16th International Coeliac Disease Symposium
21-24 June, Prague, Czech Republic

Pre-Conference Workshop on Gluten Sensitivity
June 21, 2015
The Evolving Planet of Gluten Related Disorders

Biomarkers for Non-Celiac Gluten Sensitivity: A Tough, but Intriguing Challenge for Researchers

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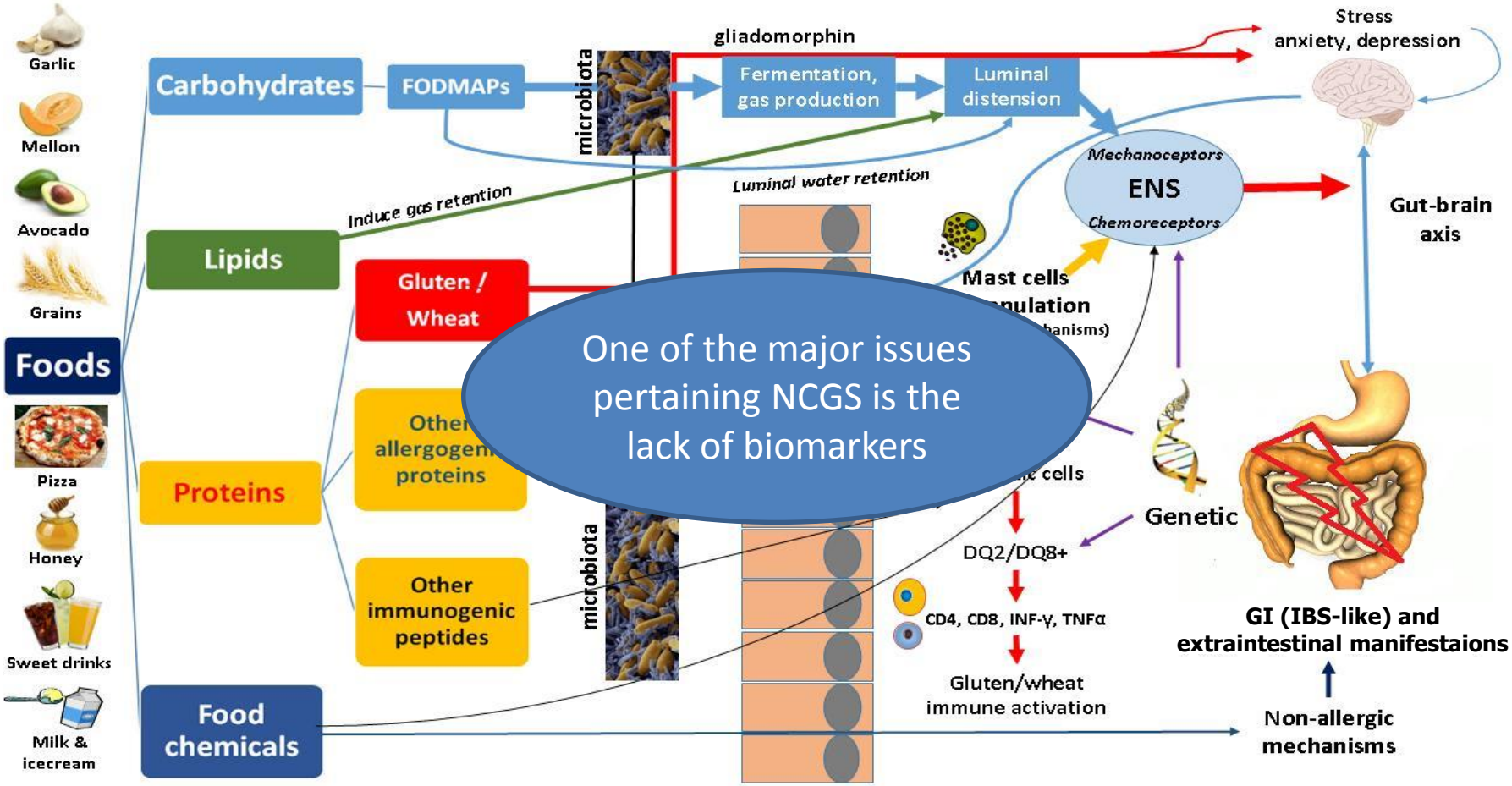
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Sensitivity to Wheat, Gluten and FODMAPs in NCGS: Facts or Fiction ?

De Giorgio R, Volta U & Gibson P, GUT 2015



Biomarkers for gluten-related disorders

Coeliac disease

R1 AGA EmA TG2 DGP TG3 TG6

1973 1980 1983 1997 2004 2005 2008

A progressive improvement

a straight and successful, but long road!!!!

