

Inflammation intestinale et dysbiose dans la mucoviscidose

Emmanuel Mas, 28 mars 2019

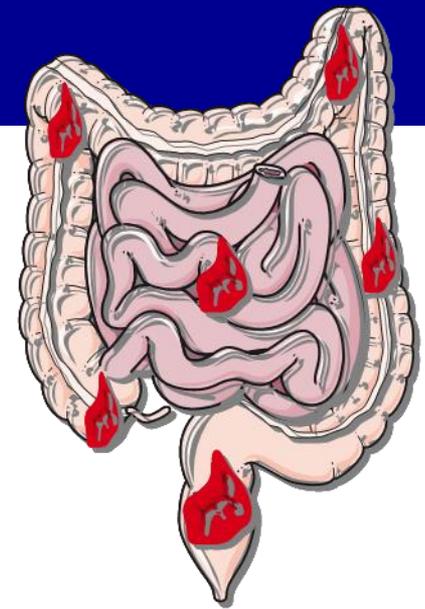
Douleurs abdominales



- Pancréatites,
- Foie,
- IIA,
- Pullulation,
- SOID,
- ...

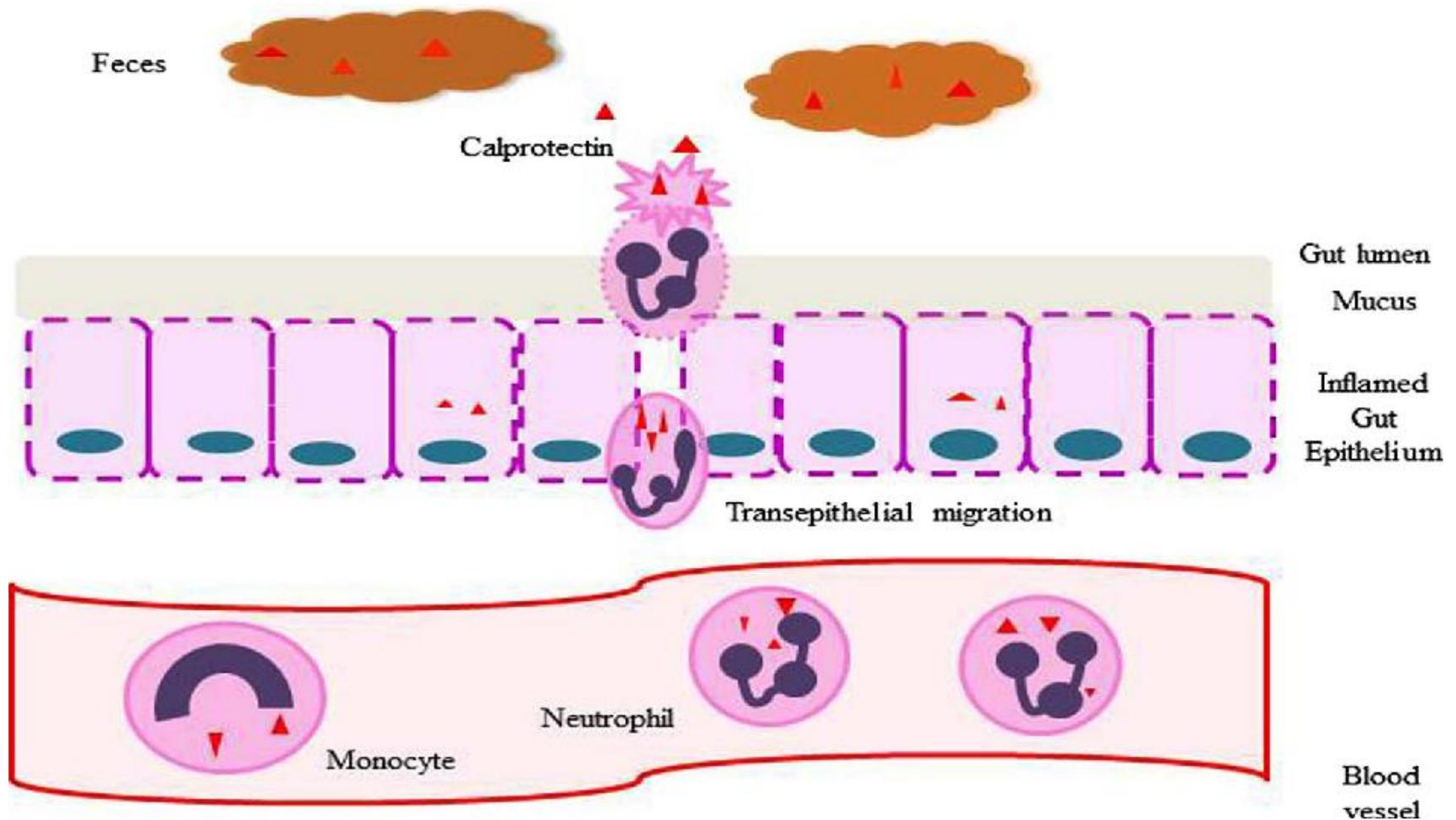
➔ **inflammation et dysbiose**

Rationnel

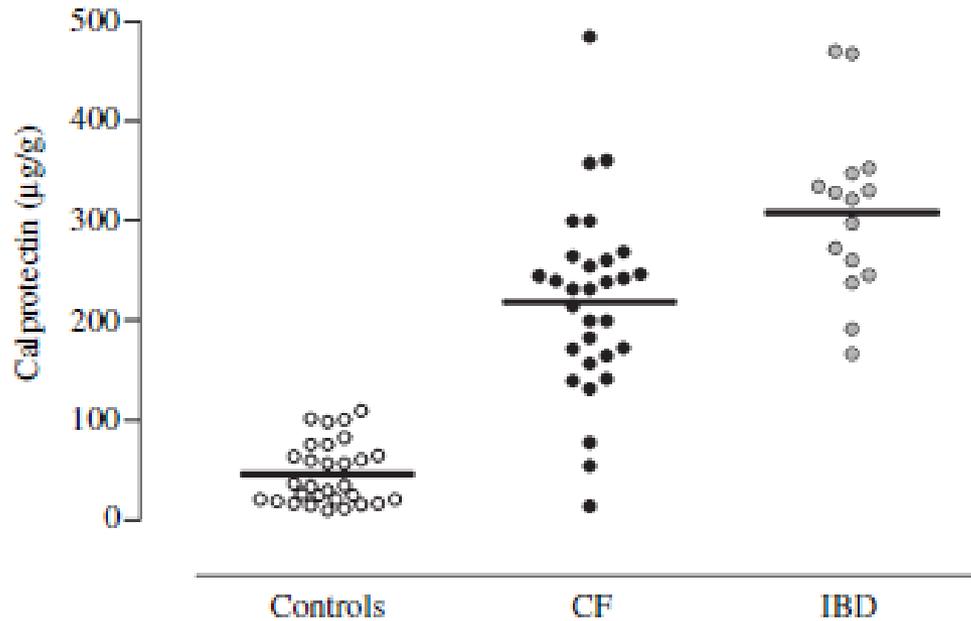


- Smyth, Croft et al. 2000 :
 - IPE
 - augmentation des pertes digestives en albumine, immunoglobulines, protéine cationique éosinophile, élastase, IL-1 β , IL-8
 - augmentation de la perméabilité intestinale ?
- Raia, Maiuri et al. 2000 :
 - inflammation chronique au niveau de biopsies duodénales de patients ayant la mucoviscidose

Marqueur non invasif = **calprotectine**



Calprotectine



- 30 contrôles : 46 ± 31 µg/g
- 30 CF : 219 ± 94 µg/g
- 15 IBD : 309 ± 86 µg/g

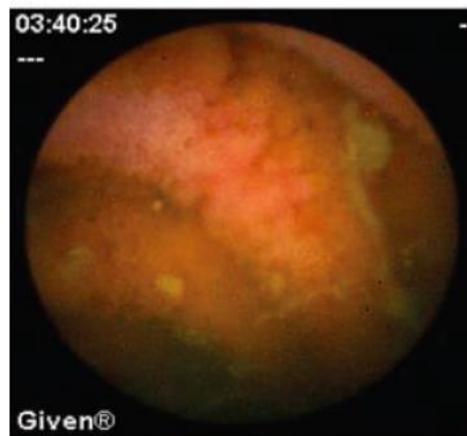
Calprotectine

- 30 contrôles : normal ($<50 \mu\text{g/g}$)
- 30 CF :
 - SP (n=9) = $23,2 \pm 15,2 \mu\text{g/g}$
 - IPE (n=21) = $257,7 \pm 302,5 \mu\text{g/g}$

TABLE 1. WCE in 41 patients with CF showing genotype, BMI, Maiden classification, and fecal calprotectin

Patient no.	Mutation	BMI	Pancreatic function	Maiden grading	Abdominal pain	Diarrhea	Calprotectin (<50 µg/g)
1	3849 + 10KbC → T/ΔF508	27.1	PS	0	N	N	
2	-/-	18.9	PS	0	Y	N	20
3	ΔF508/3849 + 10KbC → T	18.4	PS	4	N	N	48
4	ΔF508/3849 + 10KbC → T	15.4	PS	4	Y	N	
5	3849 + 10KbC → T/405 + 1 G → A	24.7	PS	0	N	N	
6	ΔF508/-	21	PS	4	Y	N	17
7	-/-	15.7	PS	2	Y	N	42
8	-/-	21.4	PS	0	N	N	38
9	-/-	23.1	PS	0	N	N	10
10	-/-	23.1	PS	3	N	N	10
11	-/-	33.7	PS	4	N	N	10
12	-/-	18.8	PS	0	Y	N	14
13	-/-	26.3	PS	0	N	N	
14	G542X/ΔF508	20.7	PI	4	N	N	
15	W1282X/D1152H	27	PI	0	Y	Y	
16	W1282X/ΔF508	16.8	PI	4	Y	N	59
17	W1282X/-	19.2	PI	2	Y	N	
18	W1282X/W1282X	21.3	PI	0	N	N	73
19	W1282X/ΔF508	17.9	PI	2,4	N	N	
20	ΔF508/G542X	20.9	PI	2,4	N	Y	103
21	W1282X/G542X	19.9	PI	4	N	N	
22	G542X/ΔF508	18.7	PI	2	N	N	
23	W1282X/ΔF508	17.9	PI	4	Y	N	95
24	W1282X/-	17.3	PI	4	N	N	208
25	W1282X/W1282X	18.6	PI	2,4	Y	N	74
26	W1282X/W1282X	14.1	PI	2,4	Y	N	349
27	ΔF508/W1282X	20.1	PI	2,4	Y	N	190
28	ΔF508/G542X	16.8	PI	0	N	N	245
29	-/-	16.4	PI	0	Y	N	316
30	ΔF508/G542X	15.6	PI	0	Y	N	282
31	ΔF508/ΔF508	21.3	PI	3	N	N	24
32	W1282X/ΔF508	21	PI	2	N	N	21
33	ΔF508/ΔF508	19.6	PI	2,3	N	N	1075
34	G542X/ΔF508	21.3	PI	0	Y	Y	975
35	W1282X/W1282X	31.3	PI	0	N	N	750
36	W1282X/W1282X	13.9	PI	0	N	Y	180
37	W1282XΔF508	20.3	PI	3	N	N	42
38	W1282X/ΔF508	21.4	PI	3	N	N	125
39	W1282XΔF508	20.6	PI	2,4	N	N	160
40	W1282XΔF508	22.7	PI	4	N	N	65
41	G542X/ΔF508	18.8	PI	2,3,4	N	Y	

