Celiac Disease: Managing, Monitoring and Research

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Milestone #1 (1997): What is the Recipe to Develop Celiac Disease
The Holy Trinity of the Autoimmune Mechanisms in Celiac Disease

Three factors underlie celiac disease: an environmental trigger, a genetic susceptibility and, according to the author’s research, an unusually permeable gut (below). The author suspects that the same basic triad contributes to other autoimmune diseases, although each disorder will have its own triggers and genetic components.

A TRIO OF CAUSES

Three factors underlie celiac disease: an environmental trigger, a genetic susceptibility and, according to the author’s research, an unusually permeable gut. The author suspects that the same basic triad contributes to other autoimmune diseases, although each disorder will have its own triggers and genetic components.

TRIGGER

The gluten protein, abundant in the endosperm of wheat kernels, sets off the aberrant immune response. Related proteins in barley and rye (hordein and secalin) do the same.

GENETIC PREDISPOSITION

Almost all patients harbor the genes HLA-DQ2 or HLA-DQ8, or both. These genes give rise to proteins of the same name that display gluten fragments to immune system cells, which then attack the intestinal lining. Other genes are likely to be involved as well, but these additional culprits may differ from person to person.

LEAKY SMALL INTESTINE

In most people, links known as tight junctions “glue” intestinal cells together. In those with celiac disease, the junctions come apart, allowing a large amount of indigestible gluten fragments to seep into the underlying tissue and incite an immune response. This can lead to potentially serious consequences, not only for celiac disease but also for other autoimmune disorders involving unusually permeable intestines.
Several genes are involved

The most consistent genetic component depends on the presence of HLA-DQ (DQ2 and/or DQ8) genes

Other genes (not yet identified) account for 60% of the inherited component of the disease

HLA-DQ2 and/or DQ8 genes are necessary (No DQ2/8, no Celiac Disease!) but not sufficient for the development of the disease
Intestine: Interesting Facts

~20 ft long

~3,000 sf!!!
The Paracellular Pathway

Tight junctions are a 'dark horse' implicated in a host of disease states, ranging from acute injury to chronic inflammation and autoimmune diseases.
Zonulin Gene Is Located on Chromosome 16

Chromosome 16 contains about 98 million bases, or some 3% of the human genome, encoding for ~1,300 genes.

Fasano A. Physiol Rev. 2011 Jan;91(1):151-75
MILESTONE #2: Understanding Why Gluten is Toxic
Dietary Factors of CD
The Grass Family - (GRAMINEAE)

Subfamily
- Festucoideae

Tribe
- Hordeae
- Oryzeae
- Zizaneae
- Aveneae
- Festuceaea
- Chlorideae

Grains:
- Wheat
- Rye
- Barley
- Rice
- Wild Rice
- Oat
- Finger Millet
- Teff

Years
- 2.5 M
  - The Human race appears on the face of hearth

Diet
- Fruits, nuts, tubers
- Occasional meat

Change from nomadic to settled life style
- 10,000

Advent of agriculture
- Development of gluten containing grains
- 2009

Advent of agriculture
- Development of gluten containing grains
- 2009
How Agriculture Evolved?
Grain Evolution

T. turgidum  AABB
28 chromosomes
100,000 genes

Aegilops tauschii  DD
14 chromosomes
50,000 genes

T. aestivum  AABBDD
42 chromosomes
150,000 genes
Making Bread in Egypt
THE GRAINS IN THE PAST WERE DIFFERENT FROM THE CURRENT GRAINS

Pieter Bruegel, 1565
What is so Special About Gluten?

Gliadin

Glutenin

Gluten (gliadin+glutenin)
Mapping of α-gliadin motifs exerting cytotoxic activity (red), immunomodulatory activity (light green), zonulin release and gut permeating activity (blue) and CXCR3-dependent IL8 release in CD patients (dark green).


