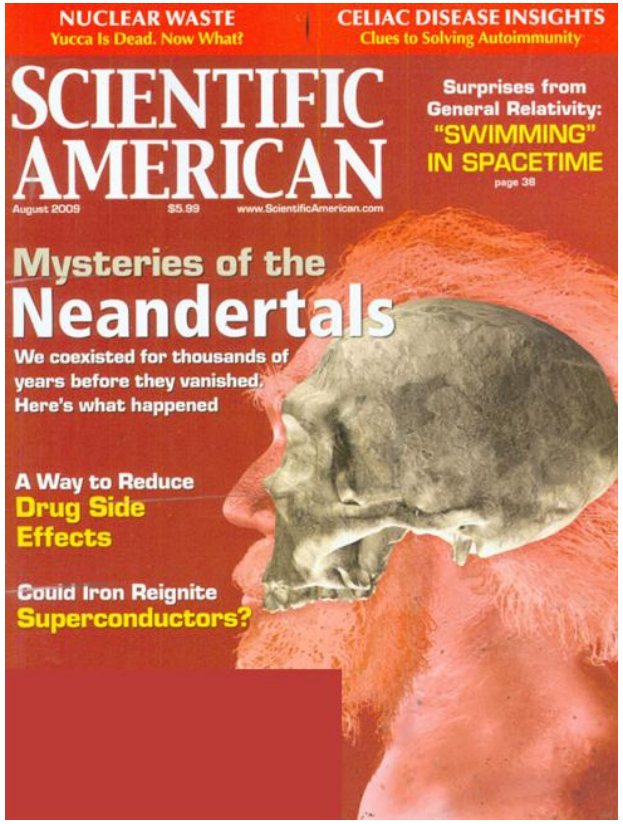
Multicenter Trial Italy-USA GS



Anna Sapone, MD, PhD

Gluten Sensitivity: Definition

Cases of gluten reaction in which both allergic and autoimmune mechanisms have been ruled out (diagnosis by exclusion criteria)

- Negative immuno-allergy tests to wheat;
- Negative CD serology (EMA and/or tTG) and in which IgA deficiency has been ruled out;
- Negative duodenal histopathology;
- Presence of biomarkers of gluten immune-reaction (AGA+);
- Presence of clinical symptoms that can overlap with CD or wheat allergy symptomatology;
- Resolution of the symptoms following implementation of a GFD (double blind)

MUCOSAL BIOLOGY RESEARCH CENTER

Original Paper

International Archives of Allergy and Immunology

Int Arch Allergy Immunol 2010;152:75–80
DOI: 10.1159/000260087

Differential Mucosal IL-17 Expression in Two Gliadin-Induced Disorders: Gluten Sensitivity and the Autoimmune Enteropathy Celiac Disease

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Maria Cartenì[a] Vincenzo Casolaro[b, e] Alessio Fasano[b]