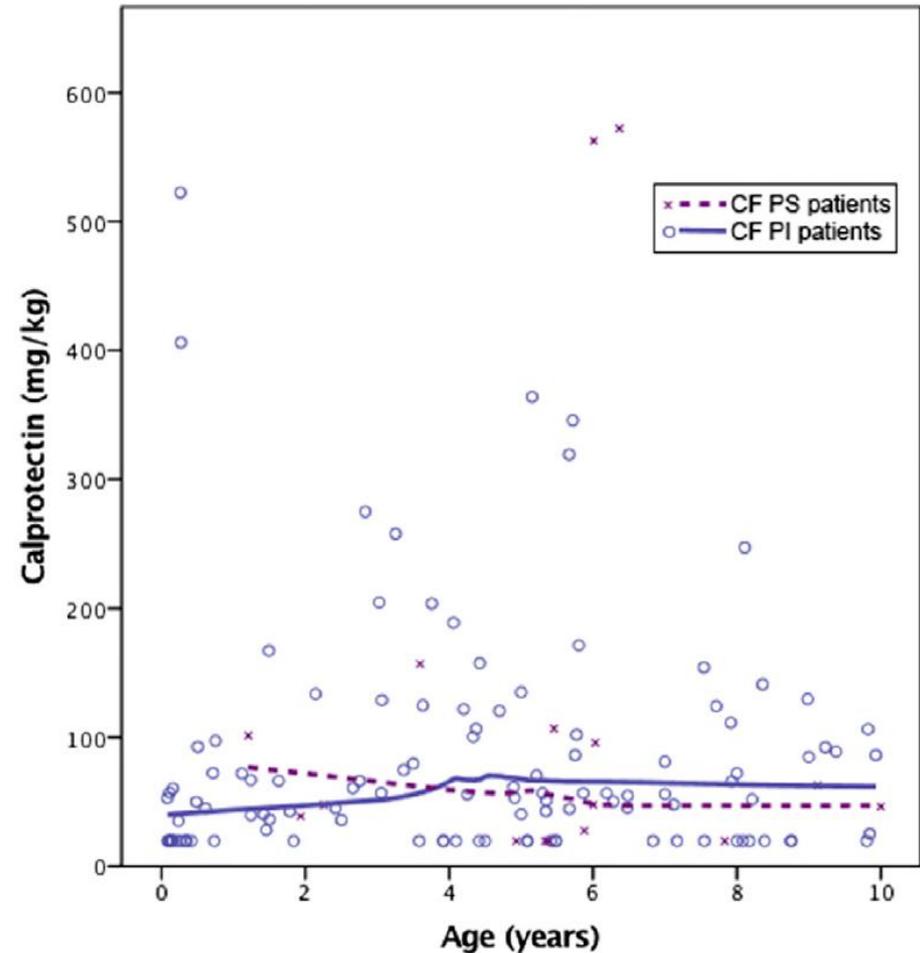
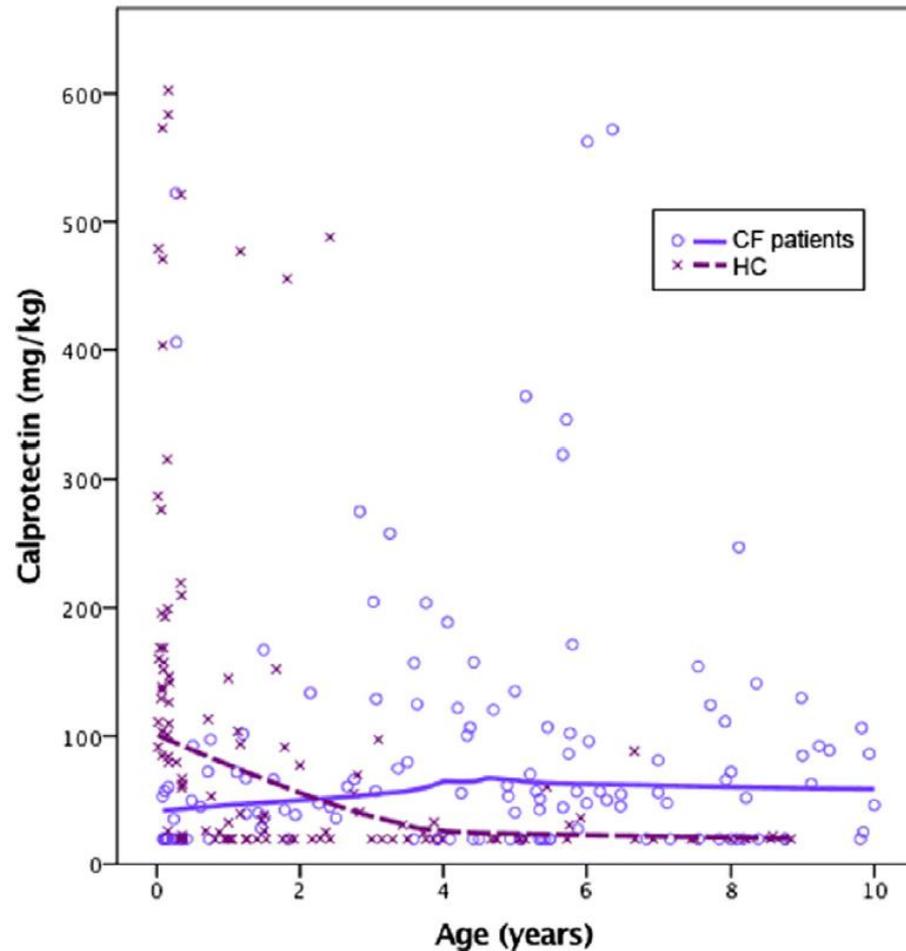
BMI = body mass index; CF = cystic fibrosis; N = no; PI = pancreatic insufficient; PS = pancreatic sufficient; WCE = wireless capsule endoscopy; Y = yes.



	Normal	Pétéchie s	Erythème	Zone « lisse »	Erosions/ulcérations
SP (n=13)	7	0	1	1	4
IPE (n=28)	8	0	11	3	12

➔ Atteinte épithéliale de l'intestin grêle dans la mucoviscidose (physiopathologie ?)

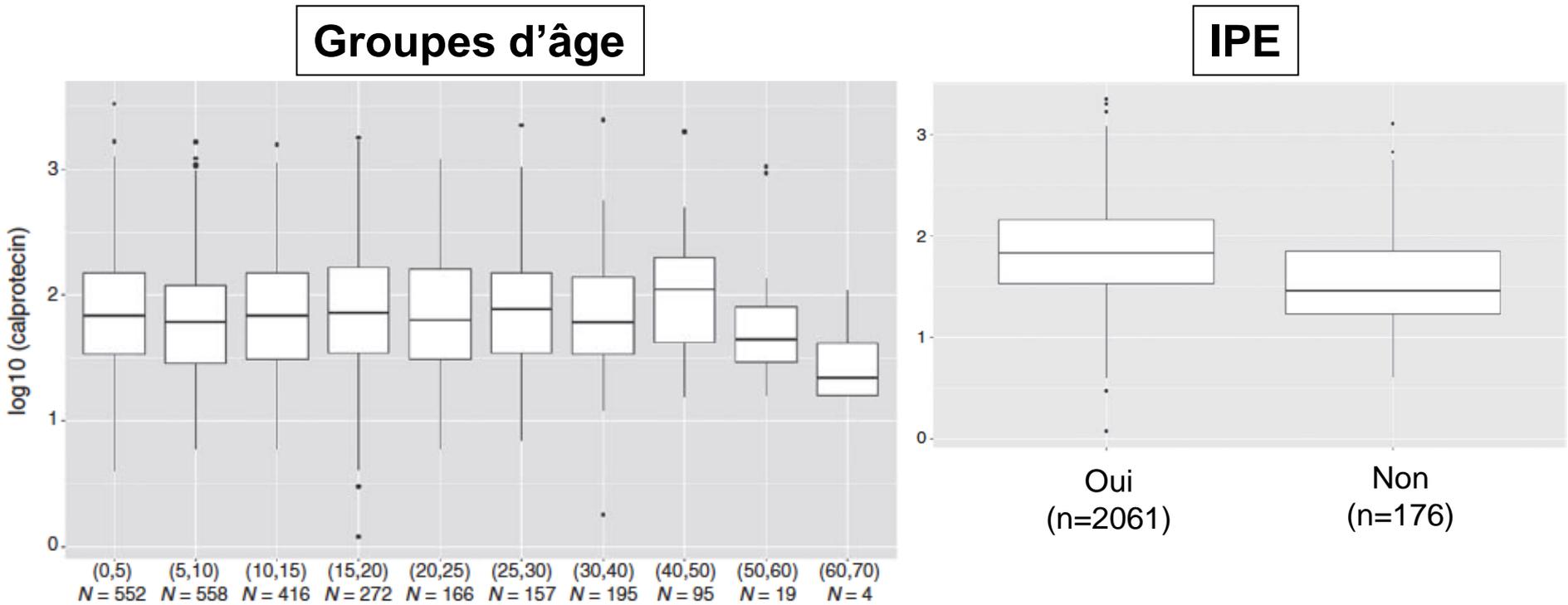
Variations de la calprotectine et CF



- ↓ 0 à 1 an = 19,7 vs.127,7 ($p = 0,03$)
- ↑ 4 et 10 ans = 55,9 vs.19,5 ($p = 0,007$)

- Calprotectine > 50 mg/kg après 4 ans :
- 41,7% des SP
 - 60,3% des IPE

Variations de la calprotectine et CF



- Etude longitudinale de 2003-2015 ; 171 patients (0-61 ans)
- Calprotectine :
 - Médiane 60,9 $\mu\text{g/g}$
 - 61% des valeurs $> 50 \mu\text{g/g}$
 - IPE $>$ SP (68 vs. 29 $\mu\text{g/g}$)

Small intestine bacterial overgrowth does not correspond to intestinal inflammation in cystic fibrosis

Scandinavian Journal of Clinical & Laboratory Investigation, 2010; 70: 322–326

Table III. Fecal calprotectin concentrations (mg/L) in cystic fibrosis (CF) patients and healthy subjects (HS).

Studied subjects (<i>n</i>)	Range	Median	Abnormal values (%)
HS (35)	ND–15.5	2.5	1 (2.9)
CF Whole group (25)	1.8–302.5	80.0	21 (84)
SIBO positive (10)	1.8–302.5	98.8	9 (90)
SIBO negative (15)	7.5–158.0	72.5	12 (80)

*ND, not detectable.

