Rapporto tra nutrienti, intestino e benessere

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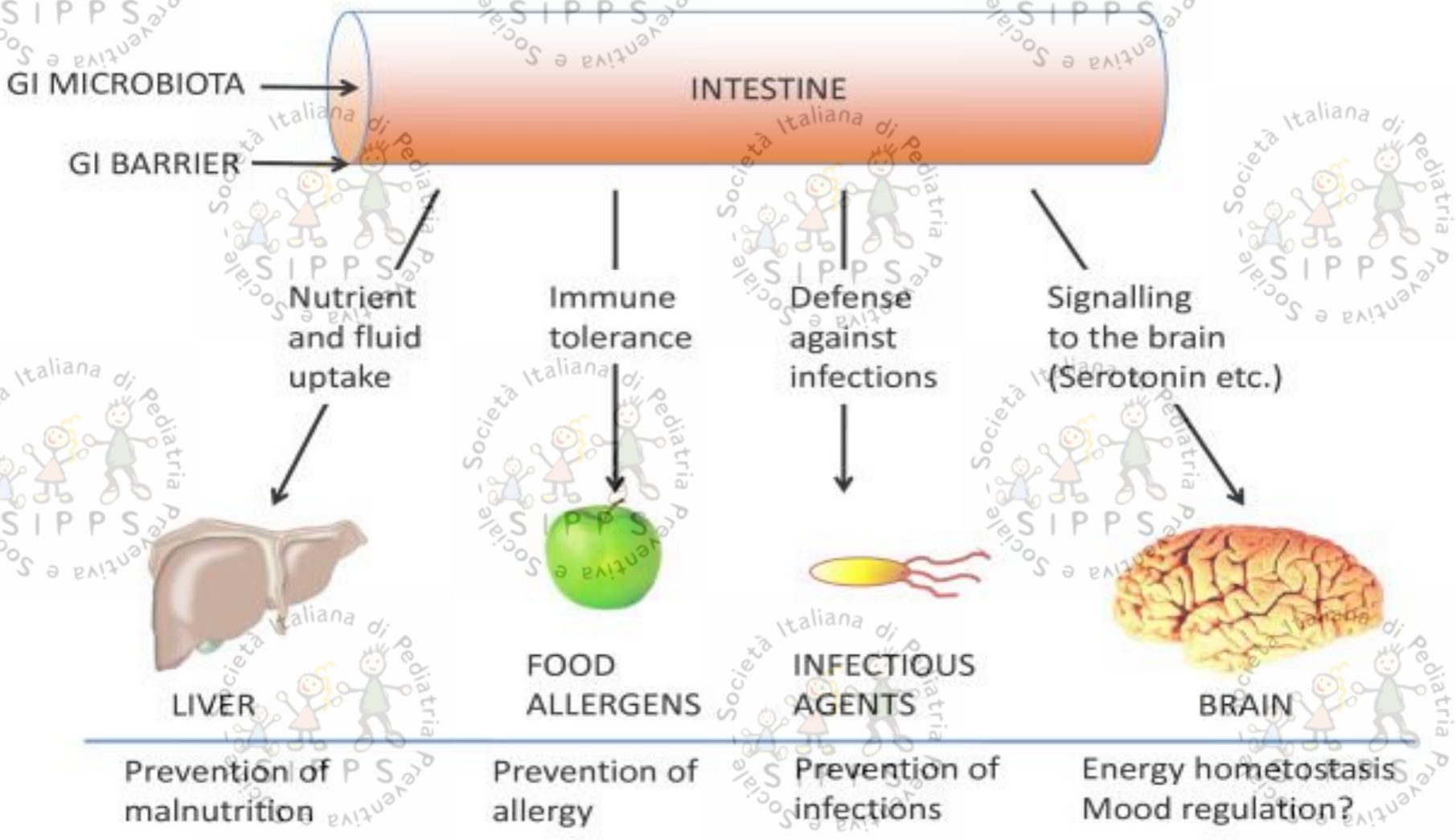
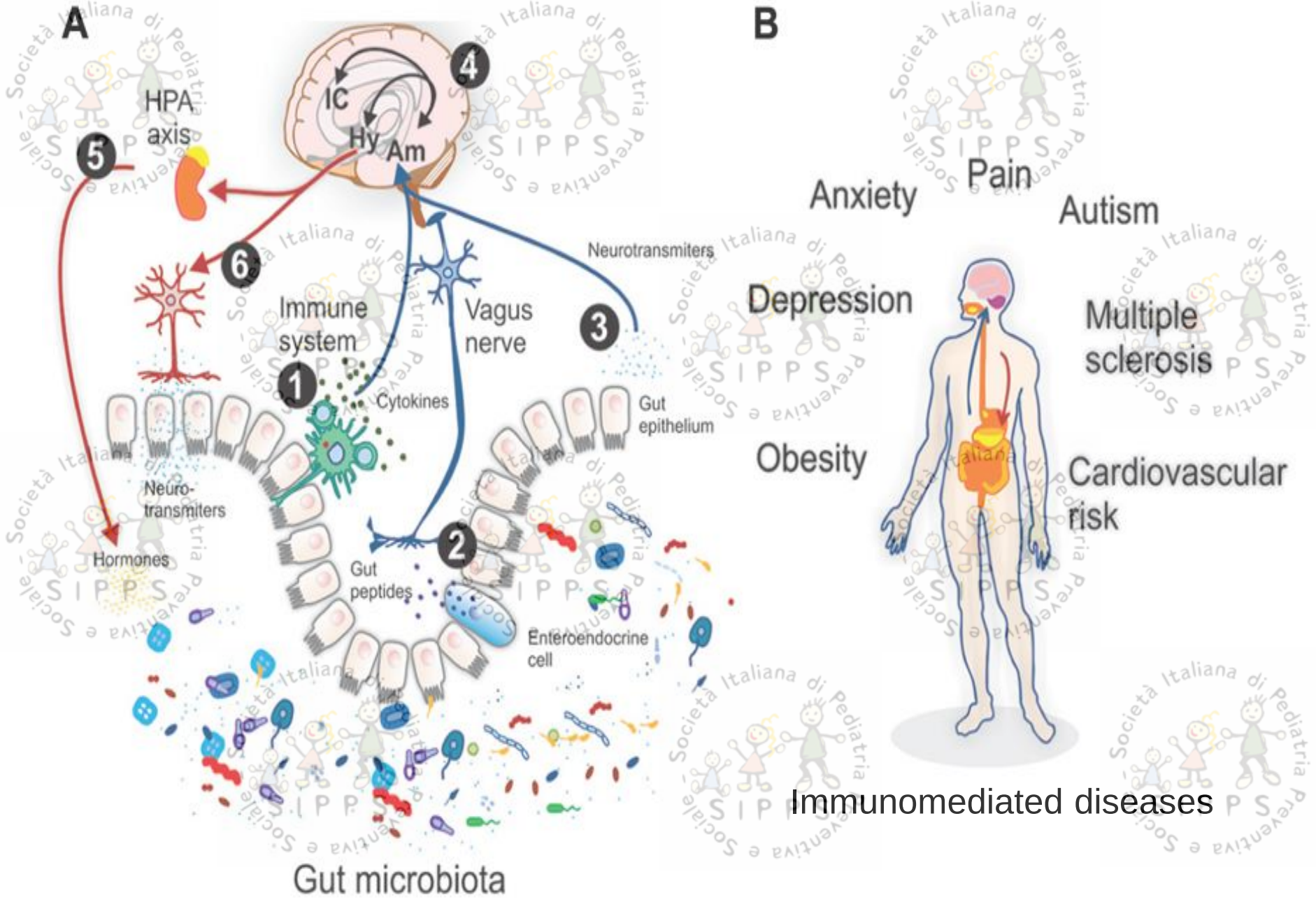
Department of Pediatrics, University of Chicago, IL, USA

La distanza tra ricerca di laboratorio ed applicazione clinica si è molto ridotta

L’impatto dell’intestino sulla salute

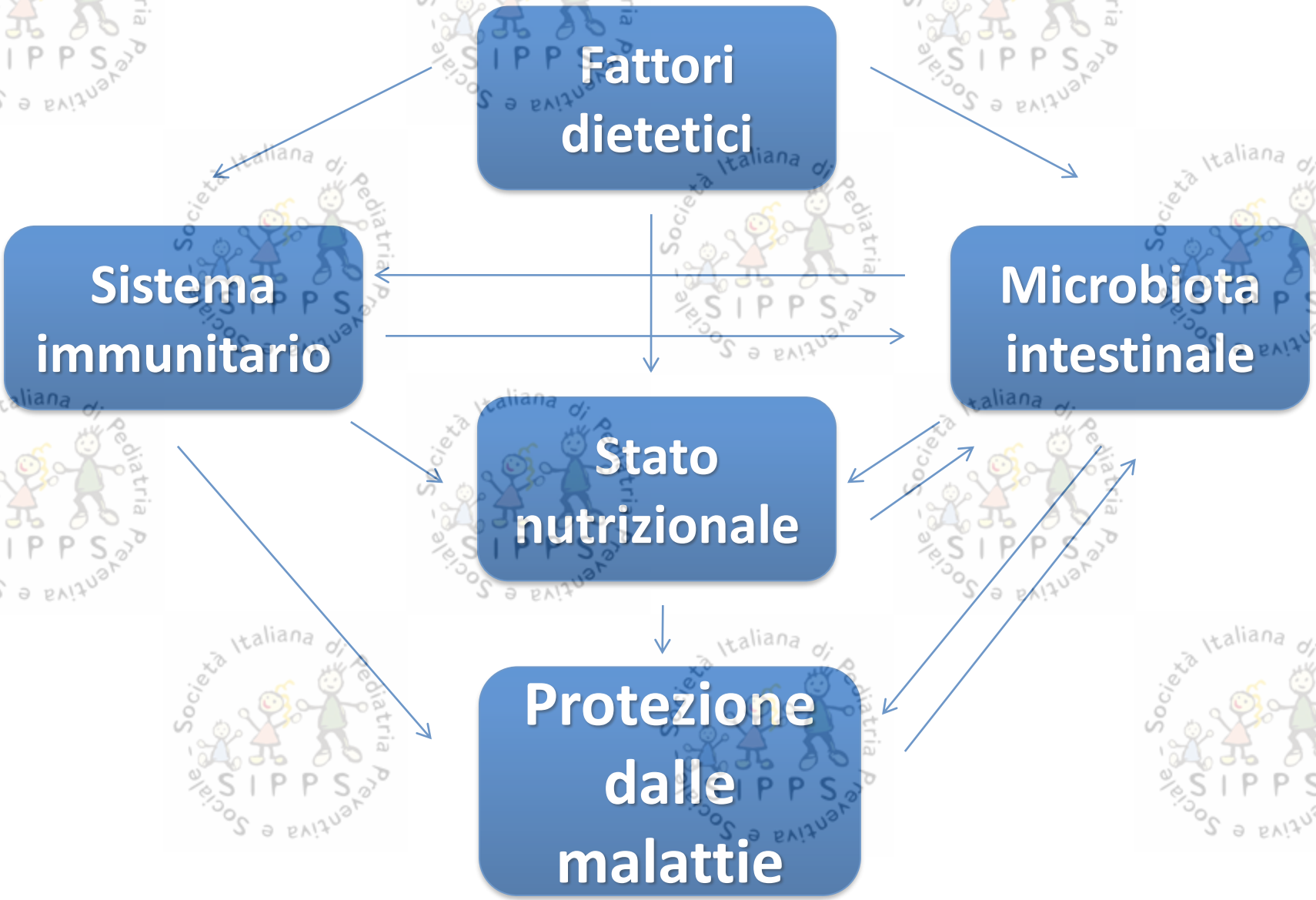


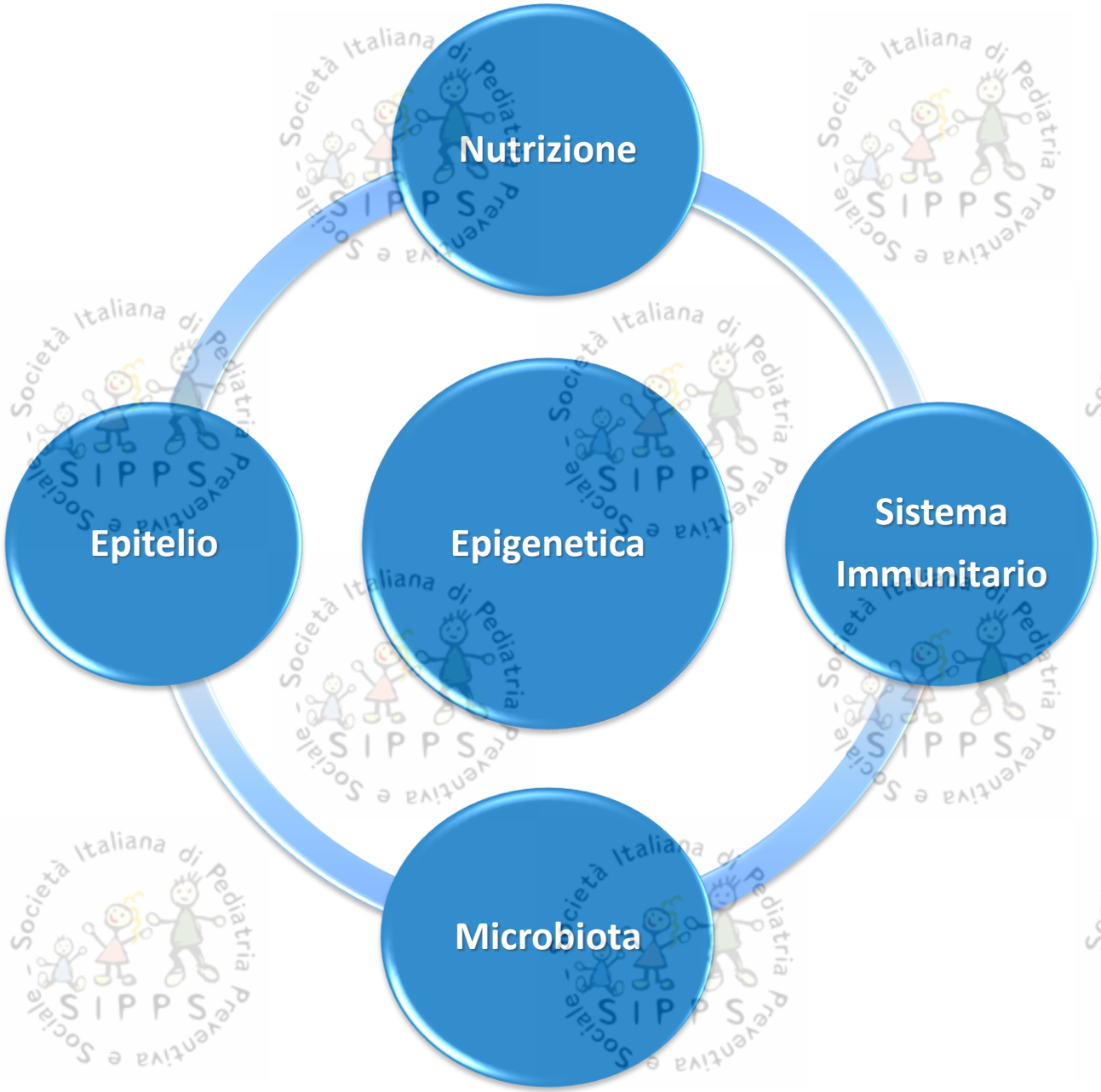

A
HPA axis
IC
Hy
Am
4
5
6
Immune system
Vagus nerve
Neurotransmiters
3
1
Cytokines
Gut epithelium
Neuro-transmiters
Hormones
2
Gut peptides
Enteroendocrine cell
Gut microbiota
B
Anxiety
Pain
Autism
Depression
Multiple sclerosis
Obesity
Cardiovascular risk
Immunomediated diseases

Microbiomes impact behaviors

Animal	Microbial species or consortium	Interaction with behavior	Implication
 Fruit fly (*Drosophila melanogaster*)	Gut microbiota	Diet-specific microbiota influence mating preferences	Microbes could drive speciation
 Mosquito (*Anopheles gambiae*)	Human skin microbiota	Skin microbes of humans influence attraction to mosquitoes	Differential attraction could impact disease spread
 Mouse (*Mus musculus*)	*Lactobacillus rhamnosus*	The probiotic *L. rhamnosus* decreases anxiety in mice	Suggests bacteria can alter mood

Immunonutrizione

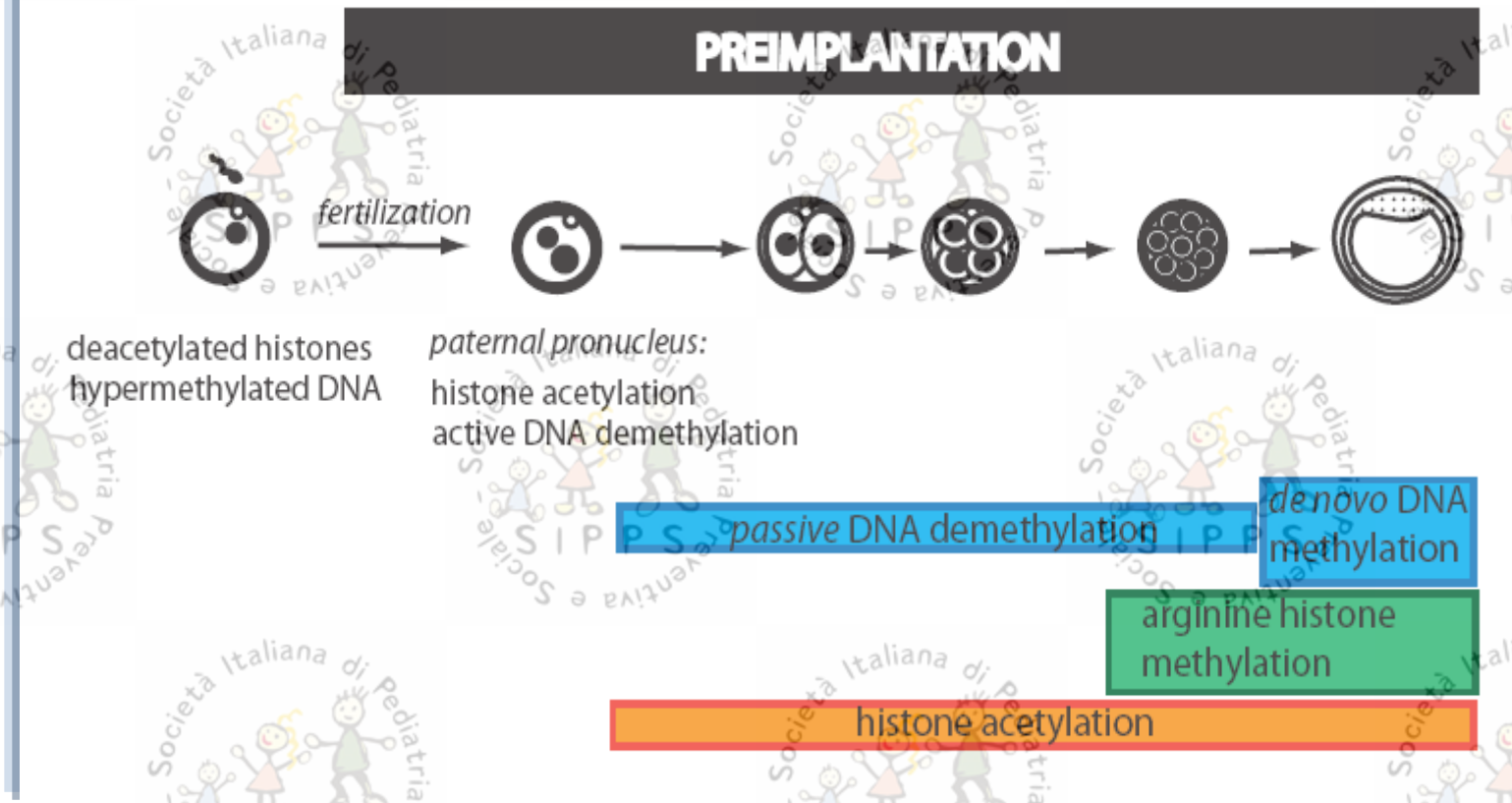


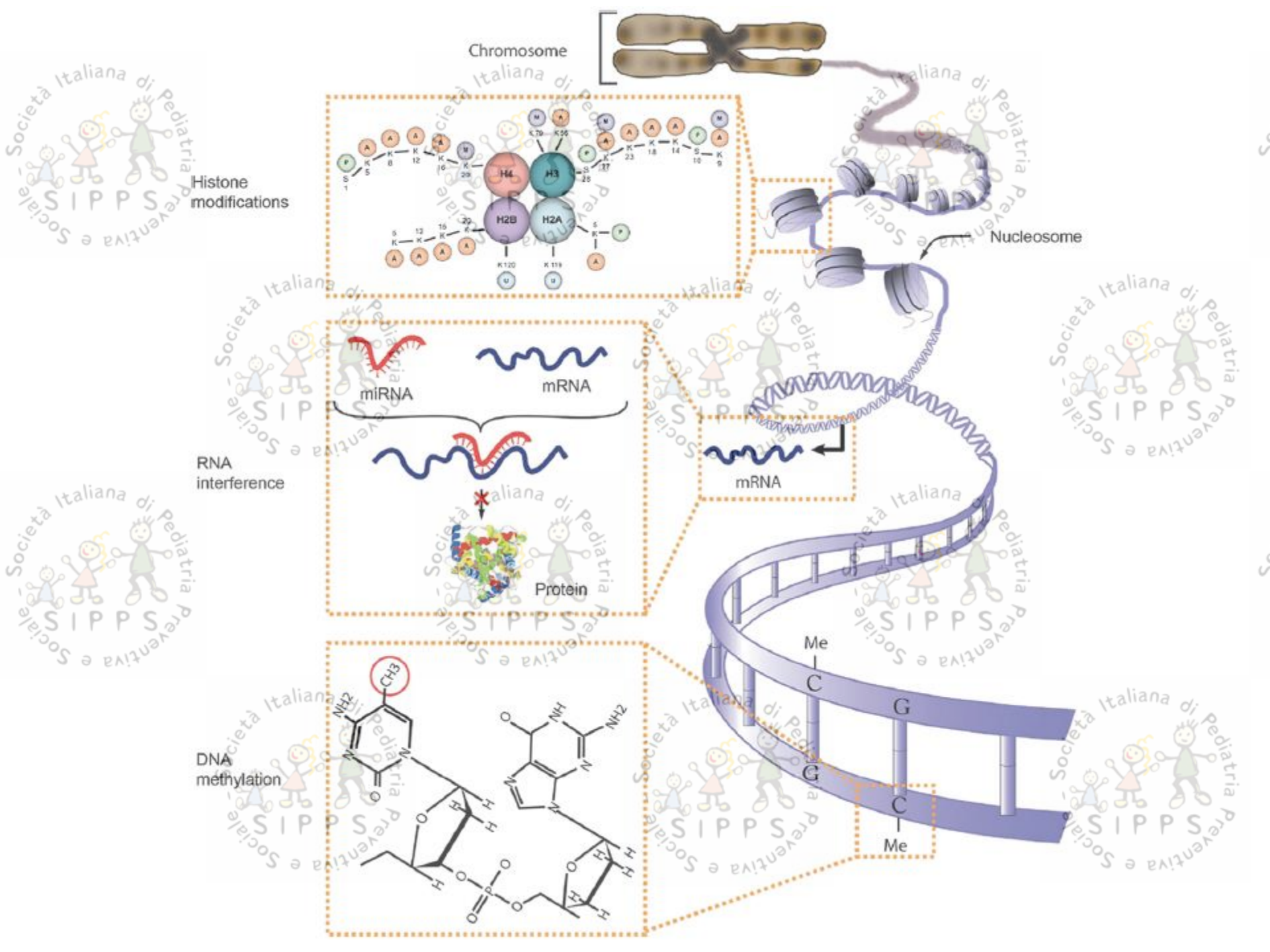

Nutrizione
Epitelio
Epigenetica
Sistema Immunitario
Microbiota

Epigenetica

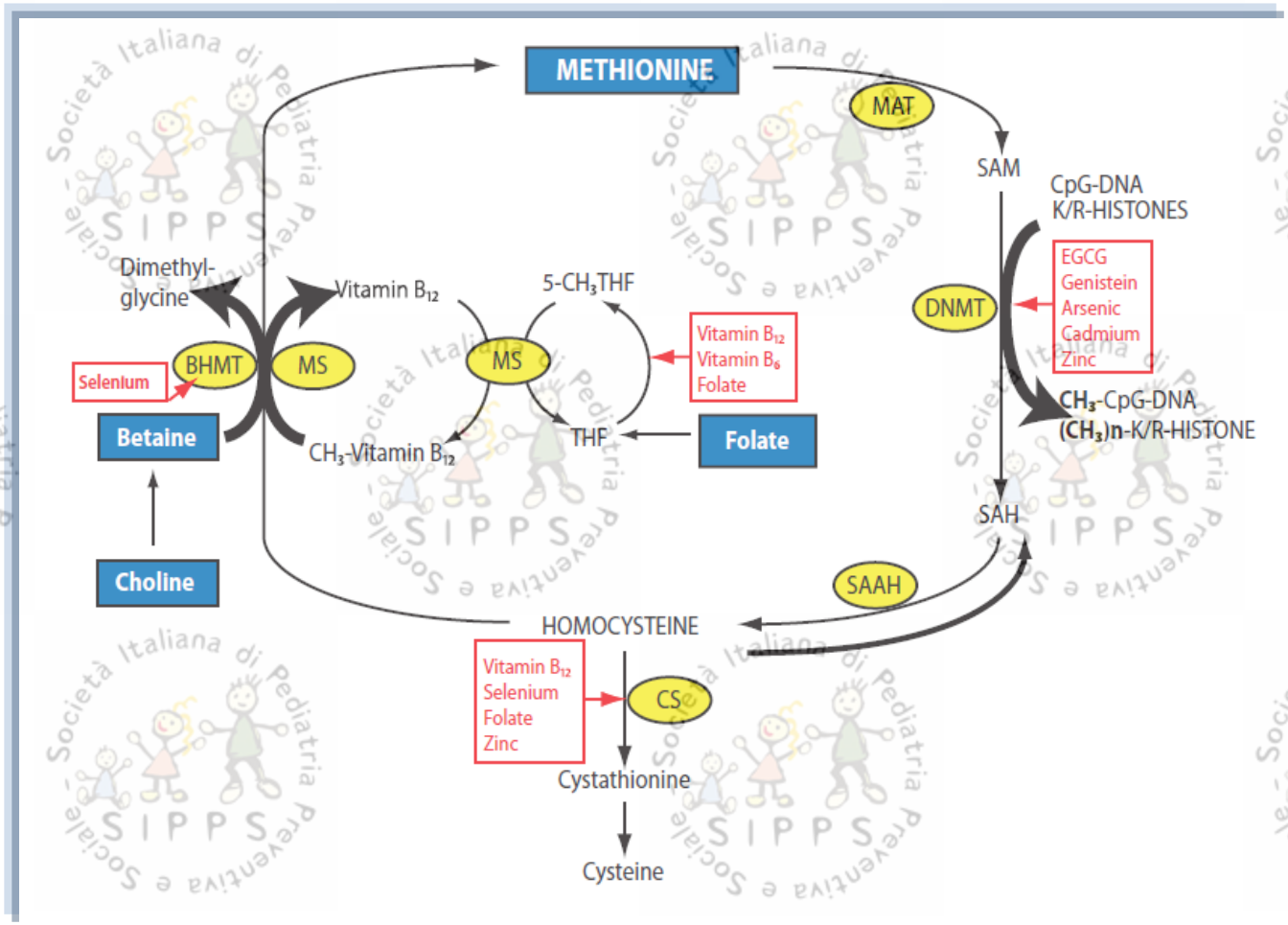
Cambiamenti del genoma ereditabili (*e potenzialmente reversibili*), trasmessi da una generazione cellulare alla successiva, che alterano l'espressione genica ma non comportano cambiamenti nella sequenza primaria del DNA.

L’ epigenetica è una parte essenziale del normale sviluppo

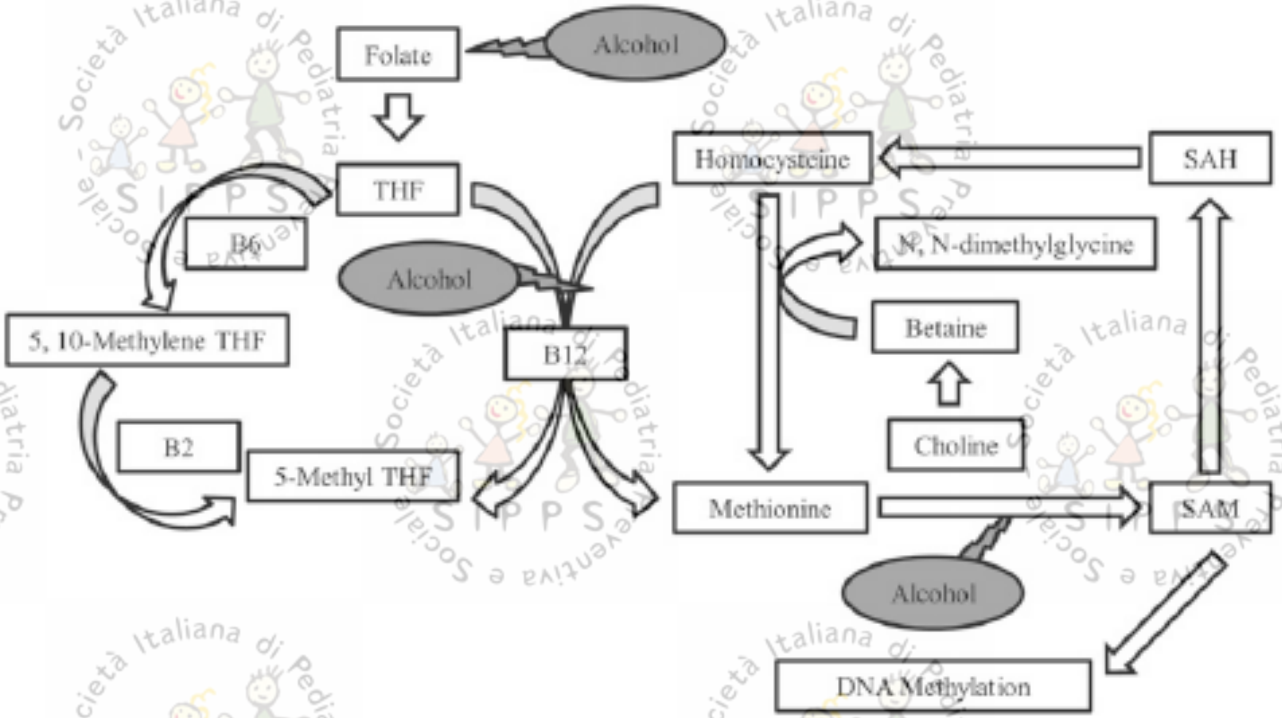



Chromosome
Histone modifications
H4
H3
H2B
H2A
Nucleosome
miRNA
mRNA
RNA interference
mRNA
Protein
Me
C
G
G
C
Me
DNA methylation
CH3
NH2
NH
NH2

Nutrienti come “metil donatori” per le metiltransferasi (DNMT)



Consumo di Alcool ed Epigenetica

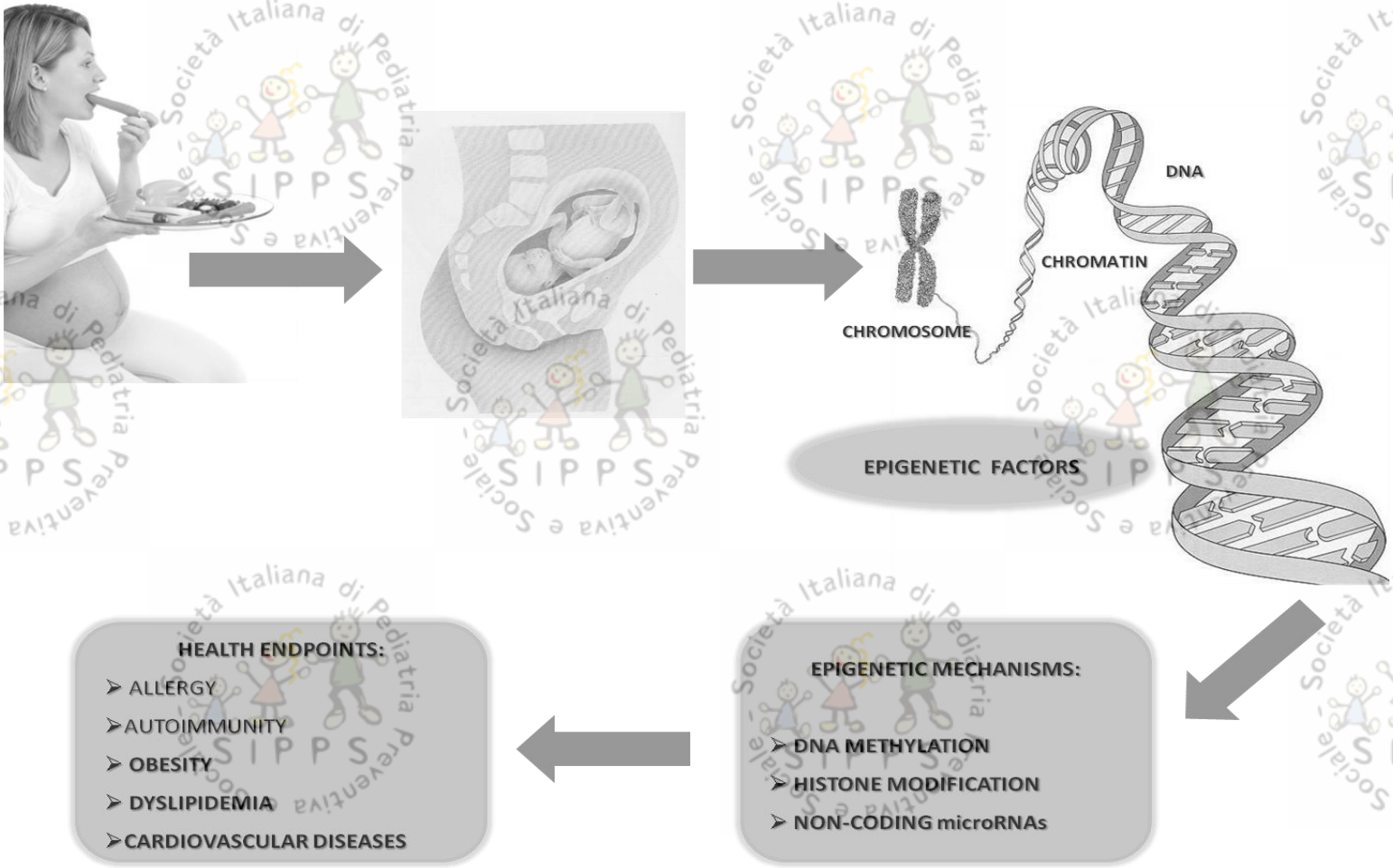


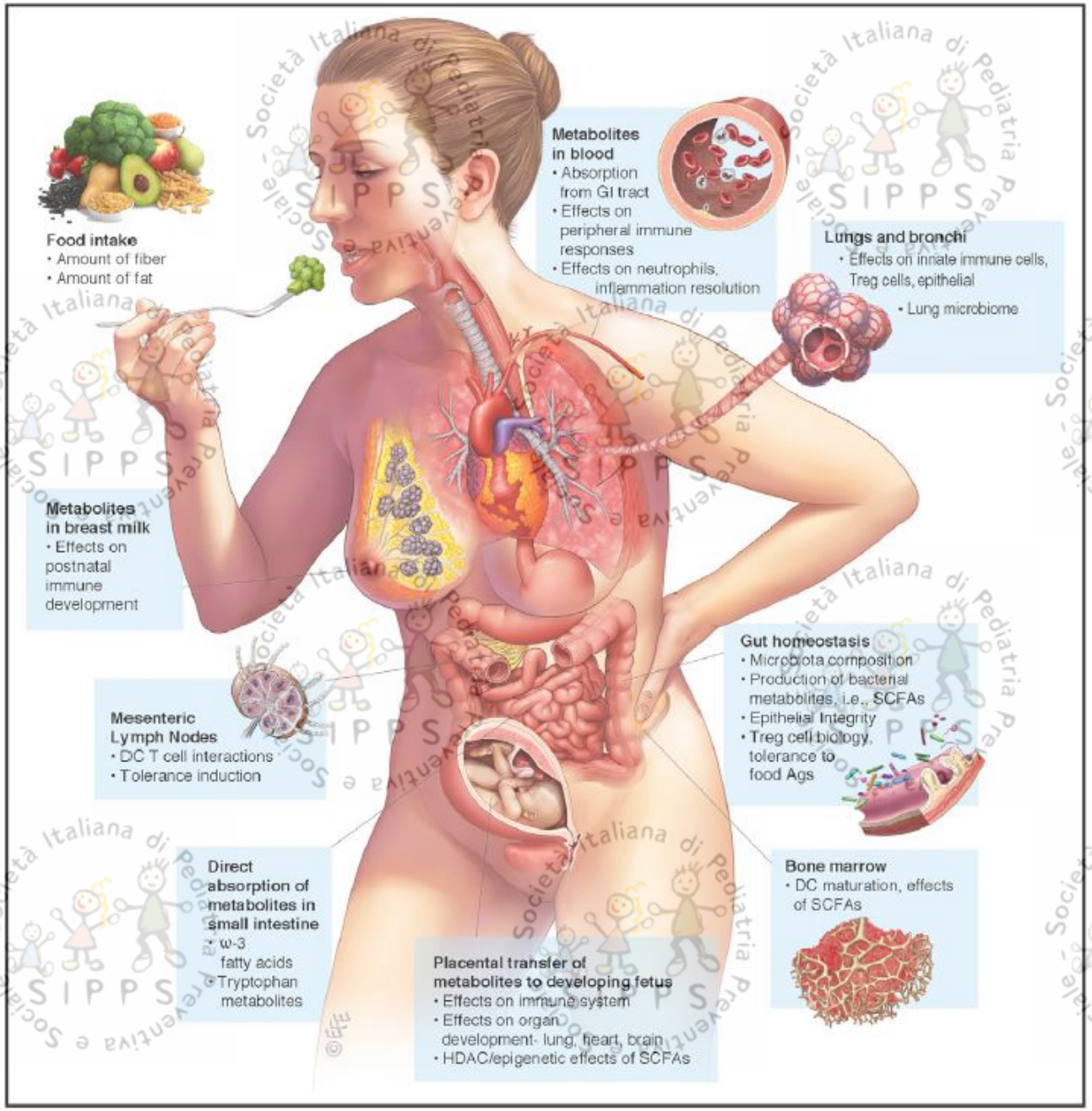
Nutrition Research Reviews, page 1 of 8

doi:10.1017/S0954422411000102

Epigenetic mechanisms elicited by nutrition in early life

Roberto Berni Canani[1,2*], Margherita Di Costanzo[1], Ludovica Leone[1], Giorgio Bedogni[2,3,4], Paolo Brambilla[2,5], Stefano Cianfarani[2,6], Valerio Nobili[2,7], Angelo Pietrobelli[2,8] and Carlo Agostoni[2,4]

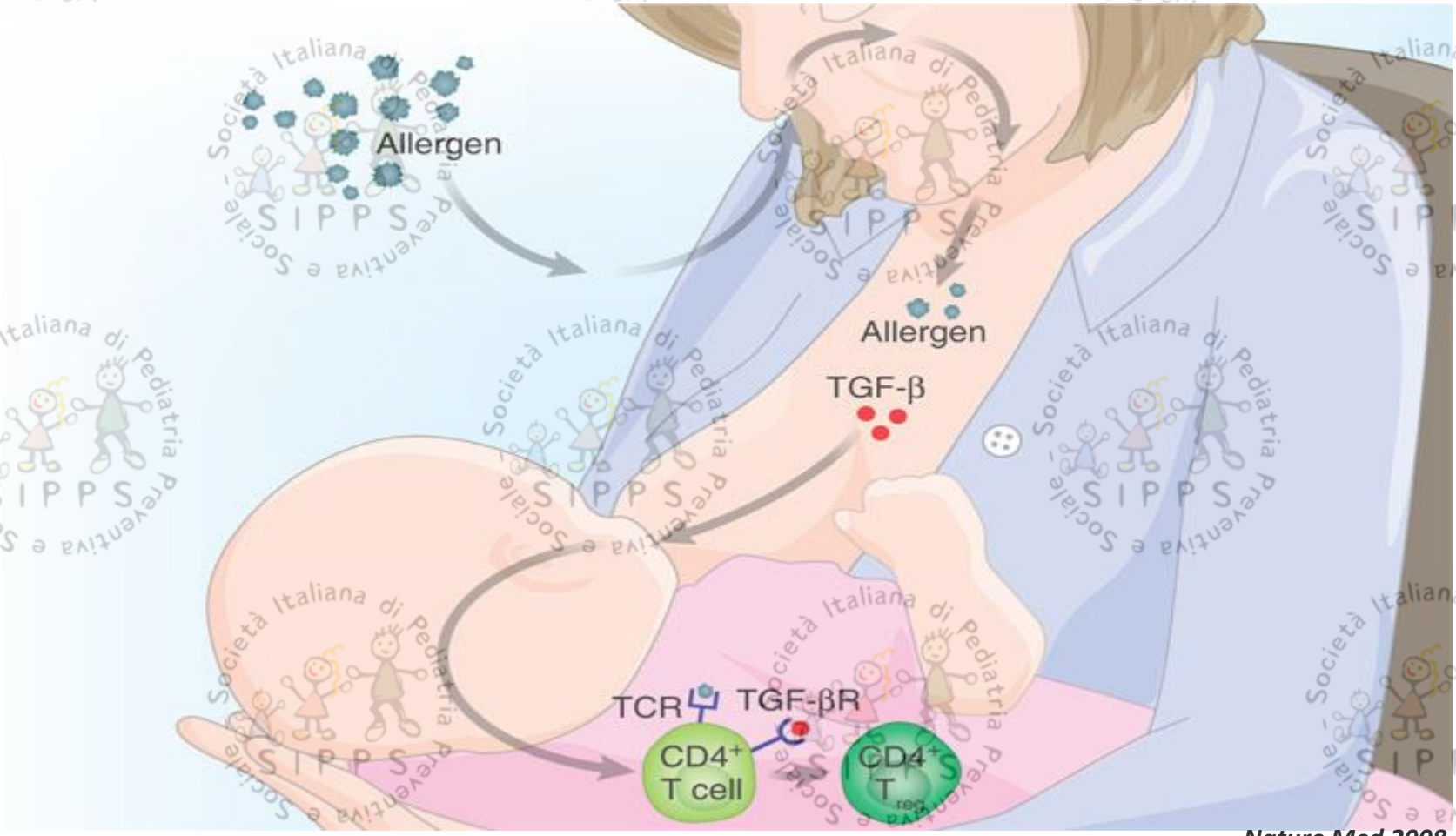



Food intake
• Amount of fiber
• Amount of fat
Metabolites in blood
• Absorption from GI tract
• Effects on peripheral immune responses
• Effects on neutrophils, inflammation resolution
Lungs and bronchi
• Effects on innate immune cells, Treg cells, epithelial
• Lung microbiome
Metabolites in breast milk
• Effects on postnatal immune development
Mesenteric Lymph Nodes
• DC T cell interactions
• Tolerance induction
Gut homeostasis
• Microbiota composition
• Production of bacterial metabolites, i.e., SCFAs
• Epithelial Integrity
• Treg cell biology, tolerance to food Ags
Direct absorption of metabolites in small intestine
• ω-3 fatty acids
• Tryptophan metabolites
Bone marrow
• DC maturation, effects of SCFAs
Placental transfer of metabolites to developing fetus
• Effects on immune system
• Effects on organ development- lung, heart, brain
• HDAC/epigenetic effects of SCFAs

Breathing easier with breast milk

Lynn Puddington & Adam Matson

Breast milk protects young mice from developing symptoms of asthma. The effect occurs through the induction of regulatory T cells after ingestion of allergen and TGF-β in breast milk (pages 170–175).



