UMC Utrecht

Common Variable immunodeficiency and the digestive tract

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Dept Rheumatology & Clinical Immunology

Outpatient clinic
2019 new patients: 1532 (immunology 346)
2019 patients in fu: 9736 (immunology 1999)

Clinical beds capacity n=6

Large out-patient treatment facility

Internist-Clinical Immunologists n=4
Rheumatologists n=5
Residents n=12-16
Nurse Specialists n=3

Primary Immunodeficiency
Center for immune diseases

Dept Clinical Immunology
Dept Infectious Diseases
Dept of Pediatric Immunology

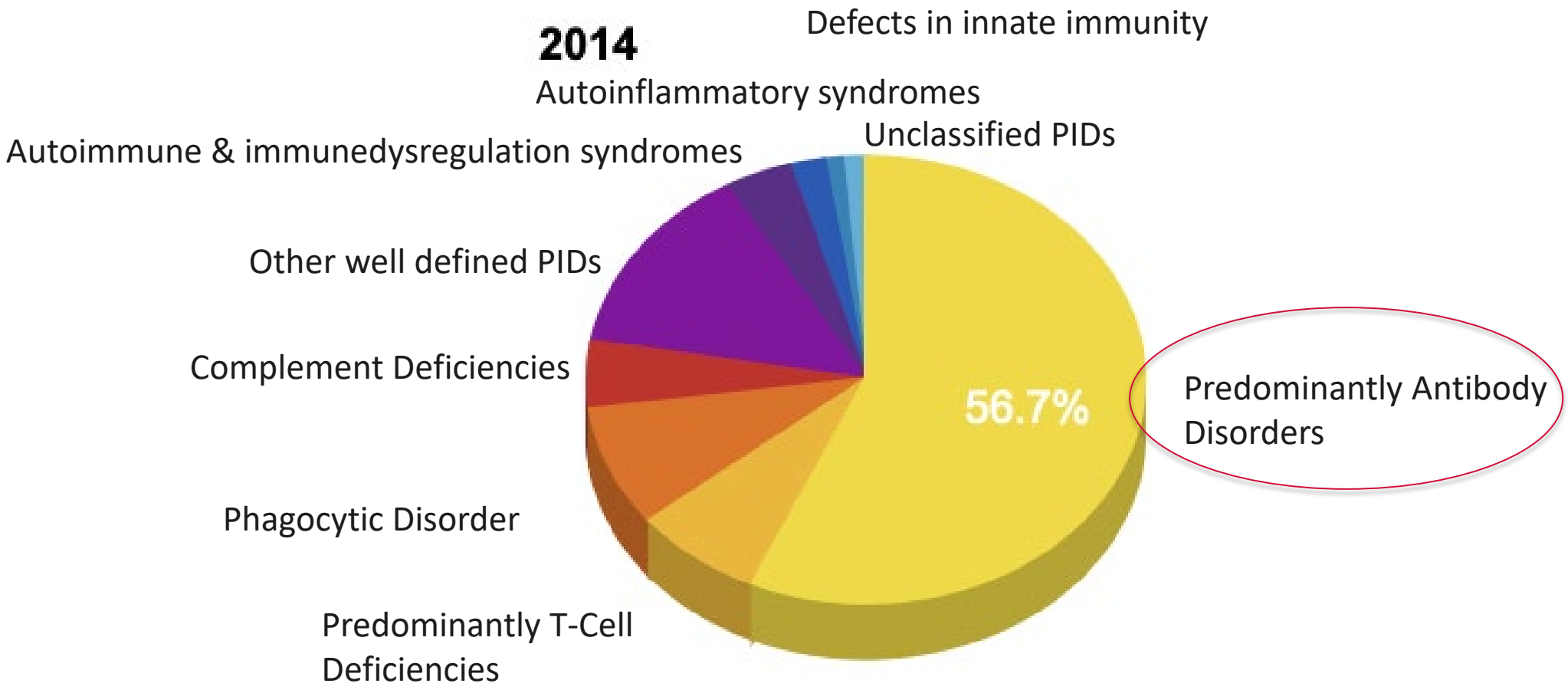
Dr Joris van Montfrans, pediatrician
Dr Pauline Ellerbroek, infectiologist
Dr Anke Bruns, infectiologist

n~500

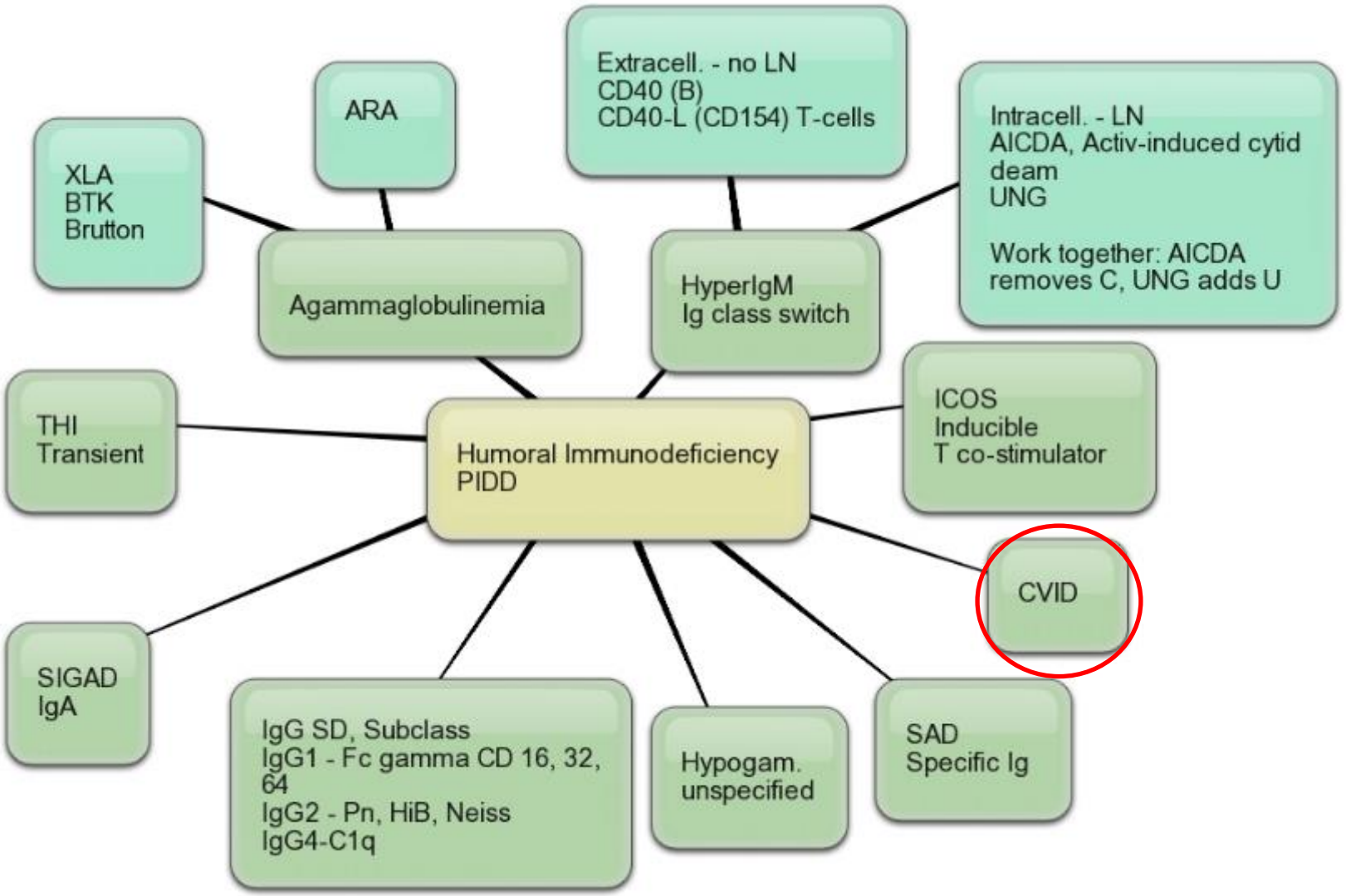
Covered topics

- IEI/PID and CVID
- CVID immunology
- Case
- Gastrointestinal complications
- Enteropathy
- Liver: nodular regenerative hyperplasia and portal hypertension/cirrhosis
- CVID and complication: distinct cytokine profile and T-cell repertoire
- Dysbiosis and outgrowth of pathobionts?

Inborn Errors of Immunity



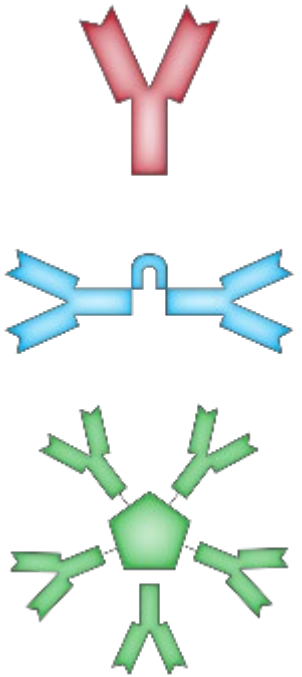
from www.esid.org



Curr Allergy Asthma Rep. 2009

CVID

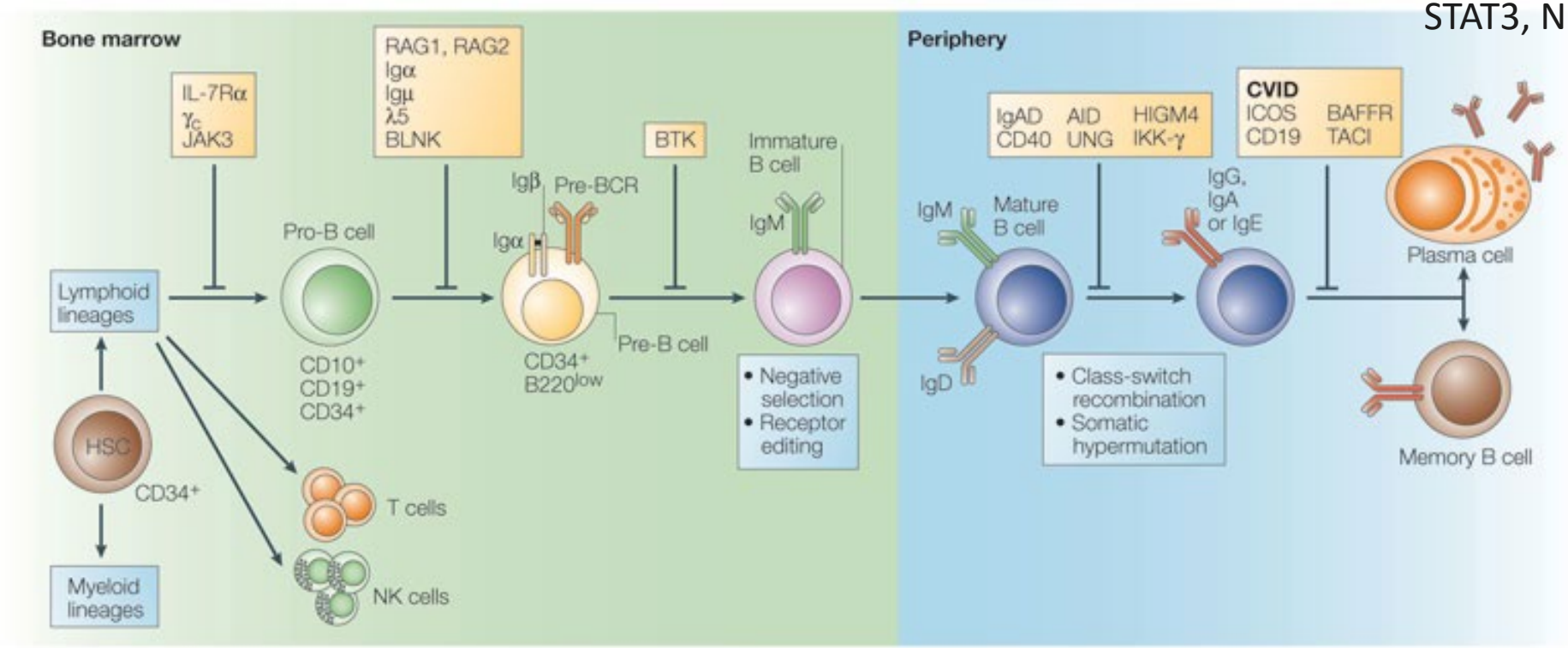
- **C**ommon
  - Prevalence 1:20.000 – 1:50.000
- **V**ariable
  - Clinically heterogeneous
  - Age of onset
- **I**mmuno**d**eficiency Disorders
  - Low IgG, and low IgA or IgM
  - Poor response to polysaccharide vaccines



ESID criteria vs Ameratunga criteria

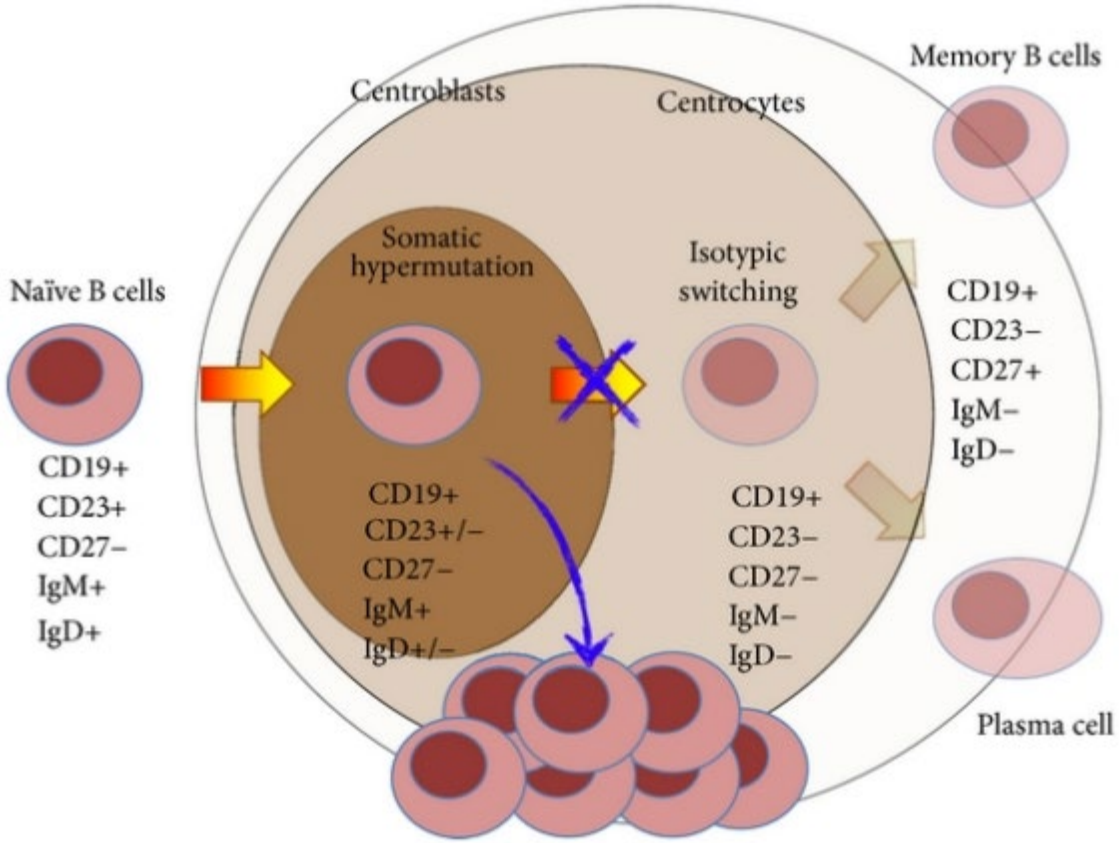
CVID: Disturbed terminal B cell differentiation

