Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*

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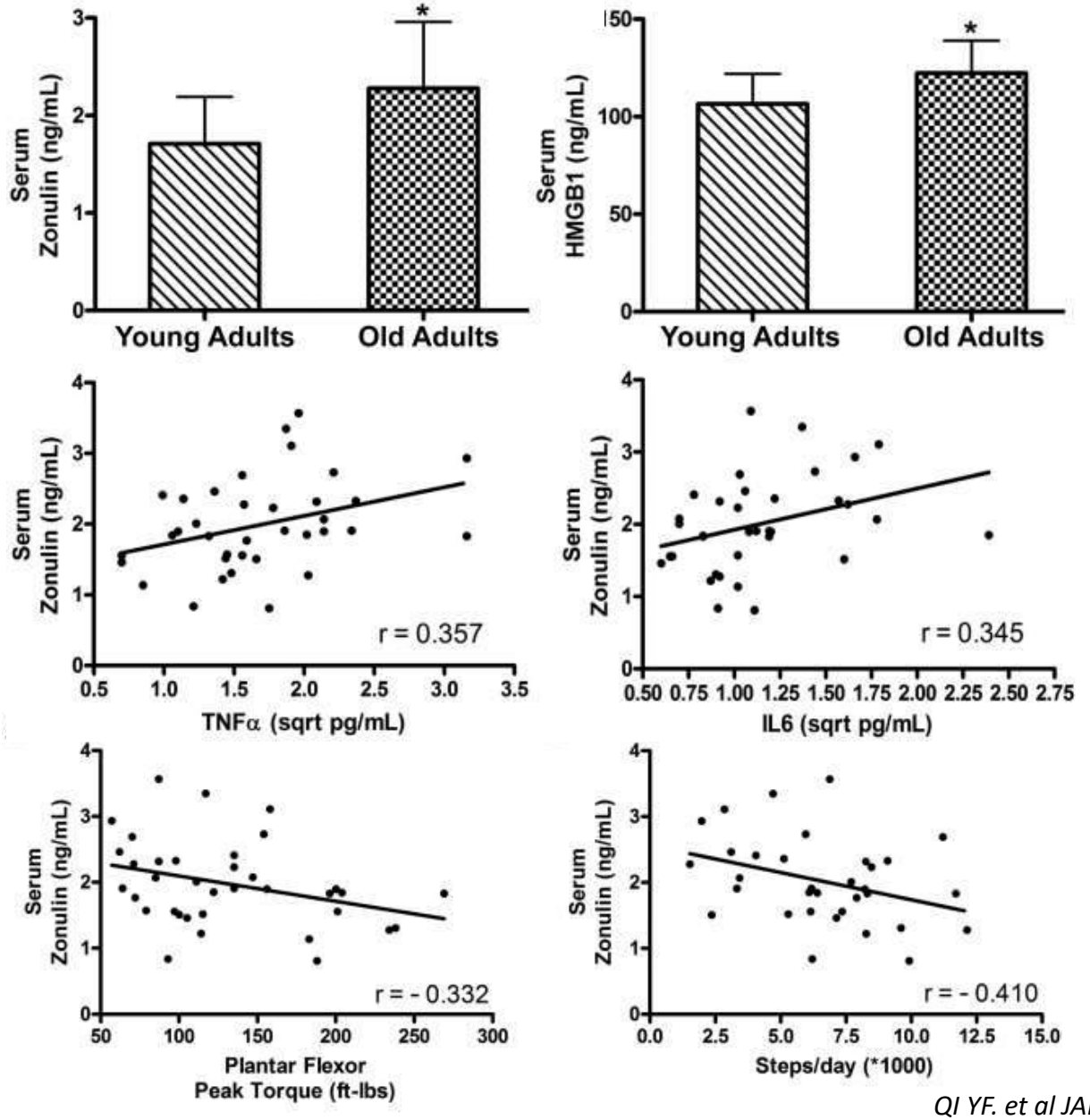
Aging is characterized by a growing risk of disease and death, yet the underlying pathophysiology is poorly understood. Indeed, little is known about how the functional decline of individual organ systems relates to the integrative physiology of aging and probability of death of the organism. Here we show that intestinal barrier dysfunction is correlated with lifespan across a range of *Drosophila* genotypes and environmental conditions, including mitochondrial dysfunction and dietary restriction. Regardless of chronological age, intestinal barrier dysfunction predicts impending death in individual flies. Activation of inflammatory pathways has been linked to aging and age-related diseases in humans, and an age-related increase in immunity-related gene expression has been reported in *Drosophila*. We show that the age-related increase in expression of antimicrobial peptides is tightly linked to intestinal barrier dysfunction. Indeed, increased antimicrobial peptide expression during aging can be used to identify individual flies exhibiting intestinal barrier dysfunction. Similarly, intestinal barrier dysfunction is more accurate than chronological age in identifying individual flies with systemic metabolic defects previously linked to aging, including impaired insulin/insulin-like growth factor signaling, as evidenced by a reduction in Akt activation and up-regulation of dFOXO target genes. Thus, the age-dependent loss of intestinal integrity is associated with altered metabolic and immune signaling and, critically, is a harbinger of death. Our findings suggest that intestinal barrier dysfunction may be an important factor in the pathophysiology of aging in other species as well, including humans.

in aged mammals (9) and *Drosophila* (10–13). Moreover, we (13) and others (14–16) have shown that the intestine represents an important target organ with respect to genetic interventions that promote longevity in both *C. elegans* and *Drosophila* (17). One interpretation of these findings is that maintaining intestinal integrity is an important determinant of health and viability at the organismal level; however, given that most assays of intestinal homeostasis are based on imaging techniques that require killing the flies (10–12), it has not been possible to determine how the onset of intestinal degeneration in individual flies relates to other aspects of aging and/or subsequent mortality.

We recently developed a noninvasive assay to determine intestinal integrity in individual flies (13). In the present work, we used this assay to further explore the role of intestinal barrier dysfunction in *Drosophila* aging. Our results show that loss of intestinal integrity accompanies aging across a range of *Drosophila* genotypes and environmental conditions. Interventions that extend lifespan, such as reduced temperature or dietary restriction, delay the onset of intestinal barrier defects, whereas loss of subunit b of mitochondrial complex II (*sdhB*) accelerates the onset of intestinal barrier defects and shortens the lifespan. Critically, intestinal barrier dysfunction is a better predictor of age-onset mortality than chronological age. Furthermore, we show that flies with intestinal barrier dysfunction display increased expression of antimicrobial peptides (AMPs), impaired IIS and reduced metabolic stores compared with age-matched animals without intestinal barrier defects. Thus, in a population of aging flies, we can now identify individuals showing systemic metabolic defects,

Serum Zonulin Concentrations Among Healthy Young and Older Adults Correlate With Inflammatory And Aging Markers



QI YF. et al JAMDA 2017;18:810e1-810e4

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Short Communications

Serum Zonulin and Endotoxin Levels in Exceptional Longevity versus Precocious Myocardial Infarction