Nature Med 2008

Diet and food allergy development during infancy: Birth cohort study findings using prospective food diary data

Kate E. C. Grimshaw, PhD, RD,[a] Joe Maskell, MSc,[b] Erin M. Oliver, MSc,[a] Ruth C. G. Morris, Dip HE, RN(Child),[c] Keith D. Foote, MBBS, FRCPCH,[d] E. N. Clare Mills, PhD,[e] Barrie M. Margetts, PhD, FFPH,[f]* and Graham Roberts, DM, MRCPCH[a]* *Southampton, Winchester, and Manchester, United Kingdom*

ALL'ETA' DI 2 ANNI

MAGGIORE PREVALENZA DI AA

MINORE PREVALENZA DI AA

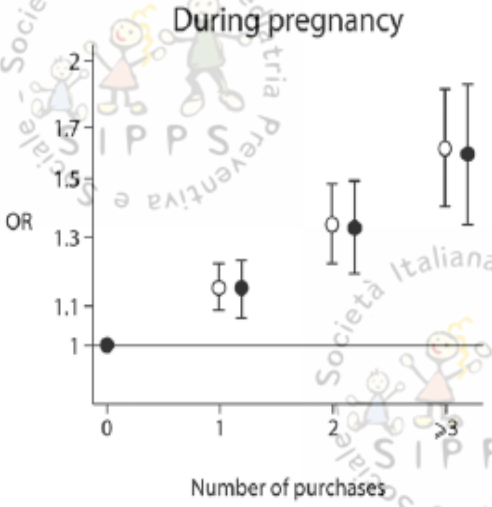
J Allergy Clin Immunol 2014;133:511-9.

Mother's and Offspring's Use of Antibiotics and Infant Allergy to Cow's Milk

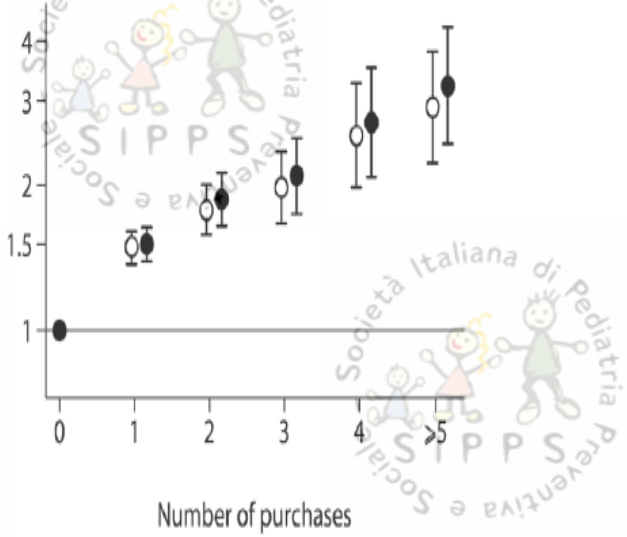
Johanna Metsälä,[a,b] *Annamari Lundqvist,*[c] *Lauri J. Virta,*[d] *Minna Kaila,*[e] *Mika Gissler,*[f,g] *and Suvi M. Virtanen*[a,b,h]

16.237 cases

Antibiotic use by the mother



Antibiotic use by the child



Epidemiology • Volume 24, Number 2, March 2013

Single children

Caesarean delivery

Microbial related factors increasing risk of food allergy

Disinfectant and antiseptic agents

Antibiotics

Gastric Acidity inhibitors

Pre-natal immune state

Immunological pathways precipitating allergy

Food allergy

Exposure to increased variety and abundance of organisms

Increasing family size

Microbial related factors reducing risk of food allergy

Farming lifestyle

Raw milk and fermented foods

Probiotics

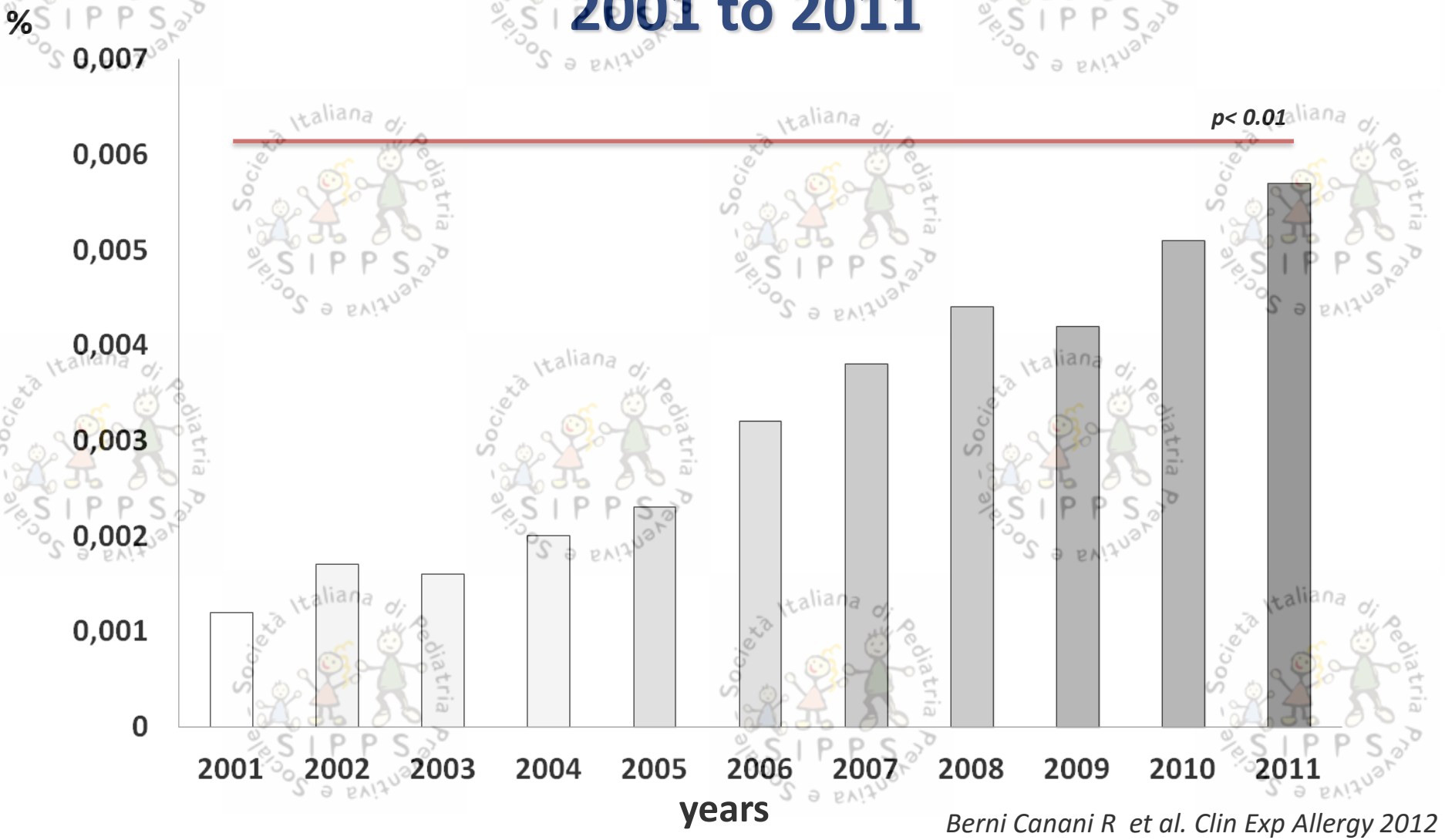
Communal childcare

Domestic animals

High fiber diet/home made foods

Maars T et al. PAI 2013

Increasing trend of hospitalization rate for food-induced anaphylaxis in Italian children from 2001 to 2011



Berni Canani R et al. Clin Exp Allergy 2012
Nocerino R et al. JACI 2015

Italian Ministry of Health, Health planning, Essential levels of care and Ethical principles of the system - Office VI. http://www.salute.gov.it/ricoveriOspedalieri/ric_informazioni/sceltadia.jsp.

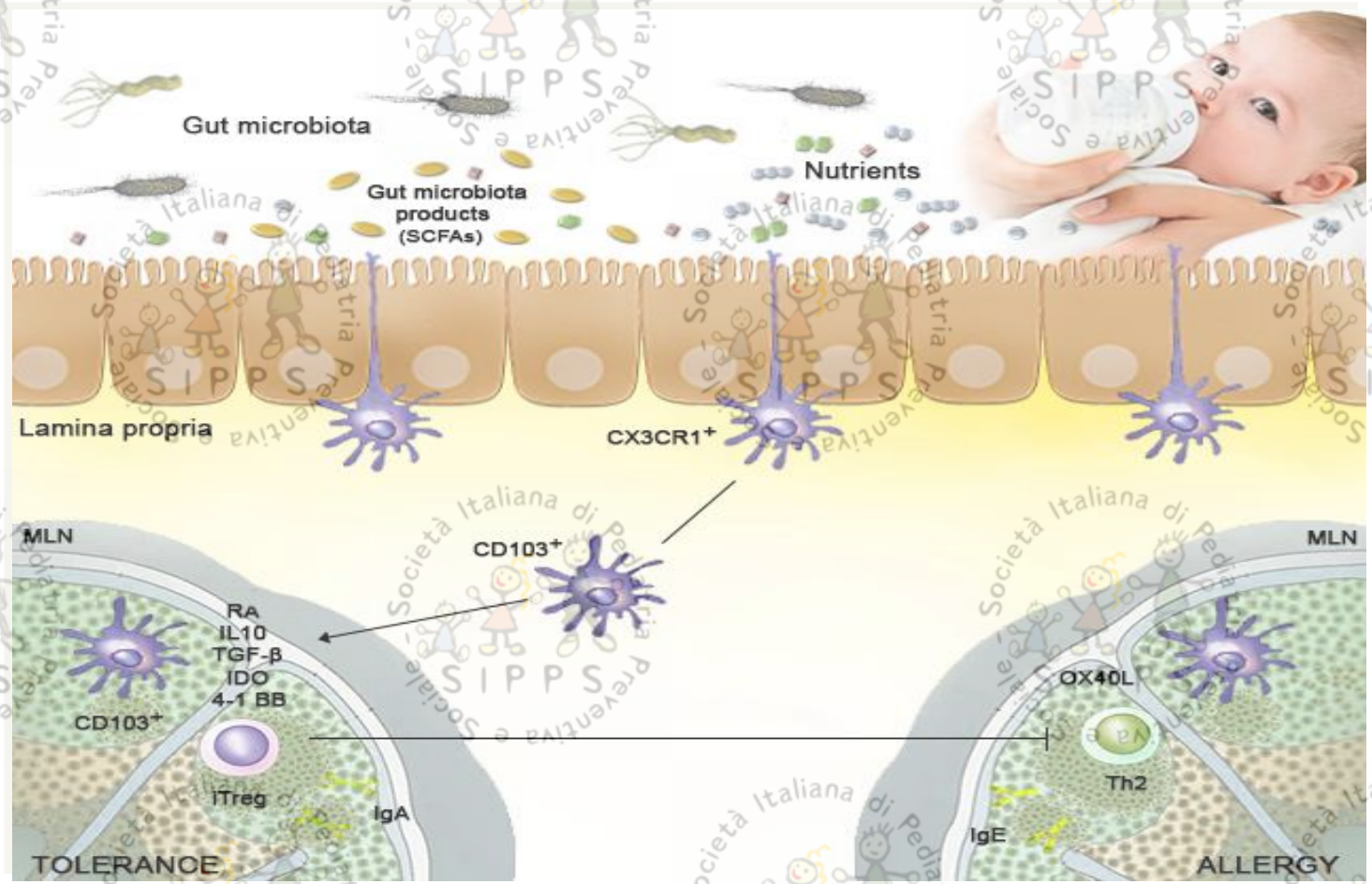
The Changing Pattern of Food Allergy

+ Increased risk of persistence until later ages
+ Increased severity of clinical manifestations
+ Increased need of hospitalization
+ Increased economic impact
+ Increased risk of atopic march *(>50% asthma, atopic eczema, allergic rhinitis)*

= Strong need to develop effective strategies to stimulate oral tolerance acquisition

Skripak JM, et al. JACI 2007
Ross MP, et al. JACI 2008
Wang J, et al. JCI 2011
Chen FM, et al. JMII 2012
Virta LJ et al. JPGN 2013
Gupta R, et al. JAMA Ped 2013
Prescott SL et al. WAO J 2013
Berni Canani R, et al. Clin Exp All 2013
Nocerino R, et al. JACI 2015

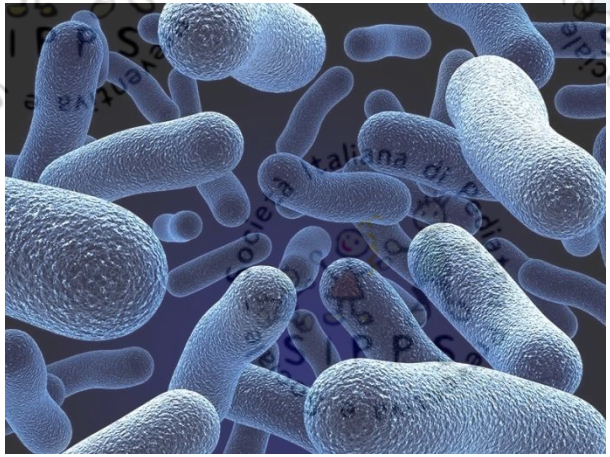
Oral tolerance: suppression of immune response to dietary antigens mediated by Ag-specific regulatory T cells

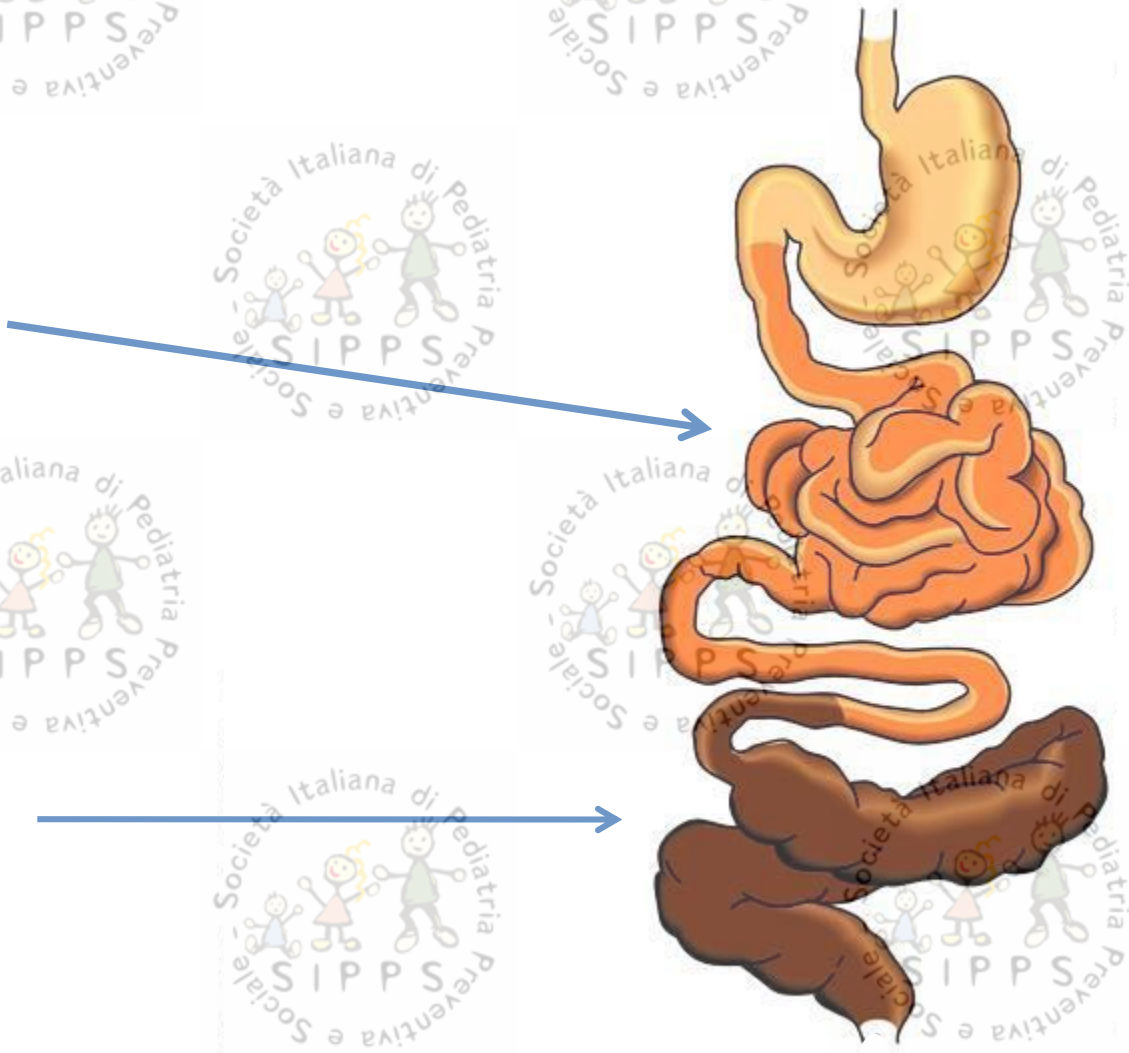


A dynamic modulation of this network is introduced at birth by exposure to enviromental factors and by acquisition of gut microbiota

Di Costanzo M and Berni Canani R JPGN 2016, in press

Two crucial players are involved in oral tolerance: dietary factors and gut microbiota



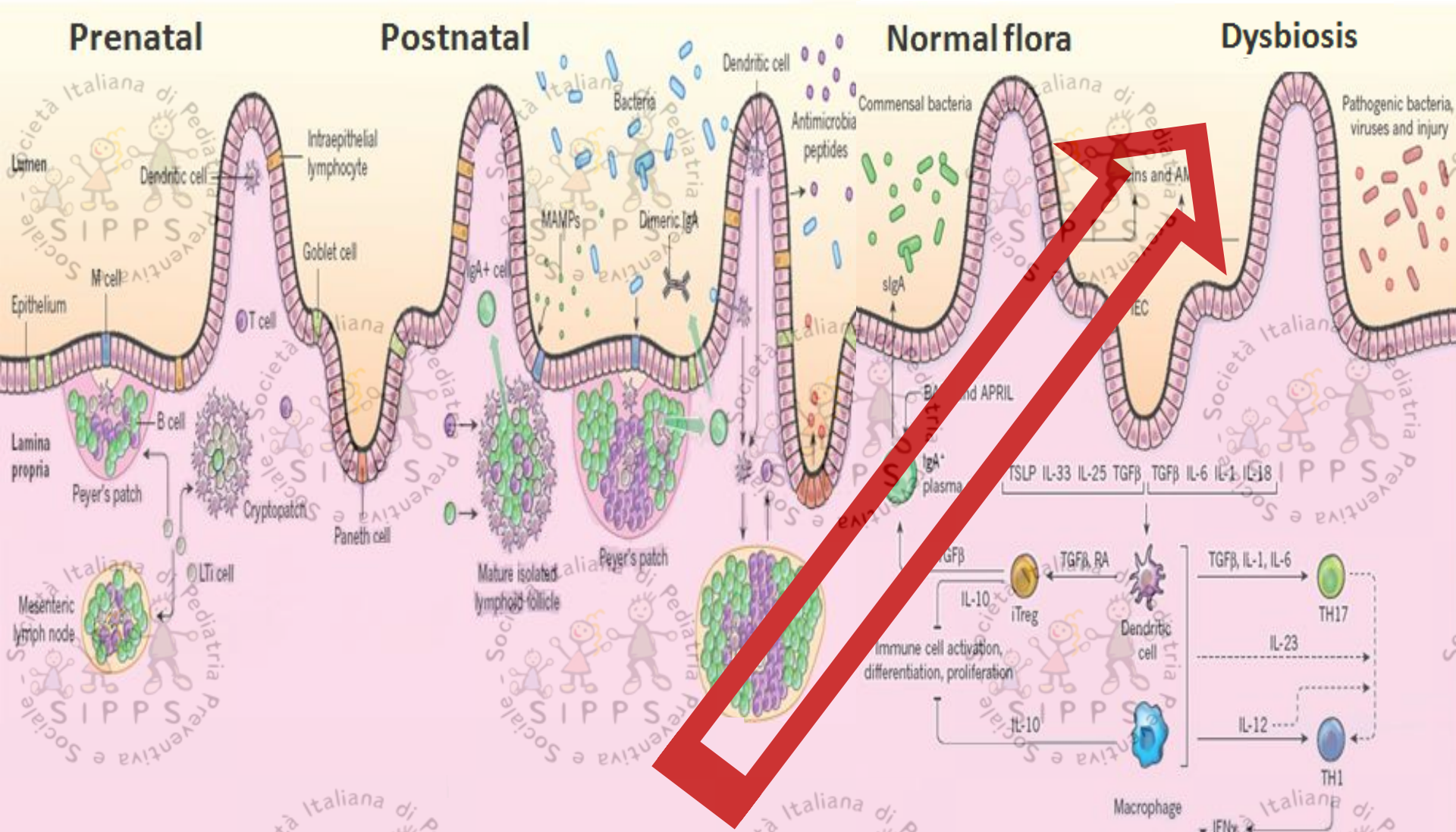


Kim KS et al. Science, 2016

Depletion of Dietary Peptides inhibits Oral Tolerance: Effects of Amino Acid-based diet

- Lower serum Ig
- Lower lymphocytes count in siLP
- Decreased size of Payer's patches
- Lower number of FoxP3+ CD4+ Treg cells
- Higher level of anti-dietary Ag sIgE
- Higher severity of allergic manifestations

Pereira P, et al. Eur J Immunol 1986
Wastmann BS, et al. Proc Soc Exp Biol Med 1991
Kim KS et al. Science 2016

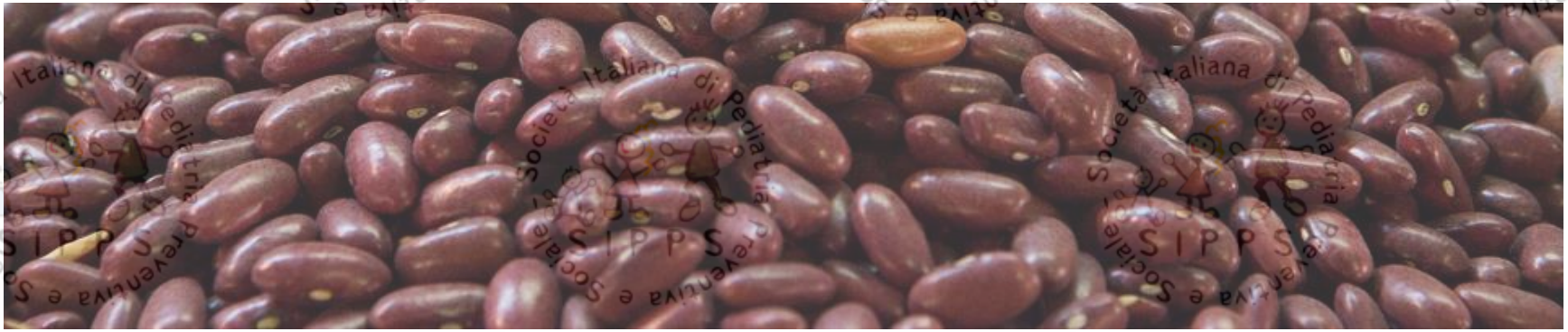


- Caesarean birth
- Lack of breast milk
- Antibiotics
- Gastric acidity inhibitors
- High fat, low plant-based fibers Western-type diet

Modified from Maynard CL, et al. Nature 2012

Food and Agriculture Organization of the United Nations

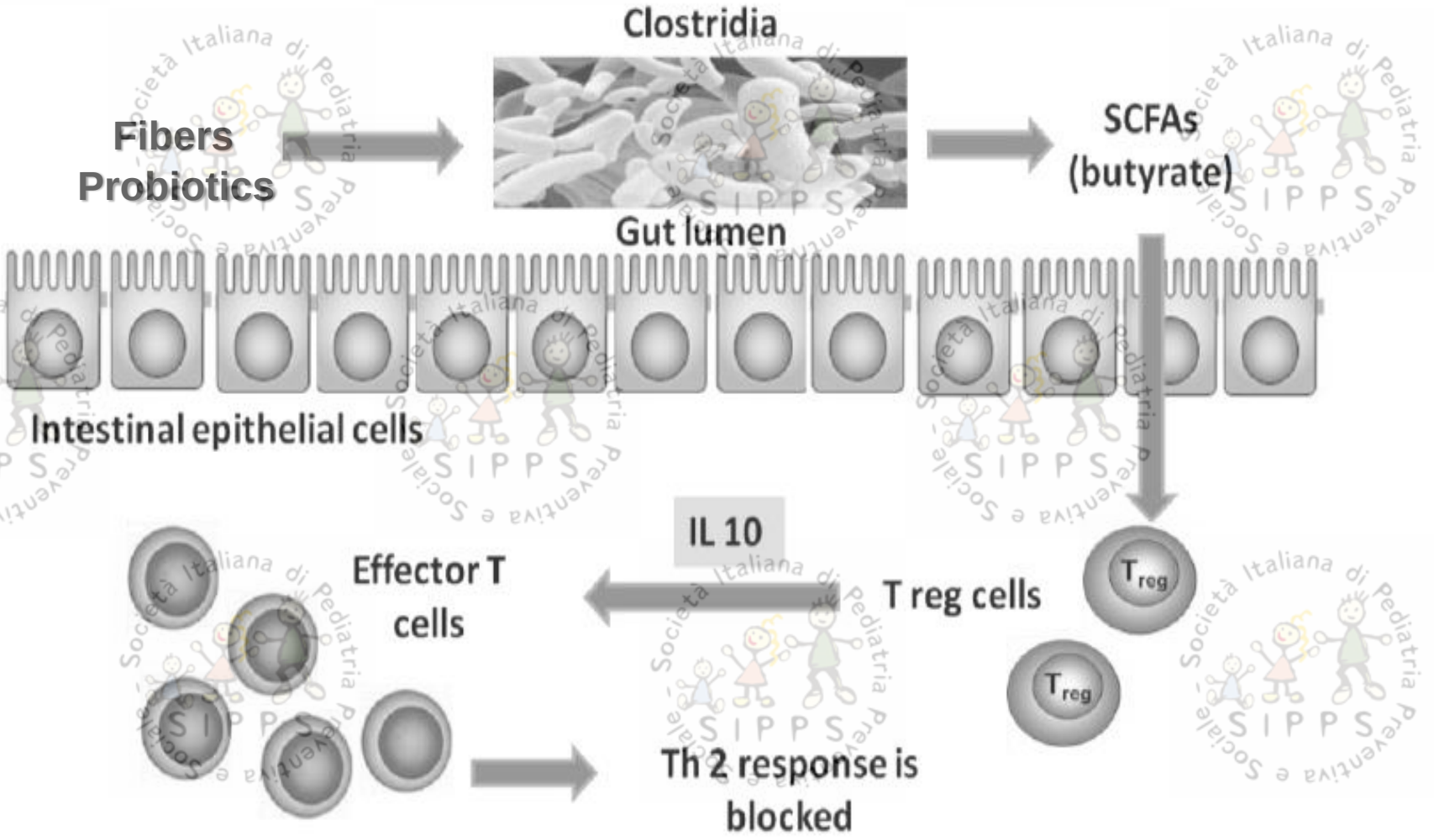
2016
ANNO INTERNAZIONALE DEI LEGUMI



The 68th UN General Assembly declared 2016 the International Year of Pulses (IYP) (A/RES/68/231)
The Food and Agriculture Organization of the United Nations (FAO) has been nominated to facilitate the implementation of the Year in collaboration with Governments, relevant organizations, non-governmental organizations and all other relevant stakeholders.
The IYP 2016 aims to heighten public awareness of the nutritional benefits of pulses as part of sustainable food production aimed towards food security and nutrition. The Year will create a unique opportunity to encourage connections throughout the food chain that would better utilize pulse-based proteins, further global production of pulses, better utilize crop rotations and address the challenges in the trade of pulses.

POTENTIAL BENEFICIAL EFFECTS OF BUTYRATE AGAINST FOOD ALLERGY

Margherita Di Costanzo[1], Lorella Paparo[1], Rosita Aitoro[1], Linda Cosenza[1], Rita Nocerino[1], Tommaso Cozzolino[1], Vincenza Pezzella[1], Gianfranco Vallone[2] and Roberto Berni Canani[1,3,]*



In: Butyrate: Food Sources, Functions and Health Benefits


PROBIOTICS
OK, WHO WANTS TO COLONIZE MY INTESTINES?!
ME!
stickycomics.com

EXPERT CONSENSUS DOCUMENT

The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic

Colin Hill, Francisco Guarner, Gregor Reid, Glenn R. Gibson, Daniel J. Merenstein, Bruno Pot, Lorenzo Morelli, Roberto Berni Canani, Harry J. Flint, Seppo Salminen, Philip C. Calder and Mary Ellen Sanders

Rare

Strain-specific effects

Neurological effects
Immunological effects
Endocrinological effects
Production of specific bioactives

Frequent

Observed at species level

Vitamin synthesis
Direct antagonism
Gut barrier reinforcement
Bile salt metabolism
Enzymatic activity
Neutralization of carcinogens

Widespread

Among studied probiotics

Colonisation resistance
Enterocytes growth
Regulation of intestinal transit
Normalization of perturbed microbiota
Competitive exclusion of pathogens

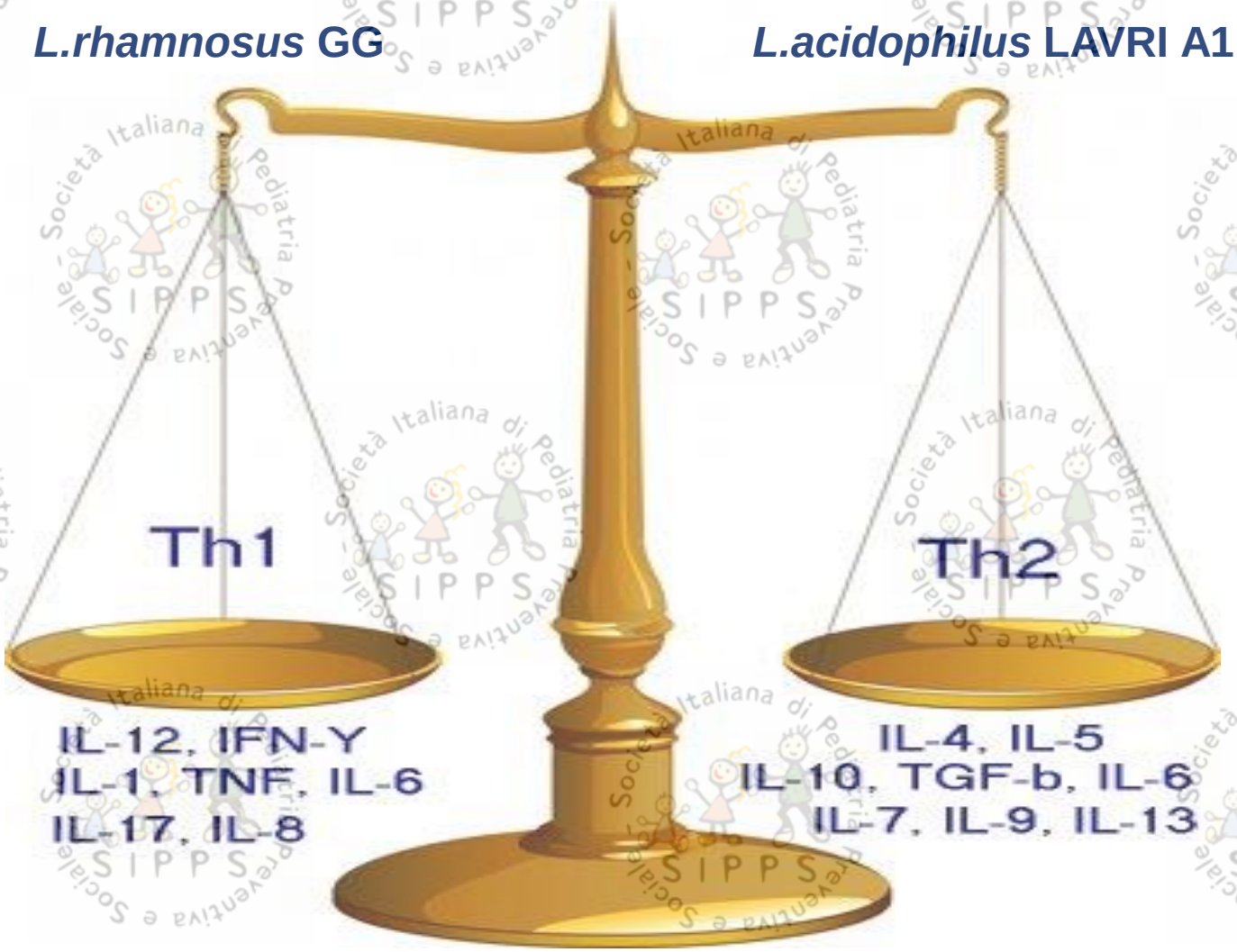
NATURE REVIEWS | GASTROENTEROLOGY & HEPATOLOGY 2014;11:506–14

Engineering the microbiome with probiotics?

***L.casei* CRL431/*B. lactis* Bb12**

***L.rhamnosus* GG**

***L.acidophilus* LAVRI A1**

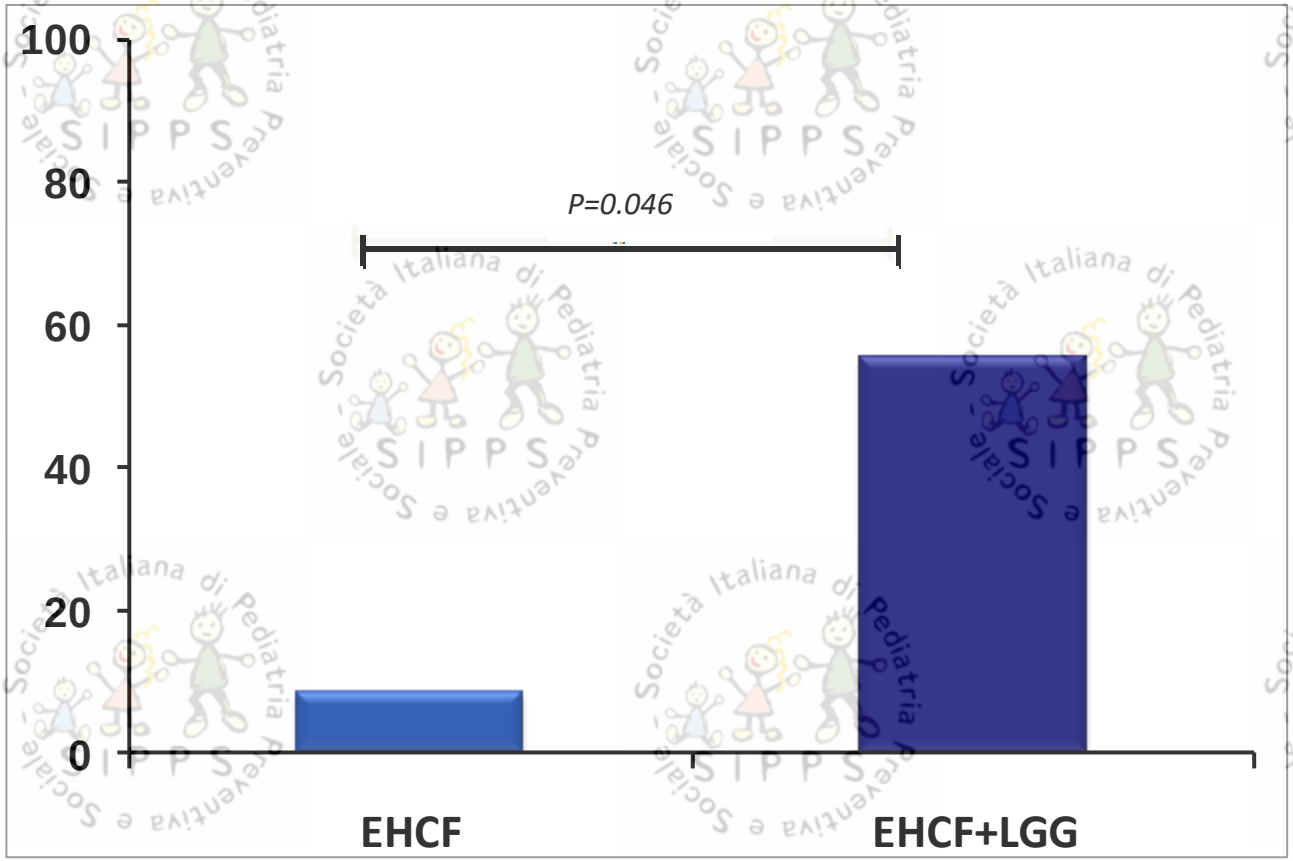


Berni Canani R et al. Curr Opin Allergy Clin Immunol 2015

LGG accelerates cow's milk protein tolerance acquisition

THE JOURNAL OF Allergy AND Clinical Immunology

Rate of children with IgE-mediated CMA acquiring tolerance at 12 m

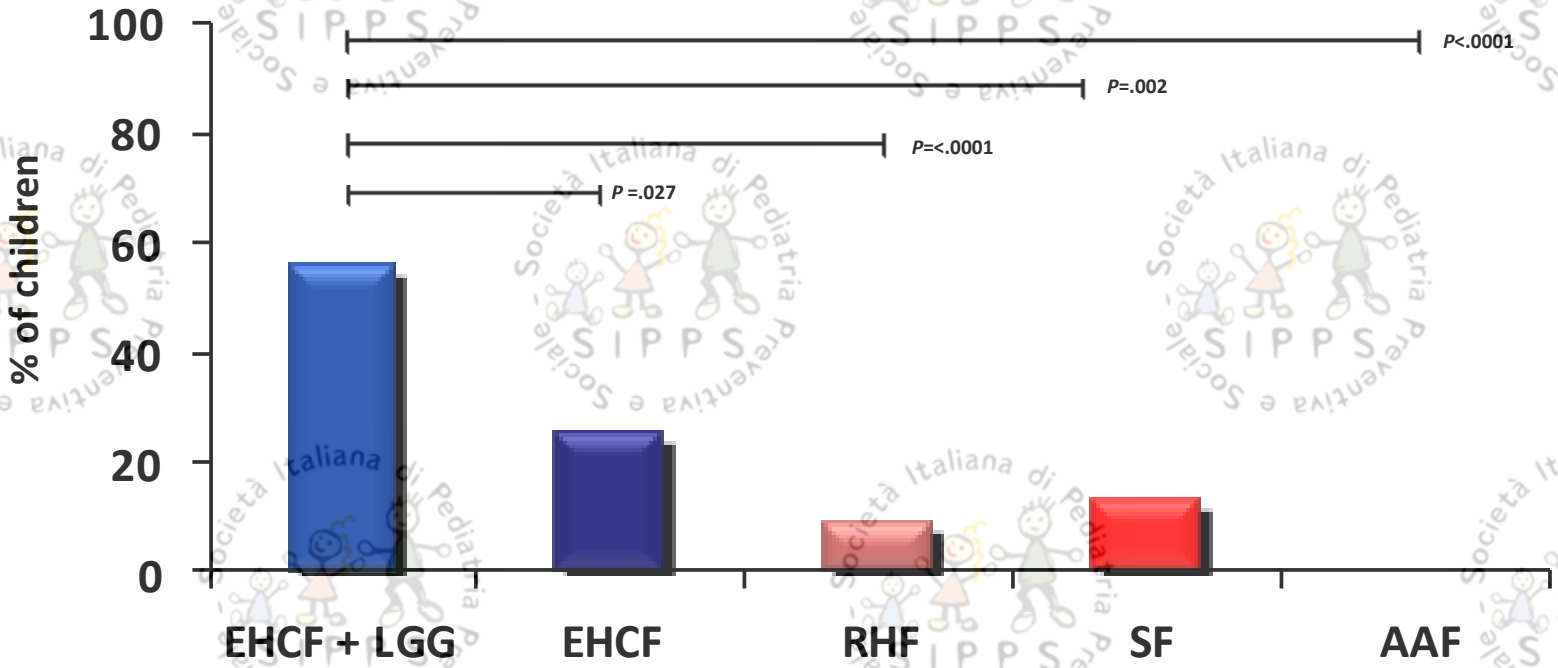


Berni Canani R, et al. 2012

EHCF+LGG leads to tolerance at an earlier age than other formulae

The Journal of Pediatrics

Rate of children with IgE-mediated CMA acquiring tolerance at 12 m

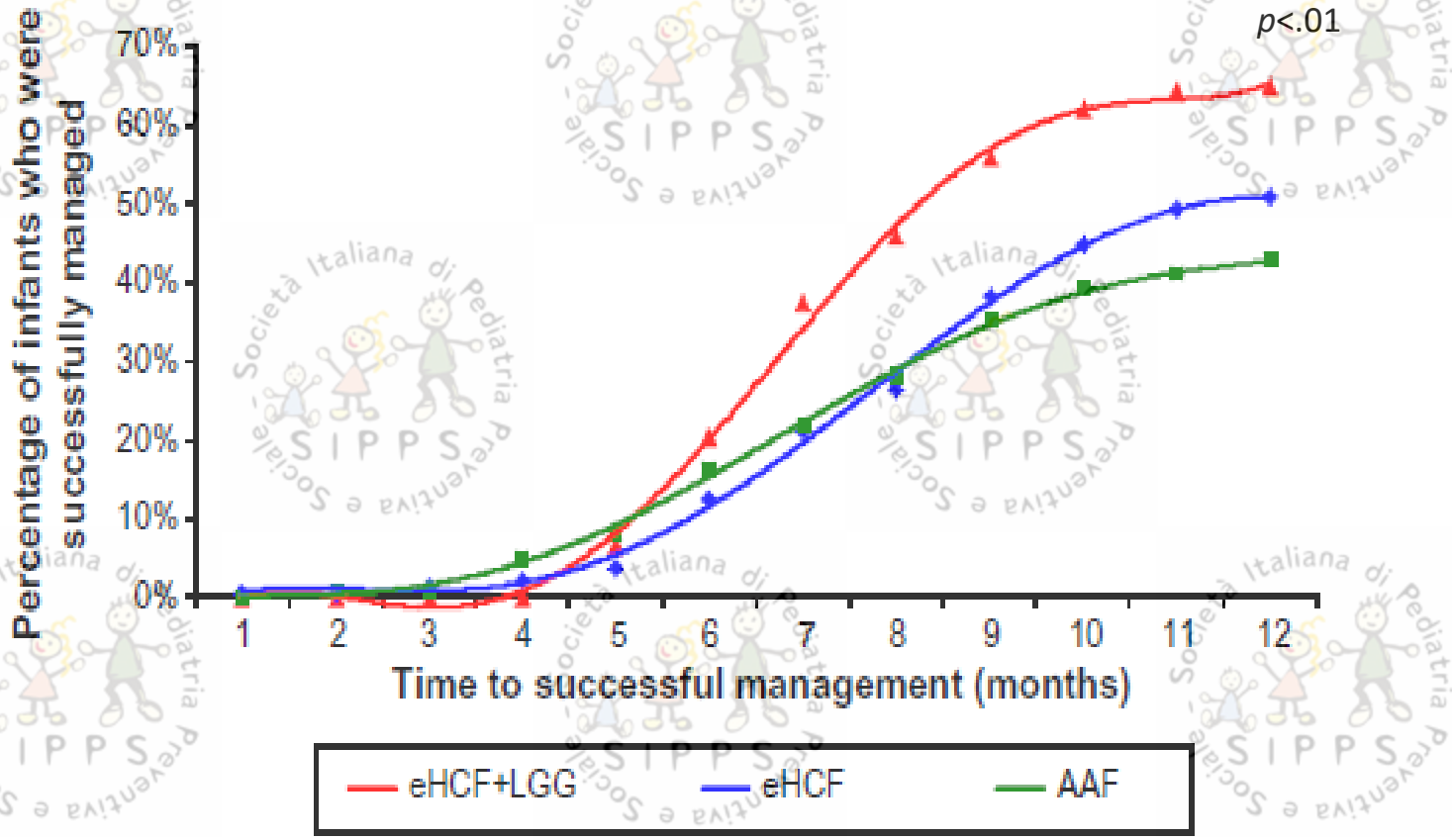


Berni Canani R, et al. 2013

Cost-effectiveness of using an extensively hydrolyzed casein formula plus the probiotic *Lactobacillus rhamnosus* GG compared to an extensively hydrolyzed formula alone or an amino acid formula as first-line dietary management for cow's milk allergy in the US