CTLA4, LRBA, PI3KCD, STAT3, NFKB1

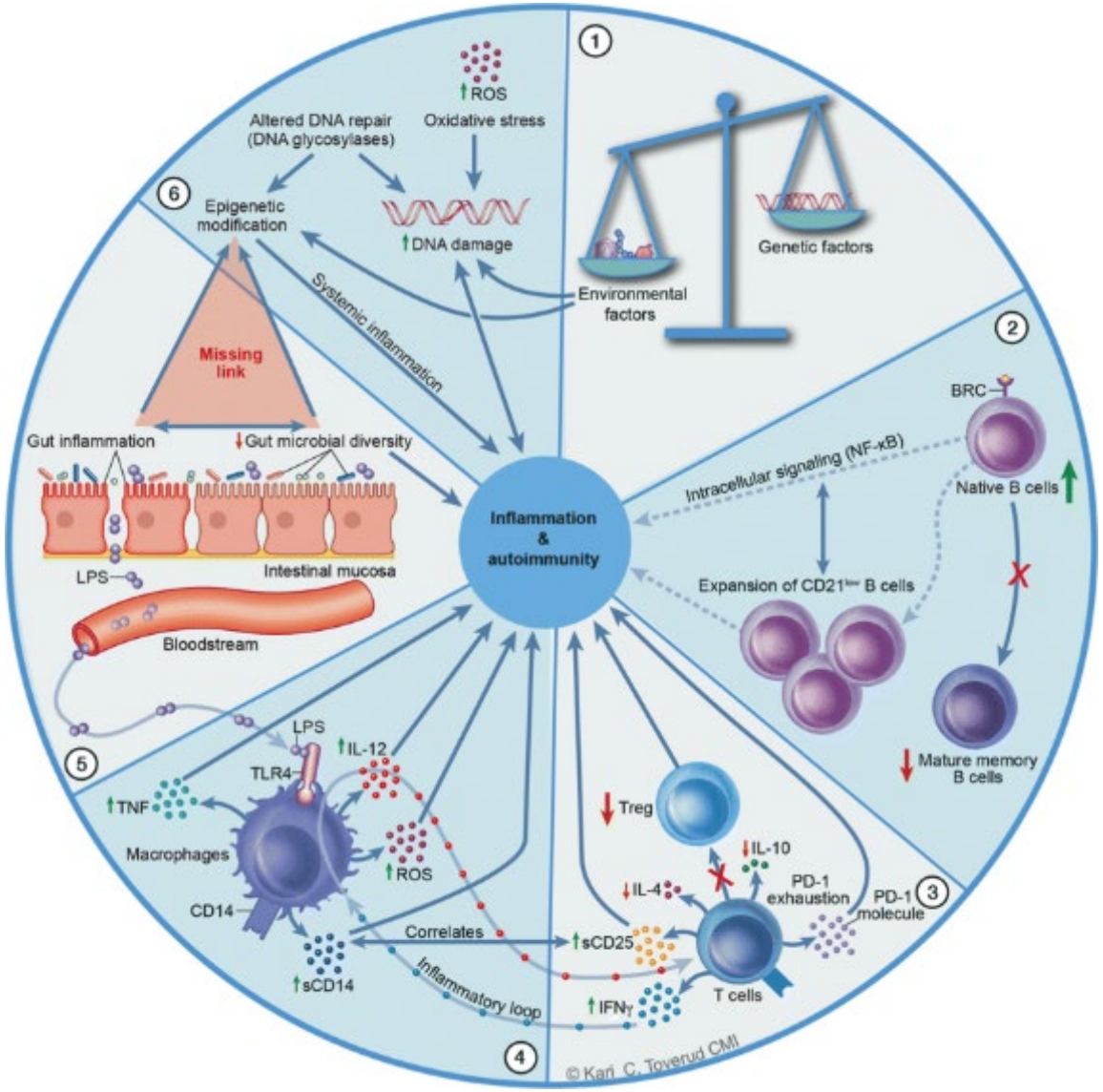


- IgG usually <3g/L, IgA <0.05, 50% IgM reduced
- ~90% normal peripheral B cells

More than defective B cells

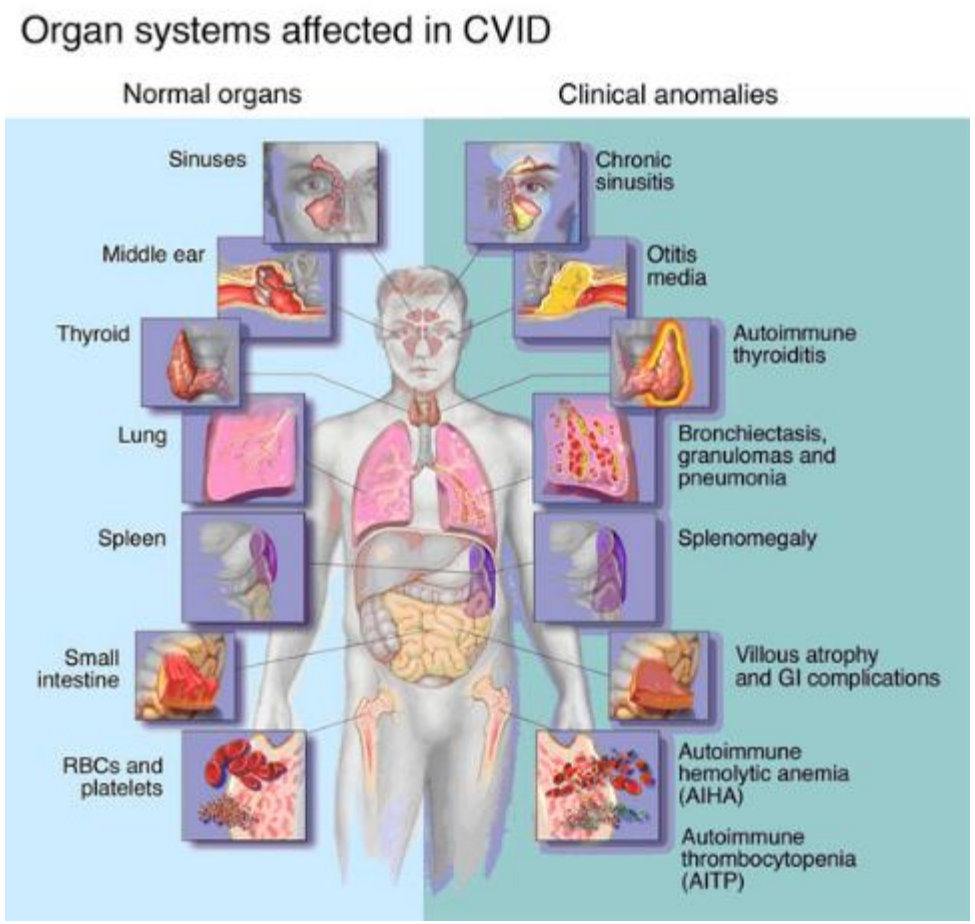


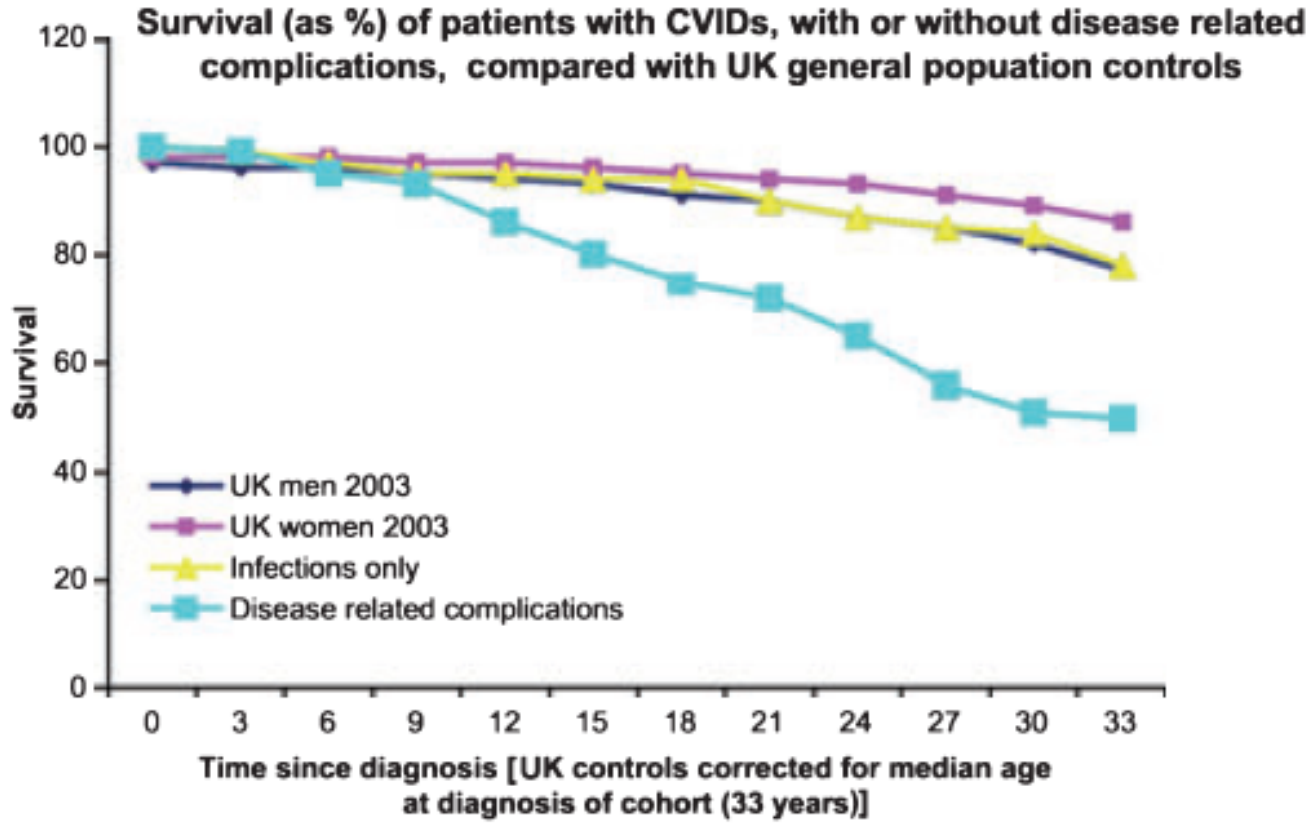
ISRN Immunol 2013



J Clin Immunol 2019

CVID: non infectious complications in IgA-





British Journal of Haematology, **145**, 709–727

- Recurrent respiratory- and GI tract infections
  → bronchiectasis
- ~50% inflammatory complications: Autoimmunity, Lymphoproliferation, Granulomas, Malignancy

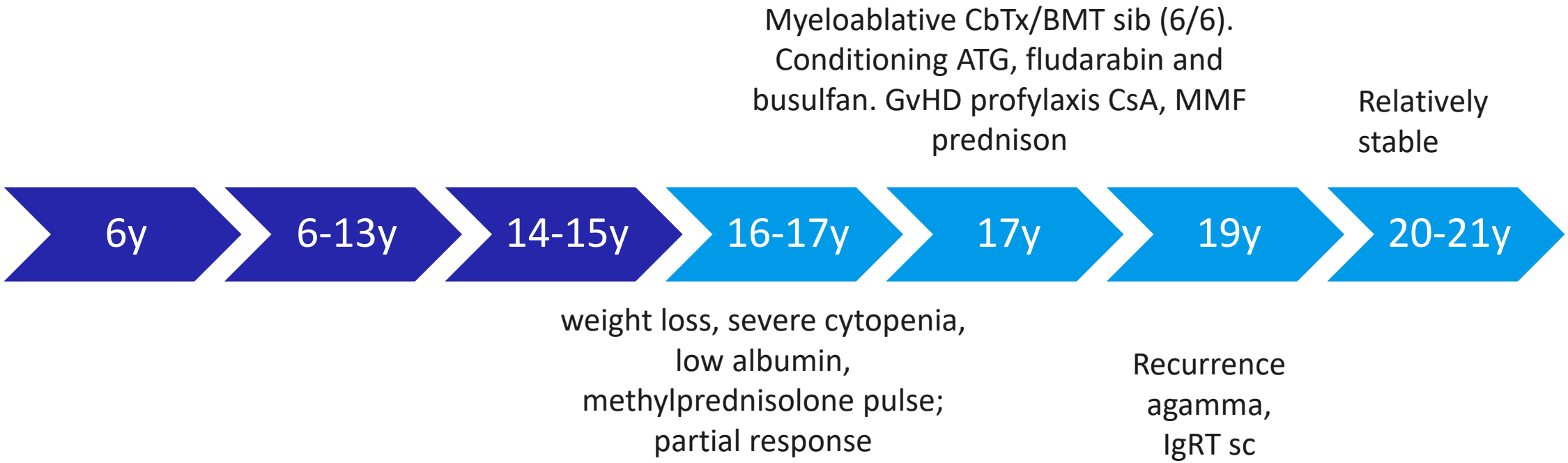
Male, currently 27y old; First 2 decades CVID

normal growth/development, few infections

CVID: recurrent RTIs, empyema
B and T cell count n,
IgG 0.25 g/L,
IgA/IgM low
IgRT

weight loss, diarrhea, low albumin:
PLE, Noro + parecho +: AI enteropathy
AI granulocytopenia, ITP, splenomegaly
CS, Tacrolimus
Middle lobe syndrome, no restriction or obstruction

Allo HSCT


Myeloablative CbTx/BMT sib (6/6). Conditioning ATG, fludarabin and busulfan. GvHD profylaxis CsA, MMF prednison
Relatively stable
6y
6-13y
14-15y
16-17y
17y
19y
20-21y
weight loss, severe cytopenia, low albumin, methylprednisolone pulse; partial response
Recurrence agamma, IgRT sc

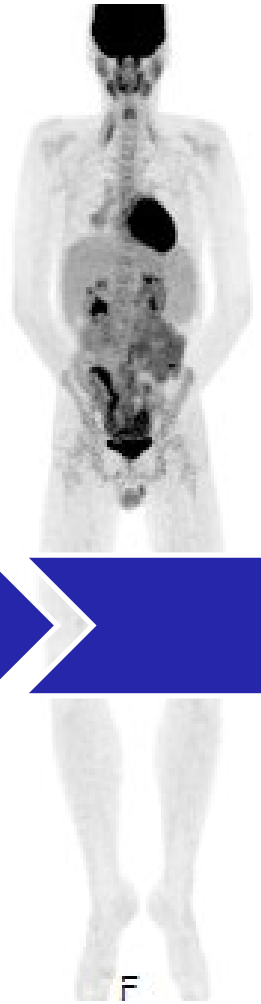
Recurrent CVID with immune dysregulation

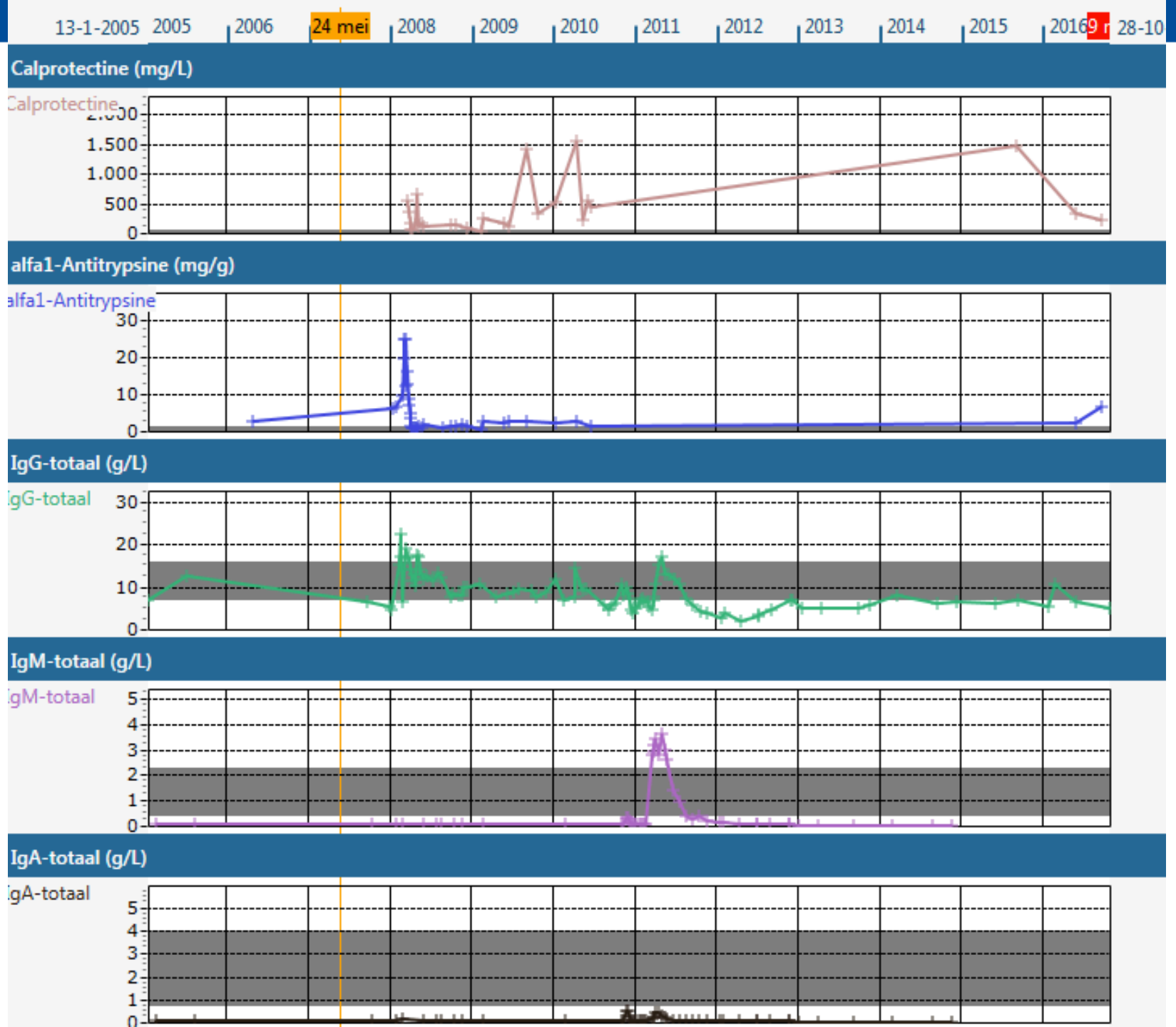
liverbiopsy: conserved architecture, severe ductopenia consult hepatologist (DD cGVHD?)

22-23y → 23y → 24y

repeated ileo-ileal invaginations
lymphoid nodular hyperplasia
increased fecal calpro, diffuse FDG uptake short bowel, lnn and ileum on PET; persistent noro/adeno, prednison and MTX w good response

Relatively well
stop MTX

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Calprotectine
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1.000
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alfa1-Antitrypsine (mg/g)
alfa1-Antitrypsine
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IgG-totaal (g/L)
gG-totaal
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IgM-totaal (g/L)
gM-totaal
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IgA-totaal (g/L)
gA-totaal
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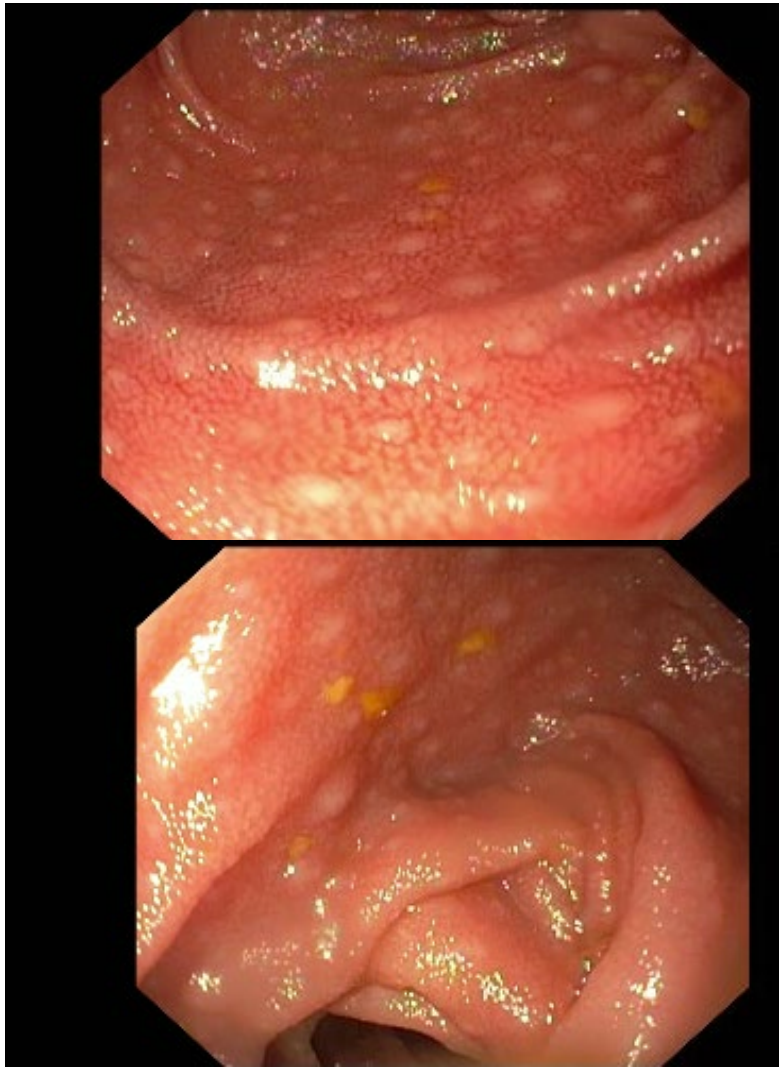
Lymphoid nodular hyperplasia



microscopy ileum:

- blunted villi, loss of globletcells due to reactive/ regenerative changes
- intra-epithelial lymphocytosis related to CVID
- lymphoid aggregates with reactive germinal centers due to nodular hyperplasia
- reactive changes possibly secondary to invagination, no cause found

Progressive inflammation

recurrence diarrhea, no invaginations, significant wasting, failure to MMF and adalimumab, PET CT no lymphoma suspected

gastroscopy: oesophageal varices, PA CVID enteropathy also in stomach Osteoporotic vertebral fractures

admitted wasting, therapy refractory diarrhoea due to CVID enteropathy noro + adeno +, also meningococcal bacteremia (no sepsis), ascites, spontaneous bacterial peritonitis despite azithromycine/cotrim 480; remission induction prednison

COVID period: only phone contact, no visit to out patient clinic

Recurrence initial CVID + IF

- CVID enteropathy, noro + adeno +
- Suspected cirrhosis, low serum albumin, yet normal INR and factor V
- AI cytopenia
- Failed to different lines immunosuppression
- Recurrent CMV reactivation, clostridium diarrhoea, GNS bacteraemia

- Interferonopathy?
  - Galectin-9 / CXCL10 +++
  - Interferon type 1 signature 15,08 (ref 7)
- Warnatz Freiburg: multipele pt gut biopsies CVID type 2 IFN signature (ESID 2020)
- Activation IFN pathways: Shulzhenko et al. Clin Immunol 2018, https://pubmed.ncbi.nlm.nih.gov/30240602/