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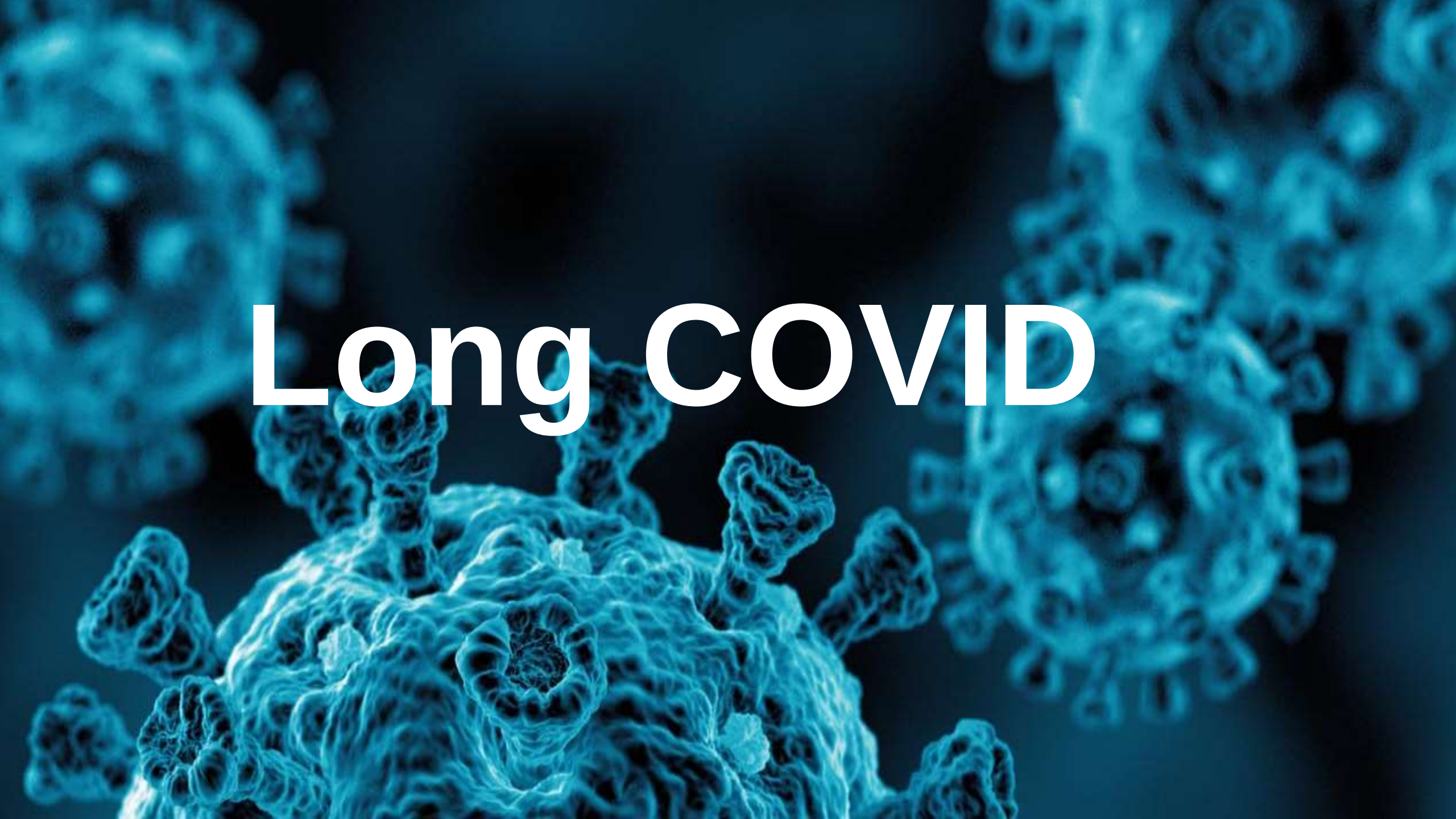
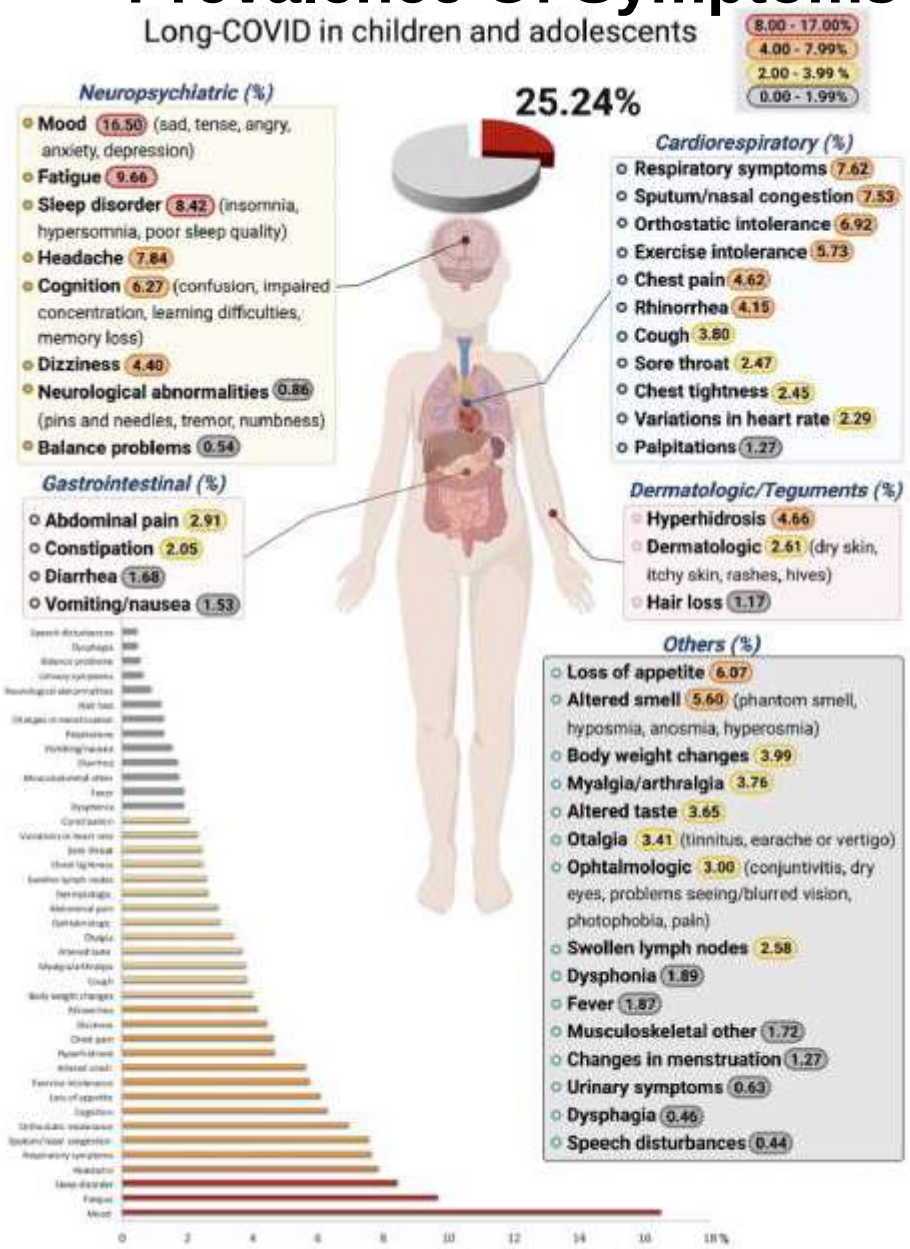
ABSTRACT: Endotoxemia-induced inflammation has been associated with insulin resistance and atherosclerosis, ultimately increasing the risk of coronary heart disease. Increased intestinal permeability is an important event leading to endotoxemia. This study aims to elucidate the possible association between endotoxin (lipopolysaccharide) and zonulin (a biomarker of intestinal permeability) levels and the risk of coronary heart disease, and thus healthy aging. Serum levels of zonulin, lipopolysaccharide and soluble CD14 (a protein that binds lipopolysaccharide) were measured in disease-free centenarians, young healthy controls and patients with precocious acute myocardial infarction. Disease-free centenarians had significantly lower levels of serum zonulin ($P<0.01$) and lipopolysaccharide ($P<0.001$) than young patients with acute myocardial infarction, and had significantly lower concentrations of serum lipopolysaccharide than young healthy controls ($P<0.05$). No significant differences were found for soluble CD14 between groups. Our findings may stimulate further research into the role played by intestinal permeability and endotoxemia not only in coronary heart disease but also in lifespan modulation.


Long COVID

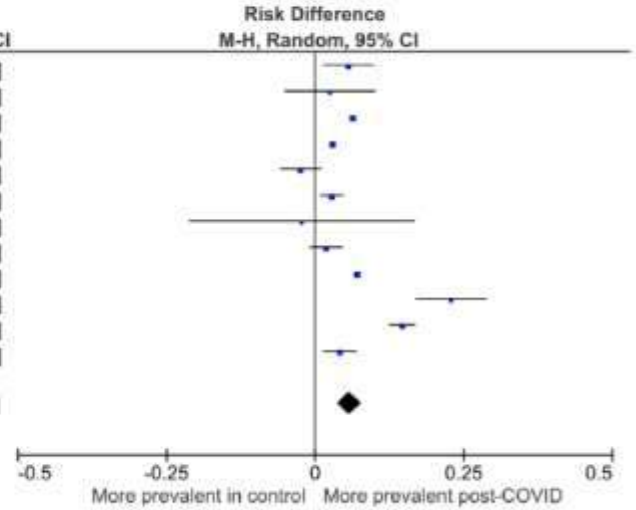
Prevalence Of Symptoms Following COVID In Children And Adolescents

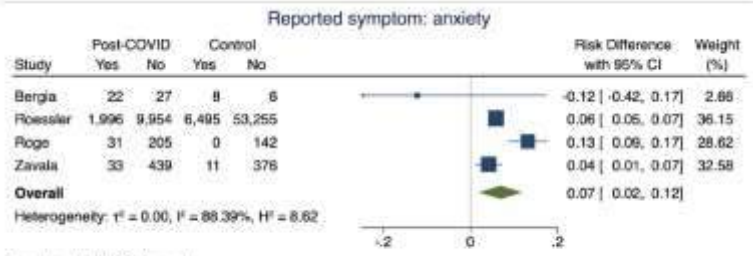


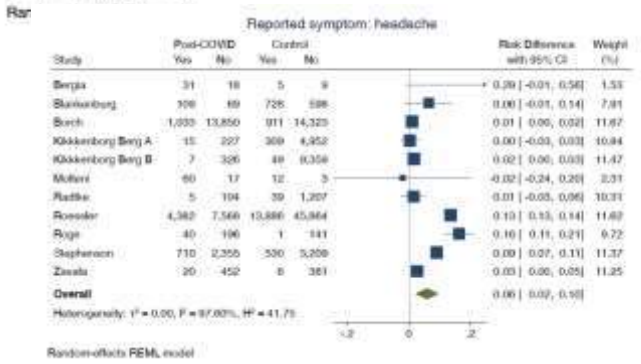
Reported symptom: fatigue

Study or Subgroup	Post-COVID Events	Post-COVID Total	Controls Events	Controls Total	Weight	Risk Difference M-H, Random, 95% CI
Bergia 2022	39	451	3	98	7.6%	0.06 [0.01, 0.10]
Blankenberg 2021	71	178	496	1327	4.4%	0.03 [-0.05, 0.10]
Borch 2022	1578	14883	654	15234	11.2%	0.06 [0.06, 0.07]
Ewan Donnachie 2022	3211	43903	3226	73873	11.3%	0.03 [0.03, 0.03]
Kikkenborg Berg 2022a	19	242	542	5261	8.5%	-0.02 [-0.06, 0.01]
Kikkenborg Berg 2022b	12	333	78	9408	10.2%	0.03 [0.01, 0.05]
Molteni 2021	65	77	13	15	1.1%	-0.02 [-0.21, 0.17]
Radtke 2021	2	109	0	1246	9.4%	0.02 [-0.01, 0.05]
Roessler 2021	1503	11950	3292	59750	11.2%	0.07 [0.06, 0.08]
Roge 2021	59	236	3	142	5.7%	0.23 [0.17, 0.29]
Stephenson 2021	1196	3065	911	3739	10.0%	0.15 [0.12, 0.17]
Zavala 2021	33	472	11	387	9.3%	0.04 [0.01, 0.07]
Total (95% CI)		75899		170480	100.0%	0.06 [0.04, 0.08]
Total events	7788		9229			

Heterogeneity: Tau² = 0.00; Chi² = 372.72, df = 11 (P < 0.00001); I² = 97%
Test for overall effect: Z = 5.50 (P < 0.00001)