Lammer K et al, *Immunology*. 2011;132:432-40


Gluten Causes Gut Leakiness in Everybody

MILESTONE #3 (2000): The Evolving Spectrum of CD Diagnosis
Serological Test Comparison

**Table 1. Serum Tests for the Diagnosis of Celiac Disease.**

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (Range)</th>
<th>Specificity (Range)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA anti-tTG antibodies</td>
<td>&gt;95.0 (73.9–100)</td>
<td>&gt;95.0 (77.8–100)</td>
<td>Recommended as first-level screening test</td>
</tr>
<tr>
<td>IgG anti-tTG antibodies</td>
<td>Widely variable (12.6–99.3)</td>
<td>Widely variable (86.3–100)</td>
<td>Useful in patients with IgA deficiency</td>
</tr>
<tr>
<td>IgA anti-endomysial antibodies</td>
<td>&gt;90.0 (82.6–100)</td>
<td>98.2 (94.7–100)</td>
<td>Useful in patients with an uncertain diagnosis</td>
</tr>
<tr>
<td>IgG DGP</td>
<td>&gt;90.0 (80.1–98.6)</td>
<td>&gt;90.0 (86.0–96.9)</td>
<td>Useful in patients with IgA deficiency and young children</td>
</tr>
<tr>
<td>HLA-DQ2 or HLA-DQ8</td>
<td>91.0 (82.6–97.0)</td>
<td>54.0 (12.0–68.0)</td>
<td>High negative predictive value</td>
</tr>
</tbody>
</table>

* Data are from Husby et al. and Giersiepen et al. DGP denotes deaminated gliadin peptides, and tTG tissue trans-glutaminase.

*Fasano & Catassi NEJM 2012;367:2419-26*
Diagnosis: The Future to Come

• Diagnostic algorithms to avoid intestinal biopsy;

• Biomarkers to predict onset of celiac disease in genetically susceptible individuals;

• Host-intestinal microbiome interaction;

• Pharmacometabonomics.
Celiac Disease Diagnosis: Simple Rules Are Better Than Complicated Algorithms

Carlo Catassi, MD, MPH, a,b Alessio Fasano MD a

aMucosal Biology Research and Center for Celiac Research, University of Maryland, School of Medicine, Baltimore; bUniversità Politecnica delle Marche, Ancona, Italy.

ABSTRACT

Celiac disease is the only treatable autoimmune disease, provided that a correct diagnosis is achieved and a strict, lifelong gluten-free diet is implemented. The current diagnostic algorithm for celiac disease includes initial screening serological tests, followed by a confirmatory small intestinal biopsy showing the autoimmune insult typical of celiac disease. The biopsy, considered the diagnostic gold standard, has been recently questioned as a reliable and conclusive test for every case. Indeed, the wide variability of celiac disease-related findings suggests that it is difficult to conceptualize the diagnostic process into rigid algorithms that do not always cover the clinical complexity of this disease. Instead we find clinically useful the shifting to a quantitative approach that can be defined as the “4 out of 5” rule: the diagnosis of celiac disease is confirmed if at least 4 of the following 5 criteria are satisfied: typical symptoms of celiac disease; positivity of serum celiac disease immunoglobulin, A class autoantibodies at high titer; human leukocyte antigen (HLA)-DQ2 or DQ8 genotypes; celiac enteropathy at the small bowel biopsy; and response to the gluten-free diet.
The 4 out of 5 Signs Rule

1. Presence of signs or symptoms compatible with CD;

2. Positive serology (TTG +/- EMA);

3. Compatible HLA (DQ2 e/o DQ8 positive);

4. Positive intestinal biopsy (enteropathy typical of CD);

5. Resolution of symptoms following implementation of a gluten-free diet
Diagnostic Developments

2006
Deamidated gliadin

2005
Quick TTG
HLA on a drop of whole blood

2000
Human TTG

1990
EMA

1980
AGA

1970

CD Epidemiology Milestones

2005
Quick Screening in Developing Countries (Lybia, Egypt, Iran)

2000
Screening in the US

1990
Screening in developing countries (W Sahara)
Screening in healthy population (Italy)

1980

1970
Screening in blood donors (Sweden)
The “old” Celiac Disease Epidemiology:

- A rare disorder typical of infancy
- Wide incidence fluctuates in space (1/400 Ireland to 1/10000 Denmark) and in time
- A disease of essentially European origin
“Mines” of CD have been found among