Current state

- Currently on baricitinib; pt very weak, shaky equilibrium, TPF
- IFNopathy jDM: galectin 9 en CXCL10 validated as disease activity markers

  Wienke et al. Arthritis Rheumatol 2019
- Monogenetic (autoinflammatory) interferonopathy case series: JAKi good response

  Hoffman and Broderick JCI 2018
- JAKi registered for therapy of colitis in IBD, RA and currently positioned in SLE, pSS and other CTDs in trials

Gastrointestinal complications in CVID

- Acute or chronic infectious diarrhoea (20-60%)
- Non-infectious enteropathy (9-32%)
  - Small bowel villous atrophy
  - Inflammatory Bowel Disease like
- Liver disease: Nodular Regenerative Hyperplasia +- portal hypertension/cirrhosis, (chronic and autoimmune) hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis (10 -> 32%?)
- Granulomatous disease (10-20%): non caseating, mostly lungs, liver, lymph node, also in GI tract
- Malignancies: NHL, mostly EBV-negative often extranodal incl. GI tract

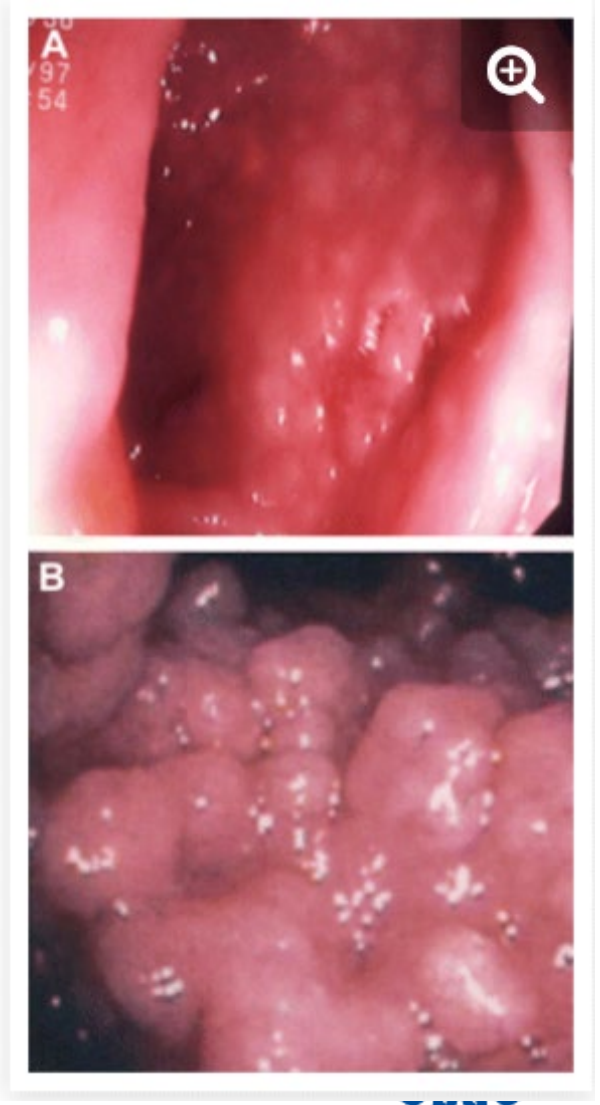
Immunol Allergy Clin N Am 2019
JACI 2014

Infections

- *Giardia lamblia*
- Campylobacter
- Salmonella
- Norovirus, adenovirus
- CMV (very rare)
- Helicobacter pylori ~ gastritis, gastric dysplasia and gastric cancer

Non-infectious inflammation: enteropathy

- Gut biopsy villous atrophy, no real coeliac disease
- many lymphocytes, lymph node aggregates, granuloma, distorted crypts, lack/reduction of plasmacells, intraepithelial CD8+ T cells, Increased IL12 and IFNg production
- Often nodular lymphoid hyperplasia: if massive > chronic diarrhoea and weight loss
- Bacterial overgrowth
- IBD like: ulcers and cobblestone



Blood 2010
Curr Gastroenterol Rep 2016
Gastroenterology 2006

How to diagnose?

- Difficult to distinguish
- Culturing/PCR
- Biopsies
- Test treatment
  - Antibiotics
  - immunosuppressant: budesonide, prednison, azathioprine

How to treat?

- Treat underlying cause
- Treat bacterial overgrowth
- Impossible to restore mucosal immunity completely
- Screen for vitamin deficiency and supplement

- glutenfree diet?
  - 20% improve, mostly w HLA coeliac disease risk profile
  - Usually no cure, sometimes **more** weight loss

- Norovirus: ribavirin/nitazoxanide?? enteral IgG?; target inflammation?
- Enteropathy/IBD: budesonide, azathioprine, mercaptopurine, TNFi, ustekinumab
- Vedolizumab probably not so effective

Curr Gastroenterol Rep 2016

ELSEVIER

Clinical Immunology

Volume 212, March 2020, 108362

Brief communication

Vedolizumab therapy in common variable immune deficiency associated enteropathy: A case series

Travis Sifers [a], Robert Hirten [b], Saurabh Mehandru [b, d], Huaibin Mabel Ko [c], Jean-Frederic Colombel [b], Charlotte Cunningham-Rundles [a, d]

Vedolizumab blocks the interaction of α4β7 integrin with MAdCAM-1, preventing leukocytes from adhesion with endothelial surfaces, thereby reducing T cell trafficking to the gut.

N=6/7 not effective;

Subset with benefit?

Complications?

- Infections could be difficult to cure: giardiasis, norovirus
- IgG loss due to leaky gut > difficult to achieve reasonable IgG through levels
- nasogastric feeding or even parental feeding

Future?

- Immunoglobuline enteral?
- Faecal microbial transplant? > take care of introducing new infections…

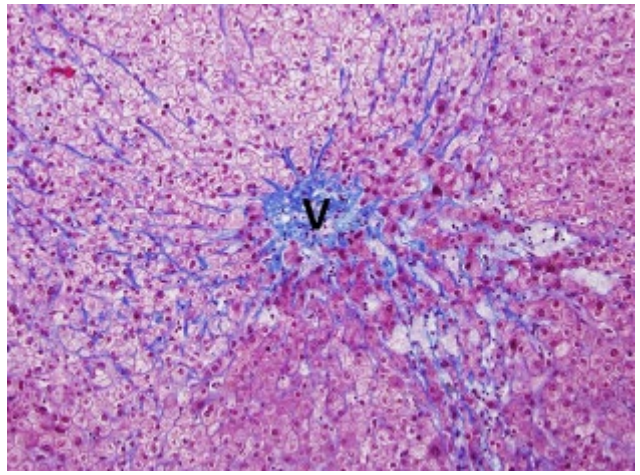
- Study of Safety, Tolerability, and Efficacy of Ustekinumab for Symptomatic Gastrointestinal Inflammation Associated With Common Variable Immunodeficiency NCT02199496 (NIAID) completed aug 2020
- Blocks interleukin (IL)-12/23p40

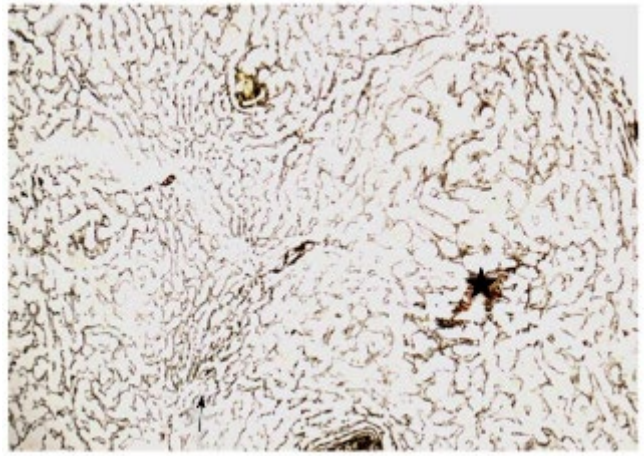
- Abatacept? (GLILD n=10, JACI Practice 2021)
- JAKi?

Increased Liver enzymes and CVID

- Cohort 108 CVID: 44% >6m aberrant liver functions

- Frequent:
  - Medication
  - Alcohol
  - Steatosis
  - Galstones in liver
  - Viral hepatitis: hep C
- Nodular regenerative hyperplasia (NRH)
- Granulomatous hepatitis



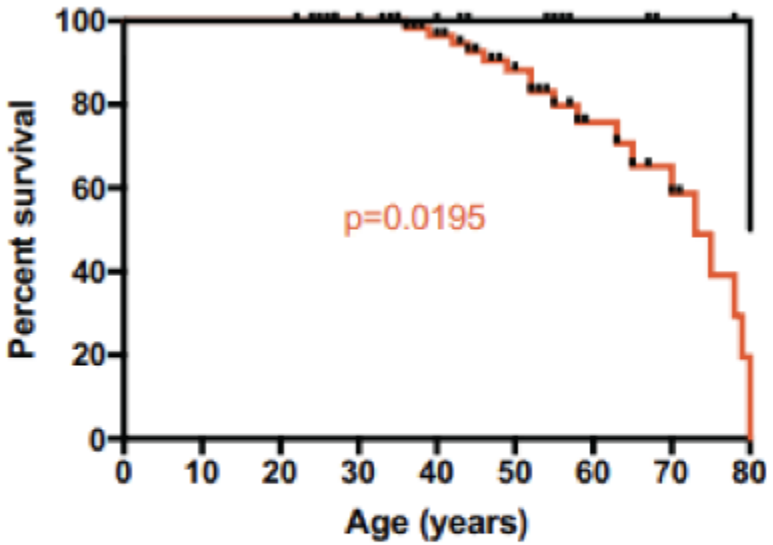


Ward et al. Clin Exp Immunol. 2008

Nodular regenerative hyperplasia

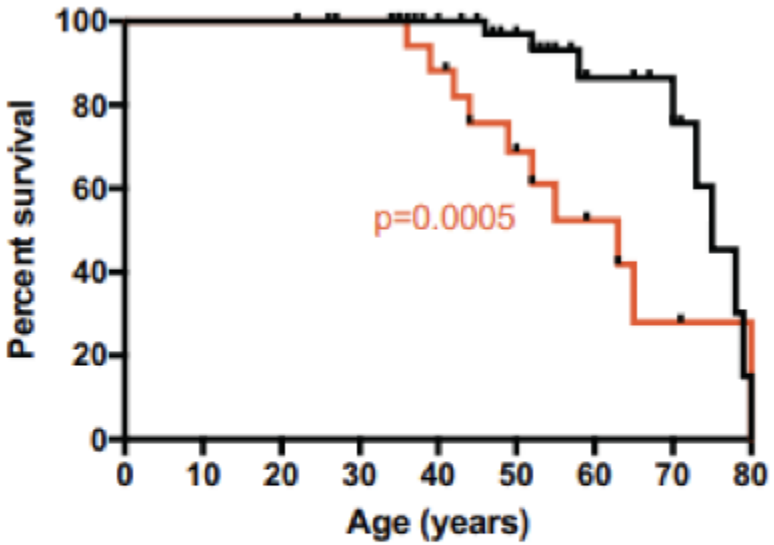
- Nodularity liver, no fibrosis, usually no inflammation
- Reaction liver high pressure in blood vessels
- Challenging pathologic diagnosis, high index of suspicion: reticulin staining
- Often only mild elevation liver enzymes; mostly **alkaline phosphatase (ALP)**
- Associated with splenomegaly and thrombocytopenia and other autoimmune phenomena
- **More frequent than previously reported: 32% UK series (n=28/86)**
- **More severe than previously thought: 6 noncirrhotic portal hypertension; 9 cirrhotic; death rate 28% vs 6%; especially after 35y**
- **CVID vs normal population with liver disease: standardized mortality ratio 25.9 vs 8.8**

Azzu et al. JACI Pract 2019



	Death rate	Median survival	Hazard ratio (95% CI)
No liver-related abnormality	1/18, 5.5%	83 years	
Liver-related abnormality	19/68, 28%	73 years	3.5 (1.2-9.9)

A



	Death rate	Median survival	Hazard ratio (95% CI)
No cirrhosis or PHTN	9/50, 18%	75 years	
Cirrhosis and/or PHTN	10/18, 56%	63 years	4.2 (1.5-11.9)

B

Azzu et al. JACI Pract 2019

Therapy?

- Exclude other causes of liver disease
- Granuloma: may respond to immune suppression (i.e. corticosteroids)
- NRH Screening if >30y or abnormal liver tests
  - yearly liver tests, clotting time, annual abdominal imaging
  - Liver biopsy
  - Screen: varices (echo duplex, gastroscopy), ascites, encephalopathy
- NRH: LiTx? Recurrence NRH in donor liver; concurrent HSCT (high mortality)?

Causes of complications in CVID

- ~10% genetic susceptibility markers
  - **Complex genetics,** epigenetics
  - Viral trigger
  - **Microbiome**
- Options for therapy?

Somatic mutations and T-cell clonality in patients with immunodeficiency

Paula Savola,[1,2] Timi Martelius,[3] Matti Kankainen,[1,2,4,5] Jani Huuhtanen,[1,2] Sofie Lundgren,[1,2] Yrjö Koski,[1,2] Samuli Eldfors,[4] Tiina Kelkka,[1,2] Mikko A.I. Keränen,[1,2] Pekka Ellonen,[4] Panu E. Kovanen,[6] Soili Kytölä,[7] Janna Saarela,[4] Harri Lähdesmäki,[8] Mikko R.J. Seppänen[2,3,9] and Satu Mustjoki[1,2,10]

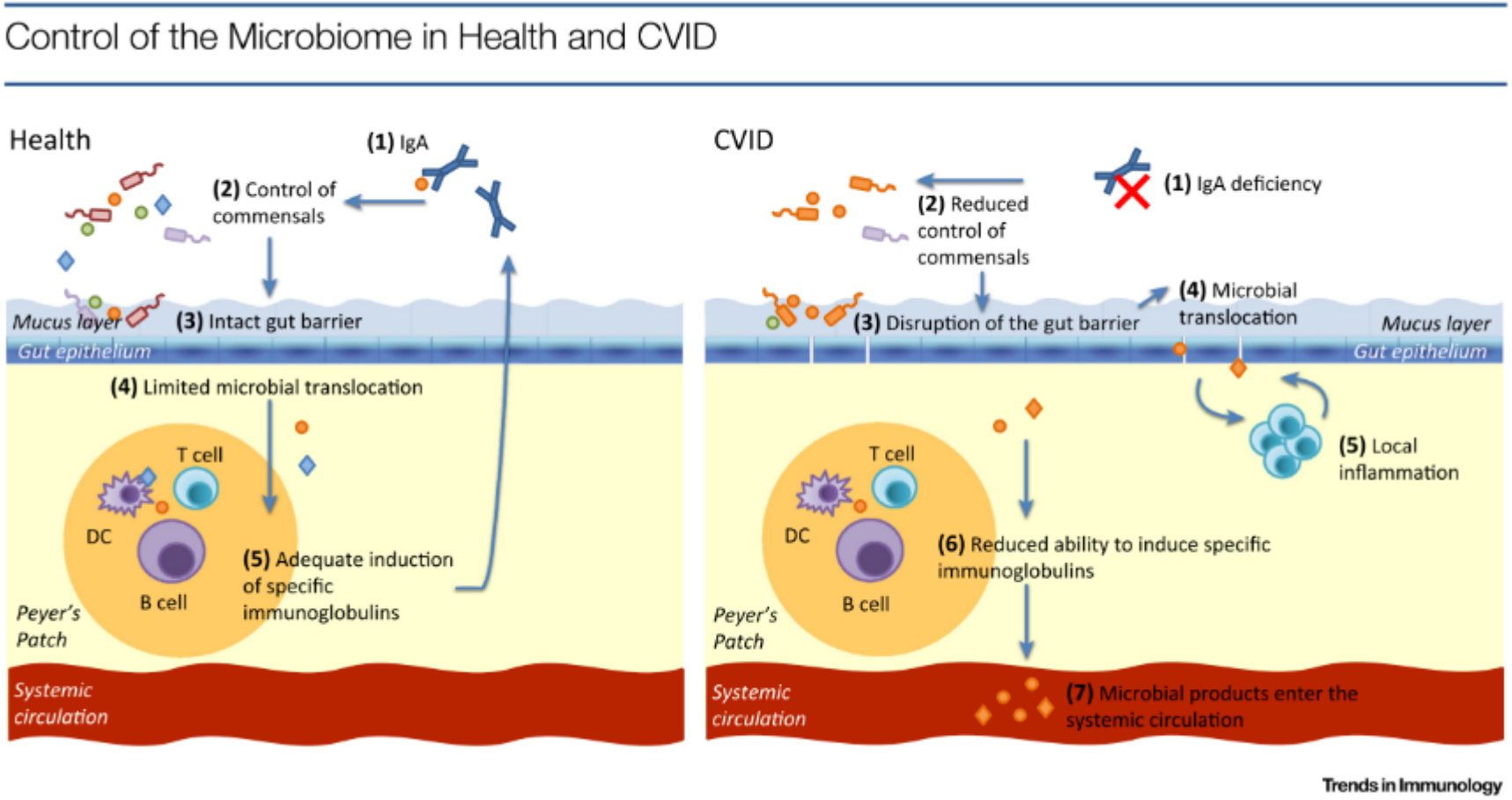
Haematologica 2020
Volume 105(12):2757-2768

N_{tot}=18, N_{CVID}=8; age 30-70
Som mut in CVID 75%, HC 48%
CD4+CD8 som mut in n=4 age 54-70
TCR convergence in CVID

Table 2. Selected somatic mutations identified in CD4⁺ and CD8⁺ cells.

Pt. ID	Disease	Cells	HGVS	AA change	COSMIC identifier	Gene	VAF	SIFT	Polyphen2	ExAC All
1	CVID	CD8⁺	1:g.1804503C>G	NM_001282539:exon6:c.G346C:p.G116R		*GNB1*	0.07	D	D	NA
2	CVID	CD4⁺	4:g.105235713delC	NM_001127208:exon3:c.1771delC:p.Q591fs		*TET2*	0.056	NA	NA	NA
		CD4⁺	4:g.105243618G>T	NM_001127208:exon6:c.G3643T:p.E1215X	COSM3719016	*TET2*	0.053	NA	NA	NA
		CD4⁺	16:g.50699619G>T	NM_022162:exon2c.G205T:p.E69X		*NOD2*	0.06	NA	NA	NA
		CD4⁺	4:g.105269703C>T	NM_001127208:exon9:c.C4138T:p.H1380Y	COSM87161	*TET2*	0.027	D	D	0.000092
		CD4⁺	17:g.42217380A>T	NM_012448:exon10:c.T1254A:p.N418K		*STAT5B*	0.036	D	D	NA
3	CVID	CD8⁺	7:g.100677602C>G	NM_005273:exon6:c.C372G:p.Y124X		*GNB2*	0.051	NA	NA	NA
		CD8⁺	11:g.75290010T>G	NM_004041:exon2:c.A50C:p.K17T		*ARRB1*	0.025	D	D	NA
4	CVID	CD4⁺	9:g.5073770G>T	NM_001322195:exon13:c.G1849T:p.V617F	COSM12600	*JAK2*	0.017	D	D	0.0007

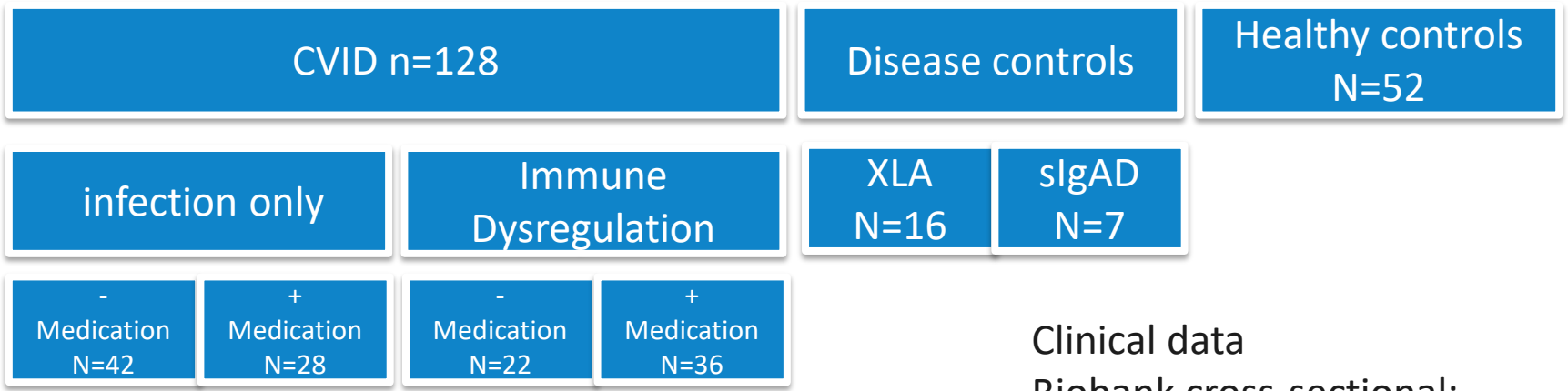
Hypothesis: role for microbiome in inflammatory complications in CVID



Berbers, R.M. et al. (2016) Microbial Dysbiosis in Common Variable Immune Deficiencies: Evidence, Causes, and Consequences. Trends Immunol. DOI: 10.1016/j.it.2016.11.008

- Can we identify CVIDid from CVIDio based on cytokine profiles and T cell repertoire?
- Can we identify pathobionts associated with CVIDid?