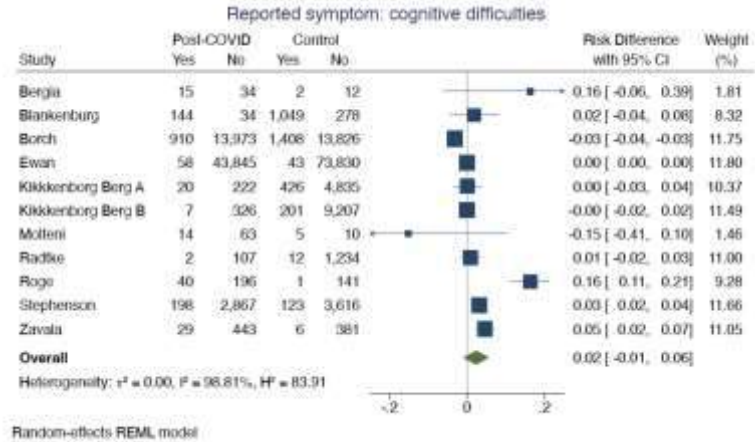
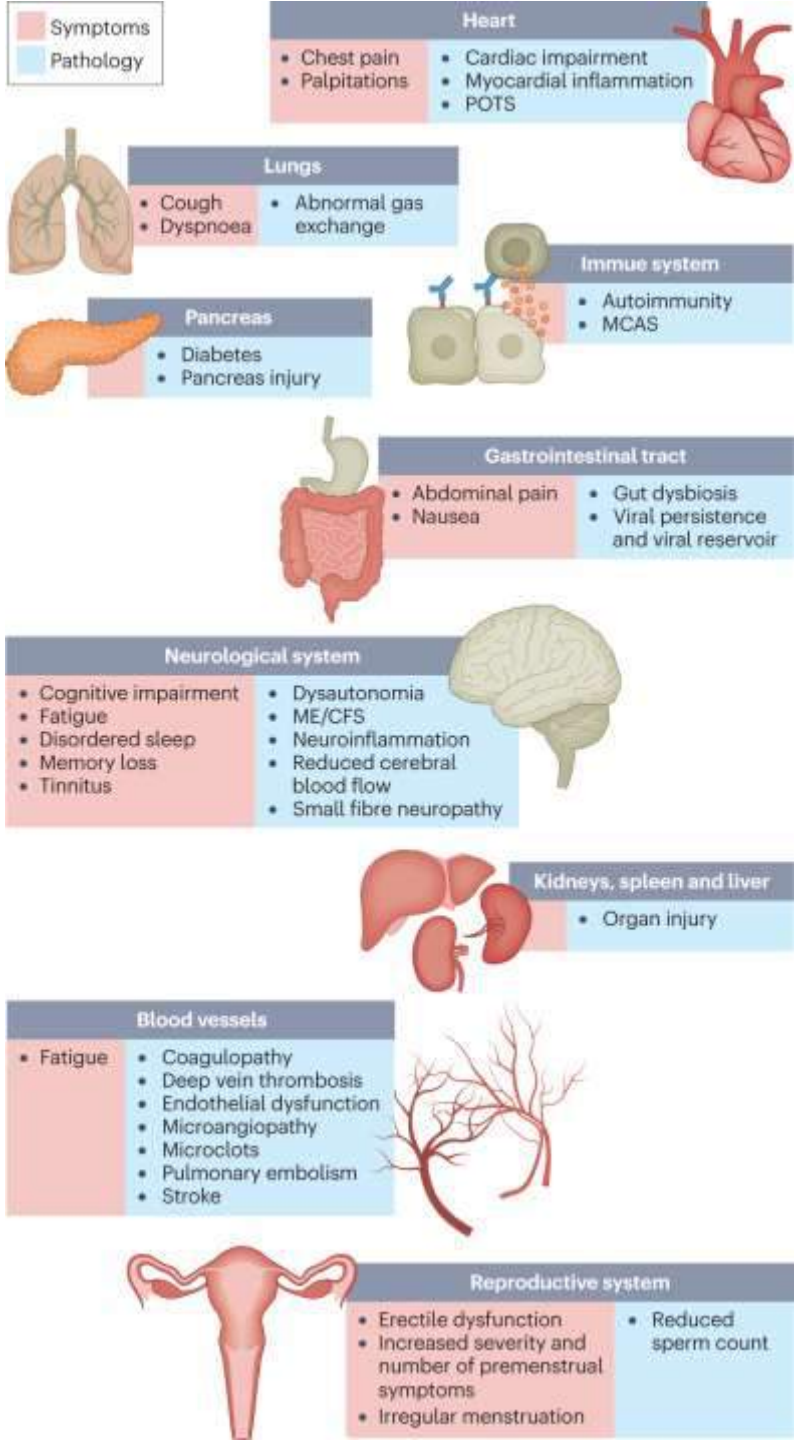
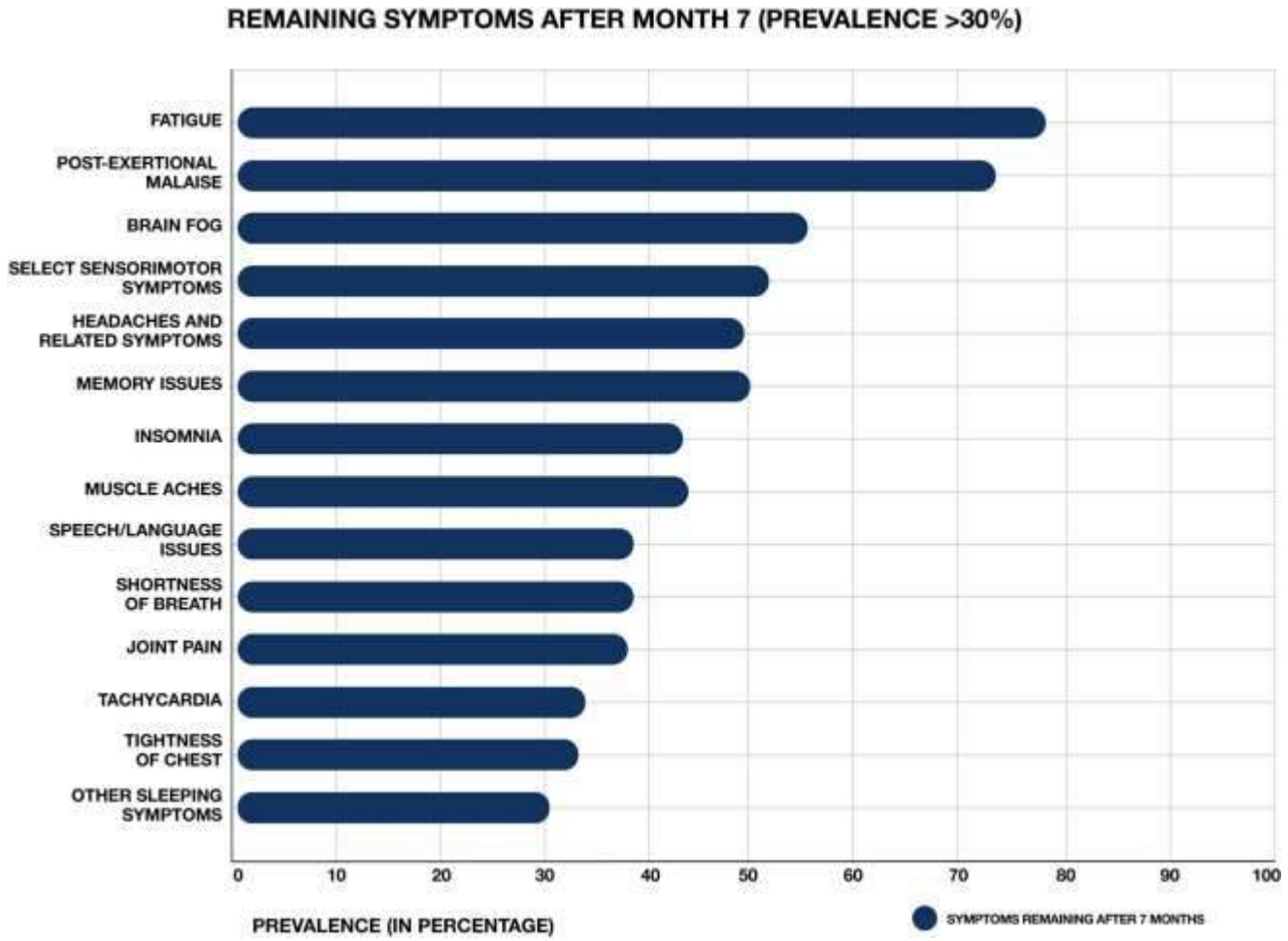


Figure 2. The pooled prevalence of long-COVID by symptoms in children and adolescents. Meta-analyses revealed that the prevalence of more than 40 long-COVID symptoms in children and adolescents. The presence of one or more symptoms following a SARS-CoV-2 infection was 25.24%.

Lopez-Leon, et al *Sci Rep* **12**, 9950 (2022).