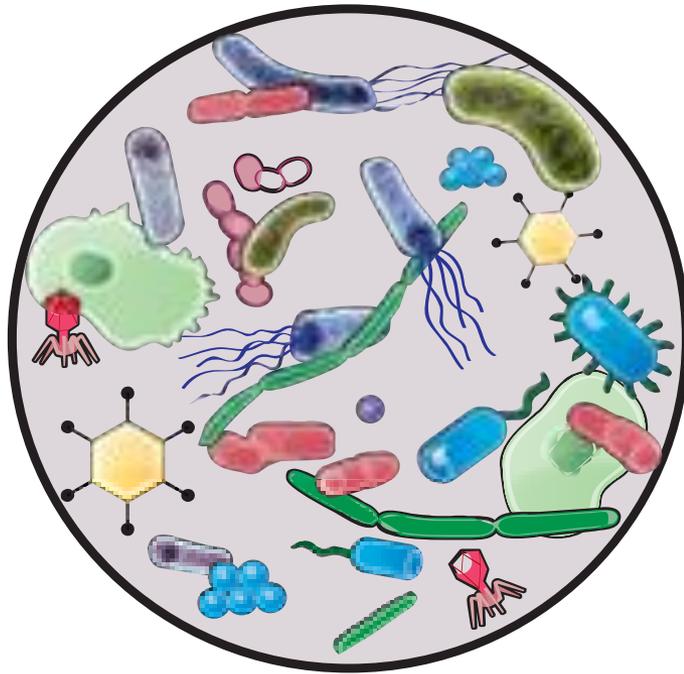
N < 15 mg/L

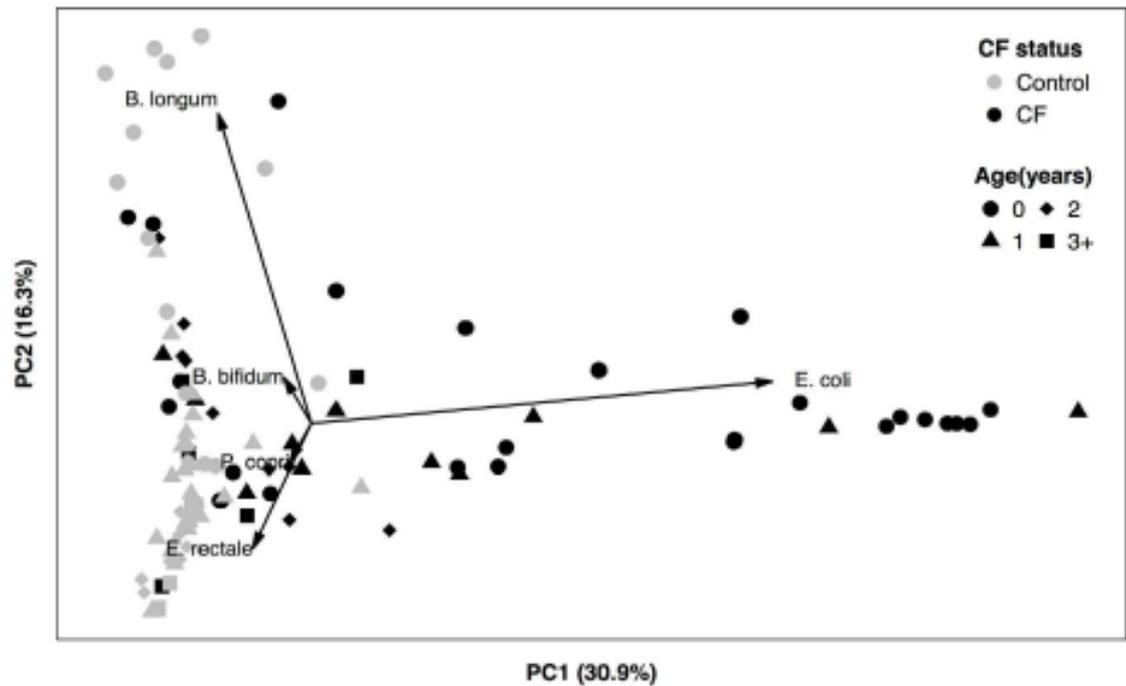
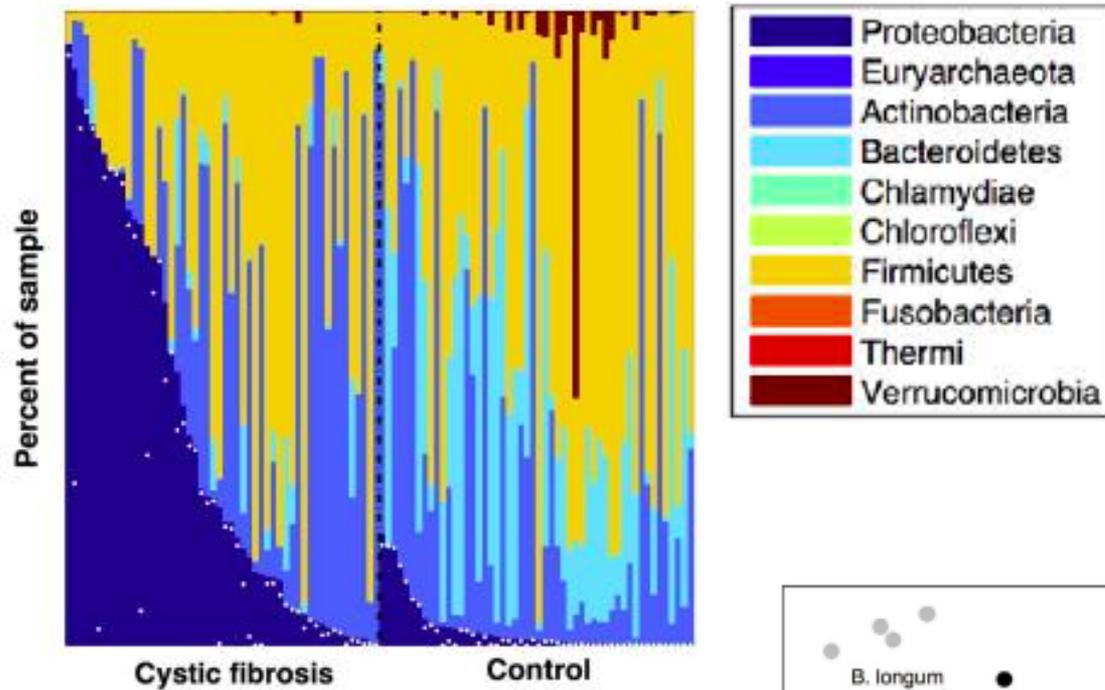
Small intestine bacterial overgrowth does not correspond to intestinal inflammation in cystic fibrosis

Scandinavian Journal of Clinical & Laboratory Investigation, 2010; 70: 322–326

- 25 mucoviscidose dont 21 calprotectine augmentée
- 10 avec une pullulation bactérienne
- élévation de la calprotectine fécale est mieux corrélée à une inflammation du côlon que de l'intestin grêle

Microbiote intestinal



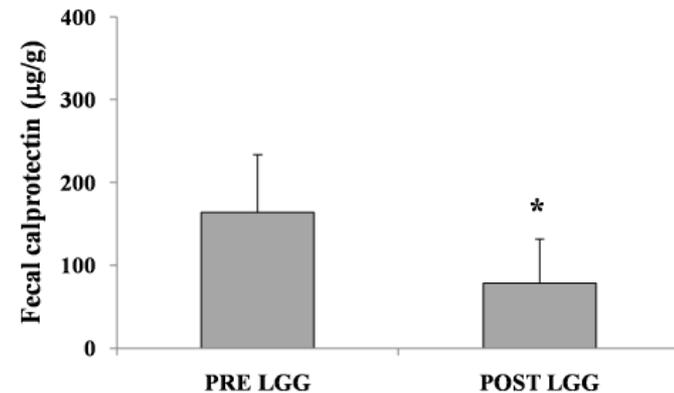
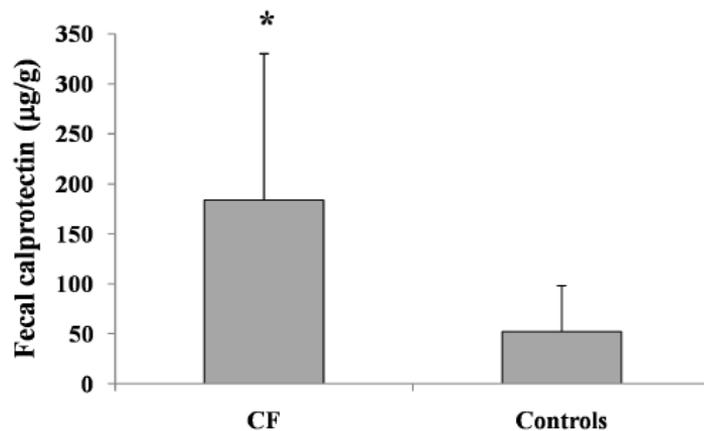


Metagenomic evidence for taxonomic dysbiosis and functional imbalance in the gastrointestinal tracts of children with cystic fibrosis. Manor O, Scientific Reports 2016

Disrupted Intestinal Microbiota and Intestinal Inflammation in Children with Cystic Fibrosis and Its Restoration with Lactobacillus GG: A Randomised Clinical Trial

Bruzzese E, et al. Plos One 2014

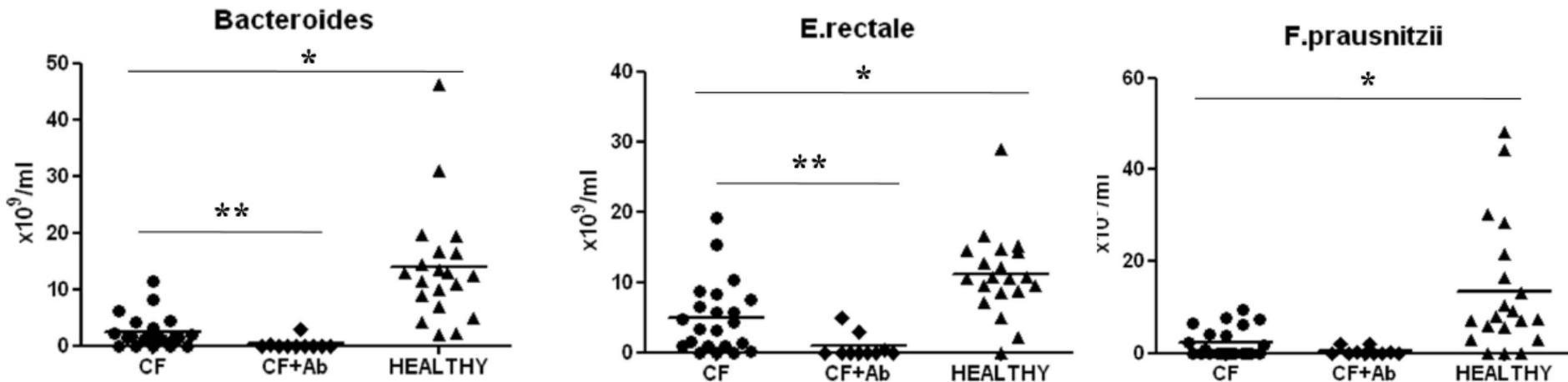
- 22 enfants (13 M)
- Âge médian = 7 ans (2-9)
- 12 F508del hom, 10 F508del het (1 mild)
- Stables (pas d'exacerbation, de CTC ou ATB depuis > 2 sem)



- $184 \pm 146 \mu\text{g/g}$ vs. $52 \pm 46 \mu\text{g/g}$
- $n = 19$; 63% > 100 $\mu\text{g/g}$

Disrupted Intestinal Microbiota and Intestinal Inflammation in Children with Cystic Fibrosis and Its Restoration with Lactobacillus GG: A Randomised Clinical Trial

Bruzzese E, et al. Plos One 2014



Perturbations du microbiote dans CF (aggravées par ATB)

Lack of efficacy of *Lactobacillus* GG in reducing pulmonary exacerbations and hospital admissions in children with cystic fibrosis: A randomised placebo controlled trial

Bruzzese E, et al. JCF 2017

- 95 CF (44 M)
- Âge médian = 103 ± 50 mois
- LGG (6x10⁹ CFU) ou placebo pendant 12 mois
- Inclusion : IPE, VEMS > 50%
- Pas d'exacerbation depuis 2 sem

