Differentiating Gluten-Related Disorders Through Diagnostic Methods

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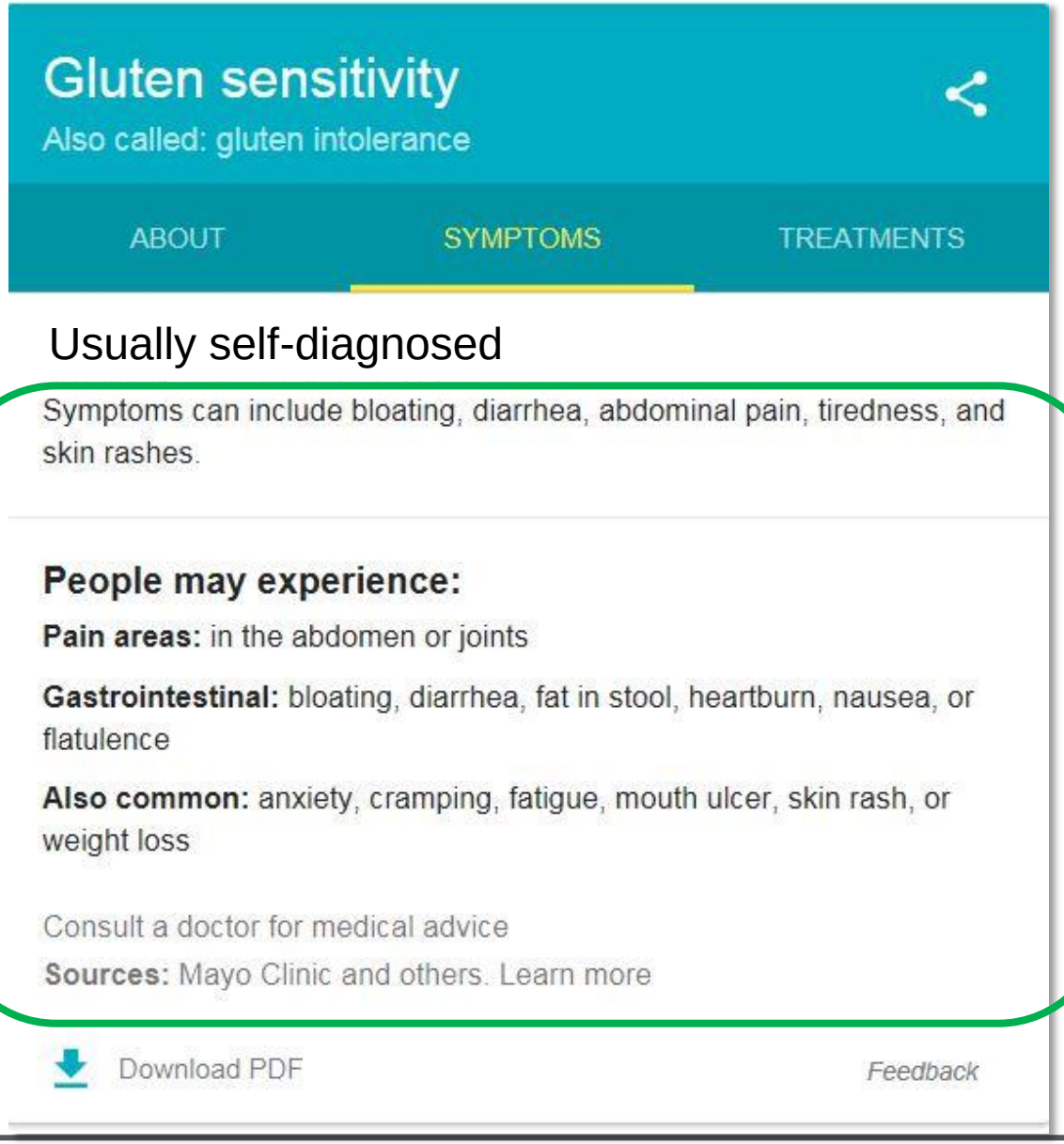
Gluten Sensitivity

