)	0.084
SM(d36:0)	0.012
SM(d38:1)	0.080
PC(O-38:4)	0.80

2-way ANOVA on lipids which are significantly impacted by prenatal exposure to PFAS

McGlinchey A, …,, Orešič M, Knip M, Hyötyläinen T. Environ Int. 2020

Establishing causal relationship between prenatal PFAS exposure and lipids in offspring

- Two studies in NOD mice:
  - **Model 1:** prenatal exposure to PFUnDA at three concentrations
  - **Model 2:** prenatal exposure to mixture of persistent organic pollutants (POP), mimicking human exposure, 2 concentration levels

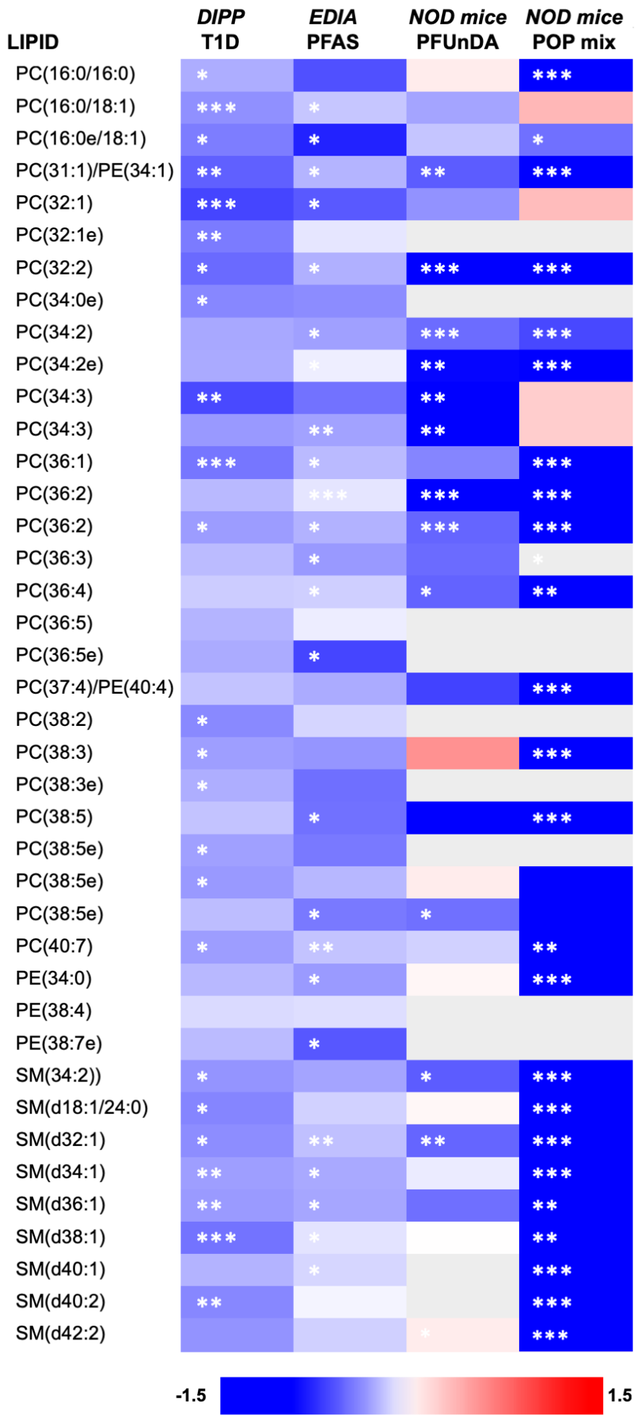
Brominated compounds	Chlorinated compounds	Perfluorinated compounds
BDE 100	PCB 101	PFDA
BDE 153	PCB 118	PFHxS
BDE 154	PCB 138	PFNA
BDE 209	PCB 153	PFOA
PBDE 47	PCB 180	PFOS
PBDE 99	PCB 28	PFUnDA
	PCB 52	
	p,p´-DDE	
	Dieldrin	
	HBCD	
	HCB	
	α, β, γ-HCH	
	α, oxy and trans- chlordane	

PFAS/POPmix exposure accelerates development of insulitis and autoimmune diabetes

Bodin J et al, *Toxicol Rep* (2016)

Comparing multiple T1D and exposure studies at the T1D-associated lipid level

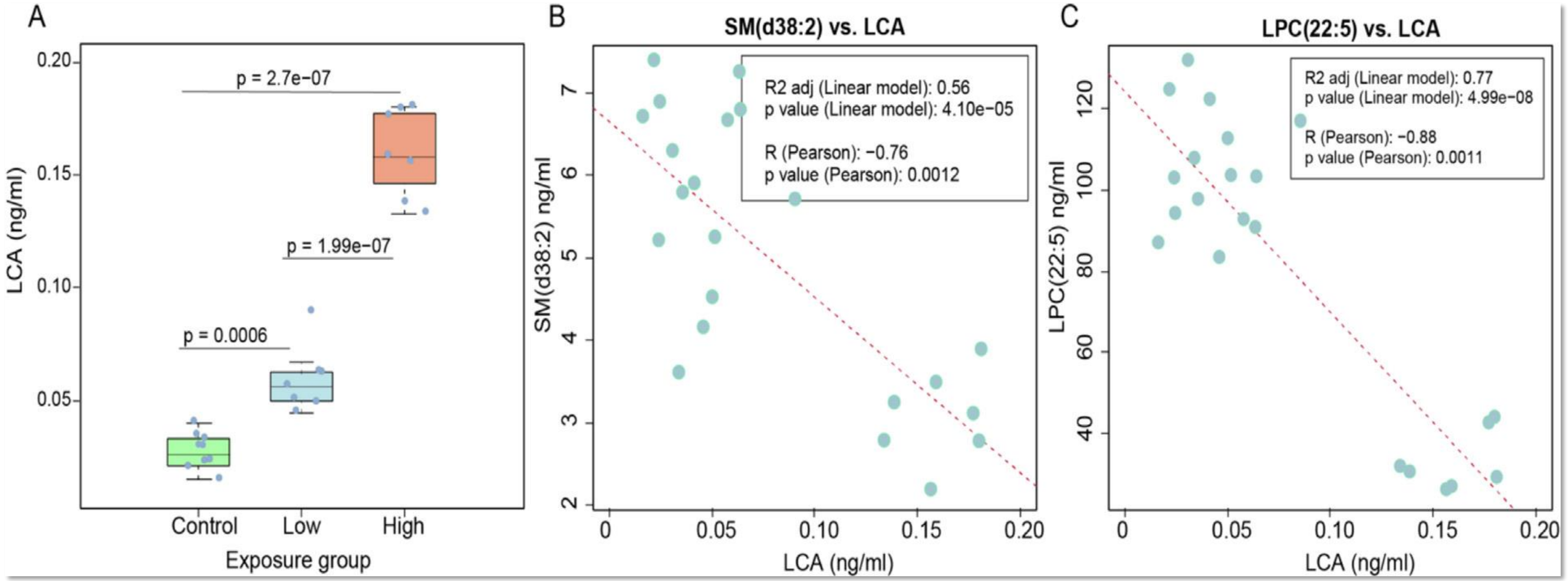
Lipids found earlier associated with risk of T1D are also down-regulated due to prenatal PFAS exposure (in humans as well as in NOD mice)



McGlinchey A, *et al.* Environ Int. 2020 Oct;143:105935.

Lithocholic acid (LCA)

Marked impact of PFAS exposure on LCA levels in NOD mice



McGlinchey A, *et al.* Environ Int. 2020 Oct;143:105935.

Litocholic acid (LCA) – secondary bile acid

LCA suppresses phospholipids and sphingolipids in the liver

Lithocholic Acid Disrupts Phospholipid and Sphingolipid Homeostasis Leading to Cholestasis in Mice

Tsutomu Matsubara, Naoki Tanaka, Andrew D. Patterson, Joo-Youn Cho, Kristopher W. Krausz, and Frank J. Gonzalez

HEPATOLOGY, Vol. 53, No. 4, 2011

LCA and its metabolites control Th17 and Treg differentiation

Article

Bile acid metabolites control T_H17 and T_{reg} cell differentiation

https://doi.org/10.1038/s41586-019-1785-z

Received: 24 October 2018

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Published online: 27 November 2019

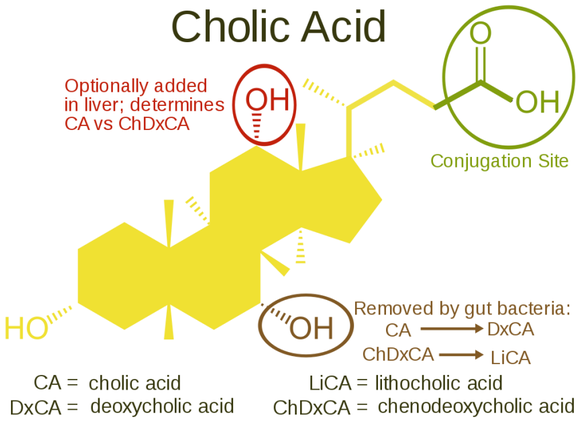
Saiyu Hang[1,12], Donggi Paik[1,12], Lina Yao[2], Eunha Kim[1], Trinath Jamma[3], Jingping Lu[4], Soyoung Ha[1], Brandon N. Nelson[5], Samantha P. Kelly[5], Lin Wu[6], Ye Zheng[7], Randy S. Longman[8], Fraydoon Rastinejad[4], A. Sloan Devlin[2], Michael R. Krout[5], Michael A. Fischbach[9]*, Dan R. Littman[6,10]* & Jun R. Huh[1,11]*