Non-coeliac gluten sensitivity

AGA ?

2011 2015

Serological tests in gluten sensitivity
Volta U et al

only a few years of research…we need more time!!

Biomarkers for NCGS: Where are we?

- Antibodies to native gliadin
- Chemokine secretion from PBMC stimulated by gluten/wheat
- Serum zonulin levels
- Submucosal mast cell and neuron density
- IgA intestinal deposits to TG2

Volta U et al, Best Practice Research and Clinical Gastroenterology 2015

IgG Anti Gliadin Antibodies: an "Old" Test for a "New" Diagnosis

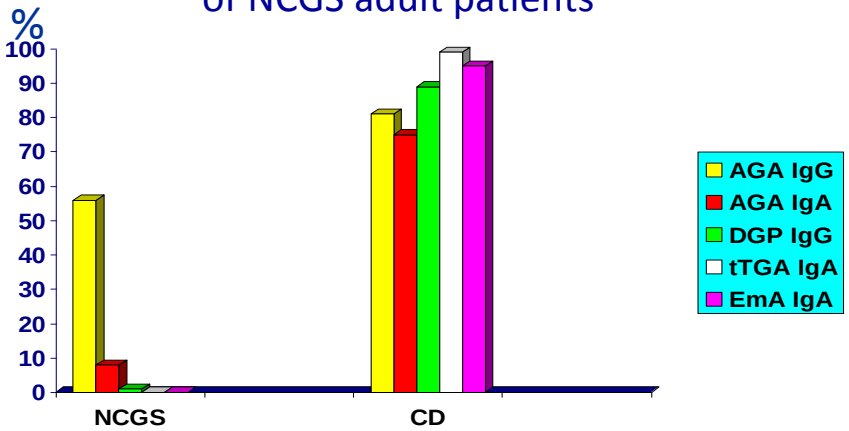
ORIGINAL ARTICLE

J Clin Gastroenterol 2012

Serological Tests in Gluten Sensitivity (Nonceliac Gluten Intolerance)

Umberto Volta, MD, Francesco Tovoli, MD, Ronny Cicola, MD, Claudia Parisi, MD, Angela Fabbri, MD, Maria Piscaglia, MD, Erica Fiorini, MD, and Giacomo Caio, MD

IgG antigliadin antibodies (AGA): positive in 56% of NCGS adult patients



THE JOURNAL OF PEDIATRICS • www.jpeds.com

ORIGINAL ARTICLES

J Pediatr. 2013 Nov 16. Epub ahead of print

Clinical, Serologic, and Histological Features of Gluten Sensitivity in Children

Ruggiero Francavilla, MD, PhD[1], Fernanda Cristofori, MD[1], Stefania Castellaneta, MD[2], Carlo Polloni, MD[3], Veronica Albano, MD[4], Stefania Dellatte[5], Flavia Indrio, MD[1], Luciano Cavallo, MD[1], and Carlo Catassi, MD, PhD[4]

IgG antigliadin antibodies (AGA): positive in 66% of NCGS children

Table I. Clinical and laboratory characteristics of the study subjects

	Controls	GS	CD
Number of children	15	15	15
Age at referral, y, mean ± SD	8.6 ± 2.7	9.6 ± 3.9	9.1 ± 3.1
Males/females, n	6/9	10/5	5/10
Intestinal symptoms	Abdominal pain, dyspepsia	Abdominal pain, chronic diarrhea, bloating, failure to thrive, vomiting, constipation	Abdominal pain, chronic diarrhea, bloating, failure to thrive
Extraintestinal symptoms	None	Tiredness, headache, limb pain	Anemia, hypertransaminasemia
Celiac serology	All negative	AGA-IgG positive in 10, AGA-IgA positive in 1; rest all negative	tTG-IgA and EmA-positive in all
HLA	Not determined	7 DQ2	All DQ2/8
Wheat IgE	All negative	All negative	All negative

AGA prevalence in other diseases and healthy controls

- Connective tissue disorders 9%
- IBS without NCGS 20%
- Autoimmune liver diseases 21%
- Healthy controls 2%

AGA IgG is not a marker neither specific nor highly sensitive for NCGS, but for the time being its positivity (especially at a high titer) in patients with suspected NCGS can contribute to this diagnosis

Volta U et al. J Clin Gastroenterol 2009; Caio G et al, Abstract 15st ICDS, Chicago 2013

Effect of gluten free diet on immune response to gliadin in patients with non-celiac gluten sensitivity

Giacomo Caio[1,2,3], Umberto Volta[1,2,3*], Francesco Tovoli[1,2,3] and Roberto De Giorgio[1,2,3]