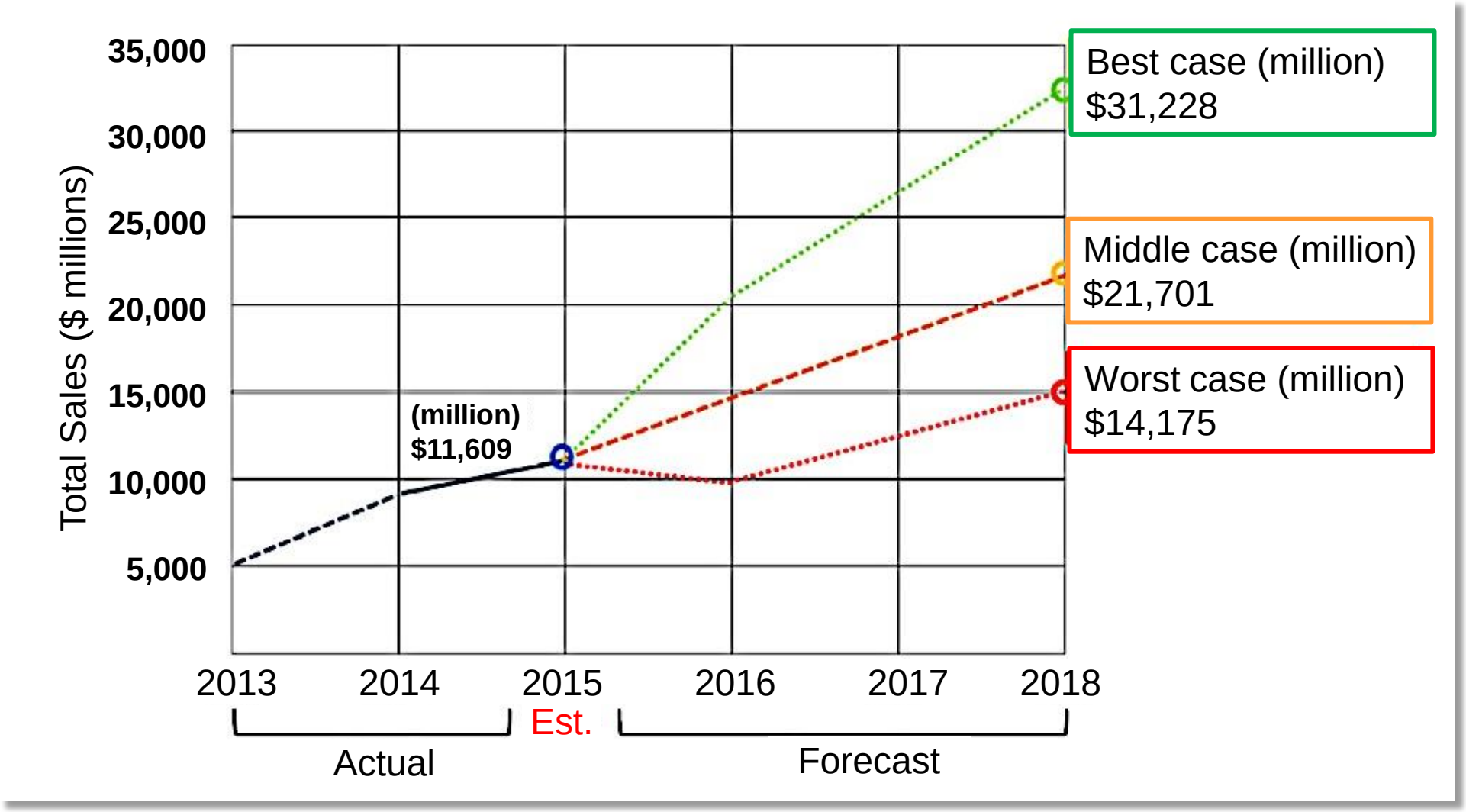


The Controversy on Who Should Be on a GFD

Only People With Celiac Disease

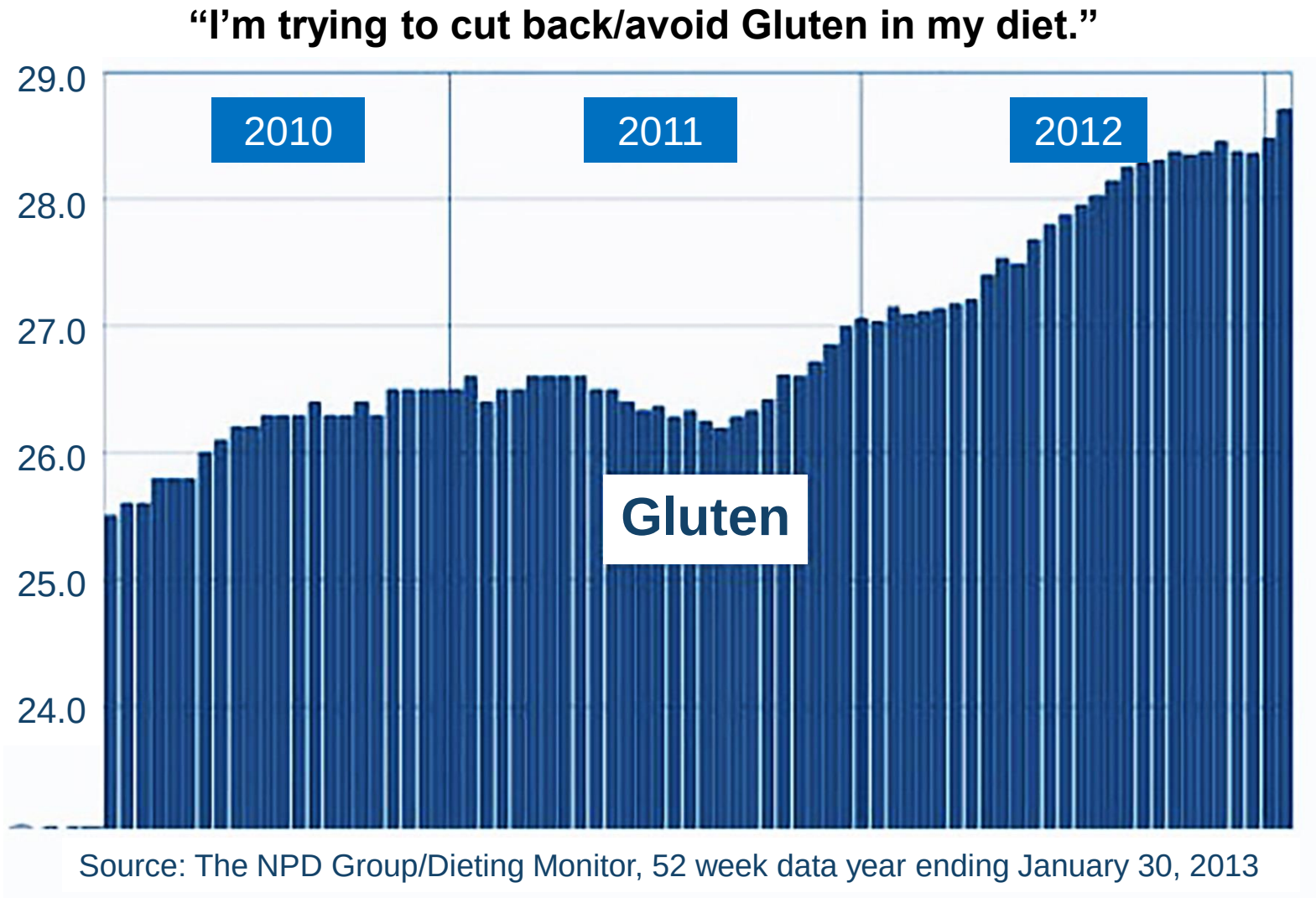
Everybody

Sales of GFD Products in the US



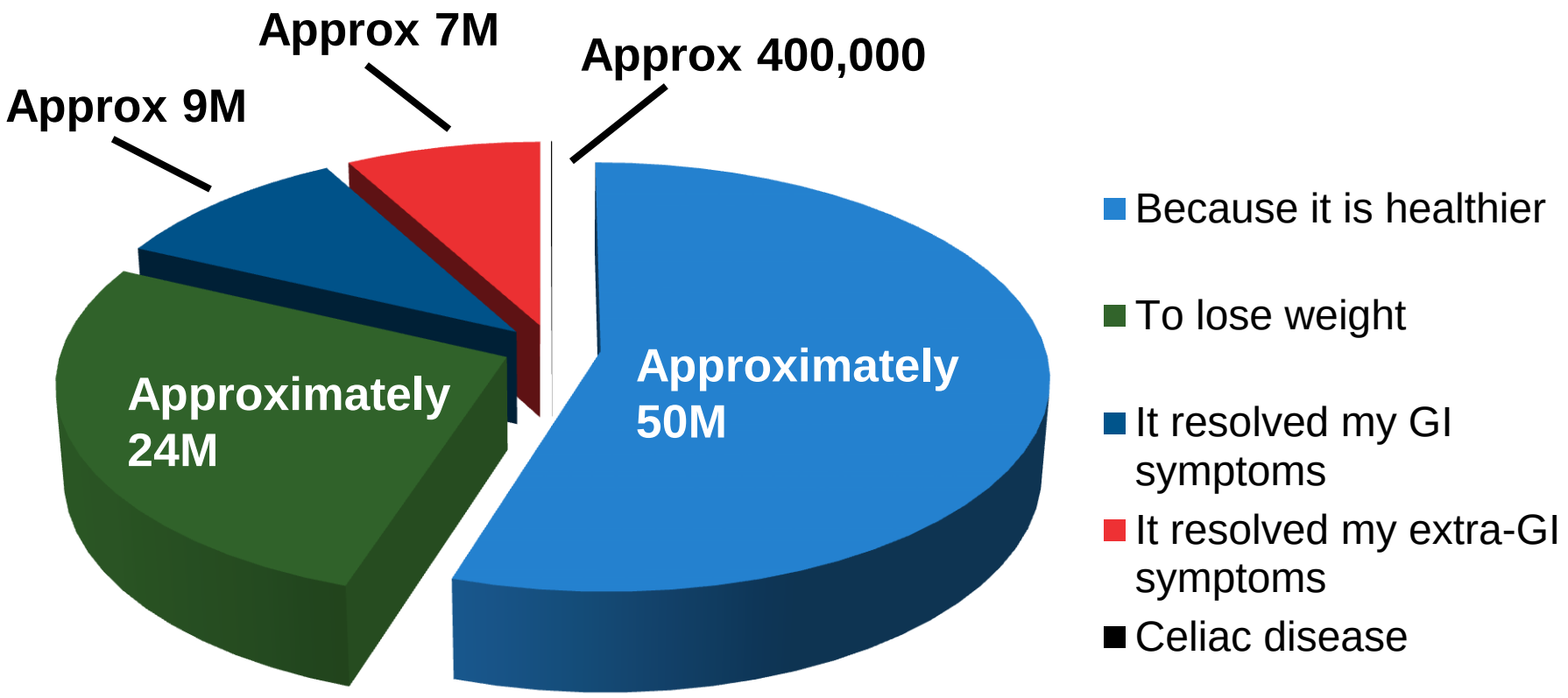
How Many People in the US are Embracing a GFD

- Percentage of U.S. adults trying to cut down or avoid gluten in their diets reaches new high in 2013, Reports NPD



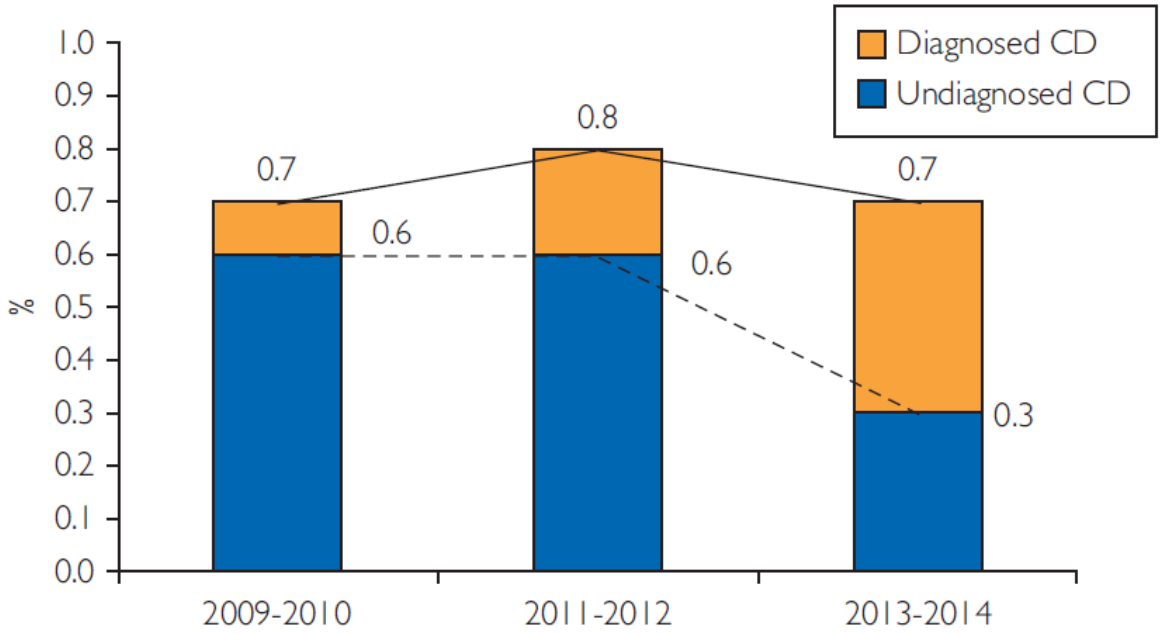
Want to Order Gluten-free Food at this Café? Better Show Some Medical Proof