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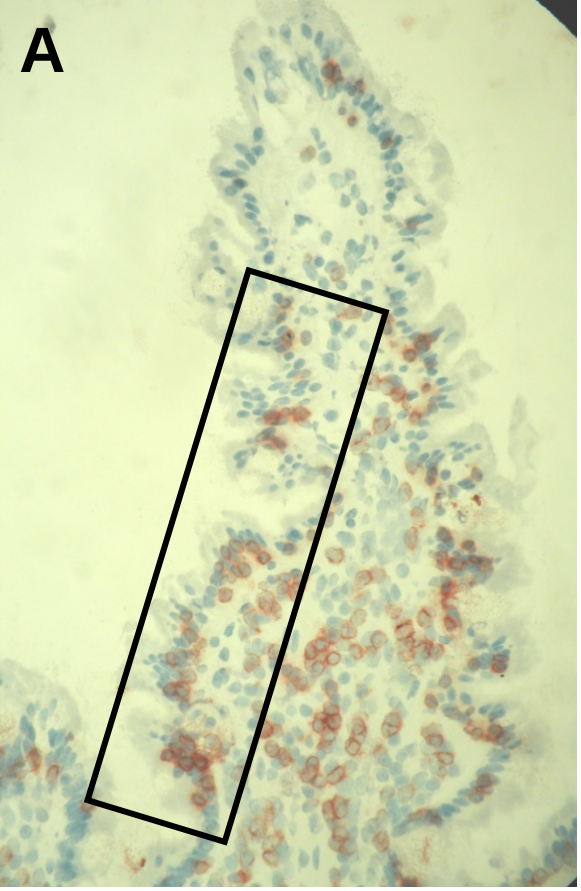
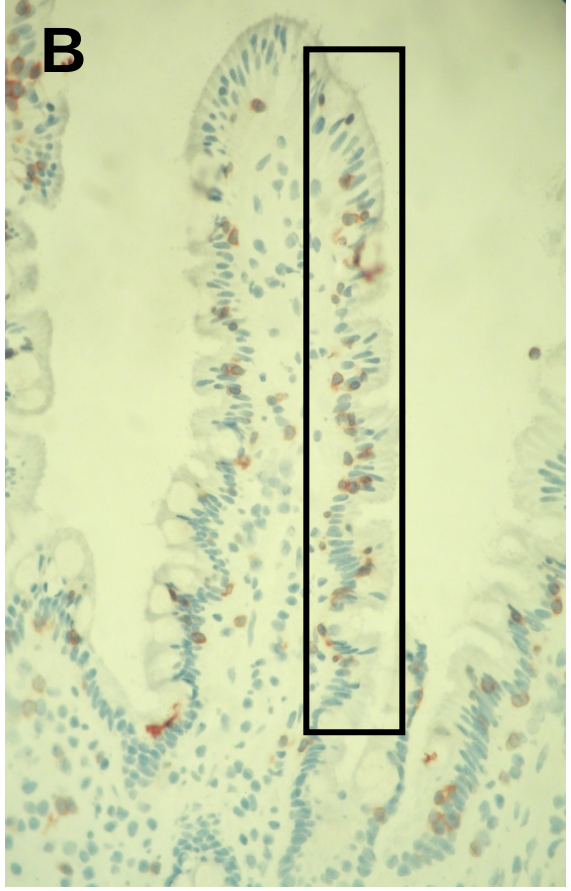
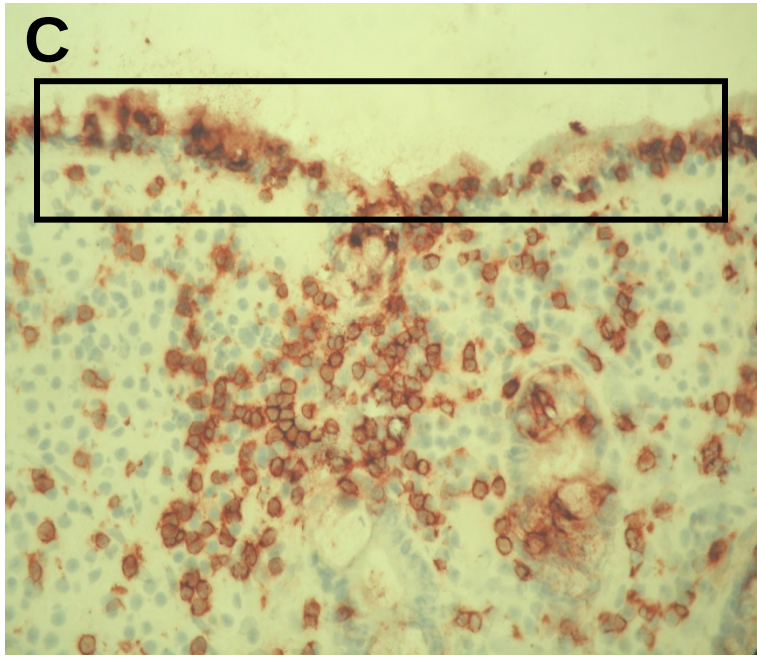
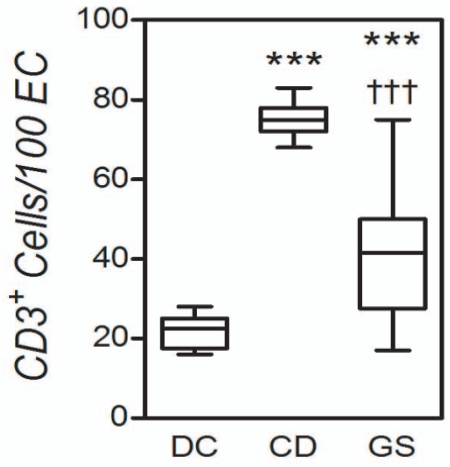
Duodenal IHC CD3+ IELs