BMC Gastroenterology 2014, 14:26

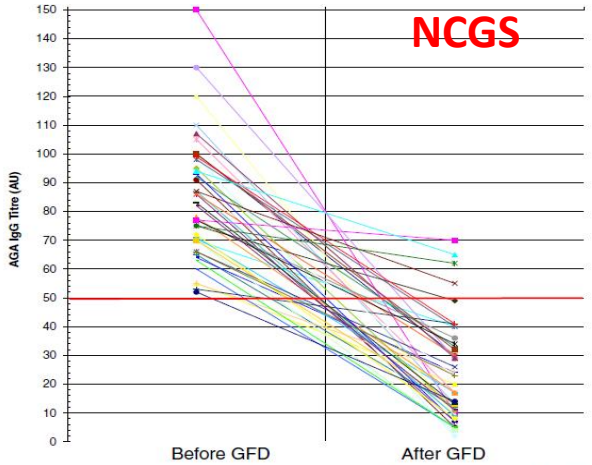


Figure 1 IgG antigliadin antibodies before and after GFD in NCGS patients: anti-gliadin antibodies (AGA) of IgG class before and after gluten free diet (GFD) in patients with non-celiac gluten sensitivity (NCGS). Only three of the 44 patients studied showed persistence of AGA IgG at a low titre after gluten withdrawal.

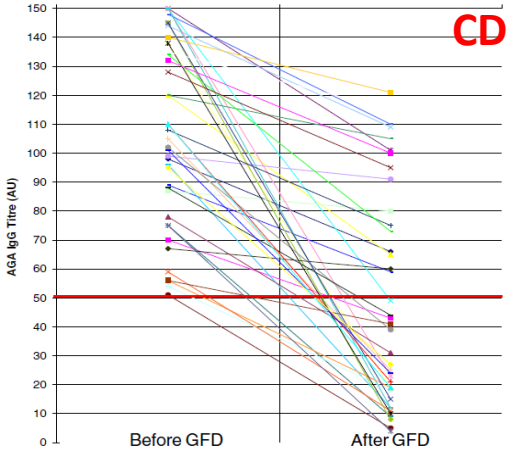
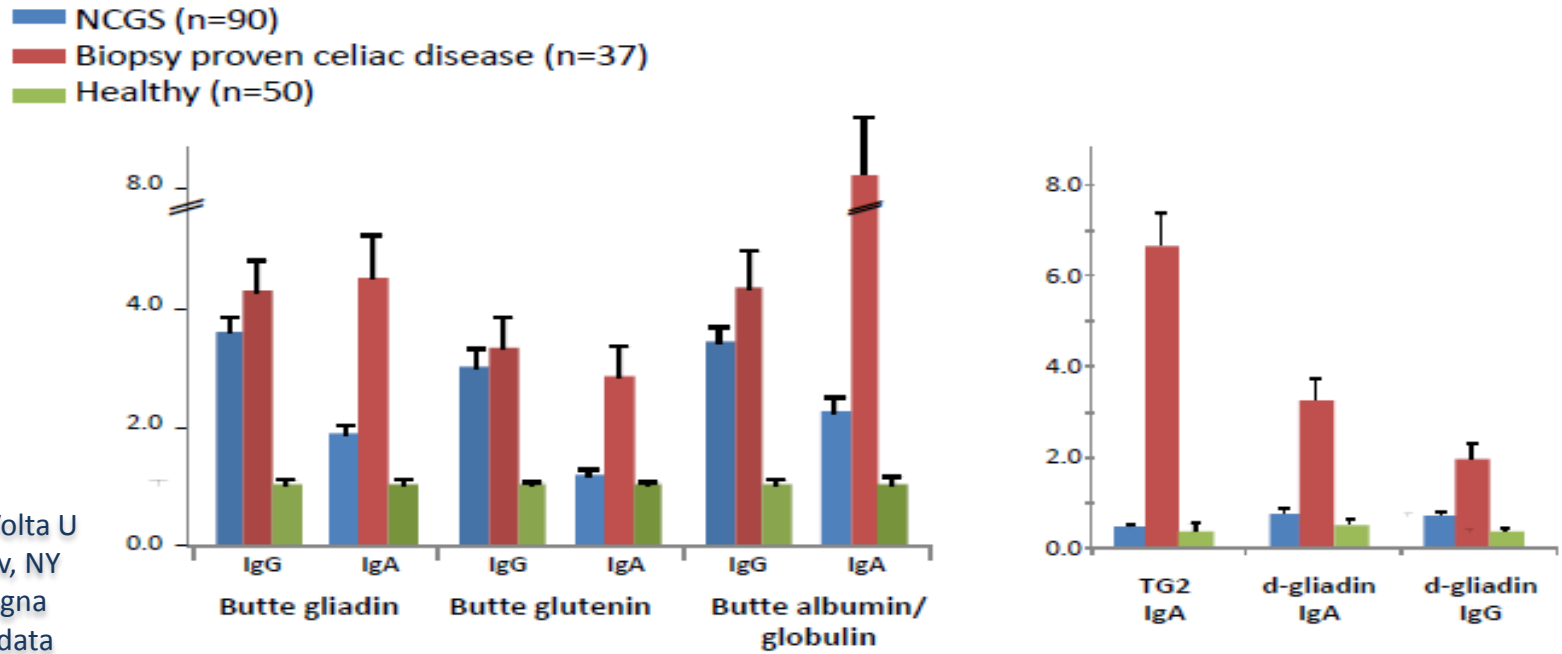