Why People in the US Embrace a GFD



Based on internet interview users age 18y+ who eats GF food

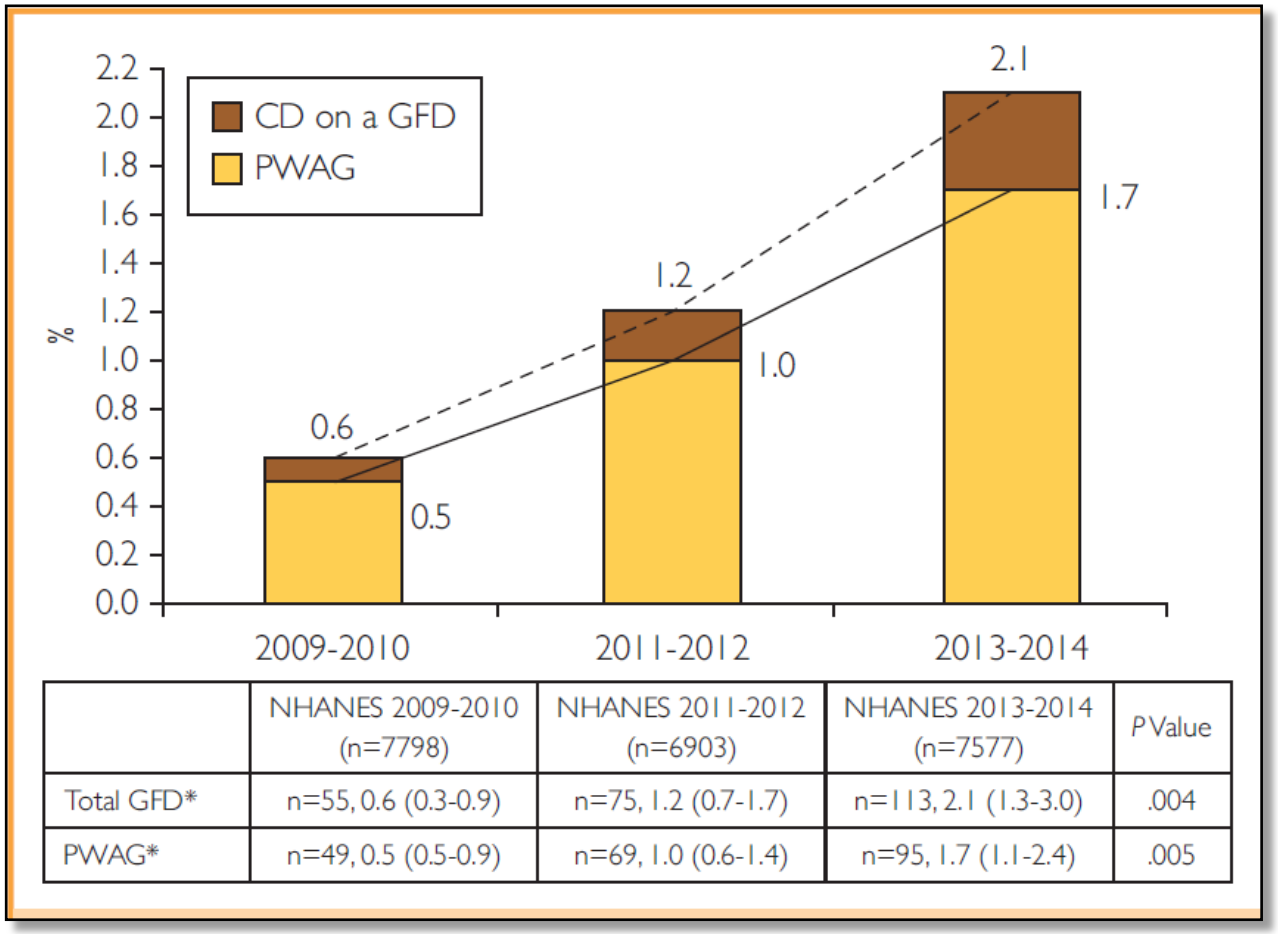
Trends in the prevalence of total CD and undiagnosed CD from 2009 to 2014



	NHANES 2009-2010 (n=7798)	NHANES 2011-2012 (n=6903)	NHANES 2013-2014 (n=7577)	*P* Value
Total CD*	n=35, 0.7 (0.6-0.8)	n=33, 0.8 (0.4-1.2)	n=41, 0.7 (0.3-1.0)	.91
Undiagnosed CD*	n=30, 0.6 (0.5-0.8)	n=27, 0.6 (0.3-1.0)	n=24, 0.3 (0.1-0.4)	.01

Choung RS et al., *Mayo Clinic Proc* 2017

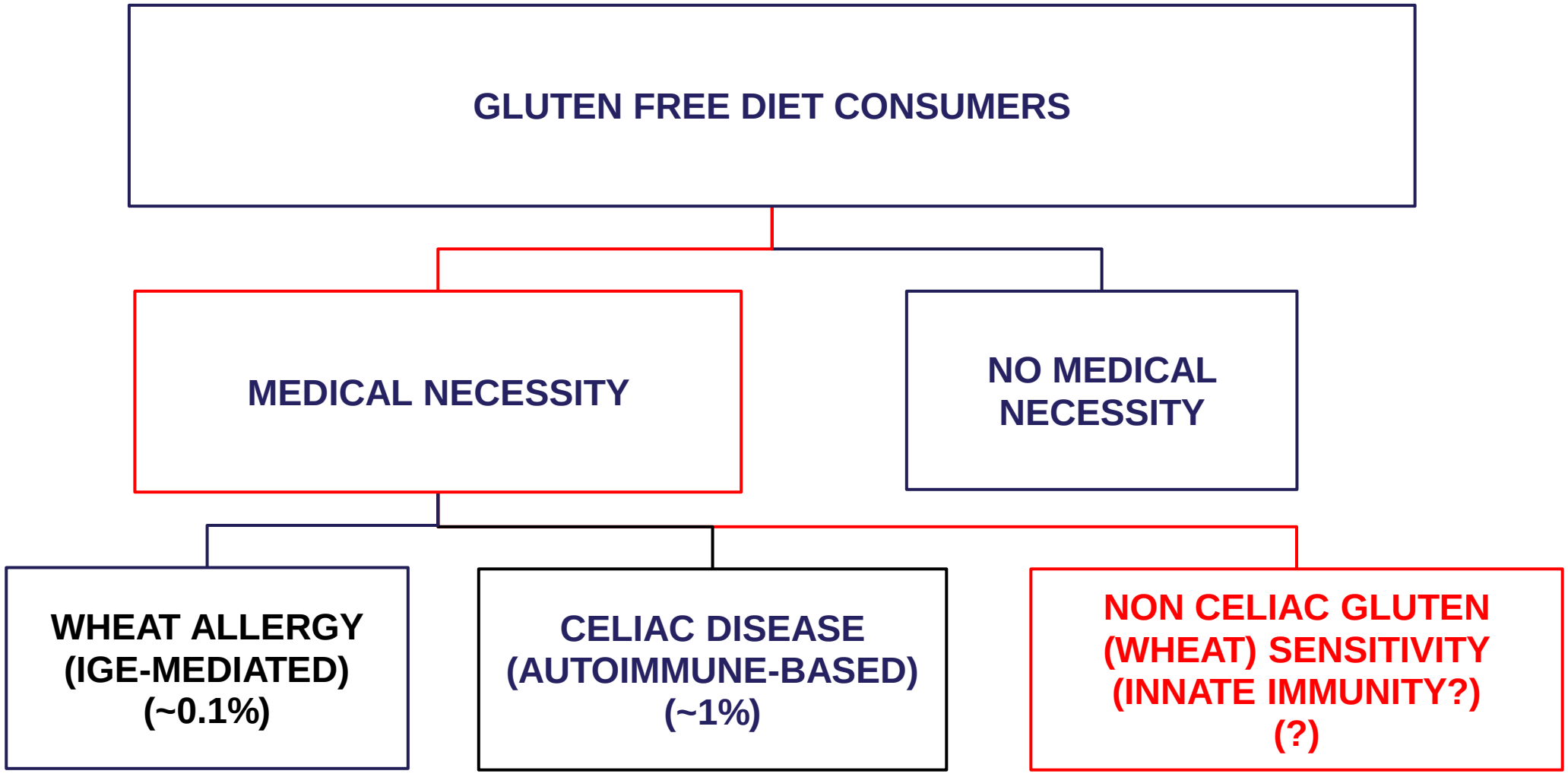
Trends in the prevalence of GFD in CD and in people without celiac disease avoiding gluten from 2009 to 2014



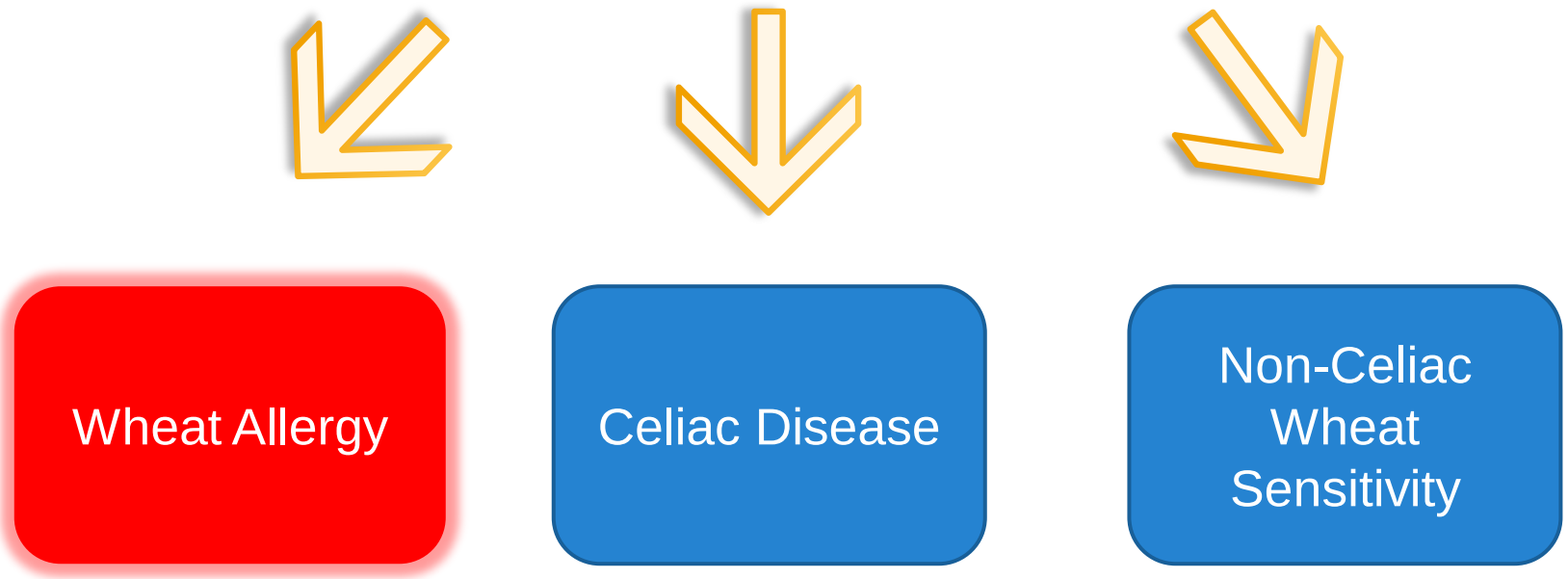
	NHANES 2009-2010 (n=7798)	NHANES 2011-2012 (n=6903)	NHANES 2013-2014 (n=7577)	*P* Value
Total GFD*	n=55, 0.6 (0.3-0.9)	n=75, 1.2 (0.7-1.7)	n=113, 2.1 (1.3-3.0)	.004
PWAG*	n=49, 0.5 (0.5-0.9)	n=69, 1.0 (0.6-1.4)	n=95, 1.7 (1.1-2.4)	.005