	LGG group (n = 41)	Placebo group (n = 40)
Age (months)		
Median [min; max]	104 [29; 201]	88 [25; 188]
Genotype, n (%)		
DF508/DF508	19 (46.3)	17 (42.5)
DF508/other mutation	22 (53.7)	23 (57.5)
Sex, n (%)		
Female	20 (48.8)	20 (50)
Male	21 (51.2)	20 (50)
BMI (kg/m ²)		
Median [min; max]	16.34 [14.46; 24.6]	17.1 [13.5; 26.7]
FEV1 (%)		
Median [min; max]	94.7 [57; 129.4]	97 [61; 151]
MMEF 25-75 (%)		
Median [min; max]	86.2 [32.5; 161.9]	73 [35.4; 140.5]

	LGG	Placebo	p
Exacerbations pulmonaires	2,3 ± 2,1	2,2 ± 1,7	NS
Hospitalisations	0,3 ± 0,6	0,3 ± 0,5	NS
Jours de TTT	33,6 ± 34,6	31,5 ± 27,5	NS
Episodes sous ATB oral	1,9 ± 1,8	1,8 ± 1,5	NS

Improvement of digestive health and reduction in proteobacterial populations in the gut microbiota of cystic fibrosis patients using a *Lactobacillus reuteri* probiotic preparation: A double blind prospective study☆

del Campo, et al. JCF 2014

- 30 CF, âge médian = 17.7 ans (8-44)
- exclusion = exacerbation
- 2 groupes 6 mois placebo puis 6 mois LR (et inverse)

Table 1

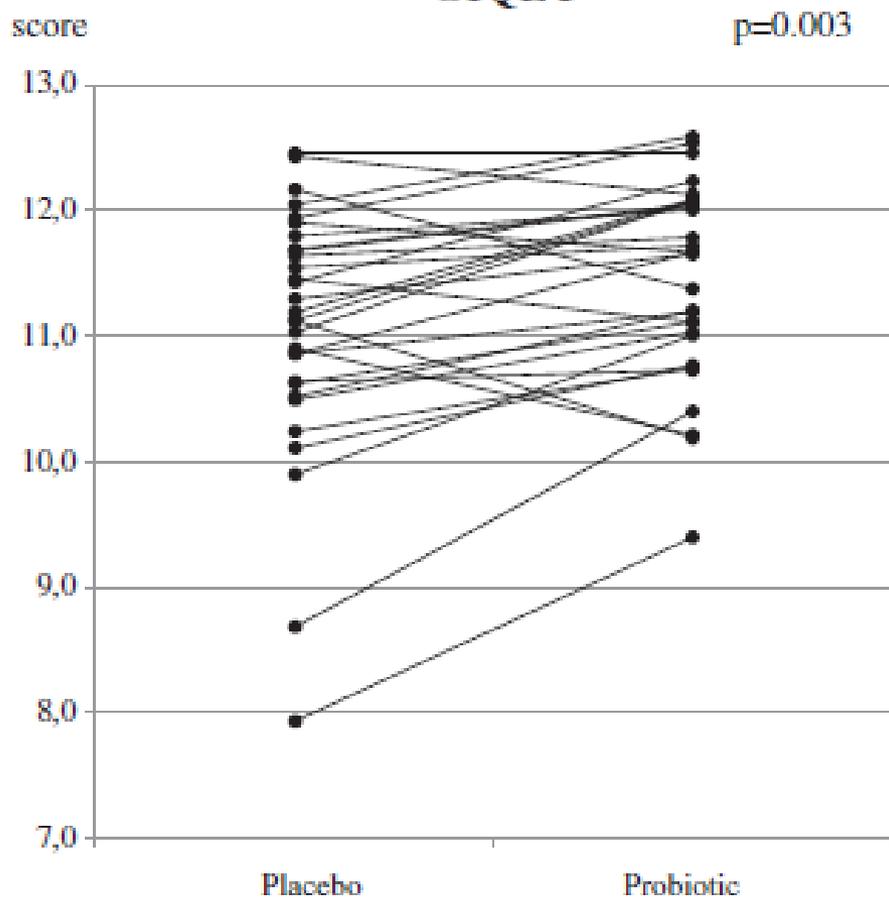
Summary of data collected after 6 months of probiotic or placebo intake.

Variable	Placebo	Probiotic	P value
Weight ^a (kg)	48.2 ± 15.2	48.5 ± 14.9	0.95
Height (m)	1.6 ± 0.1	1.6 ± 0.1	0.18
BMI ^a	19.19 ± 2.9	19.26 ± 2.8	0.98
FEV ₁ ^a (%)	77.0 ± 24.1	74.6 ± 22.6	0.73
NIRA test (gr%)	4.9 ± 4.2	4.6 ± 4.2	0.20
Fat absorption coefficient (%)	96 ± 3.5	95 ± 2.9	0.38
SF-12 (range 0–100)	1.7 ± 0.2	1.6 ± 0.3	0.66
GIQLY	11.2 ± 0.3	11.4 ± 0.3	0.003
Calprotectin (µg/ml)	33.8 ± 23.5	20.3 ± 19.3	0.003
IL-8 (pg/ml)	4.0 ± 28.5	3.6 ± 4.4	0.38
IL-1β (pg/ml)	4.4 ± 41.5	4.4 ± 23.8	0.26
IL-6 (pg/ml)	2.3 ± 2.9	2.4 ± 3.5	0.91
IL-10 (pg/ml)	1.8 ± 2.4	1.9 ± 2.5	0.72
TNFα (pg/ml)	3.3 ± 6.4	2.7 ± 3.0	0.40
IL-12p70 (pg/ml)	3.0 ± 3.0	3.2 ± 4.8	0.33