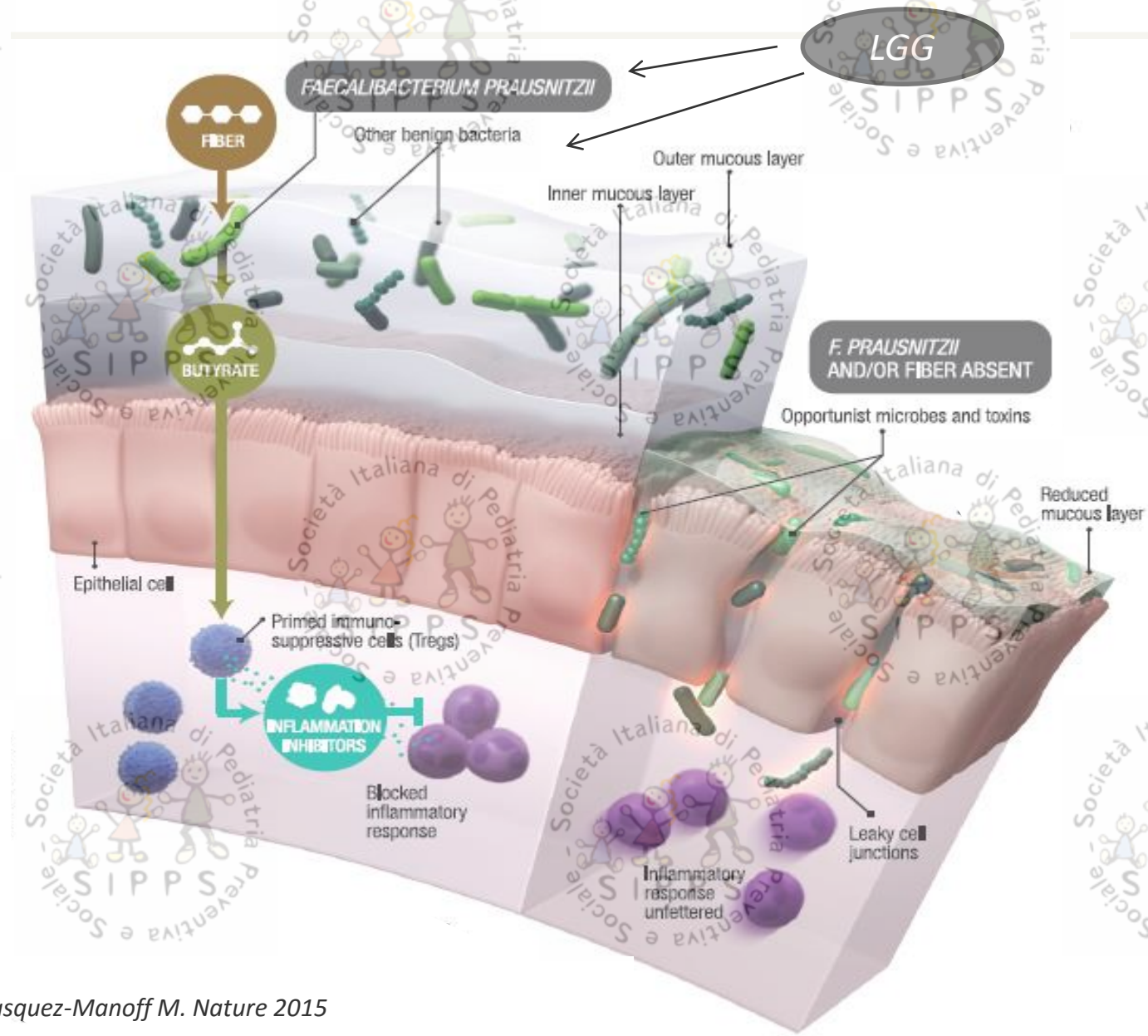
Olga Ovcinnikova[1]
Monica Panca[1]
Julian F Guest[1,2]

[1]CATALYST Health Economics Consultants, Northwood, London, [2]Faculty of Life Sciences and Medicine, King's College, London, UK



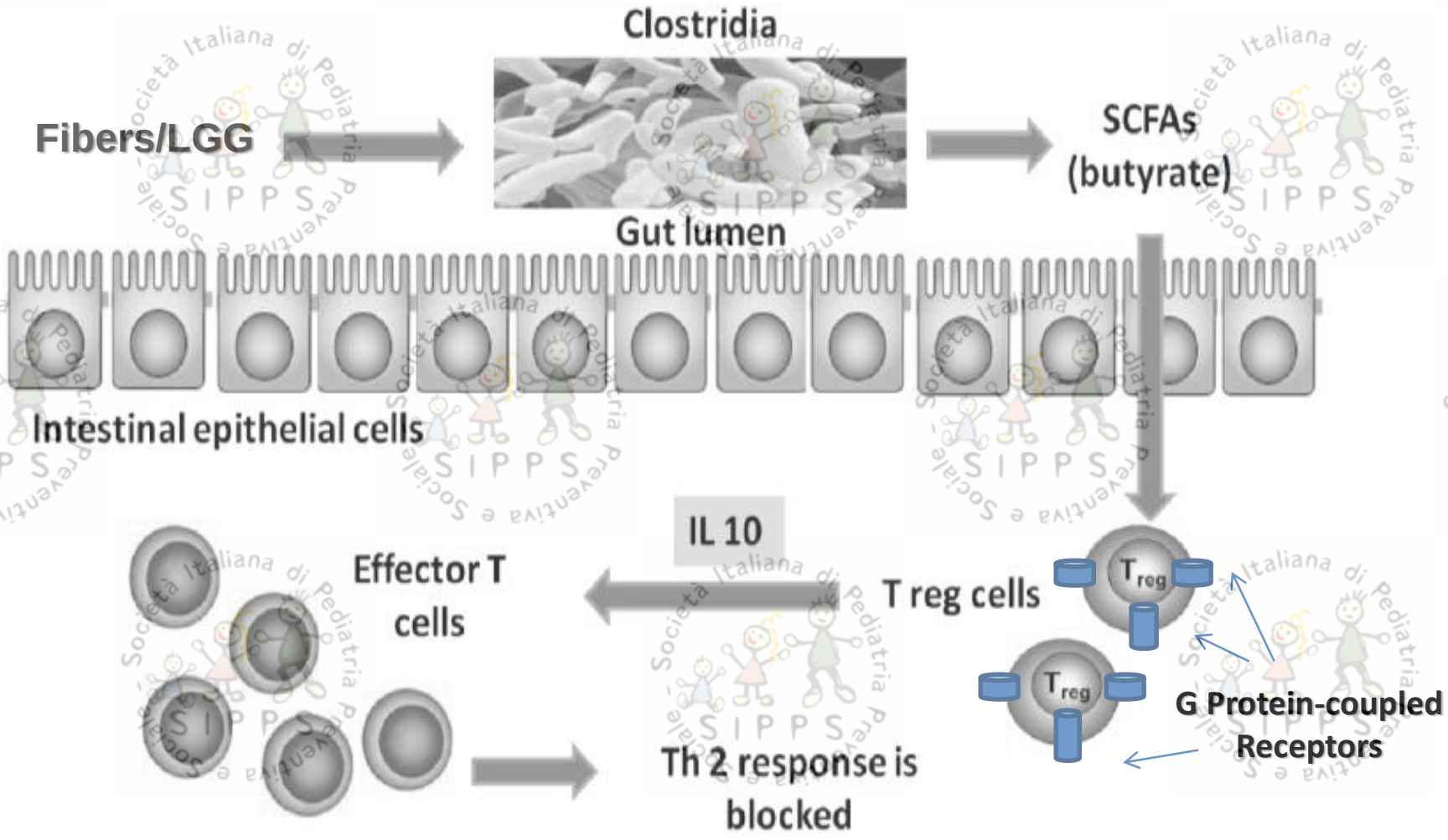
ClinicoEconomics and Outcomes Research 2015:7 145–152

LGG: The Peace-keeper for CMA

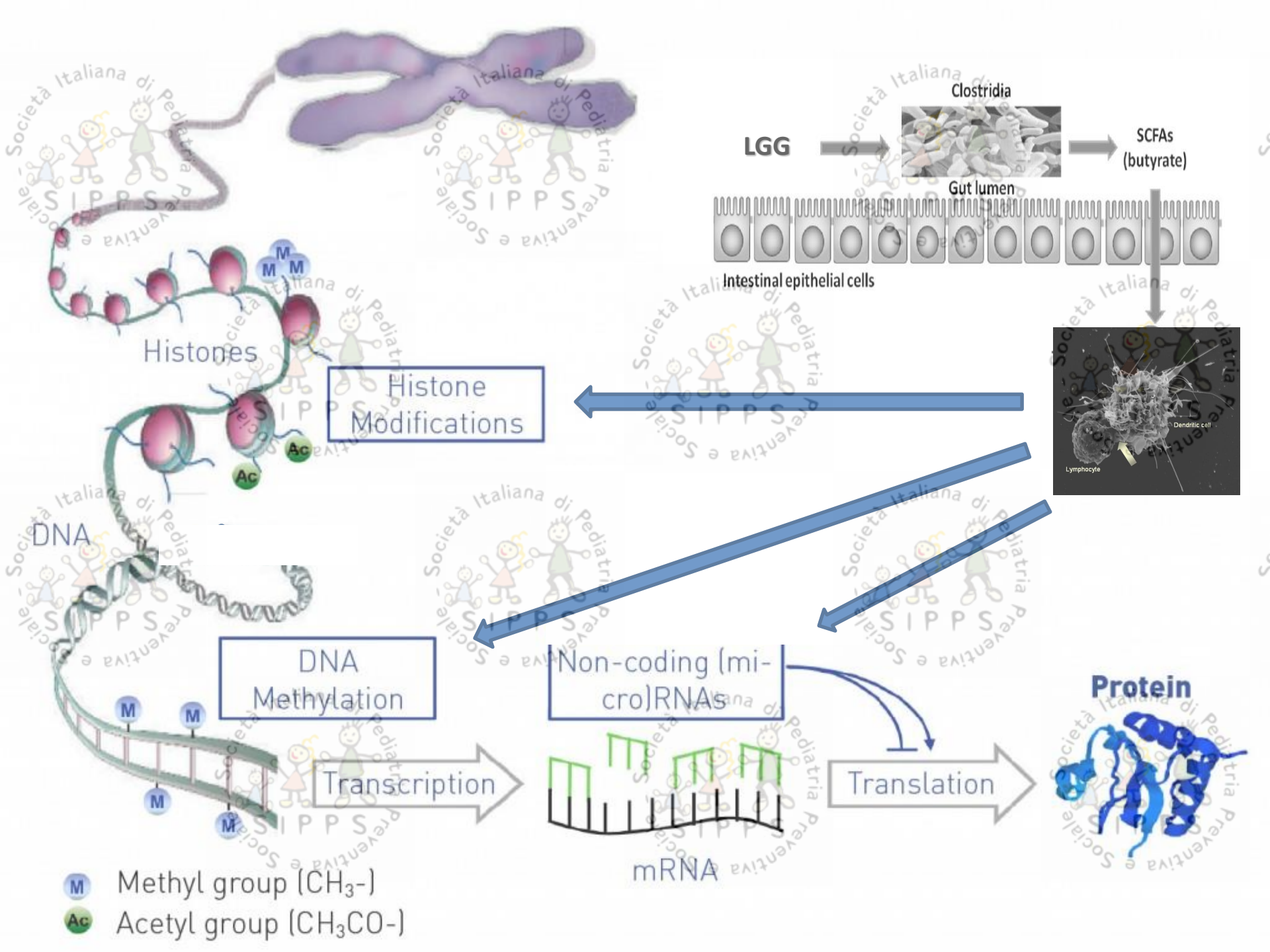


Modified from Velasquez-Manoff M. Nature 2015

EHCF+LGG significantly increases butyrate-producing bacteria: the epigenetics pathways



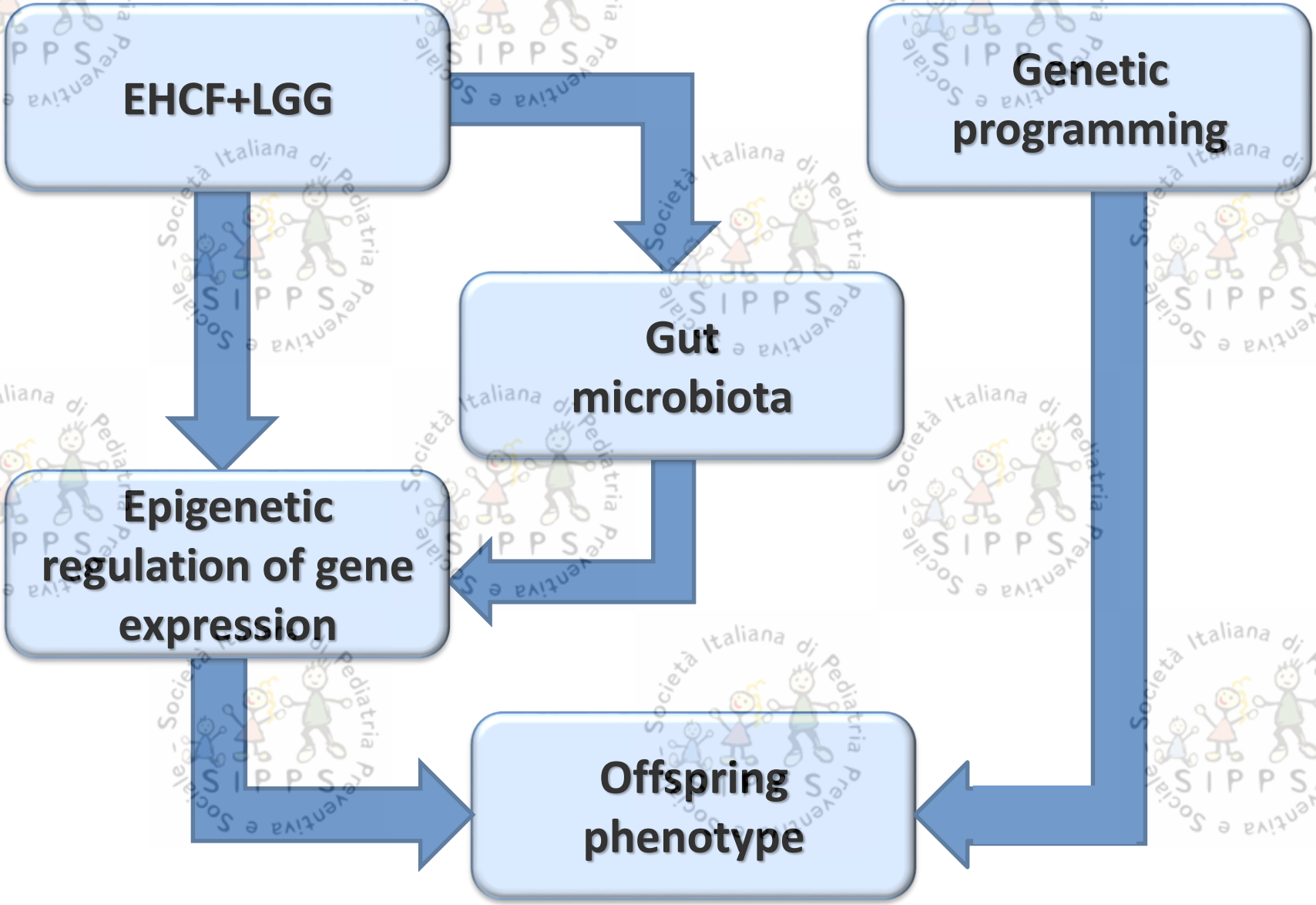
In: Butyrate: Food Sources, Functions and Health Benefits


Clostridia
LGG
SCFAs
(butyrate)
Gut lumen
Intestinal epithelial cells
Dendritic cell
Lymphocyte
Histones
Histone
Modifications
DNA
DNA
Methylation
Non-coding (mi-
cro)RNAs
Transcription
Translation
Protein
mRNA
Methyl group (CH3-)
Acetyl group (CH3CO-)

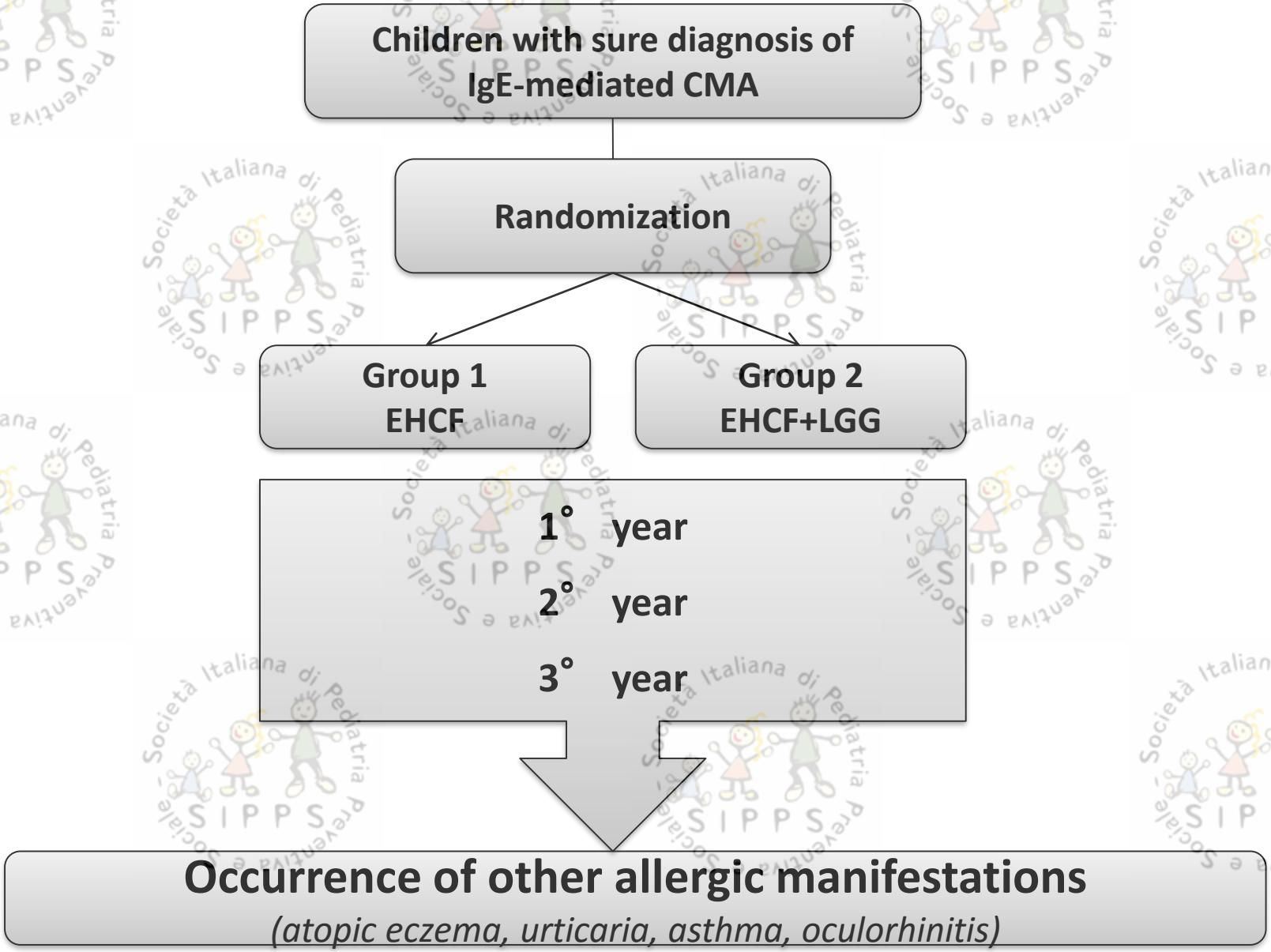
I meccanismi epigenetici regolano la differenziazione sia in senso Th1 sia Th2 e i cambiamenti nella metilazione dei promoter sono un prerequisito per l'espressione di FoxP3 e la differenziazione delle Treg.

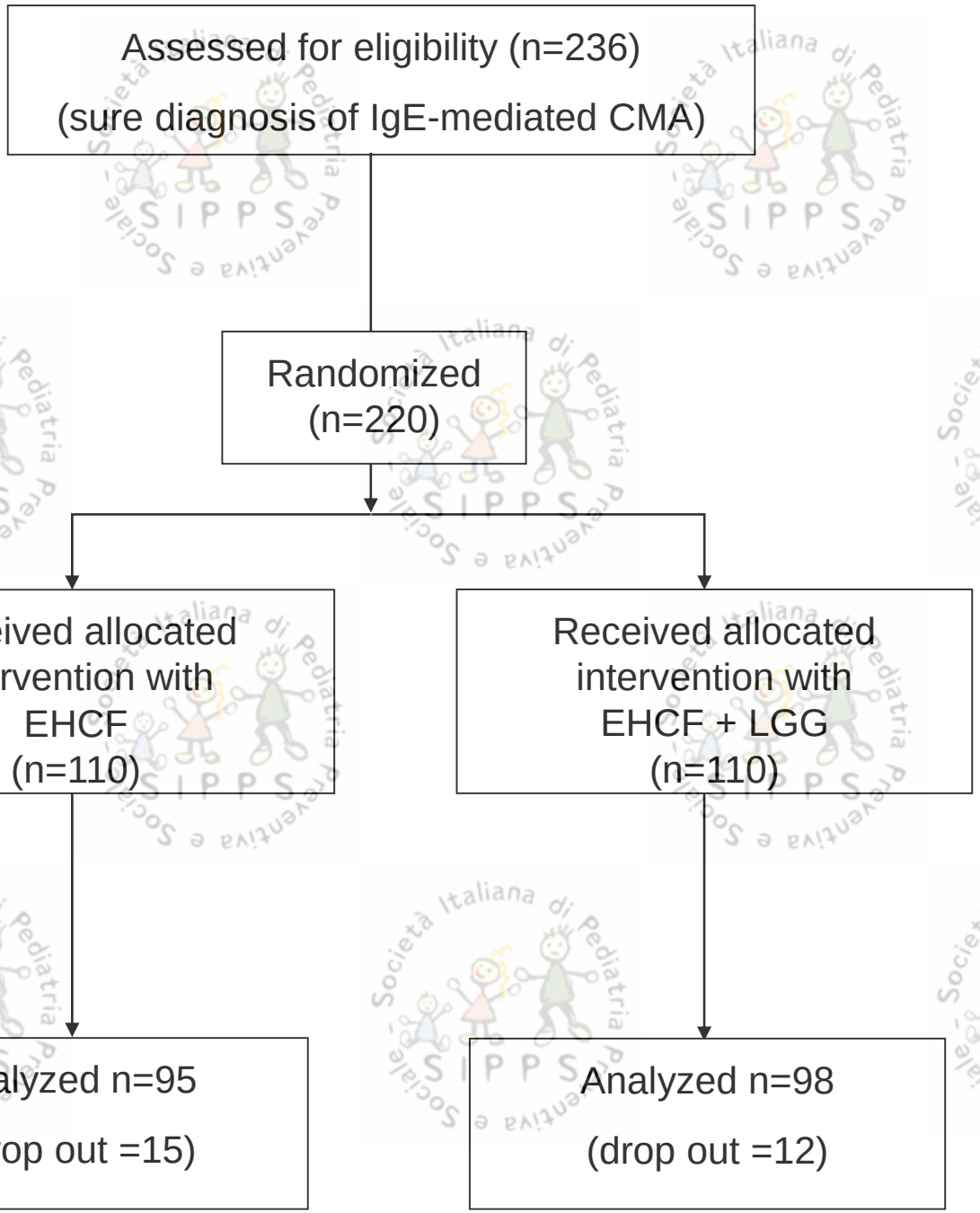
Berni Canani R et al. Nutr Res Rev 2011

Epigenetics: the interface between genes and nutrition

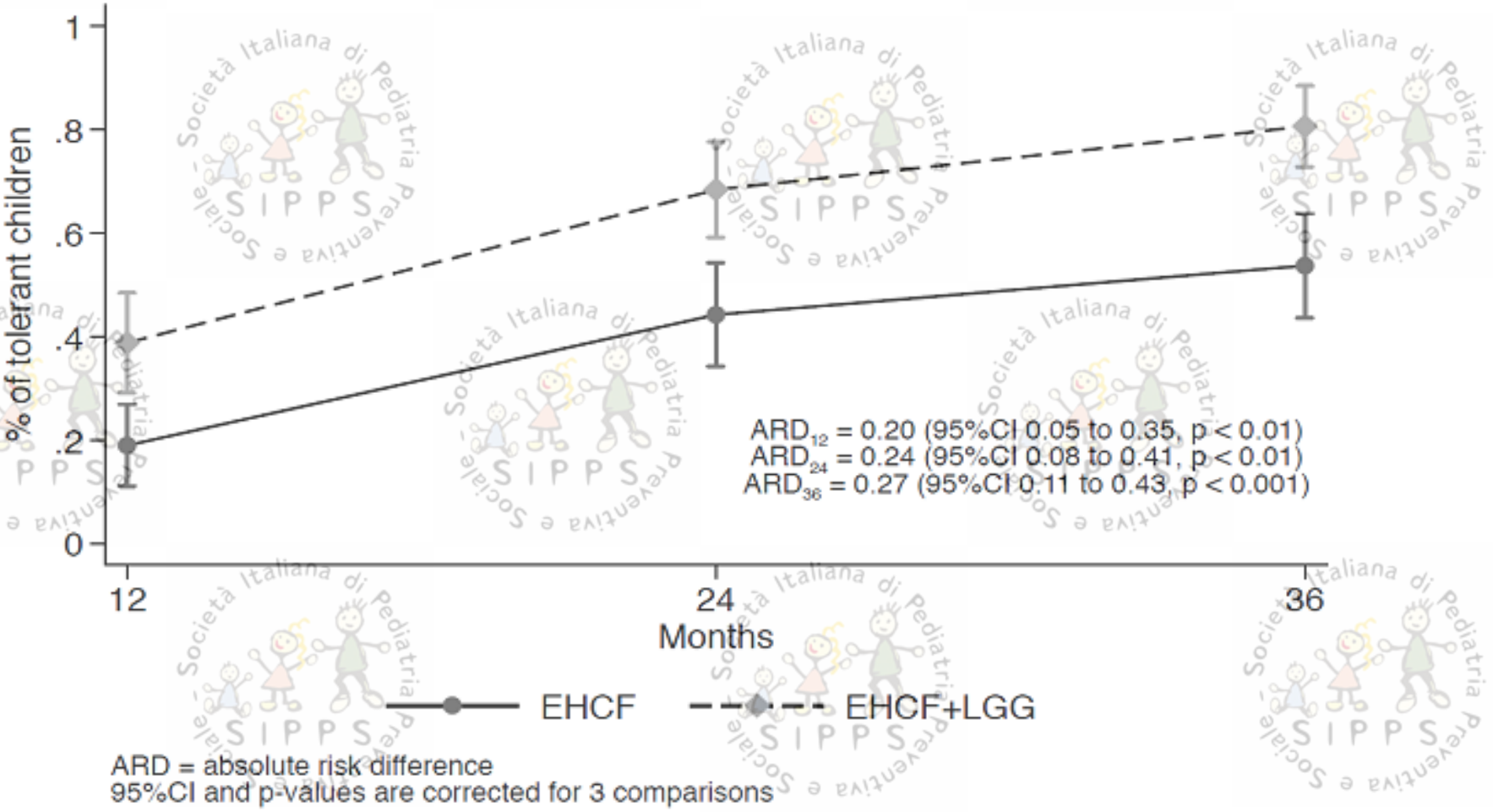


Prospective study: preventive effect elicited by LGG on atopic manifestations in CMA children



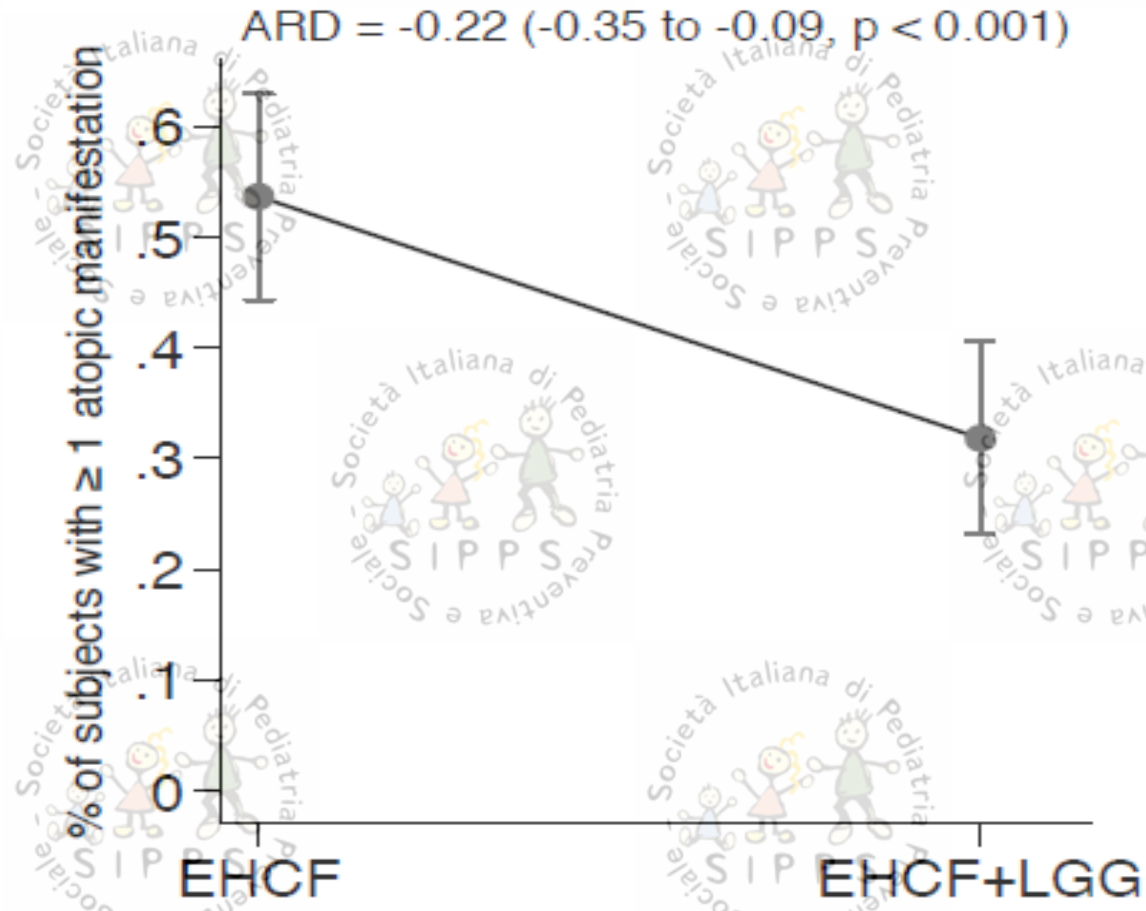

Assessed for eligibility (n=236)
(sure diagnosis of IgE-mediated CMA)
Randomized
(n=220)
Received allocated intervention with EHCF
(n=110)
Received allocated intervention with EHCF + LGG
(n=110)
Analyzed n=95
(drop out =15)
Analyzed n=98
(drop out =12)

Oral Tolerance Acquisition



Berni Canani R 2016 submitted

Rate of subjects experienced ≥ 1 atopic manifestation ITT, n=110/group



Berni Canani R 2016 submitted

Alimenti funzionali fermentati

Comuni malattie infettive dell'infanzia

- I bambini scolarizzati di età <4 aa hanno un rischio sino a 4 volte maggiore di sviluppare infezioni a carico del tratto respiratorio e gastrointestinale

- Giorni di assenza da scuola per I bambini e di lavoro persi dai genitori

- Aumento dell'utilizzo di farmaci

Costi stimati= **$1.800** milioni/anno

Lo studio è stato condotto nei 3 mesi invernali in collaborazione con i pediatri di famiglia

Visita 1 (arruolamento)

- Valutaz. anamestica e clinica
- Consenso informato
- Randomizzazione
- Consegna dei prodotti
- Consegna diario clinico
- Raccolta feci

Visita 2 (1 m)

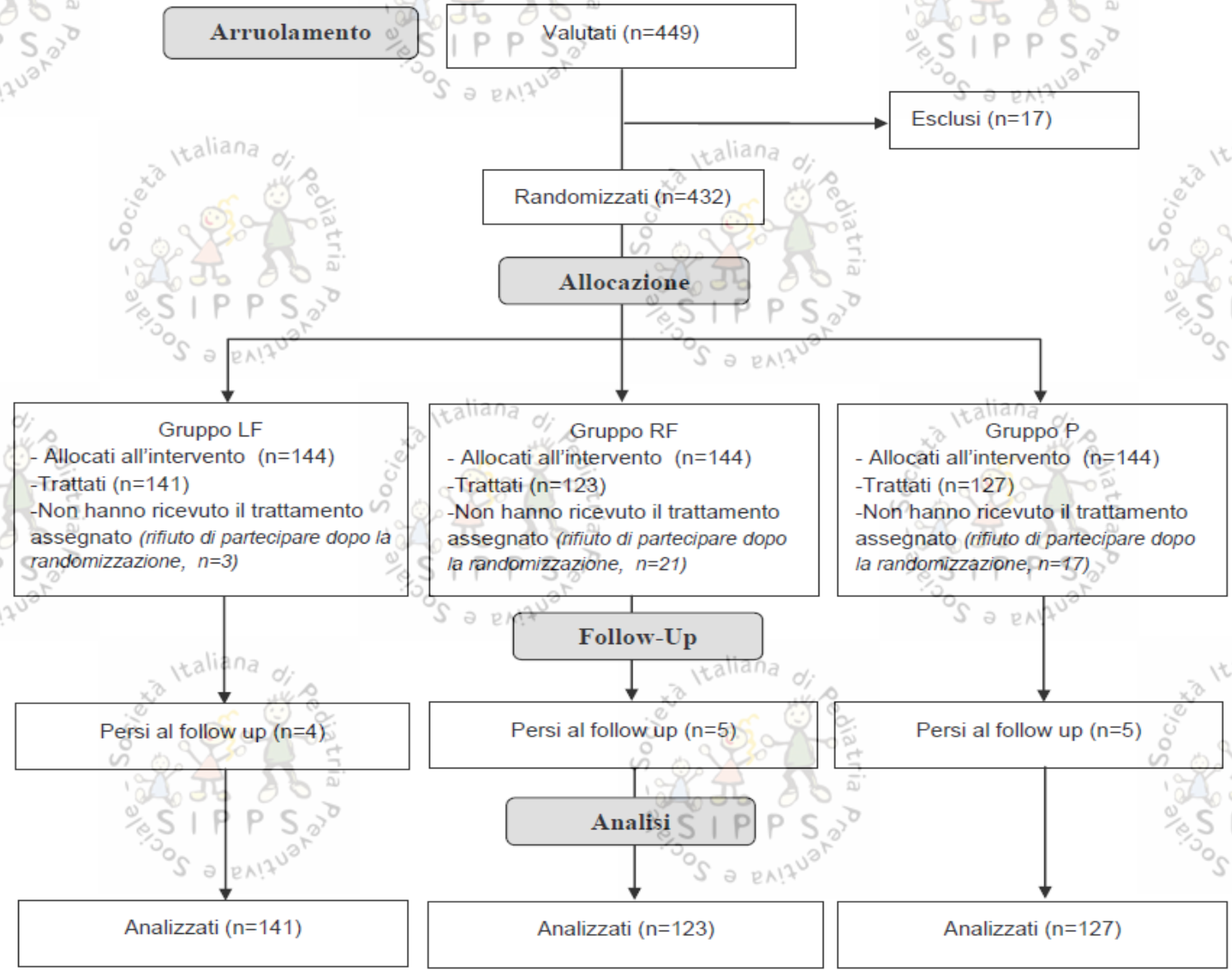
- Valutazione clinica
- Raccolta e valutazione del diario clinico
- Valutazione aderenza

Visita 3 (2 m)

Visita 4 (3 m)

- Valutazione clinica
- Raccolta e valutazione del diario clinico
- Valutazione aderenza
- Raccolta feci

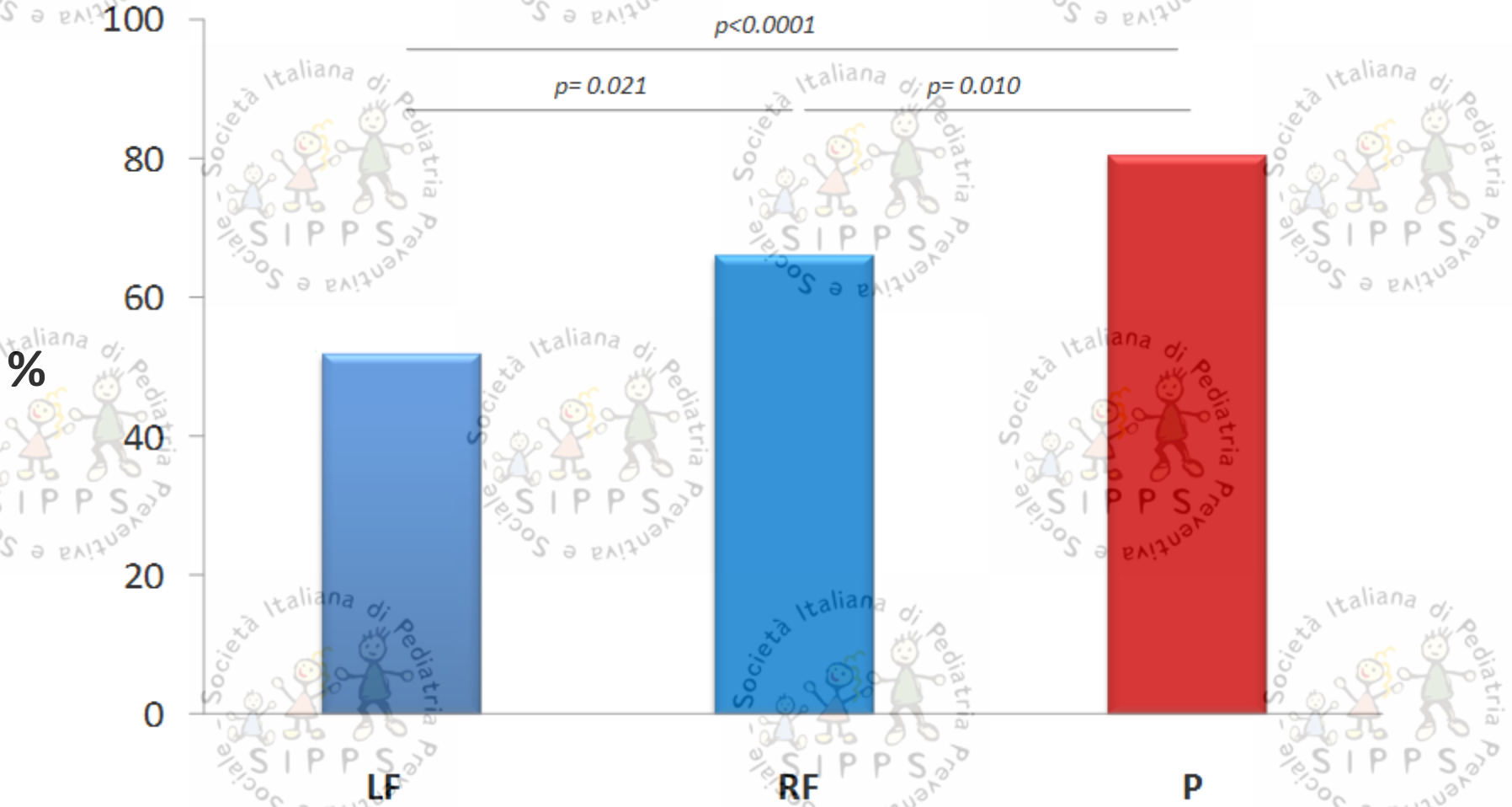
Un elevato numero di bambini ha partecipato allo studio