Nature | Vol 576 | 5 December 2019 |

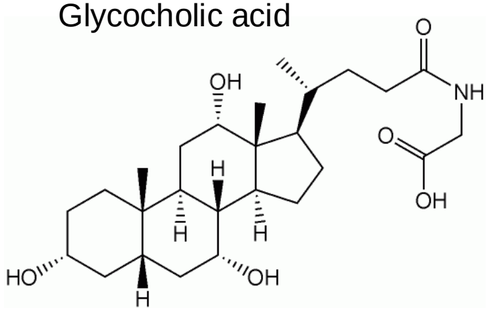
Conclusions (prenatal PFAS exposure)

- high prenatal exposure to PFAS alters lipid profiles in newborn children, which, in turn, may increase the risk of T1D.
- we identified a potential role for a gene-environment interaction (HLA risk genotype and prenatal PFAS exposure), which may lead to altered lipid profiles in newborn children at-risk of developing T1D. Role of HLA in mediating the impact of chemical exposures needs to be investigated further.
- the impact of PFAS may be mediated via secondary bile acids such as LCA (and consequently, by the gut microbiome).
- our findings may offer an explanation for the changing trend in T1D incidence in Western countries.

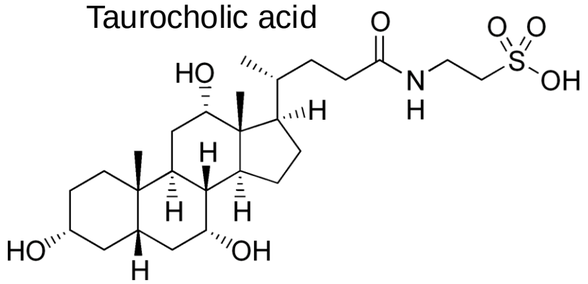
Bile acids



Glycocholic acid

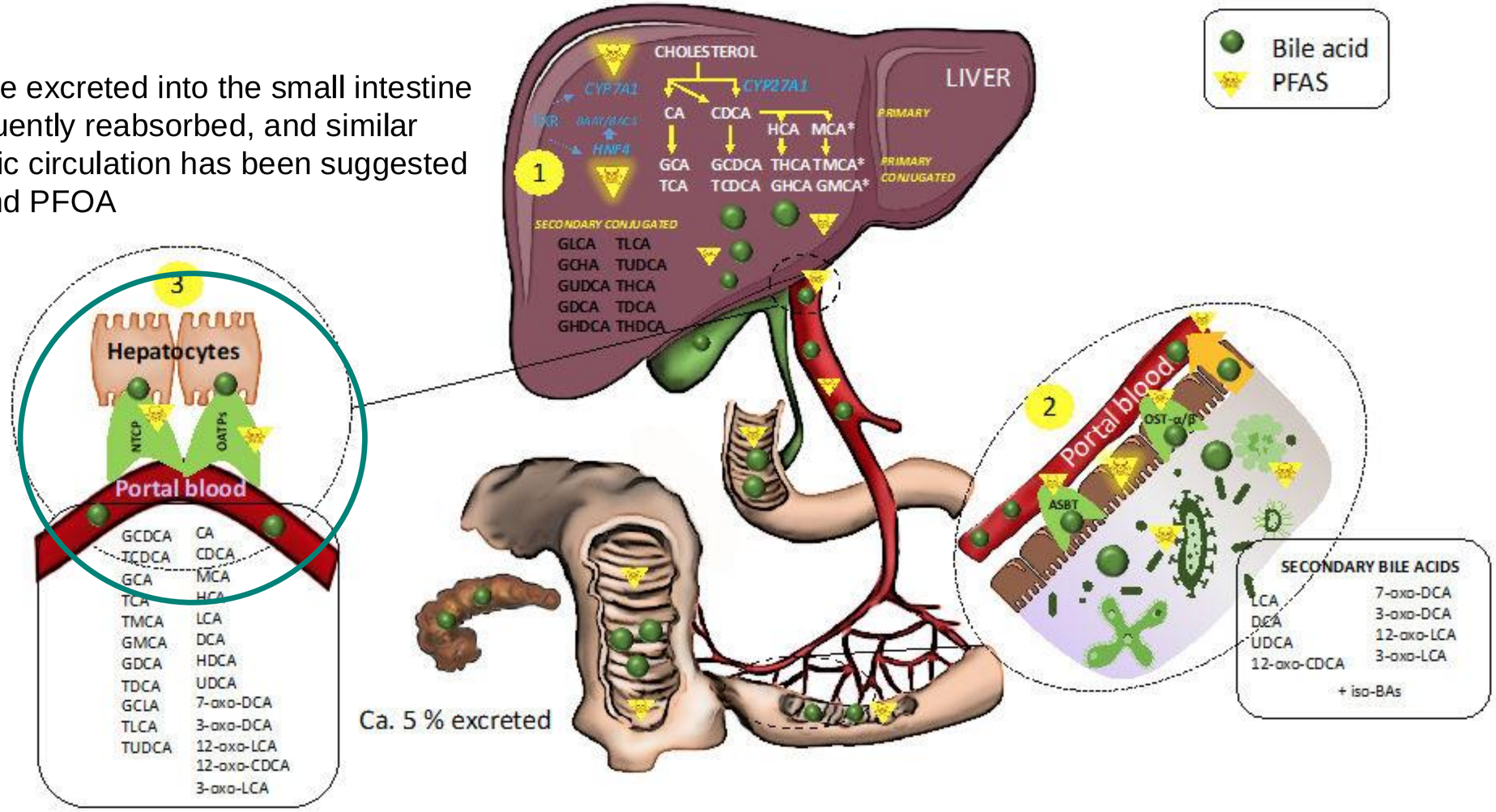


Taurocholic acid



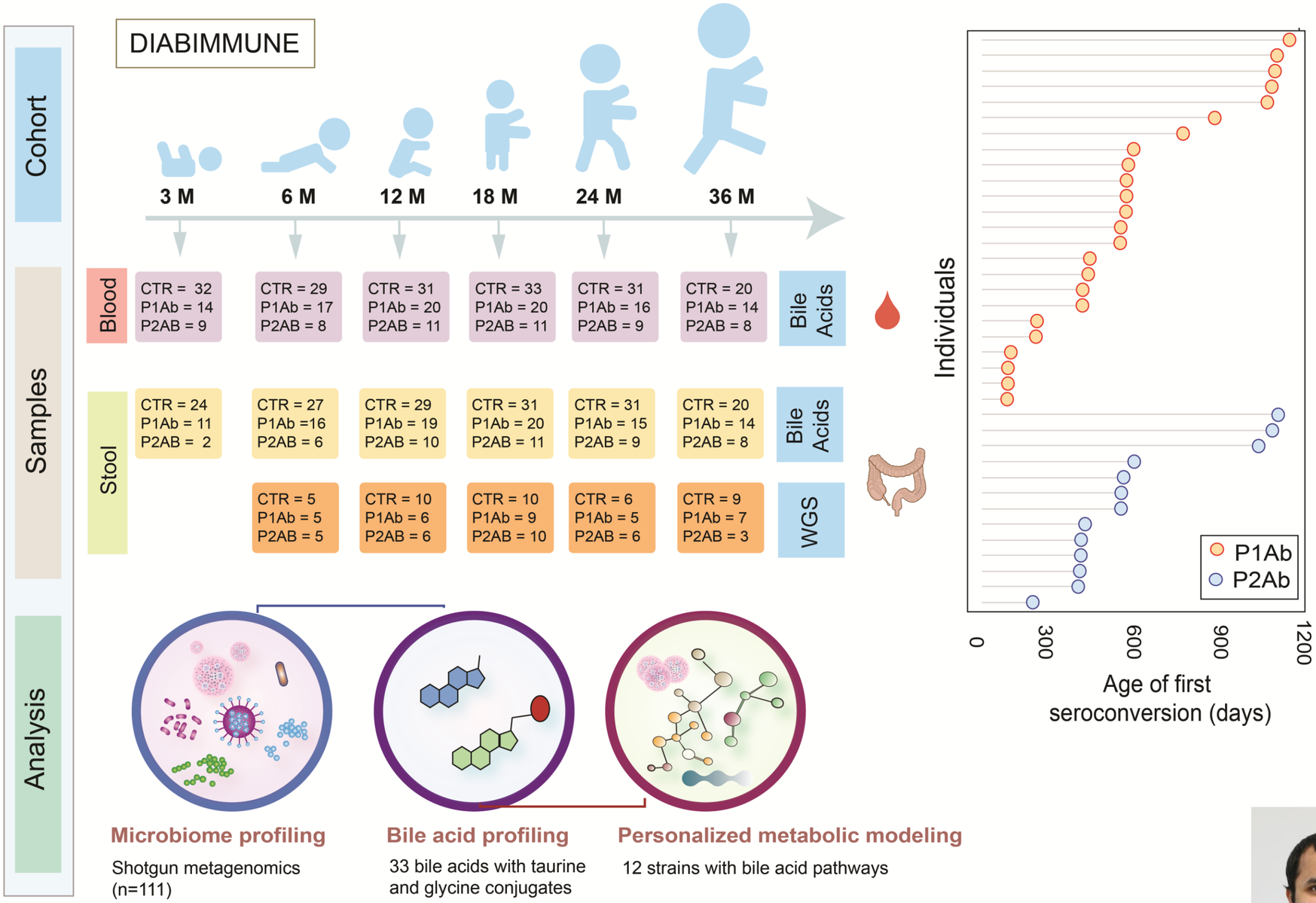
PFAS interfere with bile acid metabolism and impact gut microbiome

Bile acids are excreted into the small intestine and subsequently reabsorbed, and similar enterohepatic circulation has been suggested for PFOS and PFOA



The bile acid transporters found in the liver are also found in the **intestine** and **placenta**.