Relatives
- short stature, anemia, fatigue, hypertransaminasemia

Patients with
- Autoimmune disorders, Down’s, IgA deficiency, neuropathies, osteoporosis, infertility

Associated diseases

“Healthy” groups
- Blood donors, students, general population
Celiac Disease Epidemiological Study in USA

Population screened 13145

Healthy Individuals 4126
- Positive 31
- Negative 4095
  - Prevalence 1:133

Risk Groups 9019
- Symptomatic subjects 3236
  - Positive 81
  - Negative 3155
    - Prevalence 1:40
- 1st degree relatives 4508
  - Positive 205
  - Negative 4303
    - Prevalence 1:22
- 2nd degree relatives 1275
  - Positive 33
  - Negative 1242
    - Prevalence 1:39

Projected number (conservative) of celiac disease patients in the U.S.A.: 2,115,954
MAJOR PUBLIC HEALTH PROBLEM NATIONWIDE WITH SOME REGIONAL DIFFERENCES

The Global Village of Celiac Disease

- In many areas of the world Celiac Disease is one of the commonest, lifelong disorders affecting around 1% of the general population worldwide;
- Continents traditionally considered «immune» from Celiac Disease, including the Americas, Middle East, and Asia, show prevalence similar to Europe;
- Most cases escape diagnosis and are exposed to the risk of complications;
- Active Celiac Disease case-finding is needed but mass screening should be considered;
- The impact of Celiac Disease in the developing world needs further evaluation.
MILESTONE #5 (2005): Alternative/Integrative Treatments to The GFD

Genetically Engineered Grains

Proline Prolyl Endopeptidases

Zonulin Inhibitor

CCR9 Inhibitor

Peptide-Based Vaccine

Depiction of the intestinal mucosa with emphasis on the factors involved in the development of celiac disease in individuals with HLA-DQ2/DQ8 positive.
Cumulative Number of Celiac Disease Clinical Trials

Cumulative number of clinical trials

- 2005
- 2007
- 2009
- 2011
- 2013
Depiction of the intestinal mucosa with emphasis on the factors involved in the development of celiac disease in individuals with HLA-DQ2/DQ8 positive...
Tight junctions are inter-cellular “gates” that open and close in response to internal and external stimuli:

- Allows for immune surveillance
- Modulates immune function
- Regulates exchange of small molecules, proteins and cells across these barriers

Paradigm shift in the treatment of immune mediated and inflammatory diseases (e.g. Celiac Disease, T1D, MS, IBD, IBS, etc.)
Alba Clinical Trial Summary in Celiac Disease with Larazotide Acetate – Tight Junction Regulator

- **Phase Ib - Single Dose (CLIN1001-002)**
  - 21 Celiac disease subjects
  - Double blind, placebo controlled
  - 3 days QD, single gluten challenge on day 2
  - In-patient study
  - Completed March 2006

- **Phase IIa - Multiple Dose (CLIN1001-004)**
  - 86 celiac disease subjects
  - Double blind, placebo controlled
  - 2 weeks TID dosing and gluten challenge
  - Dose ranging - 7 arms
  - Multi-center Outpatient Study
  - Completed March 2007

- **Phase IIb - Multiple Dose (CLIN1001-006)**
  - 184 celiac disease subjects
  - Double blind, placebo controlled
  - 6 weeks TID dosing and gluten challenge
  - Dose ranging - 4 arms
  - Multi-center Outpatient Study