Choung RS et al., *Mayo Clinic Proc* 2017

The Gluten Free Diet: Not Only Celiac Disease



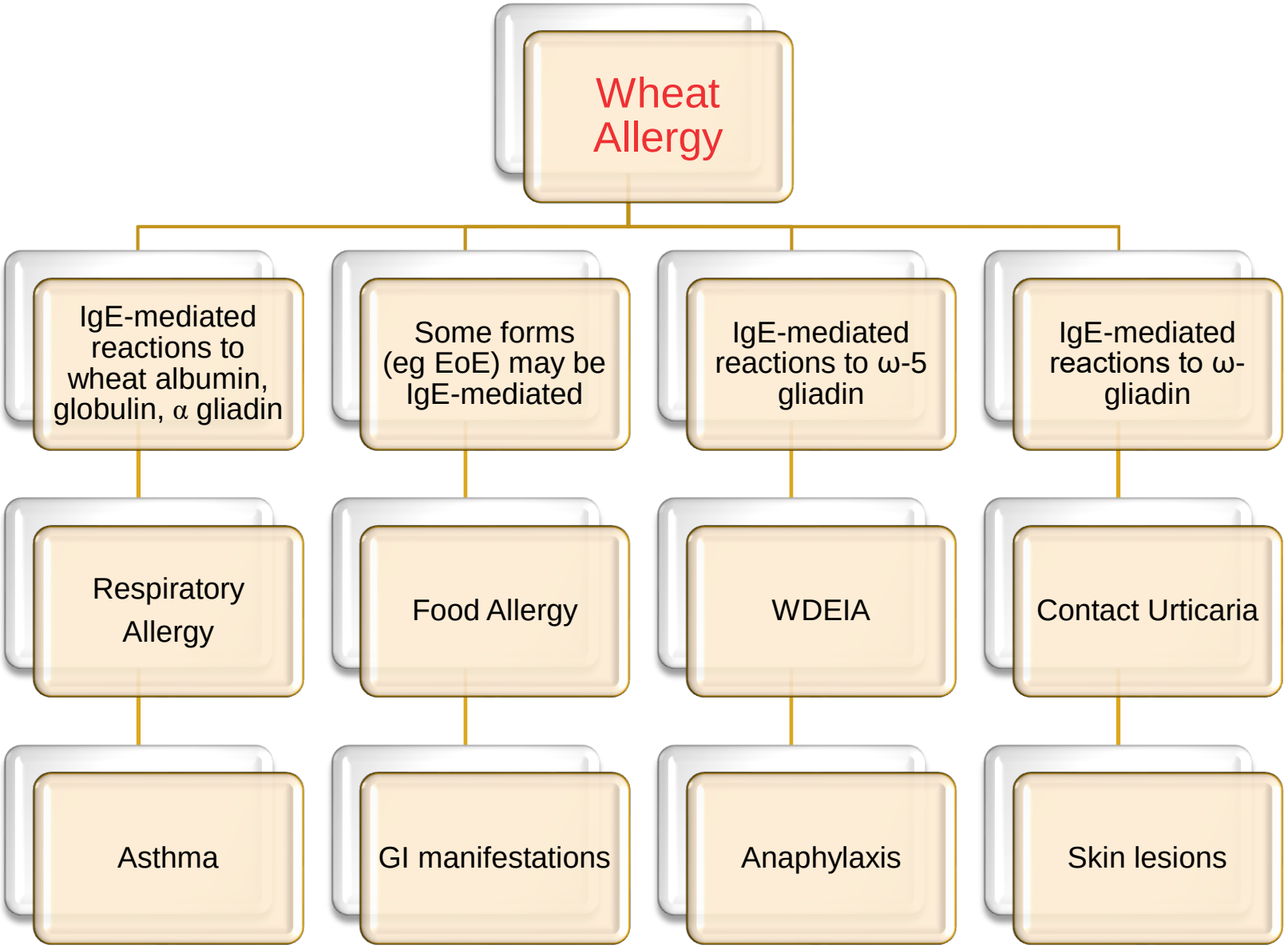
Adverse Effects of Wheat Ingestion in Humans – Wheat Allergy



Wheat Allergy

- A hypersensitivity reaction to wheat proteins mediated through immune mechanisms and involving mast cell activation.
- The immune response can be IgE mediated, non-IgE mediated, or both.
- Most commonly a food allergy, but wheat can become a sensitizer when the exposure occurs through the skin or through the airways (Baker's asthma)

Hill ID, Fasano A, Guandalini S, Hoffenberg E, Levy J, Reilly N, Verma R.
NASPGHAN Clinical Report on the Diagnosis and Treatment of Gluten-related disorders.
J Pediatr Gastroenterol Nutr 2016

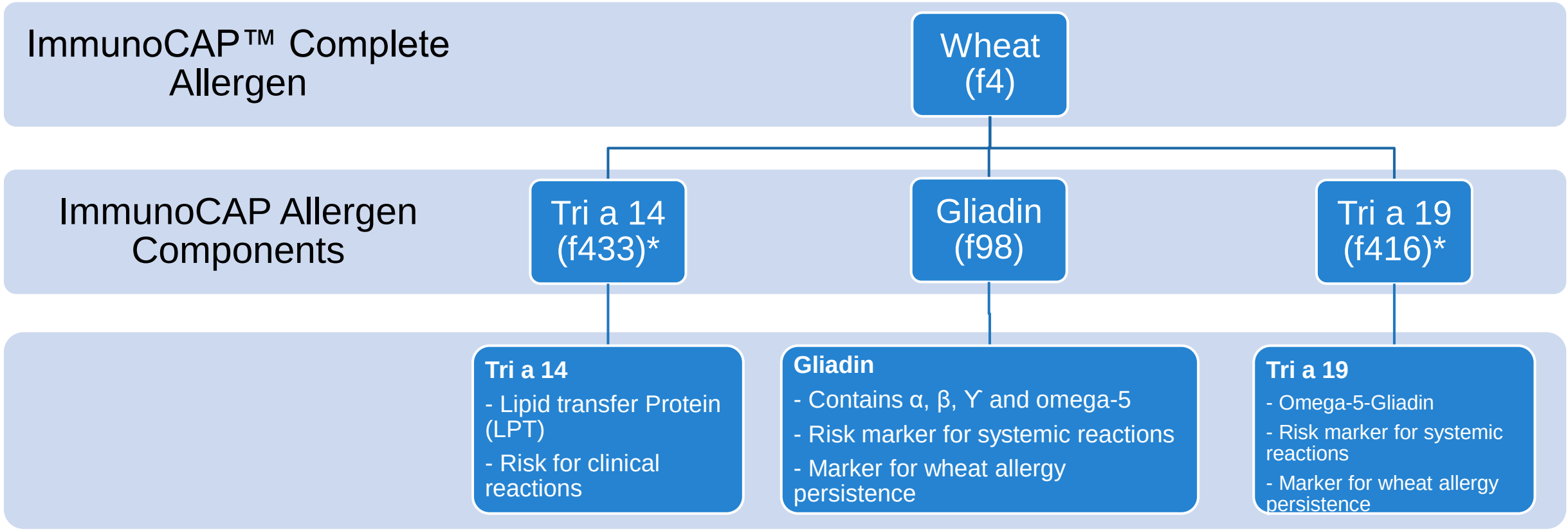

Wheat Allergy
IgE-mediated reactions to wheat albumin, globulin, α gliadin
Some forms (eg EoE) may be IgE-mediated
IgE-mediated reactions to ω-5 gliadin
IgE-mediated reactions to ω-gliadin
Respiratory Allergy
Food Allergy
WDEIA
Contact Urticaria
Asthma
GI manifestations
Anaphylaxis
Skin lesions

Mr. Phillips

- 28 year old man, c/o watery eyes, itchy rash, occasional wheezing.
- Works at a bakery

Sounds like wheat allergy

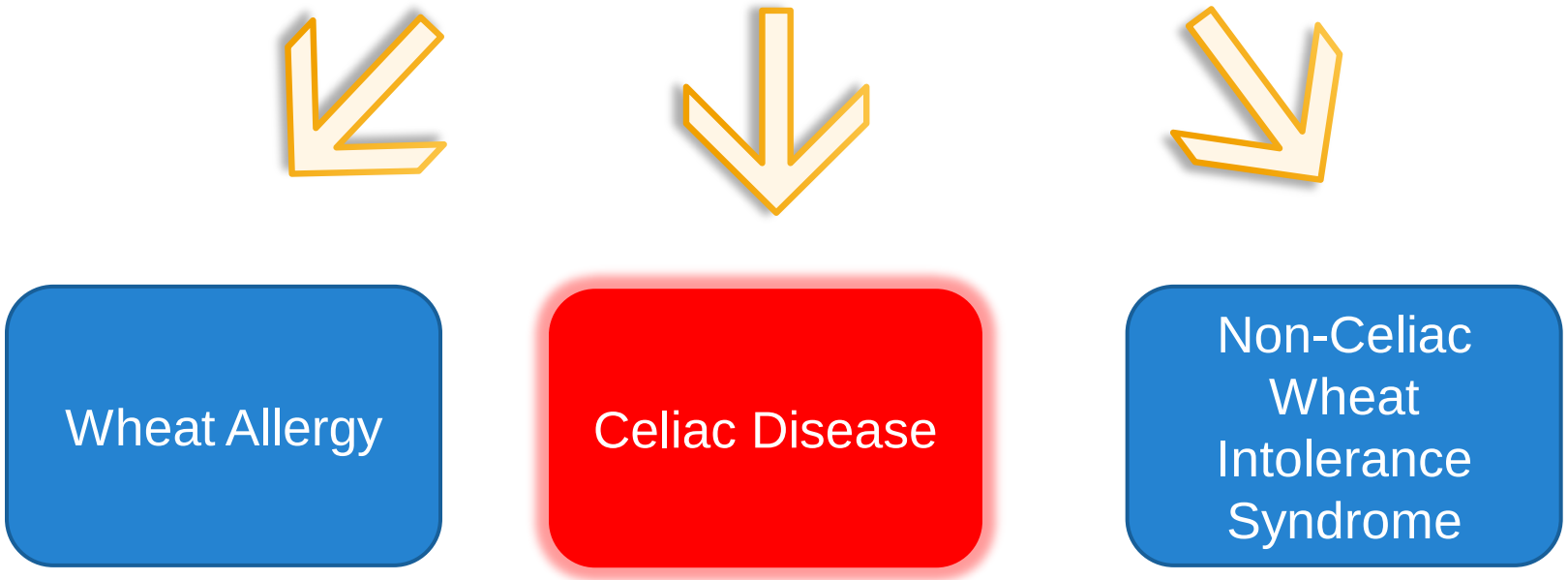
Potential Testing Cascade



Gliadin gives high sensitivity for detecting wheat food allergy while Tri a 19 provides higher specificity

*These assays are only available in the United States through Phadia immunology Reference Laboratory (PiRL) as Laboratory Developed Tests.