Cross-sectional cohort
UMC Utrecht, Erasmus MC, UMC Groningen



Clinical data
Biobank cross-sectional:
- Gut biopsies
- Serum, plasma
- PBMCs
- Oral swabs
- Feces samples
- HRCT (5 yearly at least)

Medication: antibiotics, immune suppression, inhaled corticosteroids, proton pump inhibitors Past 3 months

Erasmus MC Universitair Medisch Centrum Rotterdam

umcg

Cytokines: Cohorts

Training Cohort	HC	CVIDio	CVIDid
Total N	15	16	14
Age (years), median (IQR)	38 (35-57)	38.5 (28.25-50.5)	41.5 (34-51.75)
Male N (%)	7 (47%)	8 (50%)	7 (50%)
Inclusion site N (%)			
Utrecht, the Netherlands	9 (60%)	11 (69%)	10 (71%)
Rotterdam, the Netherlands	6 (40%)	5 (31%)	4 (29%)
Clinical phenotype N (%)			
AI disease	0		
GLILD	0		
Granulomatous disease other	0		
Enteritis	0		
Lymphoproliferation	0		
Malignancy	0		
Splenomegaly	0		
Medication use during 3 months prior to sampling N (%)			
Antibiotics	0		
Immune suppressive therapy	0	0	0
Genetics N (%)			
Genetics done	0	1 (6%)	5 (35%)
Nothing found	0	0	1 (7%)
VUS found	0	0	2 (14%)
Relevant pathogenic mutations	0	1 (6%)	2 (14%)

Testing cohort	HC	CVIDio	CVIDid
Total N	27	24	23
Age (years), median (IQR)	43 (37-49)	37 (25-57.25)	37 (23-49)
Male N (%)	9 (33%)	11 (46%)	16 (70%)
Inclusion site N (%)			
UMCU	19 (70%)	11 (46%)	13 (57%)
EMC	4 (15%)	7 (29%)	3 (13%)
		0	0
		6 (24%)	7 (30%)
		0	13 (57%)
		0	8 (35%)
		0	1 (4%)
		0	10 (43%)
		0	6 (26%)
		0	1 (4%)
		0	10 (43%)
Medication use during 3 months prior to sampling N (%)			
Antibiotics	0	8 (33%)	5 (22%)
Immune suppressive therapy	0	0	4 (17%)
Genetics N (%)			
Genetics done	0	7 (29%)	7 (30%)
Nothing found	0	7 (29%)	2 (9%)
VUS found	0	0	1 (4%)
Relevant pathogenic mutations	0	0	4 (17%)

180 serum markers:
Olink Panels
Inflammation
Immune Response

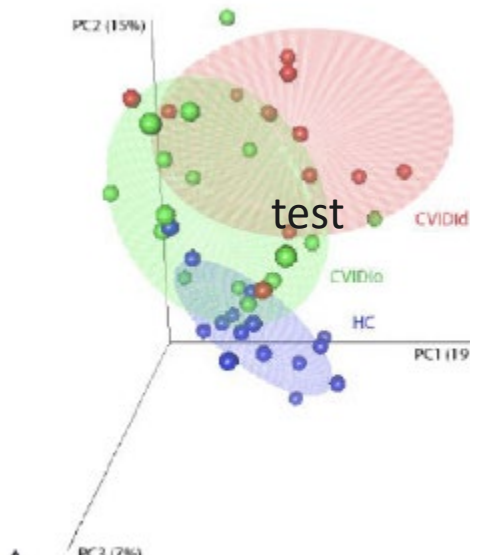
Training cohort → classification algorithm → Testing cohort → performance

Random forest, Elastic Net Regression, Extreme Gradient Boosting

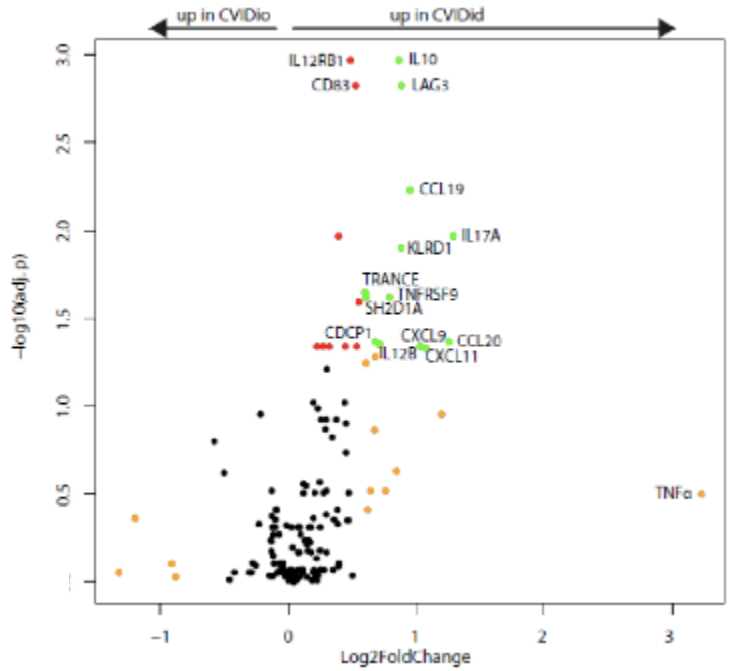
Berbers et al. Journal of Clinical Immunology 2020

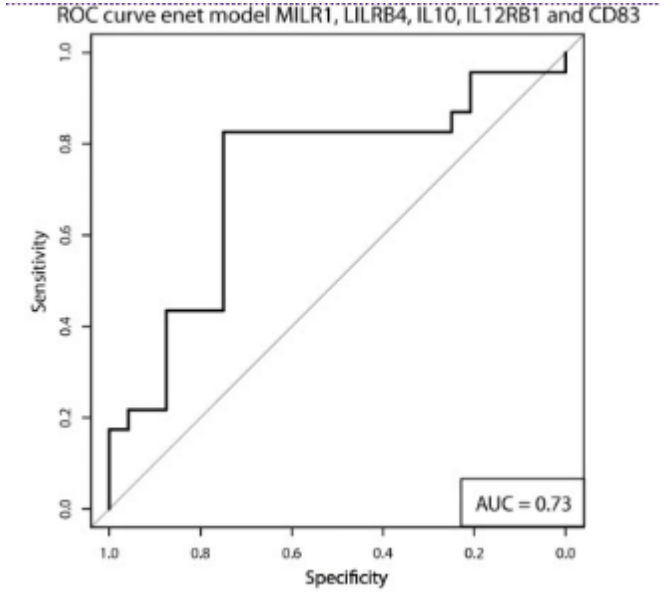
Targeted Proteomics reveals inflammatory pathways that classify immune dysregulation in Common Variable Immunodeficiency

--Manuscript Draft--

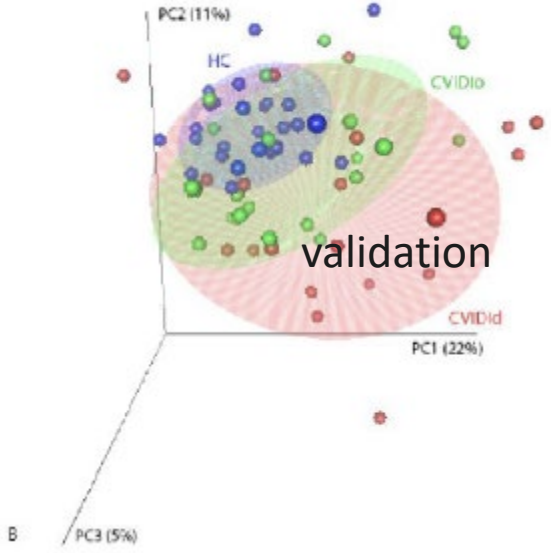


test





CVID ID have distinct cytokine patterns

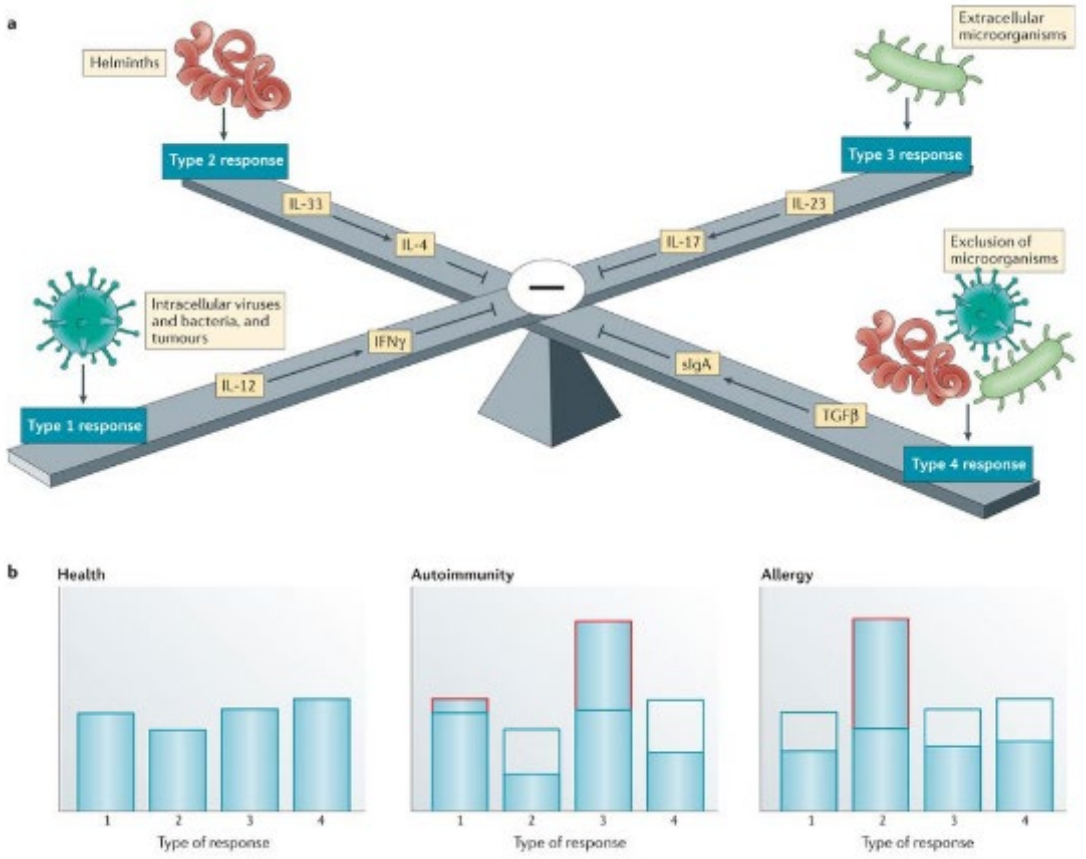


validation

elastic net classifier

Activated pathways (fold change > 1.5, FDR-adjusted $p < 0.05$) in CVIDid included Th1 and Th17-associated signaling, as well as IL10 and other immune regulatory markers (LAG3, TNFRSF9, CD83)

- Inflammation of the innate compartment
  - CD83
  - IL12B
  - IL10
  - TRANCE/RANK-L
- Activated Th1/Th17 profile
  - IL17
  - IL12
  - IL18
  - CCL19/CCL20
  - CXCL9/CXCL10
- Chronic activation markers
  - IL10
  - LAG3
  - CD137/4-1BB
  - CD83

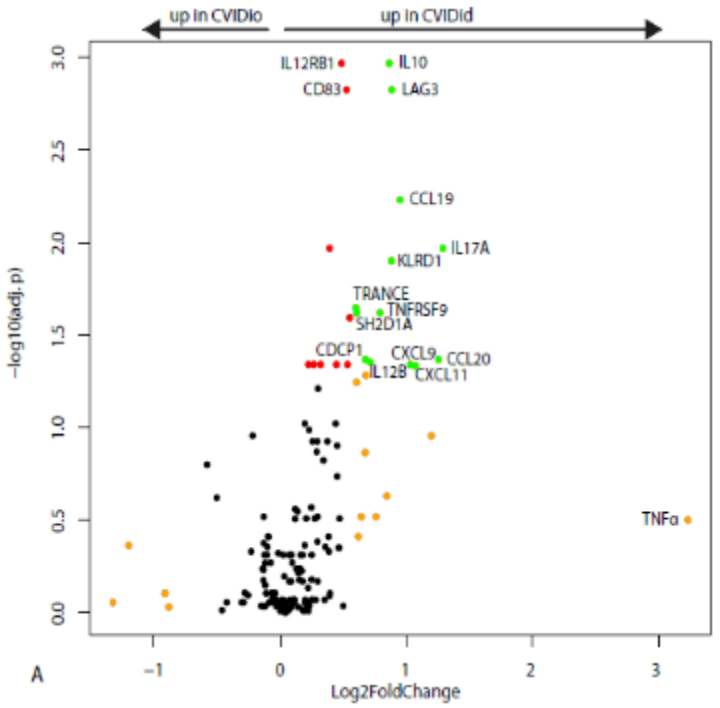


Eberl. 2016 Nature reviews Immunology

Berbers et al. Journal of Clinical Immunology 2020

Flow cytometry cohort: ex-vivo PBMCs

- Serum cytokines (this cohort)
  - Th1
  - Th17
  - Immune regulatory proteins
  - Homing to inflamed tissues



→ Characterizing the T-cell phenotype in CVIDid peripheral blood

Summary Statistics	HC (n=12)	CVIDio (n=12)	CVIDid (n=20)
Characteristics			
age (median, IQR)	45.50 (40.25, 52.00)	38.50 (29.00, 58.25)	38.50 (35.75, 43.50)
sex (male)	5 (41.67%)	4 (33.33%)	11 (55.00%)
center (Utrecht)	9 (75.00%)	11 (91.67%)	15 (75.00%)
antibiotics	0 (0.00%)	3 (25.00%)	8 (40.00%)
immunosuppressive medication	0 (0.00%)	0 (0.00%)	0 (0.00%)
IgA <0.1 g/L	0 (0.00%)	2 (16.67%)	13 (65.00%)
Immune dysregulation complications			
Pulmonary	0 (0.00%)	0 (0.00%)	8 (40.00%)
Hematological	0 (0.00%)	0 (0.00%)	2 (10.00%)
Gastrointestinal	0 (0.00%)	0 (0.00%)	8 (40.00%)
Rheumatological	0 (0.00%)	0 (0.00%)	6 (30.00%)
Dermatological	0 (0.00%)	0 (0.00%)	4 (20.00%)
Hematological malignancy	0 (0.00%)	0 (0.00%)	2 (10.00%)
Lymphoproliferation (incl splenomegaly)	0 (0.00%)	0 (0.00%)	10 (50.00%)
Other	0 (0.00%)	0 (0.00%)	4 (20.00%)
Genetics			
Not done	12 (100.00%)	12 (100.00%)	14 (70.00%)
Nothing found	0 (0.00%)	0 (0.00%)	2 (10.00%)
VUS found	0 (0.00%)	0 (0.00%)	2 (10.00%)
Pathogenic mutations found	0 (0.00%)	0 (0.00%)	2 (10.00%)

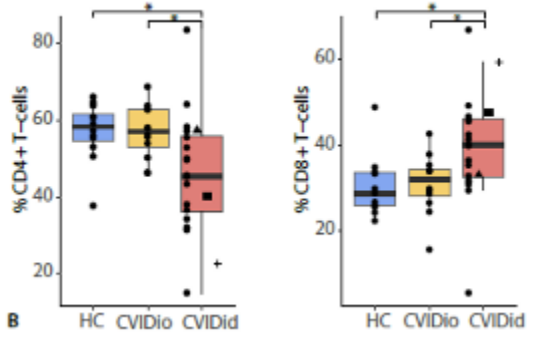
→ Patients in remission or before treatment!
12 DMARD naive / pre-clinical
8 in remission

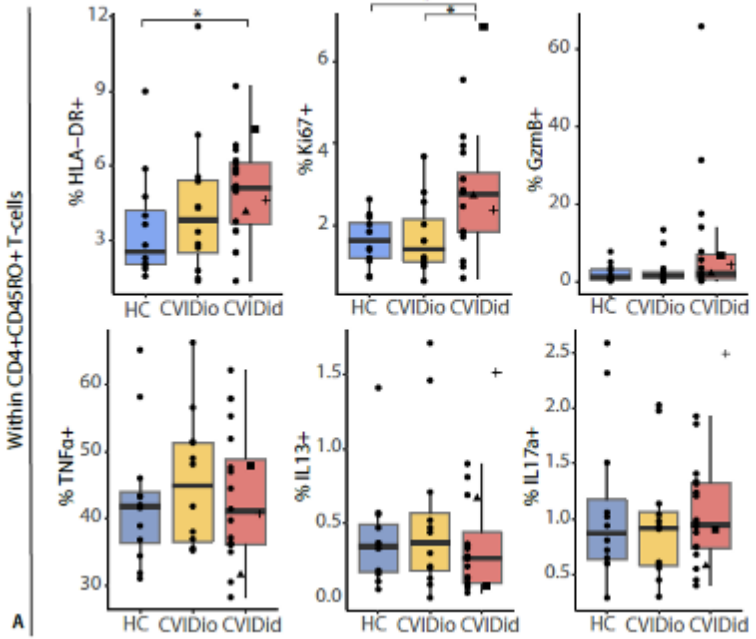
→ GLILD

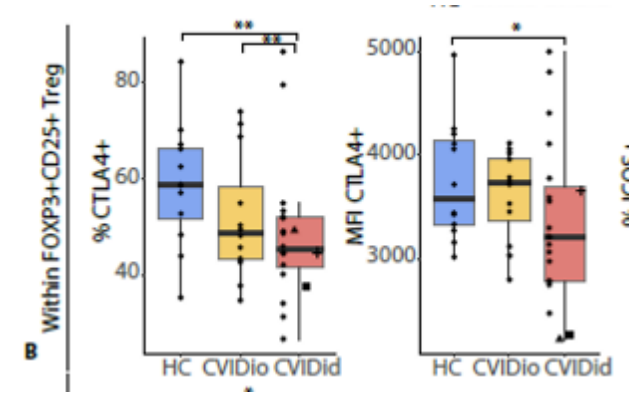
→ IBD, gastritis, coeliac, hepatitis

→ CTLA4
PIK3R1

T cells: chronic activation, no exhaustion, impaired Treg function?