0% Bioavailability

No Adverse Safety Trends

- Larazotide acetate acts locally in the gastrointestinal tract
- No systemic exposure, no measurable plasma drug levels in any clinical study
- No immunogenicity, no antibody development in any clinical study
- No toxicity observed to date in 24 completed animal toxicology studies
- No safety signals in ~500 celiac subjects exposed to larazotide acetate up to 8 weeks
- To date, safety comparable to placebo
Larazotide Acetate Consistently Reduced Gastro-Intestinal Symptoms in 3 Gluten-Challenge Clinical Trials

**CLIN1001-002 Study**
(Baseline to Day 3, 12 mg QD)

**CLIN1001-004 Study**
(Baseline to Day 14, pooled active)

**CLIN1001-006 Study**
(Baseline to LDBTP, 1 mg TID)

% of patients with symptoms

<table>
<thead>
<tr>
<th>Domain</th>
<th>Placebo</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flatulence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
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Change in GSRS domains

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<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux</td>
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</tbody>
</table>

LDBTP, Last Double-Blind Treatment Period Visit

Placebo  Active
MILESTONE #6 (2010):
We Are Not Born with Gluten Related Disorders, Including Celiac Disease
Increased Prevalence Over Time in U.S.A. (in Line with Other Autoimmune Diseases)

During the past 35 years the true prevalence of CD in USA doubled every 15 years.

C. Catassi et al, Annal Med 2010
Celiac Disease
Autoimmune Pathogenesis

Necessary but **NOT** Sufficient
Key Questions in CD Pathogenesis

1. What kind of tricks were used by people genetically at risk for CD that were able to tolerate gluten for decades?

2. What happened to them that caused the shift from tolerance to immune response to gluten?

How to Re-Write the Natural History of CD?
The Epidemics Of Celiac Disease:
Which Additional Factors are Driving this Epidemics?

- Quality of gluten;
- Quantity of gluten;
- Breast Feeding;
- Maturity of gut functions influencing Ag trafficking and handling:
  - GALT
  - PRRs
  - Mucous production
  - Barrier function
- Timing of gluten introduction.
- Changes in microbiome composition.
The Complexity of the Human Body

Over the years we came to appreciate the complexity of the human body.

Only 25,000 genes, 99.5% identical to chimpanzee, cannot explain such complexity and difference with other primates.

However, it would be inappropriate to describe the human body without considering the 300,000,000,000 bacteria (collectively defined as microbiome) gladly living inside us and that express ~100 fold more genes that the human genome.
The Real Story of Our Genetic Complexity:
We Inherit two Parallel Genomes

**Human Genome:**
Inherited from both parents, stable, never change in its composition

**Microbiome:**
Inherited from the mother, extremely dynamic, changes from individual to individual and in the same individual over time
C-Section Delivery and Increased Risk of Celiac Disease

- **Decker et al. Pediatrics 2010;125:e1433-40**
  
  Key message: Celiac disease children were more likely born by cesarean delivery compared with control subjects (odds ratio: 1.8, 95% confidence interval: 1.13-2.88).

- **Marild K et al. Gastroenterology 2012;142:39-45**
  
  Key message: Positive association between elective cesarean delivery and later celiac disease (adjusted odds ratio: 1.15, 95% confidence interval: 1.04-1.26).
Proof of Concept of Microbiome-Metabolome Analysis and Delayed Gluten Exposure on Celiac Disease Autoimmunity in Genetically At-Risk Infants

Maria Sellitto, Guoyun Bai, Gloria Serena, W. Florian Fricke, Craig Sturgeon, Pawel Gajer, James R. White, Sara S. K. Koenig, Joyce Sakamoto, Dustin Boothe, Rachel Gicquelais, Deborah Kryszak, Elaine Puppa, Carlo Catassi, Jacques Ravel, Alessio Fasano