Adverse Effects of Wheat Ingestion in Humans – Celiac Disease



Celiac Disease

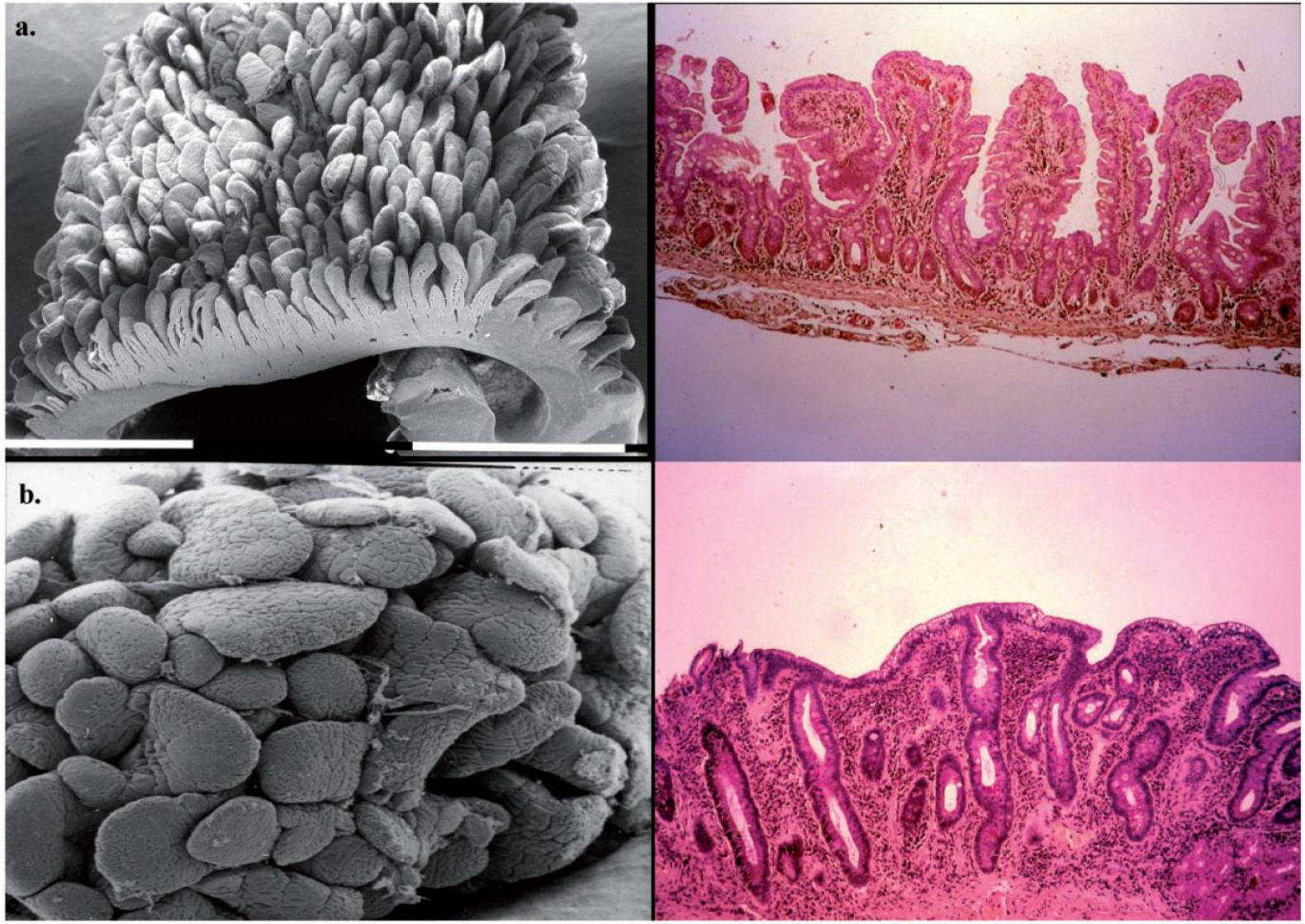
- An immune-mediated systemic disorder triggered by gluten and related prolamines in genetically susceptible individuals (HLA-DQ2 or HLA-DQ8 haplotypes)
- Characterized by:
  - Inflammatory Enteropathy of variable severity
  - A wide range of gastrointestinal and/or systemic complaints
  - CD-specific antibodies

ESPGHAN Guidelines – JPGN 2012 ***and***
NASPGHAN clinical report – JPGN 2016

Microscopic Images and Histology

(a) normal cytoarchitectonic villus-crypt and absorbent epithelium of the small intestine scanning electron microscopy (left) and histology (right. Emat.cos.80x)

(b) subtotal villous atrophy in scanning electron microscopy (left) associated with hyperplasia of the crypts (right. Emat.cos.x80)

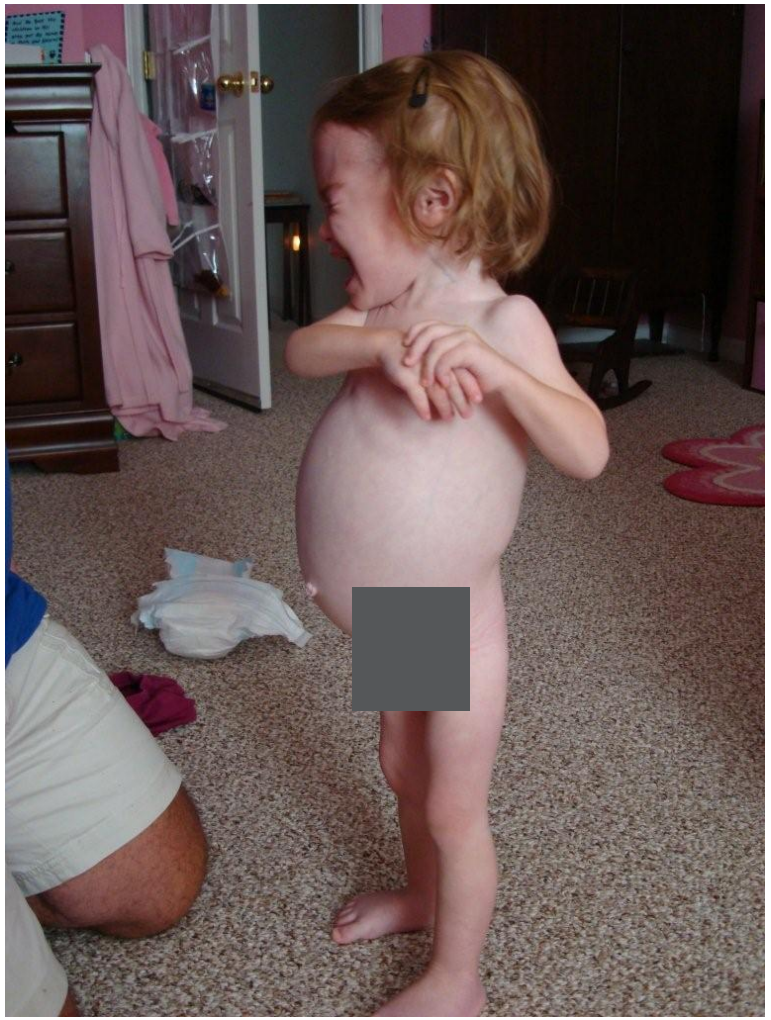


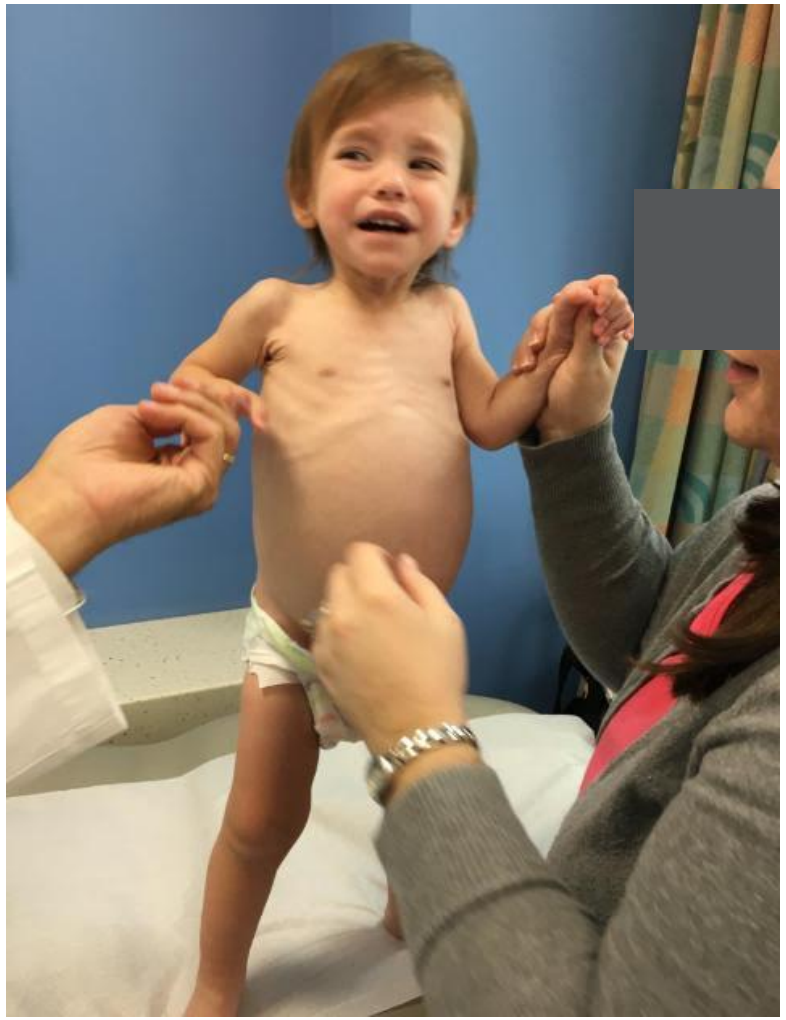
Gasbarrini GB and Mangiola F - *UEG Journal* 2014. DOI: 10.1177/2050640614535929