Orešič M, et al. Metabolites. 2020;10(11):454.

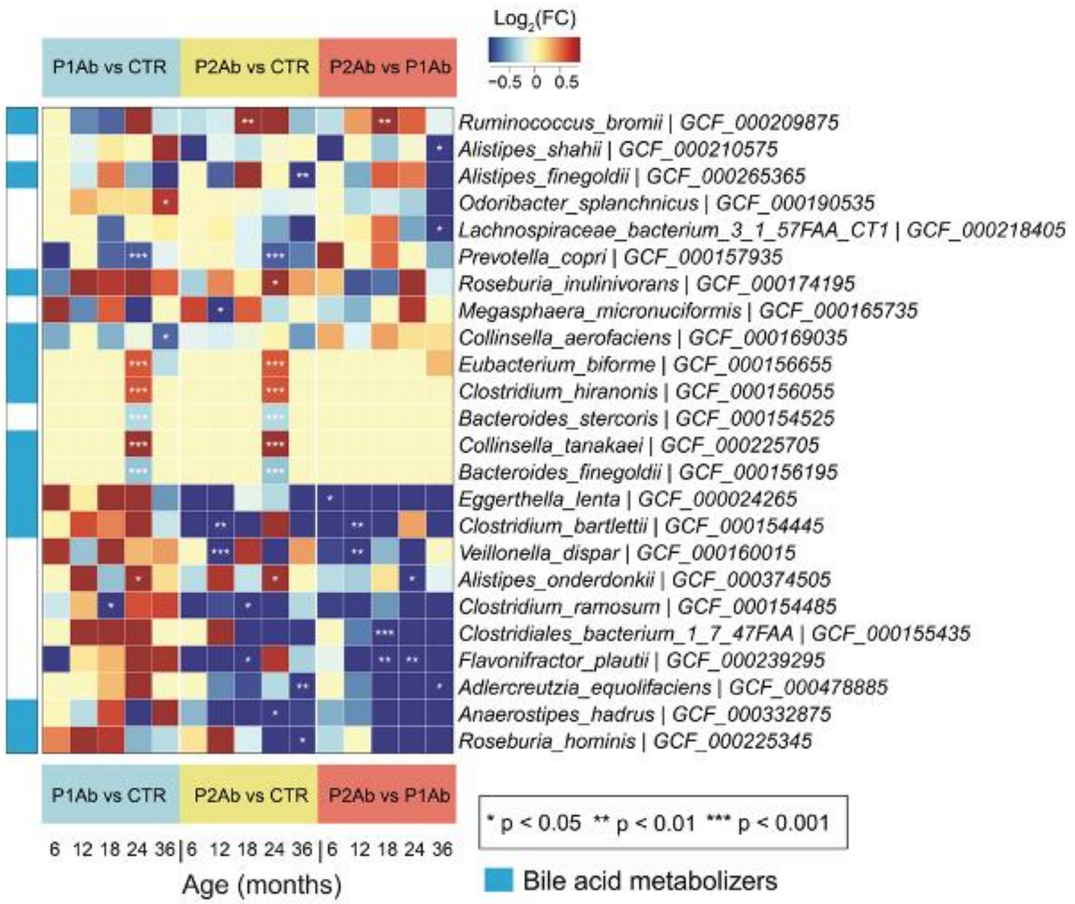


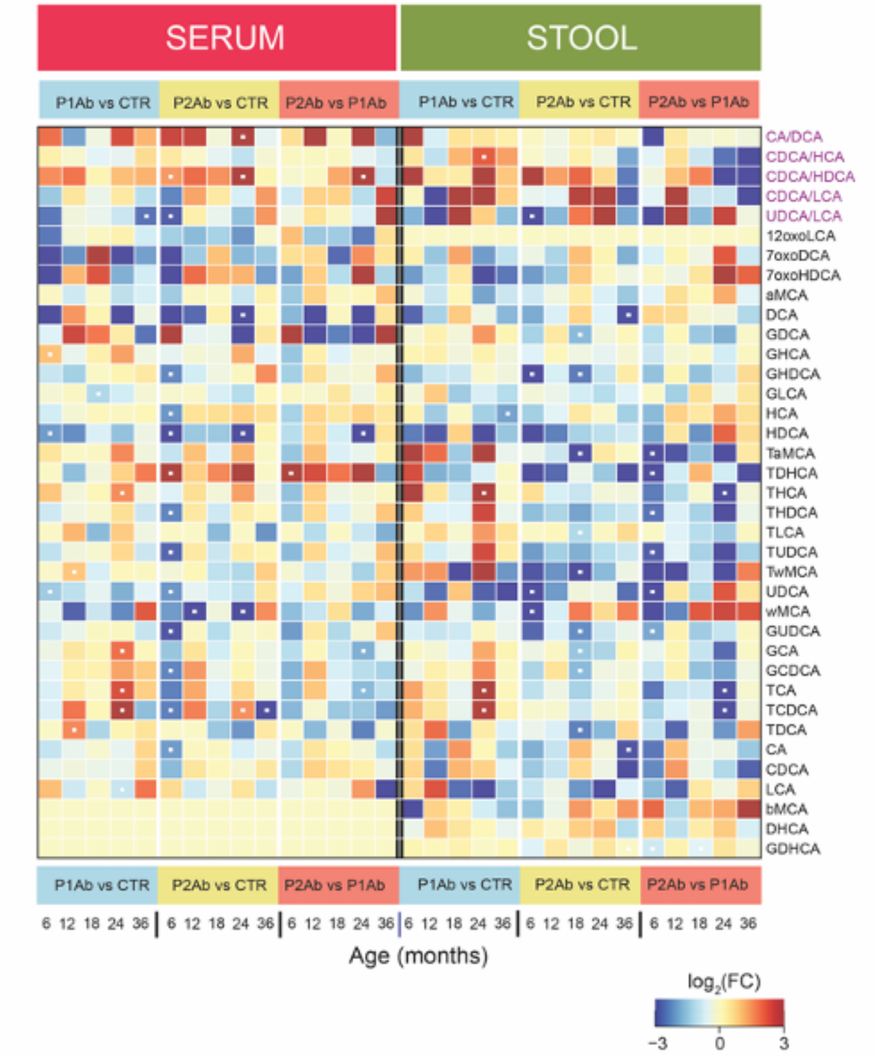
Santosh Lamichhane.

Partho Sen

Lamichhane S, Sen P, …Knip M, Orešič M. Cell Rep Med. 2022

Several microbes metabolizing bile acids are decreased in P2Ab group





Genome scale metabolic modeling of gut microbial communities predicted decreased production of secondary bile acids in P2Ab group.