Caratteristiche simili nelle 3 popolazioni di studio

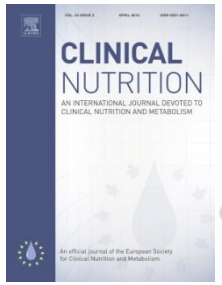
	LF *n=141*	RF *n=123*	P *n=127*
Maschi, n (%)	72 (51.1)	64 (52)	65 (51.2)
Età, mesi (±DS)	32 (10)	31 (11)	34 (9)
Peso, kg (±DS)	14.6 (2.8)	14.5 (2.7)	14.8 (2.9)
Altezza, cm (±DS)	93.3 (9)	92.7 (9)	94.3 (7.2)
Allattamento materno, n (%)	99 (70.2)	88 (71.5)	97 (76.4)
Durata dell'allattamento, mesi (±DS)	7.6 (6.1)	6.2 (4)	6.4 (5.1)
Età alla scolarizzazione, mesi (±DS)	13.4 (2.4)	12.9 (2.2)	12.8 (2.3)
Fratelli, n (%)	108 (76.6)	96 (78)	100 (78.7)
N. di fratelli, (±DS)	1.30 (0.6)	1.4 (0.6)	1.4 (0.6)
Fumo passivo, n (%)	65 (46.1)	56 (45.5)	59 (46.5)

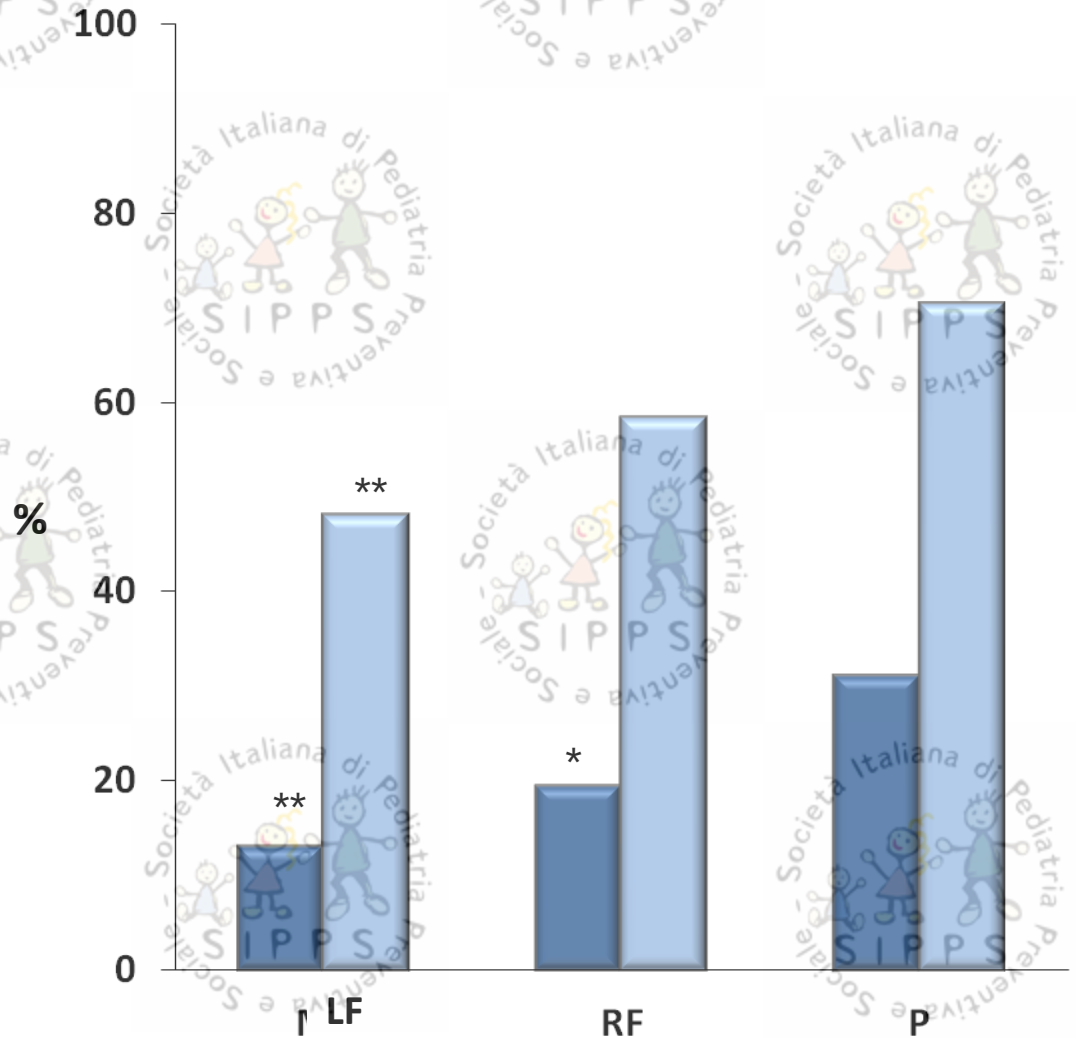
Rate soggetti che hanno presentato ≥ 1 episodio infettivo



Nocerino R, et al. Clin Nutr 2015

Soggetti che hanno presentato ≥1 episodio di gastroenterite acuta o infezione alte vie del respiro





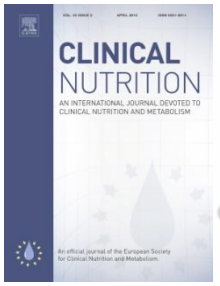
*p<0.05, RF vs P
**p<0.0001, LF vs P

Gastroenterite acuta

Infezione alte vie del respiro

Nocerino R, et al. Clin Nutr 2015

I bambini che hanno assunto latte o riso fermentato hanno presentato meno episodi infettivi



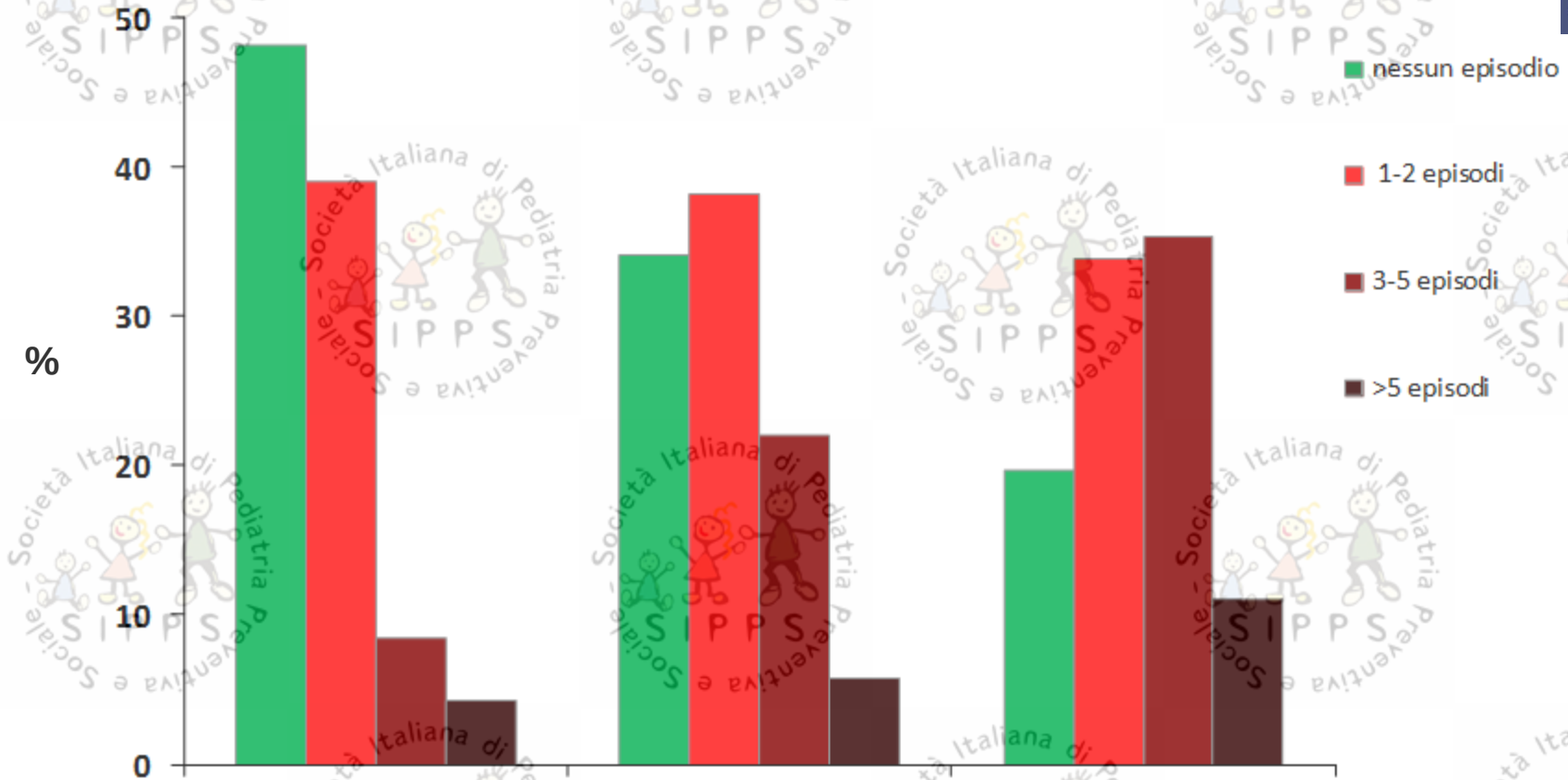
Numero totale di infezioni
Riduzione del 60% nei bambini che assumevano latte fermentato e del 40% nei bambini che assumevano riso fermentato

Numero di episodi di diarrea acuta
Riduzione del 55% nei bambini che assumevano latte fermentato e del 45% nei bambini che assumevano riso fermentato

Numero di episodi di infezioni a carico delle alte vie del respiro
Riduzione del 60% nei bambini che assumevano latte fermentato e del 40% nei bambini che assumevano riso fermentato

Nocerino R, et al. Clin Nutr 2015

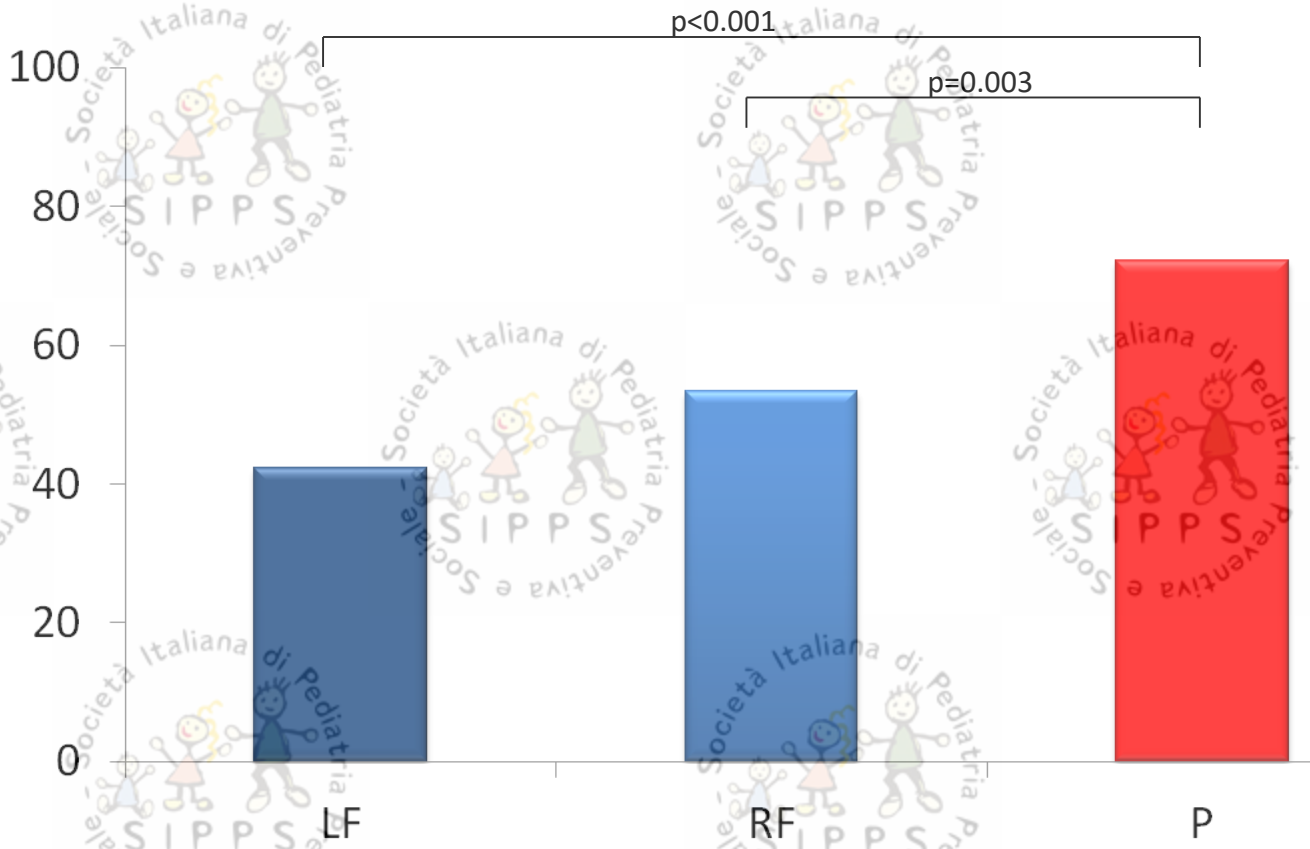
Soggetti che hanno presentato episodi infettivi ricorrenti



LF vs RF	LF vs P	RF vs P
p	*P*	*p*
0.010	*<0.0001*	*0.010*

Nocerino R, et al. Clin Nutr 2105

Rate oggetti che hanno richiesto ≥ 1 visita pediatrica



Nocerino R, et al. Clin Nutr 2015

Utilizzo di farmaci

100

80

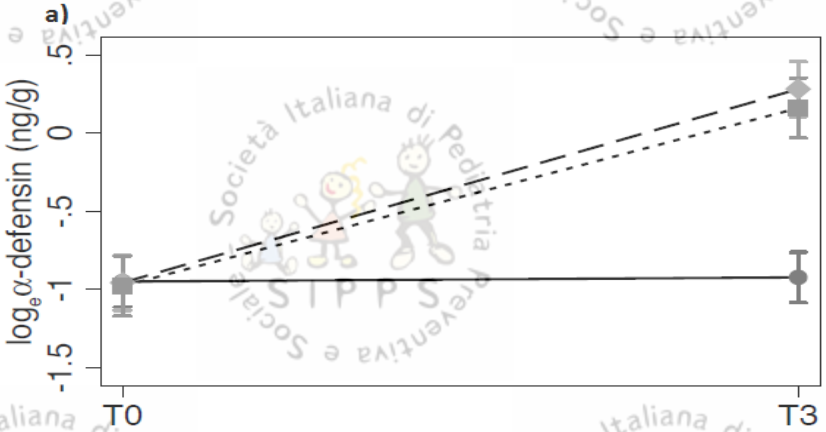
Riduzione dell'utilizzo di antibiotici del 75% nei bambini che assumevano latte fermentato e del 58% nei bambini che assumevano riso fermentato

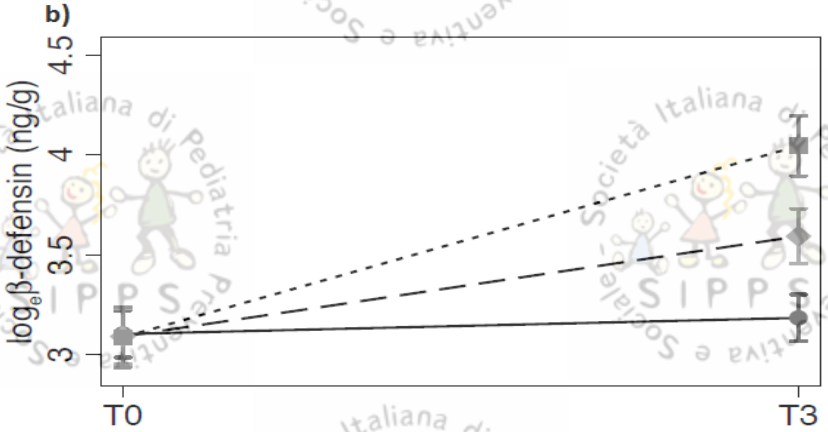
	LF	RF	P		
	LF vs RF *p*	LF vs P *p*	RF vs P *p*		
Almeno 1 farmaco	*0.004**	*<0.0001**	*0.021*		
Antibiotici	*0.003**	*<0.0001**	*0.001**		
Antipiretici	*0.163*	*0.001**	*0.058*		
Steroidi	*0.141*	*0.001**	*0.070*		

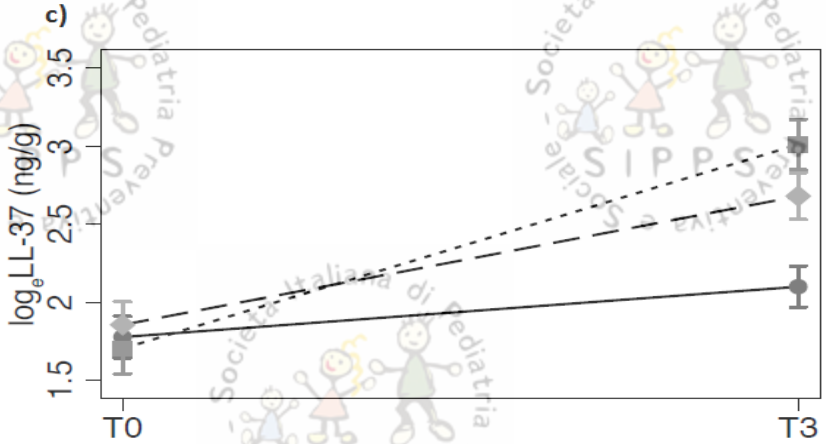
**I valori di p restano significativi dopo la correzione di Bonferroni*

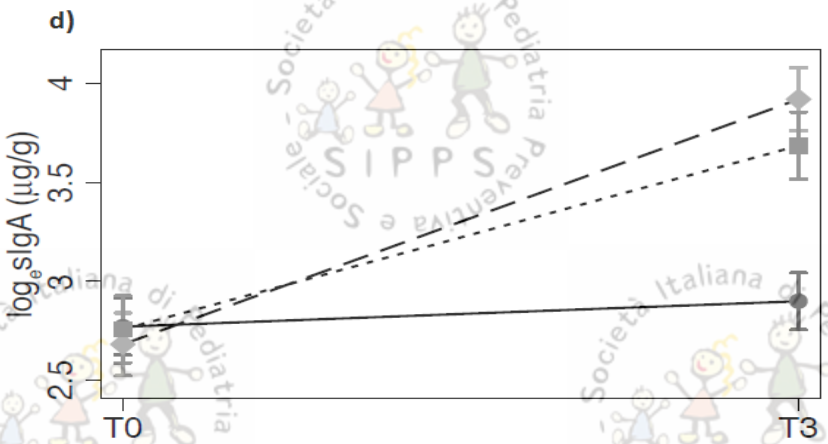
Nocerino R, et al. Clin Nutr 2015

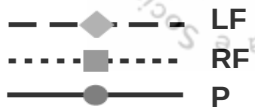
Latte fermentato e riso fermentato con *L.paracasei* CBA L74 esercitano una significativa immunomodulazione











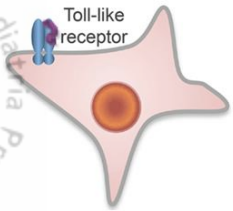
Nocerino R, et al. Clin Nutr 2015

Peptidi dell'immunità innata

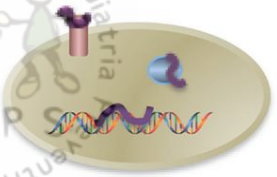
Inibizione legame patogeno cellule bersaglio

Proteolisi

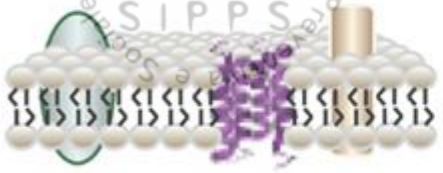
Attivazione sistema immunitario

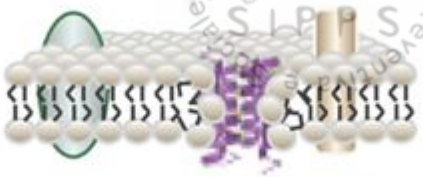


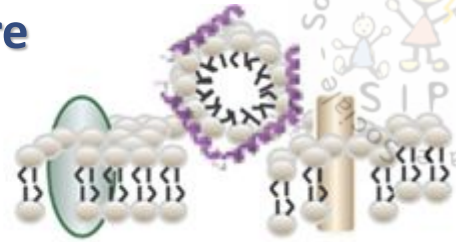
Interazione diretta con espressione dei geni del patogeno



Alterazione membrana cellulare







Modified from Duplantier AJ Front Immunol 2013

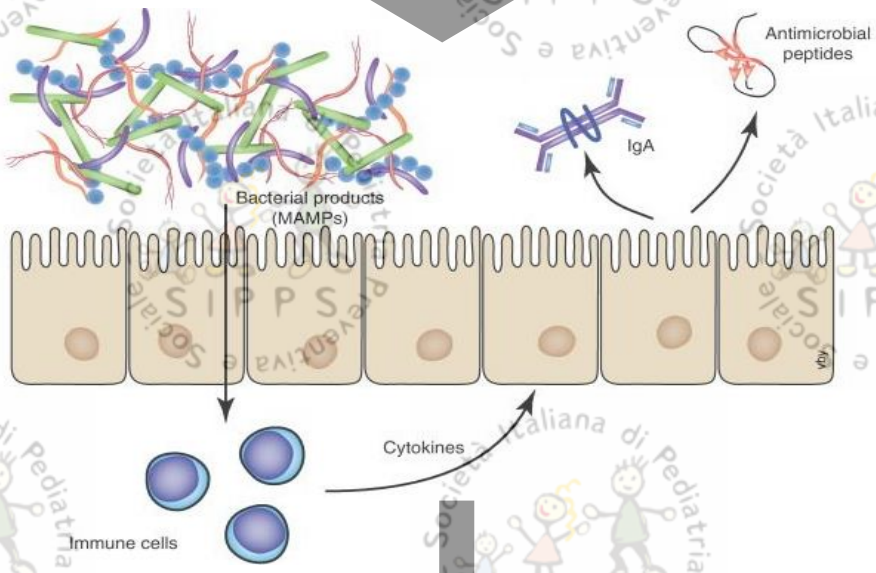
Latte fermentato e riso fermentato con CBA-L74

Peptidi

Microbiota intestinale

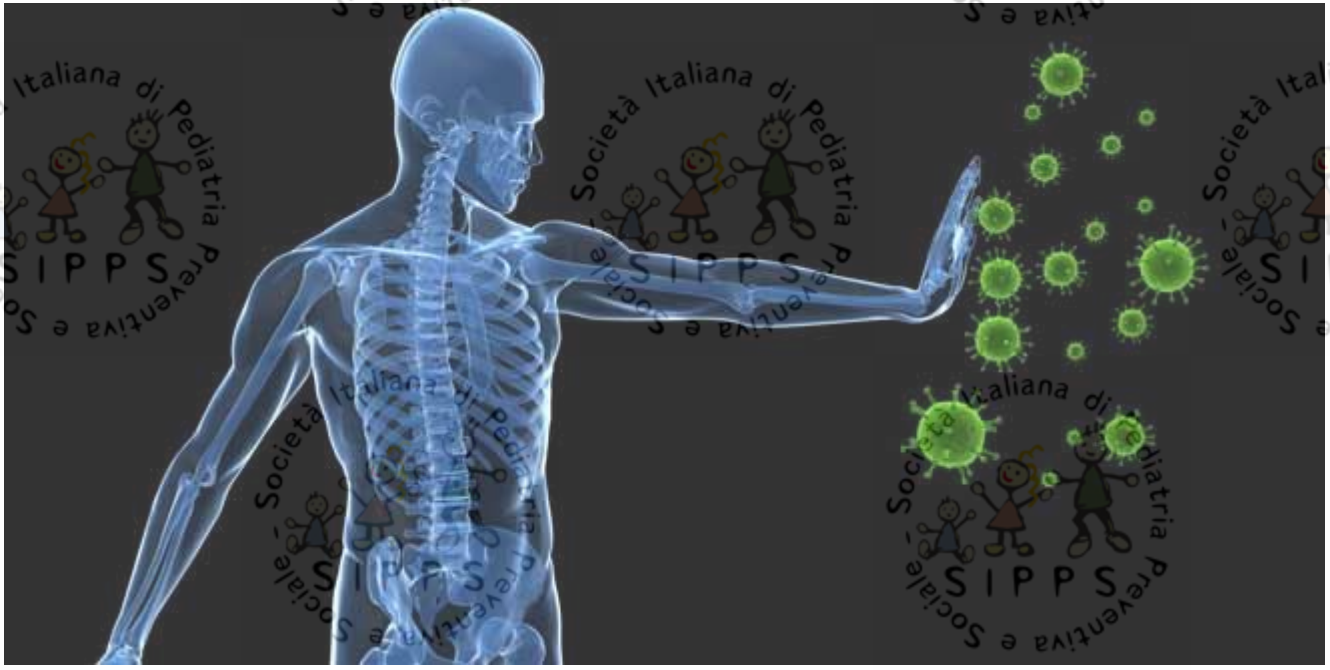
Prodotti batterici

Butirrato



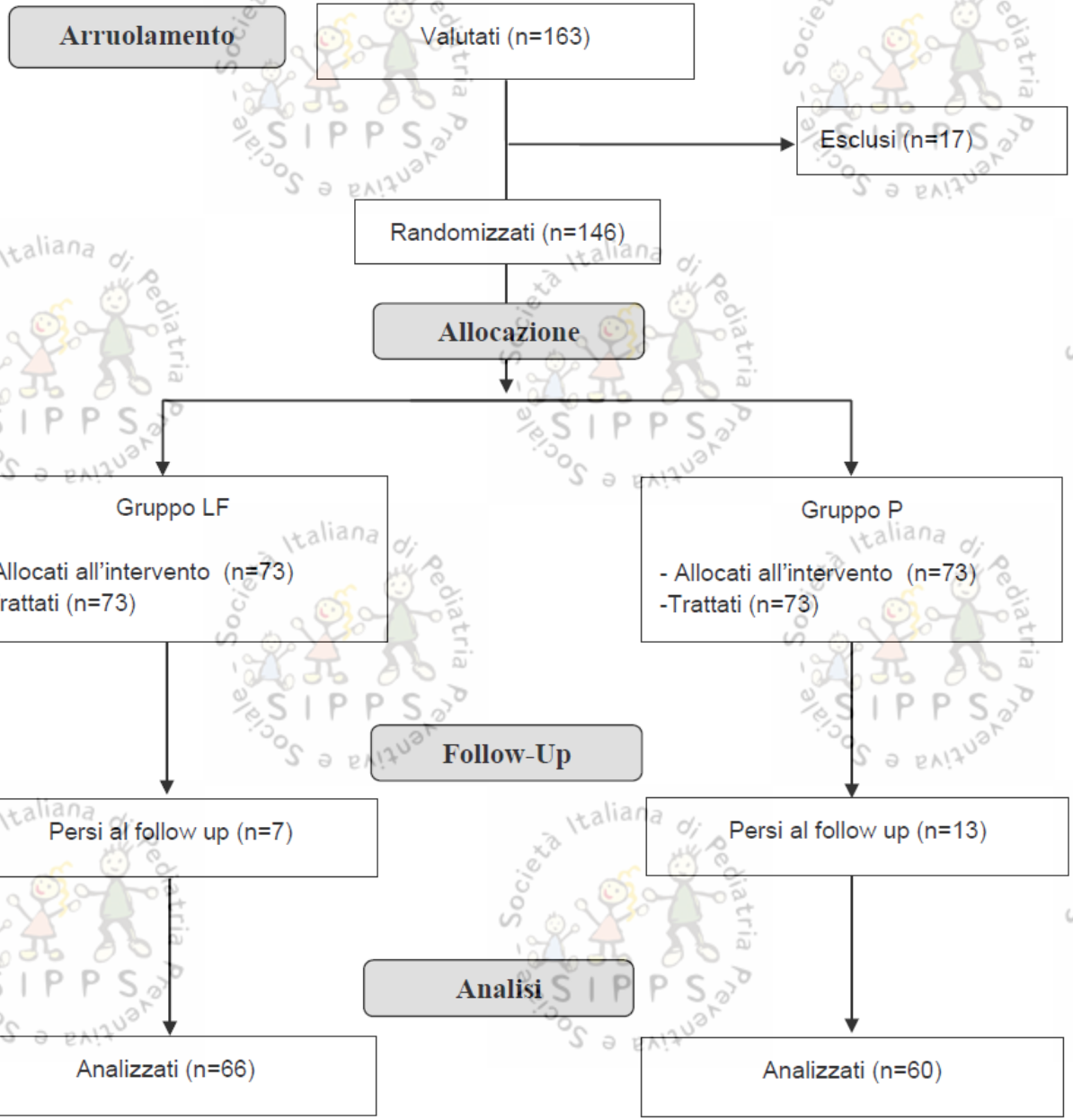
Stimolazione dei meccanismi di difesa immunologici e non-immunologici

EFFICACIA DI LATTE FERMENTATO CON *L.PARACASEI* CBA-L74 SULLA COMPARSA DI INFEZIONI GASTROINTESTINALI E RESPIRATORIE IN BAMBINI SCOLARIZZATI: STUDIO MULTICENTRICO RANDOMIZZATO



Centri partecipanti

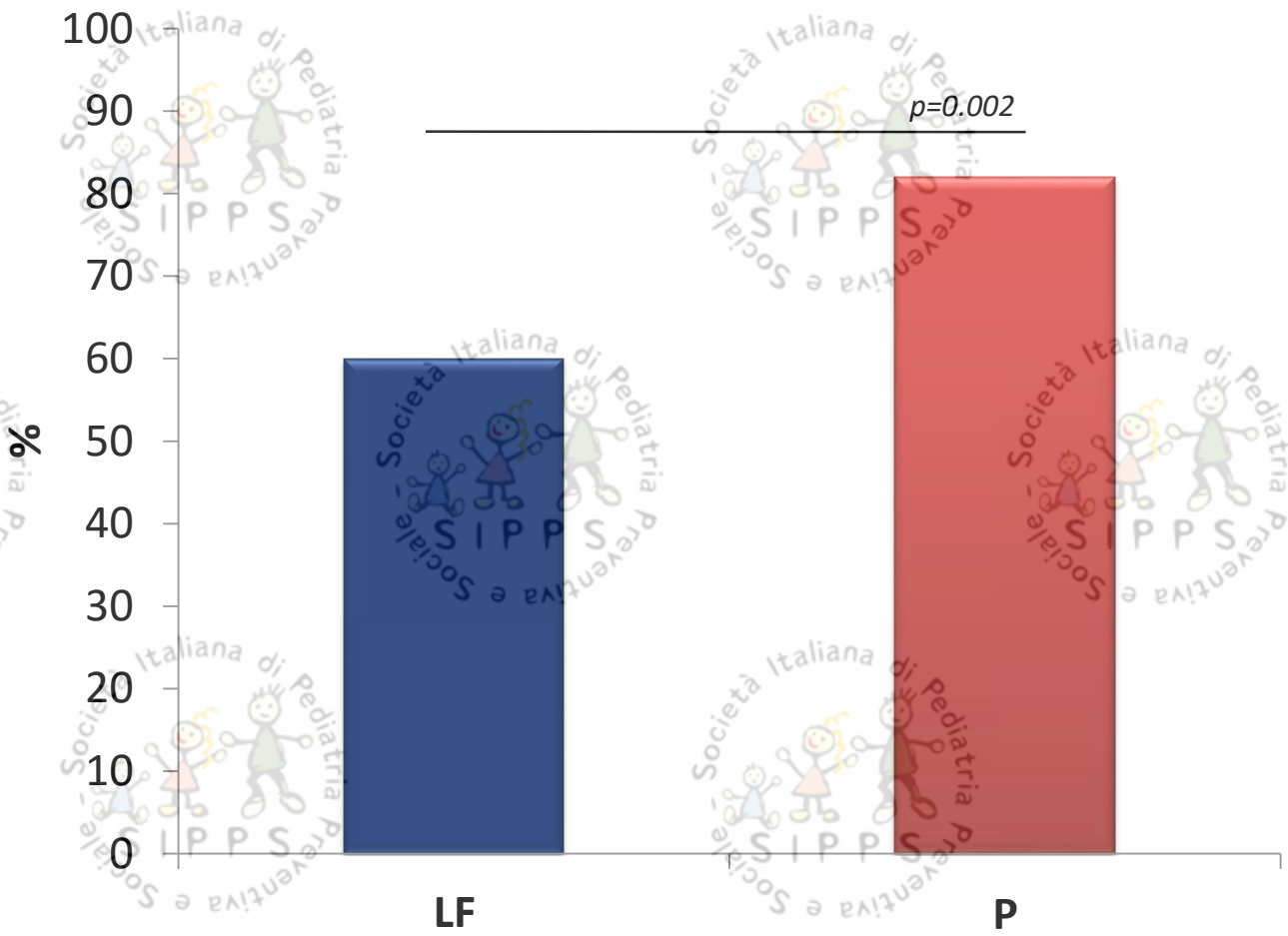
Flusso dei soggetti durante le fasi dello studio



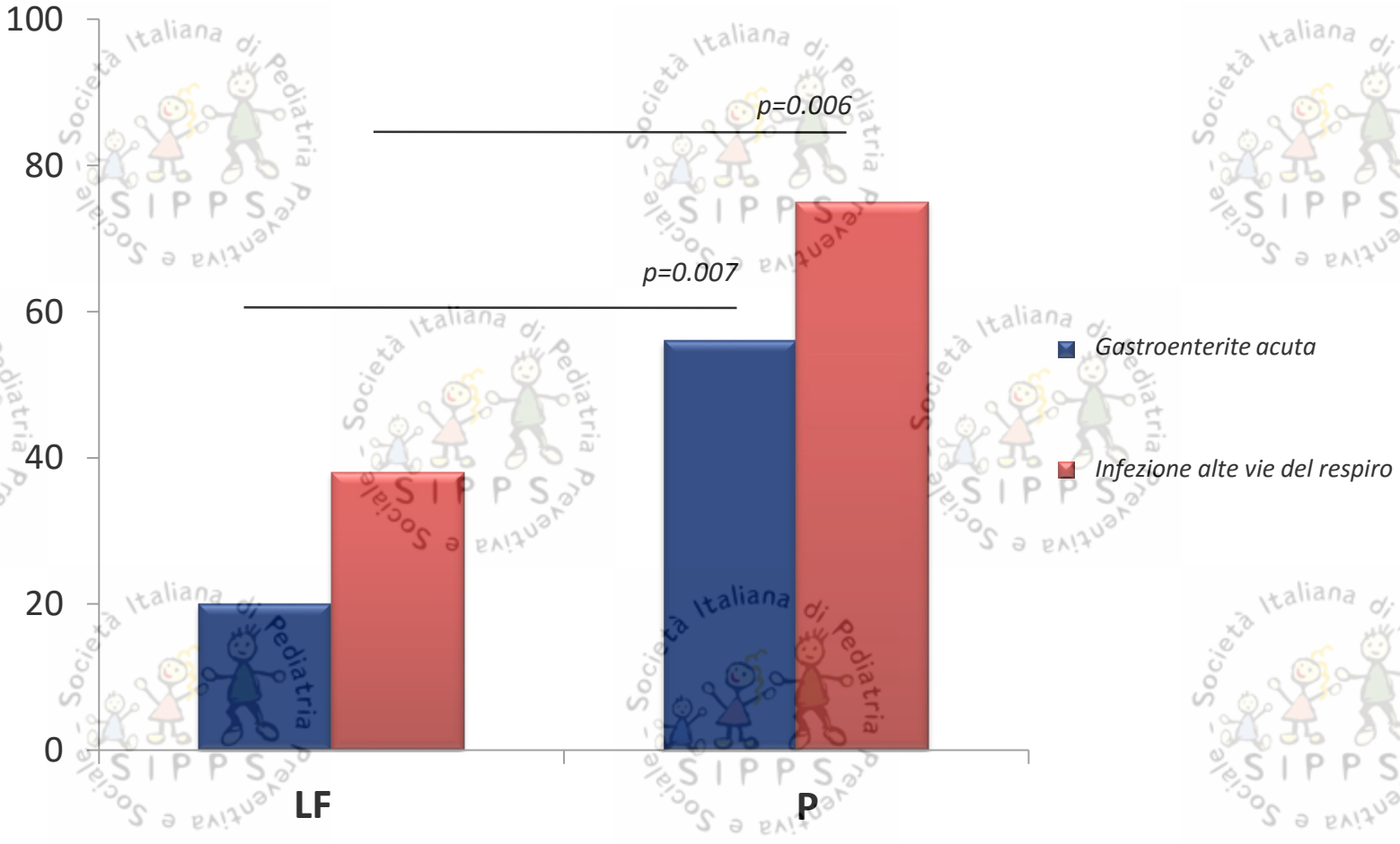
Caratteristiche simili delle due popolazioni di studio all'arruolamento

	LF	P
	n=73	***n=73***
Maschi, n (%)	39 (53.4)	45 (61.6)
Età, mesi (±DS)	32.5 (9.7)	33.7 (8.6)
Peso, kg (±DS)	14.8 (3.2)	15 (3)
Altezza, cm (±DS)	92 (8.1)	94 (7.6)
Allattamento materno, n (%)	50 (68.5)	41 (56.2)
Durata dell'allattamento, mesi (±DS)	7.1 (6)	7 (8.5)
Età alla scolarizzazione, mesi (±DS)	23.2 (9)	25.9 (8.5)
Fratelli, n (%)	60 (82.2)	52 (71.2)
N. di fratelli, (±DS)	1.4 (0.6)	1.4 (0.6)
Fumo passivo, n (%)	28 (38.4)	30 (41.1)

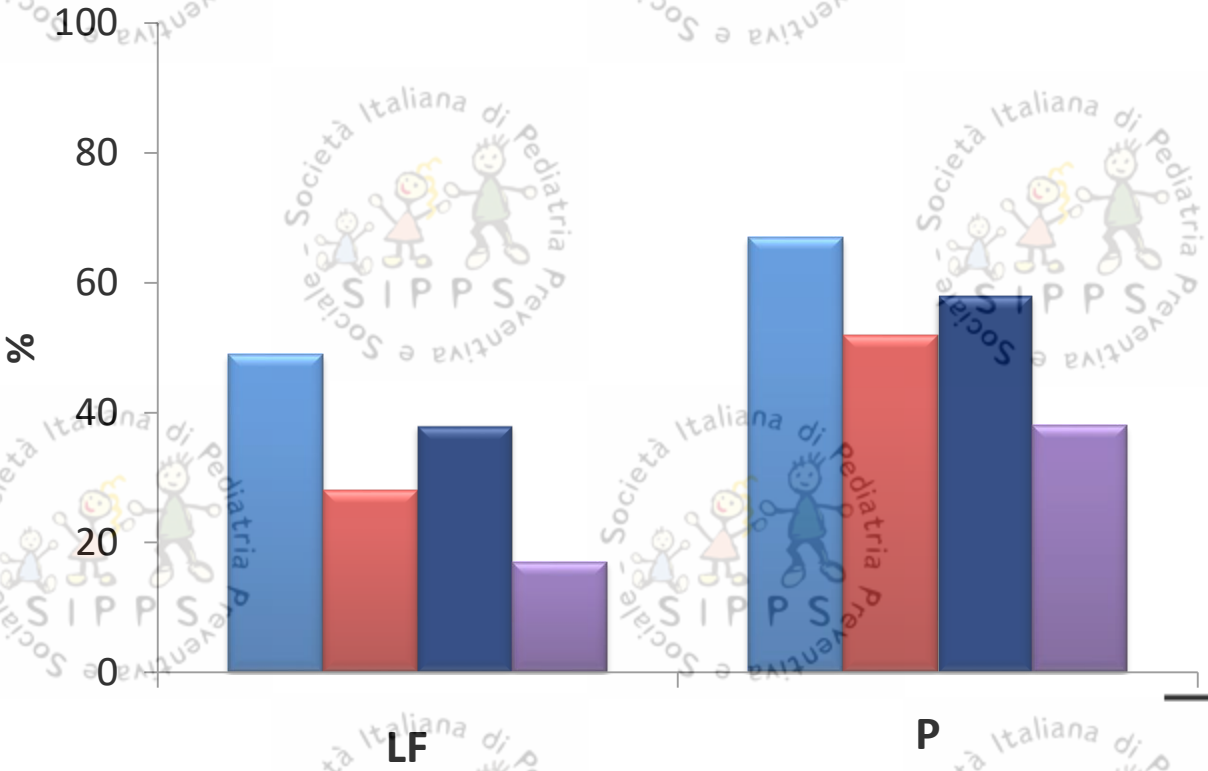
Soggetti che hanno presentato ≥1 episodio infettivo *(analisi ITT)*



Soggetti che hanno presentato ≥1 episodio di gastroenterite acuta/infezione alte vie del respiro