Prevalence Of Symptoms Following COVID In Adults

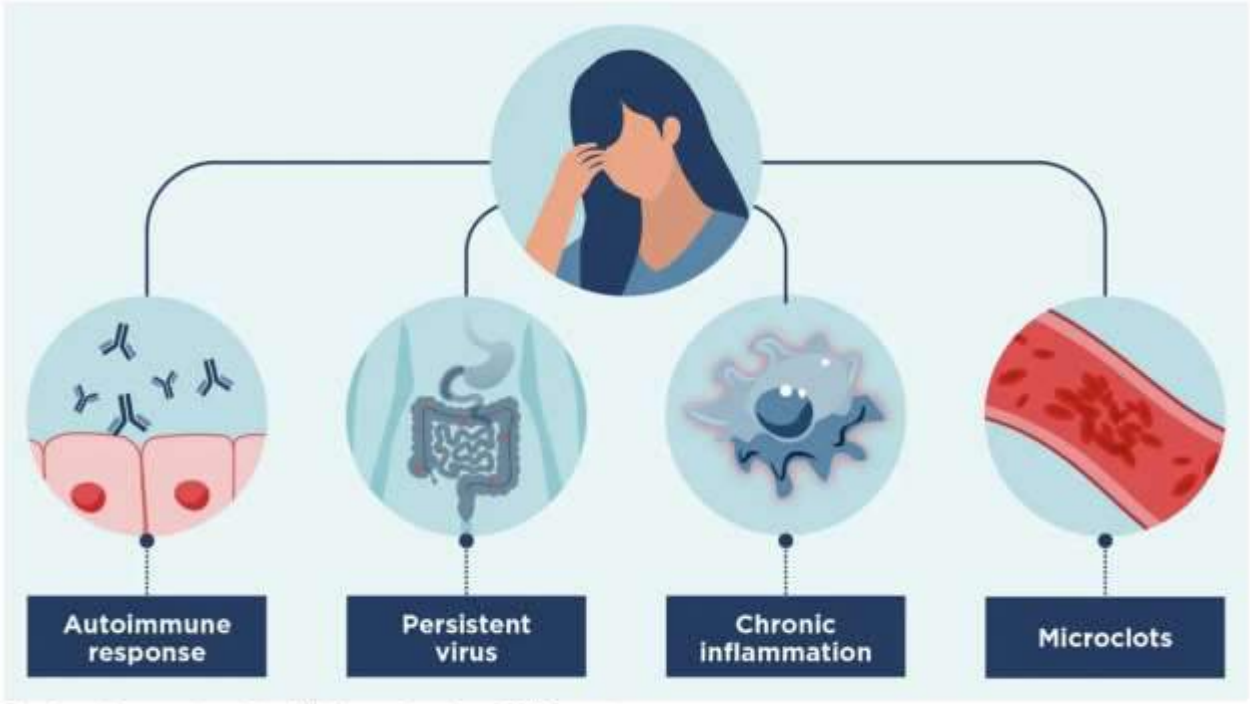




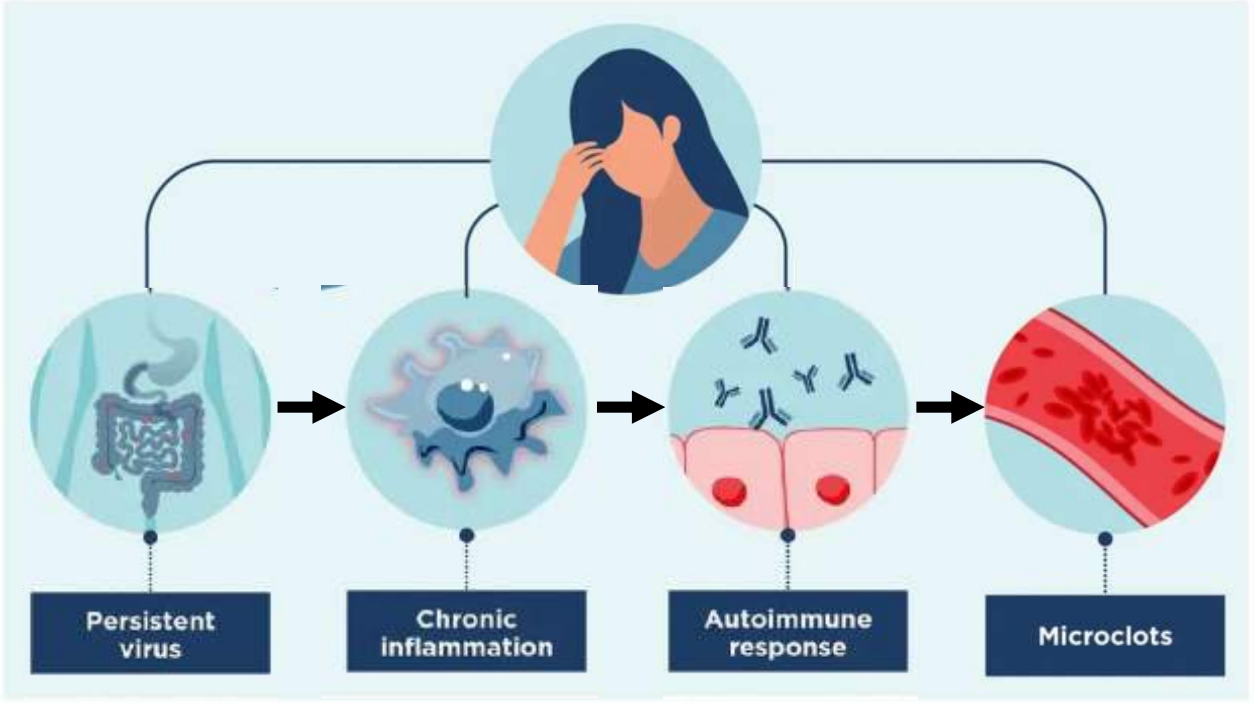
Davis HE, et al *Nat Rev Microbiol* **21**, 133–146 (2023)

What Causes Long COVID?

Four Hypothesis As To What Might Be Causing Long COVID Symptoms

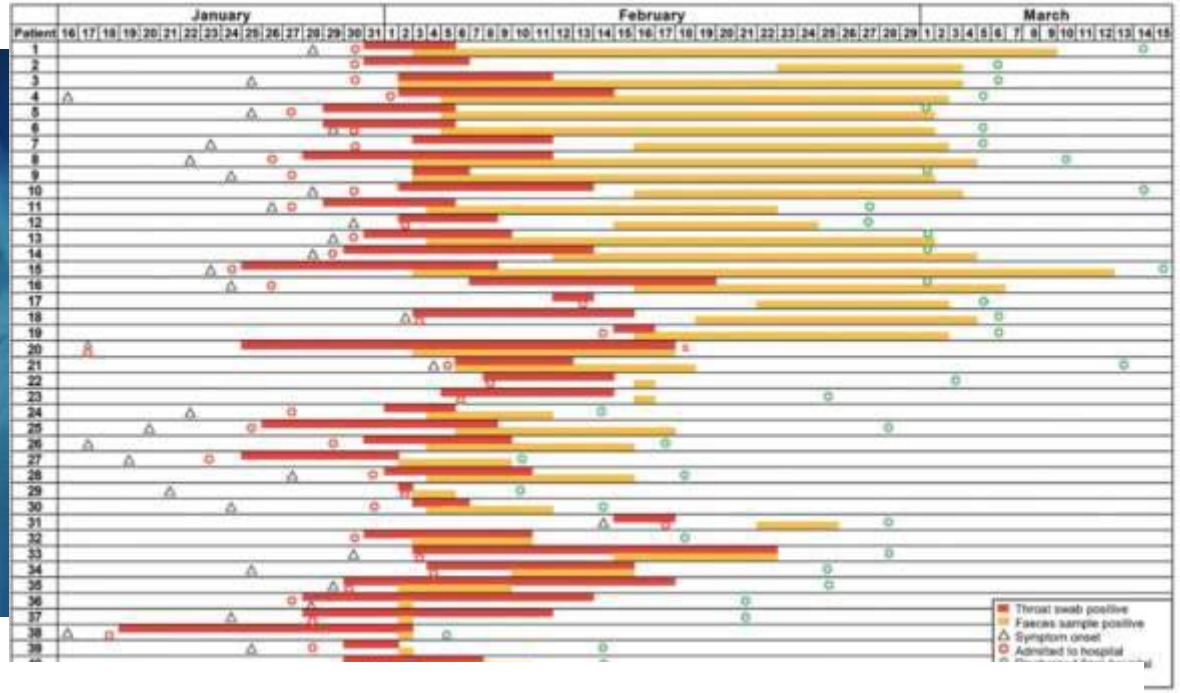


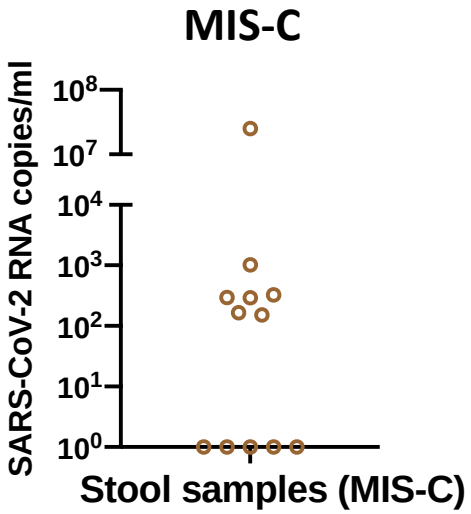
Four Interconnected Steps Leading To Long COVID Symptoms