Lamichhane S, Sen P, …Knip M, Orešič M. Cell Rep Med. 2022

Discovery of microbially conjugated bile acids (MCBAs)

Article

Global chemical effects of the microbiome include new bile-acid conjugations

https://doi.org/10.1038/s41586-020-2047-9

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Check for updates

Robert A. Quinn[1,2], Alexey V. Melnik[1], Alison Vrbanac[3], Ting Fu[4], Kathryn A. Patras[3], Mitchell P. Christy[1], Zsolt Bodai[5], Pedro Belda-Ferre[3], Anupriya Tripathi[1,3], Lawton K. Chung[3], Michael Downes[4], Ryan D. Welch[4], Melissa Quinn[6], Greg Humphrey[3], Morgan Panitchpakdi[1], Kelly C. Weldon[1,19], Alexander Aksenov[1], Ricardo da Silva[1], Julian Avila-Pacheco[7], Clary Clish[7], Sena Bae[8,9], Himel Mallick[7,8], Eric A. Franzosa[7,8], Jason Lloyd-Price[7,8], Robert Bussell[10], Taren Thron[11], Andrew T. Nelson[1], Mingxun Wang[1], Eric Leszczynski[6], Fernando Vargas[1], Julia M. Gauglitz[1], Michael J. Meehan[1], Emily Gentry[1], Timothy D. Arthur[3,7], Alexis C. Komor[5], Orit Poulsen[3], Brigid S. Boland[12], John T. Chang[12], William J. Sandborn[12], Meerana Lim[3], Neha Garg[13,14], Julie C. Lumeng[15], Ramnik J. Xavier[7], Barbara I. Kazmierczak[16], Ruchi Jain[16], Marie Egan[17], Kyung E. Rhee[3], David Ferguson[6], Manuela Raffatellu[3], Hera Vlamakis[7], Gabriel G. Haddad[3], Dionicio Siegel[1], Curtis Huttenhower[7,8], Sarkis K. Mazmanian[11], Ronald M. Evans[4,18], Victor Nizet[1,3,19], Rob Knight[3,19,20,21] & Pieter C. Dorrestein[1,3,19] ✉

Article

Bile salt hydrolase catalyses formation of amine-conjugated bile acids

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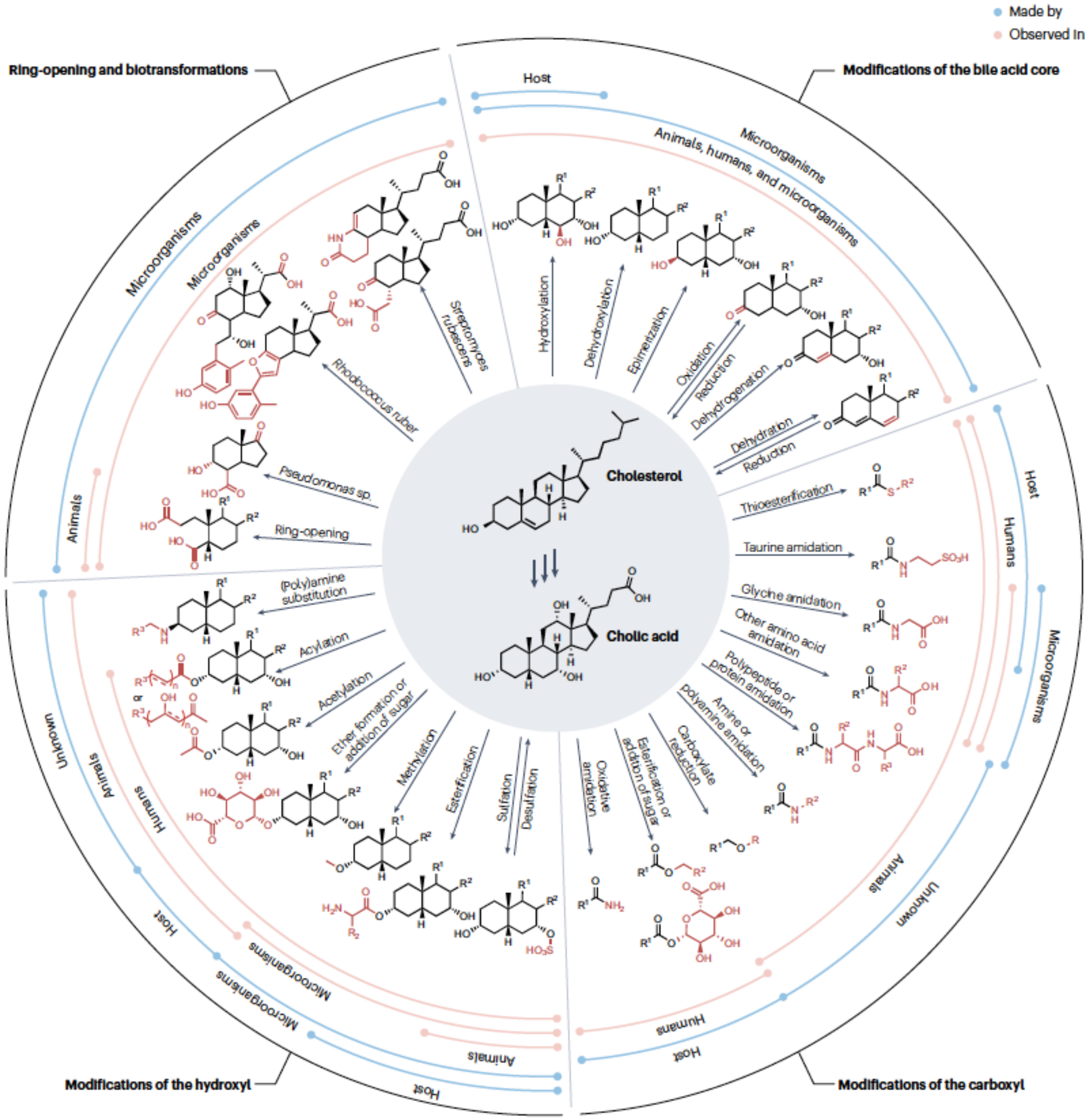
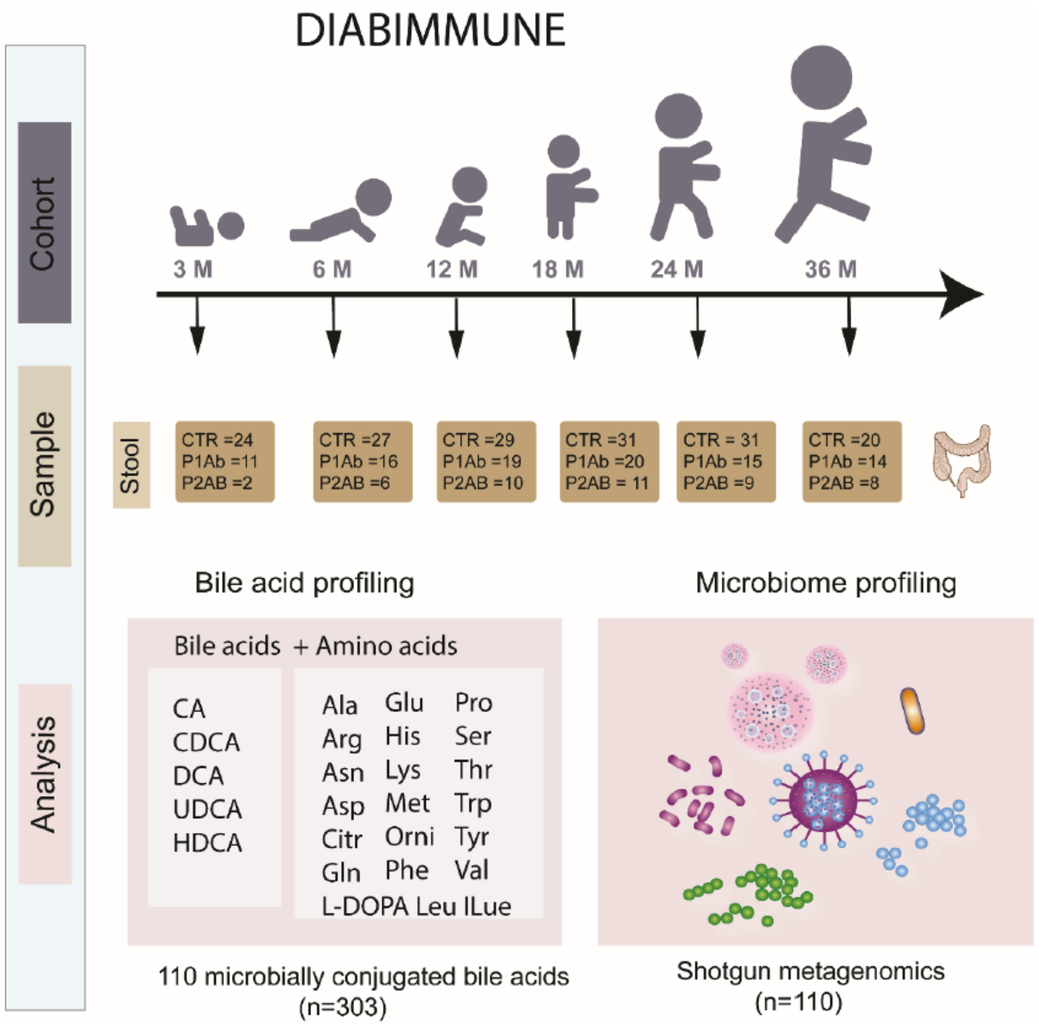


Diversity of known bile acid chemical modifications

Mohanty I et al. *Nat Rev Gastroenterol Hepatol* (2024)