40 X



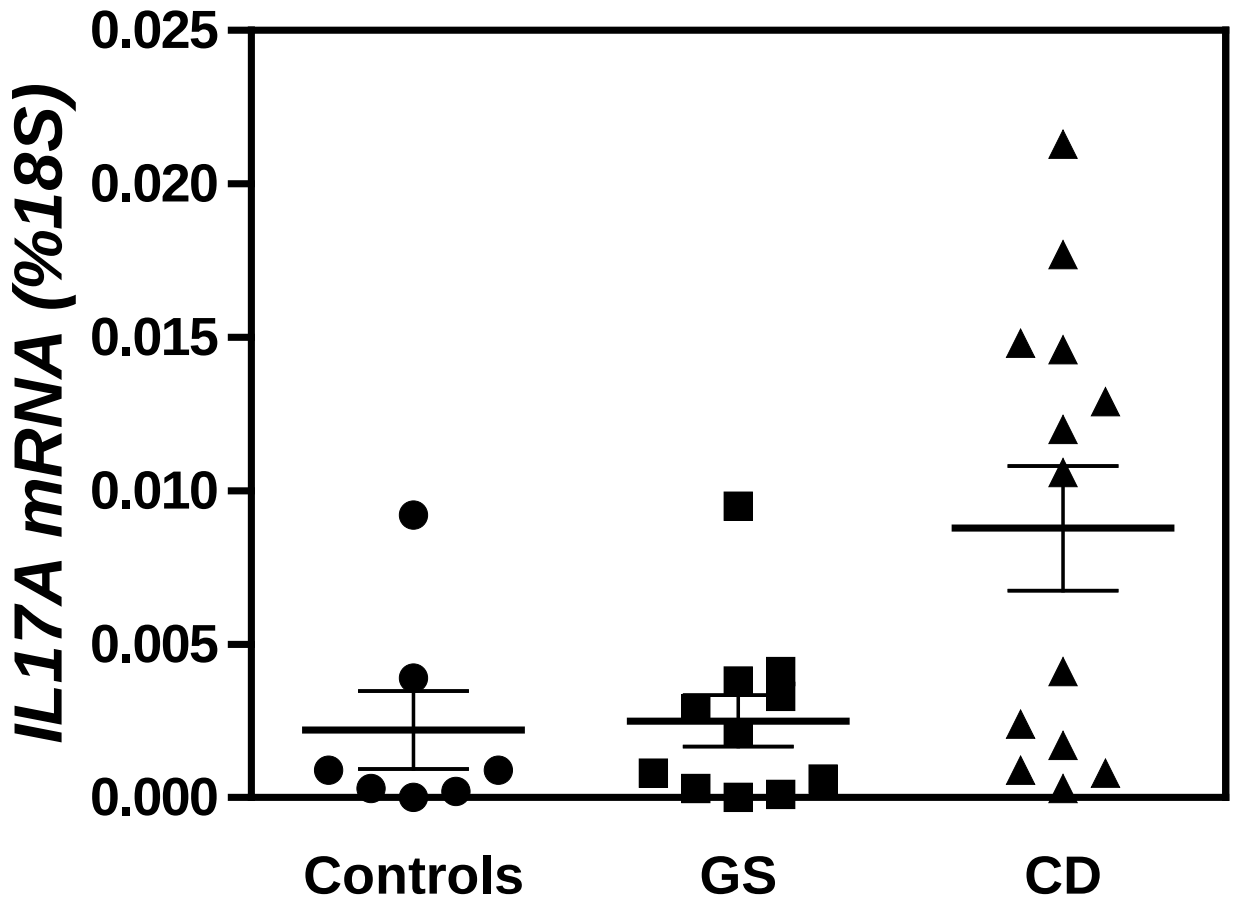
A= Gluten Sensitive

B= Controls

C= CD Active

Sapone A. et al. *Intern Arch Aller Immunol* 2010

The Autoimmune-Related Cytokine IL17 is Elevated in Celiac Disease but not Gluten Sensitivity



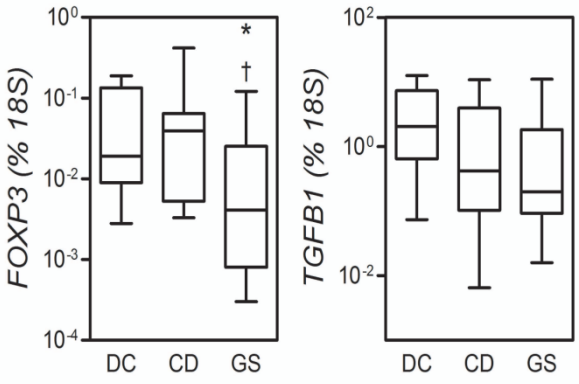
Sapone A. et al. *Intern Arch Aller Immunol* 2010