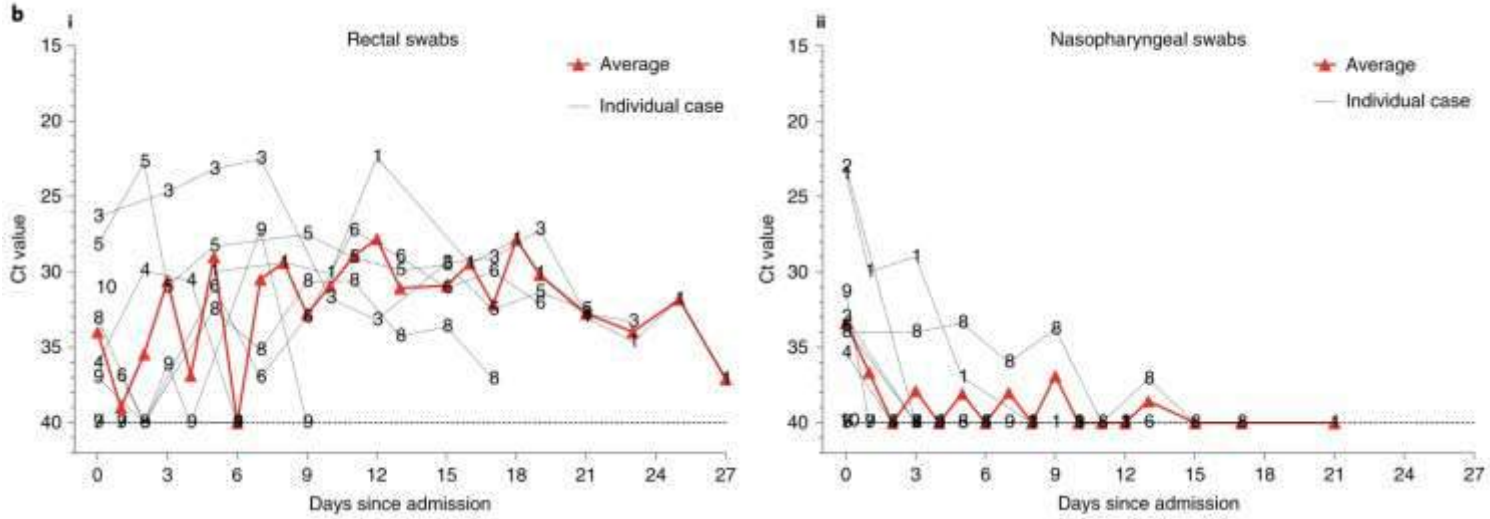


1. Persistent Virus:
The Role of The Gut in COVID-19

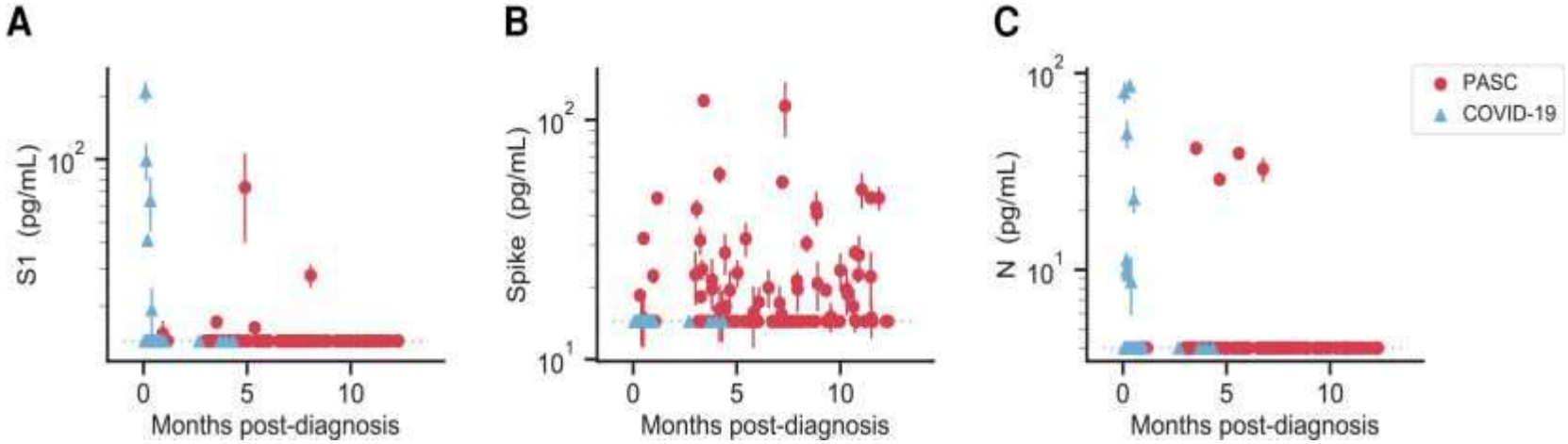
- Prolonged shedding of virus in stools
- Role in acute illness (?)
- Role in long COVID
- Role in MIS

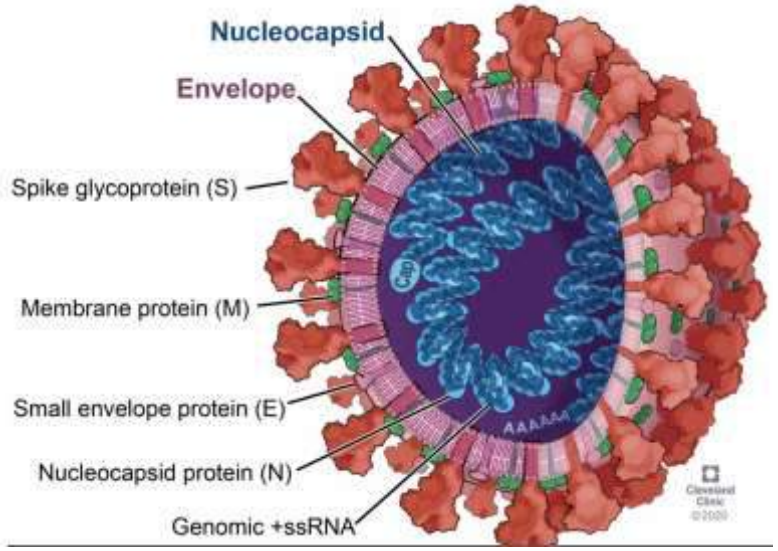






Spike protein present in the serum of long COVID patients despite no virions are present in circulation



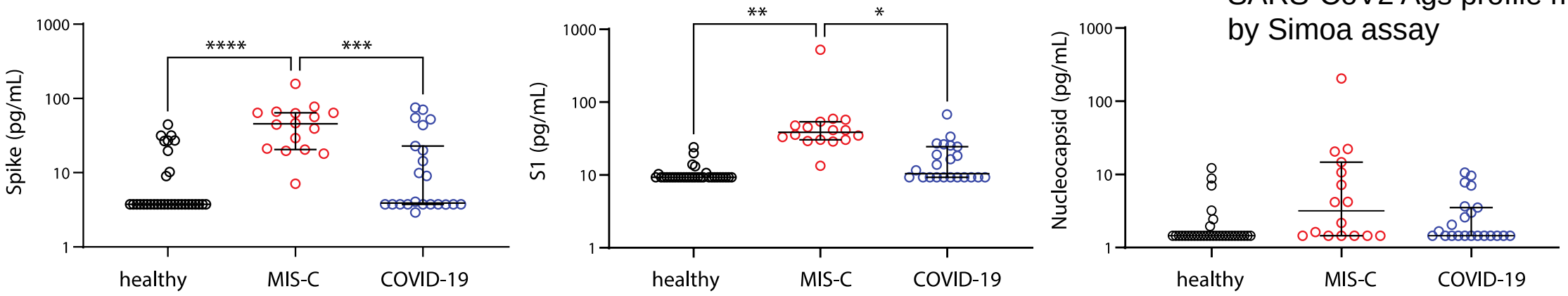


Clin Infect Dis, Volume 76, Issue 3, 1 February 2023, Pages e487–e490, https://doi.org/10.1093/cid/ciac722

Why? How?

Spike protein is present in the serum of MIS-C patients despite no virions are present in circulation

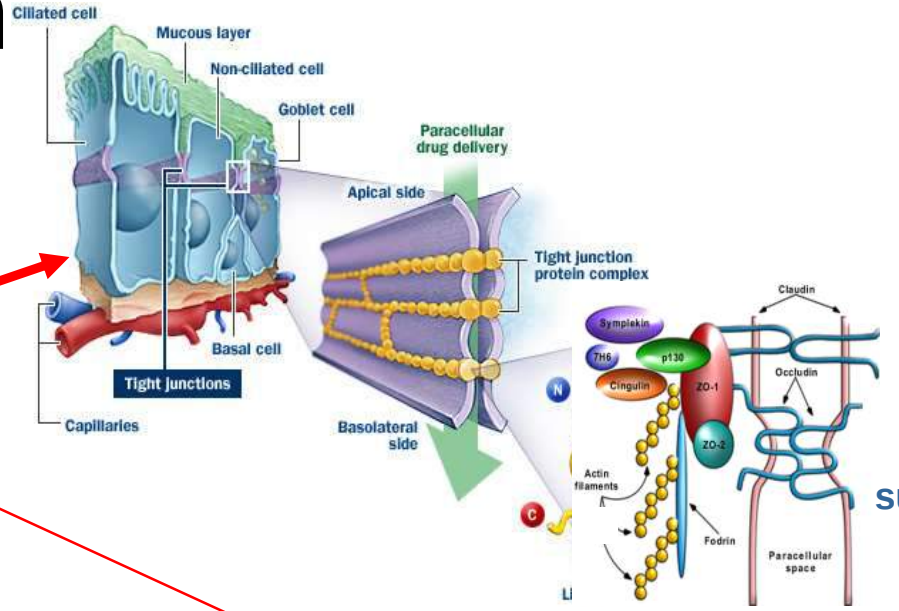
SARS-CoV2 Ags profile measured by Simoa assay



Yonker, Gilboa, Ogata et al, JCI, July 2021

2. Chronic Inflammation

Loss of Mucosal Immune Homeostasis

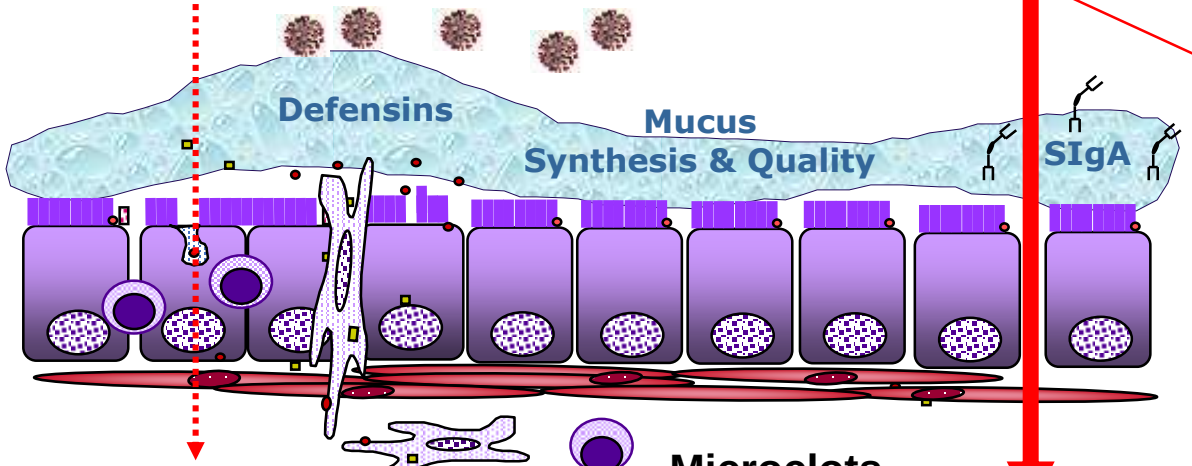


❶ Normal/physiologically controlled permeability

❷ SARS-CoV2 in the GI tract causes dysbiosis and Increased zonulin release

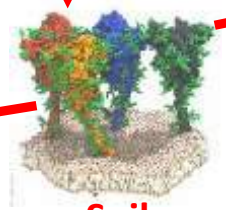
❸ Zonulin-dependent barrier defect leads to SARS-CoV2 spike influx

❹ SARS-CoV2 superantigen triggers T cell proliferation, cytokines storm, and microclots



Zonulin Inhibitor
AT1001 (Larazotide)