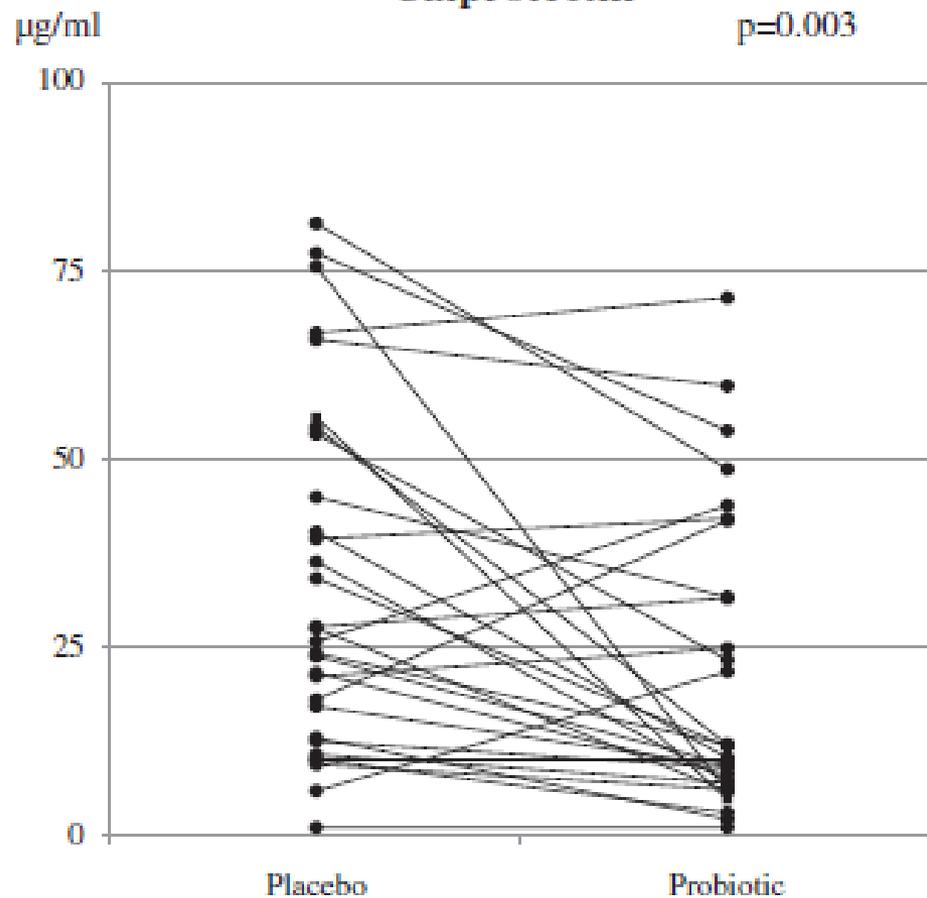
GIQLY

$p=0.003$

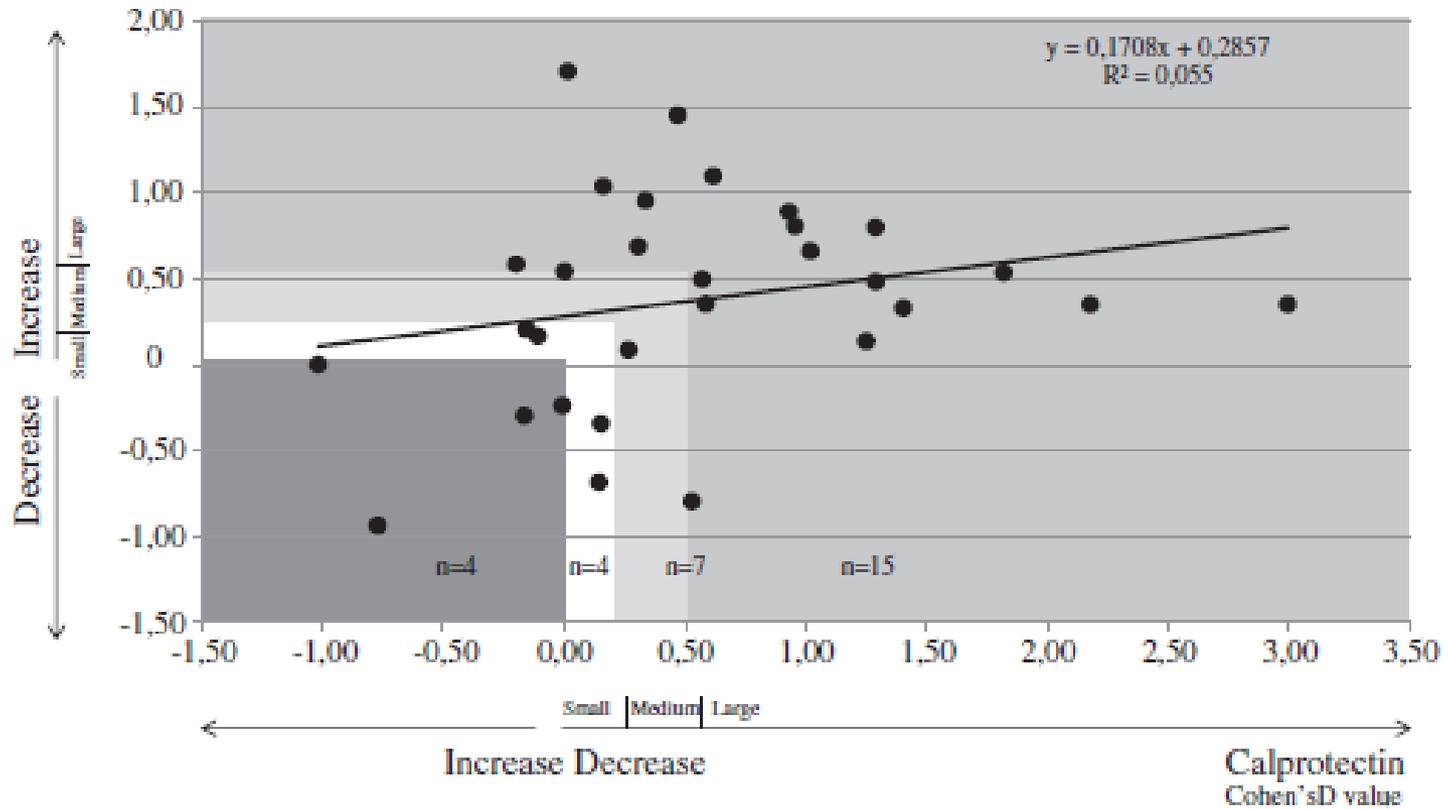


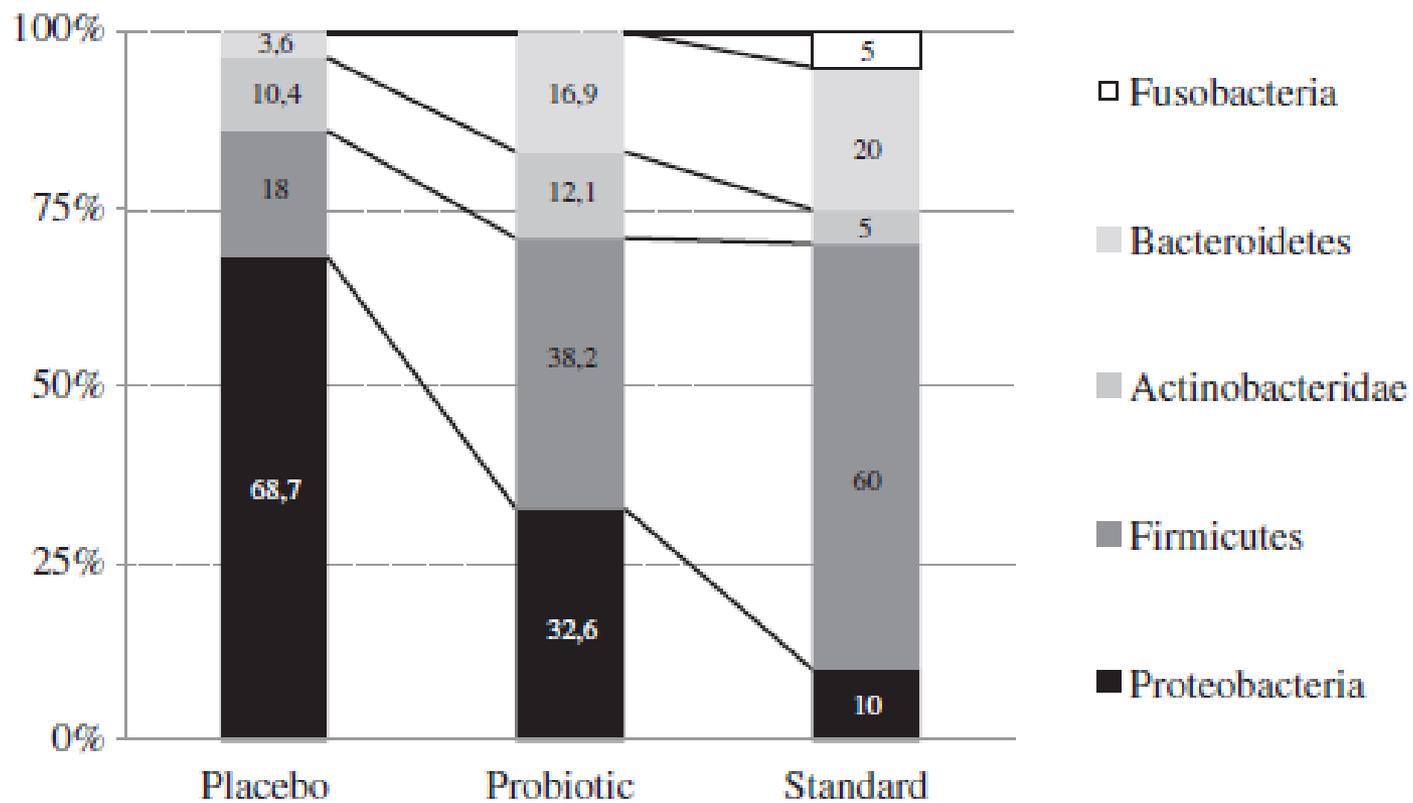
Calprotectin

$p=0.003$



GIQLY
Cohen's d value





Lactobacillus reuteri ATCC55730 in Cystic Fibrosis

Di Nardo, et al. JPGN 2014

- 61 CF (39 M)
- VEMS > 70%, pas de CTC ni AINS, pas d'exacerbation < 2 mois
- LR 5 gouttes/j (10^{10} CFU) ou placebo pendant 6 mois
- Âge médian = 17,5 ans (6-29)

	LR	Placebo	p
Exacerbations pulmonaires	1/30	11/30	<0,01
Infections respiratoires hautes	1/30	6/30	<0,05

- Pas de différences : hospitalisation pour exacerbations respiratoires (nombre et durée) et infections digestives
- Baisse de la calprotectine non différente entre les 2 groupes (-60 vs -37)

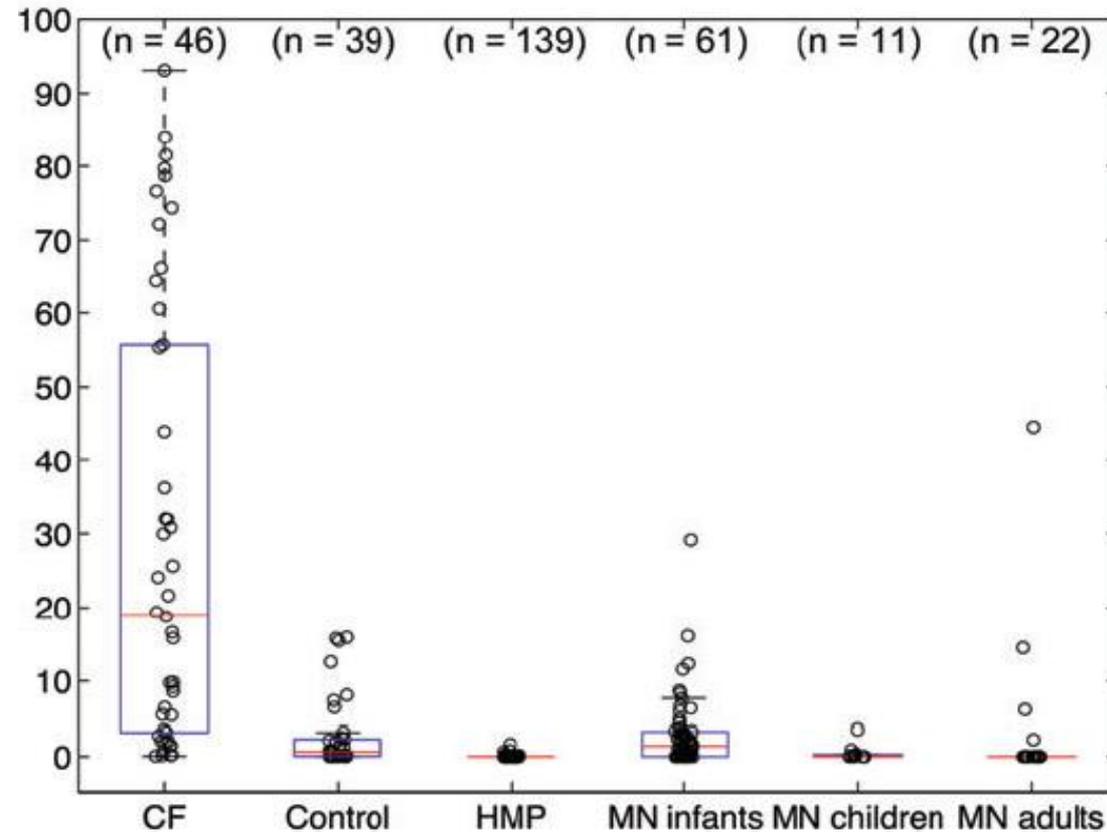
Dysbiose : *E.coli*

Abondance relative *E.coli*

- *E.Coli* < 5% à 1 mois puis <1%
- 12 enfants CF, 39 témoins

Abondance *E.Coli* corrélée :

- calprotectine
- stéatorhée

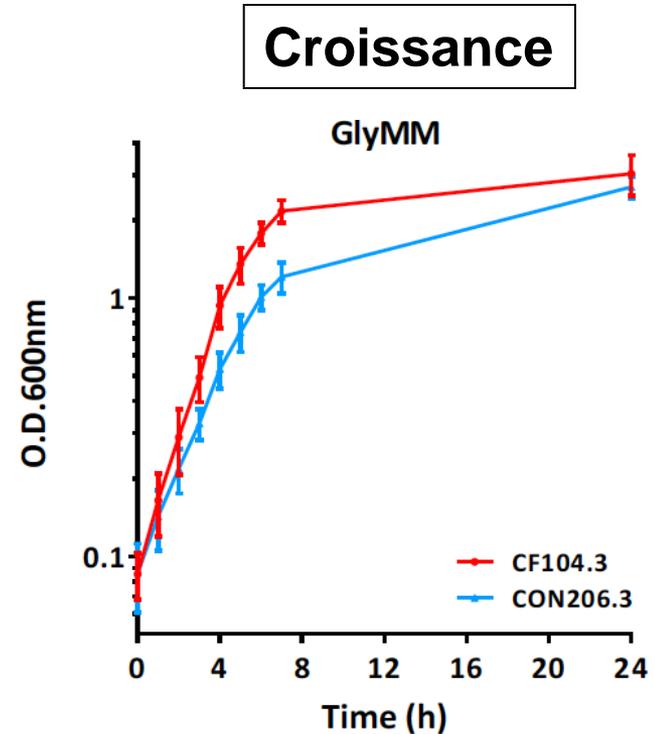
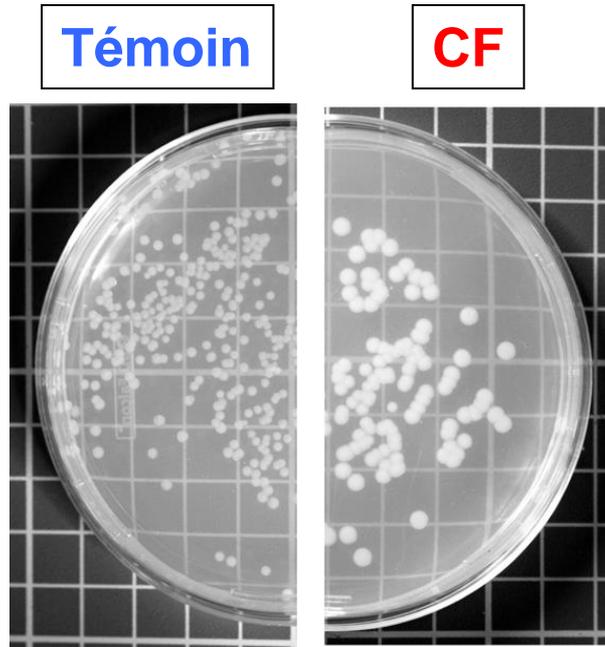


Augmentation d'*E.coli* pourrait contribuer à inflammation intestinale, altérer le métabolisme lipidique et l'absorption, aggravant l'atteinte digestive et la dénutrition