Figure 3 IgG antigliadin antibodies before and after GFD in CD patients: anti-gliadin antibodies (AGA) of IgG class before and after gluten free diet (GFD) in patients with celiac disease (CD).

Table 1 AGA IgG in NCGS and CD related to compliance to the GFD and clinical picture

44 NCGS patients (all with AGA IgG when untreated) after 6-months-GFD	**Good response to GFD 39 /44**		**Mild response to GFD 5/44**	
Compliance	AGA IgG +	AGA IgG-	AGA IgG +	AGA IgG-
Strict 41/44	0	38	1	2
Low 3/44	0	1	2	0
40 CD patients (all with AGA IgG on a gluten containing diet) after 6-months-GFD	**Good response to GFD 30/40**		**Mild response to GFD 10/40**	
Compliance	AGA IgG+	AGA IgG-	AGA IgG +	AGA IgG-
Strict 32/40	11	14	2	5
Low 8/40	2	3	1	2

Levels of antibodies to gliadin, glutenin, and albumin/globulin proteins of wheat, and to TG2 and deamidated gliadin in NCGS patients and controls



Alaedini A & Volta U
Columbia Univ, NY
Univ. of Bologna
unpublished data

GASTROENTEROLOGY 2013;144:903–911

CLINICAL—ALIMENTARY TRACT

A Controlled Trial of Gluten-Free Diet in Patients With Irritable Bowel Syndrome-Diarrhea: Effects on Bowel Frequency and Intestinal Function

MARIA I. VAZQUEZ–ROQUE,[1,2] MICHAEL CAMILLERI,[1] THOMAS SMYRK,[3] JOSEPH A. MURRAY,[1] ERIC MARIETTA,[1] JESSICA O'NEILL,[1] PAULA CARLSON,[1] JESSE LAMSAM,[4] DENISE JANZOW,[5] DEBORAH ECKERT,[1] DUANE BURTON,[1] and ALAN R. ZINSMEISTER[6]

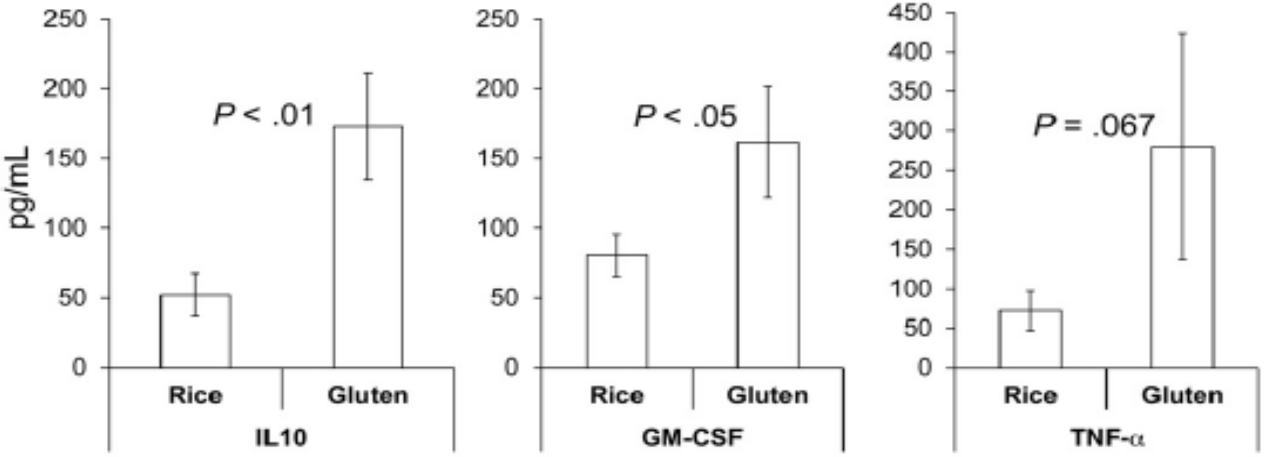


Figure 3. In vitro cytokine production by PBMCs in response to stimulation with either rice (control) or gluten. Note the increased production of IL-10 and GM-CSF, and the borderline increased TNF-α in response to gluten, compared with rice.