Microclots



Spike

Endothelial vessels

SIgA

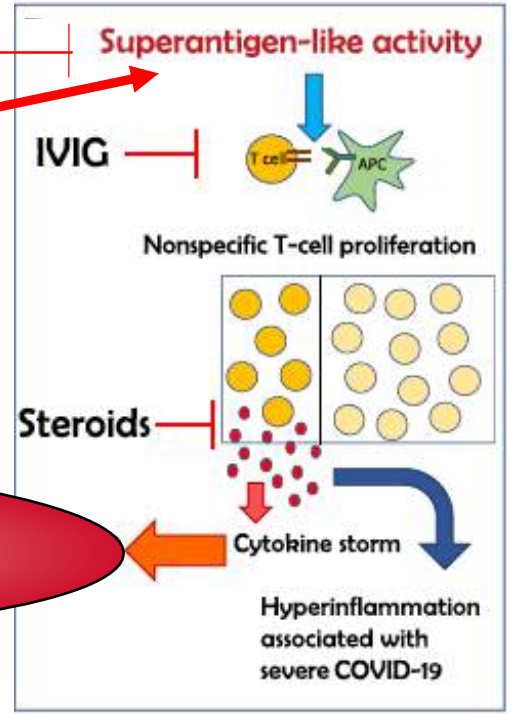
C3 & C4 activation

IL-1β, IL-6, IL-8, IL-10, IP-10, IFN-γ, IL-17, TNF-α

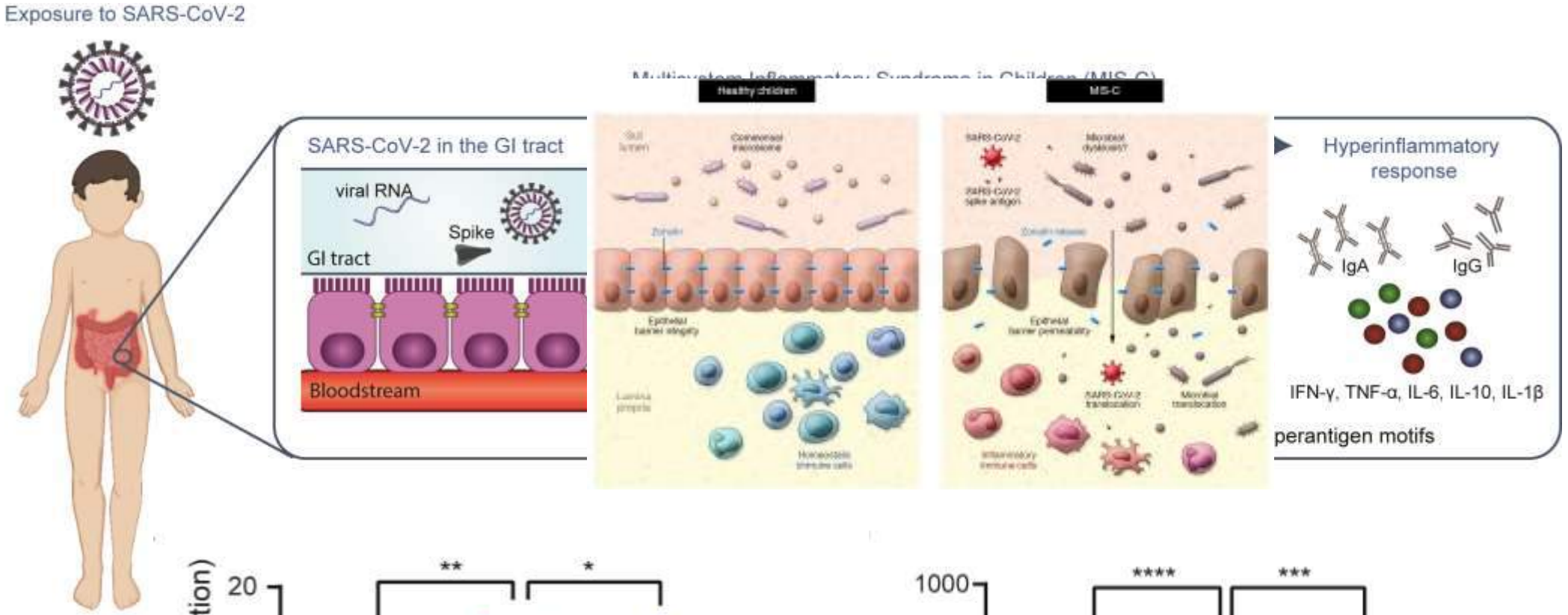
Mucosal Tolerance
Homeostasis
Anergy

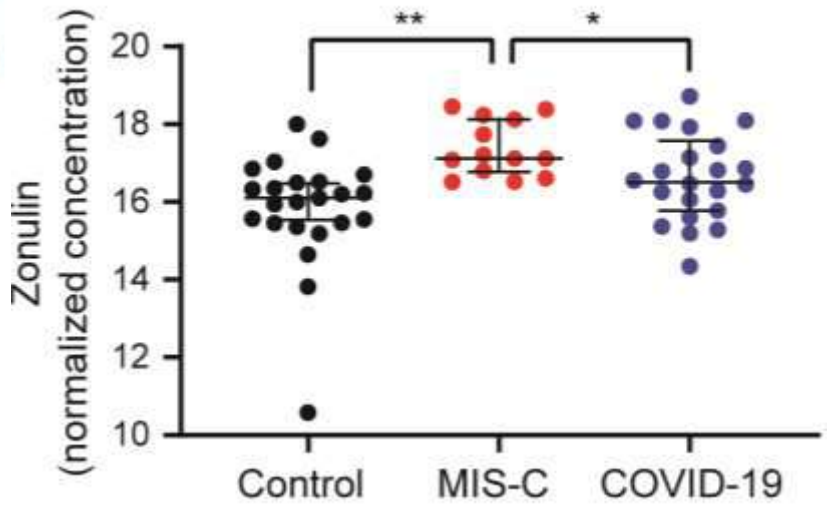
Regulatory DC's
Macrophages
Tregs
IL-10/TGF-β

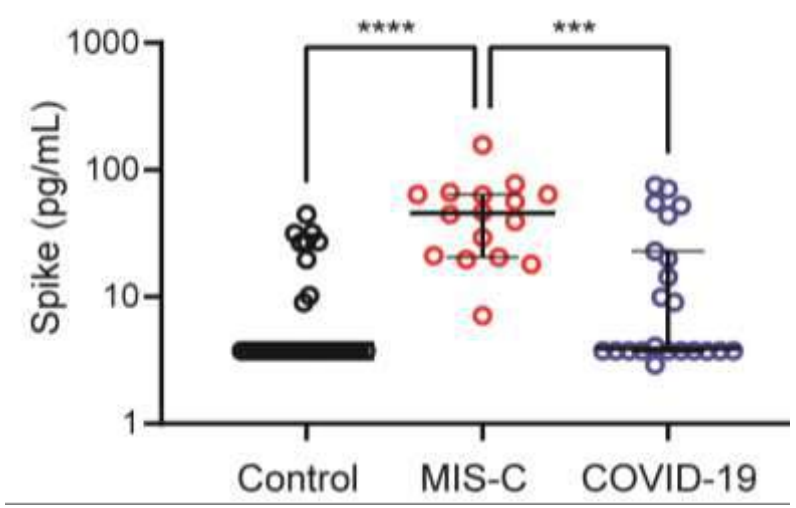
MIS-C
Long COVID



MIS-C is driven by zonulin-dependent loss of gut mucosal barrier







Yonker, Gilboa, Ogata et al, JCI, July 2021

Pathogenesis of MIS-C

- SARS-CoV-2 detected in the stool weeks-months after COVID-19 first encounter.
- SARS-CoV2 presence in GI tract causes microbiome imbalance (dysbiosis)
- Zonulin release detected in MIS-C plasma.
- Highly inflammatory viral particles leak into circulation.
- Current treatments target immune hyperactivation, not mucosal barrier integrity.

Increase In Zonulin And Oxidized LDL Is Associated With Post-Acute Sequelae of SARS-CoV-2

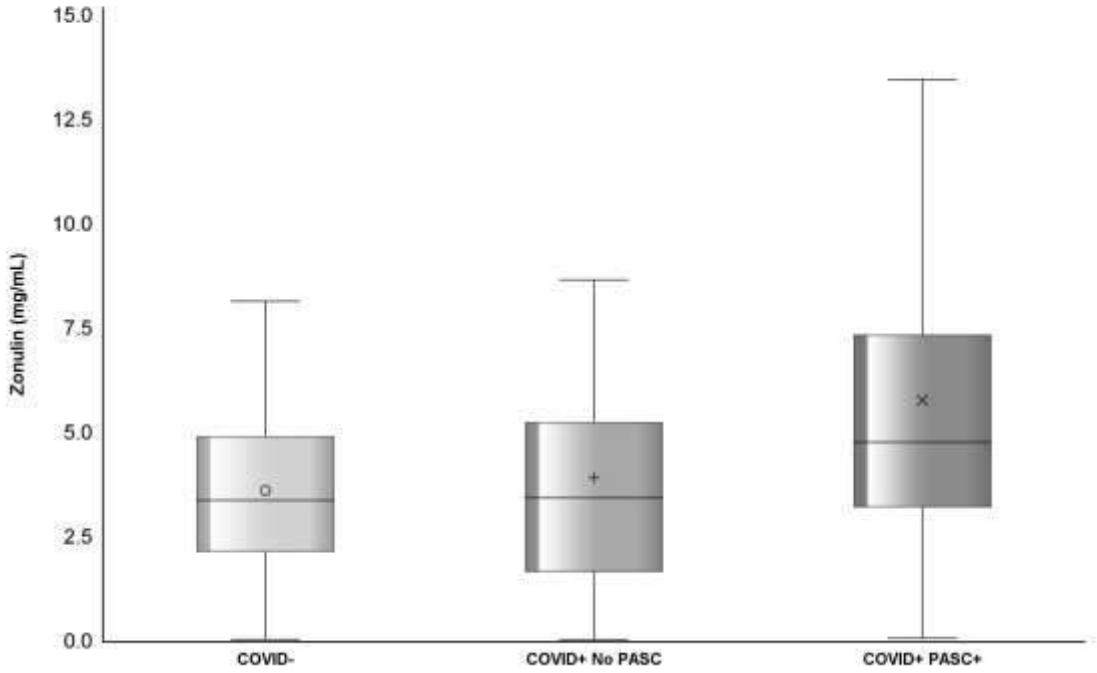
	COVID- (n=258)	COVID+		p-value
		No PASC (n=72)	PASC+ (n=85)	
	n (%) or median (IQR) / mean ± std			
Age (years)	43.68 ± 13.69	44.85 ± 13.31	47.81 ± 13.49	0.05
Female Sex	101 (24.34)	35 (8.43)	50 (12.05)	**0.01**
Non-white Race*	108 (26.02)	25 (6.02)	26 (6.27)	0.14
BMI (kg/m2)	27.91 ± 6.05	30.68 ± 9.49	31.82 ± 8.63	**0.0002**
Current Smoker	158 (38.35)	19 (4.61)	9 (2.18)	**<.0001**
Median number of days from infection	- -	292 (IQR: 172, 518)	229 (IQR: 147, 478)	0.22
Comorbidities				
Hypertension	27 (10.47)	14 (19.44)	23 (27.06)	**0.001**
Diabetes	5 (1.94)	5 (6.94)	9 (10.59)	**0.0002**
HIV infection	98 (37.98)	22 (30.56)	22 (25.88)	0.1
Medications				
Statin	5 (1.94)	11 (14.1)	10 (9.17)	0.28
Ox-LDL (U/L)	47.02 (35.52, 62.77)	57.24 (40.7, 75.37)	76.75 (59.95, 103.28)	**<.0001**
IL-6 (pg/ml)	2.65 (1.62, 4.25)	2.21 (1.44, 3.63)	2.46 (1.77, 4.01)	0.25
D-dimer (ng/mL)	423.7 (256.02, 705.09)	429.96 (243.58, 677.41)	451.57 (266.36, 626.74)	0.93
hs-CRP (ng/ml)	2720.07 (941.82, 7205.25)	2764.87 (1188.0, 7241.84)	3182.65 (1423.9, 8931.79)	0.35
sTNF-RI (pg/ml)	1103.11 (887.11, 1375.03)	966.99 (755.06, 1331.06)	1099.98 (886.19, 1316.1)	0.08
Zonulin (mg/mL)	3.37 (2.13, 4.91)	3.43 (1.65, 5.25)	4.76 (3.2, 7.35)	**<.0001**
LBP (ng/mL)	16657.37 (11899.67, 21686.01)	14441.98 (9919.42, 23111.83)	16438.56 (10640.08, 22563.73)	0.66