Dysbiose : *E.coli*

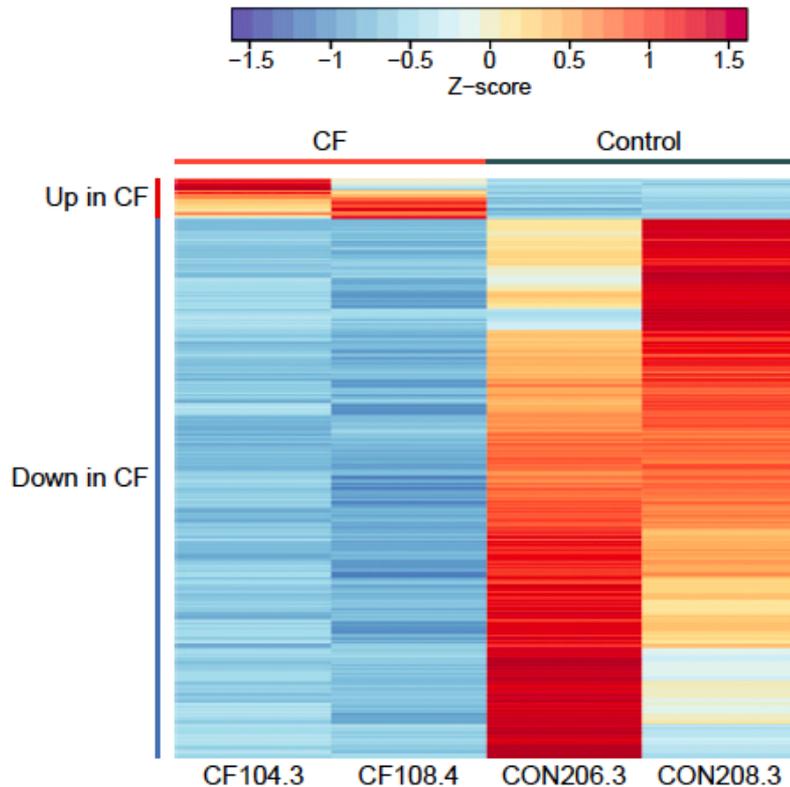
- **Hypothèse = adaptation de souches dans un milieu particulier :**
 - ✓ Riche en graisses
 - ✓ Mucus anormal
- **Etude :**
 - ✓ 6 CF et 2 témoins
 - ✓ Milieu pauvre en glucose ou glycérol

Dysbiose : *E.coli*



Différences en conditions aérobies uniquement

Dysbiose : *E.coli*



GluMM = 20 gènes exprimés différemment

GlyMM 405 gènes (377 gènes non induits dans la mucoviscidose)

Dans la mucoviscidose, l'augmentation de croissance en milieu GlyMM ne serait pas liée à une reprogrammation métabolique mais à une perte de l'inhibition de croissance et de réponse au stress.

Utilisation de probiotiques ?

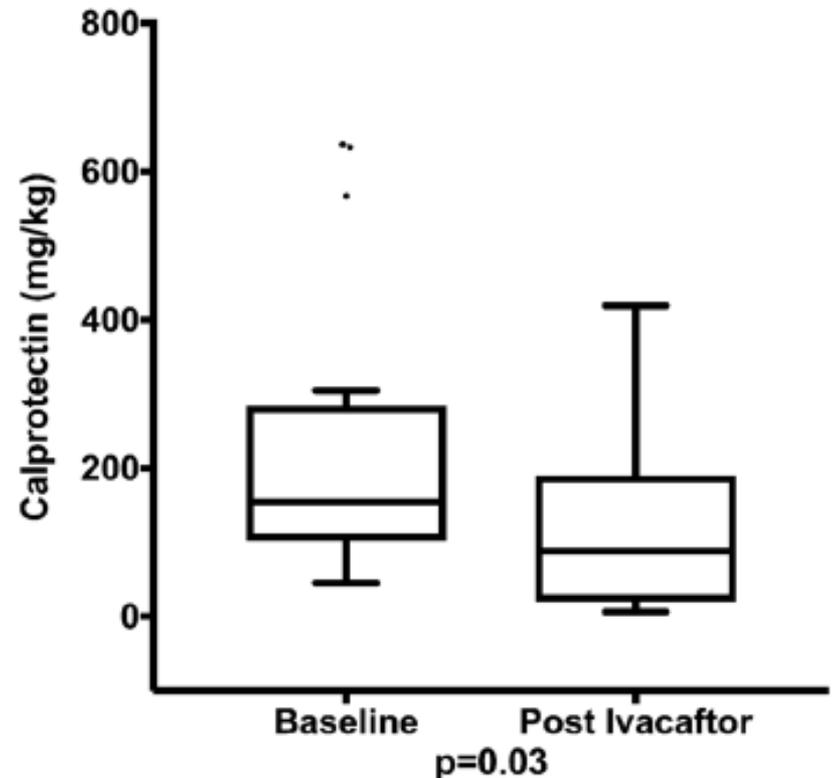
Does probiotic supplementation affect pulmonary exacerbation and intestinal inflammation in cystic fibrosis: a systematic review of randomized clinical trials

5 études incluses, 188 patients, suivis de 1 à 6 mois

Conclusion: The lack of high quality RCTs makes it impossible to support a general recommendation about the use of probiotics in the treatment of CF pulmonary exacerbation and intestinal inflammation.

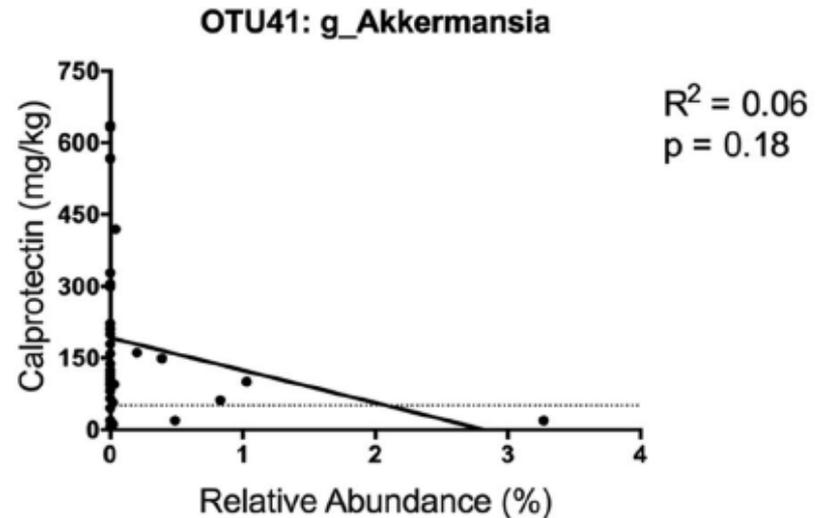
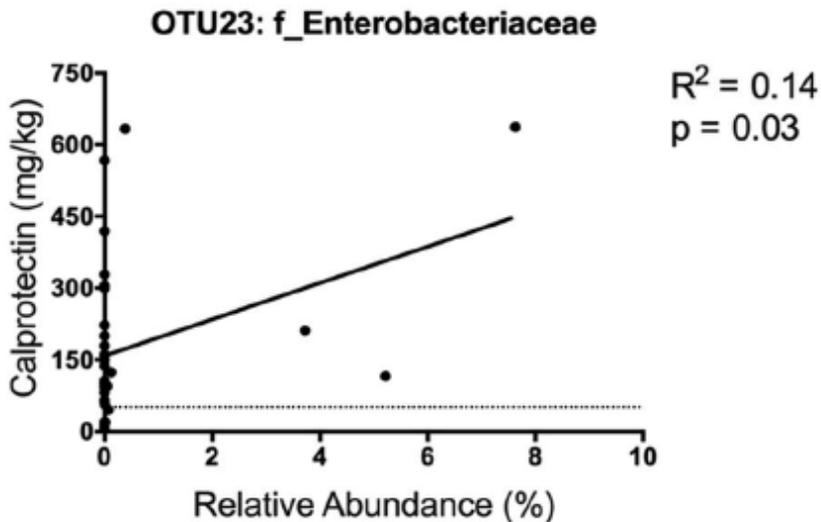
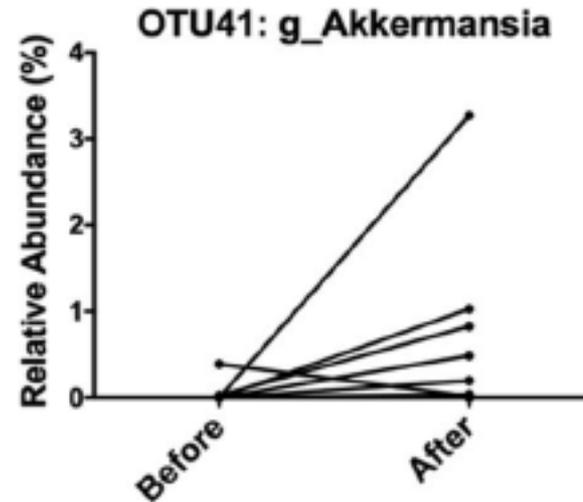
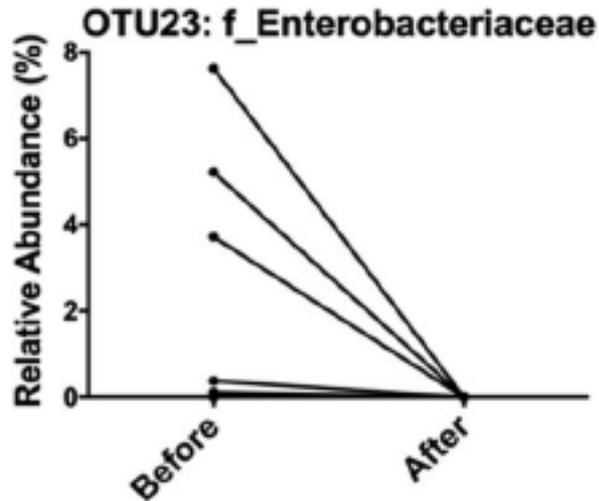
Effet de l'Ivacaftor ?

- 8 enfants et 8 adultes CF
- Inflammation et microbiote à M0-M6
- 17,1 ans (7,8-32,7)
- Enfants = 7,8 ans (6,2 – 11,5)
- Adultes = 32,3 (21,8 – 42,9)
- 14 IPE
- Prise de poids :
 - ✓ E : Z-score : 0,32 à 0,66 (p=0,012)
 - ✓ A : + 1,8 kg (p=0,028)
- Amélioration du VEMS et du Chlore

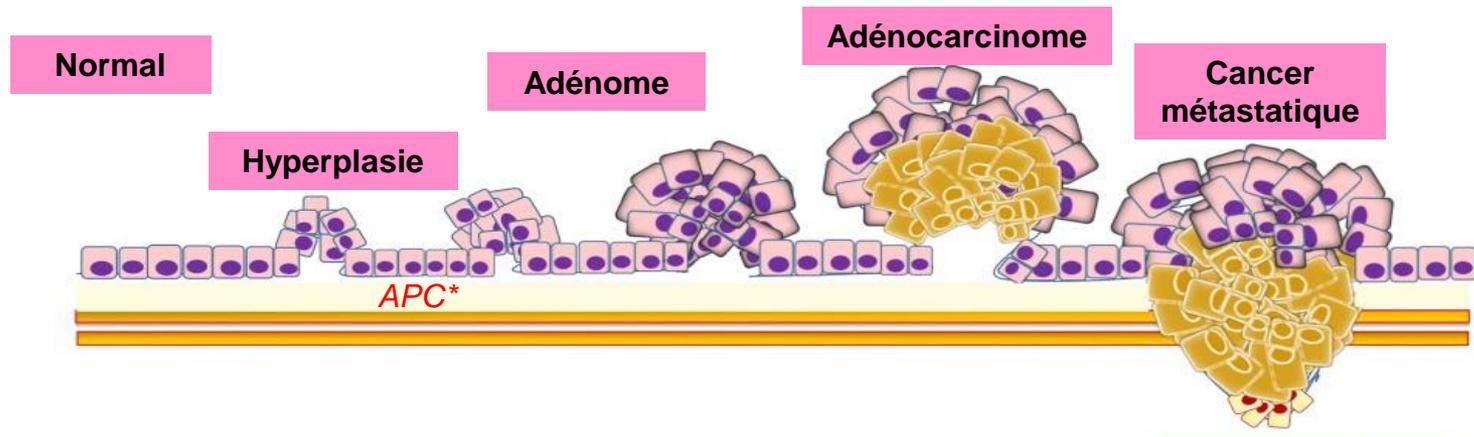


154,4 à 87,5 mg/kg

Effet de l'Ivacaftor ?