Increased levels of IL-10, TNF-α, granulocyte-colony-stimulating factor by PBMC from HLA-DQ2/DQ8+ IBS patients with NCGS after stimulation with gliadin extracts

Food Chemistry 176 (2015) 167–174

Contents lists available at ScienceDirect

Food Chemistry

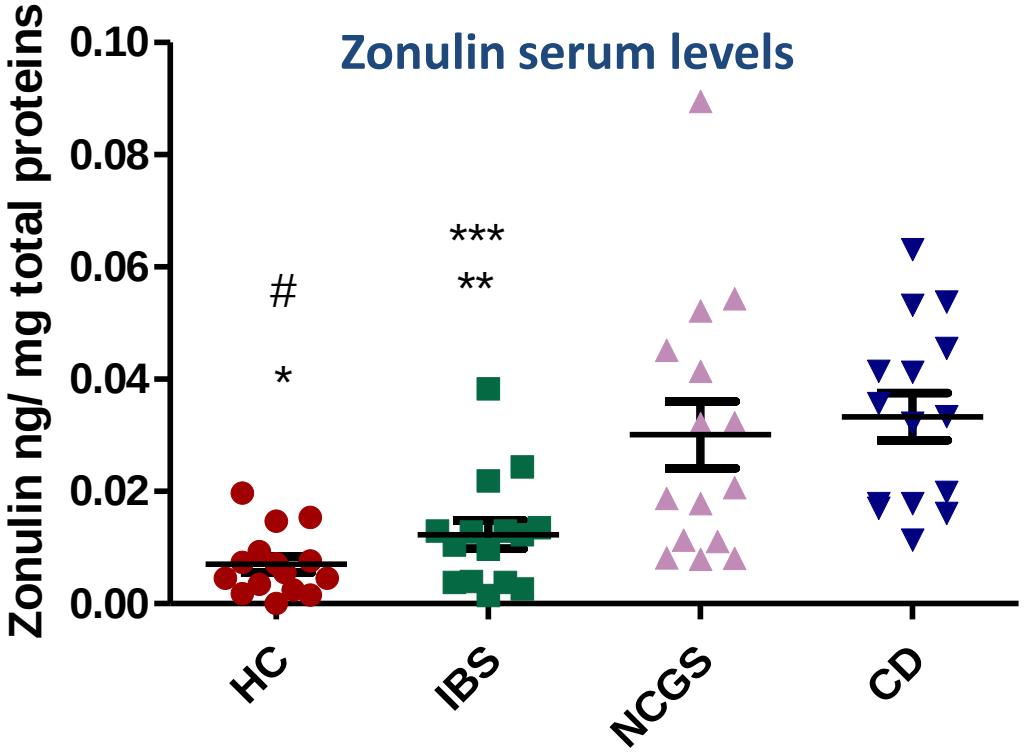
journal homepage: www.elsevier.com/locate/foodchem

Responses of peripheral blood mononucleated cells from non-celiac gluten sensitive patients to various cereal sources

Maria Chiara Valerii [a,1], Chiara Ricci [b,1], Enzo Spisni [a,*], Raffaella Di Silvestro [c], Luigia De Fazio [a], Elena Cavazza [a], Alberto Lanzini [b], Massimo Campieri [d], Alessandro Dalpiaz [e], Barbara Pavan [f], Umberto Volta [d], Giovanni Dinelli [c]

Fig. 3. CXCL10 secretion by cultured PBMC stimulated with different wheat protein extracts. PBMC obtained from healthy donors (A) or from NCGS patients (B) were stimulated for 24 h with total wheat protein extracts from different wheat cultivars. The amount of secreted CXCL10, measured by using Luminex® assay, is indicated as mean ± 1SD. a, $P < 0.001$ *vs.* Manitoba group. b, $P < 0.001$ *vs.* rice group.

Increased serum levels of zonulin in NCGS



*HC vs CD *P*< 0,0001
**IBS vs NCGS *P*<0,05
***IBS vs CD *P*< 0,001
#HC vs NCGS *P*<0,001

Zonulin serum levels were significantly different among the four groups (p<0.001).