*Includes African American, Asian, Hispanic, and Other Numbers in Bold indicates statistical significance (P < 0.05).

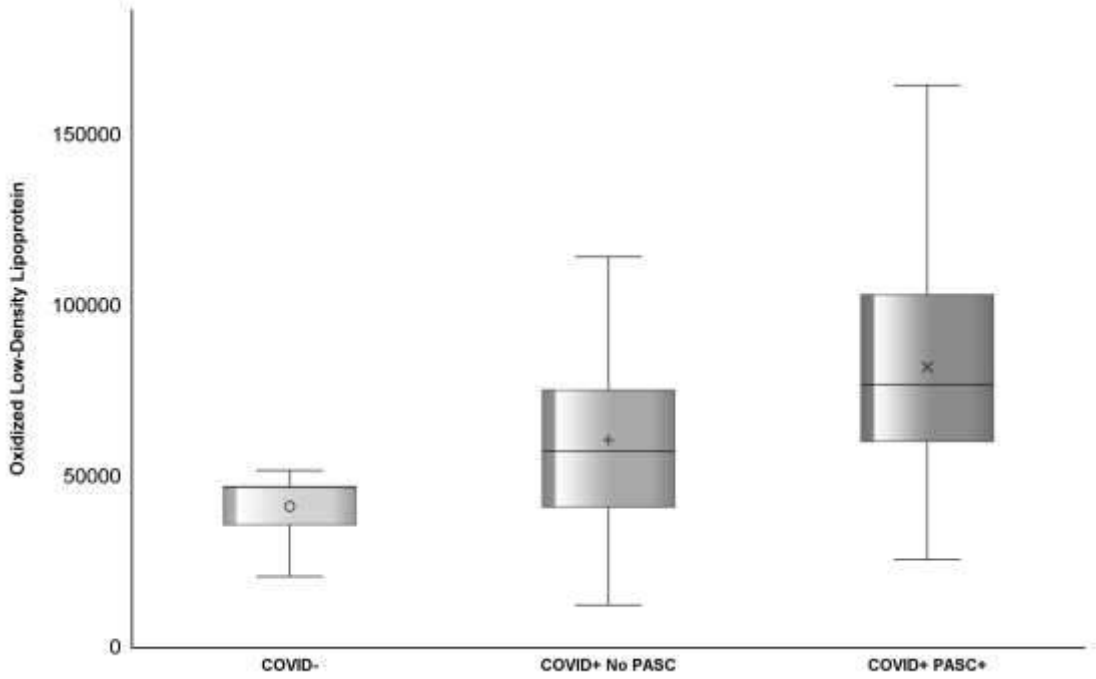
Characteristics of participants by COVID and PASC (Long COVID) Status

Mouchati C et al, Front Immunol, 2023

Distribution of Zonulin by COVID And PASC Status



Distribution of Oxidative-LDL by COVID And PASC Status



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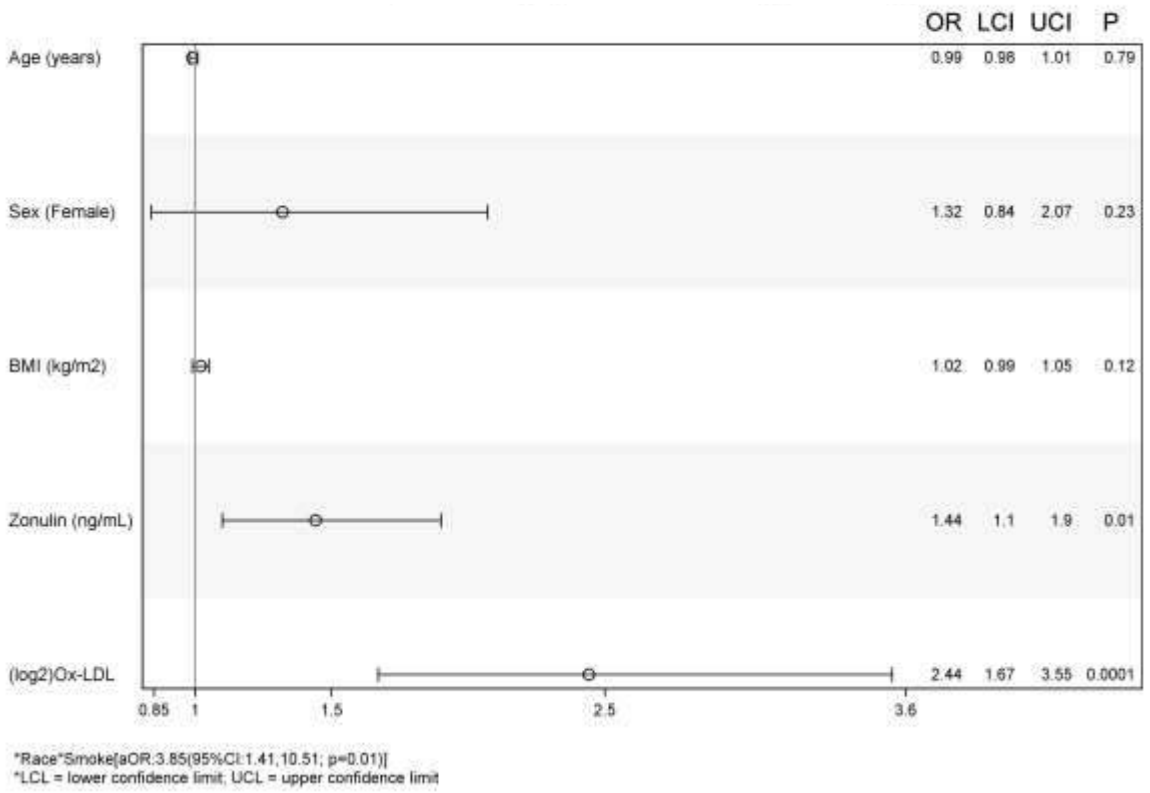
Association of Gut Permeability And Oxidated-LDL with PASC



*Race*Smoke[aOR:3.85(95%CI:1.41,10.51; p=0.01)]
*LCL = lower confidence limit; UCL = upper confidence limit

Independent Associations with COVID+ PASC+

	uOR (95% CI)*	p-value
Age	1.02 (1.002, 1.03)	**0.02**
Female *vs.* Male Sex	1.88 (1.3[illegible]2.78)	**0.001**
non-White *vs.* White Race	[illegible] (0.44, 0.99)	**0.04**
BMI	1.06 (1.03, 1.09)	**<.0001**
Current Smoker	0.13 (0.08, 0.22)	**<.0001**
IL-6	0.84 (0.65, 1.09)	0.2
hs-CRP	1.09 (0.95, 1.26)	0.23
D-dimer	0.86 (0.68, 1.07)	0.18
Ox-LDL	6.38 (3[illegible])	**<.0001**
Zonulin	1.48 (1.14, 1.91)	**0.003**
LBP	0.8 (0.61, 1.06)	0.12