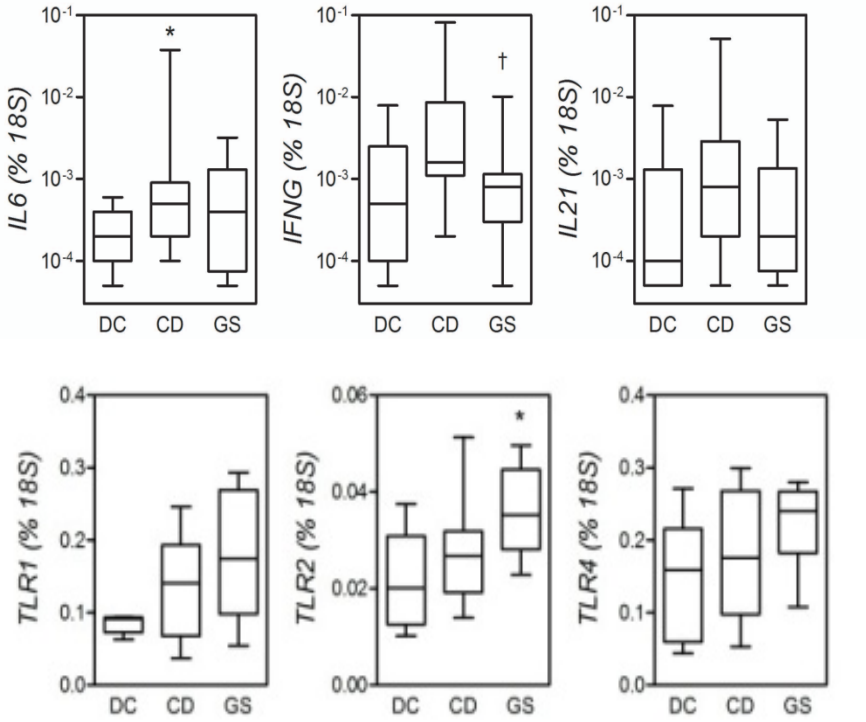
Sapone *et al. BMC Medicine* 2011, **9**:23
http://www.biomedcentral.com/1741-7015/9/23