Cancer colorectal



Cancer colorectal

- **Etude :**
 - ✓ monocentrique prospective (Minnesota)
 - ✓ 88 patients > 40 ans ; VEMS > 40%
- **Polypes :**
 - ✓ 49% initialement (n=43)
 - ✓ 7 nouveaux cas lors de la surveillance

→ total 56%
- **Sévérité :**
 - ✓ 32% des cas (n=28) = lésions histologiques sévères et ≥ 3 polypes adénomateux
 - ✓ 3 patients (3%) = un cancer colorectal (2 in situ, 1 invasif)

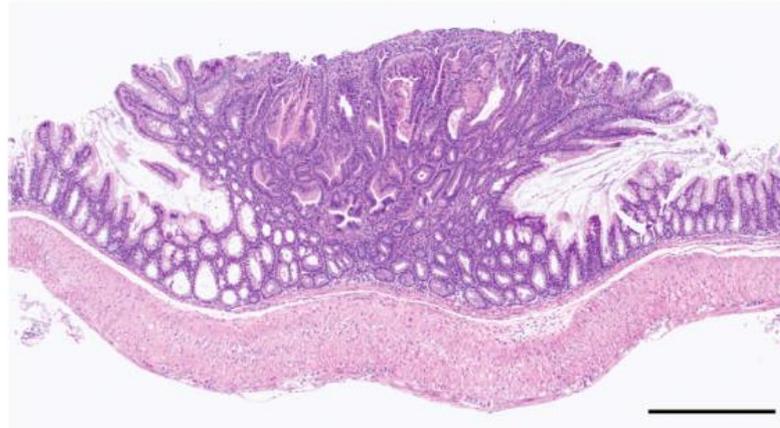
Facteurs de risque

- **polypes adénomateux**
 - ✓ diabète ($p=0,0171$)
 - ✓ une mutation F508del homozygote ($p=0,0206$)
- **≥ 3 polypes ou un polype de haut risque :**
 - ✓ sexe masculin ($p=0,0133$)
 - ✓ transplantation pulmonaire ($p=0,0350$)

**risque accru de cancer colorectal (au moins x 5)
dans la mucoviscidose, en raison d'une survenue
plus précoce d'adénomes coliques et de leur
progression**

Hypothèses : gène suppresseur de tumeur

- Rôle CFTR dans cancer intestinal :
 - souris Apc^{Min} et $Cftr^{fl/fl}$ Villin-Cre
- Développent plus de tumeurs dans grêle et côlon que $Apc^{Min} Cftr^{+/+}$
- Même $Apc^{+/+} Cftr^{fl/fl}$ Villin-Cre développent plus de tumeur



Hypothèses : gène suppresseur de tumeur

Grêle

Côlon

Table 3. Top upregulated and downregulated genes identified by microarray in the normal small intestine of *Apc^{+/+} Cfr^{fl/fl}-Villin-Cre* mice

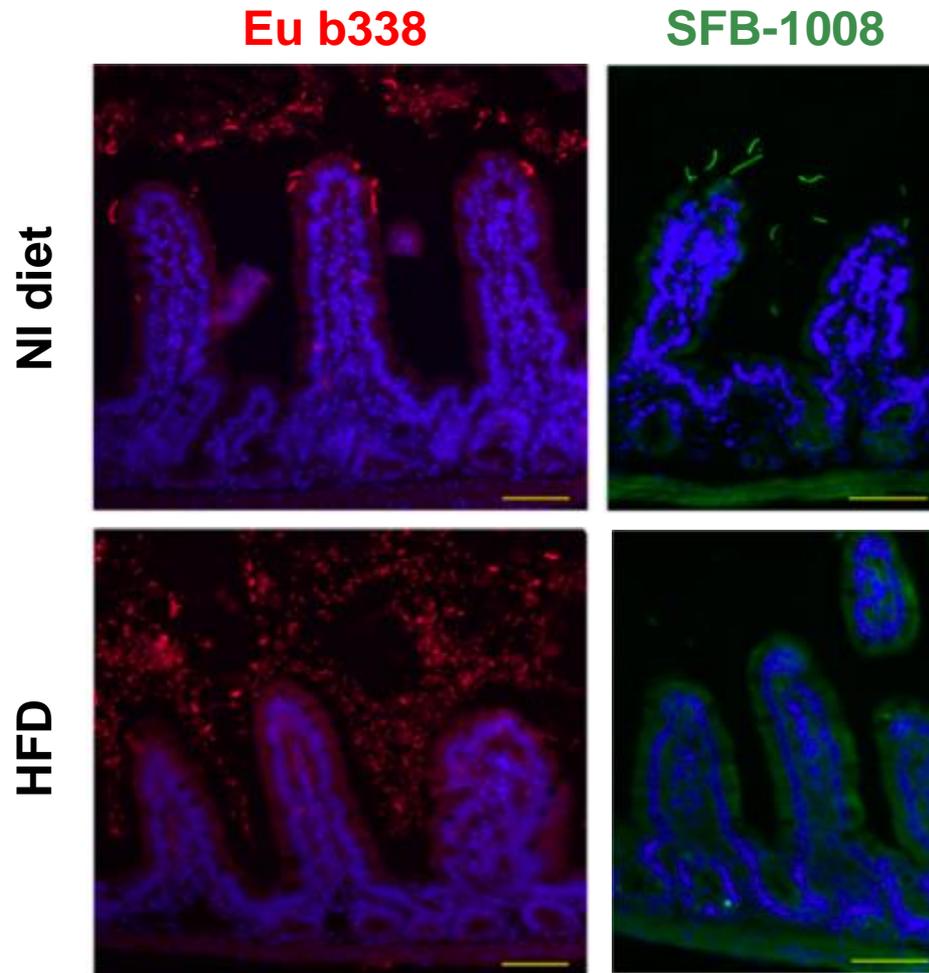
Table 4. Top upregulated and downregulated genes identified by microarray in normal colon of *Apc^{+/+} Cfr^{fl/fl}-Villin-Cre* mice

<i>Top upregulated genes</i>	<i>Top downregulated genes</i>
Reg3g	Cfd
Reg3a	Scd
Amy2a	Defa1
Retnlb	Igk-V8
Anxa4	Cyp2C9
B3galt5	Lpl
Muc3a	Slc5a4b
Cela3a	Adipoq
Defa3	Reg3g
Cldn4	Aldh1a1

<i>Top upregulated genes</i>	<i>Top downregulated genes</i>
Defa6	Sycn
Defa3	Gsdmc
Slpi	Clps
Reg3g	Fxyd4
Clca4	Ces1d
Defa-rs1	Rn18s
Lyz1/Lyz2	Cyp4b1
C3	Actb
Reg3a	Prlr
Clca6	Pbld

Gènes impliqués dans immunité cellulaire, mucines, inflammation...

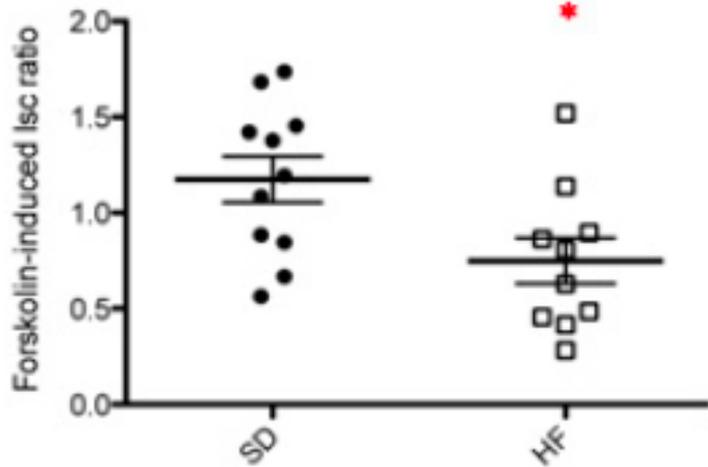
Hypothèses : diététique



**Localisation anormale
du microbiote intestinal**

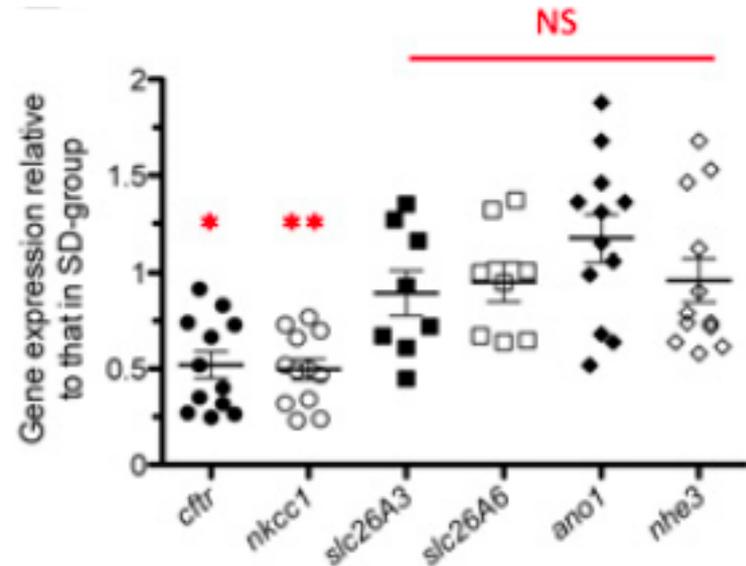
High-fat diet modifies the PPAR- γ pathway leading to disruption of microbial and physiological ecosystem in murine small intestine. Tomas J, PNAS 2016