Clinical Presentations

Symptoms	Duodenal Biopsy	Serology	Type
GI manifestations	Villous Atrophy	Positive	Typical
Extra-GI manifestations	Villous Atrophy	Positive	Atypical
Asymptomatic	Villous Atrophy	Positive	Silent
Symptoms present or absent	Normal or only increased intraepithelial lymphocytes	Positive	Potential

GI Presentations of Celiac Disease in Children





Typical CD in Children: GI Presentations

- Diarrhea
- Vomiting
- Failure to thrive or weight loss
- Abdominal bloating/pain
- Constipation

Main “Atypical”: Extra-Intestinal

- Malnutrition Related
  - Short stature
  - Delayed puberty
  - Iron-deficient anemia resistant to oral Fe
- Recurrent stomatitis
- Liver and biliary tract disease
  - Autoimmune Liver Disease
  - Benign hypertransaminasemia
- Skin disorders
  - Dermatitis Herpetiformis
  - Alopecia Areata
- Osteopenia/Osteoporosis
- Arthritis/Arthralgia
- Neurological problems
  - Headache
  - Peripheral Neuropathy
  - Seizures with occipital calcifications
  - Gluten Ataxia
- Behavioral changes & psychiatric disorders
  - Poor mood
  - Anxiety
  - Depression
- Women: sub-infertility

Who Should be tested?

- **Asymptomatic children and adolescents at increased risk for CD such as:**
  - Type 1 diabetes mellitus (T1DM)
  - Autoimmune thyroid disease
  - Down syndrome
  - Turner syndrome
  - Williams syndrome
  - Selective immunoglobulin A (IgA) deficiency
  - Autoimmune liver disease
  - First-degree relatives with CD *(overall prevalence 8.1%, varying from 13% in sisters, daughters to 3% in parents)*

Johnny

- 12 year old boy with type 1 diabetes; previously tested negative for celiac, but somewhat stunted growth in past couple years, increased irritability, some abdominal pain.

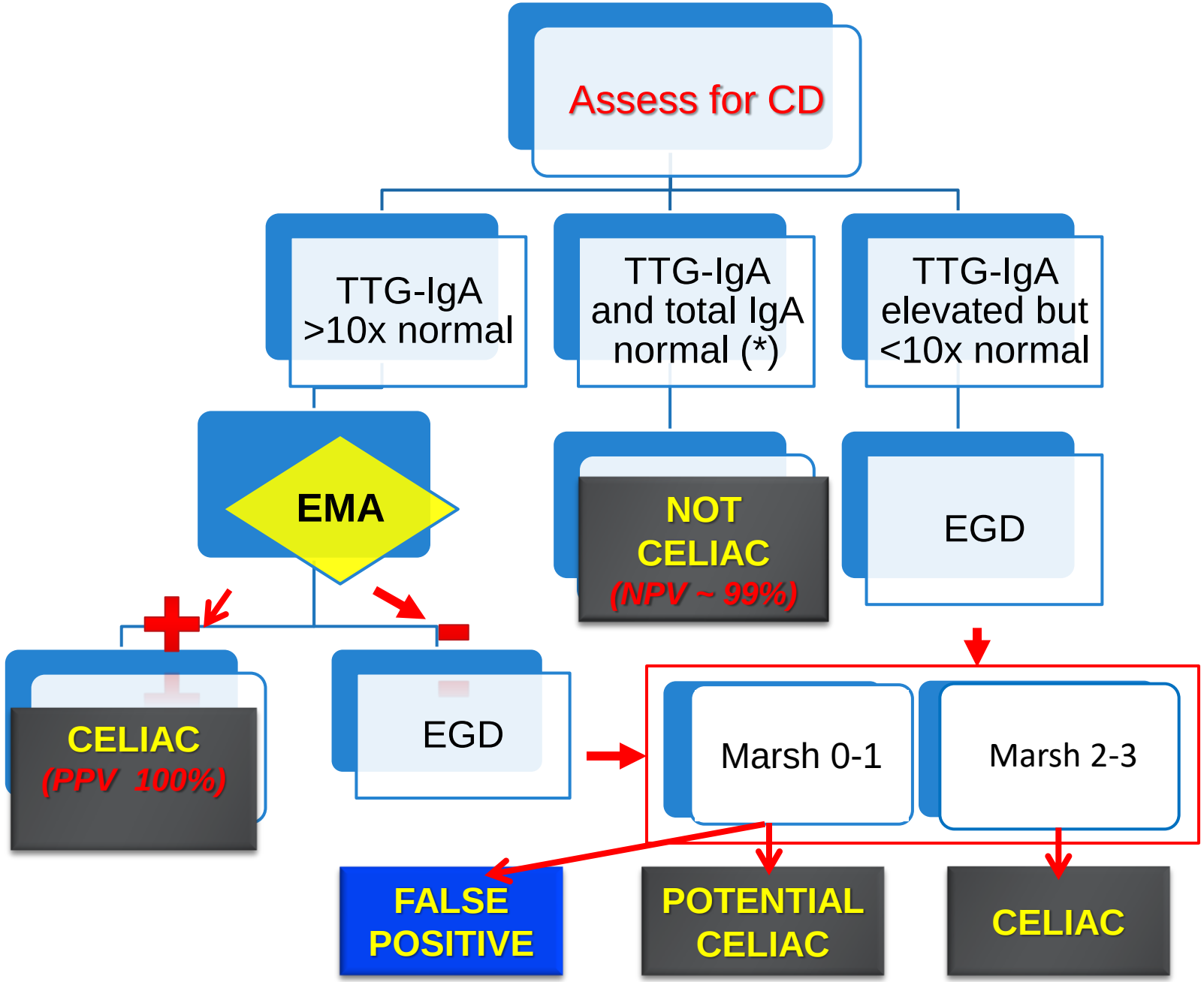
Sounds like celiac

Celiac-specific Antibodies

	Positive likelihood ratio	Negative likelihood ratio
EMA / IgA	**31.8** *(18.6 - 54.3)*	**0.067** *(0.038 - 0.118)*
Anti-TG2 / IgA	**21.8** *(12.9 - 36.8)*	**0.060** *(0.040 - 0.090)*
Anti-DGP / IgG	**13.6** (8.1 - 22.8)	**0.061** *(0.017 - 0.221)*
Anti-DGP / IgA	**9.4** (6.8 - 13.1)	**0.121** *(0.072 - 0.203)*
AGA / IgA	**7.3** *(4.5 - 11.8)*	**0.186** *(0.095 - 0.362)*

EMA: Endomysial Antibody
TG2: anti transglutaminase-2
DGP: anti-deamidated gliadin peptides
AGA: anti-gliadin antibody

Giersiepen K et al., *JPGN* 2012



Adapted from NASPGHAN *Clinical Guide for Pediatric Celiac Disease*

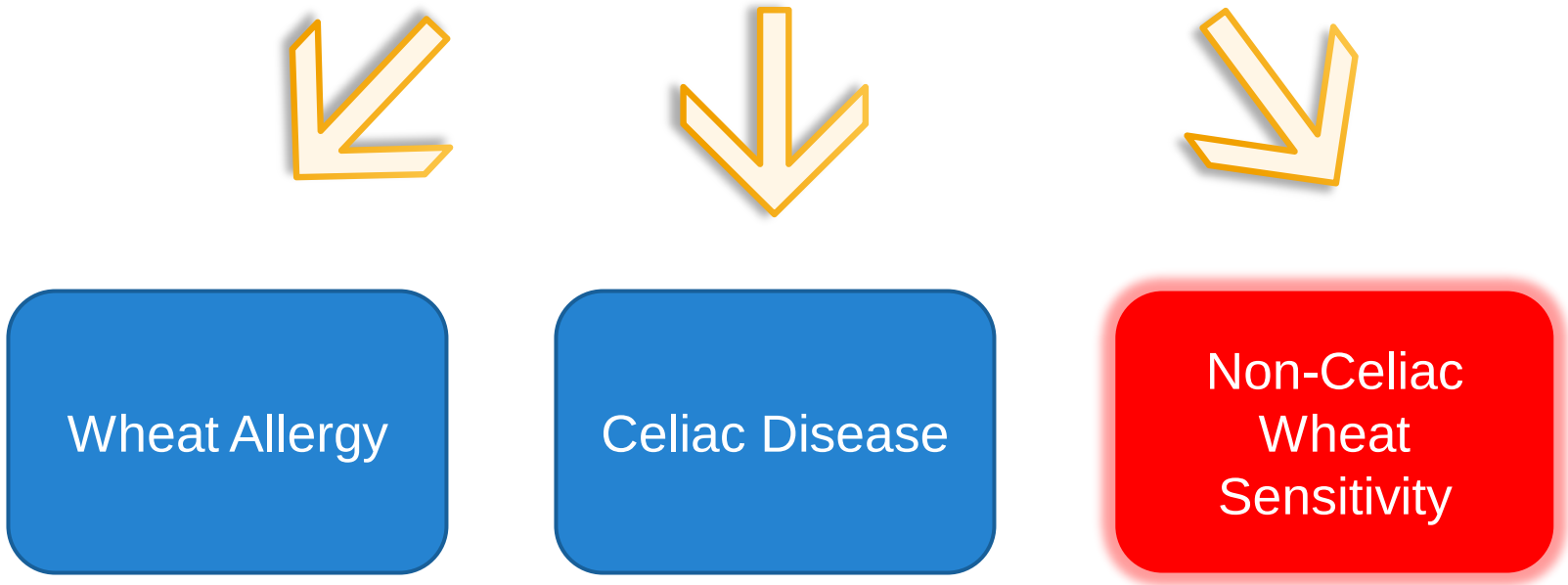
(*) if IgA-deficient: TTG-IgG or DGP-IgG normal