Patients' stratification

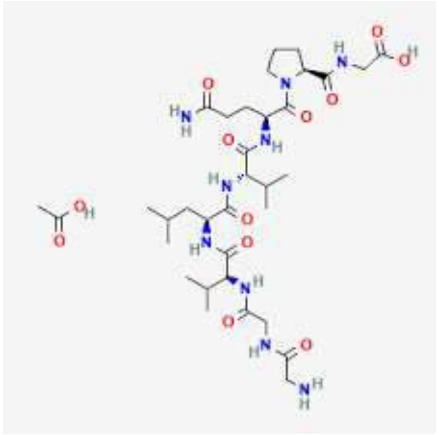
Potential Biomarkers

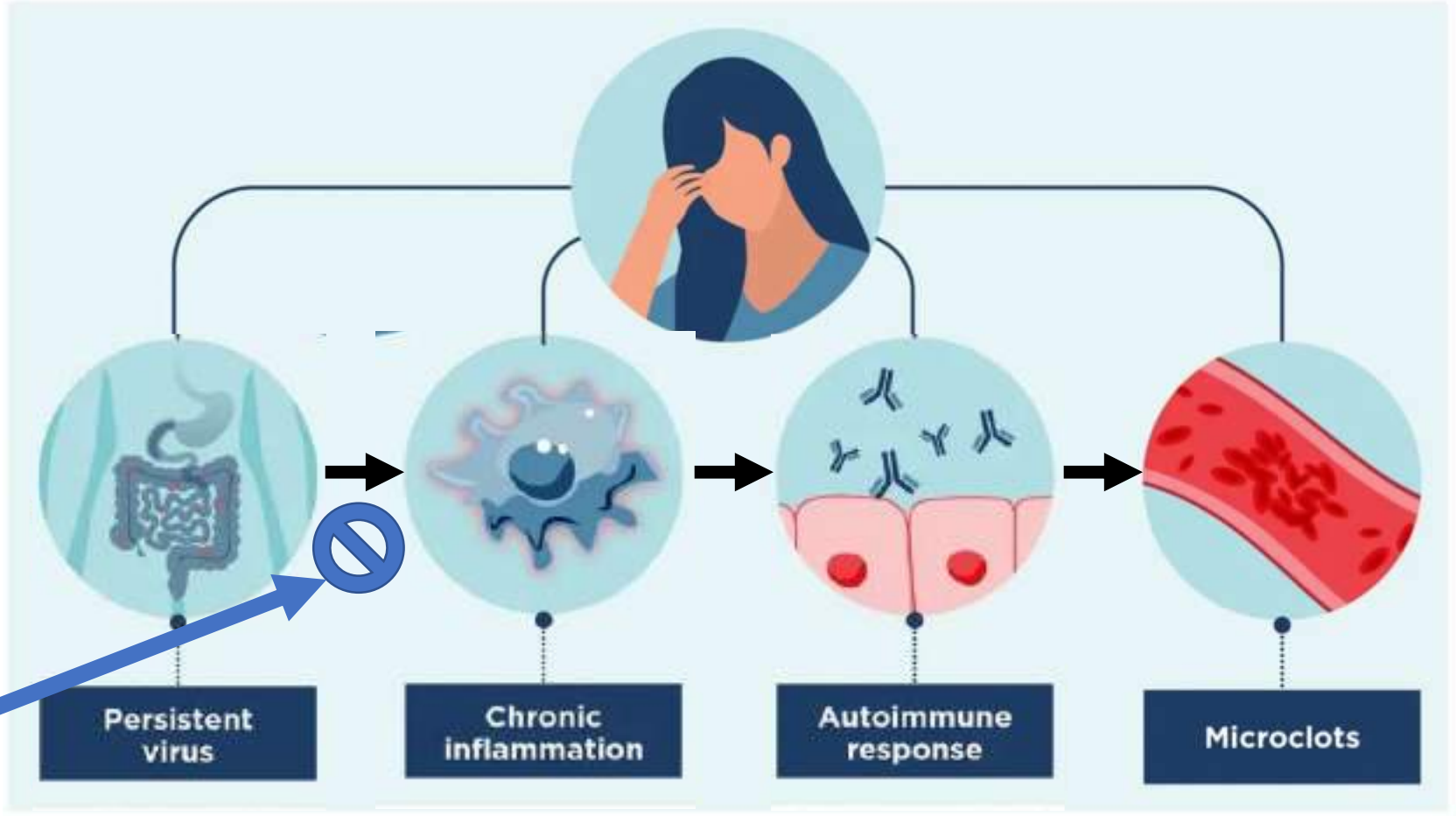
*uOR=Unadjusted Odds Ratio and 95% Confidence Intervals.
Numbers in Bold indicates statistical significance (P < 0.05).

Mouchati C et al, Front Immunol, 2023

Blocking Zonulin to Stop The March Toward Chronic Inflammation Onset (Experimental Medicine At Work)

Zonulin Inhibitor
Larazotide Acetate
(AKA AT1001)





Larazotide Expedites GI Symptoms Resolution and Spike Clearance in MIS-C

- FDA approval for compassionate use
- IRB approval
- Clinical team approval
- Patient/family consent
- Larazotide 10mcg/kg (max 500mcg/dose) four times daily x 21 days

- Larazotide treatment associated with:
- Significantly shorter time to resolution of GI symptoms
- Significantly faster clearance of Spike antigenemia
- *Trend* toward improvement in:
  - Length of stay
  - Fever duration
- No escalation of care in larazotide-treated group
- **Currently in phase 2 DBPC trial**

Patient characteristics	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**
Age (years)	17	3	6	9
Sex	F	F	F	M
Race, Ethnicity	White Non-Hispanic	Asian Non-Hispanic	Black Non-Hispanic	White Non-Hispanic
SARS-CoV-2 RT-PCR or antibody positive	Yes	Yes	Yes	Yes
SARS-CoV-2 Spike antigenemia	Yes	Yes	Yes	Yes
Cardiac involvement	None	None	coronary aneurysm	Mild dilation of coronary artery
Gastrointestinal involvement	adbominal pain, diarrhea	abdominal pain, vomiting, diarrhea	abdominal pain, vomiting	abdominal pain, vomiting, diarrhea
Highest level of care	Ward	Ward	PICU	Ward
Treatment with approved, expanded use of larazotide	Yes	Yes	Yes	Yes

MIS-C with larazotide add-on vs historic controls	Larazotide treated (n=4)	Historic controls (n=22)	*P value*
Age, mean years (range)	8.8 (3-17)	9.1 (1-21)	*ns*
LOS, mean days (range)	4.5 (3-7)	7 (2-16)	*ns*
Escalation of care, number	0	3	*ns*
Fever duration, post steroids/IVIG, mean days (range)	0.8 (0-2)	1.6 (0-6)	*ns*
Time to resolution of GI symptoms, mean days (range)	**2.3 (1-3)**	**6.7 (1-17)**	***0.03***
Time to first clearance of Spike, median days (95% CI)	**1 (1-12)**	**10 (6-190)**	***0.04***

Conclusions

- Genetic predisposition and exposure to environmental triggers are necessary but not sufficient to start the march from genetic predisposition to clinical outcome

- The human microbiome, particularly the gut microbiome, seems to be instrumental in shifting from genetic predisposition to clinical outcome

- The first 1,000 of life are crucial in setting the proper symbiotic relationship between the microbiome and its human host

- Western lifestyle seems associated to an increased risk of developing chronic inflammatory diseases

- Of all the elements that may disturb the proper engraftment and maturation of a symbiotic microbiome, diet seems to be the most impactful

- This observation outline the important of quality, and not only quantity, of food as a key determinant of the balance between health and disease

- Therefore, dietary interventions may in the near future be a therapeutic strategy to implement personalized interventions and/or mitigate the risk of developing chronic inflammatory diseases

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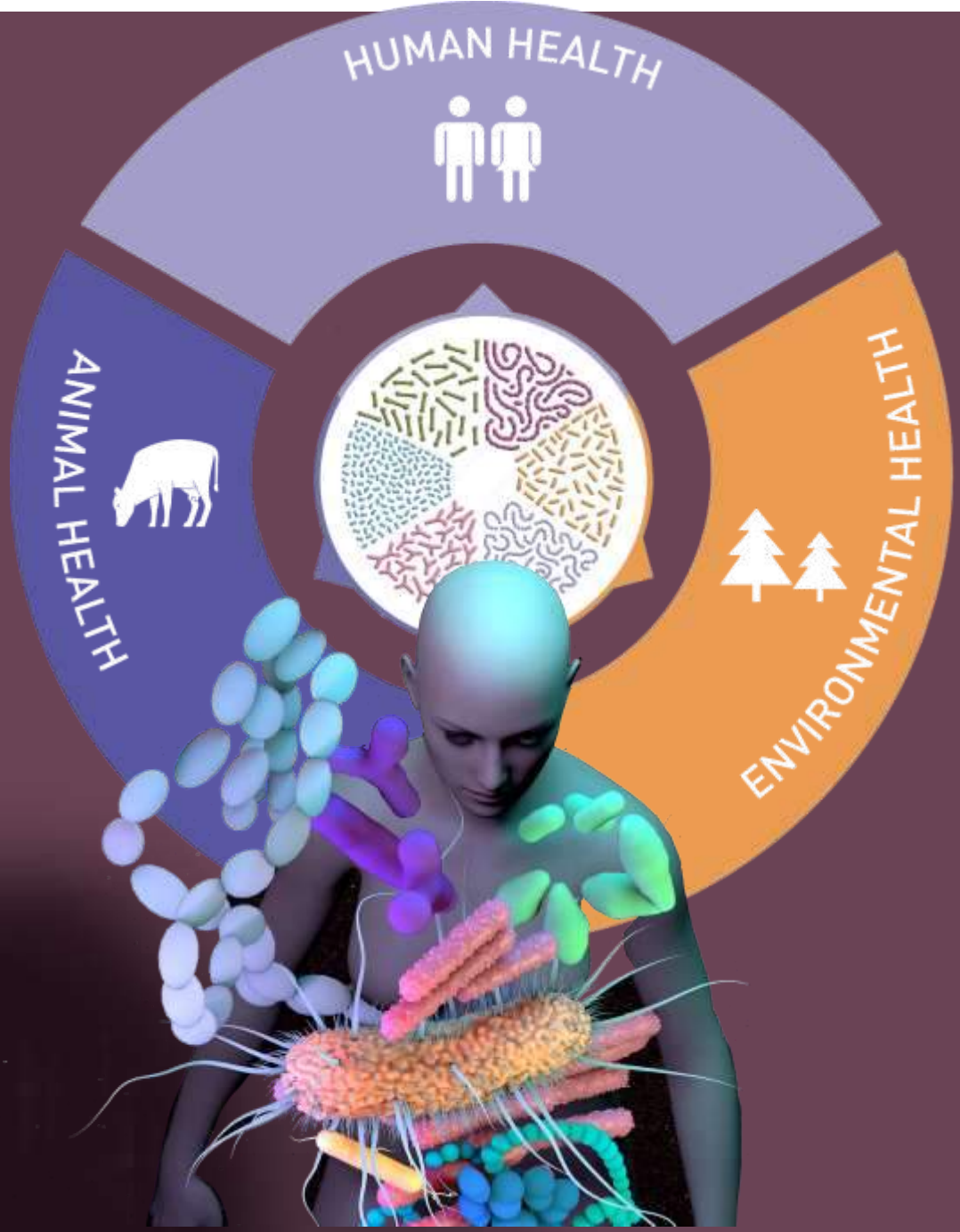

Thank you

GEMM's and their families

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