Hypothèses : diététique

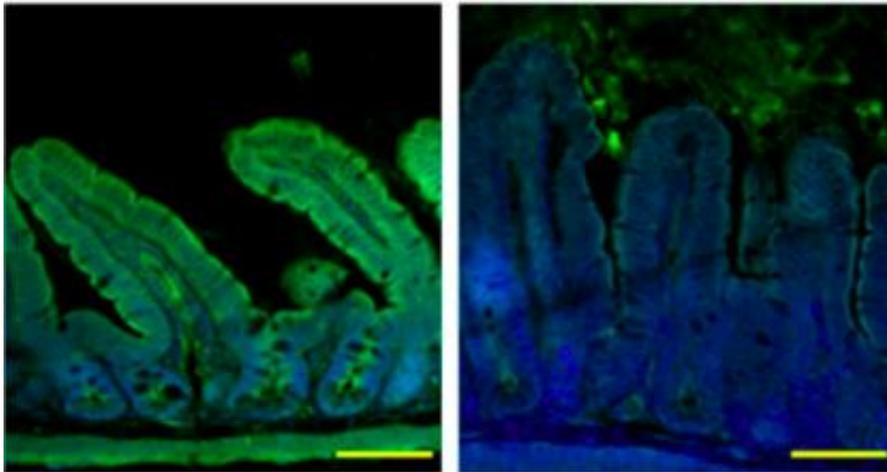


NI diet

HFD



CFTR

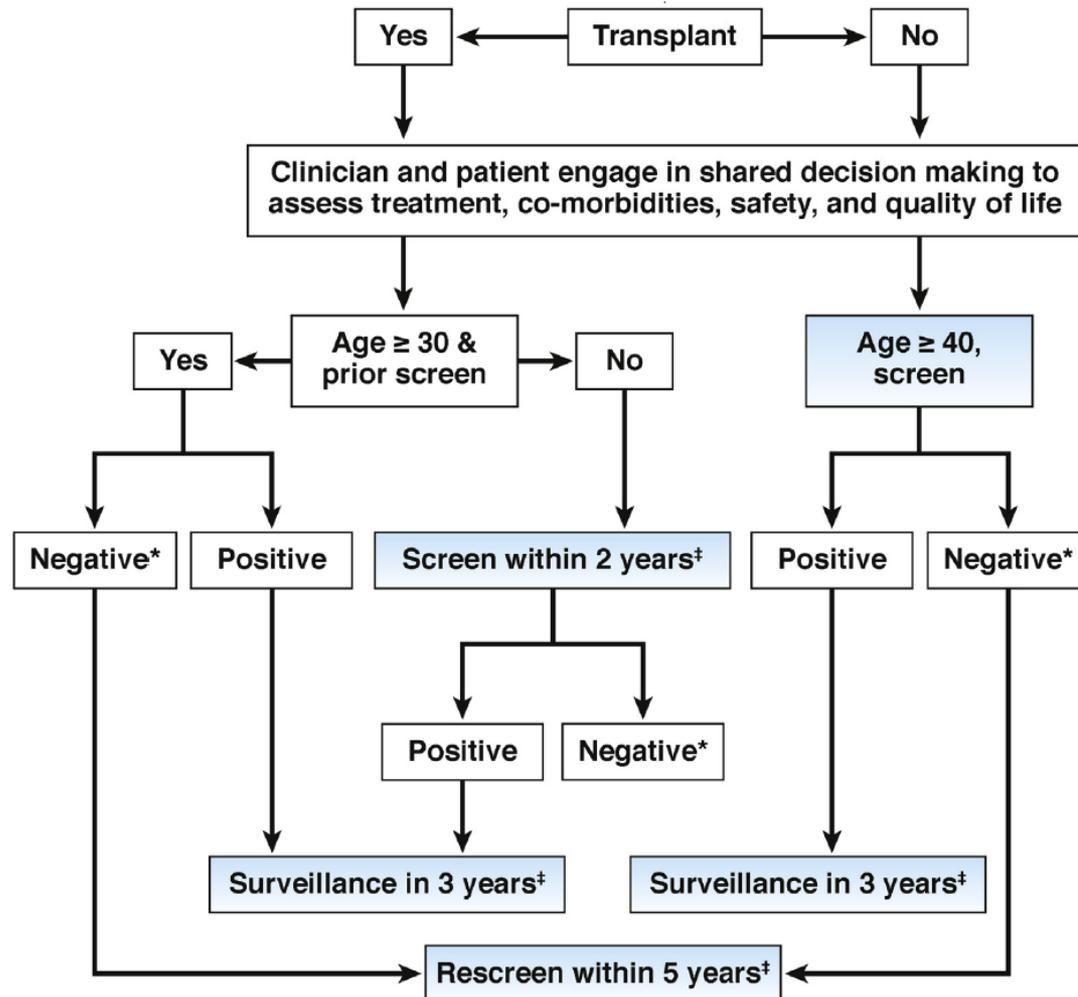


Le régime riche en graisses altère l'intégrité fonctionnelle iléale via une dysfonction CFTR

Cystic Fibrosis Colorectal Cancer Screening Consensus Recommendations

Gastroenterology 2018;154:736–745

Risque de CCR x 5-10 dans mucoviscidose (25-30 chez transplantés)

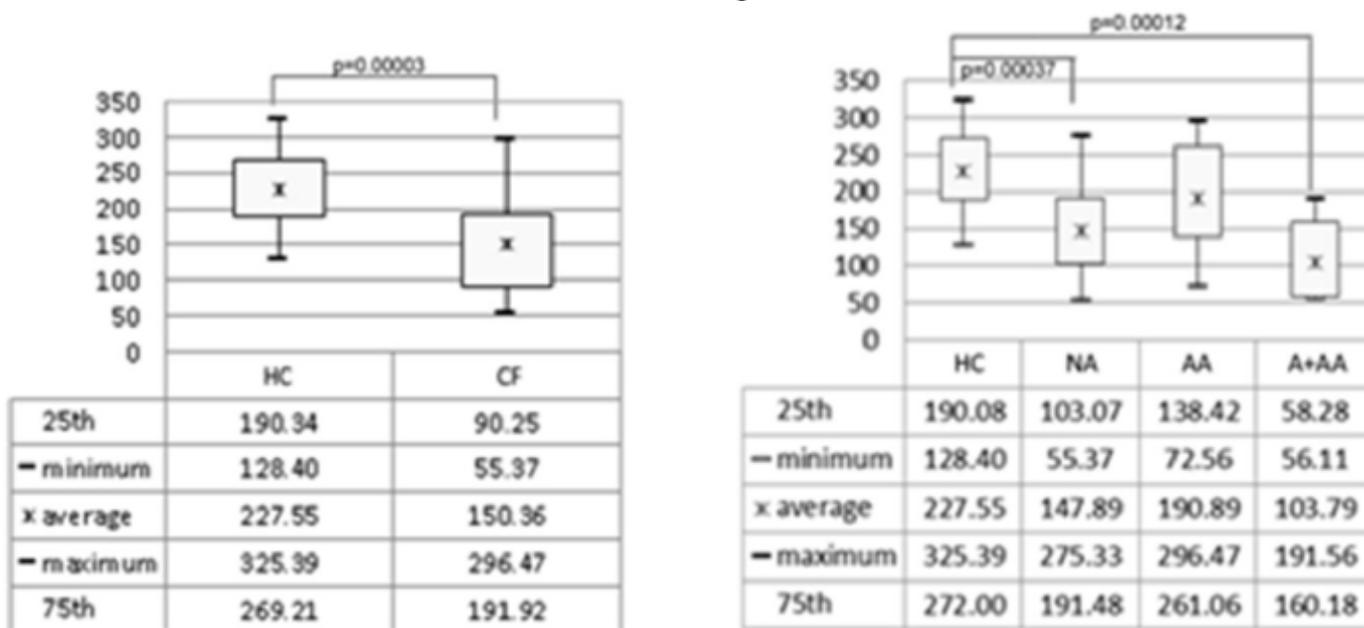


Gut microbiota signatures in cystic fibrosis: Loss of host CFTR function drives the microbiota enterophenotype

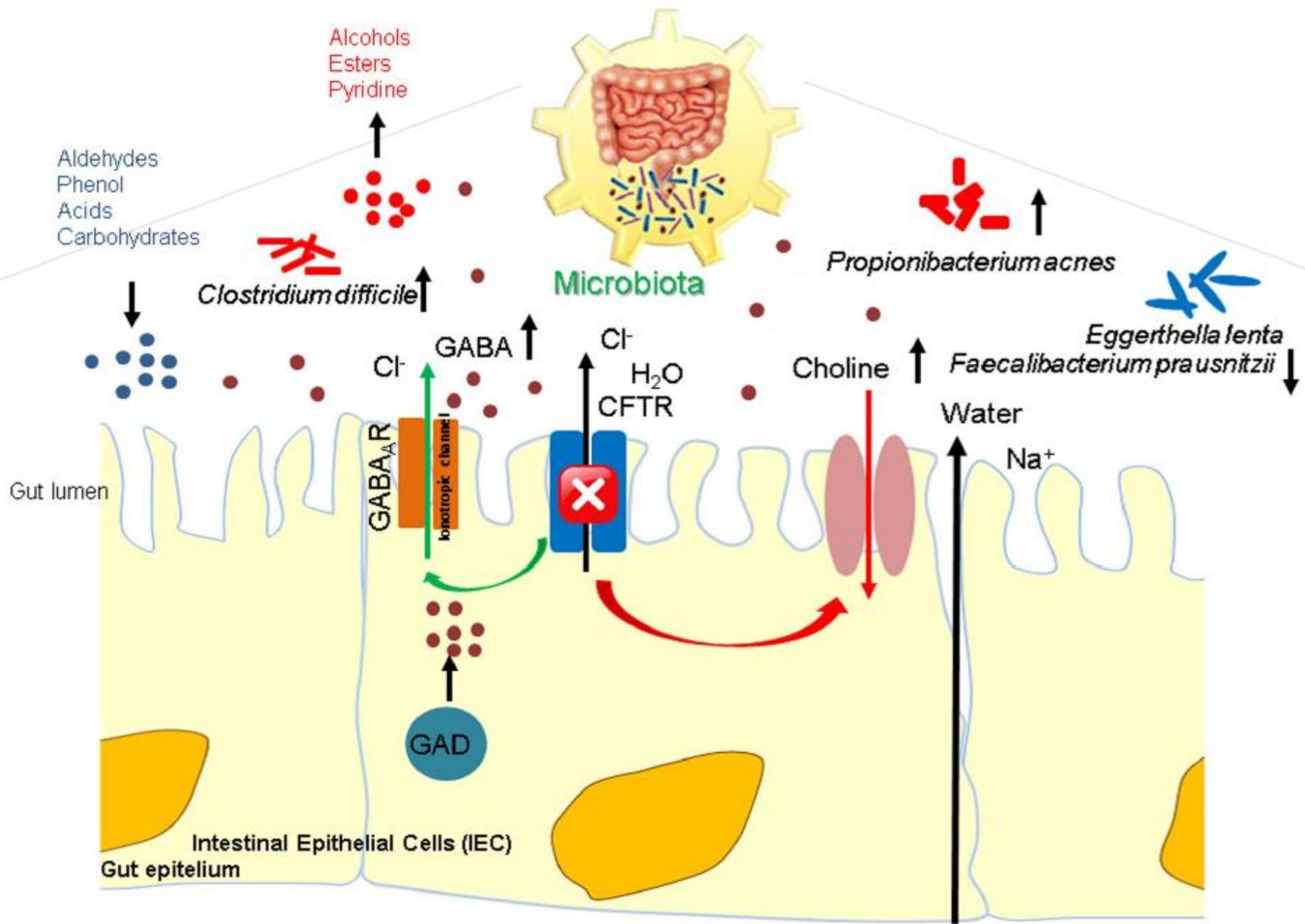
Vernocchi P

PLOS ONE | <https://doi.org/10.1371/journal.pone.0208171> December 6, 2018

31 CF de 1-6 ans



Diversité diminuée





• **Dysbiose CF ?**



• **Inflammation ?**



• **Prise en charge ?**