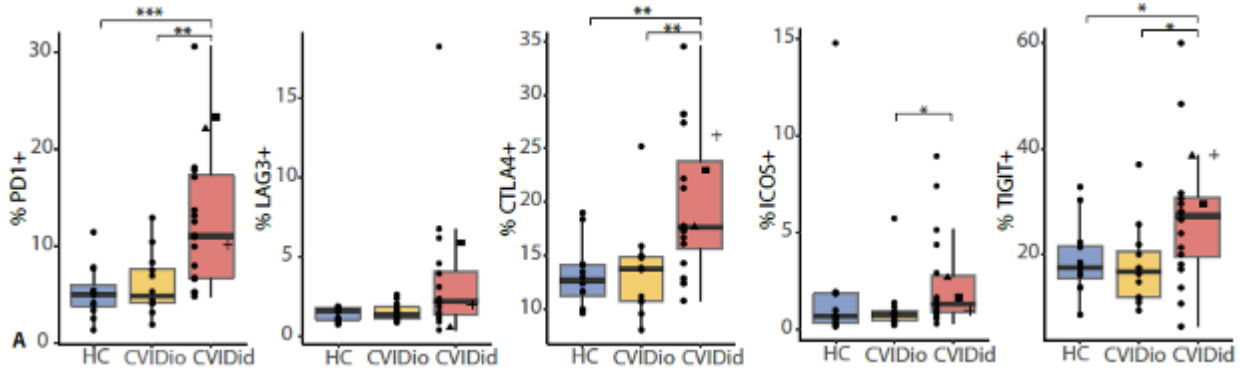


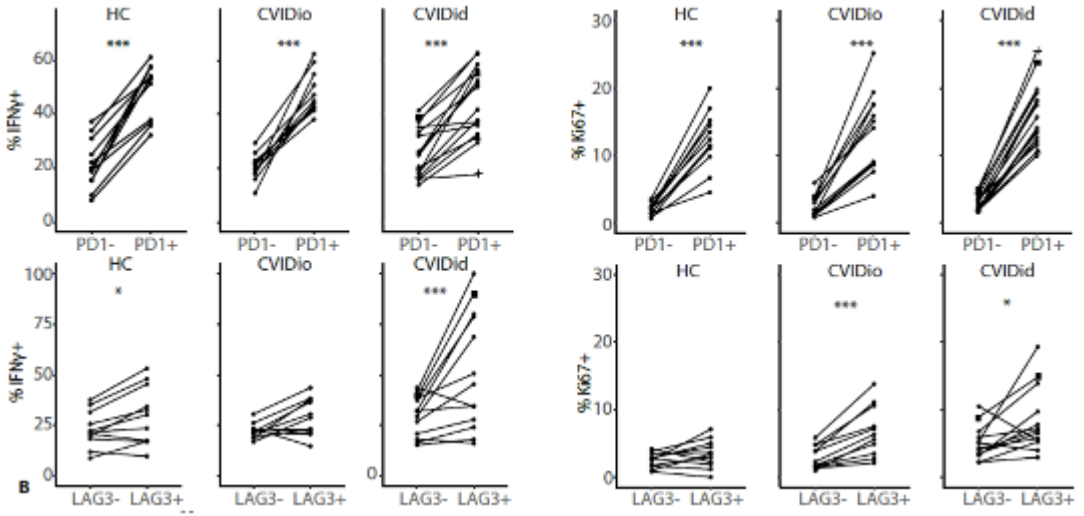


IFNg+ Th cell ~ serum levels CXCL9 CXCL10 CXCL 11

IL17+ Th norm: serum increase IL17a not correlated!!!

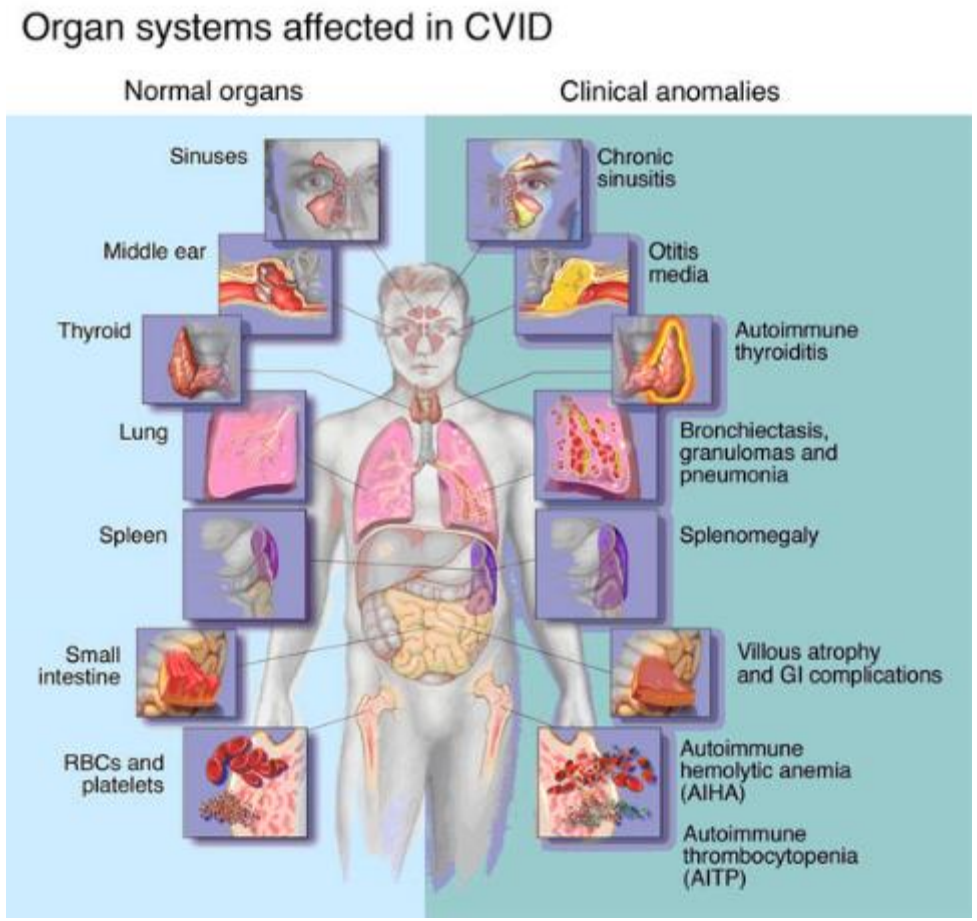
Increased expression negative regulators immune activation



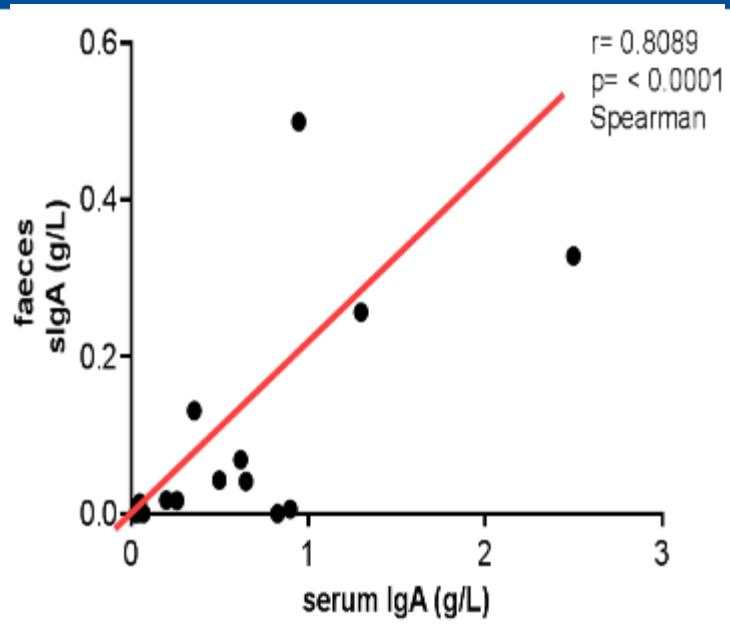


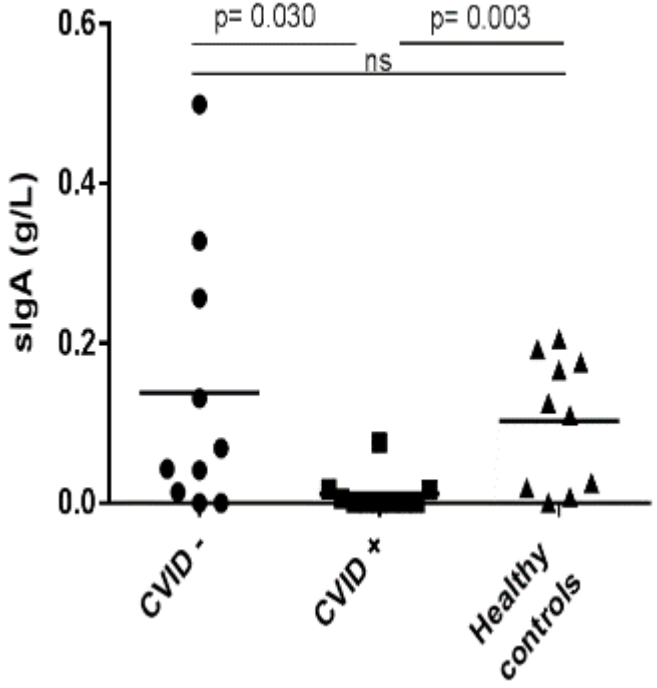
Berbers et al. JoCI 2021

CVID: clinical complications in IgA-

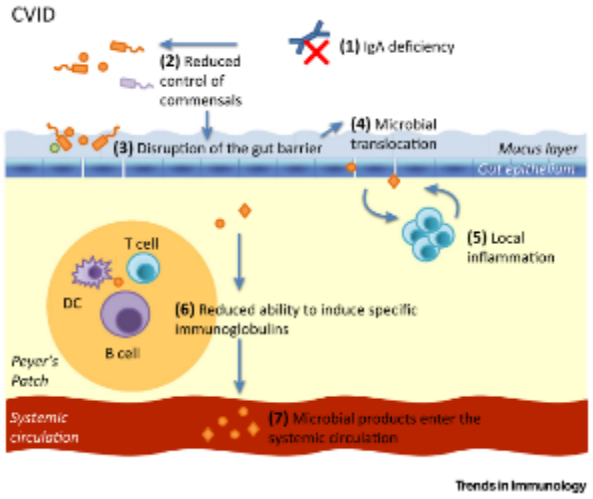


- Recurrent respiratory- and GI tract infections
  → bronchiectasis
- ~50% inflammatory complications
  - Autoimmunity
  - Lymphoproliferation
  - Granulomas
  - Malignancy



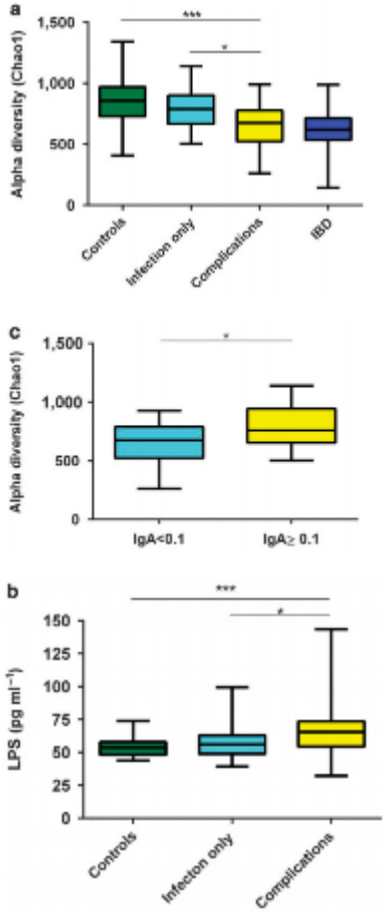


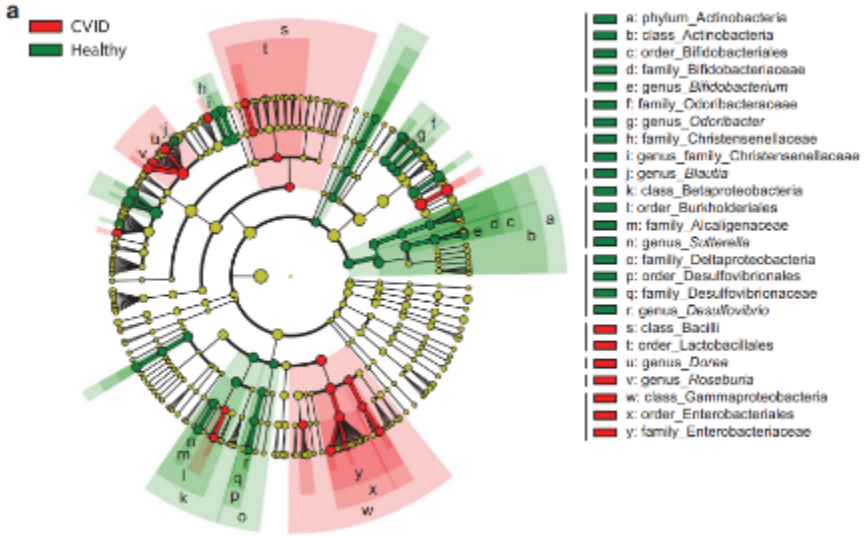
Fecal microbiota: why?



Altered gut microbiota profile in common variable immunodeficiency associates with levels of lipopolysaccharide and markers of systemic immune activation

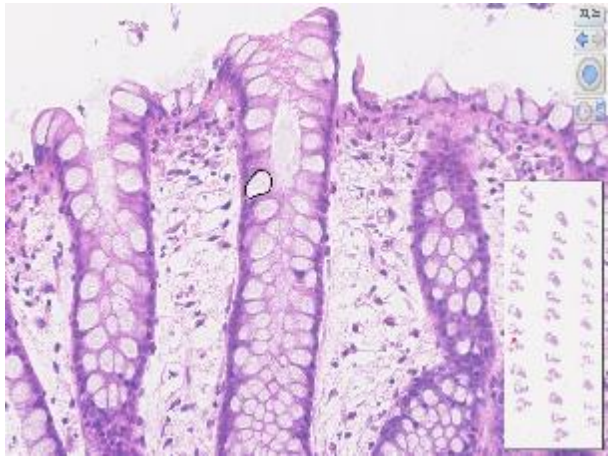
SF Jørgensen[1,2,3,4], M Trøseid[1,2,3], M Kummen[1,3,4,5], JA Anmarkrud[1,5], AE Michelsen[1,4], LT Osnes[6], K Holm[1,5], ML Høivik[7], A Rashidi[1], CP Dahl[1,8], M Vesterhus[5,9], B Halvorsen[1,3,4], TE Mollnes[3,6,10,11], RK Berge[12,13], B Moum[4,7], KEA Lundin[4,14], B Fevang[1,2,4], T Ueland[3,4,14,15], TH Karlsen[1,3,4,5,14,16], P Aukrust[1,2,3,4] and JR Hov[1,3,4,5,14]



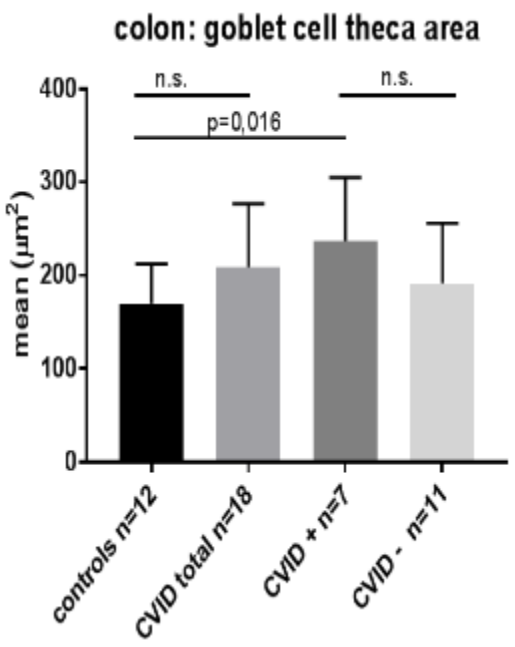


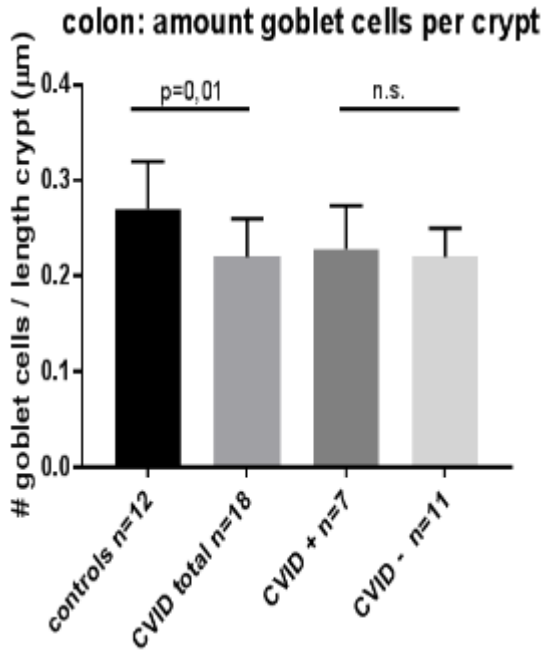
Missing: microbiota CVIDio vs CVIDid (sample size CVIDio=9)

Mucus production impaired in CVID ID?



Representative colon biopsy, with goblet cell theca area indicated (black circle)

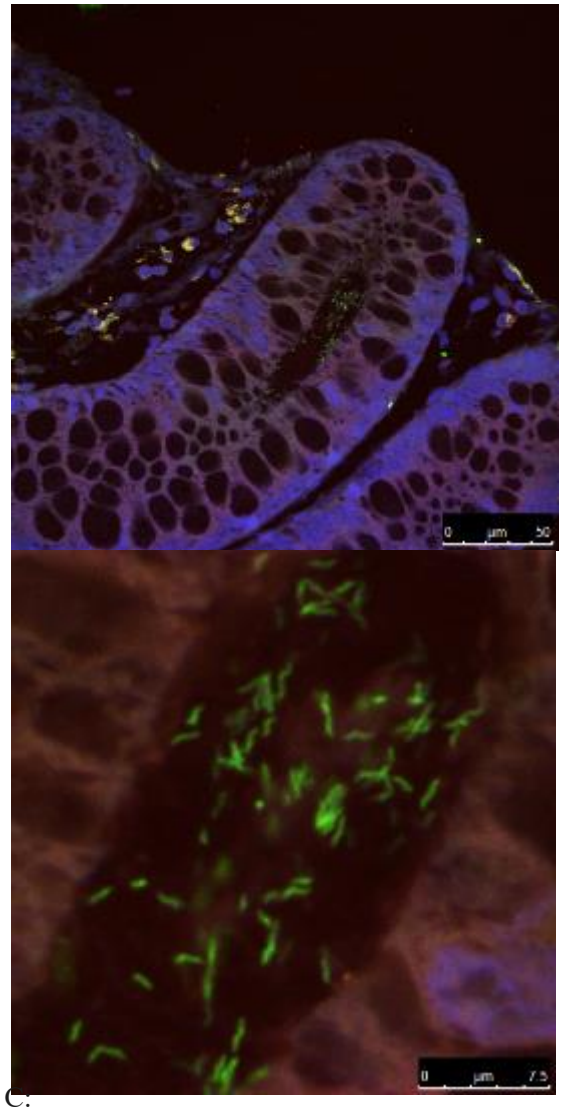




goblet cell theca area increased in colon crypts CVIDid

Average amount of cells per crypt is reduced in CVID.