BMC Medicine

RESEARCH ARTICLE Open Access

Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity

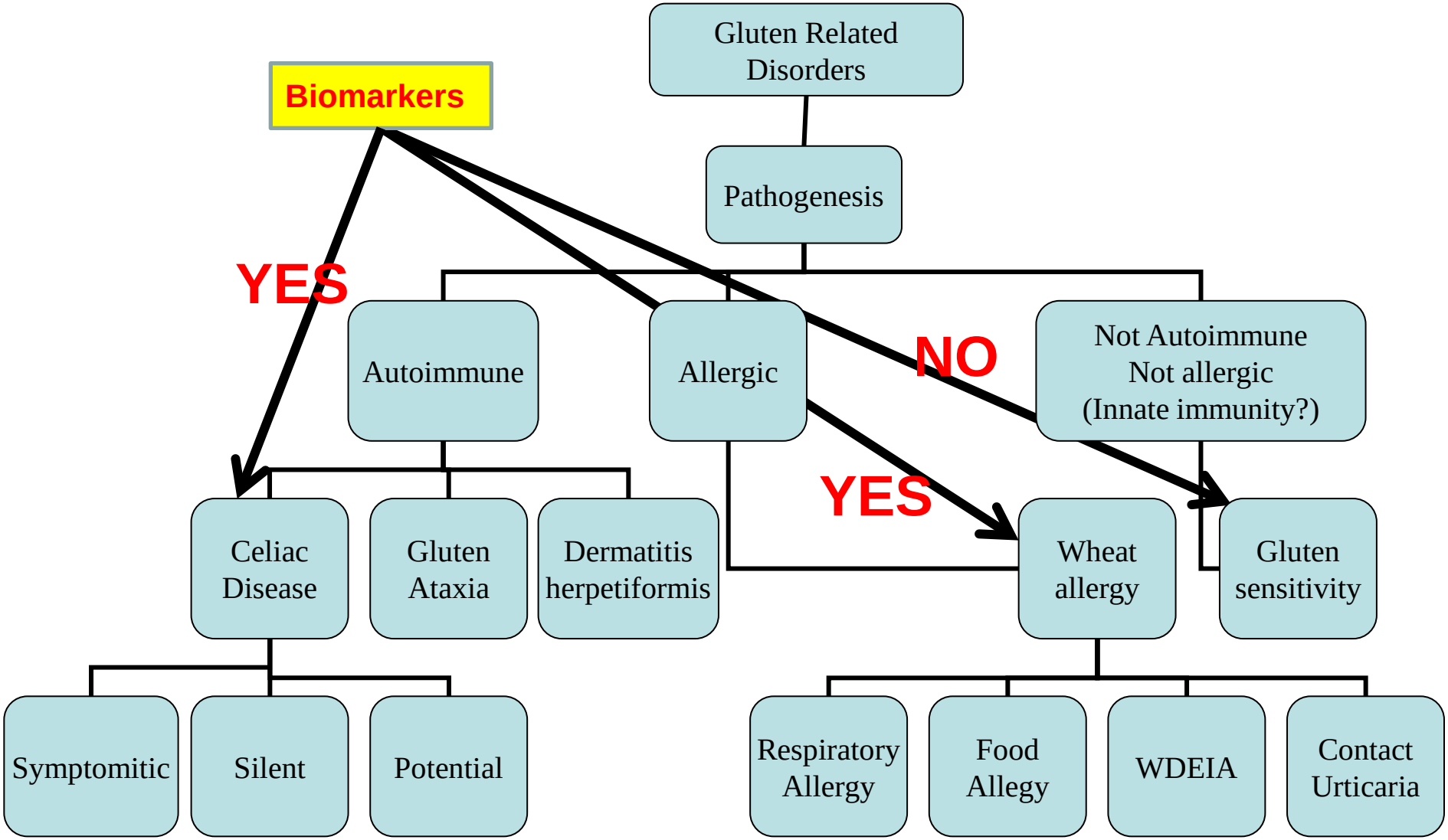




Differential Diagnosis Between CD, GS, and WA

	Celiac Disease	Gluten Sensitivity	Wheat Allergy
Time interval between gluten exposure and onset of symptoms	Weeks-Years	Hours-Days	Minutes-Hours
Pathogenesis	Autoimmunity (Innate+ Adaptive Immunity)	Immunity? (Iinnate Immunity?)	Allergic Immune Response
HLA	HLA DQ2/8 restricted (~97% positive cases)	Not-HLA DQ2/8 restricted (50% DQ2/8 positive cases)	Not-HLA DQ2/8 restricted (35-40% positive cases as in the general population)
Auto-antibodies	Almost always present	Always absent	Always absent
Enteropatia	Almost always present	Always absent (slight increase in IEL)	Always absent (eosinophils in the lamina propria)
Symptoms	Both intestinal and extra-intestinal (not distinguishable from GS and WA with GI symptoms)	Both intestinal and extra-intestinal (not distinguishable from CD and WA with GI symptoms)	Both intestinal and extra-intestinal (not distinguishable from CD and GS when presenting with GI symptoms)
Complications	Co-morbidities Long term complications	Absence of co-morbidities and long term complications (long follow up studies needed to confirm it)	Absence of co-morbidities. Short-term complications (incliuding anaphylaxis)

Proposed New Classification of Gluten Related Disorders



Gluten Sensitivity in Non-Celiac Patients (GS)

This study is currently recruiting participants.

Verified November 2012 by Second University of Naples

Sponsor:
Second University of Naples

Information provided by (Responsible Party):
Laura de Magistris, Second University of Naples

ClinicalTrials.gov Identifier:
NCT01485341

First received: November 17, 2011
Last updated: November 13, 2012
Last verified: November 2012
History of Changes

Gluten Causes Gastrointestinal Symptoms in Subjects Without Celiac Disease: A Double-Blind Randomized Placebo-Controlled Trial

Jessica R. Biesiekierski, B Appl Sci[1], Evan D. Newnham, MD, FRACP[1], Peter M. Irving, MD, MRCP[1], Jacqueline S. Barrett, PhD, BSc, MND[1], Melissa Haines, MD[1], James D. Doecke, BSc, PhD[2], Susan J. Shepherd, B Appl Sci, PhD[1], Jane G. Muir, PhD, PGrad Dip(Dietetics)[1] and Peter R. Gibson, MD, FRACP[1]

OBJECTIVES: **Despite increased prescription of a gluten-free diet for gastrointestinal symptoms in individuals who do not have celiac disease, there is minimal evidence that suggests that gluten is a trigger. The aims of this study were to determine whether gluten ingestion can induce symptoms in non-celiac individuals and to examine the mechanism.**