HC vs IBS: P=0,1 NCGS vs CD: P=0,4

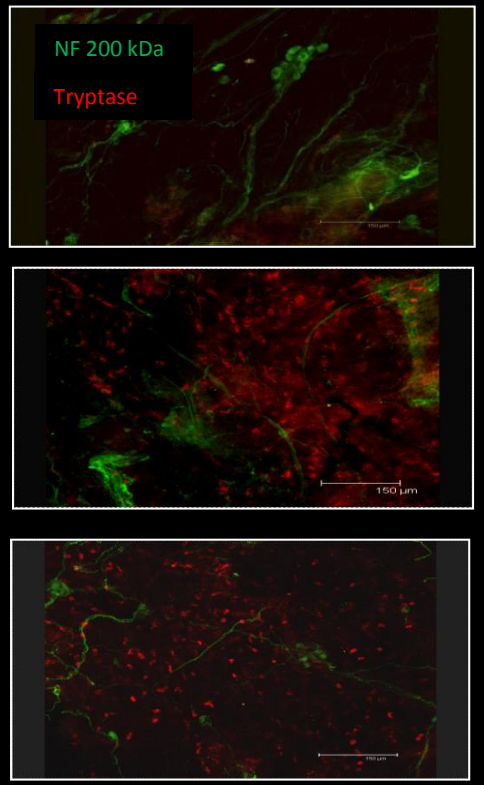
Barbaro MR , Volta U et al, UEG week 2014, DDW 2015

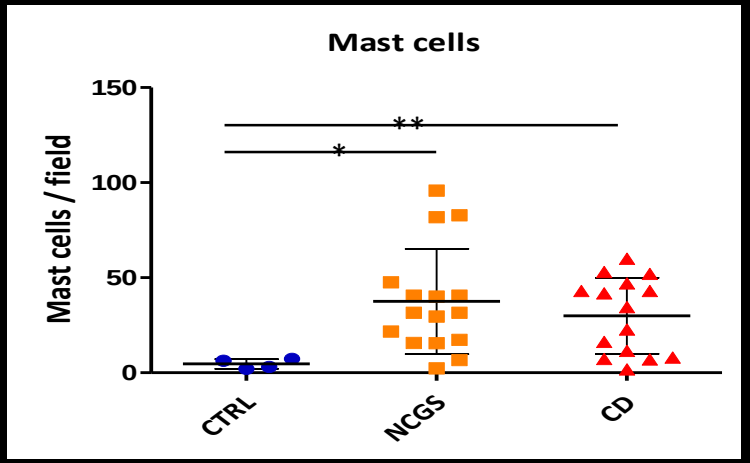
Submucosal mast cell density in NCGS

CTRL
N= 6

NCGS
N= 16

CD
N= 15





CD vs. NCGS ns; * CTRL *vs.* NCGS $P < 0.05$; ** CTRL vs. CD $P < 0.05$

Mast cell density is associated with alternating bowel habits in "IBS-like" NCGS pts

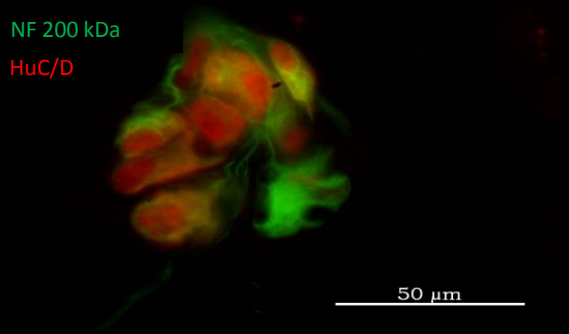
Giancola, Volta, Caio, et al. unpublished