FISH; crypt-invasive bacteria, zoom-in crypt invasive bacteria
In CVID IgA-

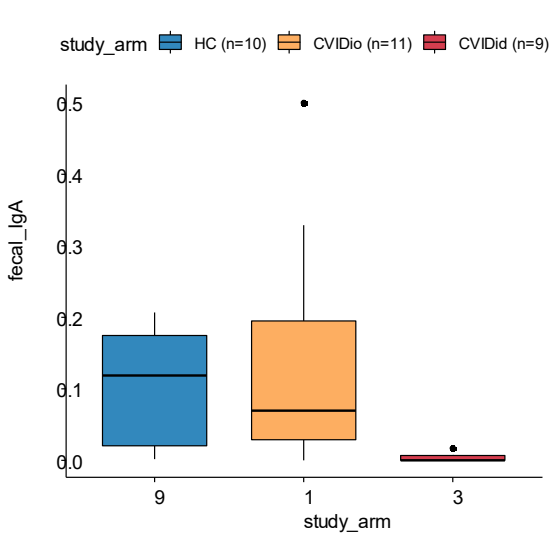


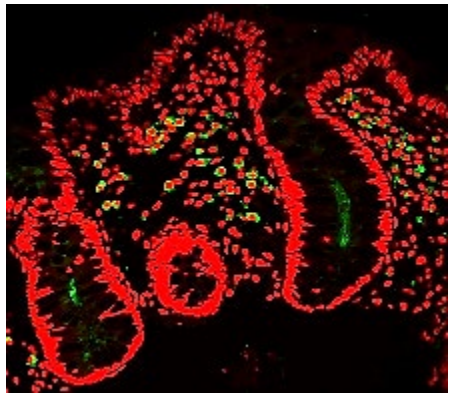
C.

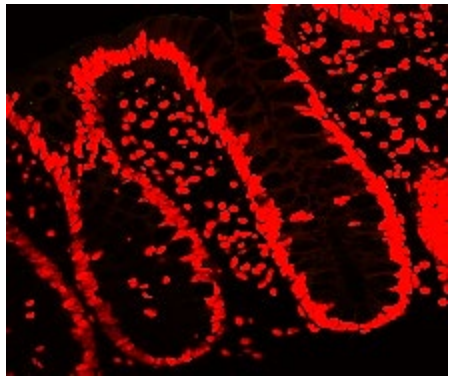
Berbers et al., submitted

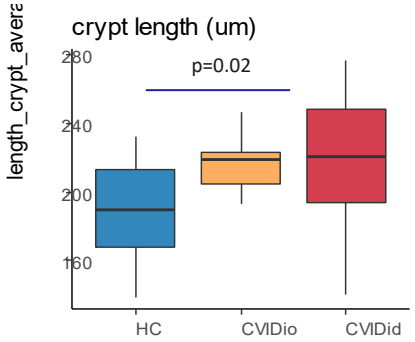
Fecal microbiota: mucosa

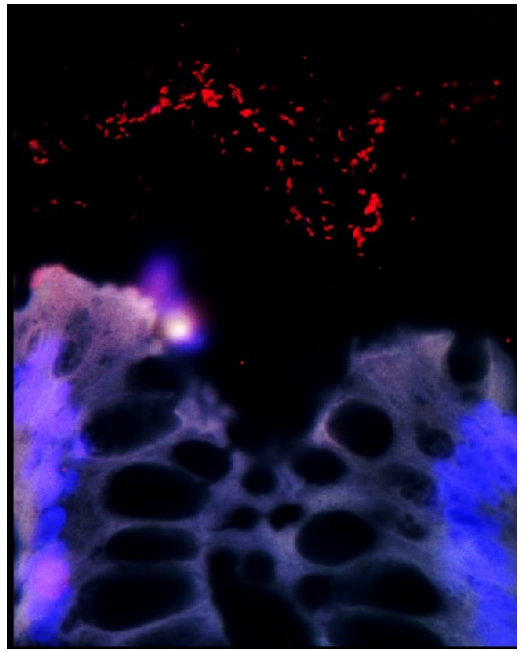
CVID with complications associated with absence of fecal IgA (ELISA and IHC), disturbed crypt architecture, and aberrant microbiota localisation (FISH)

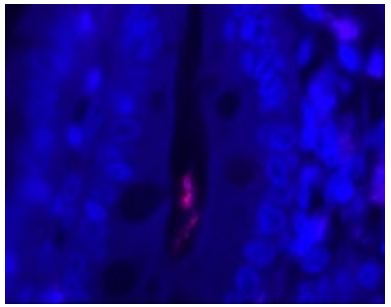


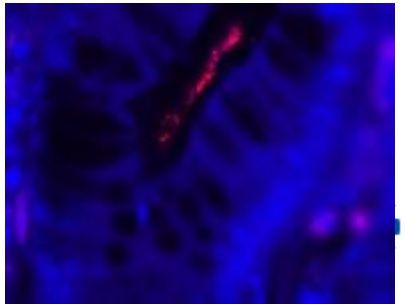




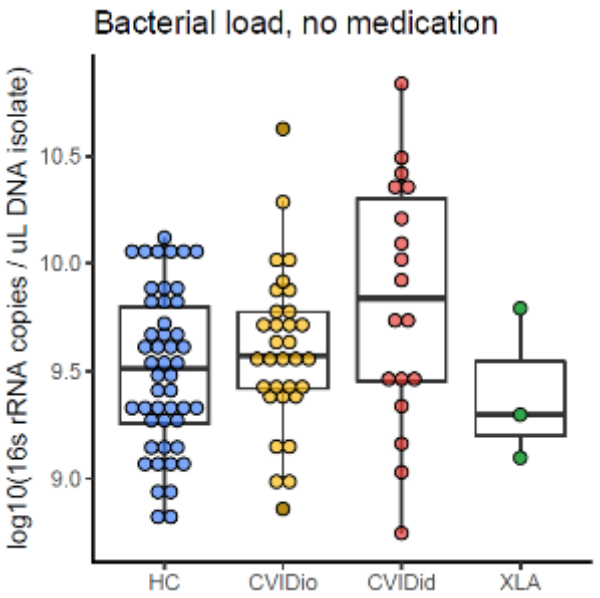


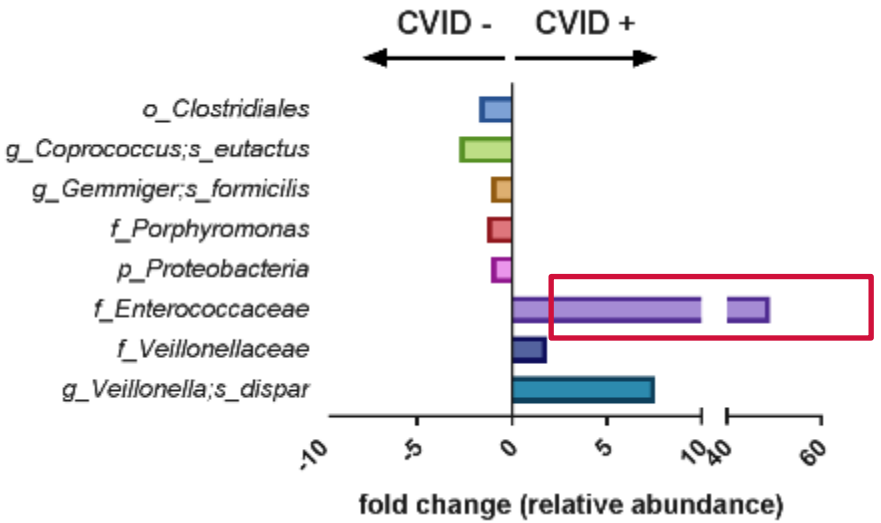


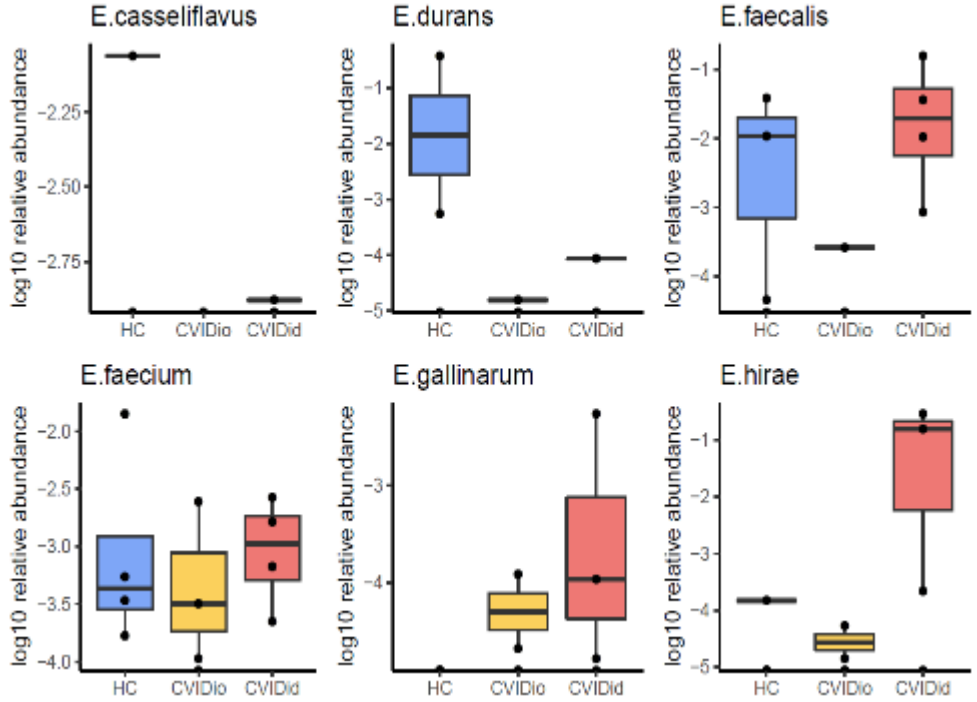


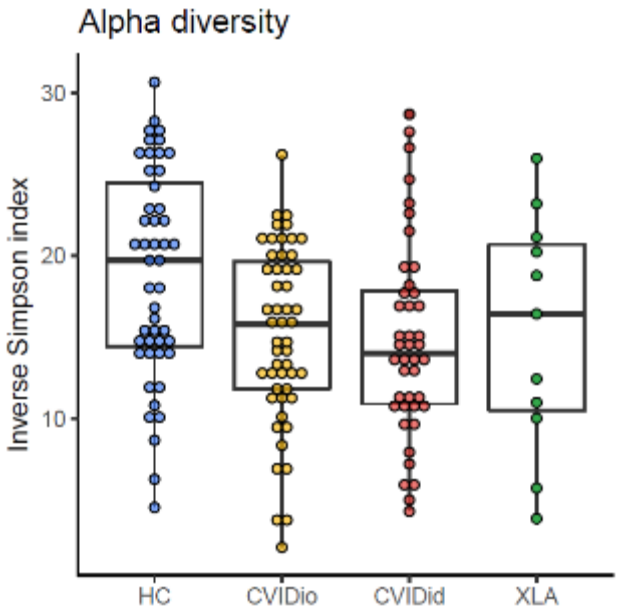


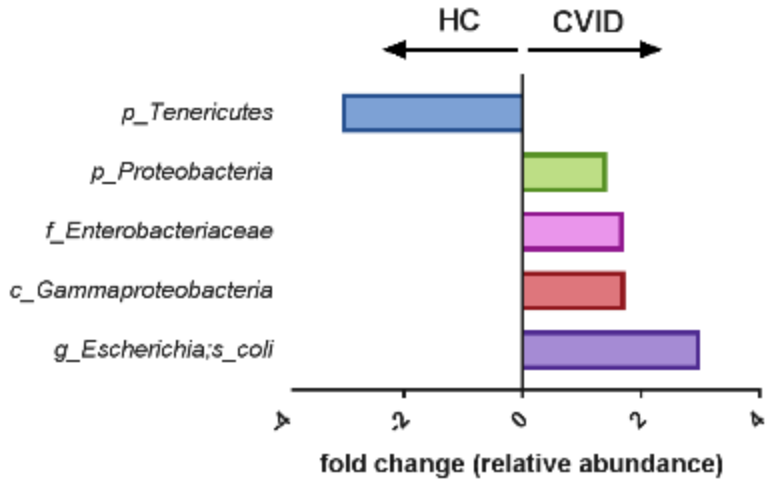
CVIDid stool MB dysregulated and enterococcal outgrowth











E. gallinarum + *E. hirae*

	CVIDio	CVIDid
Patients (n)	33	22
E. gallinarum (n, %)	2 (3.03)	5 (22.73)
E. hirae (n, %)	5 (15.15)	6 (27.28)

- Similar use of antibiotics in both CVID groups
- Role in immune dysregiulation?

Science

RESEARCH

MICROBIOTA

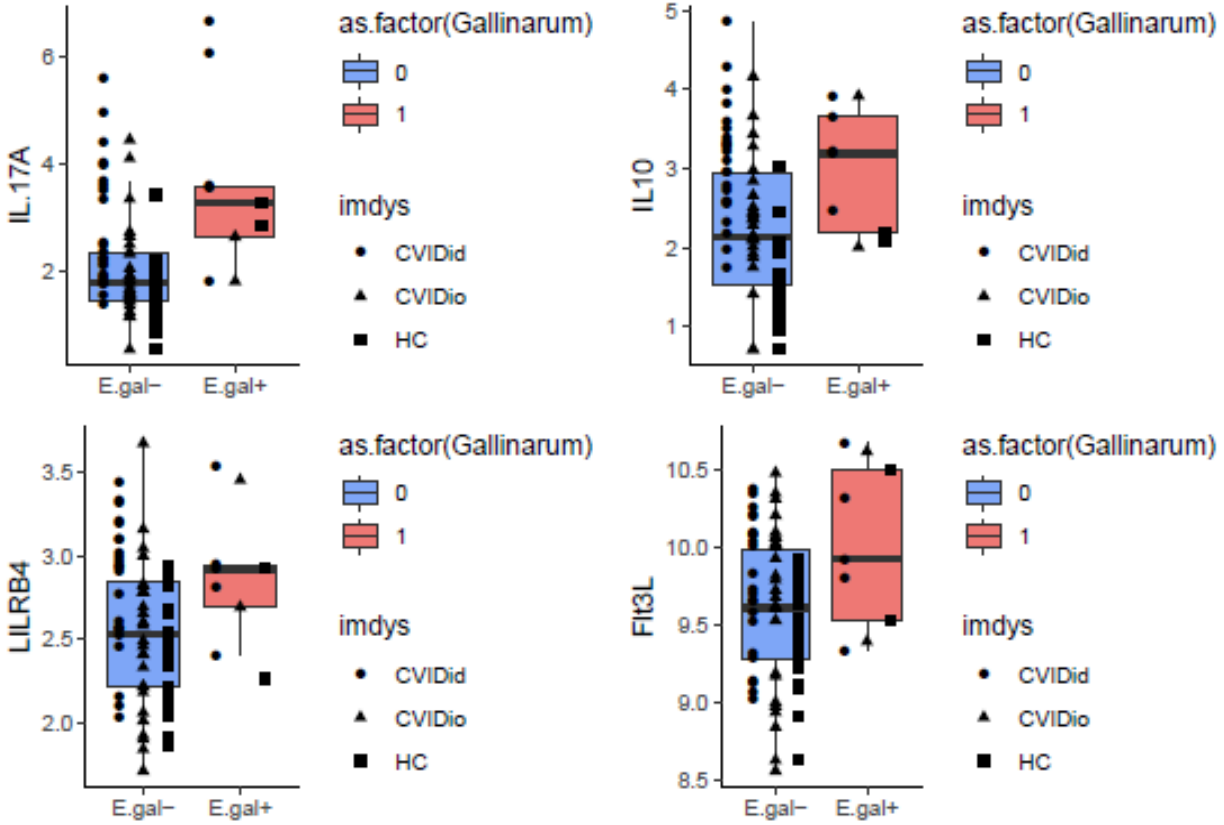
Translocation of a gut pathobiont drives autoimmunity in mice and humans

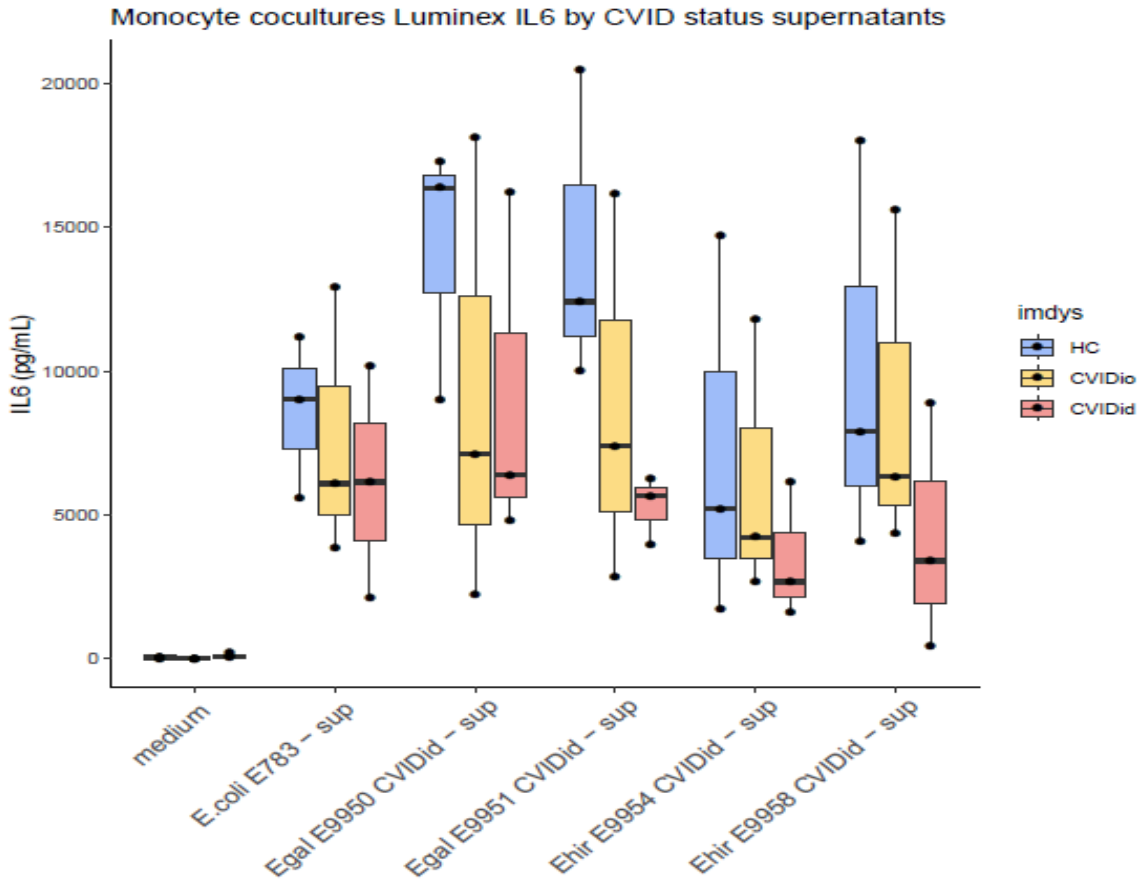
S. Manfredo Vieira,[1] M. Hiltensperger,[1] V. Kumar,[2] D. Zegarra-Ruiz,[1] C. Dehner,[1] N. Khan,[1] F. R. C. Costa,[1*] E. Tiniakou,[1†] T. Greiling,[1‡] W. Ruff,[1] A. Barbieri,[3] C. Kriegel,[1] S. S. Mehta,[4] J. R. Knight,[4] D. Jain,[3] A. L. Goodman,[5] M. A. Kriegel[1,2§]

Despite multiple associations between the microbiota and immune diseases, their role in autoimmunity is poorly understood. We found that translocation of a gut pathobiont, *Enterococcus gallinarum*, to the liver and other systemic tissues triggers autoimmune responses in a genetic background predisposing to autoimmunity. Antibiotic treatment prevented mortality in this model, suppressed growth of *E. gallinarum* in tissues, and eliminated pathogenic autoantibodies and T cells. Hepatocyte–*E. gallinarum* cocultures induced autoimmune-promoting factors. Pathobiont translocation in monocolonized and autoimmune-prone mice induced autoantibodies and caused mortality, which could be prevented by an intramuscular vaccine targeting the pathobiont. *E. gallinarum*–specific DNA was recovered from liver biopsies of autoimmune patients, and cocultures with human hepatocytes replicated the murine findings; hence, similar processes apparently occur in susceptible humans. These discoveries show that a gut pathobiont can translocate and promote autoimmunity in genetically predisposed hosts.