METHODS: **A double-blind, randomized, placebo-controlled rechallenge trial was undertaken in patients with irritable bowel syndrome in whom celiac disease was excluded and who were symptomatically controlled on a gluten-free diet. Participants received either gluten or placebo in the form of two bread slices plus one muffin per day with a gluten-free diet for up to 6 weeks. Symptoms were evaluated using a visual analog scale and markers of intestinal inflammation, injury, and immune activation were monitored.**

RESULTS: **A total of 34 patients (aged 29–59 years, 4 men) completed the study as per protocol. Overall, 56% had human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8. Adherence to diet and supplements was very high. Of 19 patients (68%) in the gluten group, 13 reported that symptoms were not adequately controlled compared with 6 of 15 (40%) on placebo ($P=0.0001$; generalized estimating equation). On a visual analog scale, patients were significantly worse with gluten within 1 week for overall symptoms ($P=0.047$), pain ($P=0.016$), bloating ($P=0.031$), satisfaction with stool consistency ($P=0.024$), and tiredness ($P=0.001$). Anti-gliadin antibodies were not induced. There were no significant changes in fecal lactoferrin, levels of celiac antibodies, highly sensitive C-reactive protein, or intestinal permeability. There were no differences in any end point in individuals with or without DQ2/DQ8.**

CONCLUSIONS: **"Non-celiac gluten intolerance" may exist, but no clues to the mechanism were elucidated.**

Am J Gastroenterol 2011; 106:508–514; doi:10.1038/ajg.2010.487; published online 11 January 2011

Am J Gastroenterol. 2012 Jul 24. doi: 10.1038/ajg.2012.236. [Epub ahead of print]

Non-Celiac Wheat Sensitivity Diagnosed by Double-Blind Placebo-Controlled Challenge: Exploring a New Clinical Entity.

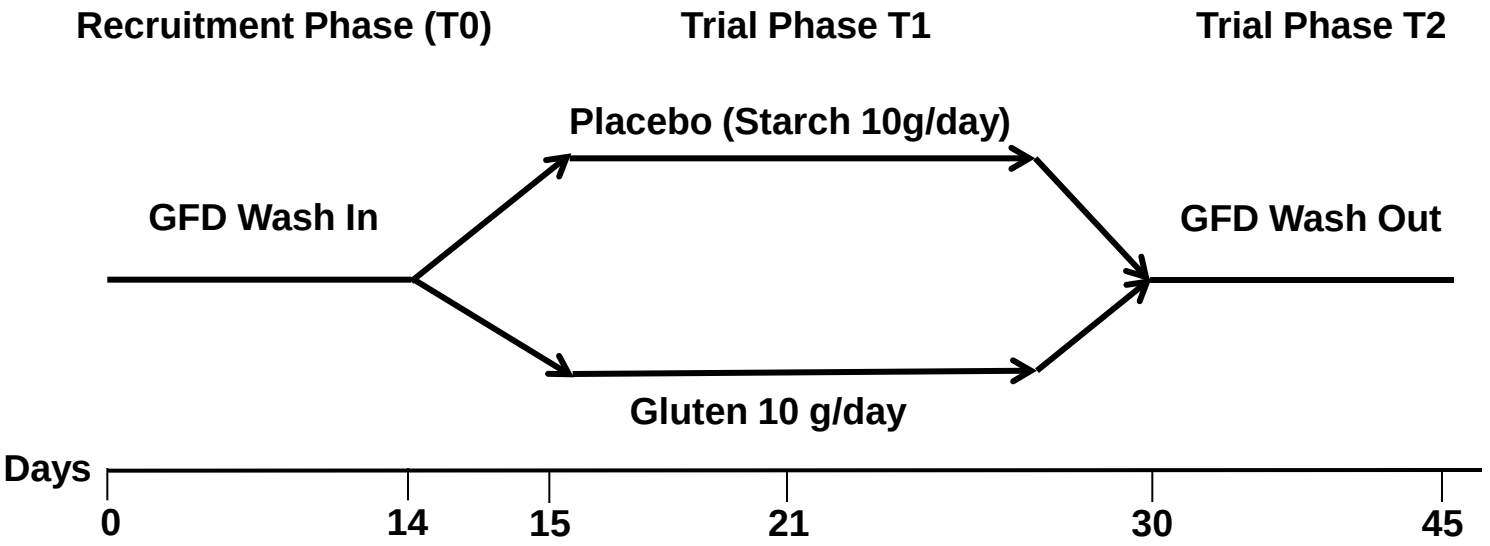
Carroccio A, Mansueto P, Iacono G, Soresi M, D'Alcamo A, Cavataio F, Brusca I, Florena AM, Ambrosiano G, Seidita A, Pirrone G, Rini GB.