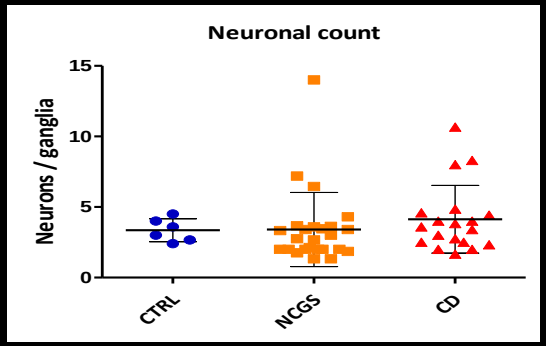
Neuro-immune analysis in NCGS

Submucosal plexus from duodenal mucosal routine biopsies during EGDS

CONTROLS N=6	NCGS N=16	CD N=15

Submucosal neuron quantitative evaluation





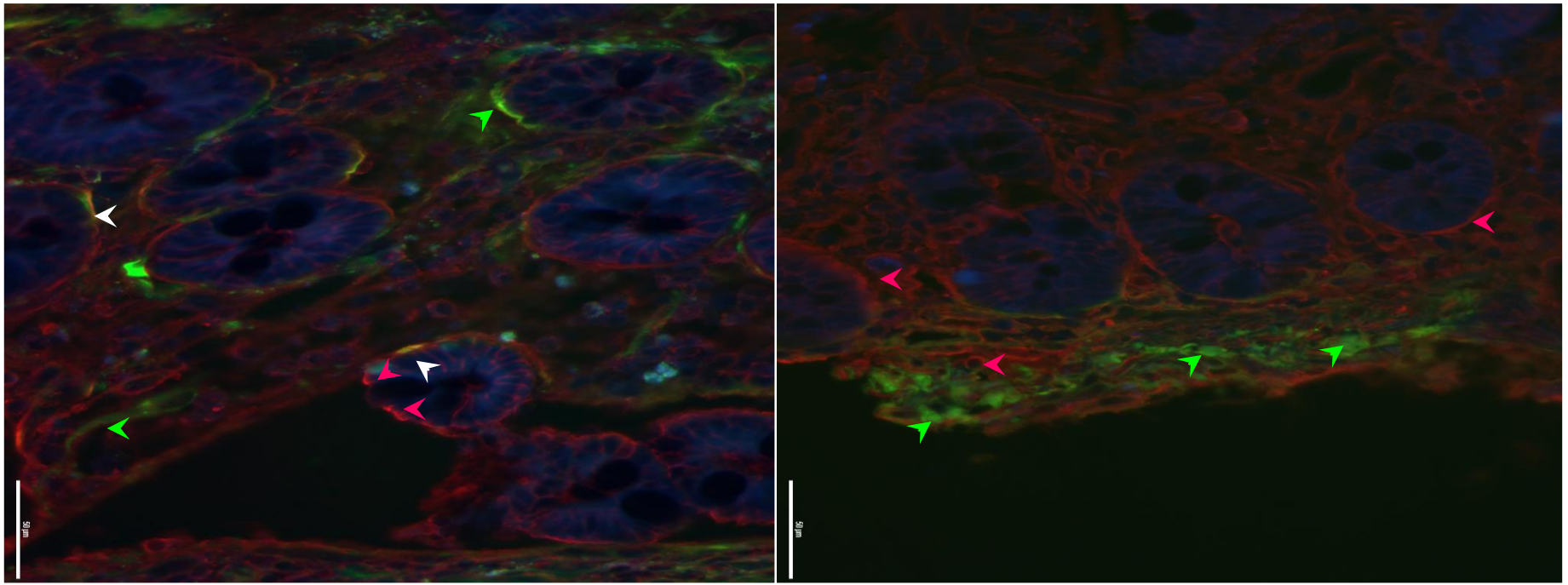
Statistical analysis by One-Way ANOVA showed no significant difference between groups

Giancola, Volta, Caio, et al. unpublished

Intestinal deposits of IgA anti-TG2

IgA anti-TG2 deposits in HLA-DQ2+ NCGS

No IgA anti-TG2 deposit in a NCGS patient



TG2 (green arrow) at the basement membrane and in the epithelium levels, IgA (red arrow) and deposits of IgA anti-TG2 (white arrow)

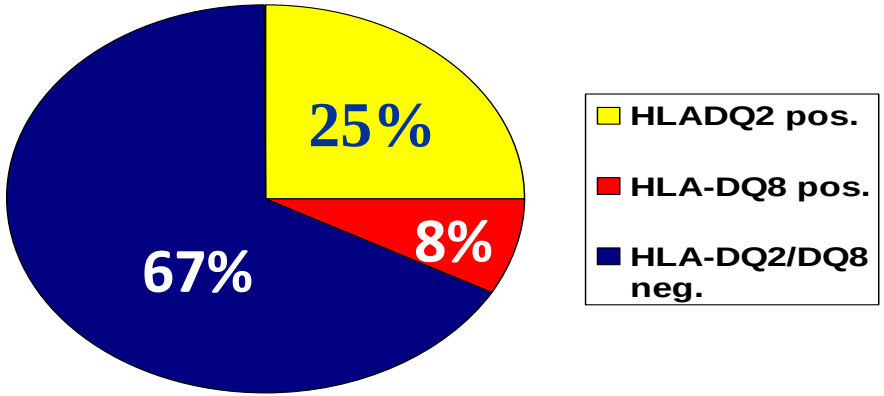
Volta et al. BMC Medicine 2014, 12:85
http://www.biomedcentral.com/1741-7015/12/85

BMC Medicine

RESEARCH ARTICLE Open Access

An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity

Umberto Volta[1*], Maria Teresa Bardella[2], Antonino Calabrò[3], Riccardo Troncone[4], Gino Roberto Corazza[5] and The Study Group for Non-Celiac Gluten Sensitivity