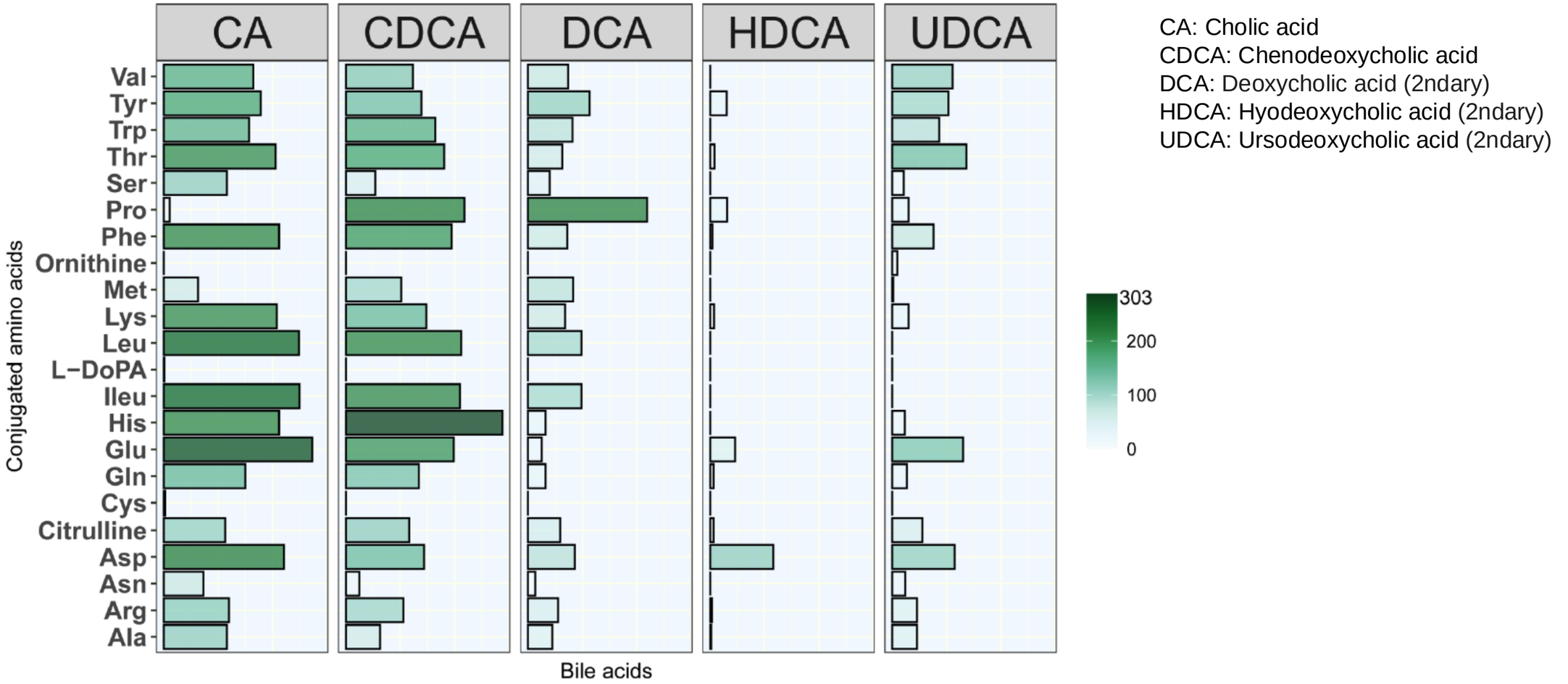
Study of MCBAs in DIABIMMUNE cohort



Aims

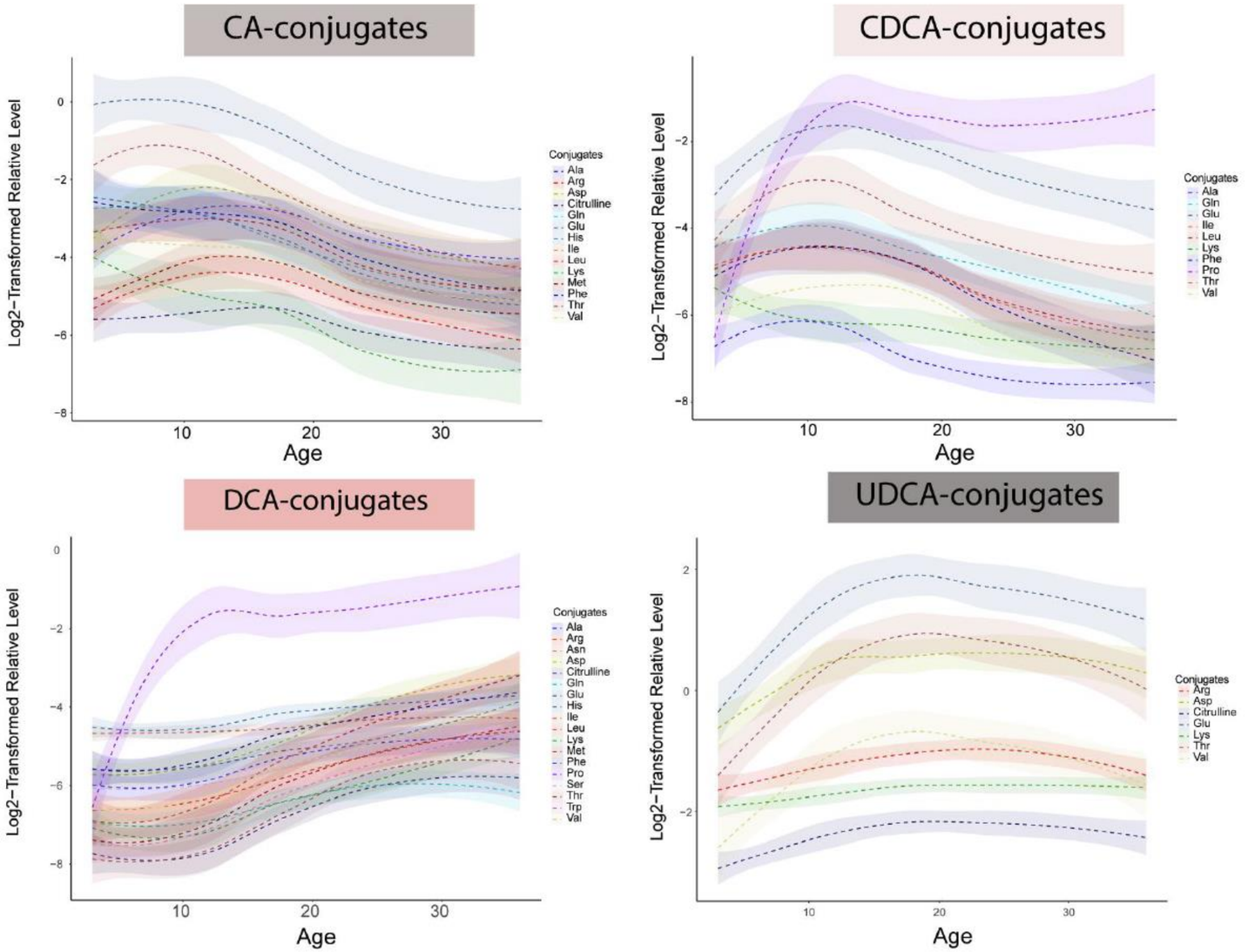
1. Define trajectories of MCBAsin early life (from fecal samples)
2. Study the associations of MCBAs with progression to one or multiple islet autoantibodies

Presence of MCBAs in early life

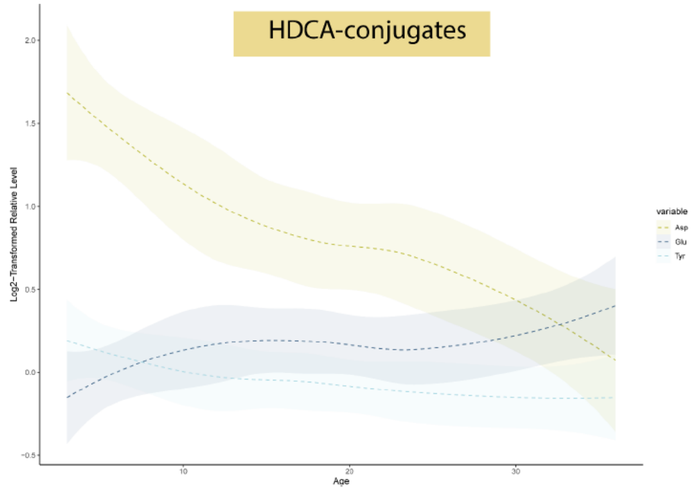


CA: Cholic acid
CDCA: Chenodeoxycholic acid
DCA: Deoxycholic acid (2ndary)
HDCA: Hyodeoxycholic acid (2ndary)
UDCA: Ursodeoxycholic acid (2ndary)