However…

- All “adult” societies recommend biopsy confirmation of diagnosis of celiac disease
  - AGA
  - ACG
  - BSG
  - NICE

Gastroenterology, 131:1981, 2006
*Am J Gastroenterol **108**, 656-76 (2013)*
*Gut **63**, 1210-28 (2014)*
*BMJ **351**, h4513 (2015)*

Adverse Effects of Wheat Ingestion in Humans – Non-Celiac Wheat Sensitivity



#5 – “Low Immunity”; #6 – “Dental issues”

Non-Celiac Wheat Sensitivity

- A poorly defined syndrome characterized by a variable combination of intestinal and extra-intestinal symptoms, typically occurring soon after the ingestion of gluten-containing foods and disappearing quickly upon their withdrawal, occurring in individuals where both CD and WA have been excluded

Hill ID, Fasano A, Guandalini S, Hoffenberg E, Levy J, Reilly N, Verma R.
NASPGHAN Clinical Report on the Diagnosis and Treatment of Gluten-related disorders.
J Pediatr Gastroenterol Nutr 2016

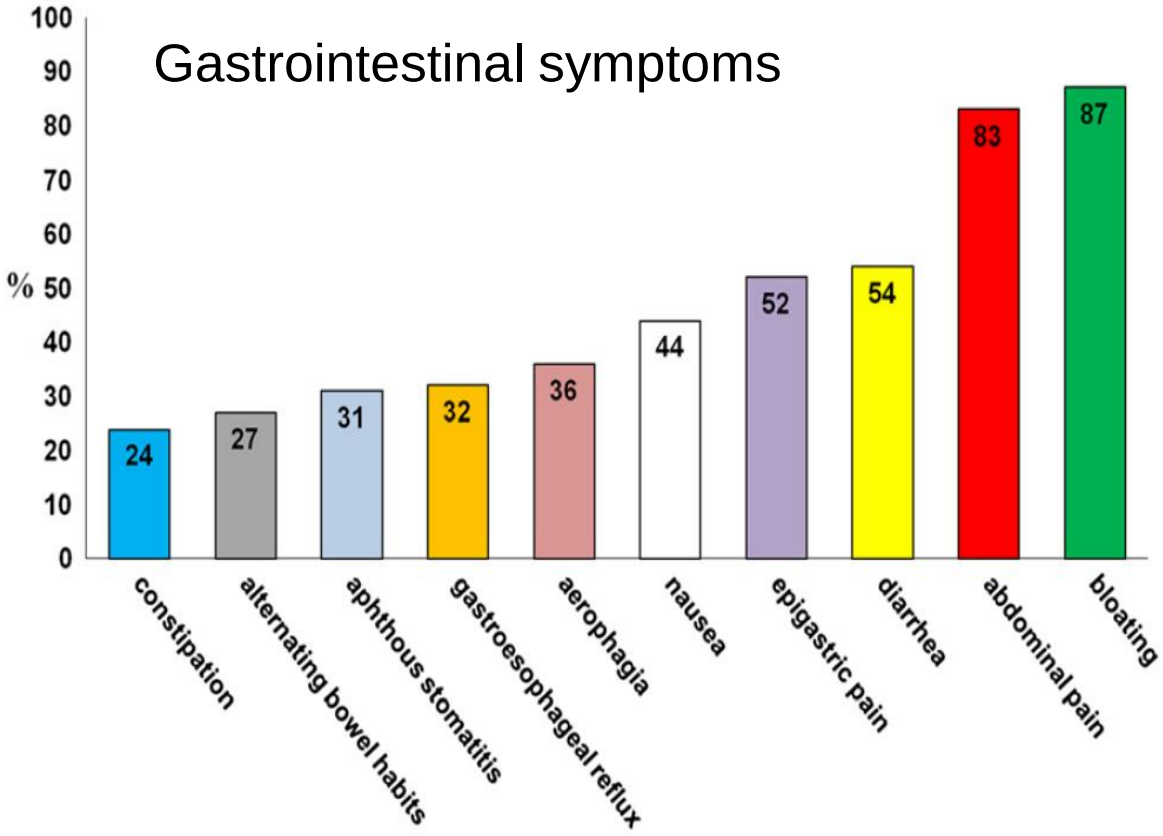
NCWS: Definition

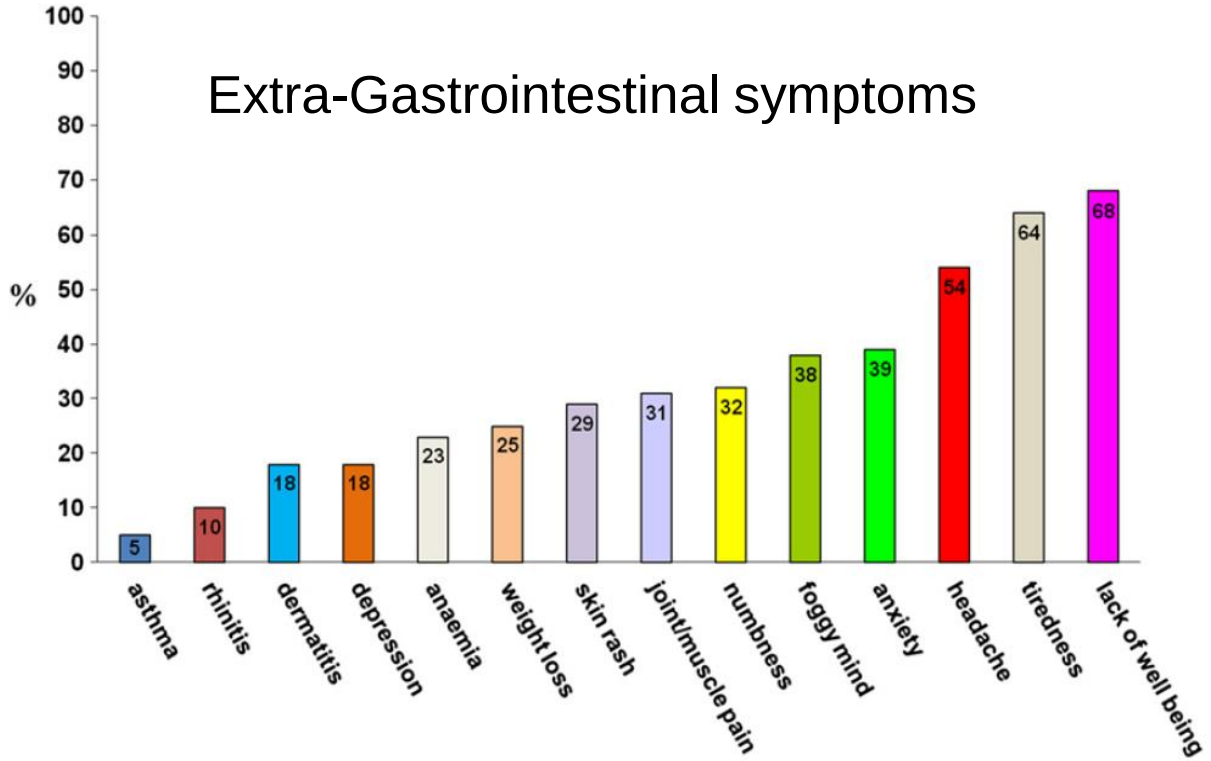
<u>Cases of reaction to ingestion of wheat and possibly gluten-containing grains</u> in which both allergic and autoimmune mechanisms have been ruled out (diagnosis by exclusion criteria)

- Triggered by the ingestion of gluten-containing grains
- 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
- Possible 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 and relapse after re-exposure to gluten-containing grains (double blind)

Sapone A. et al *BMC Med* 2012, Ludvigsson JF et al *Gut* 2013, Catassi C. Et al, *Nutrients* 2013, Catassi et al *Nutrients* 2015

An Italian survey on 486 patients





Volta U et al., *BMC Medicine* 2014

Clinical manifestations of NCWS

Frequency	Intestinal	Extra-intestinal
Very Common	Bloating	Lack of wellbeing
	Abdominal pain	Tiredness
Common	Diarrhea	Headache
	Epigastric pain	Anxiety
	Nausea	Foggy mind
	Aerophagia	Numbness
	GER	Joint/muscle pain
	Aphtous stomatitis	Skin rash/dermatitis
	Alternating bowel habits	
	Constipation	
Undetermined	Hematochezia	Weight loss
	Anal fissures	Anemia
		Loss of balance
		Depression
		Rhinitis/asthma
		Weight increase
		Interstitial cystitis
		Ingrown hairs
		Oligo or polimenorrhea
		Sensory symptoms
		Disturbed sleep pattern
		Hallucinations
		Mood swings
		Autism
		Schizophrenia

The Salerno NCGS diagnostic criteria (*Nutrients*, 2015)

Open Questions

- Prevalence? (between 0.6-6%)
- Are children affected? (only 1 open-label paper published)
- Cause? (Gluten and/or other wheat components?)
- Pathophysiology? (Leaky gut? Innate/adaptive immunity?)
- Diagnosis? (No marker available)
- Complications? (Unknown)
- Treatment? (GFD or wheat-free diet? How strict? For how long?)