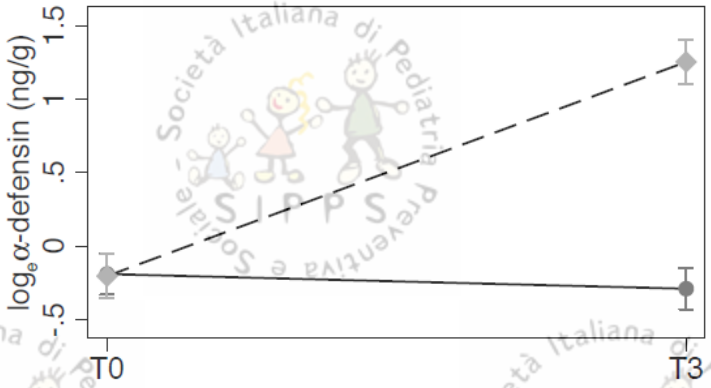
Utilizzo di farmaci

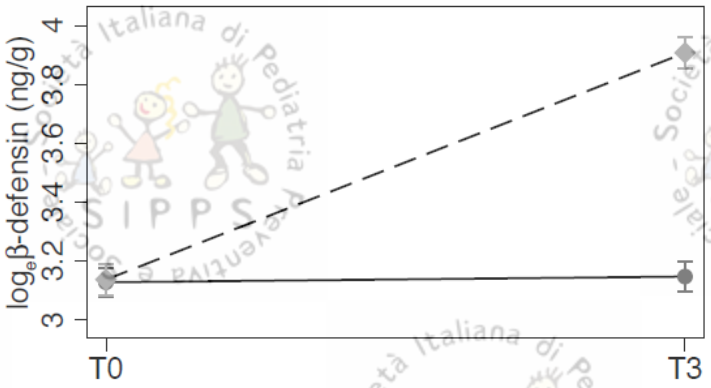


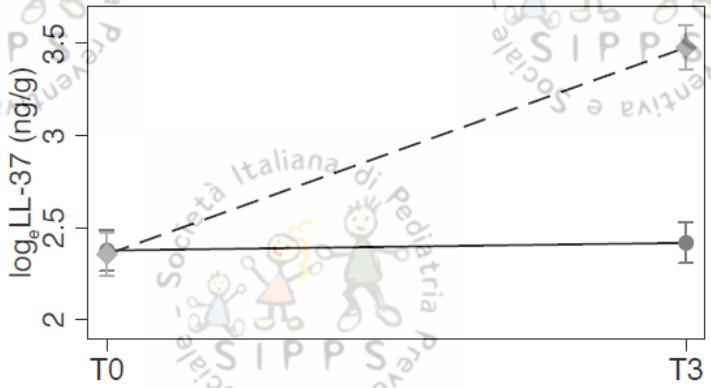
- *Soggetti che hanno assunto almeno 1 farmaco*
- *Soggetti che hanno assunto almeno 1 antibiotico*
- *Soggetti che hanno assunto almeno 1 antipiretico*
- *Soggetti che hanno assunto almeno 1 steroide*

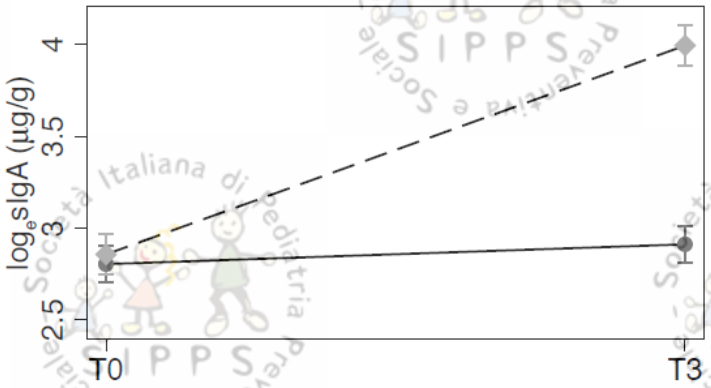
	p
Almeno 1 farmaco	*0.019*
Antibiotici	*0.03*
Antipiretici	*0.044*
Steroidi	*0.027*

Stimolazione dell'immunità innata ed acquisita dopo 3 mesi di trattamento









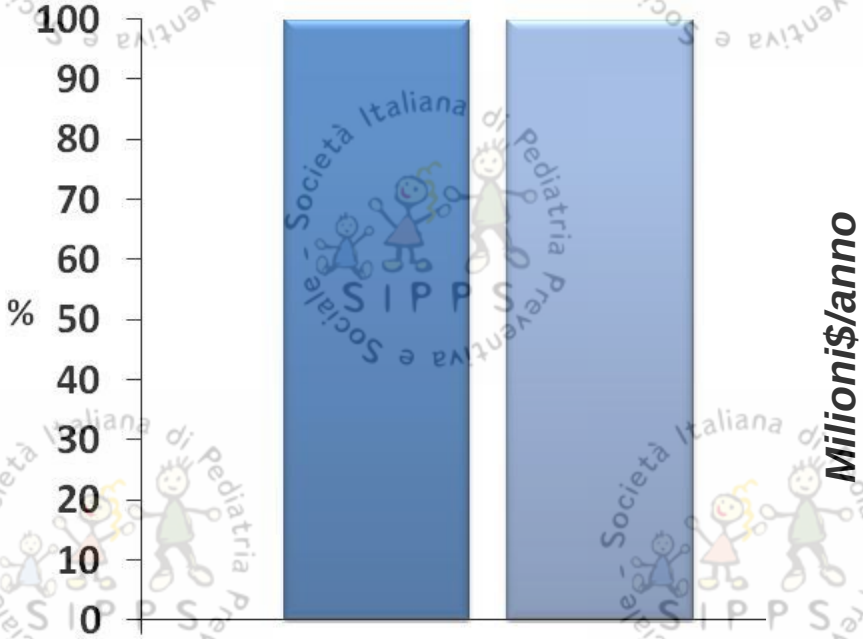
LF

P

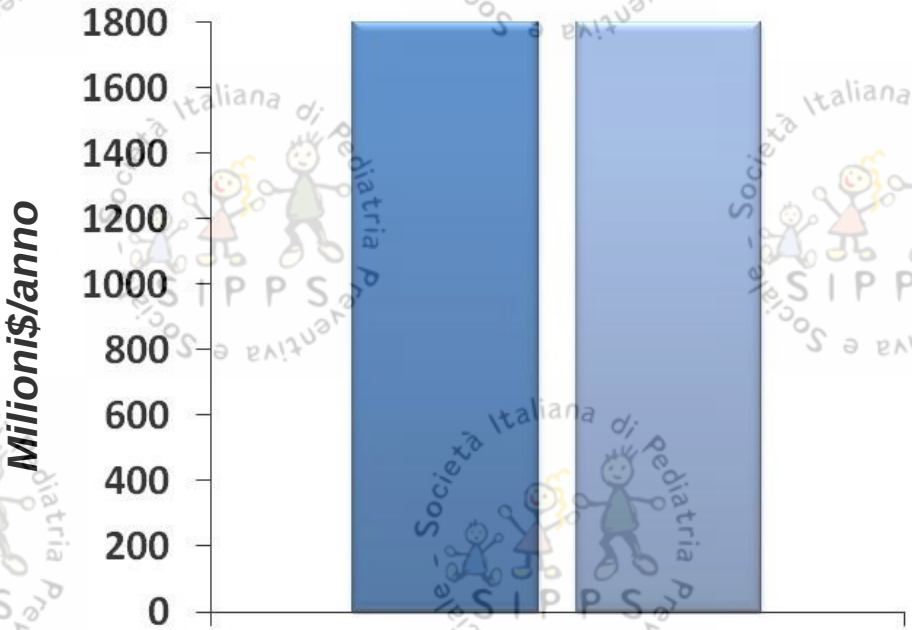
Punti di forza degli alimenti funzionali fermentati

- Contengono probiotici di origine umana
- Contengono probiotici inattivati
- Nessun rischio di traslocazione batterica
- Elevata facilità di conservazione e trasporto
- Elevata riproducibilità (monitoraggio preciso del processo tecnologico)

L’impatto



Incidenza infezioni



Costi stimati

LF

RF

- Spesa sanitaria per consumo antibiotici in Italia nel 2015 in età : **15.661.863 €**
- Utilizzo di latte fermentato con CBA-L74 riduce del 75% l'utilizzo di antibiotici

Spesa per consumo di antibiotici: 3.915.715 €

Risparmio di € 11.746.397

ISTAT 2015

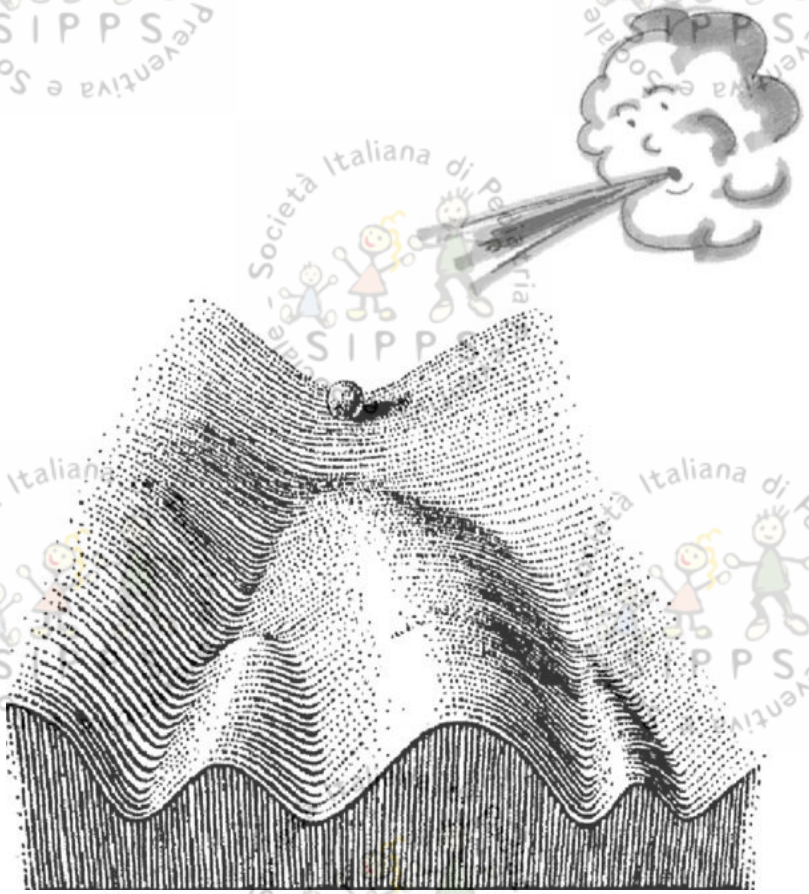
Senato della Repubblica

CONFERENZA STAMPA
"Ricerca e Nutrizione"

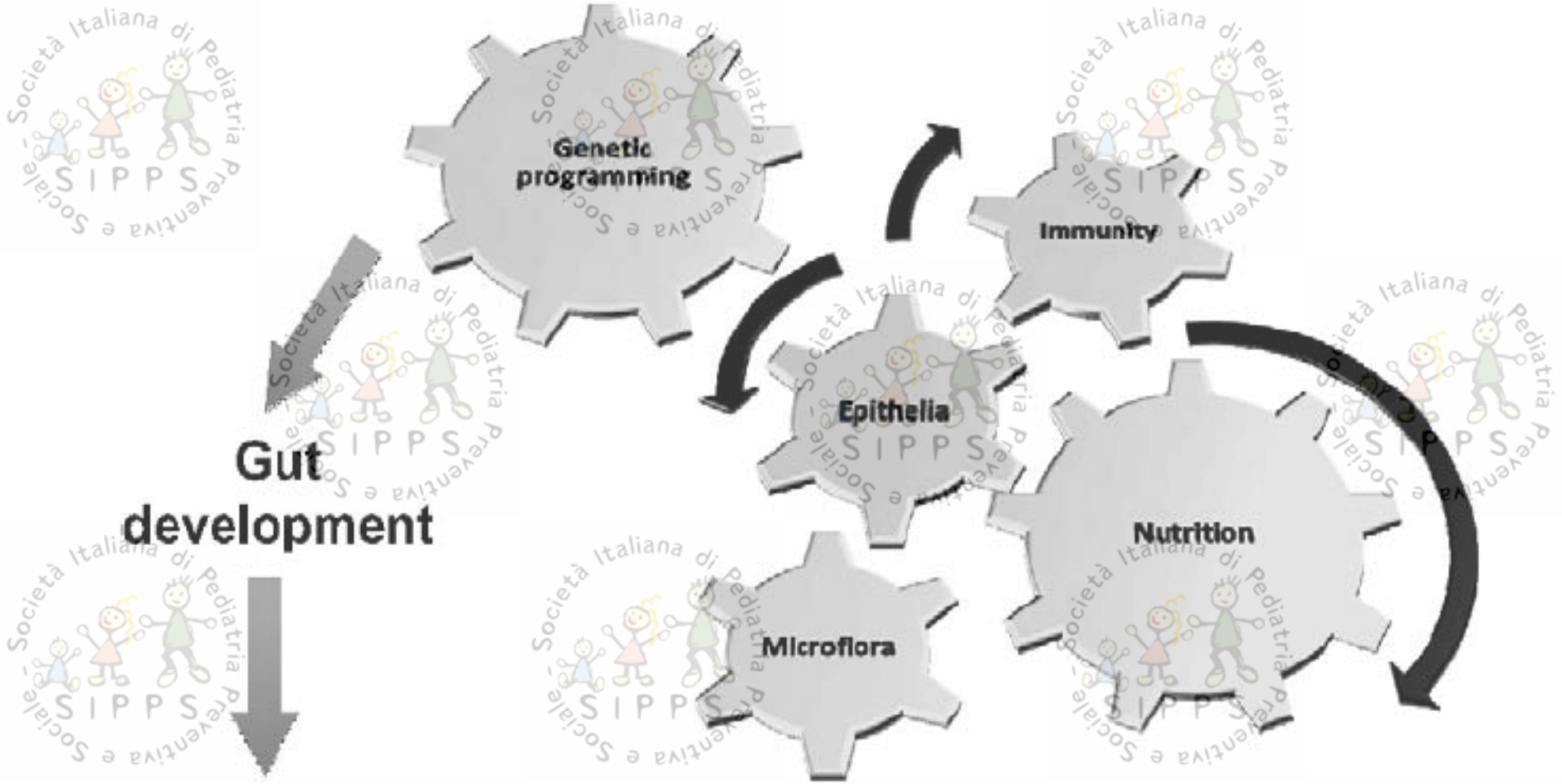
13 Aprile 2016
Ore 11:45

Senato della Repubblica
Sala "Caduti di Nassirya"
Piazza Madama, Roma

NUTRIZIONE EPIGENETICA SALUTE DEL BAMBINO



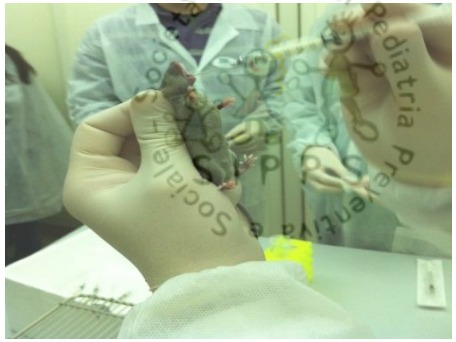
Nutrition may viewed as the wind blowing over the epigenetic landscape



Berni Canani R, et al. The nutritional modulation of the evolving intestine. J Clin Gastroenterol 2008,

L’attività di ricerca continua…..

- Attività biologiche/meccanismi di azione
- Caratterizzazione molecolare (lipidi, nucleotidi, piccoli peptidi termostabili, ecc)
- Nuove applicazioni cliniche
- Trial clinici randomizzati controllati



Team

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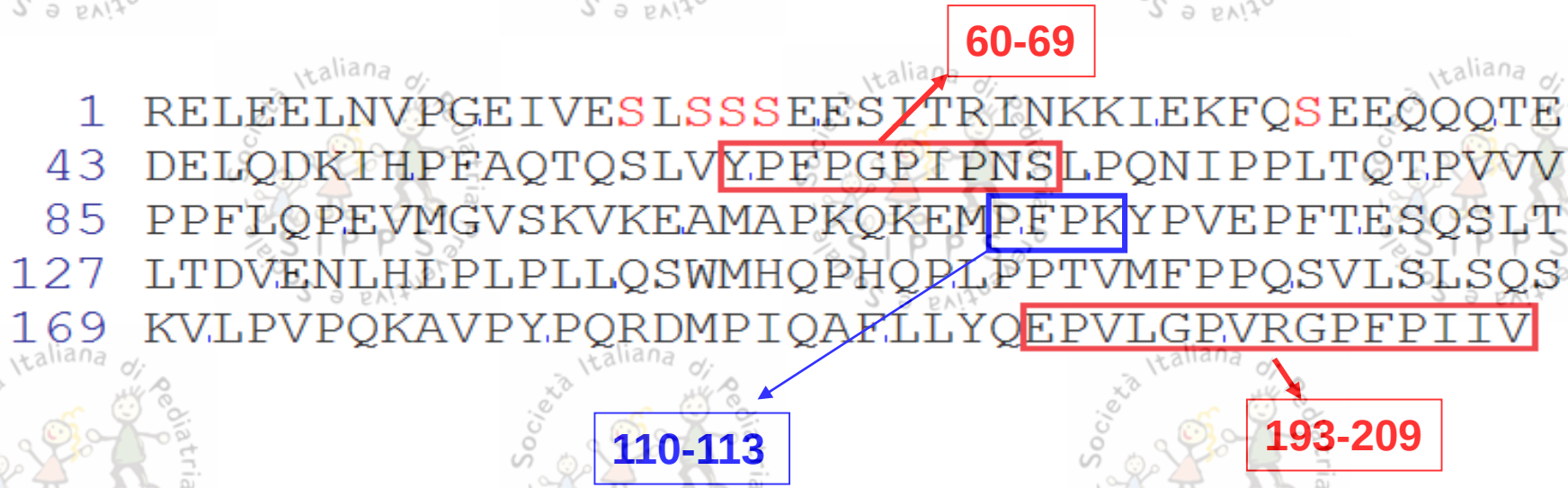
S.Guandalini, T. Patton

Exploring immunoregulatory mechanisms elicited by EHCF and EHCF+LGG

1. Beta casein peptides action	CASEIN HYDROLYSIS COULD GENERATES BIOACTIVE IMMUNOMODULATORY PEPTIDES

EHCF peptides that survive hydrolysis derive from boxed regions of β-CN

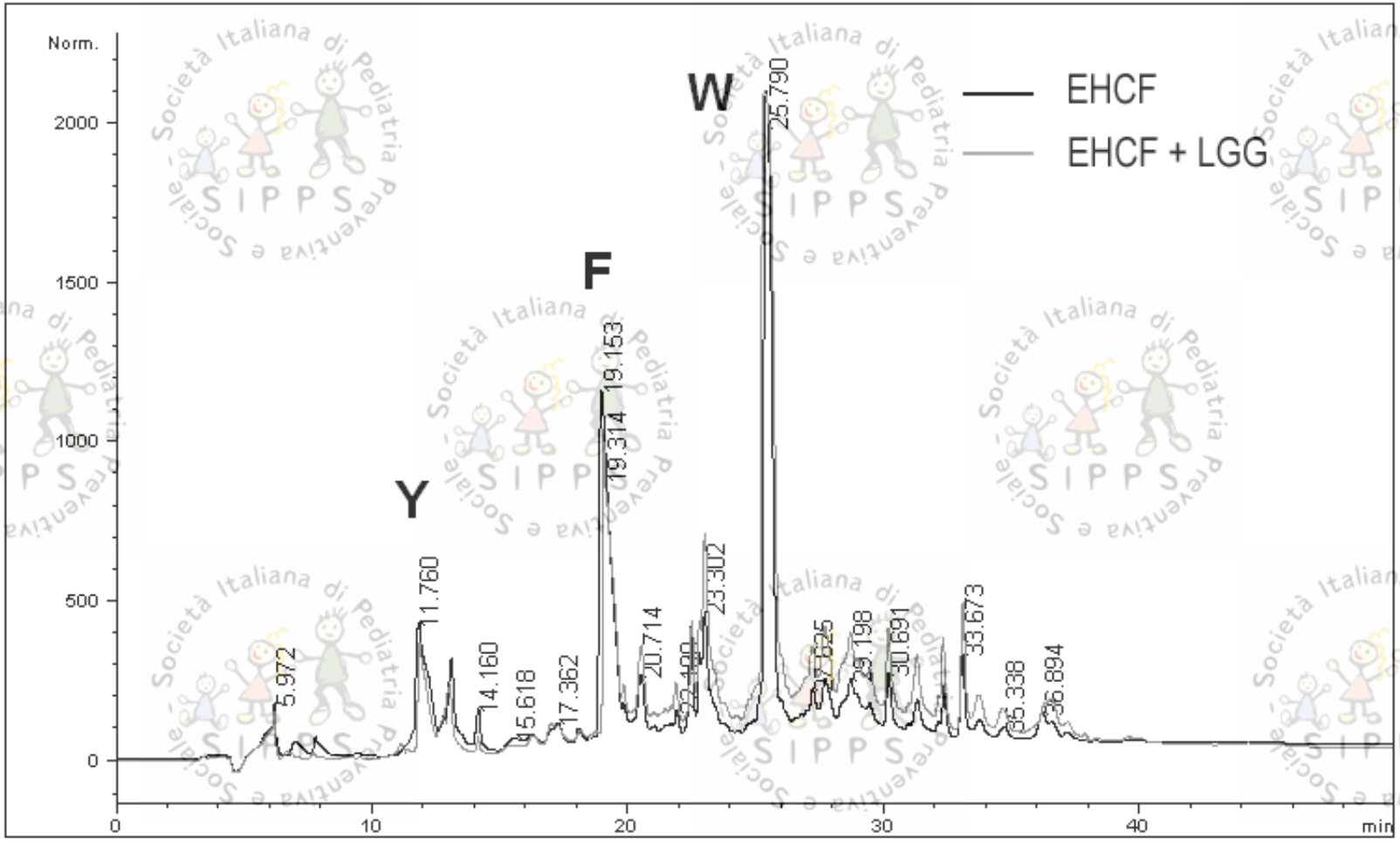


- ✓ Immunomodulatory activity of these domains is well recognized
- ✓ Pro-Phe-Pro is the main "structural determinant" for this activity

Gill HS, et al. Br J Nutr 2000
Sandrè C, et al. J Nutr 2001
Otani H, et al. Animal Science J 2004
Bonomi et al. Int Dairy J 2011
Kiewiet MB et al. Pediatr Allergy Immunol 2015

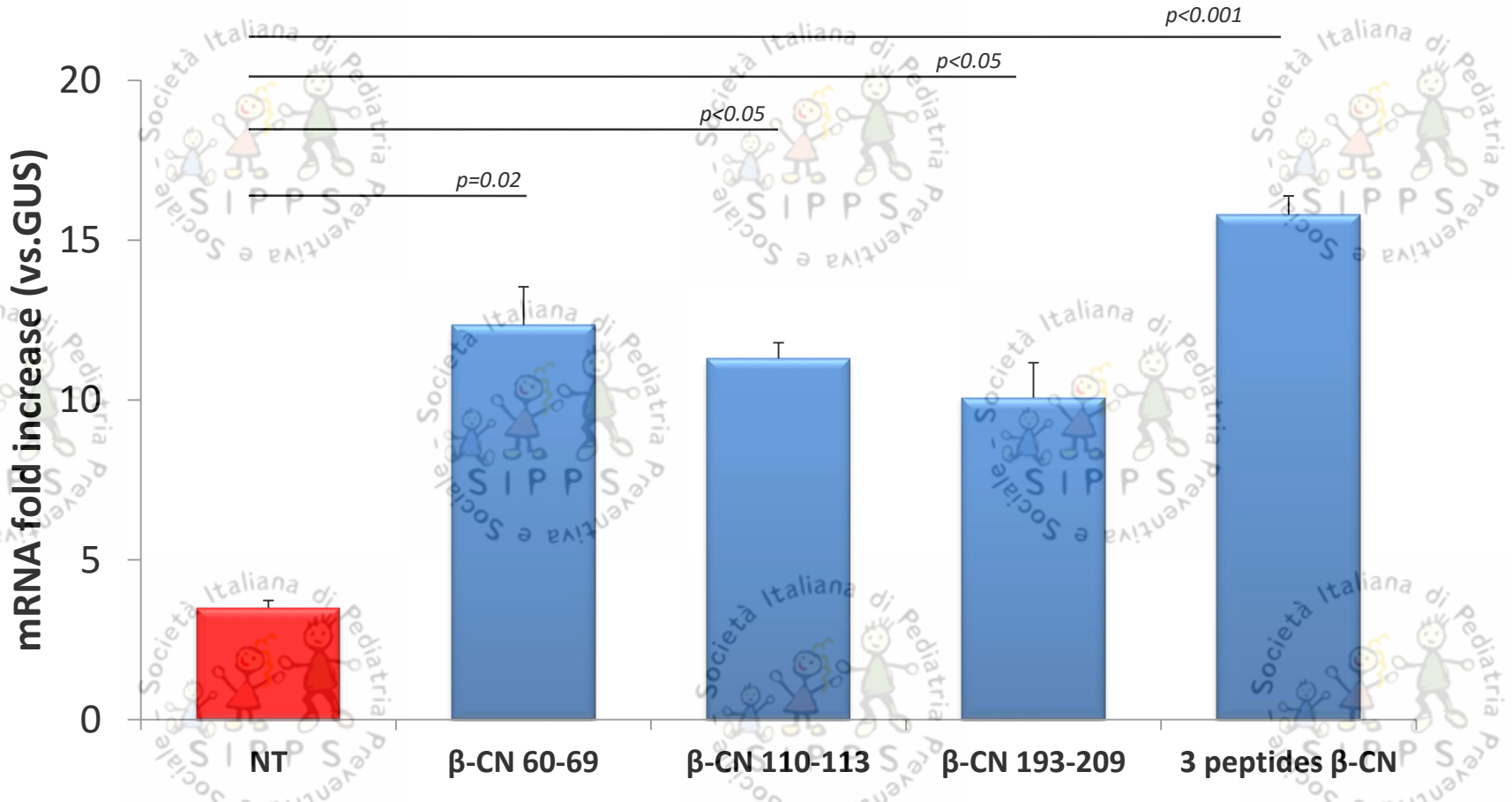
These peptides remain intact in the presence of LGG

Characterization of peptides by RP-HPLC



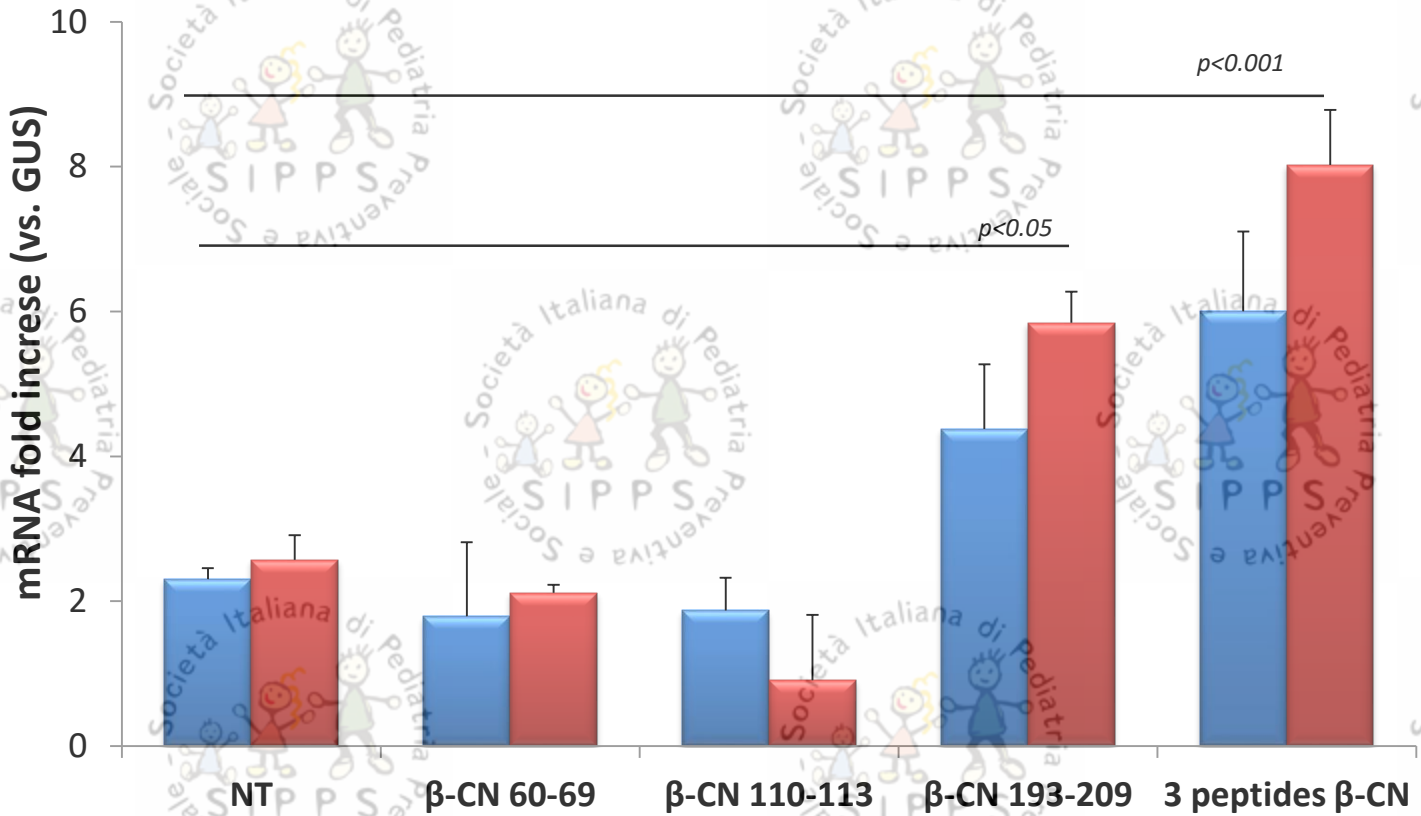
Aitoro R. et al. 2016 submitted

Direct effect of β–CN peptides on human enterocytes: mucus production (Muc 2)



Berni Canani R data on file

Direct effect of β–CN peptides on human enterocytes: tight junction proteins expression

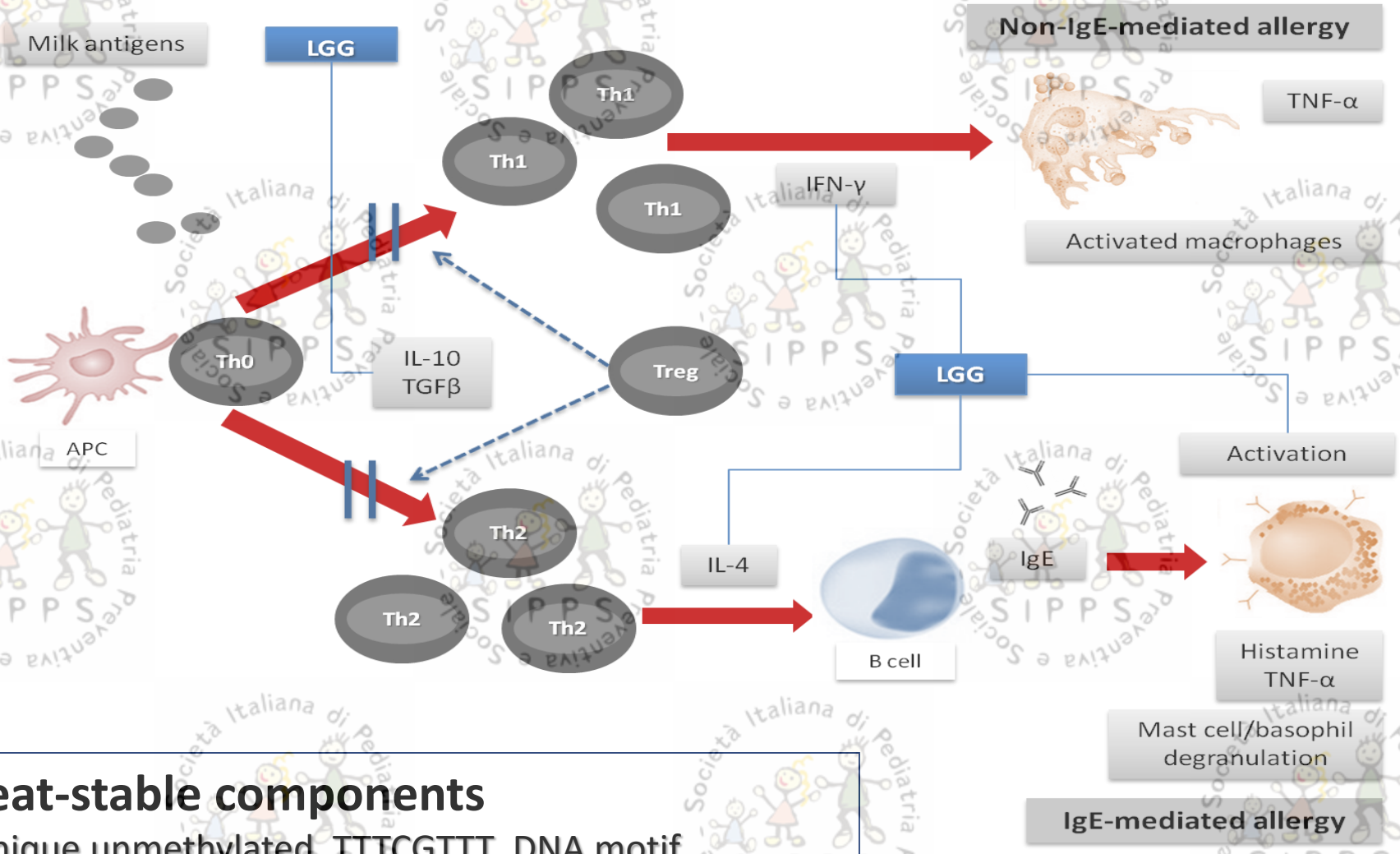


Berni Canani R data on file

Exploring immunoregulatory mechanisms elicited by EHCF and EHCF+LGG

1. β-casein peptides action	CASEIN HYDROLYSIS COULD GENERATES BIOACTIVE IMMUNOMODULATORY PEPTIDES
2. L.rhamnosus GG action	BACTERIA COMPONENTS ELICIT IMMUNOREGULATORY EFFECTS

LGG counteracts main CMA mechanisms



Heat-stable components

- Unique unmethylated TTTCGTTT DNA motif
- Lipoteichoic acid (LTA)
- Exopolysaccharides (EPS)

Maassen CBM et al. Vaccine 2000
Ghadimi D et al. Immunobiology 2008
Oksaharju A et al. WJG 2011
Segers ME et al. Microbial Cell Factors 2014
Berni Canani R, et al. Ben Microbes 2015

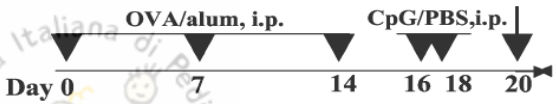
LGG

ID35 5'-ACTTTCGTTTTCTGCGTCAA-3'

Unique TTTCGTTT motif located at the 5' end of the ODN

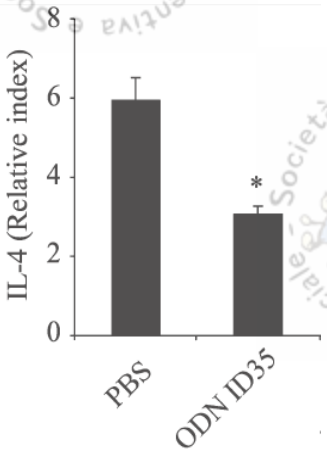
1µM

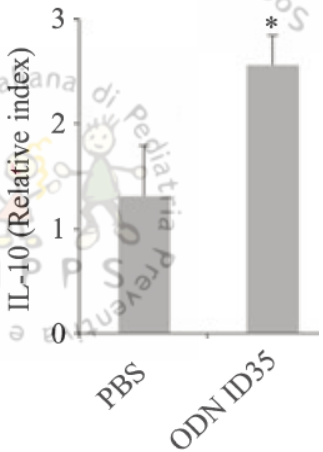
Sacrificed mice



***LGG* DNA**: 11.2 µg/ml

CpG ODN ID35: 1µM (0.225µg/ml)





Iliev ID et al., Scand J Immunol 2008
Iliev ID et al., Cellular Microbiology 2005
Klinman MD et al., PNAS 1996

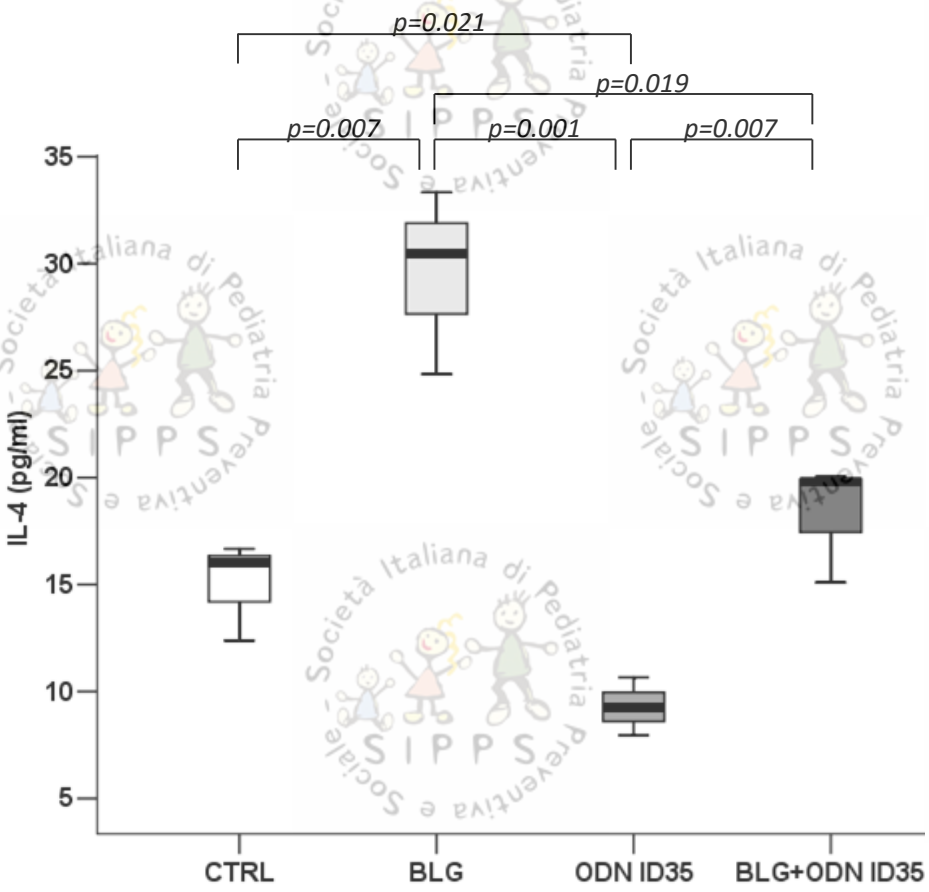
Children with challenge proven IgE-mediated CMA
All positive for SPT and specific serum IgE against β-lactoglobulin

PBMCs

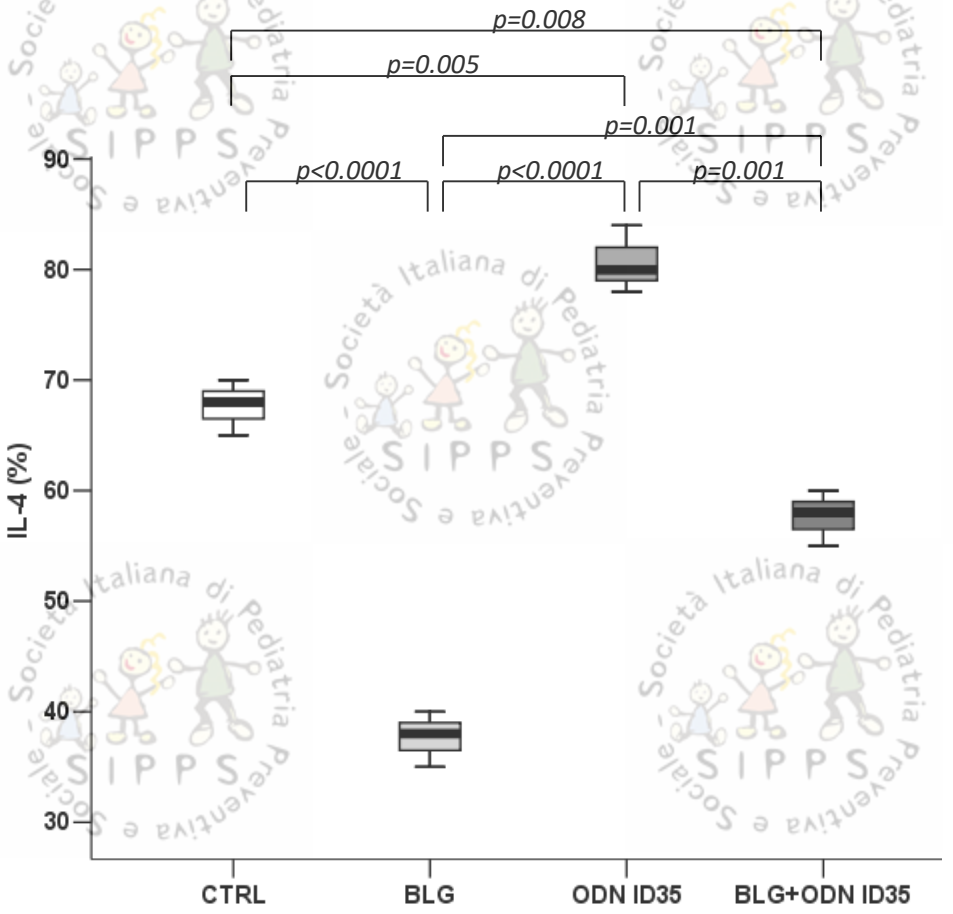
CTRL	BLG	ODN ID35	BLG +ODN ID35
	100 µg/ml	1 µM	1 µM

LGG DNA sequence ID35 inhibits IL-4 response of PBMCs from CMA children in only 24 h through an epigenetic mechanism