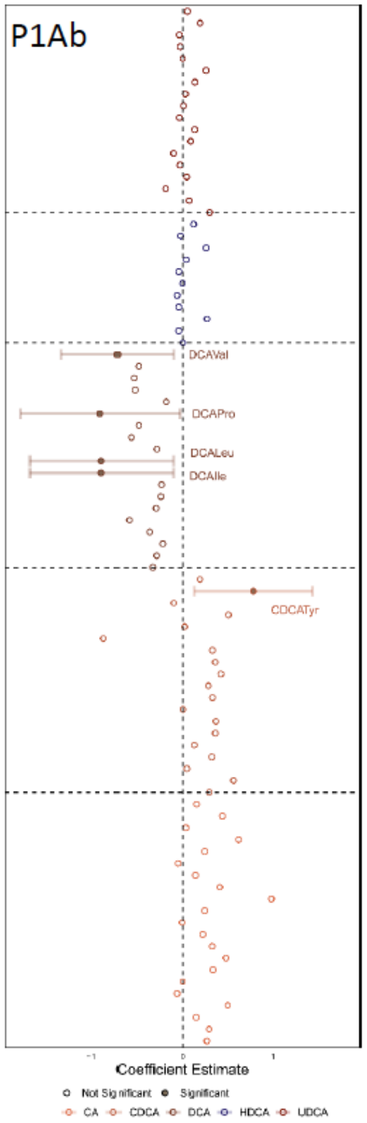
Trajectories of MCBAs in early life

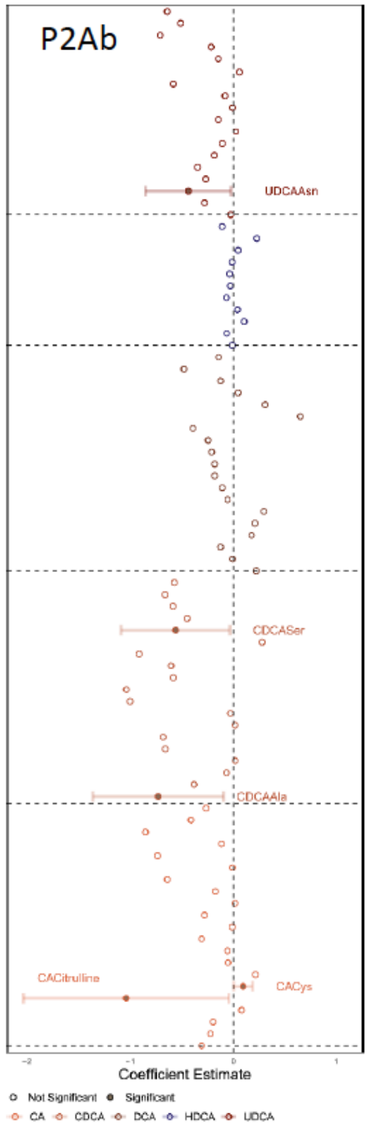


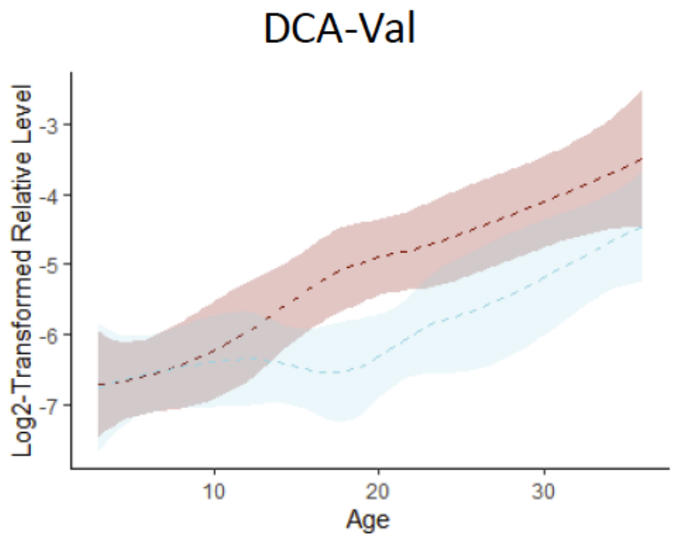
The loess curve plot of MCBA s over time (3, 6, 12, 18, 24 and 36 months).

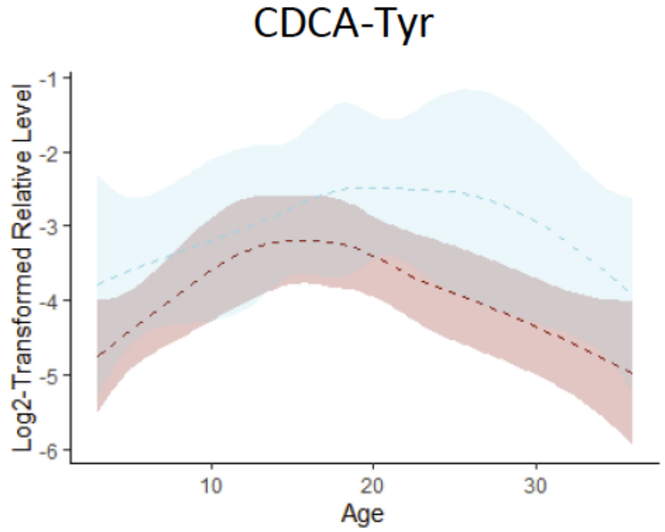


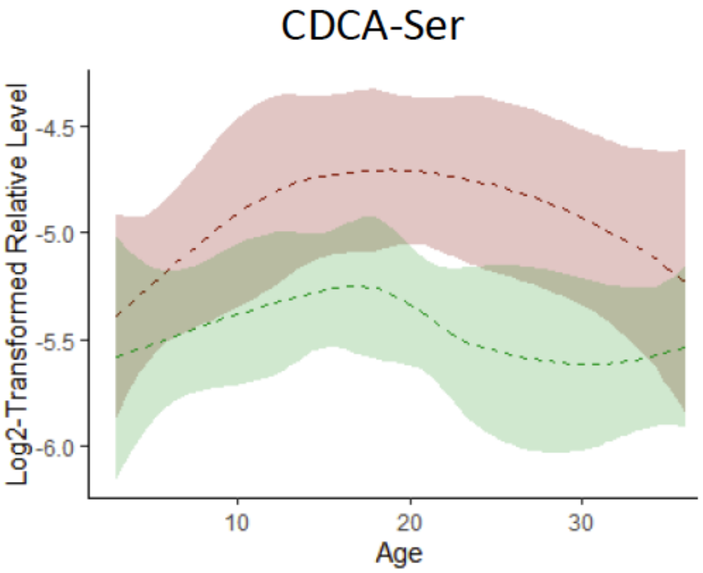
MCBAs in progression to islet autoimmunity