Manfredo Vieira *et al.*, *Science* **359**, 1156–1161 (2018) 9 March 2018

CVID: *E gallinarum* induced systemic inflammation?





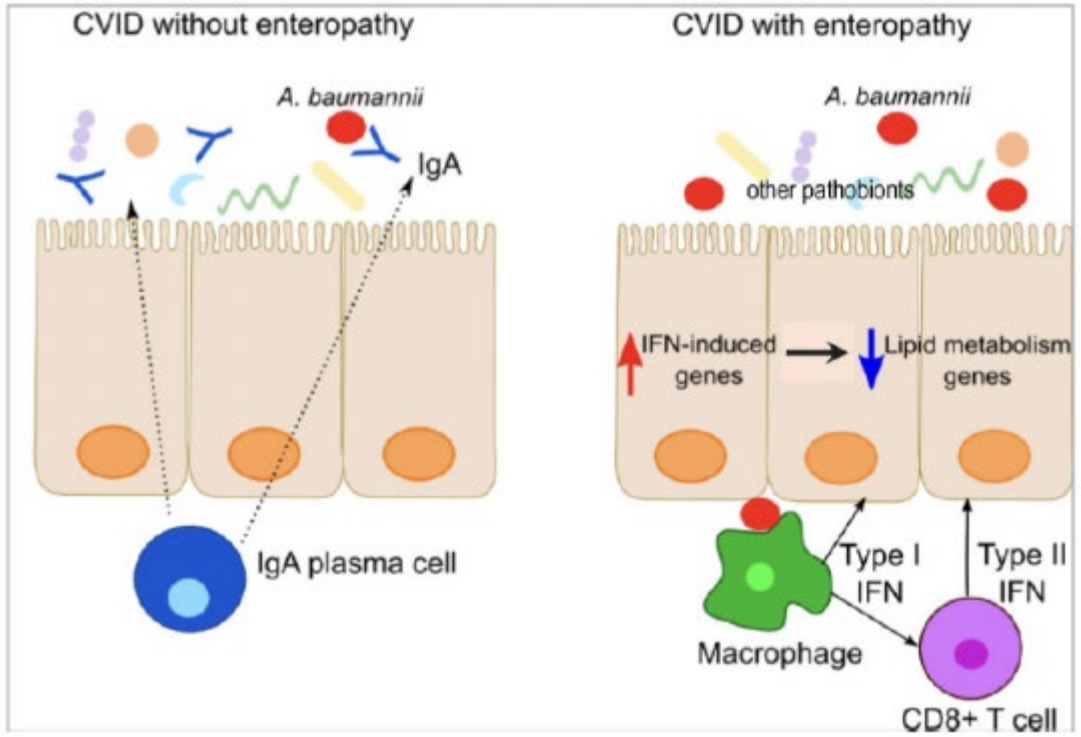
Clin Immunol. 2018 December ; 197: 139–153. doi:10.1016/j.clim.2018.09.008.

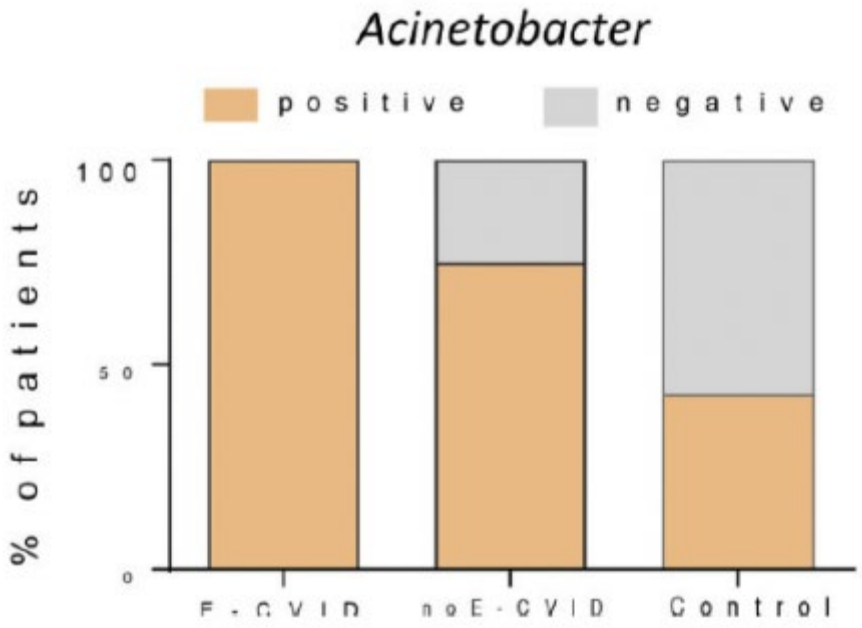
CVID Enteropathy is Characterized by Exceeding Low Mucosal IgA Levels and Interferon-Driven Inflammation Possibly Related to the Presence of a Pathobiont

Natalia Shulzhenko[#1], Xiaoxi Dong[#2], Dariia Vyshenska[#2], Renee L Greer[#1], Manoj Gurung[1], Stephany Vasquez-Perez[1], Ekaterina Peremyslova[2], Stanislav Sosnovtsev[3], Martha Quezado[4], Michael Yao[5,6], Kim Montgomery-Recht[5,7], Warren Strober[5,*], Ivan J Fuss[5,*], and Andrey Morgun[2,*]

- Biopsies duodenum E-CVID n=7, noE-CVID n=8, HC n=7
- E-CVID low IgA
- Gene profile: down lipid metabolism, up immune genes; not influenced by norovirus
- Up IFNg and CXCL9, CXCL10, CCL19, CCL20, CCL5
- Microbiome analysis: ns
- Selected bacteria based on IgA gene expression > 45 potential OTUs enriched, transkingdom network analysis
- Most abundant *Acinetobacter baumanii*; confirmed by shotgun seq

- Co-cultured THP-1 macrophages w *A. baumanii* vs *Lactobacillus plantarum*
- Production IFNB1 and CXCL9





Remaining questions:

- Identify early markers of bacterial dysbiosis and CVIDid despite heterogeneous nature of CVID
- Identify more pathobionts
- Find effective interventions to target early CVIDid in gut? (diet? Prebiotics? anti-inflammatory drugs?)
- Needed: longitudinal multinational cohorts for multi-omics approaches
  - Future: intervention studies diet / prebiotics in CVID > HTLH 2021 consortium
- NL CVID GLILD cohort multipharma funding: consortium, data and biobanking
- E GLILD initiative

Acknowledgements

Clinicans
Roos Berbers UMC
Joris van Montfrans
WKZ/UMC
Bas Smits UMC
Pauline Ellerbroek UMC
Martin van Hagen EMC
Virgil Dalm EMC
Annick van de Ven UMCG

Feces 16S microbiome And metagenomic shotgun seq
Dept Med Microbiology
Rob Willems
Fernanda Paganelli

Serum cytokine analysis
Stefan Nierkens U-DAIR
Julia Drylewicz
Center of Translational Immunology

T cell exhaustion
Gut homing T cells
Bacterial – T cell interactions
Femke van Wijk, CTI

IgA seq 16S microbiome feces
Marcel de Zoete
Dept Med Microbiology

Integration with clinical data and radiologic data
Hae Won Uh,
Biostatistics Julius
Firdaus Muhammed Hussein, dept Radiology

Respiratory 16S microbiome
~ GLILD
Dept MMB

Genetics:
Marielle van Gijn
Lars vd Veken
Manon Huibers

Gut histology
AMP production
FISH: bacterial translocation
Lodewijk Brosens,
Dept Pathology

REMAP-CAP

A Randomised, Embedded, Multi-factorial, Adaptive Platform Trial for Community-Acquired Pneumonia

17,407

Patient randomisations

15,614

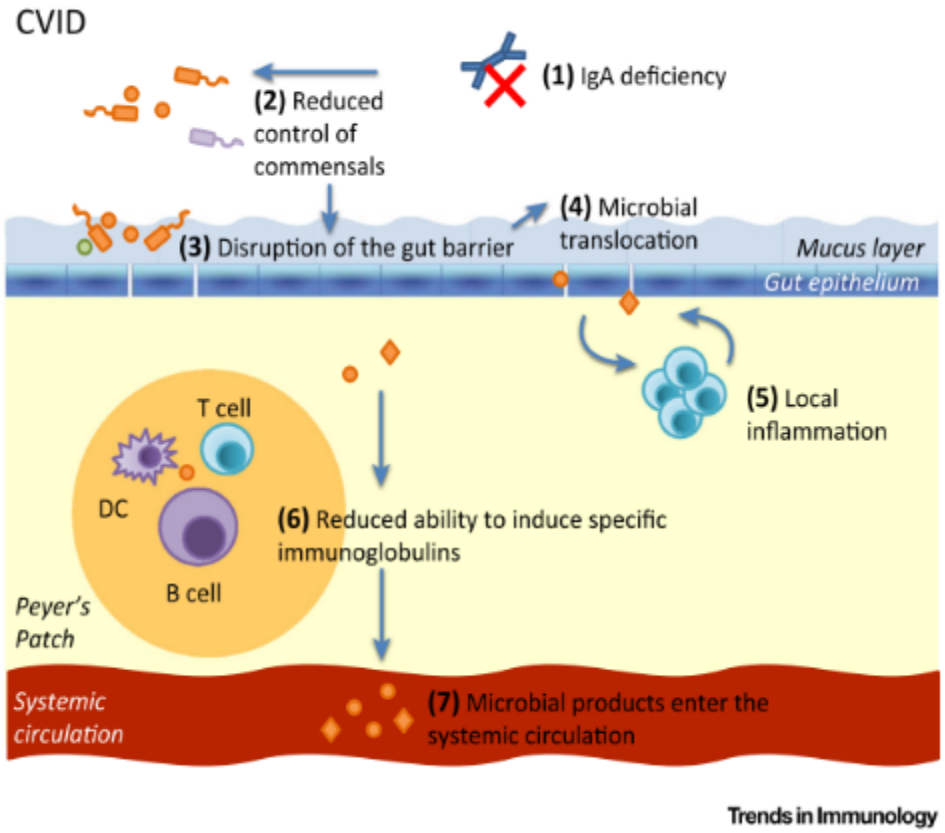
Patient randomisations with suspected or proven COVID-19

50

Current or completed interventions in 14 Domains

What might be causing this inflammation?

- Risk factor for inflammation in CVID: low IgA

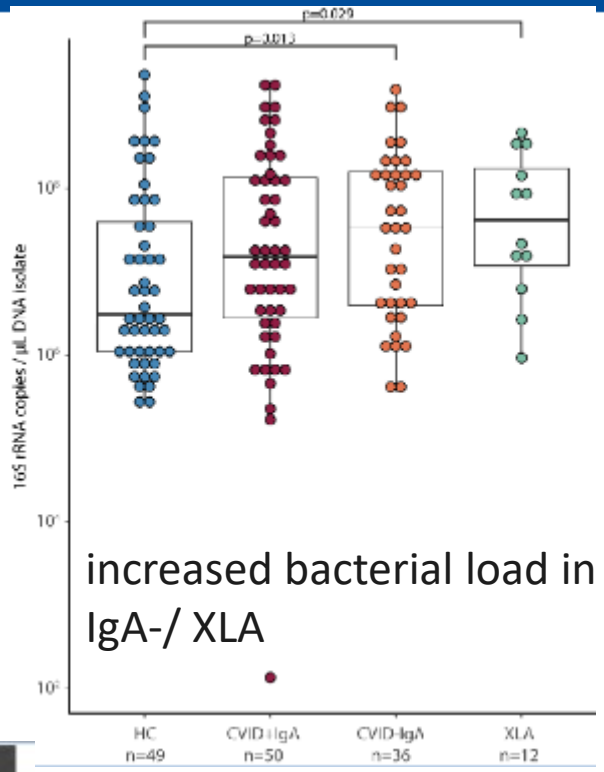


Berbers et al. Trends in Immunology 2016

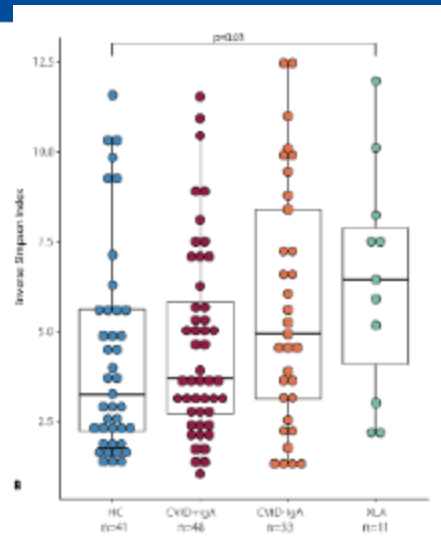
frontiers in Immunology

Low IgA Associated With Oropharyngeal Microbiota Changes and Lung Disease in Primary Antibody Deficiency

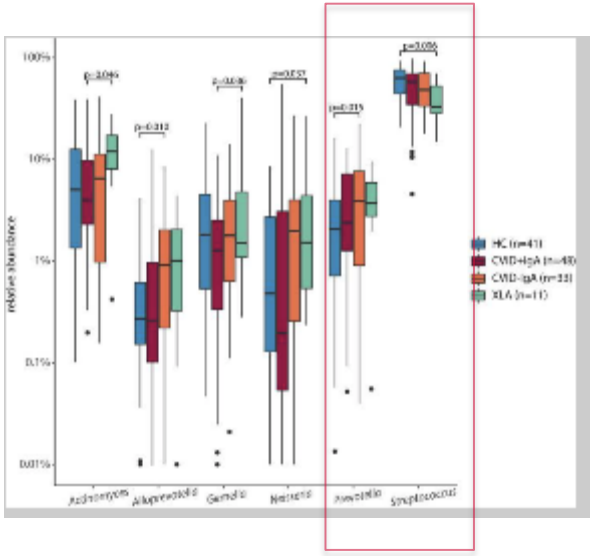
Roos-Marijn Berbers, Firdaus A. A. Mohamed Hoesein, [...], and Helen L. Leavis

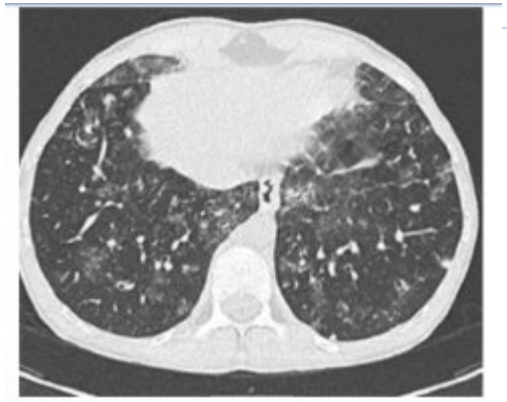


increased bacterial load in CVID IgA-/ XLA



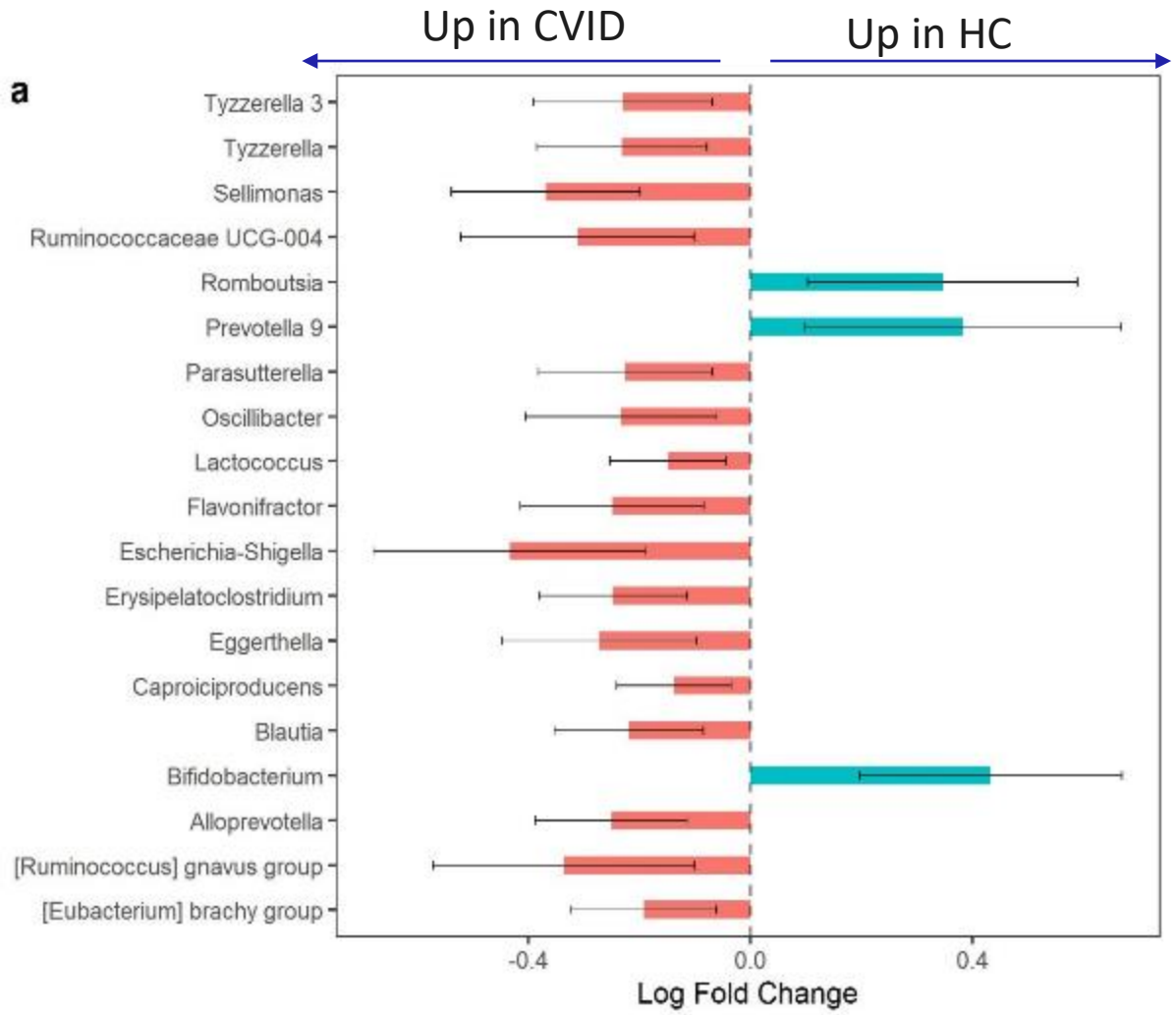
increased alpha diversity CVID IgA - def/XLA