IL-4 concentration



IL-4 DNA promoter methylation rate



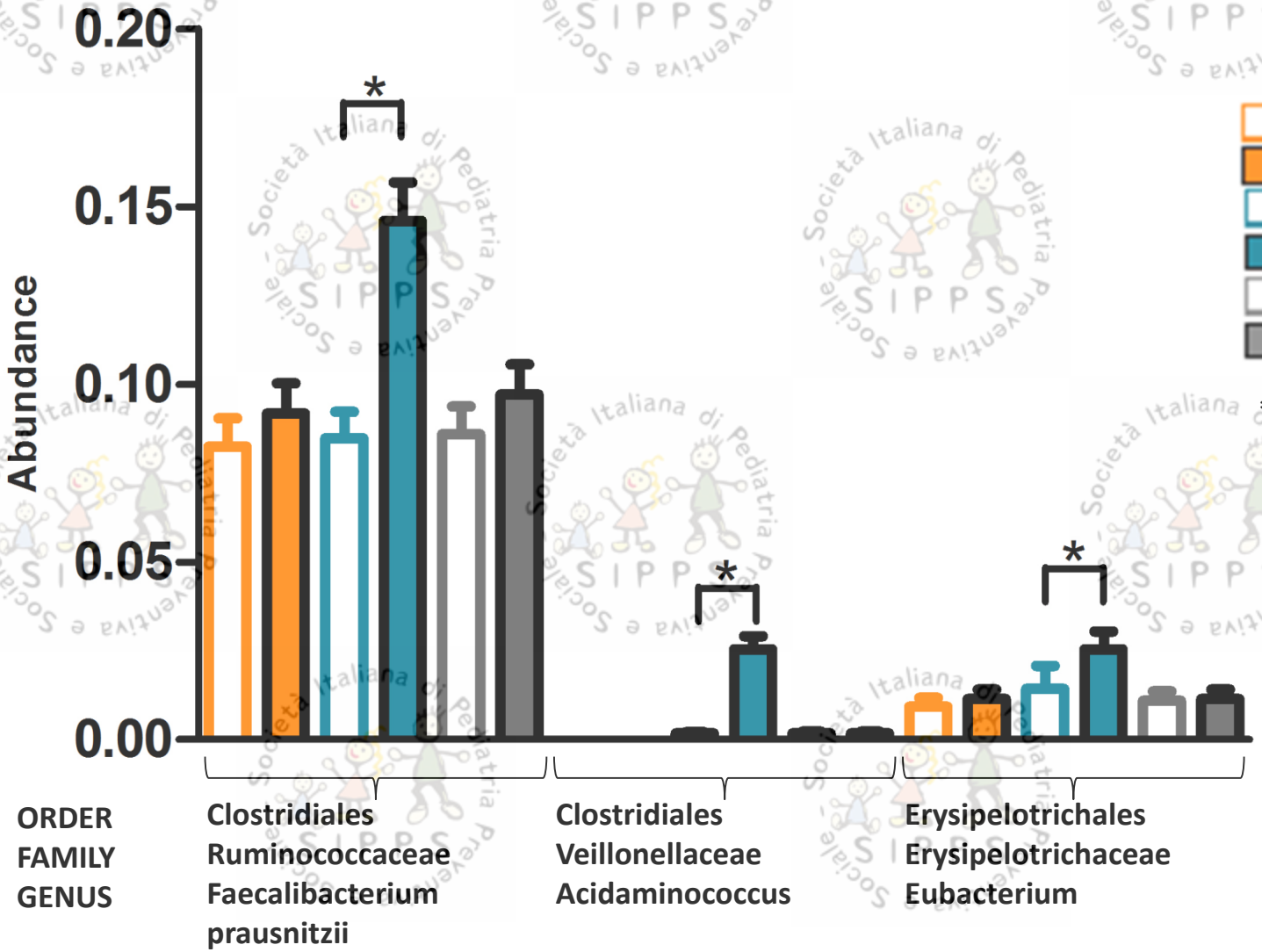
Berni Canani R data on file

Exploring immunoregulatory mechanisms elicited by EHCF and EHCF+LGG

1. β-casein peptides action	CASEIN HYDROLYSIS COULD GENERATES BIOACTIVE IMMUNOMODULATORY PEPTIDES
2. L.rhamnosus GG action	BACTERIA COMPONENTS ELICIT IMMUNOREGULATORY EFFECTS
2. Gut microbiota action	POSITIVE GUT MICROBIOTA SHAPING

LGG expands protective bacteria in the gut lumen of CMA infants

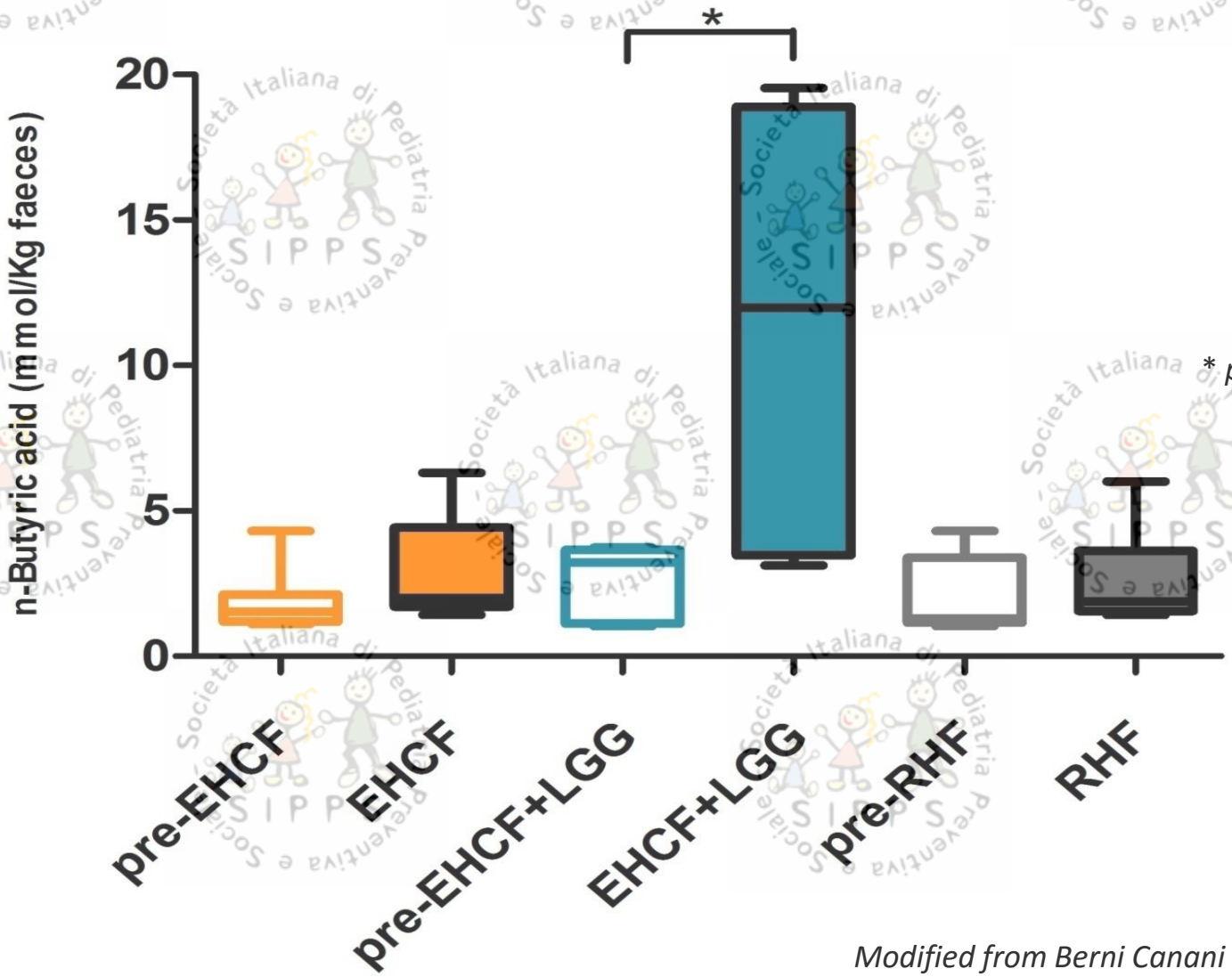
The ISME Journal
Multidisciplinary Journal of Microbial Ecology
The ISME Journal TOP TEN



Modified from Berni Canani R. et al, ISME journal 2015

LGG expands butyrate-producing bacteria in CMA infants

The ISME Journal
Multidisciplinary Journal of Microbial Ecology
The ISME Journal TOP TEN



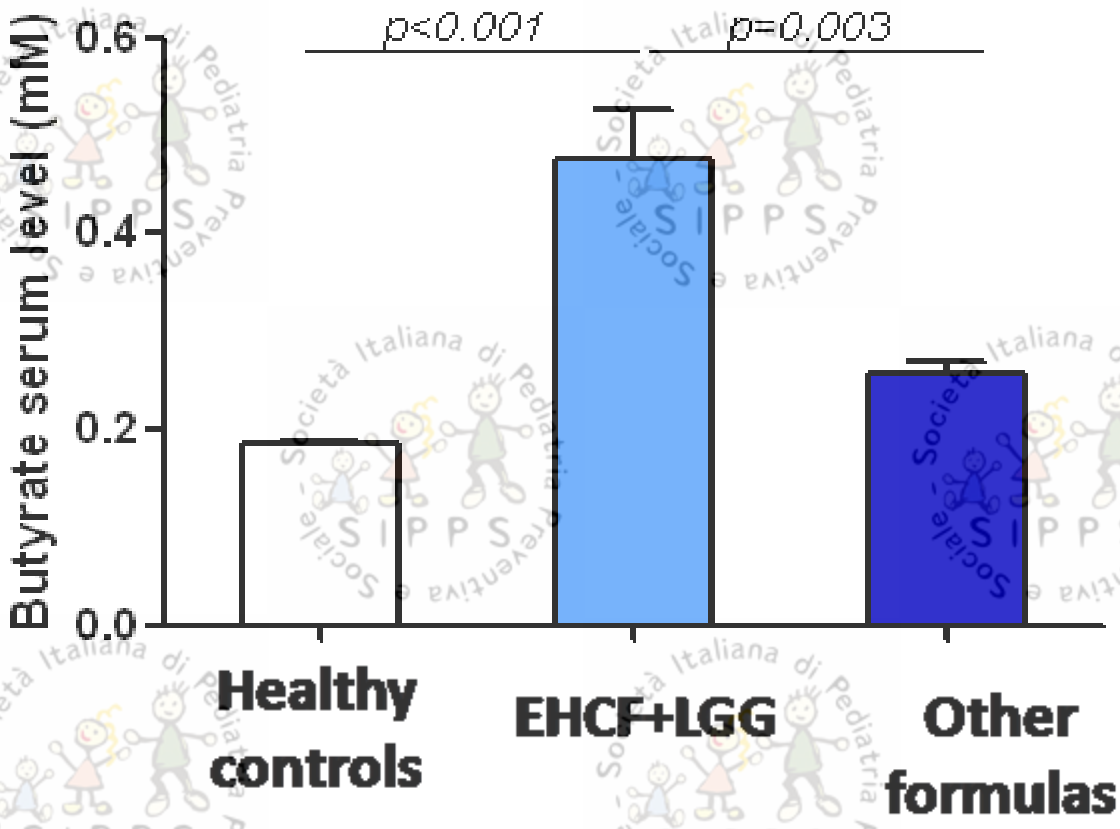
* p = <.005

Modified from Berni Canani R. et al, ISME journal 2015

Exploring immunoregulatory mechanisms elicited by EHCF and EHCF+LGG

1. β-casein peptides action	CASEIN HYDROLYSIS COULD GENERATES BIOACTIVE IMMUNOMODULATORY PEPTIDES
2. L.rhamnosus GG action	BACTERIA COMPONENTS ELICIT IMMUNOREGULATORY EFFECTS
3. Gut microbiota action	POSITIVE GUT MICROBIOTA SHAPING
4. Direct butyrate action	BUTYRATE AS MOLECULAR PLAYER INVOLVED IN THE BENEFICIAL INTERACTION BETWEEN BACTERIA AND IMMUNE SYSTEM

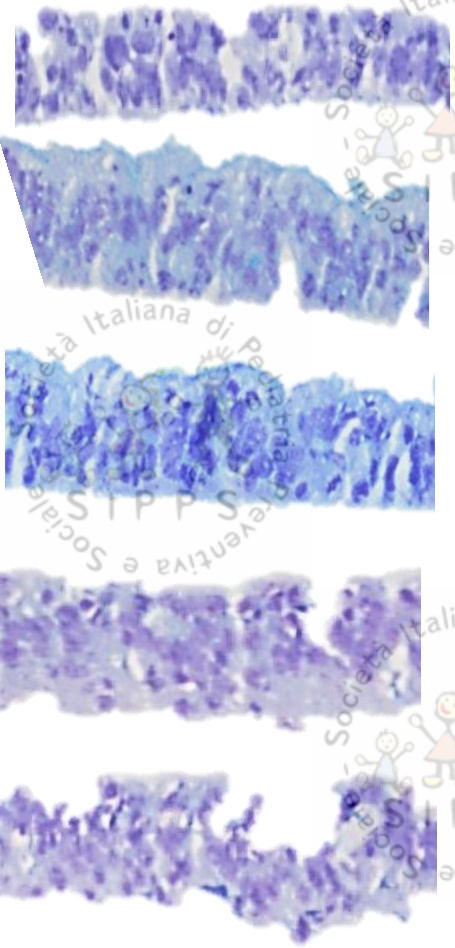
IgE-mediated CMA children treated with EHCF+LGG show higher butyrate serum levels



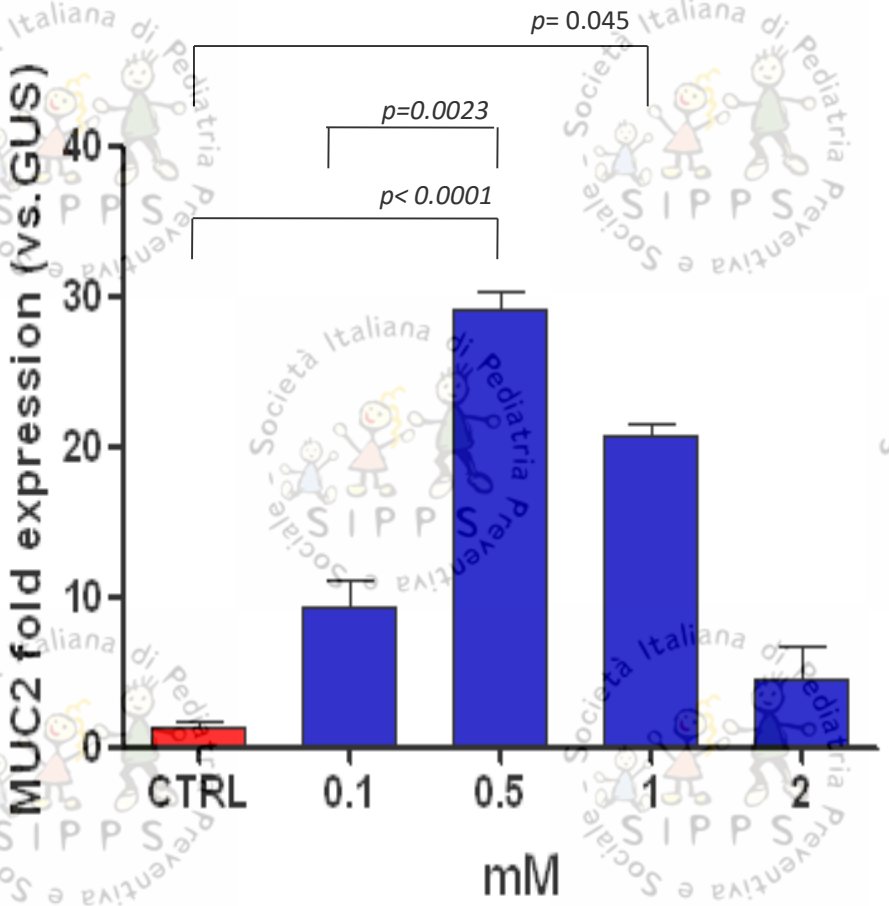
Butyrate concentration in systemic circulation = 0.1 to 1.2 mM
(*Cummings JH et al. Gut 1987)*

Berni Canani R data on file

Butyrate stimulates mucus production and MUC2 expression in human enterocytes



Dose	Thickness (µm)
CTRL	-
0.1mM	3±2
0.5mM	5±2
1mM	-
2mM	-

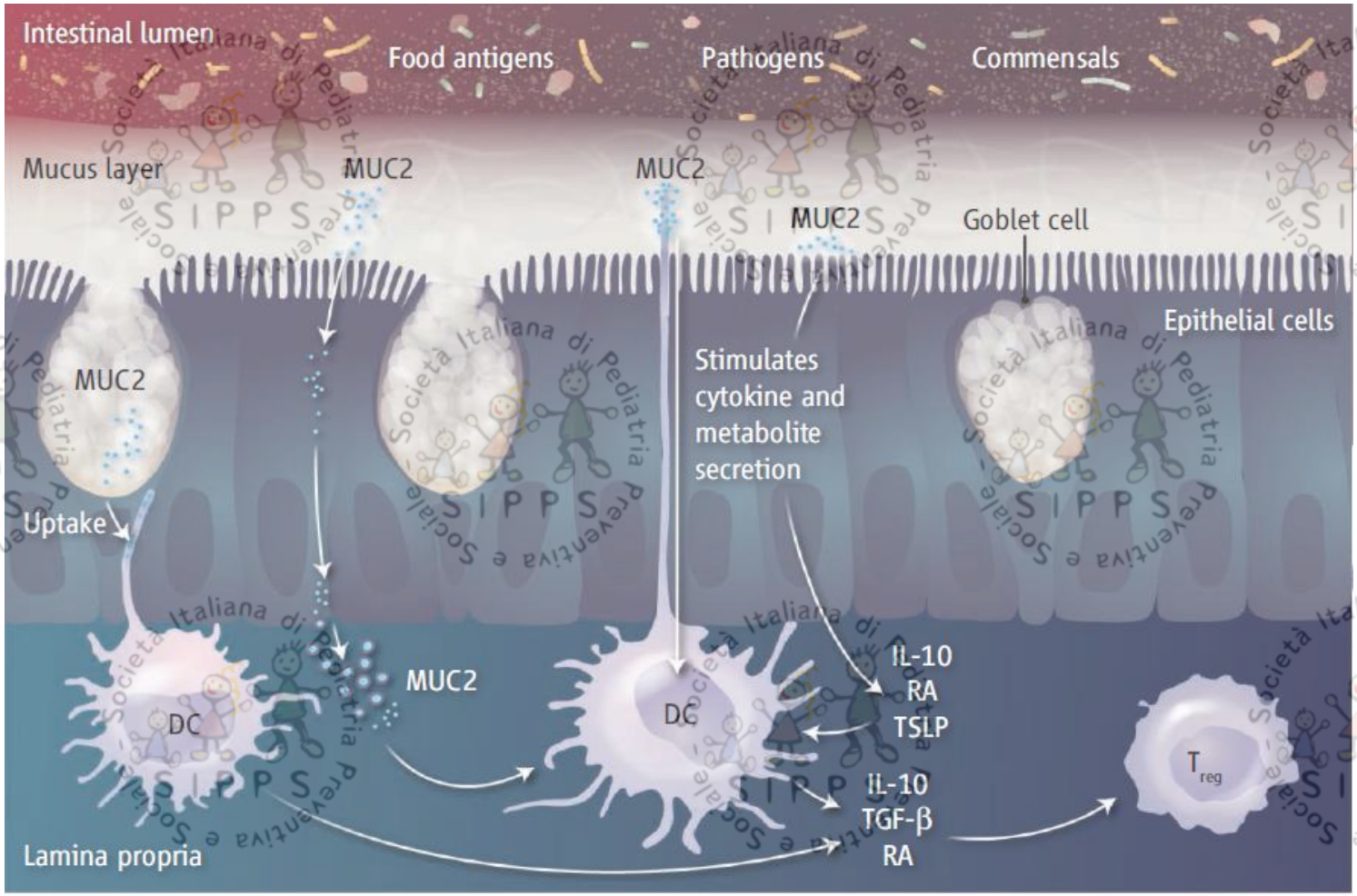


Berni Canani R data on file

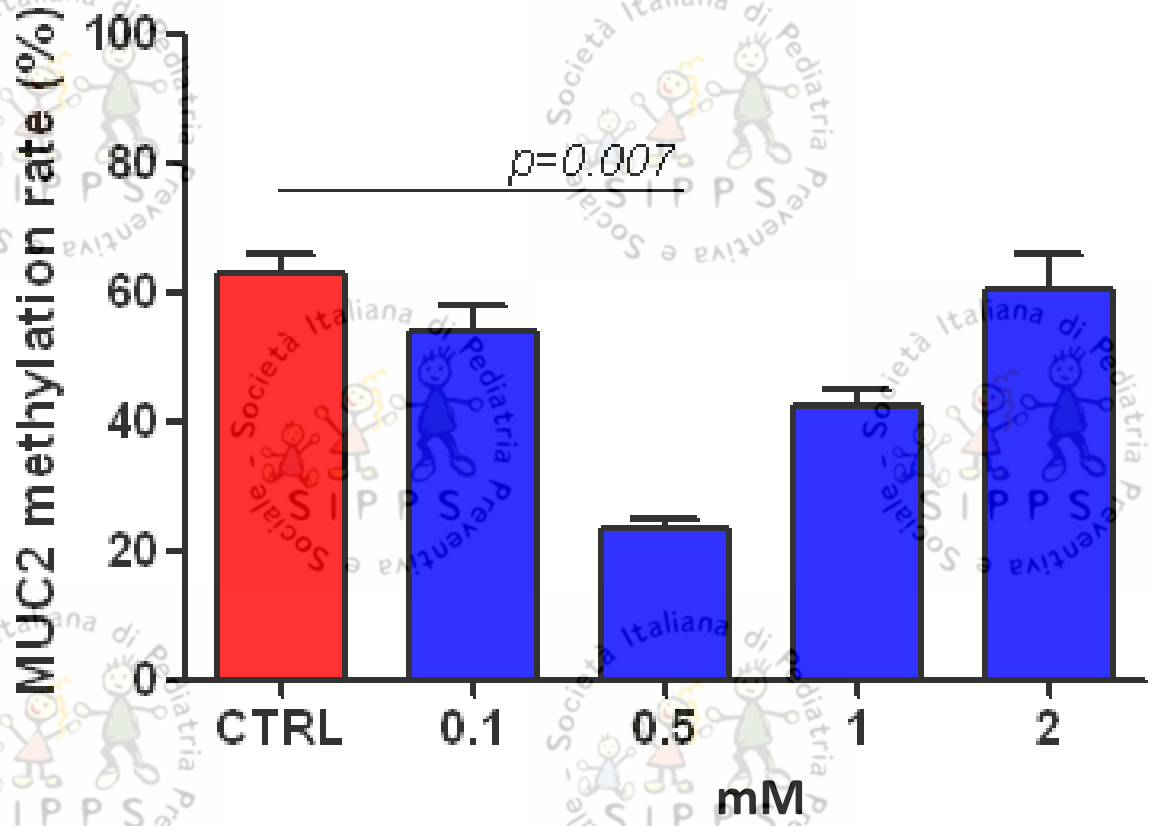
Mucus Coat, a Dress Code for Tolerance

Yasmine Belkaid and John Grainger

Mucus is a determinant of gut immune specification and immune tolerance.



Butyrate induces MUC2-DNA demethylation in human enterocytes



Berni Canani R data on file

Conclusions

- CMA persistence and severity climbed steadily during past 2 decades under the pressure of negative gene-environment interaction leading to immune-system dysfunction mediated at least in part by epigenetic mechanisms
- We are exploring the possibility to counteract this pathway modulating the interaction among dietary agents, gut microbiota, epigenetic mechanisms and immune system
- These effects lead to a positive impact on oral tolerance acquisition and on long term protection against other atopic manifestations in children with CMA

Team

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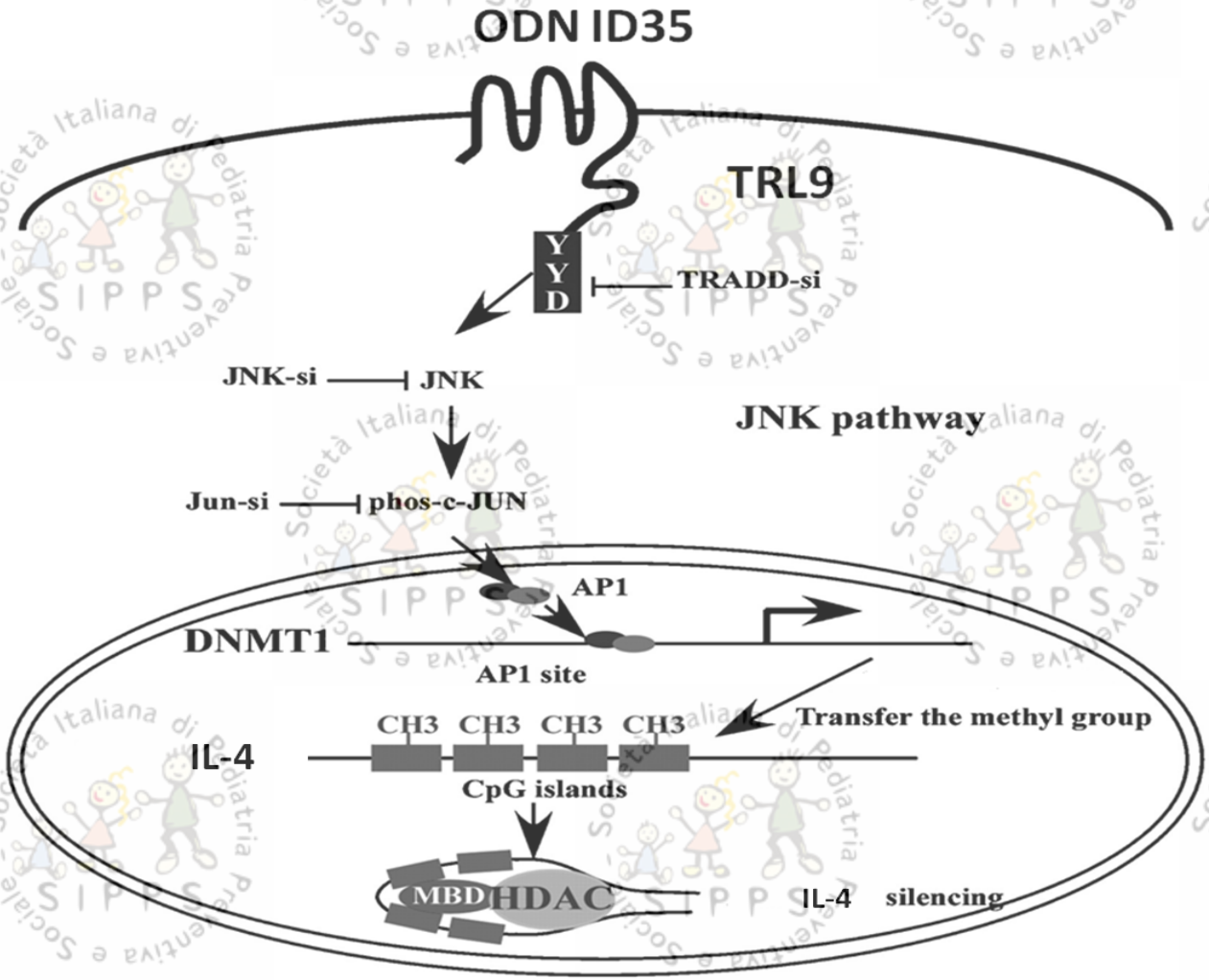
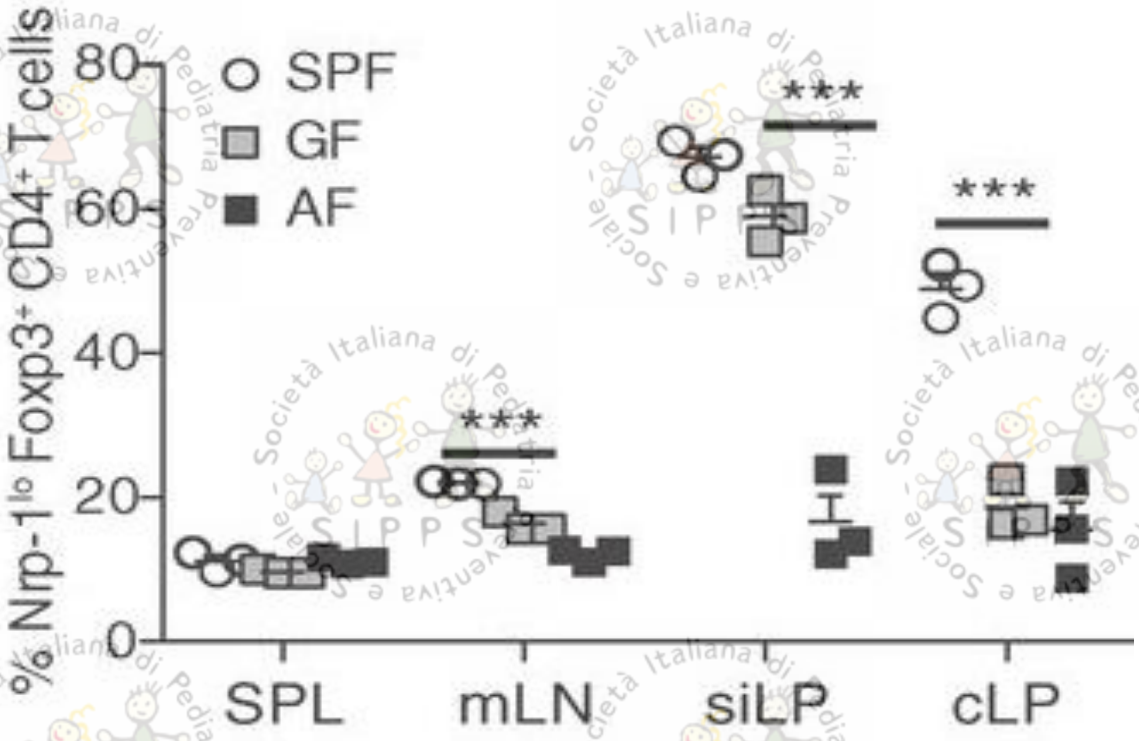
Comer Children's Hospital – Pediatric GI Unit
S.Guandalini, T. Patton

Specific bacterial DNA sequences drives epigenetic effects in human cells: intracellular pathway



Modified from Klinman DM, et al. *Drug News Perspect* 2000.

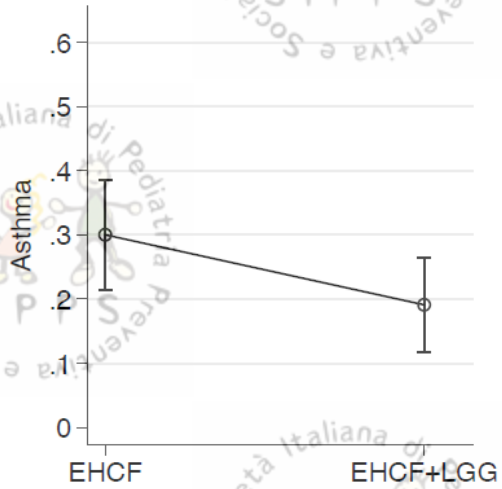
Two crucial players are involved in oral tolerance: dietary factors and gut microbiota

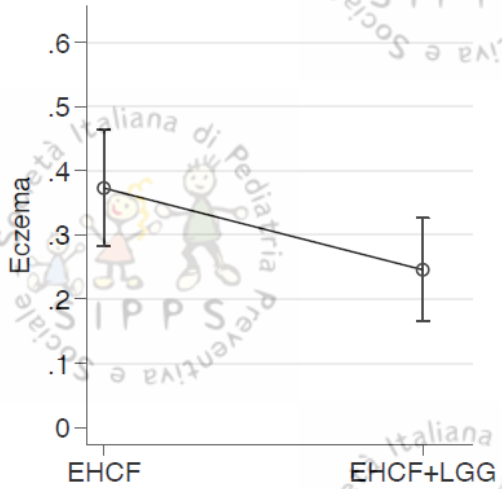


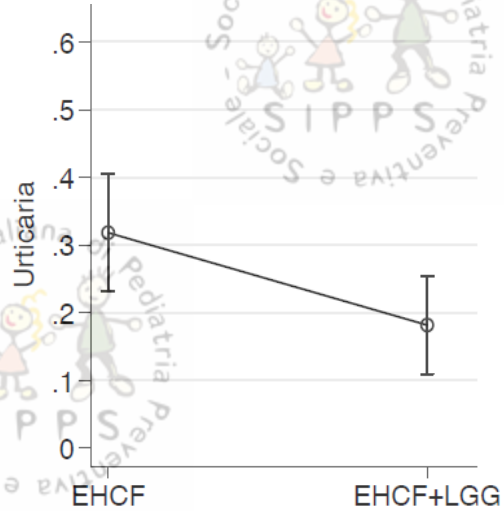
Depletion of macromolecules from the diet inhibits Treg activation in small intestine

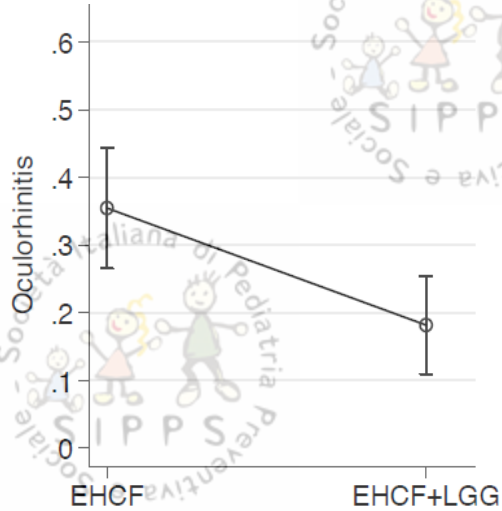
KS Kim et al. Science, 2016

Rate of subjects experienced ≥ 1 atopic manifestations during the study period



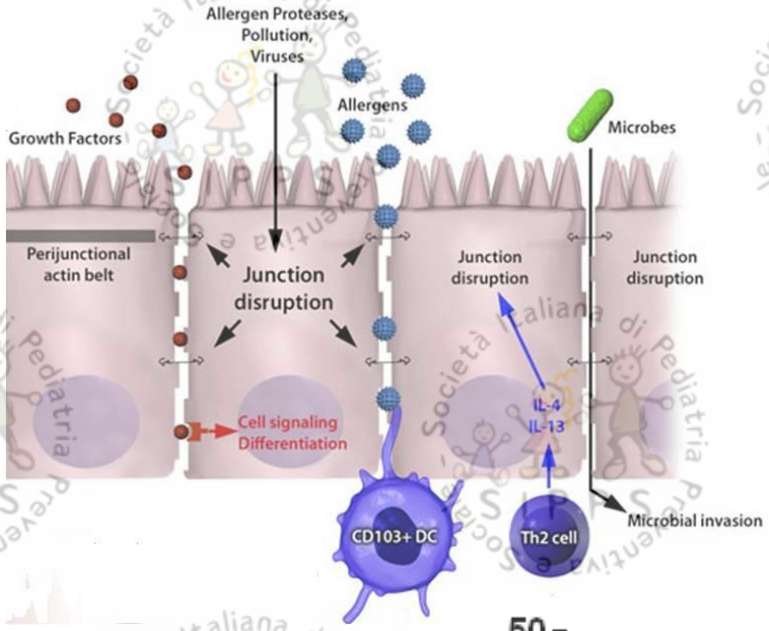


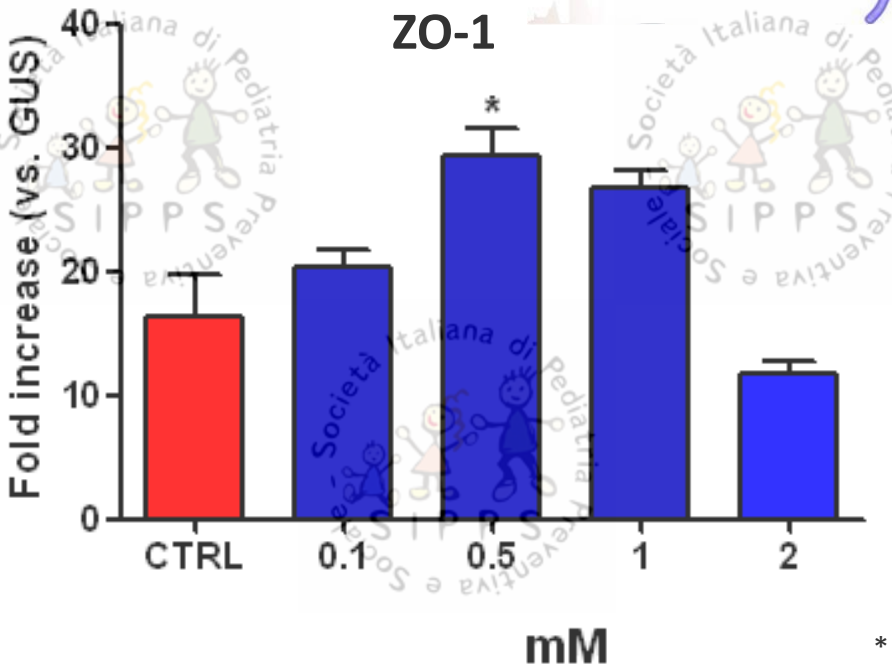


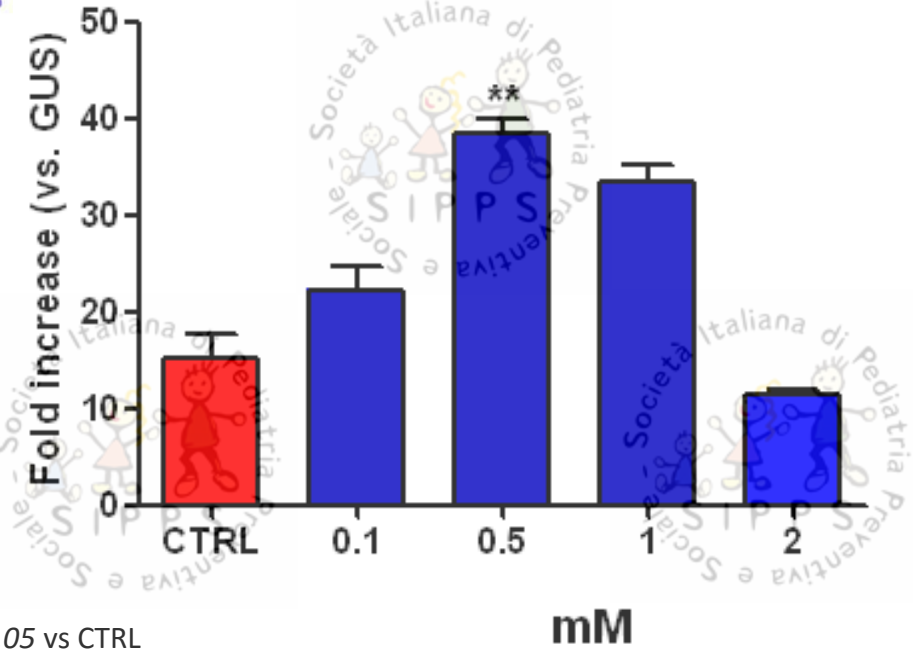


Berni Canani R 2016 submitted

Butyrate enhances TJ proteins in human enterocytes





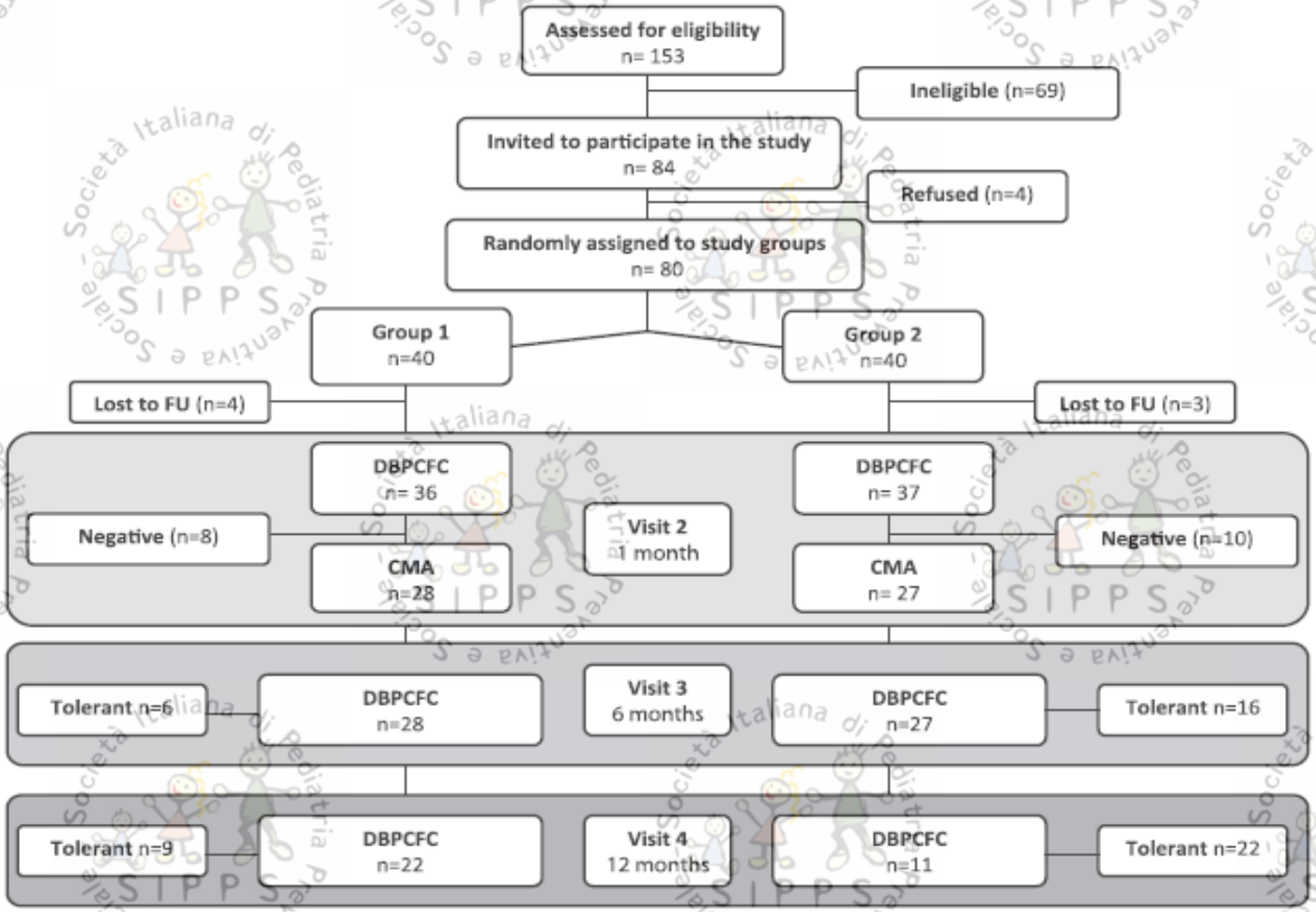


* *p<0.05* vs CTRL
* **p<0.001* vs CTRL

Berni Canani R data on file

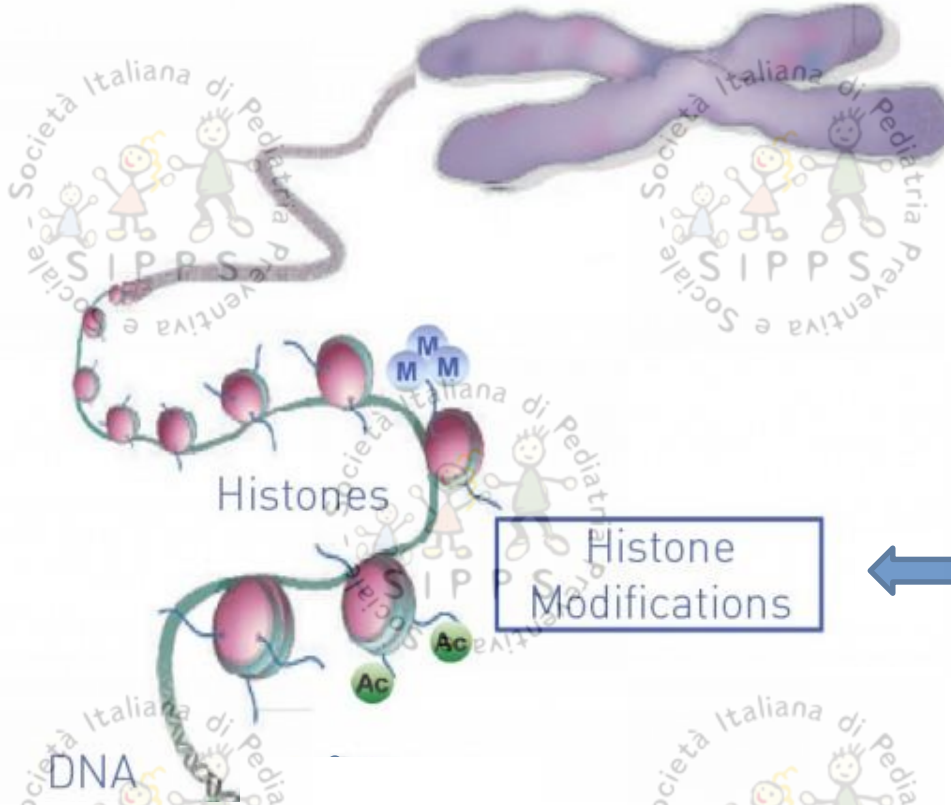
Effect of Lactobacillus GG on tolerance acquisition in infants with cow's milk allergy: A randomized trial