Division of Internal Medicine, Hospital of Sciacca, ASP, Agrigento, Italy.

Abstract

OBJECTIVES:Non-celiac wheat sensitivity (WS) is considered a new clinical entity. An increasing percentage of the general population avoids gluten ingestion. However, the real existence of this condition is debated and specific markers are lacking. Our aim was thus to demonstrate the existence of WS and define its clinical, serologic, and histological markers.METHODS:We reviewed the clinical charts of all subjects with an irritable bowel syndrome (IBS)-like presentation who had been diagnosed with WS using a double-blind placebo-controlled (DBPC) challenge in the years 2001-2011. One hundred celiac disease (CD) patients and fifty IBS patients served as controls.RESULTS:Two hundred and seventy-six patients with WS, as diagnosed by DBPC challenge, were included. Two groups showing distinct clinical characteristics were identified: WS alone (group 1) and WS associated with multiple food hypersensitivity (group 2). As a whole group, the WS patients showed a higher frequency of anemia, weight loss, self-reported wheat intolerance, coexistent atopy, and food allergy in infancy than the IBS controls. There was also a higher frequency of positive serum assays for IgG/IgA anti-

In Search of GS Biomarkers: Study Design



Interventions

- blood samples
- urine samples
- stool samples;
- symptoms evaluation scales (GSRS, extra-intestinal, modified Zung and STAY-Y1

- blood samples
- urine samples
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- Symptoms diary

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- Intestinal biopsy
- blood samples
- urine samples
- stool samples;
- symptoms evaluation scales (GSRS, extra-intestinal, modified Zung and STAY-Y1
- Symptoms diary

- blood samples
- urine samples
- stool samples;
- symptoms evaluation scales (GSRS, extra-intestinal, modified Zung and STAY-Y1
- Symptoms diary

Double Blind Randomized Placebo Controlled Multicentric Trial (gluten vs placebo) in *Gluten Sensitive* Subjects”