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Abstract

Celiac disease (CD) is a unique autoimmune disorder in which the genetic factors (DQ2/DQ8) and the environmental trigger (gluten) are known and necessary but not sufficient for its development. Other environmental components contributing to CD are poorly understood. Studies suggest that aspects of gluten intake might influence the risk of CD occurrence and timing of its onset, i.e., the amount and quality of ingested gluten, together with the pattern of infant feeding and the age at which gluten is introduced in the diet. In this study, we hypothesize that the intestinal microbiota as a whole rather than specific infections dictates the switch from tolerance to immune response in genetically susceptible individuals. Using a sample of infants genetically at risk of CD, we characterized the longitudinal changes in the microbial communities that colonize infants from birth to 24 months and the impact of two patterns of gluten introduction (early vs. late) on the gut microbiota and metabolome, and the switch from gluten tolerance to immune response, including onset of CD autoimmunity. We show that infants genetically susceptible to CD who are exposed to gluten early mount an immune
Microbiome
(140-fold Human Genome)
Dynamic

Human Genome
(~30,000 genes)
Stable

Metabonome

Jazz

Pop

Classic

Clinical Outcome
NMR Analysis

CD patient
The Best Way To Predict The Future Is To Create It
MILESTONE #7: How Much Gluten is Too Much
Why 20 ppm Threshold Makes Sense?

Choosing a GFD threshold is a complex matter that involves social, economic, and cultural factors

- **Testing toxicity of a substance**
  - Classic FDA approach (drug development): pre-clinical tests (including animal studies) followed by clinical studies. This does not apply in CD;
  - Double blind studies have shown safety of 20 ppm. Moreover, 30 years of experience (particularly in Europe) has clearly demonstrated safety of 20 ppm in millions of celiac sufferers (the strongest evidence so far);

- **Safety assessments not the best measure for celiac disease**
  - Abating the safety threshold by a factor of 10 or 100 to take into account the extrapolation of safety data from animal models to humans does not apply for CD, since we do not have yet an animal model.

- **Need for evidence-based science**
  - Choosing a safe threshold cannot be matter of “gut feeling”, rather needs to be established on hard provable and challengeable evidence

- **Margin of error of ELISA to measure gluten in foodstuff**
  - Setting the threshold too close to the level of sensitivity of the assay increase tremendously the risk of errors and, therefore, to have a product testing safe one day and unsafe at a subsequent screening

- **We leave in a gluten-free global village**
  - Having a more restrictive threshold compared to other countries will limit freedom of traveling and competition among producers to increase palatability nd abate costs of GF products
Gluten-Free Labeling of Foods

An estimated 3 million people in the United States have celiac disease. In people with celiac disease, foods that contain gluten trigger production of antibodies that attack and damage the lining of the small intestine. Such damage limits the ability of celiac disease patients to absorb nutrients and puts them at risk of other very serious health problems, including nutritional deficiencies, osteoporosis, growth retardation, infertility, miscarriages, short stature, and intestinal cancers.

On August 2, 2013, FDA issued a final rule defining “gluten-free” for food labeling, which will help consumers, especially those living with celiac disease, be confident that items labeled “gluten-free” meet a defined standard for gluten content.

- **News Release:** FDA defines “gluten-free” for food labeling
  The regulation will provide a uniform standard for manufacturers who choose to label their products as “gluten-free.” It will also help the estimated one in every 133 people - about 3 million people in the United States - who have celiac disease, a condition that can only be managed by eating a gluten-free diet.
- **Federal Register Notice for the Gluten-Free Labeling Final Rule**
- **Questions & Answers:** FDA’s Final Rule on Gluten-Free Labeling
GLUTEN FREEDOM

ALESSIO FASANO, MD
Founder and Director of the Center for Celiac Research at Massachusetts General Hospital
WITH SUSIE FLAHERTY

FOREWORD BY RICH GANNON

Order at Amazon.com: http://amzn.to/1dEtM1x