THE JOURNAL OF Allergy AND Clinical Immunology

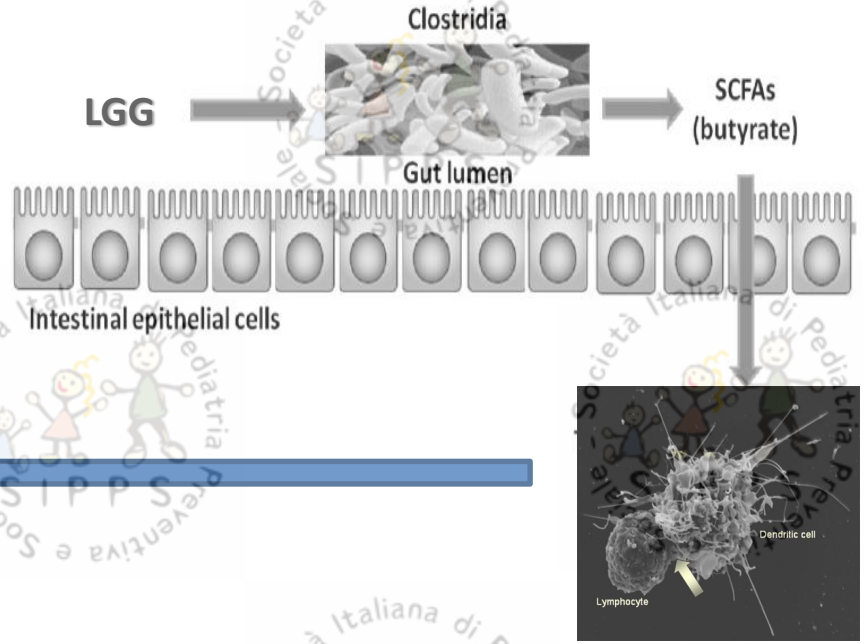


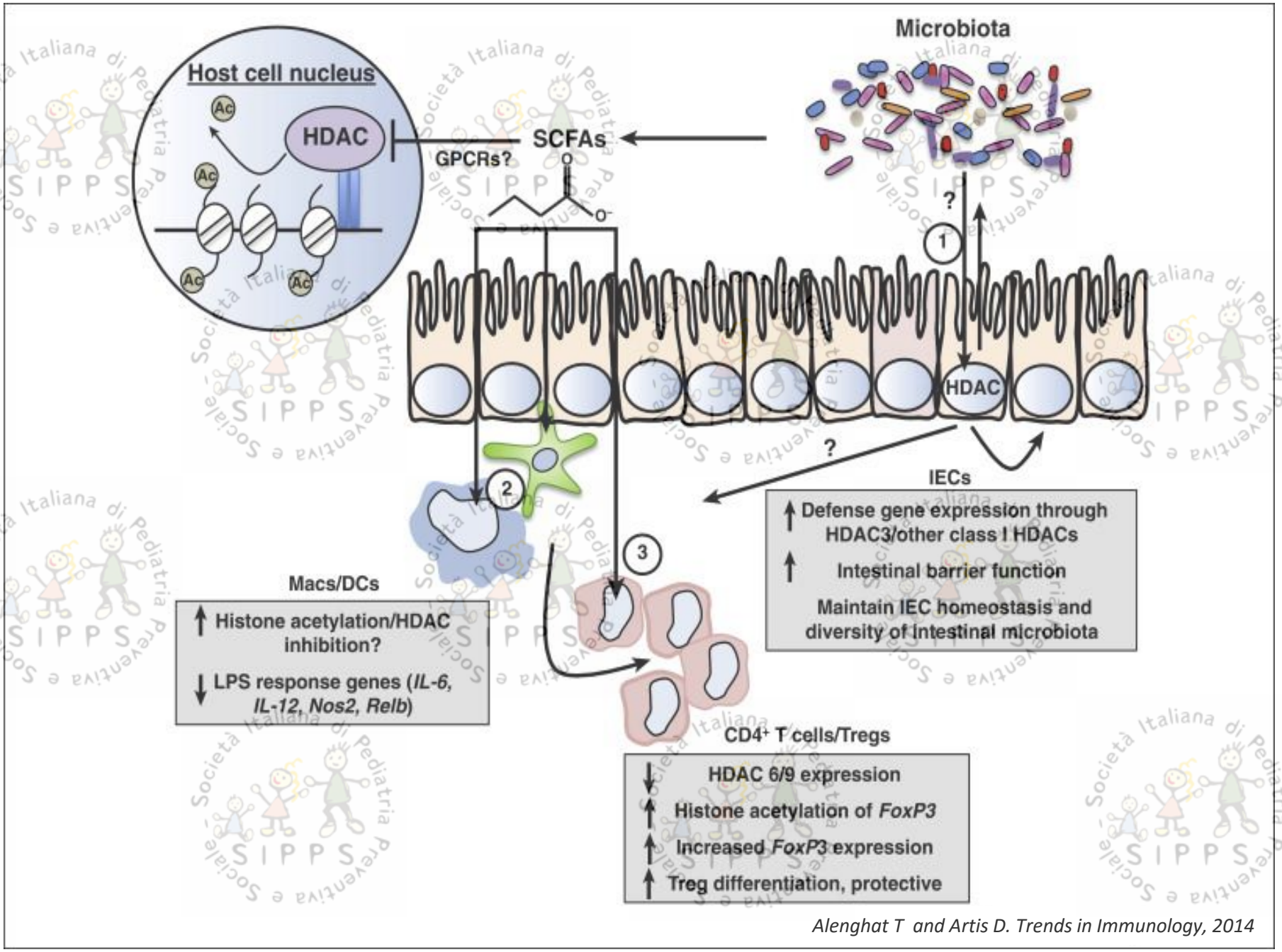
DBPCFC = double-blind, placebo-controlled food challenge.

Berni Canani R, et al. 2012



Histone Modifications

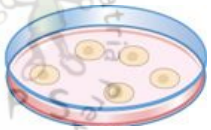


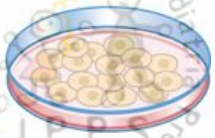


Alenghat T and Artis D. Trends in Immunology, 2014

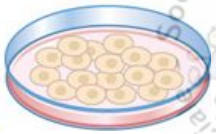
Children with challenge proven IgE-mediated CMA
All positive for SPT and specific serum IgE against β-lactoglobulin

PBMCs



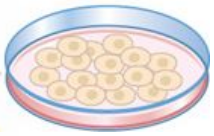


CTRL



BLG

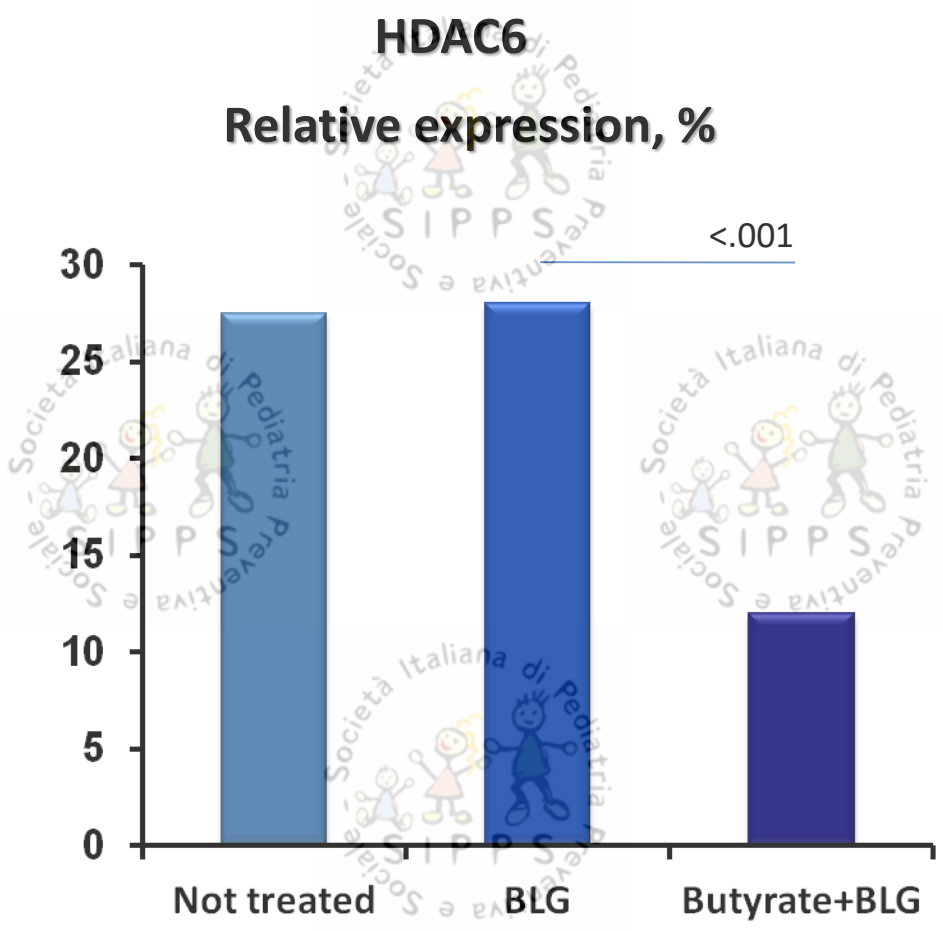
100 µg/ml

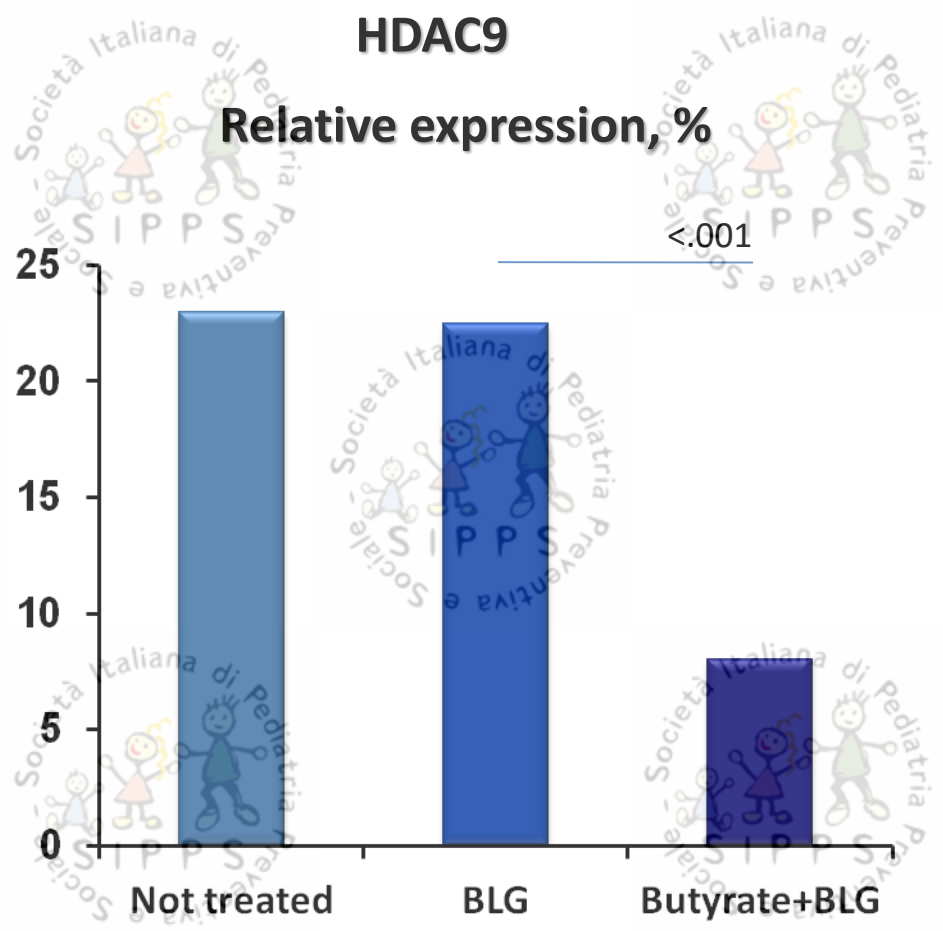


BLG + BUTYRATE

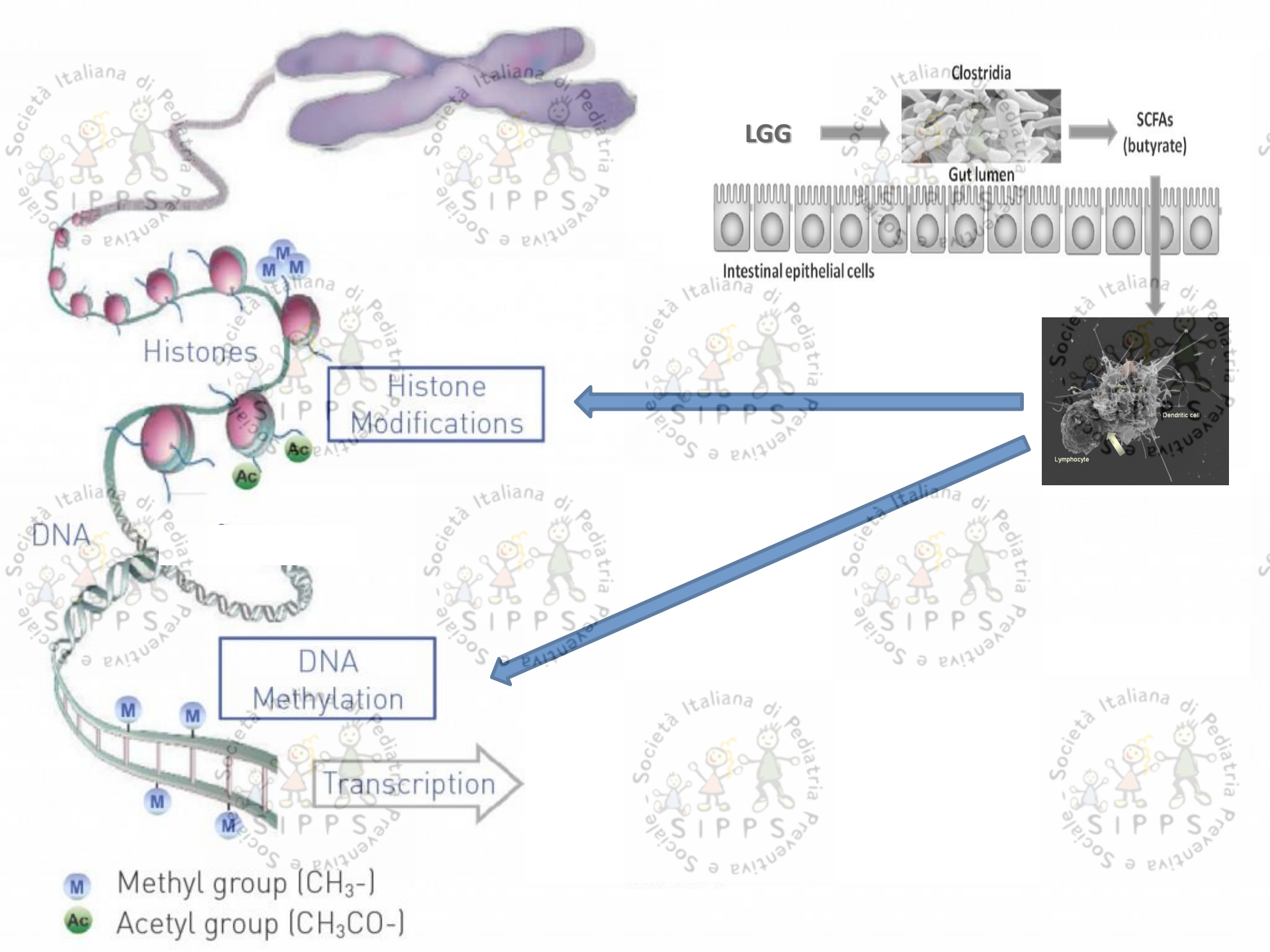
0.5 mM

Butyrate modulates histone deacetylases (HDAC) expression in PBMCs from children with IgE-mediated CMA

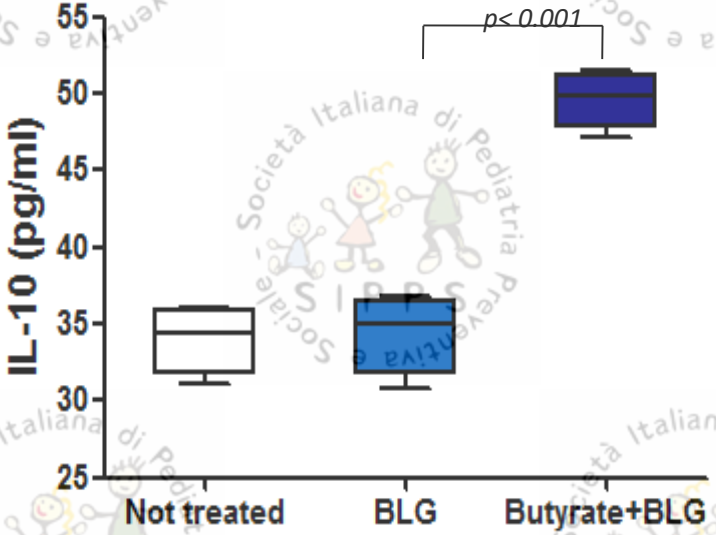


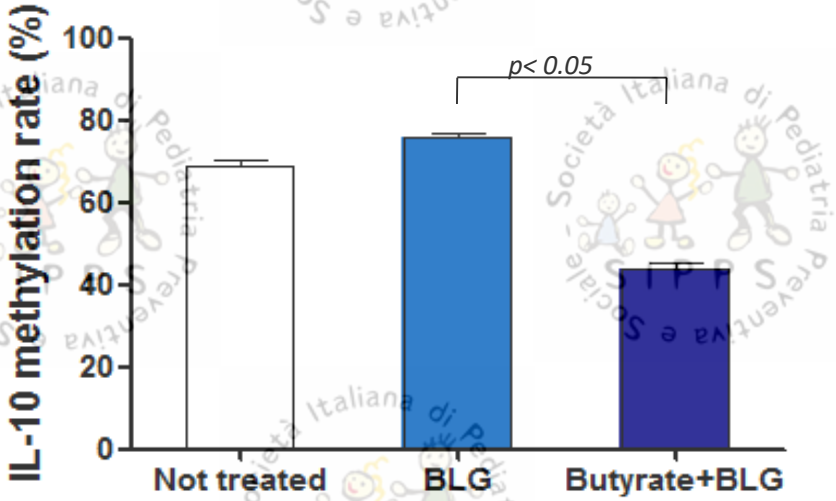


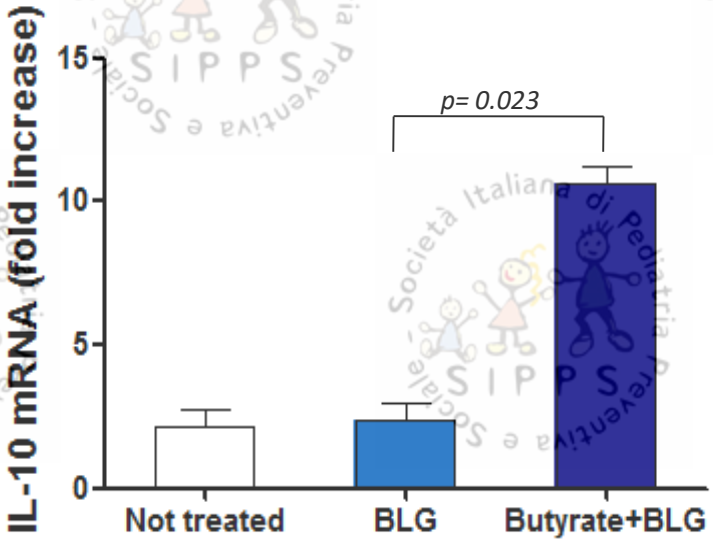
Berni Canani R data on file


Clostridia
LGG
SCFAs
(butyrate)
Gut lumen
Intestinal epithelial cells
Dendritic cell
Lymphocyte
Histones
Histone
Modifications
DNA
DNA
Methylation
Transcription
M
Ac
Methyl group (CH_3-)
Acetyl group (CH_3CO-)

Butyrate induces Th1 cytokines demethylation in PBMCs from IgE-mediated CMA children

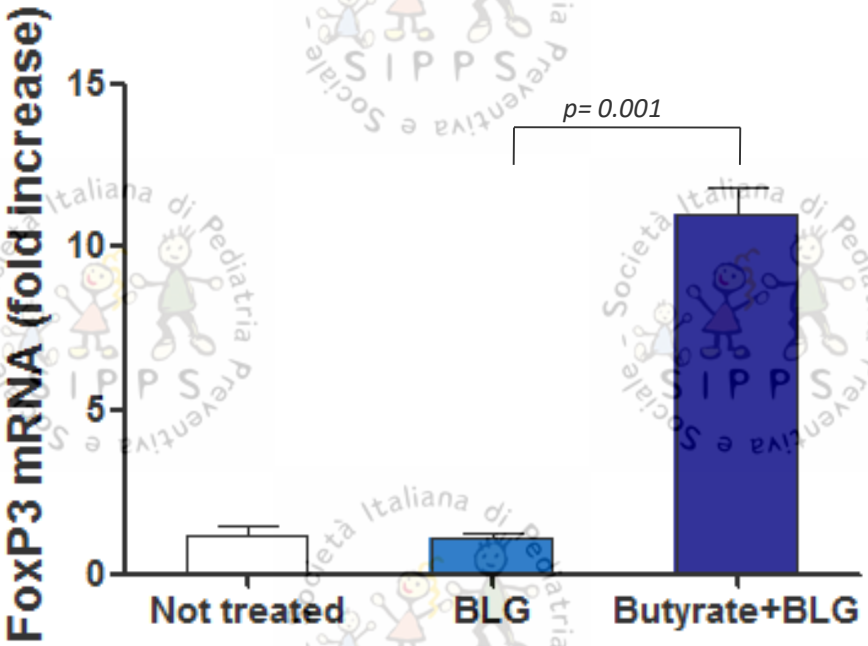


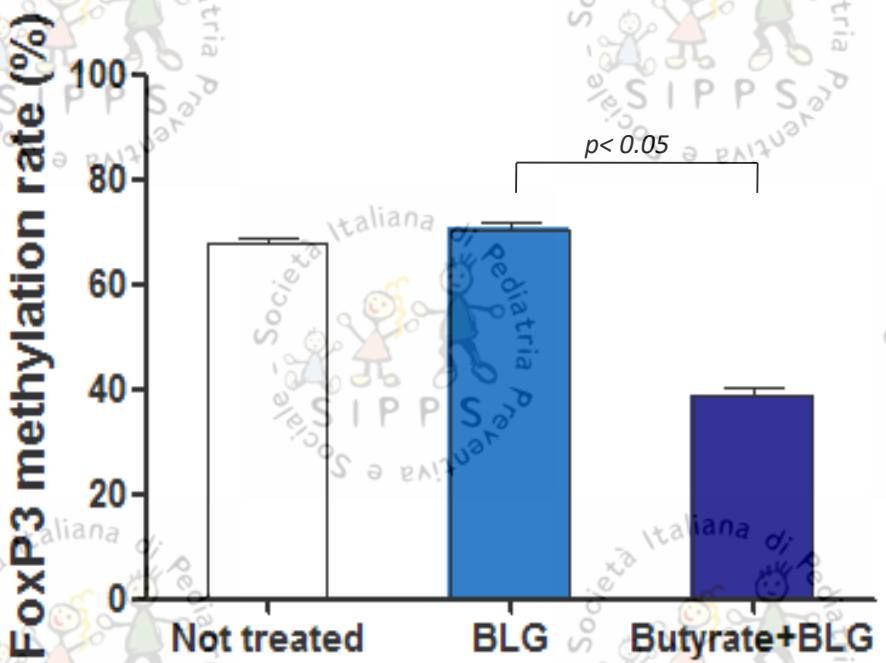




Berni Canani R data on file

Butyrate modulates Treg activation through DNA demethylation

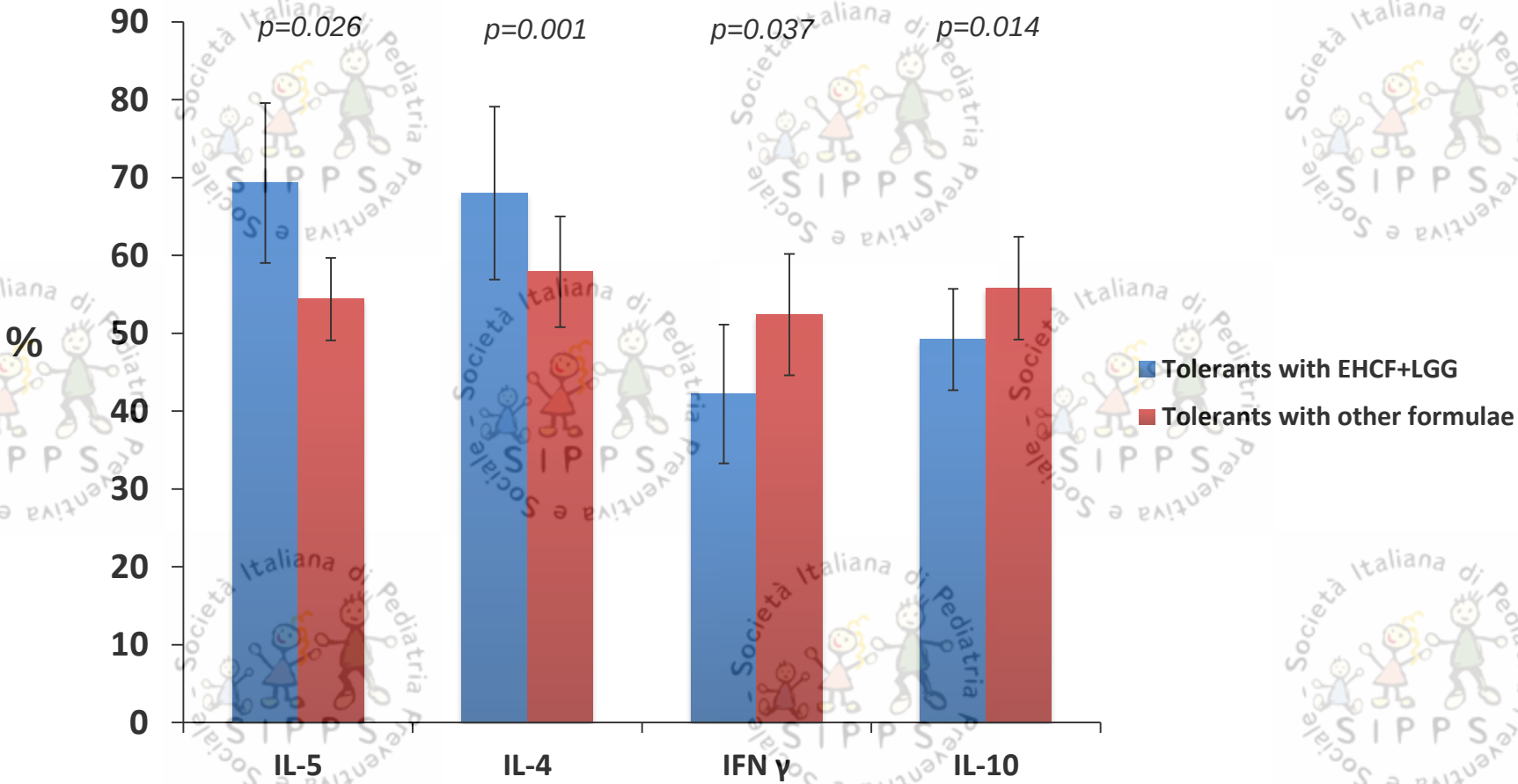




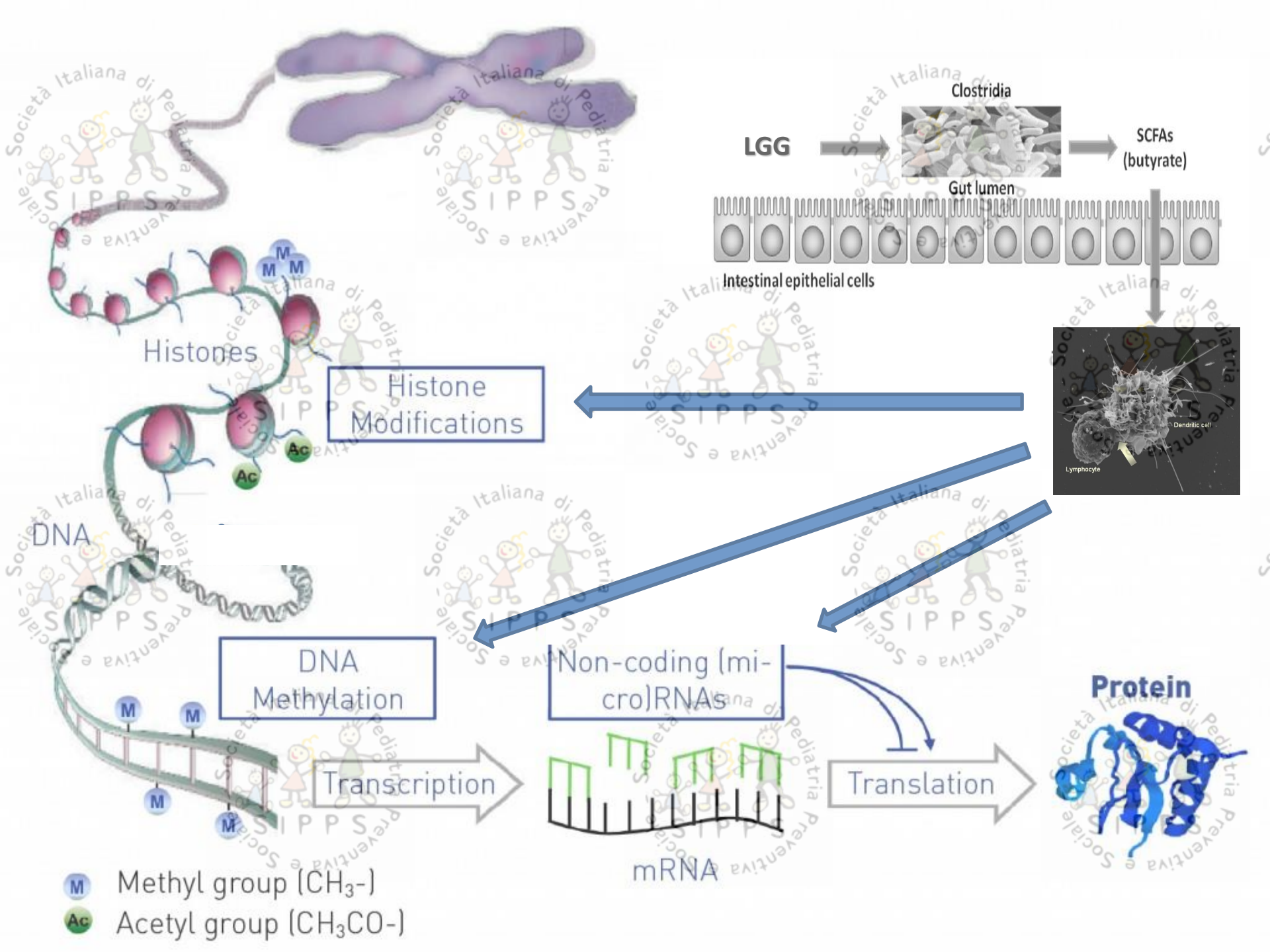
Berni Canani R data on file

Different DNA methylation rate of IL-4, IL-5, IL-10, INF-γ genes observed in children who outgrew CMA receiving different formulas

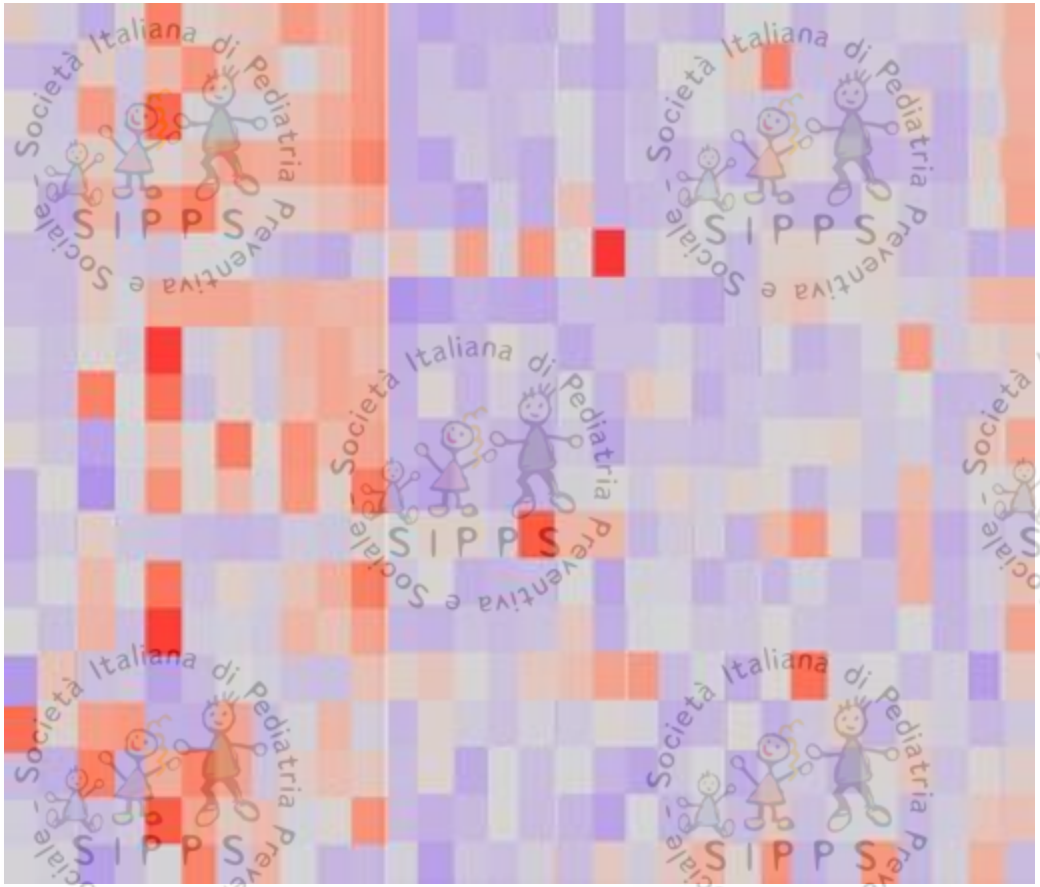
CLINICAL EPIGENETICS



Berni Canani R et al. 2015

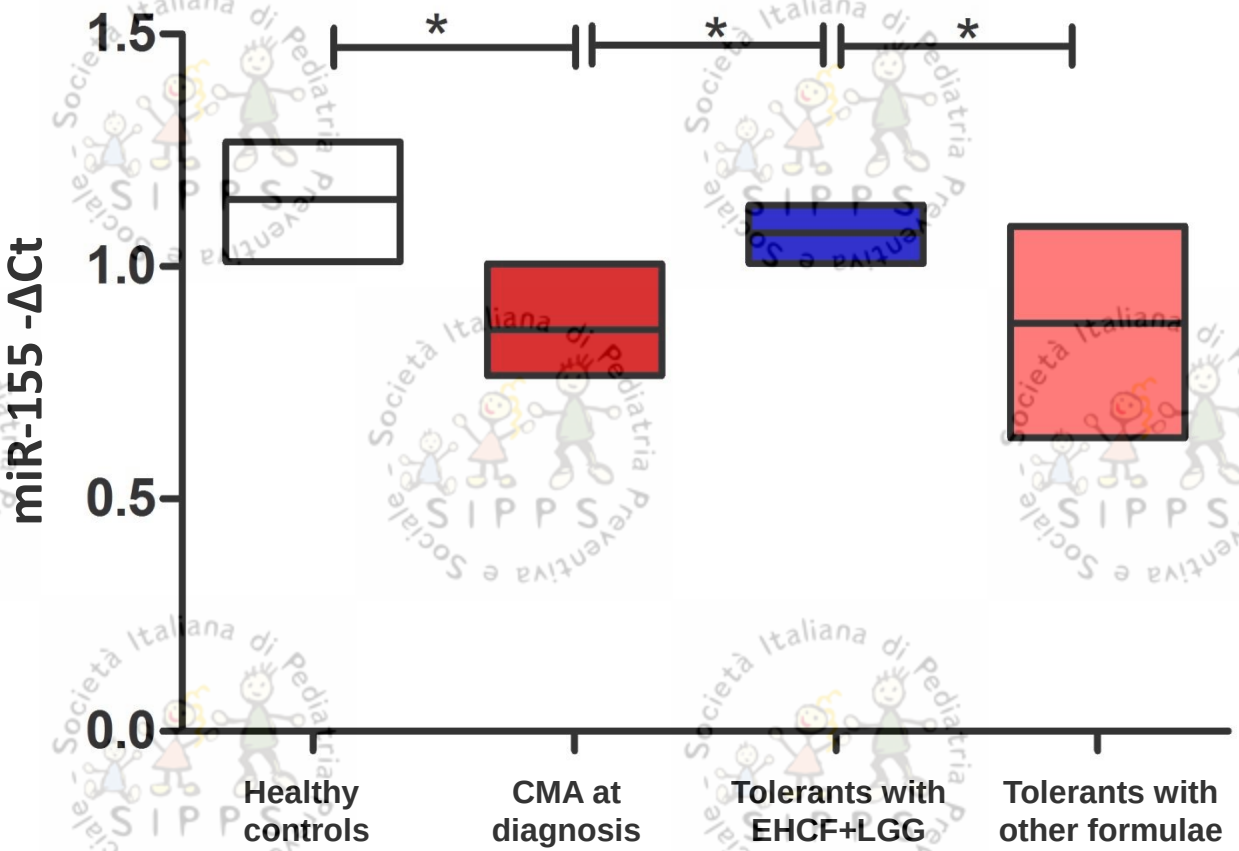

Clostridia
LGG
SCFAs
(butyrate)
Gut lumen
Intestinal epithelial cells
Dendritic cell
Lymphocyte
Histones
Histone
Modifications
DNA
DNA
Methylation
Non-coding (mi-
cro)RNAs
Transcription
Translation
Protein
mRNA
Methyl group (CH3-)
Acetyl group (CH3CO-)

miRNAs signature in PBMCs from IgE-mediated CMA children at different disease stage and from healthy controls