➢ **So far no genetic marker identified (no correlation with HLA-DQ2 and-DQ8)**

Volta et al. BMC Medicine 2014, 12:85
http://www.biomedcentral.com/1741-7015/12/85

BMC Medicine

RESEARCH ARTICLE Open Access

An Italian prospective multicenter survey on patients suspected of having non-celiac gluten sensitivity

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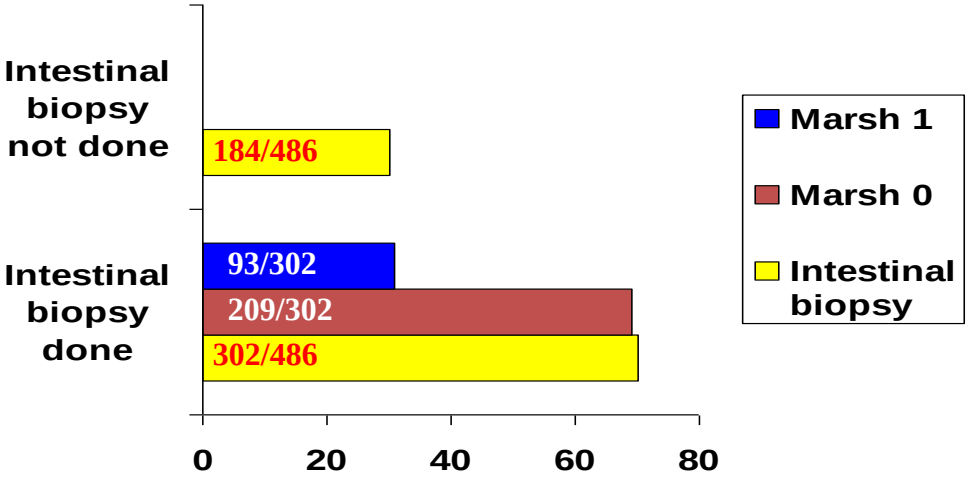

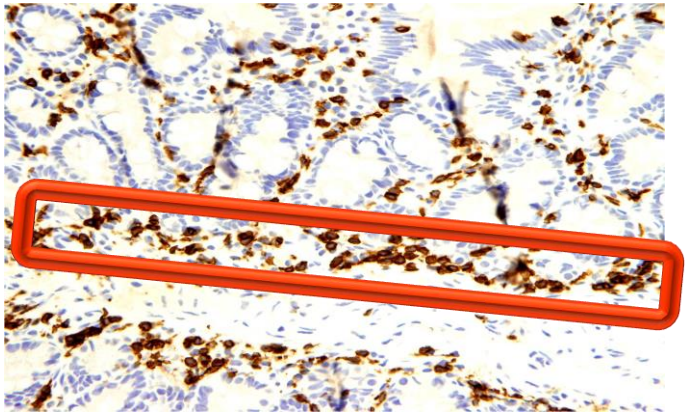

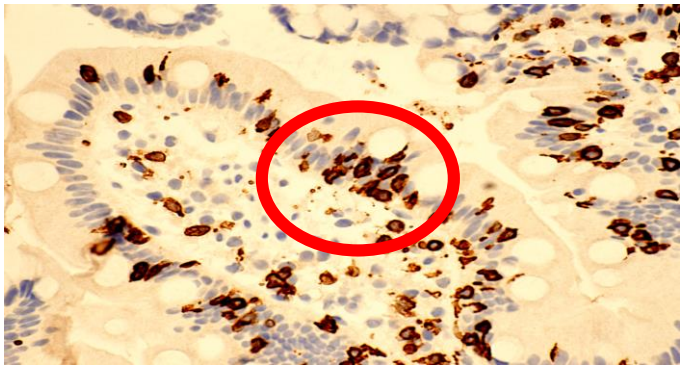

CD3+ T lymphocytes:
linear distribution in the deeper part
of the mucosa and clusters in the villi

Possible histological pattern of NCGS?

Villanacci V et al., Am J Gastroenterol 2013

Take home message

- The identification of biomarkers is a key point for confirming the diagnosis of NCGS
- Their availability would allow to switch from an exclusion diagnosis towards a diagnosis based on positive criteria
- No biomarker for NCGS is available, but there are many attempts for identifying them (AGA characterization, chemokine secretion from PBMC, serum zonulin markers, submucosal mast cell and neuron density, IgA deposits to TG2)
- About 50% of NCGS patients are positive for antibodies to native gliadin (mainly of IgG class), which disappear quickly after GFD together with symptom disappearance or improvement
- Antibodies to native gliadin are not specific nor highly sensitive for NCGS, but for the time being they are the only test whose positivity in patients with suspected NCGS can contribute to the diagnosis

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