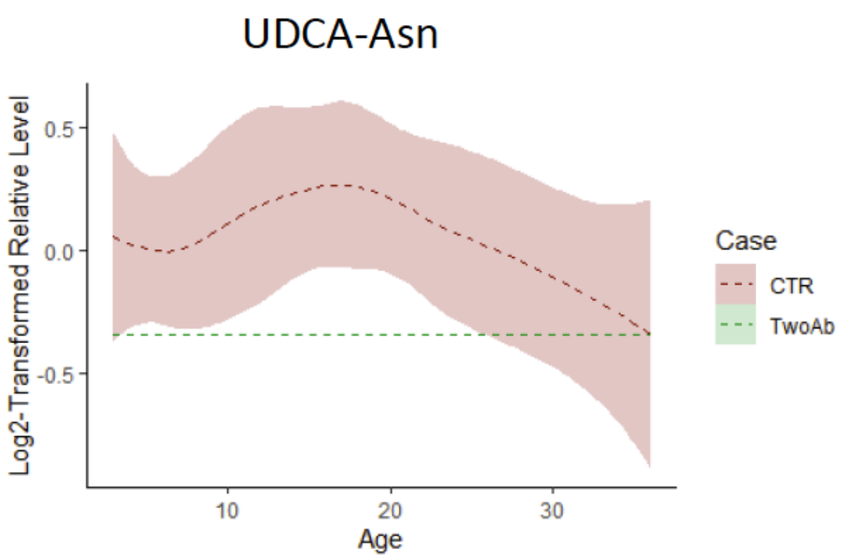




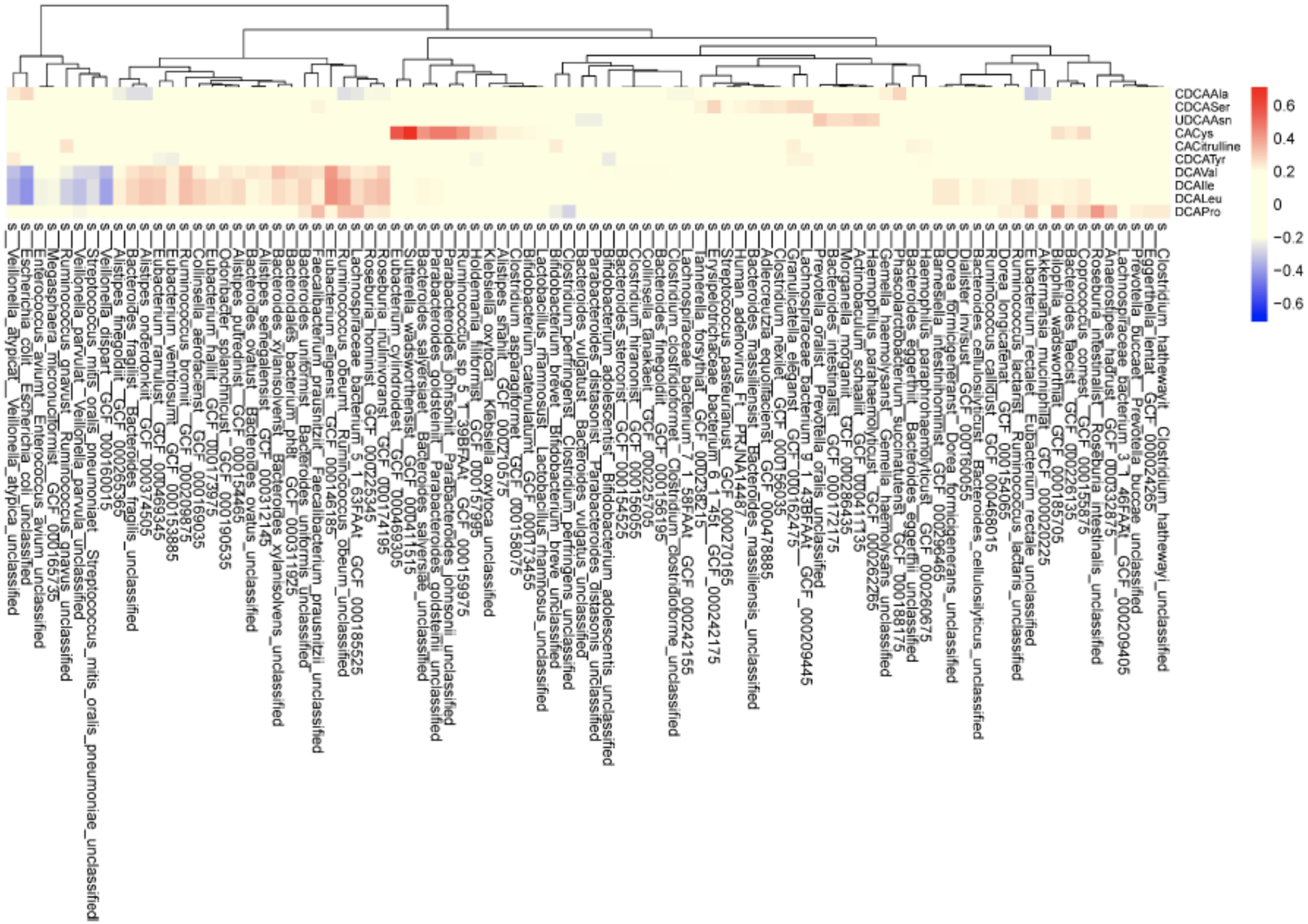




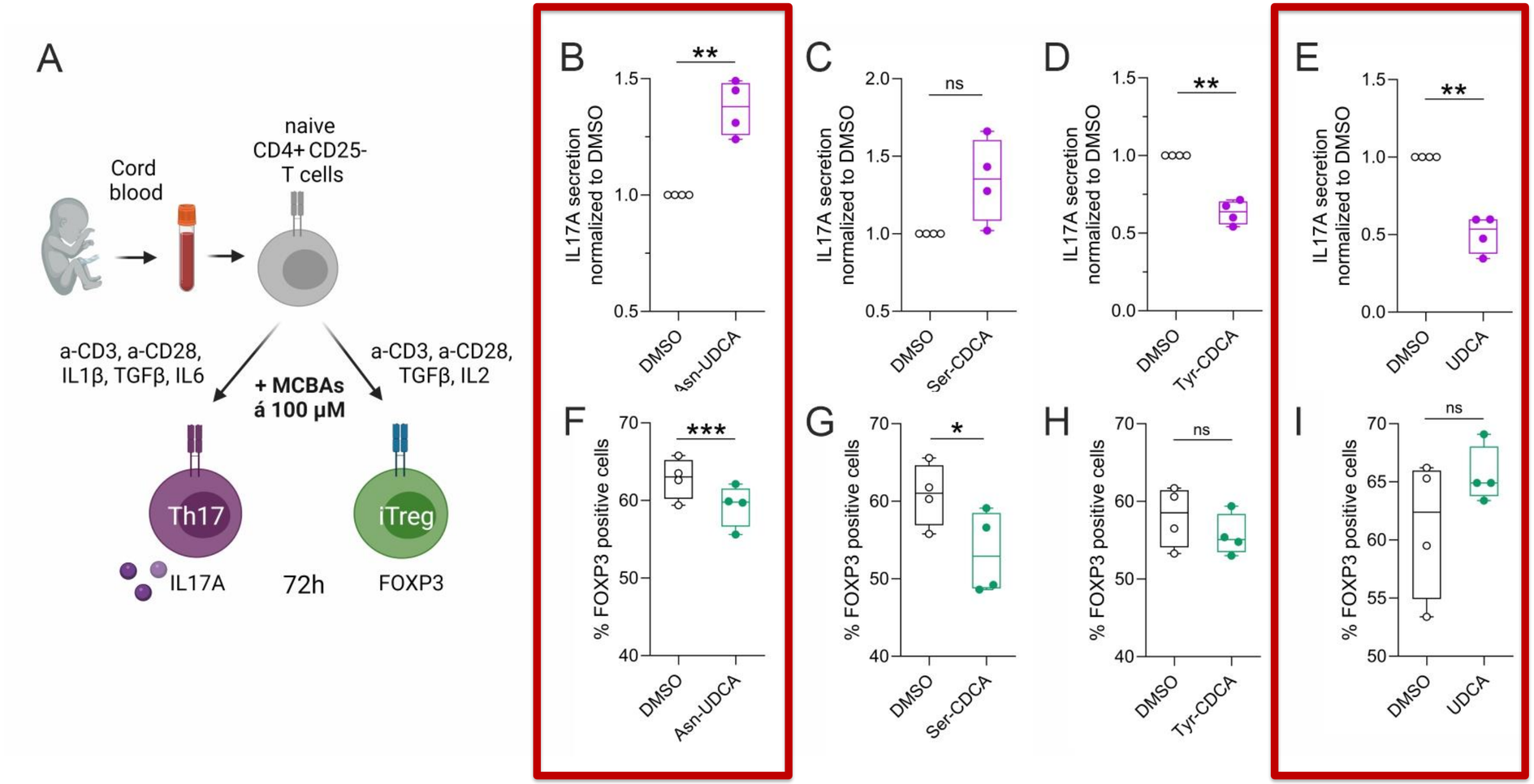




Correlations between the microbes and stool levels of MCBAs altered in progression to islet autoimmunity



Functional experiments (collaboration with Riitta Lahesmaa lab)

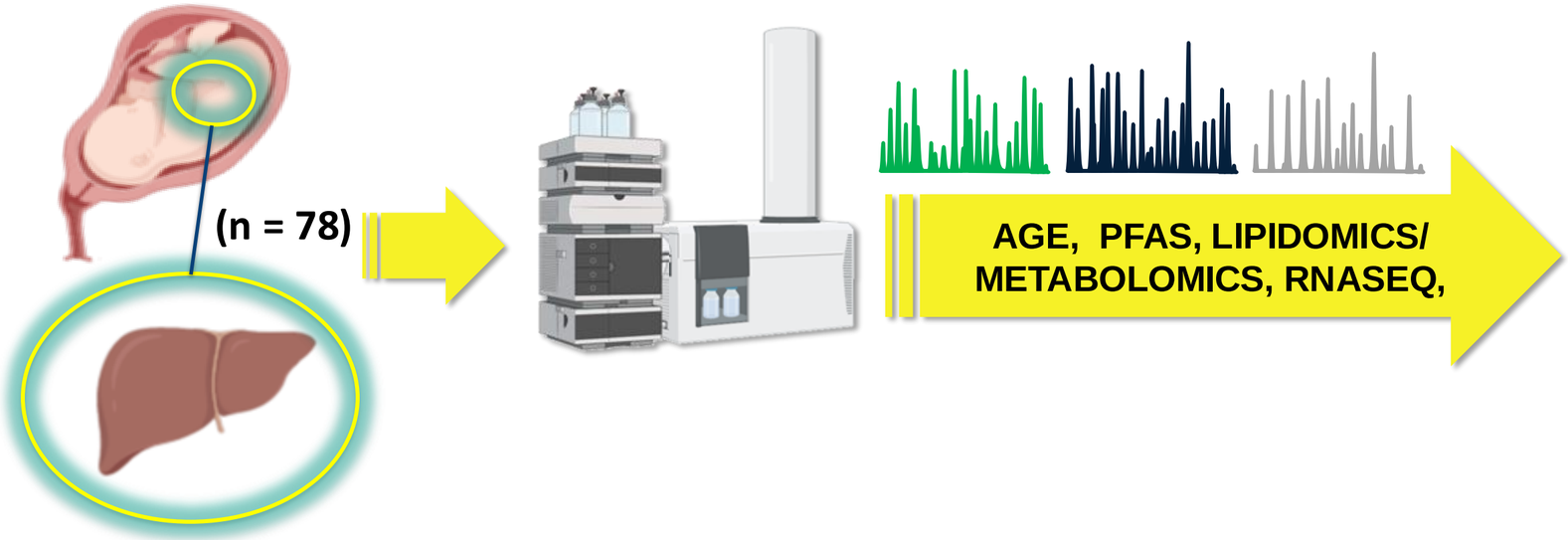


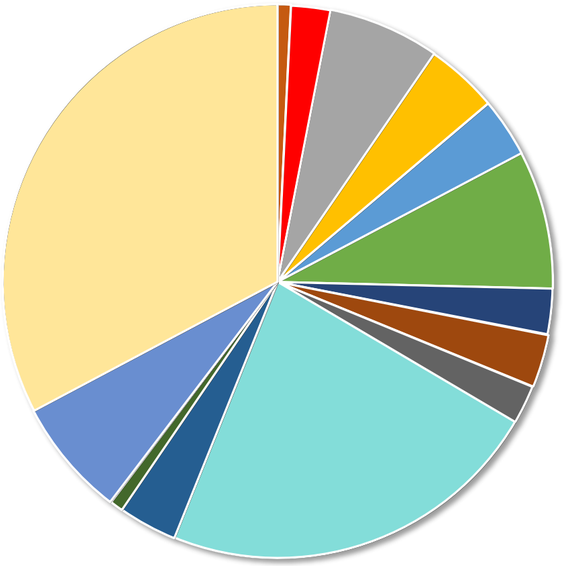
UDCA-Asn is proinflammatory while UDCA is anti-inflammatory