Berni Canani R data on file

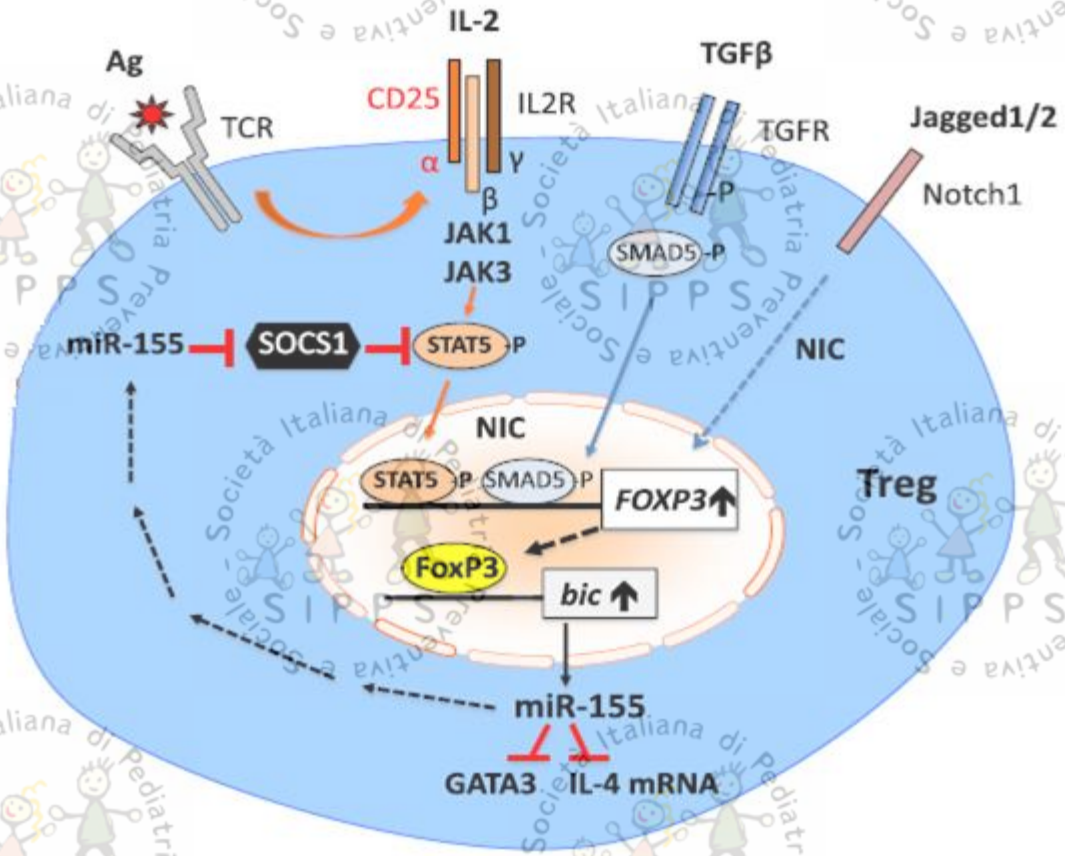
Relative expression of miRNA-155 evaluated by qRT-PCR in PBMCs from IgE-mediated CMA children and from healthy controls



* $p<.005$

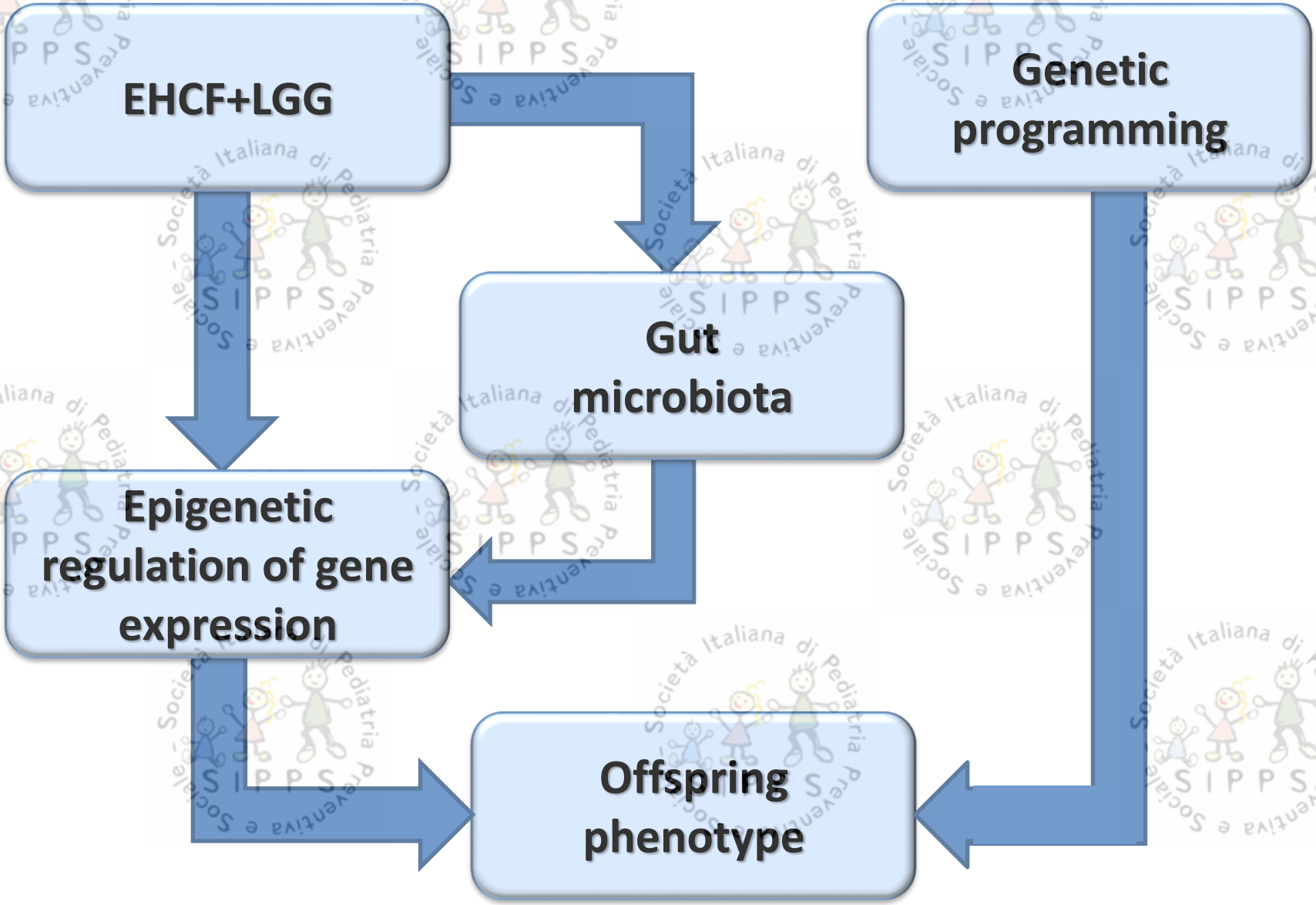
Berni Canani R data on file

miR-155 activity: downregulation of IL-4 level

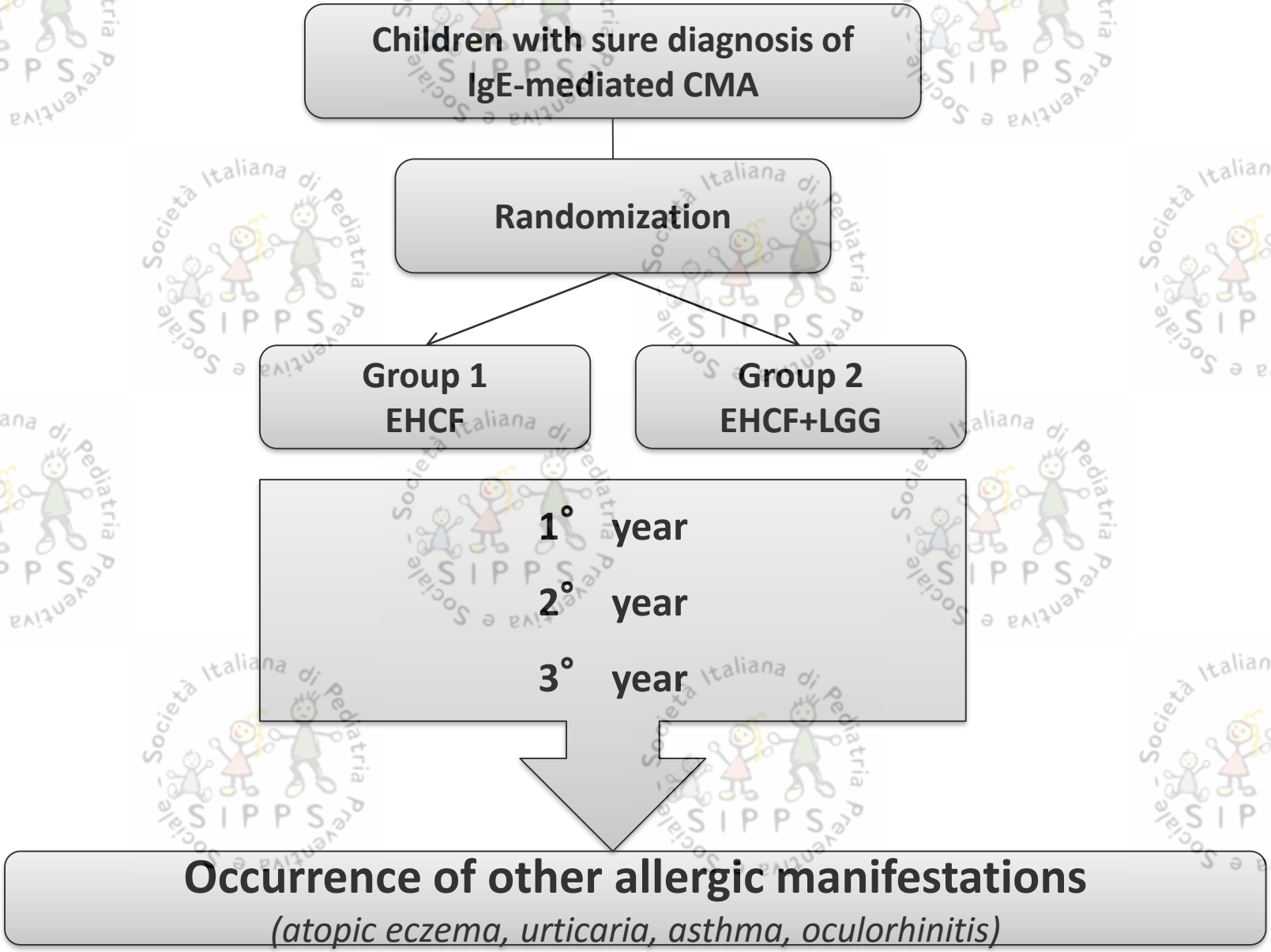


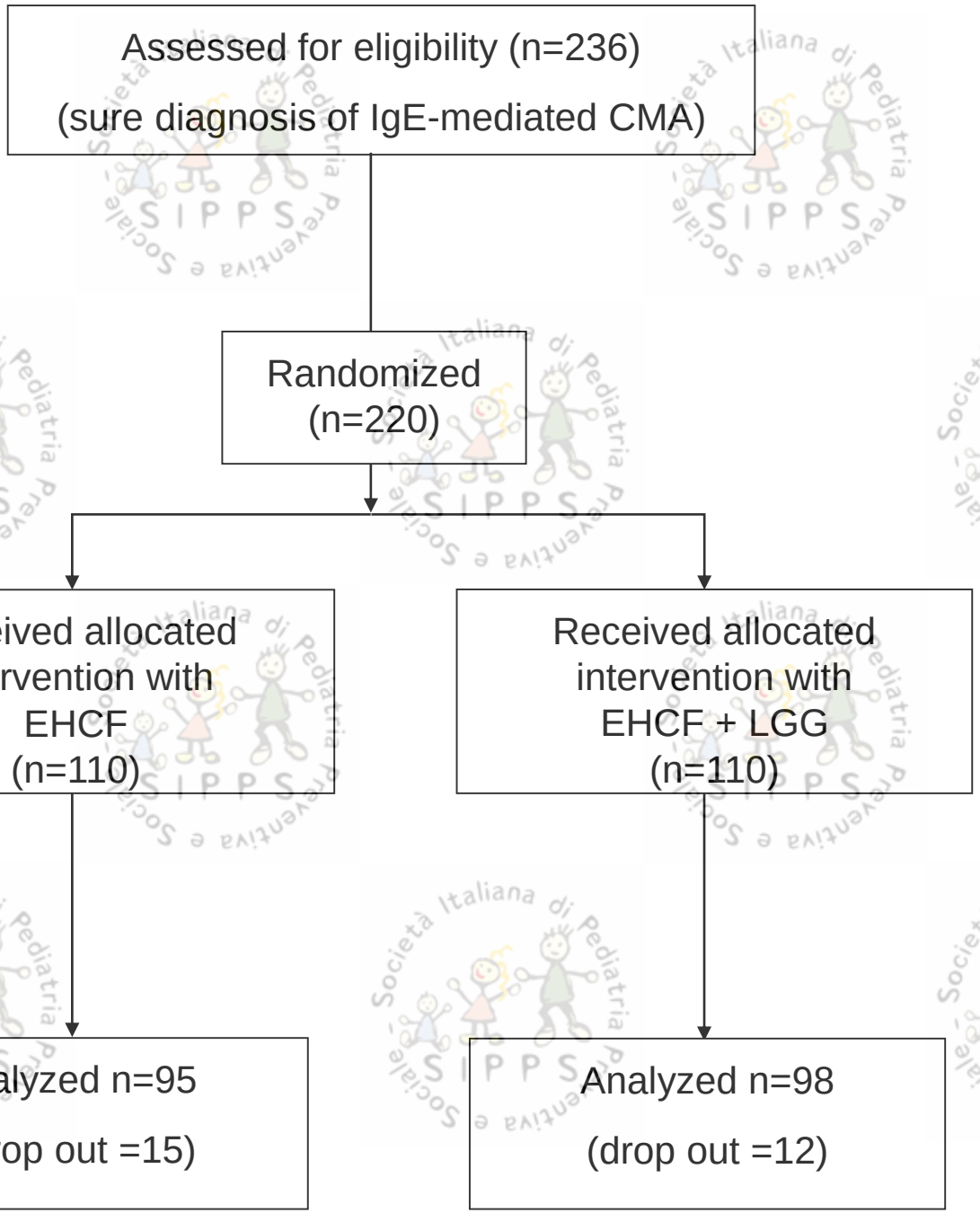
Melnik et al. J Translat Med2014

Epigenetics: the interface between genes and nutrition

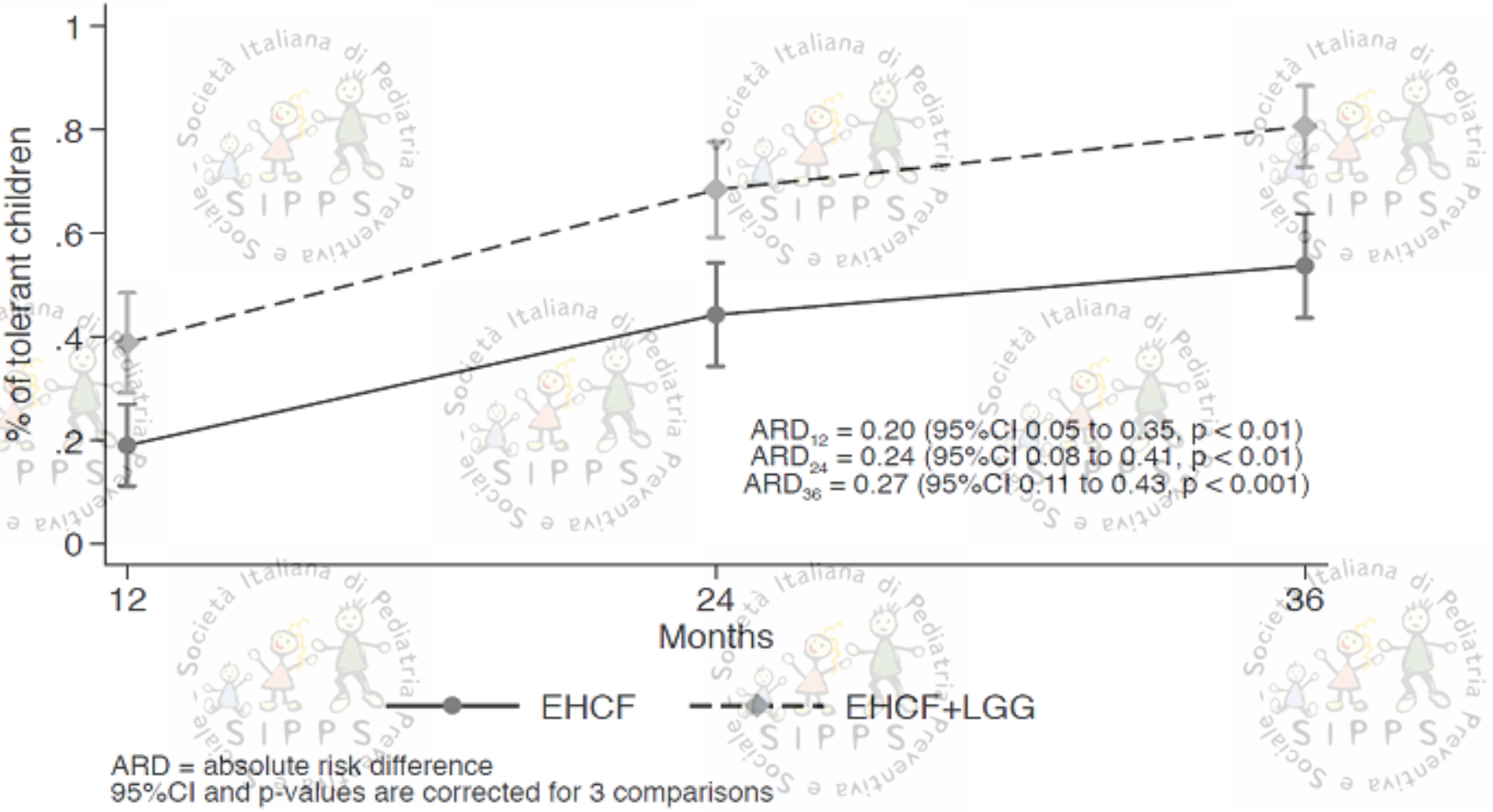


Prospective study: preventive effect elicited by LGG on atopic manifestations in CMA children



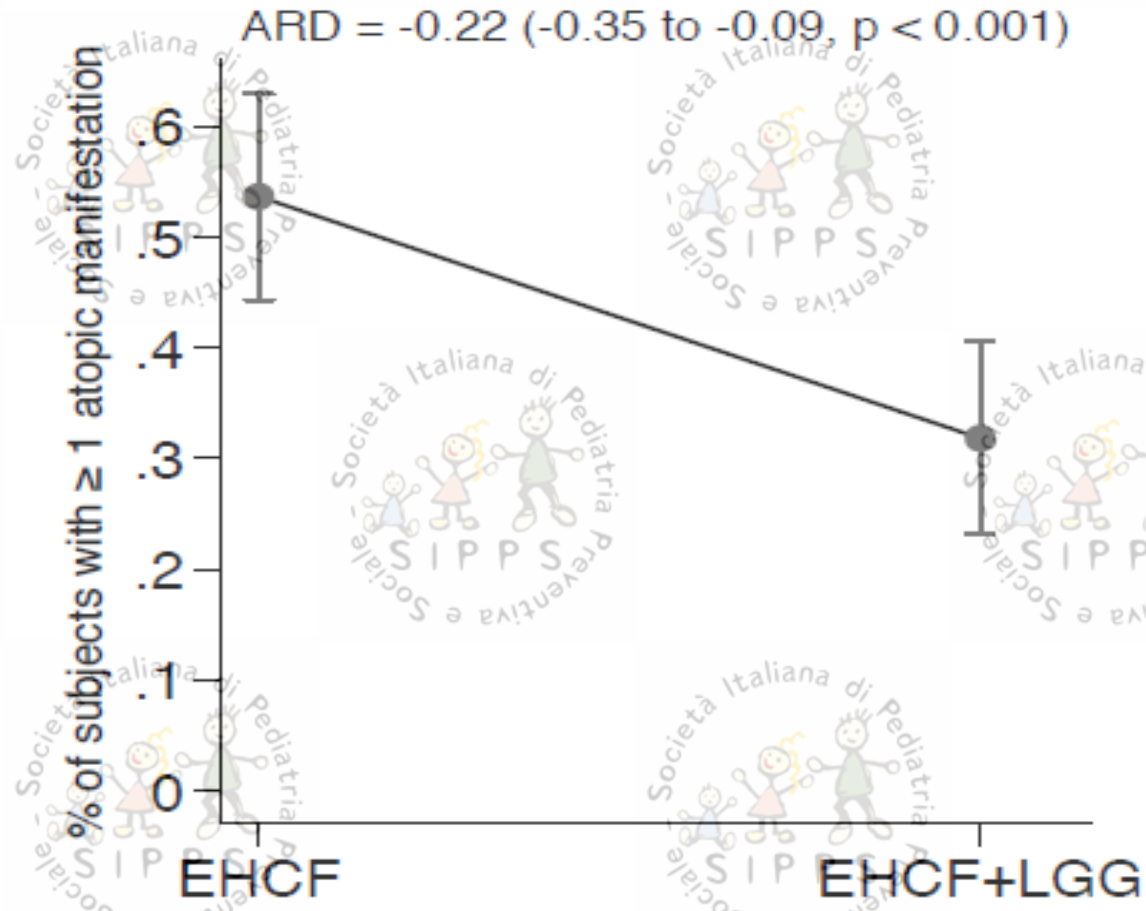

Assessed for eligibility (n=236)
(sure diagnosis of IgE-mediated CMA)
Randomized
(n=220)
Received allocated intervention with EHCF
(n=110)
Received allocated intervention with EHCF + LGG
(n=110)
Analyzed n=95
(drop out =15)
Analyzed n=98
(drop out =12)

Oral Tolerance Acquisition



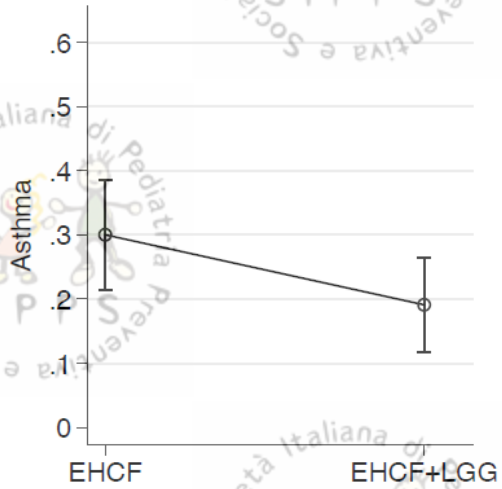
Berni Canani R 2016 submitted

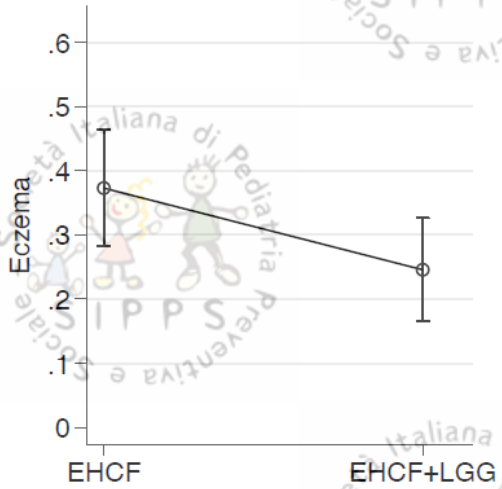
Rate of subjects experienced ≥ 1 atopic manifestation ITT, n=110/group

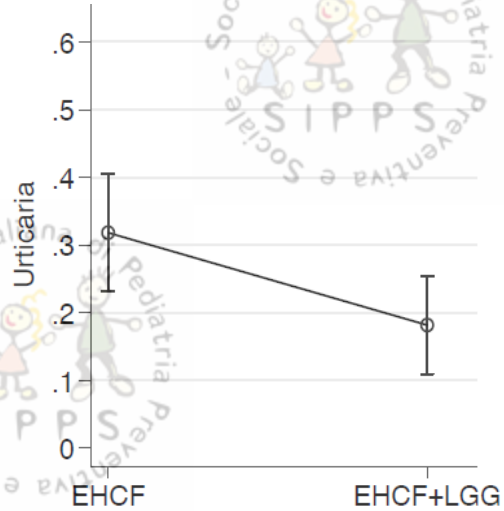


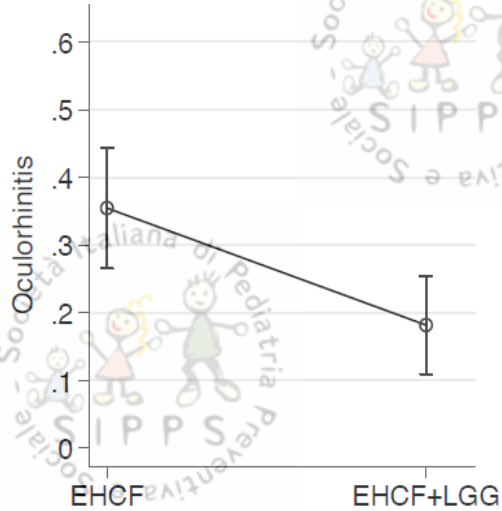
Berni Canani R 2016 submitted

Rate of subjects experienced ≥ 1 atopic manifestations during the study period









Berni Canani R 2016 submitted

Conclusions

- CMA persistence and severity have been on a rise in recent decades under the pressure of negative gene-environment interaction leading to immune-system dysfunction mediated at least in part by epigenetic mechanisms
- We are exploring the possibility to counteract this pathway modulating the interaction among dietary agents, gut microbiota, epigenetic mechanisms and immune system
- These effects lead to a positive impact on oral tolerance acquisition and on long term protection against other atopic manifestations in children with CMA

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Diet and food allergy development during infancy: Birth cohort study findings using prospective food diary data

Kate E. C. Grimshaw, PhD, RD,[a] Joe Maskell, MSc,[b] Erin M. Oliver, MSc,[a] Ruth C. G. Morris, Dip HE, RN(Child),[c] Keith D. Foote, MBBS, FRCPCH,[d] E. N. Clare Mills, PhD,[e] Barrie M. Margetts, PhD, FFPH,[f]* and Graham Roberts, DM, MRCPCH[a]* *Southampton, Winchester, and Manchester, United Kingdom*

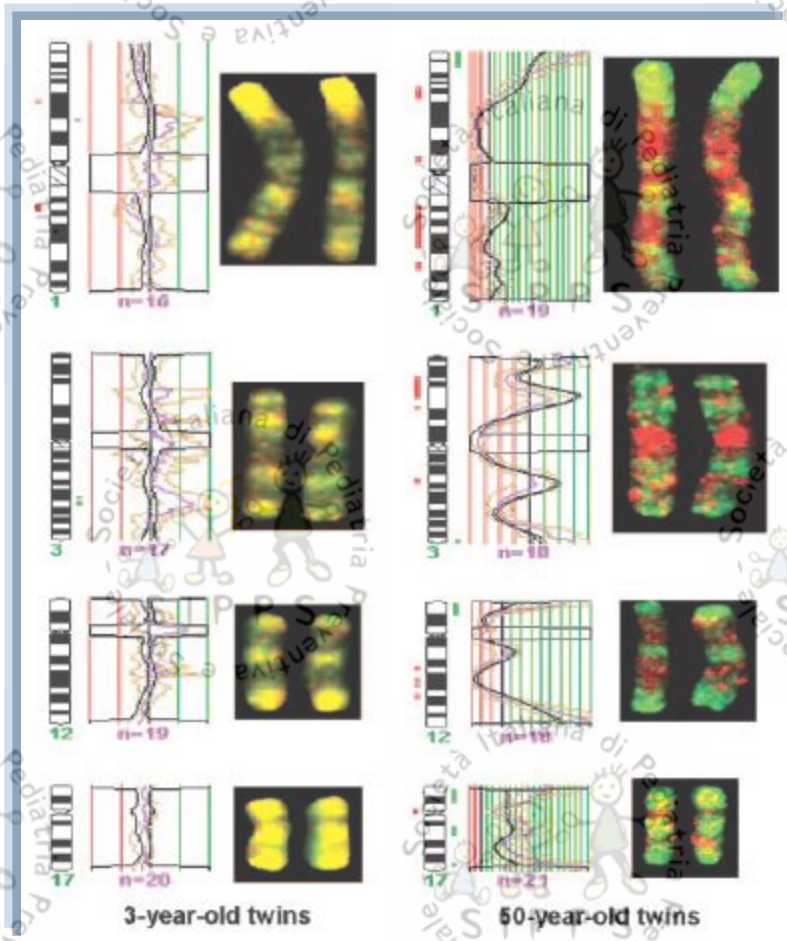
BY THE AGE OF 2 YEARS

MORE FOOD ALLERGY

LESS FOOD ALLERGY

J Allergy Clin Immunol 2014;133:511-9.

Mappatura di regioni cromosomiche con differente metilazione del DNA in gemelli monozigoti attraverso ibridazione genomica comparativa per DNA metilato



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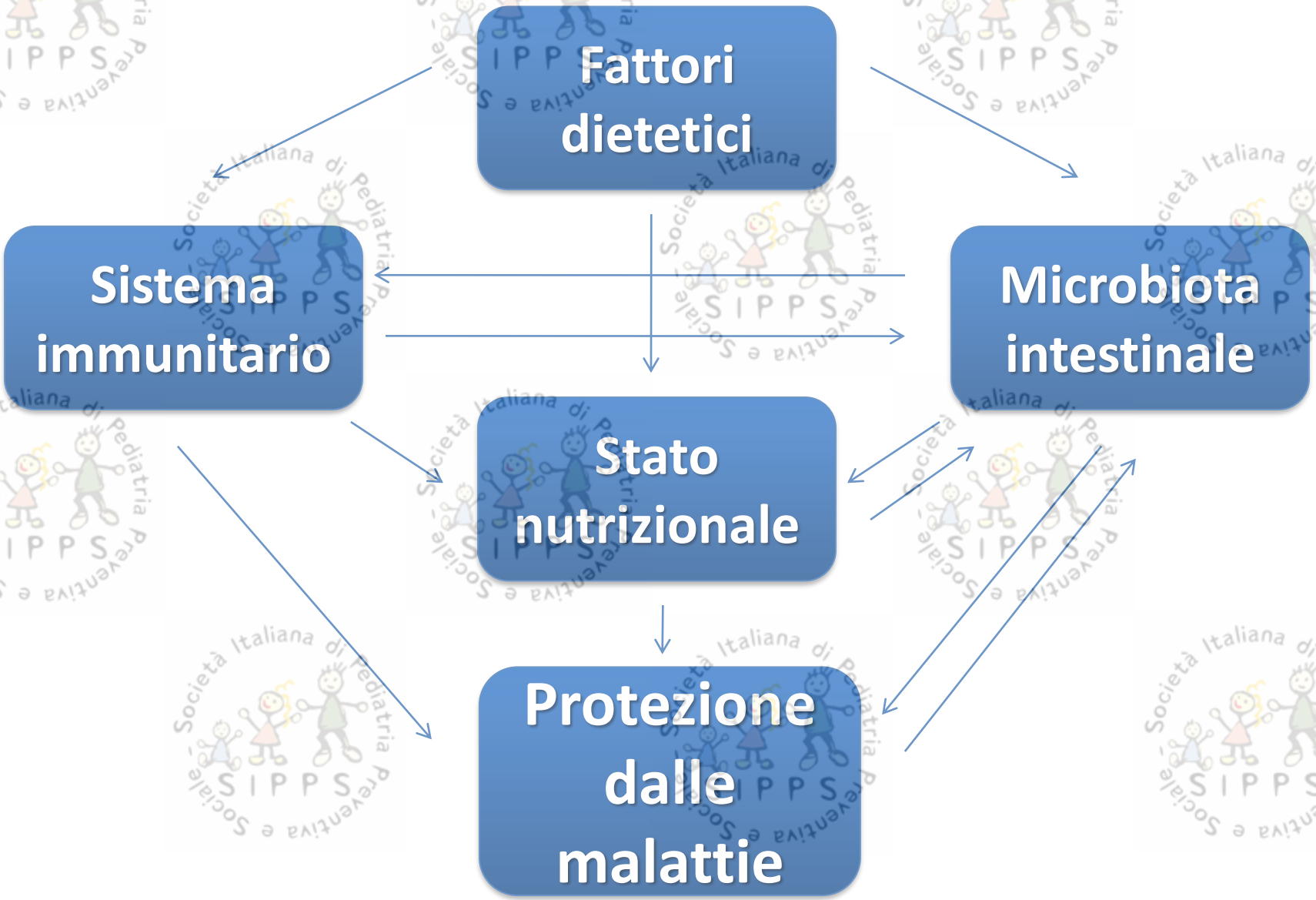
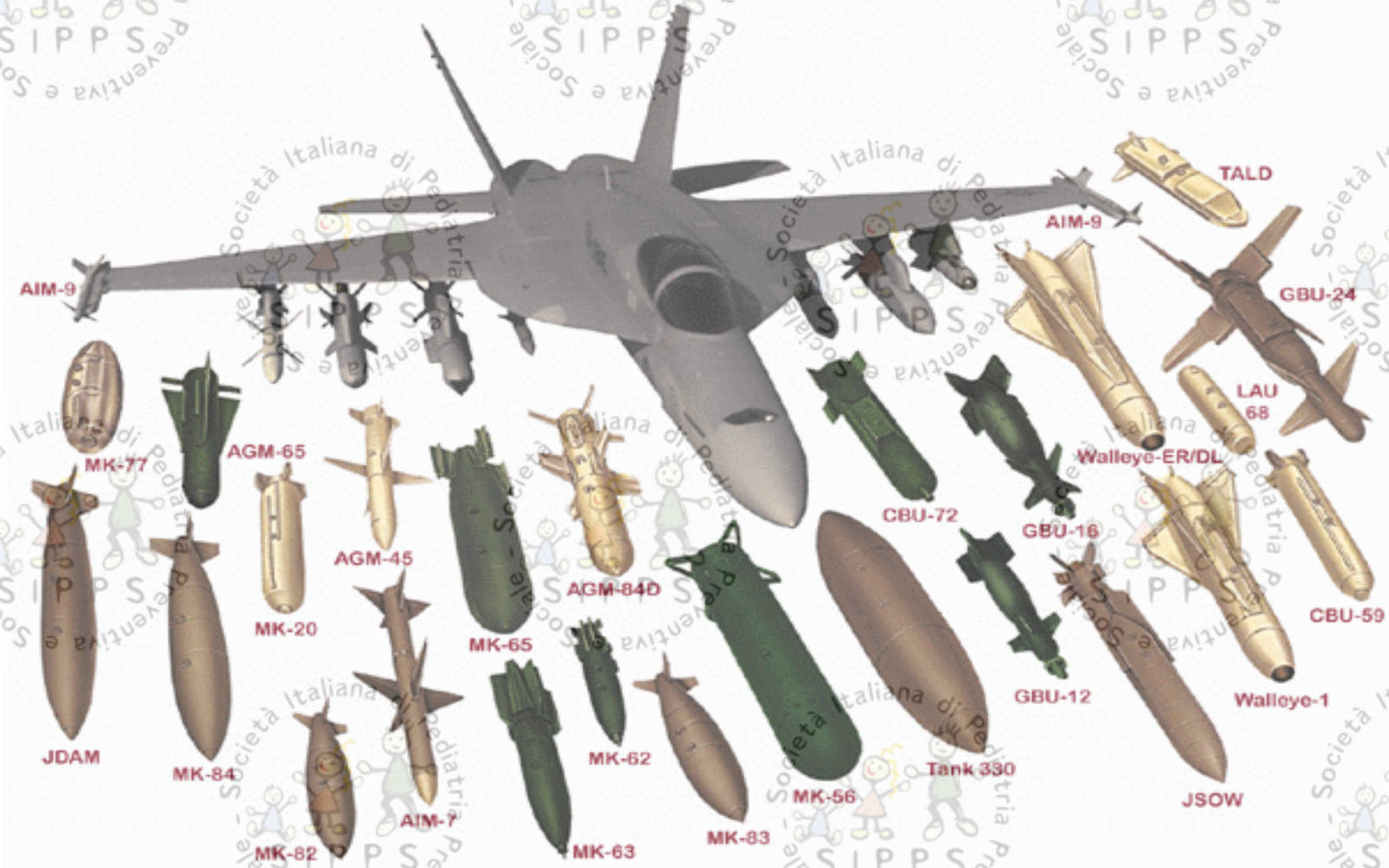
Immunonutrizione



Meccanismi di Azione



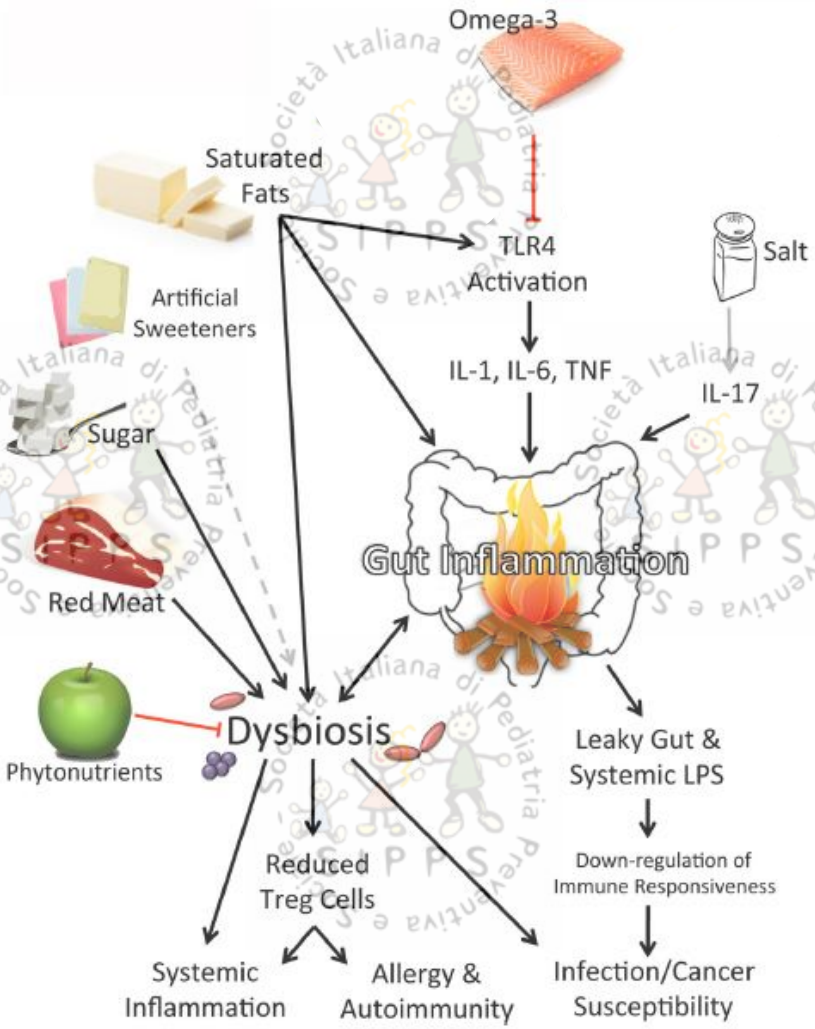
Myles *Nutrition Journal* 2014, 13:61
http://www.nutritionj.com/content/13/1/61

NUTRITION JOURNAL

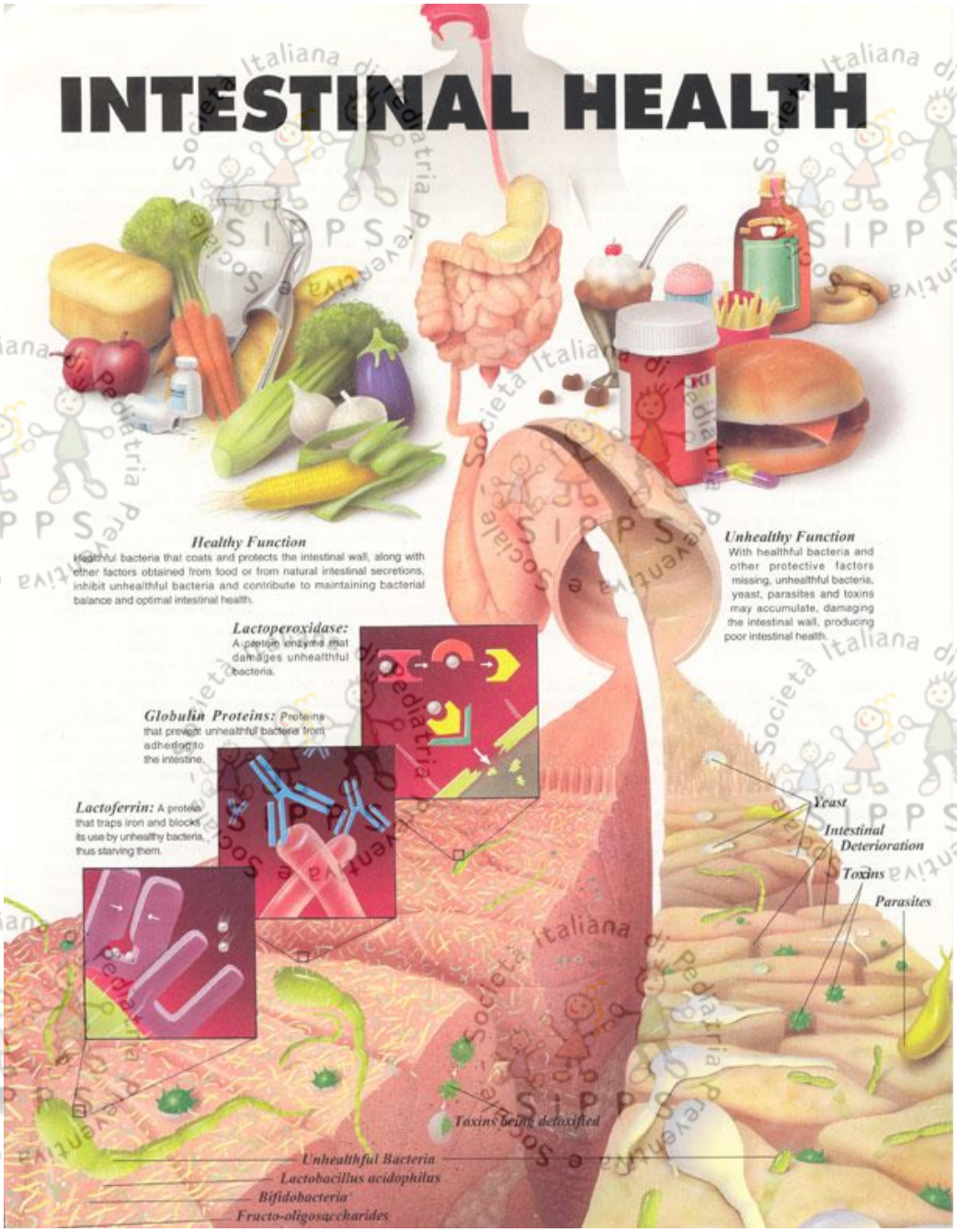
REVIEW Open Access

Fast food fever: reviewing the impacts of the Western diet on immunity

Ian A Myles



INTESTINAL HEALTH



EHCF+LGG significantly increases butyrate-producing bacteria: activation of the epigenetics pathways

- Modulation of histone deacetylases 6 and 9
- Th1 cytokine genes demethylation
- Treg activation through DNA demethylation

Up-regulation of Th1 response

- Selective miRNAs modulation

Down-regulation of Th2 response

Berni Canani R et al. Clin Epigenet 2015
Paparo L et al. Clin Epigenet in press