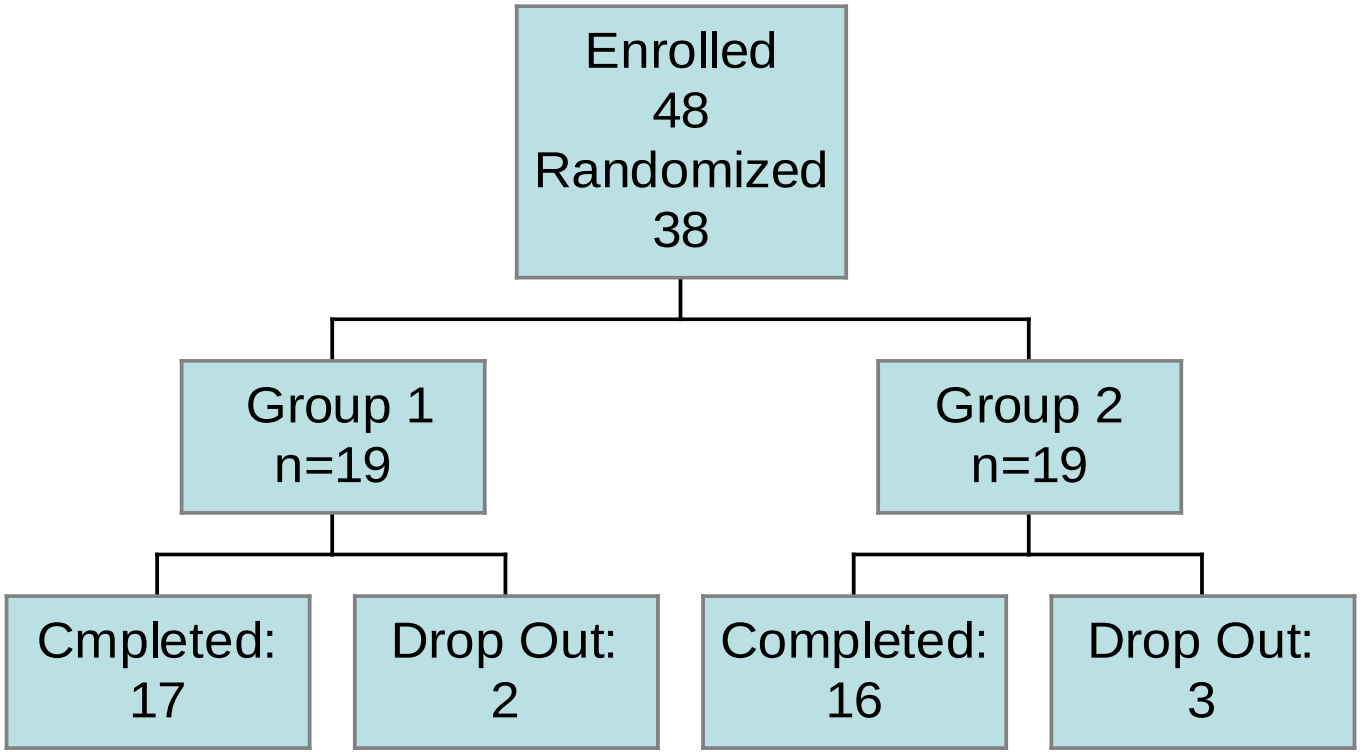
Participating Centers

- Gastroenterologia e Chirurgia Endoscopica della Seconda Università degli Studi di Napoli
- AOU S.Giovanni di Dio e Ruggi D'Aragona, Università di Salerno
- Unità di Gastroenterologia ed Endoscopia digestiva dell’Ospedale San Giuseppe Moscati di Avellino
- Ospedale "Casa Sollievo della Sofferenza" - IRCCS - San Giovanni Rotondo (Foggia)

Coordinating Center

- Center for Celiac Research, University of Maryland

Double Blind Randomized Placebo Controlled Multicentric Trial (gluten vs placebo) in *Gluten Sensitive* Subjects Update on Recruitment November 2012



PRIMARY ENDPOINT
Clinical Scores

SECONDARY ENDPOINTS
Validation of Biomarkers:

Serum, urine, feces: T0, T1, and T2
Intestinal Biopsy: T1

Double Blind Randomized Placebo Controlled Multicentric Trial (gluten vs placebo) in *Gluten Sensitive* Subjects”

Primary Endpoint

Evaluation Scales:

-GSRS (gastrointestinal symptoms);

-SeG (extra-intestinal symptoms);

-VQV (Quality of Life);

Global score/subject/observation MIN=40 MAX=158

Potential Serum GS Biomarkers To Be Validated During the Trial

- **Chemokine IL-8;**
- **Conventional (native) anti-gliadin antibodies (AGA) of both class IgA and IgG;**
- **Anti-DPG**
- **Anti- tissue transglutaminase (tTG) 6 antibodies;**
- **Amylase Trypsin Inhibitors (ATIs);**
- **α Amilase.**

Potential GS Biomarkers To Be Validated During the Trial

Peripheral Blood

- PBMC and neutrophils isolation and their stimulation with gluten to evaluate gene expression for: pro-inflammatory cytokines IL1ß, IL2, IL6, TNF- , IFN- , regulatory cytokines IL10, TGF-ß, IL4, IL23, IL17 A, chemokine IL8, regulatory molecules CD3, CD4, CD8, CD25, co-stimulatory molecules MHC-II CD40, CD80, CD86, CD28,transduction molecules HMGB1.
- Dendritic cells (DCs) isolated from the venous peripheral blood and stimulated with gliadin. DCs and Tcells areco-cultured, with a ratio 1:10, for 48 and 72 hours.IFN , IL-17, IL-15, IL-12, IL-6 production in the supernatant is determined by ELISA test.

Potential GS Biomarkers To Be Validated During the Trial

Urine

- Intestinal permeability, administration of lactulose/mannitol test (LA/MA)

Potential GS Biomarkers To Be Validated During the Trial

Intestinal Biopsies

- Tight junction genes expression (Occludin, Claudin 1, 2, 3, 4, ZO1) and Toll Like Receptors (TLR 1, 2 and 4) .
- Regulatory molecules gene expression FOXP3, HMGB1, regulatory cytokines IL10, TGF-ß, IL 1ß, IL 23, IL17 A.
- Expression of CD83, CD25, and COX-2 on dendritic cells and macrophages; MICA and HLA-E on epithelial cells; CD3 and CD94 on intraepithelial T lymphocytes as well as to a series of different markers to analyze the adaptive immune response (CD25 on T lymphocytes, ICAM1, CD80) on dendritic cells and macrophages.
- Expression of activated CD3 cells (CD3+CD25+) and activated macrophages (CD68+CD25+).

Potential GS Biomarkers To Be Validated During the Trial

Stools

- Microbiota analysis
- Metabolome analysis

Future Directions

- **Continue recruitment to reach the final number of 102 subjects completing the trial;**
- **Open recruitment in USA in 2013;**
- **Interim analysis once 50% recruitment will be reached;**
- **Multivariate analysis to validate biomarkers**