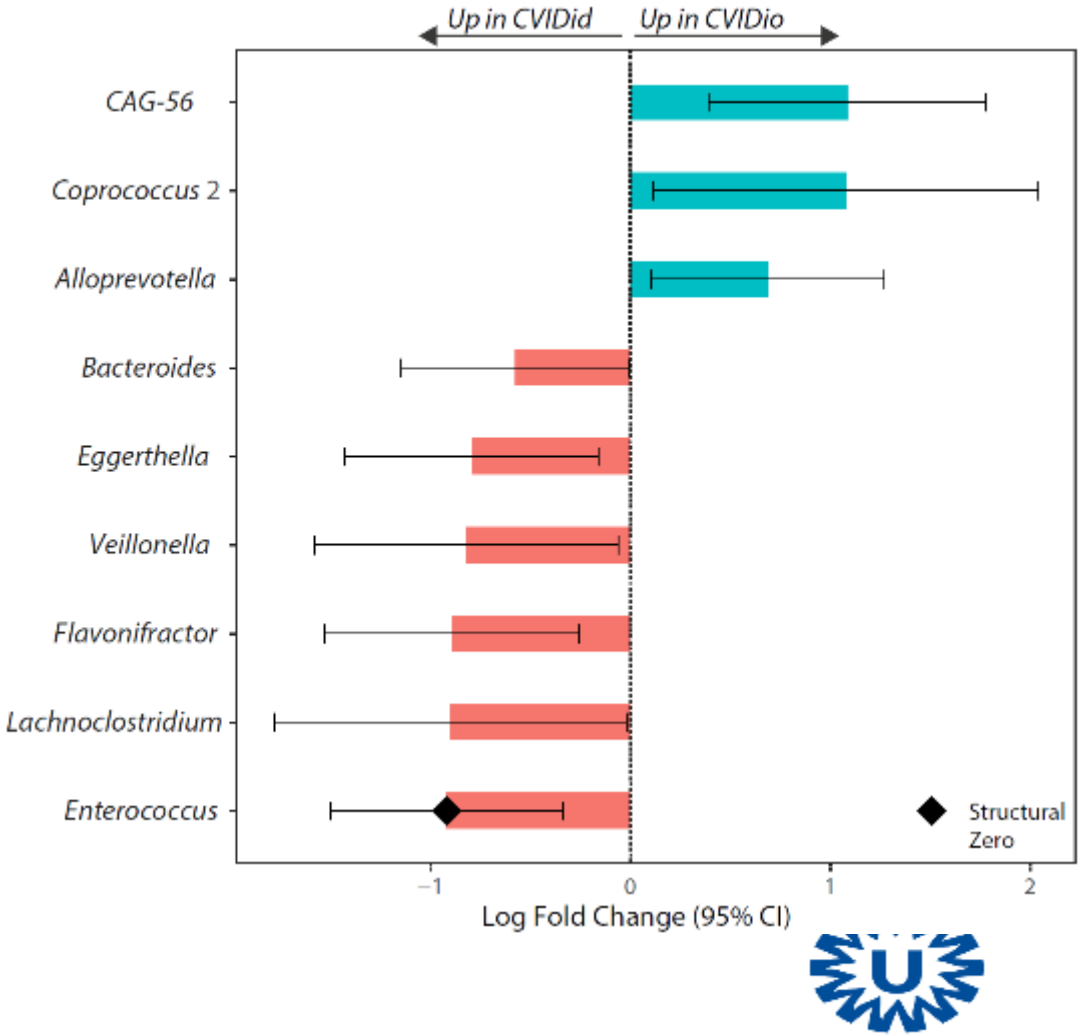




Linear regression: *Prevotellaceae* correlated with CVID – IgA *and* airway disease; streptococci with ILD

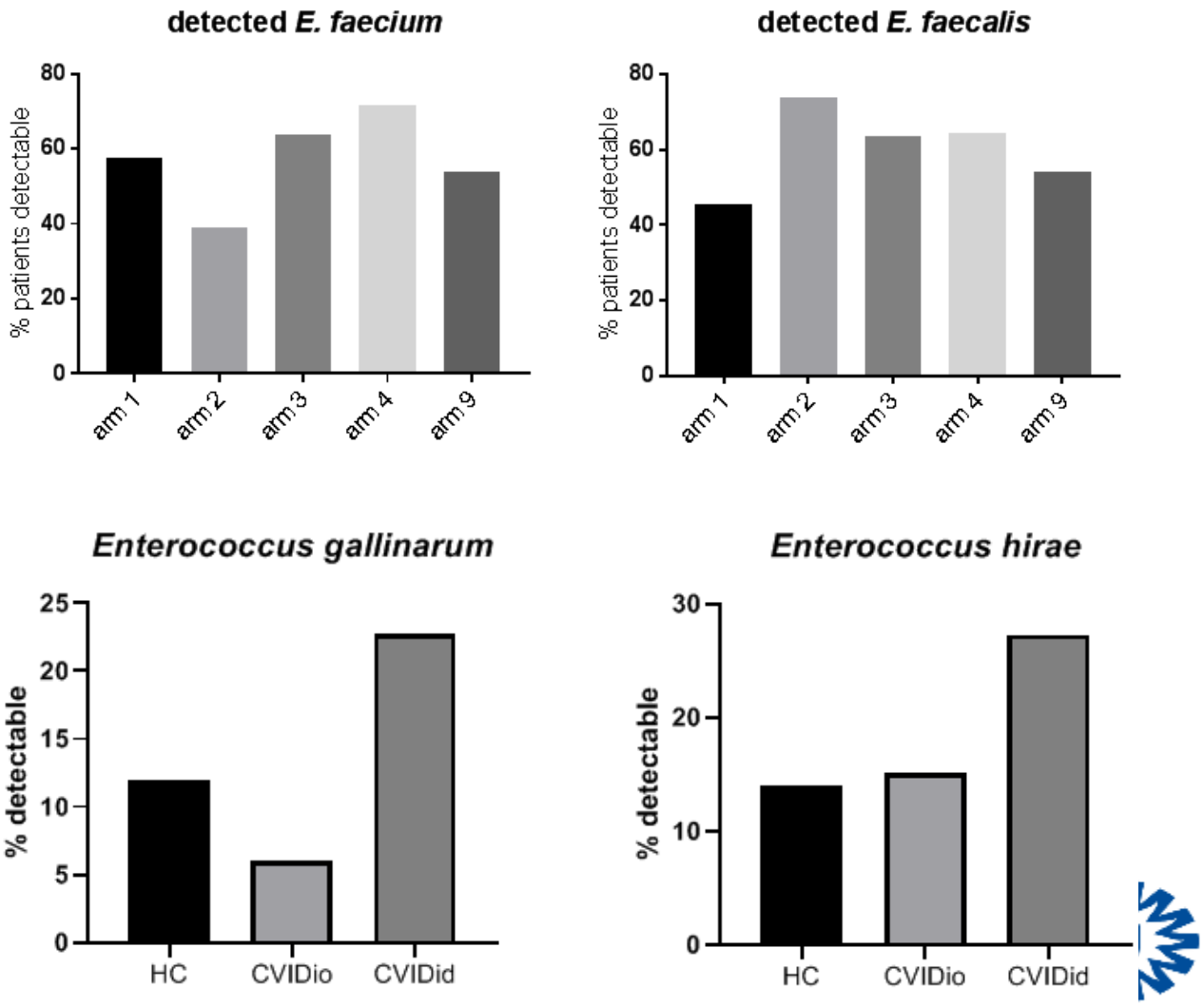
Fecal microbiota: differential abundance





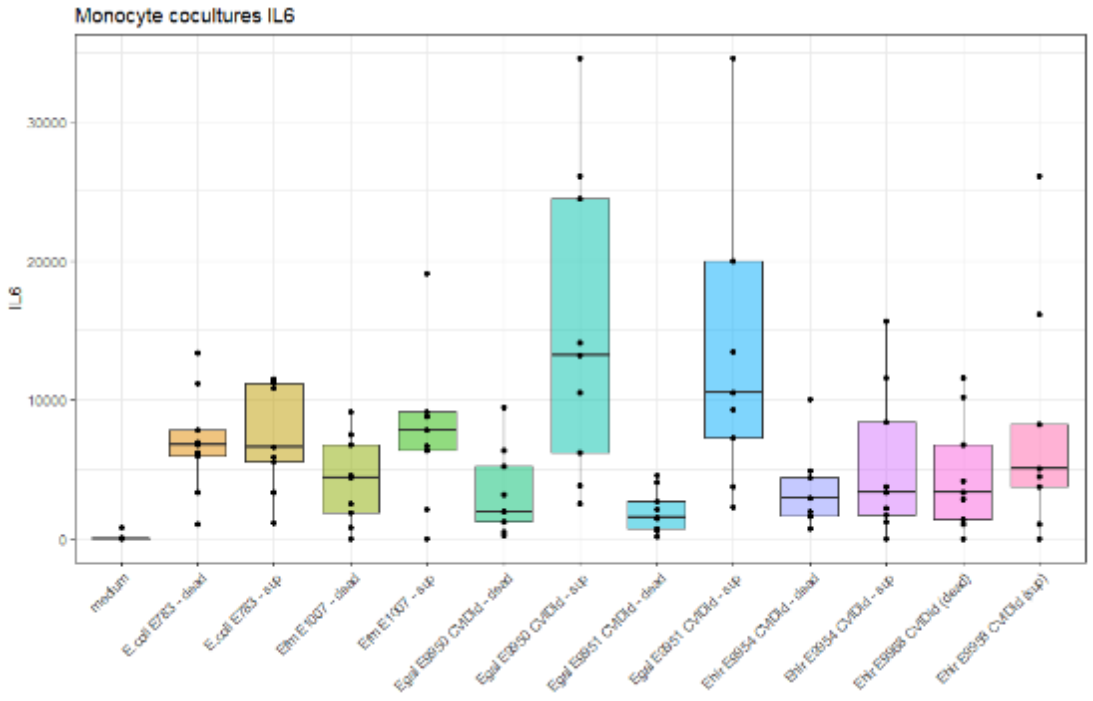
Fecal microbiota: enterococci

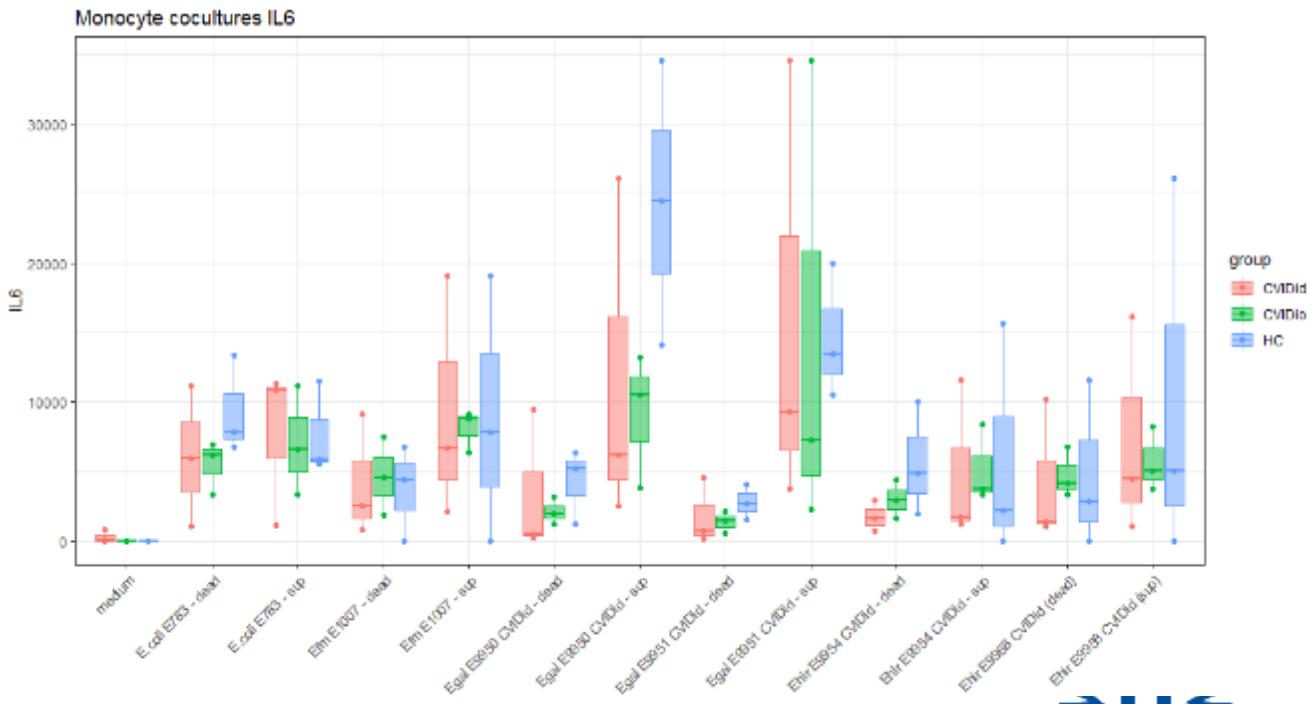
- Metagenomic shotgun sequencing
  - 4 CVIDio
  - 4 CVIDid
  - 4 HC
- Most abundant in CVIDid
  - *E. faecium*
  - *E. faecalis*
  - *E. hirae*
  - *E. gallinarum*
- *E. hirae* and *E. gallinarum* most enriched in CVIDid (qPCR and selective culturing)



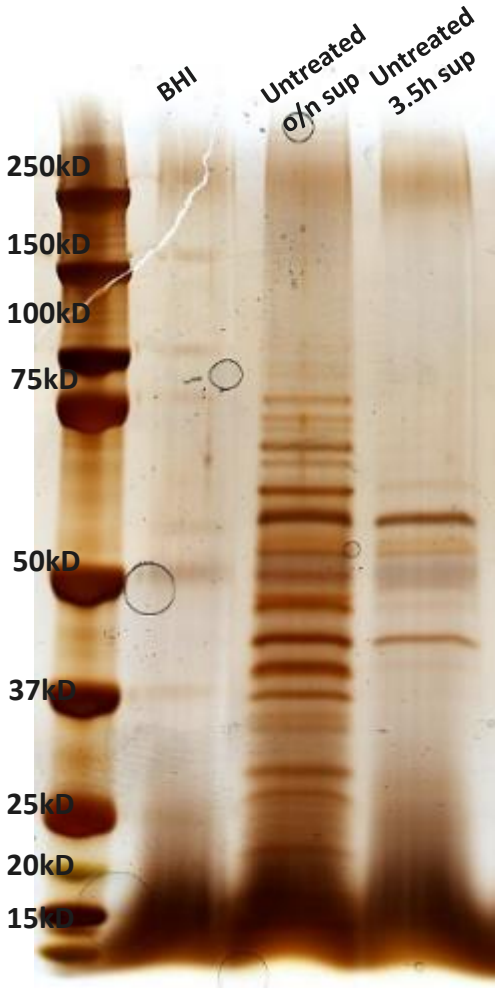
Fecal microbiota: function enterococci

- Coculture overnight with dead bacteria / supernatant and primary monocytes





Fecal microbiota: *E. gallinarum* supernatant

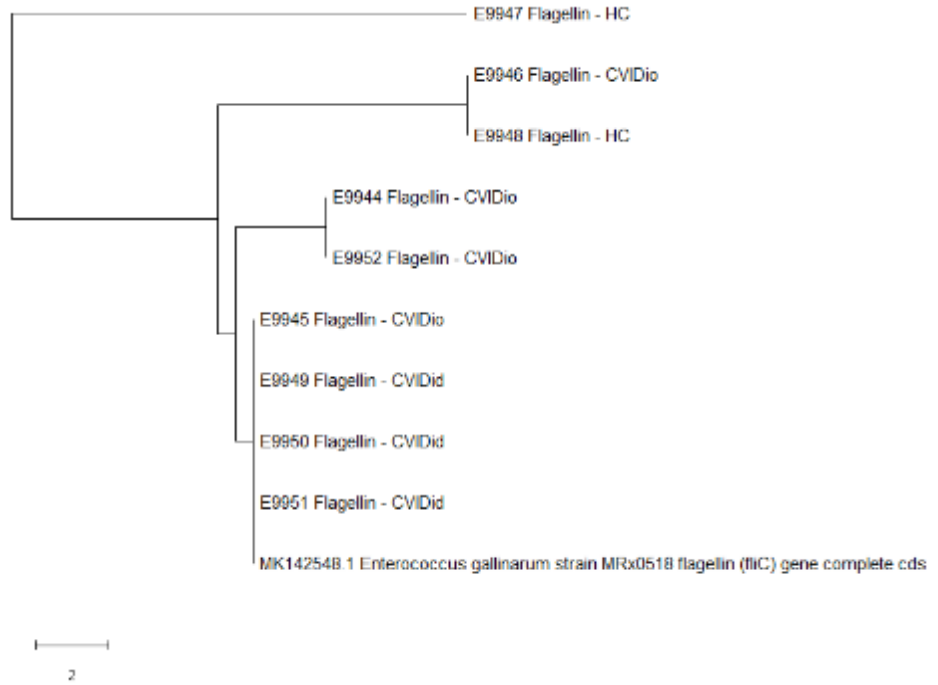


- What could it be?

The flagellin of candidate live biotherapeutic *Enterococcus gallinarum* MRx0518 is a potent immunostimulant

Delphine L. Lautié-Caly, Emma J. Raftis, Philip Cowie, Emma Hennessy, Amy Holt, D. Alessio Panzica, Christina Sparre, Beverley Minter, Eline Stroobach & Imke E. Mulder

- Final step: characterize proteins in the supernatant



Fecal microbiota: conclusions so far

- CVIDid associated with fecal IgA deficiency, disturbed architecture and increased contact of microbiota
- Increased bacterial load and decreased alpha diversity
- *Enterococcus gallinarum* is enriched in CVIDid and causes inflammation in monocytes

- TBA:
  - Mass-spec supernatant
  - More cytokine data on cocultures, also with T cells
  - More fecal IgA measurements
  - Association of cytokines with enterococcus presence?

Overall comments

CVID-related

- Inflammation in CVID seems sustained from the innate compartment
- IgA deficiency and inflammation in CVID are associated with perturbed microbiota in oropharyx and feces
- Enrichment of *Enterococcus gallinarum* was found in CVIDid feces, which strongly stimulated monocytes *in vitro*

More general microbiome-related

- IgA effect seems stronger in oropharynx than feces
- Alpha diversity in oropharynx increased, in feces decreased
- Bacterial load increased in both

Recommendations for CVID care

- Consider (local) replacement therapy for IgA as well!
- Preference for IgG therapy instead of antibiotics
- In case of stem cell transplantation; consider concurrent fecal transplant, especially with *Enterococcus*+ patients