Summary – bile acids and gut microbiome in progression to islet autoimmunity

- Systemic bile acids and the associated gut microbiota were altered in progression to islet autoimmunity.
- Quantitative analysis and community modeling of gut microbiota on a genome-scale revealed dysregulation of secondary bile acid pathways, preceding the seroconversion to islet autoantibodies.
- MCBAs have defined core BA-dependent trajectories in early life.
- Specific MCBAs associate with progression to one or multiple islet autoantibodies
- MCBAs regulate Th17/Treg balance in speciffic manner

Do chemical exposures impact metabolism of human fetus?

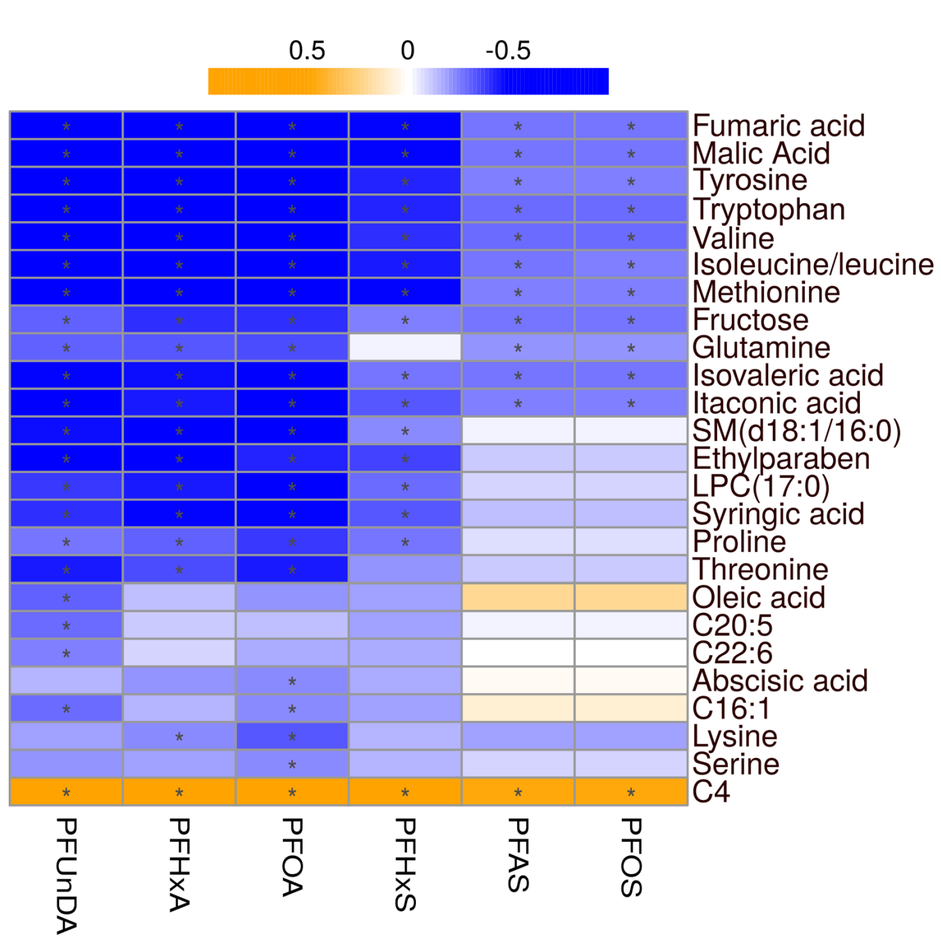


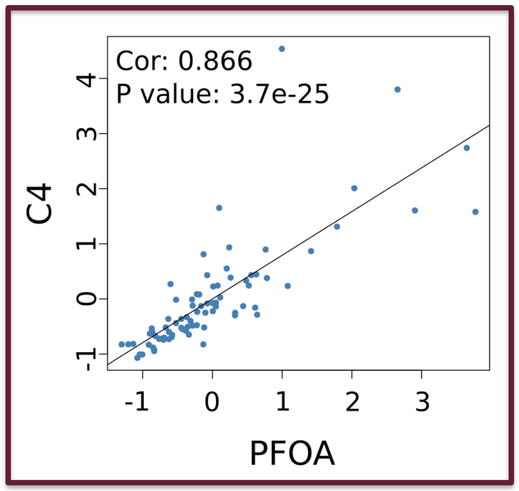


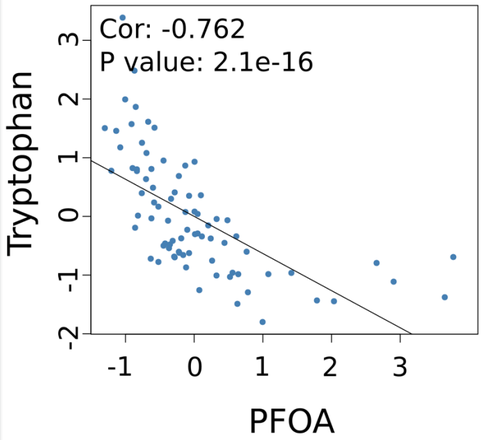
	Female	Male	p-value*
Metabolomics Cohort			
n	39	39	
Weeks of gestation	14.6±.4	15.±.4	NS
Fetal weight (g)	75.7±11.1	85.4±12.4	NS
Fetal crown-rump length (mm)	97±4.5	12±4.5	NS
Maternal age (years)	23±1	26±1	NS
Maternal BMI (kg/m^2)	24.8±.7	25.±.7	NS

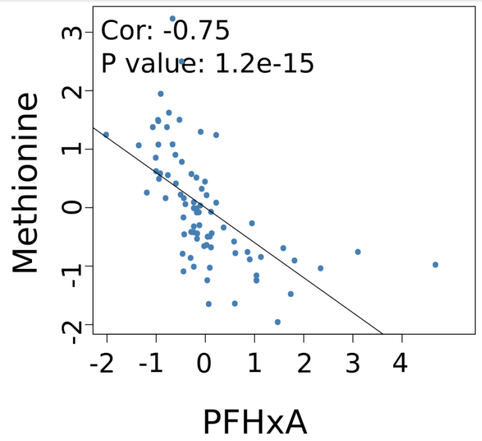
Hyötyläinen T, …, Fowler PA,, Orešič M. Lancet Planetary Health (2024)

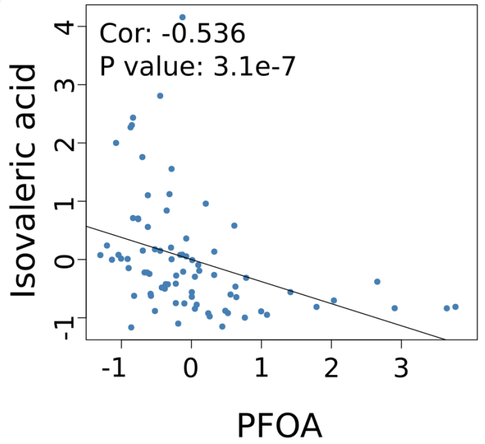
Notable associations of hepatic metabolites with PFAS exposure in human fetus











7α-hydroxy-4-cholesten-3-one (C4), a marker of BA synthesis rate, was strongly positively associated with PFAS levels and was detectable as early as gestational week 12.

Hyötyläinen T, …, Fowler PA,, Orešič M. Lancet Planetary Health (2024)

Conclusions (fetal liver study)

- The data show direct evidence for *in-utero* effects of PFAS exposure on specific key hepatic products.
- Our results provide evidence that PFAS exposure, with potential future consequences, manifests in the human fetus as early as the first trimester of gestation.
- Such exposures are already linked with susceptibility, initiation, progression and/or exacerbation of a wide range of metabolic diseases.

Special thanks!

- Systems Medicine team (Turku)
- Systems Medicine team (Örebro)

… particularly Santosh Lamichhane, Pragya Karmacharya, Matilda Kråkström, Alex M. Dickens, Tuulia Hyötyläinen

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Thank you!