Open Questions

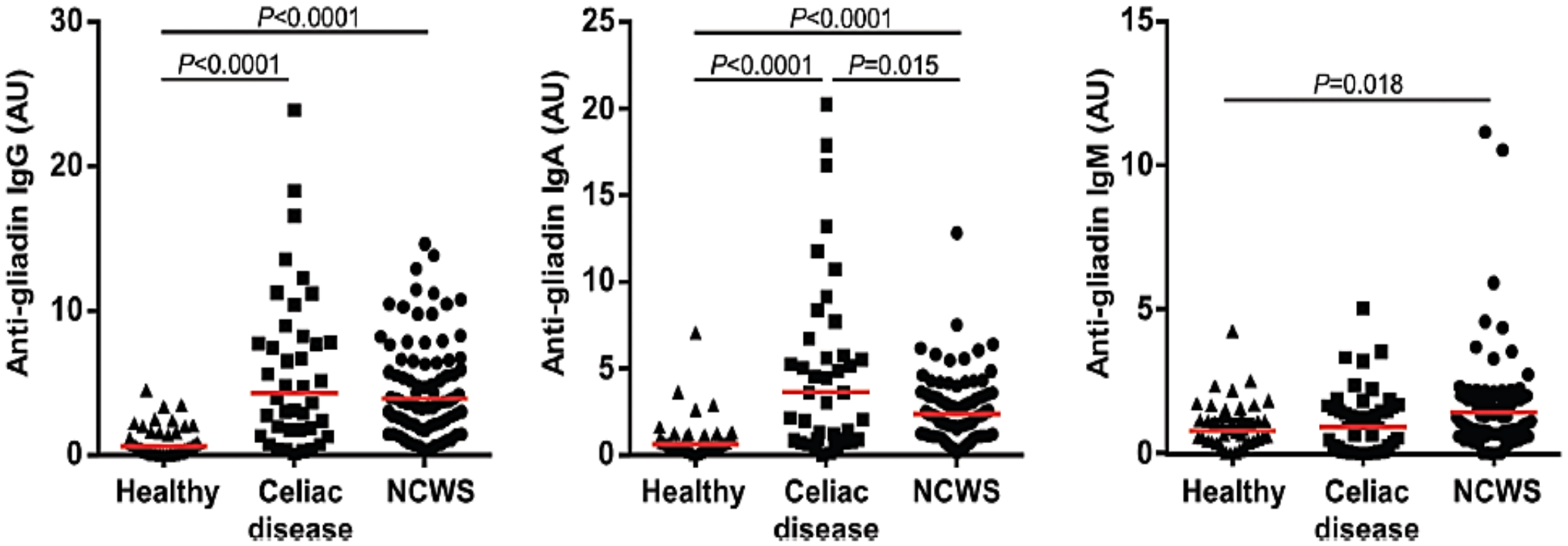
- Prevalence? (between 0.6-6%)
- Are children affected? (only 1 paper published)
- Cause? (Gluten and/or other wheat components?)
- Pathophysiology? (Leaky gut? Innate/adaptive immunity?)
- Natural history? (Permanent? Transient? Complications?)
- Diagnosis? (No marker available)
- Treatment? (GFD or wheat-free diet? How strict? For how long?)

Evidence for Gluten as Responsible for NCWS in IBS-type Adult Patients

- Di Sabatino et al., 2015: 5% of 59 pts
- Elli et al., 2016: 14% of 98 pts
- Zanini et al., 2016: 34% of 35 pts

- Weighted average: 9.8%

Antibodies to Native Gliadin in NCWS vs Celiac Disease (CD) and Healthy Controls



Both CD and NCWS pts had significantly higher levels of IgG, IgA and IgM AGA than healthy controls

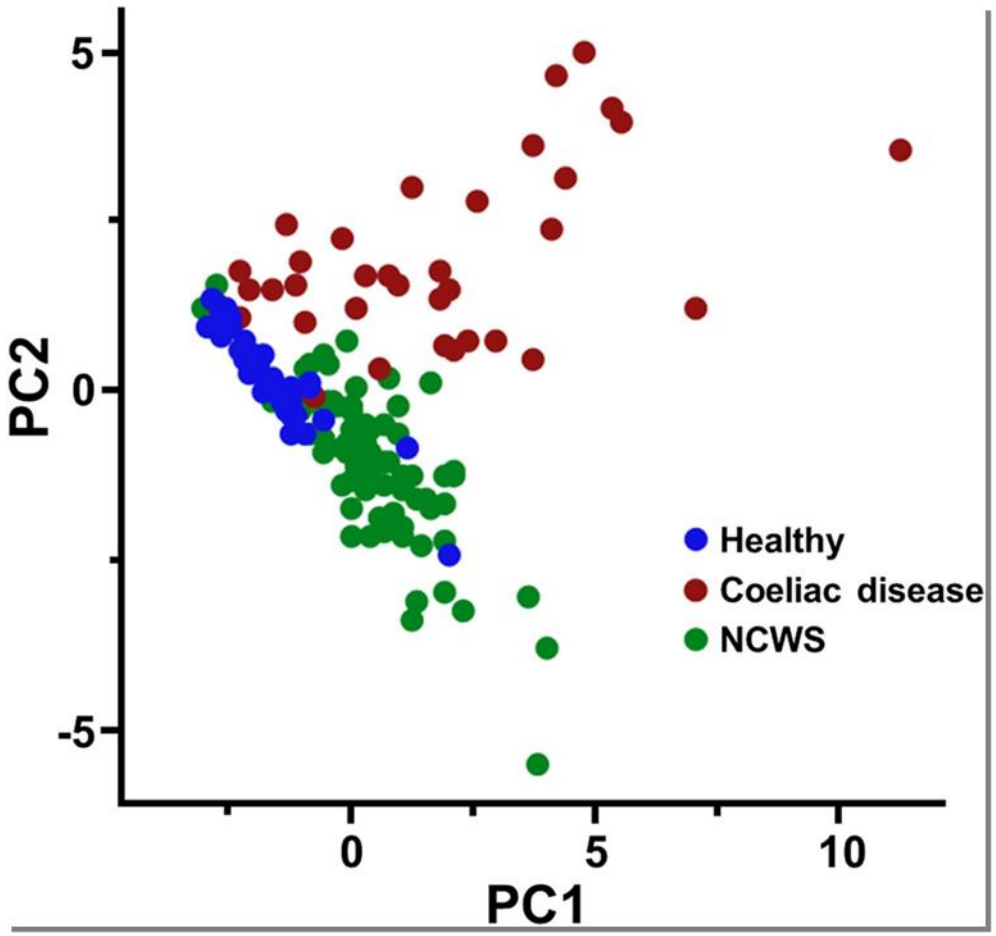
- IgA AGA significantly higher in CD than in NCWS
- IgM AGA not significantly higher in NCWS than in CD and IgG AGA in CD than in NCWS

Uhde M et al. *Gut* 2016

Principal Component Analysis (PCA)

PCA score plot for the complete dataset of serological markers

- Anti-transglutaminase 2 (anti-TG2) IgA
- Anti-deamidated gliadin IgG and IgA
- Anti-gliadin IgG, IgA and IgM
- Lipopolysaccharide-binding protein (LBP)
- Soluble CD14 (sCD14)
- Endotoxin-core antibodies (EndoCAb) IgG, IgA and IgM
- Anti-flagellin IgG, IgA and IgM
- Fatty acid-binding protein 2 (FABP2) measured in healthy controls, patients with coeliac disease and individuals with non-celiac wheat intolerance syndrome (NCWS)



Uhde M et al. *Gut* 2016

Other Potential Causes for NCWS: FODMAP

COMMON FOODS CONTAINING FODMAPs

EXCESS FRUCTOSE	LACTOSE	FRUCTANS	GALACTANS	POLYOLS
> Fruits apples, pears, nashi, mangoes, tinned fruit in natural juice, watermelon **> Sweeteners** fructose, high fructose corn syrup **> Large total fructose dose** concentrated fruit sources, large serves of fruit, dried fruit, fruit juice **> Honey**	**> Milk** cows', goats' and sheeps' milk, yoghurt, ice cream **> Cheeses** soft and fresh (eg. ricotta, cottage)	**> Vegetables** artichokes, beetroot asparagus, Brussels sprouts, cabbage, fennel, garlic, leeks, okra, onions, spring onions (white part), shallots **> Cereals** wheat and rye when eaten in large amounts (eg. bread, pasta, couscous, crackers, biscuits) **> Fruits** watermelon, custard apples, persimmons	**> Legumes** chickpeas, lentils, red kidney beans, baked beans	**> Fruits** apples, apricots, cherries, lychees, nashi, nectarines, pears, peaches, plums, prunes, watermelon **> Vegetables** avocados, mushrooms **> Sweeteners** sorbitol (420), mannitol (421), xylitol (967), maltitol (965), isomalt (953)

✓ SUITABLE ON A LOW-FODMAP DIET

FRUIT	VEGETABLES	MILK PRODUCTS	GRAIN FOODS	OTHERS
> Fruit bananas, grapefruit, blueberries, grapes, honeydew melons, kiwifruit, lemons, limes, mandarin, oranges, pawpaw, passionfruit, tangelos, raspberries, rock-melons, strawberries, tangelos	**> Vegetables** bamboo shoots, bok choy, carrots, celery, capsicums, chokos, choy sum, corn, eggplant, green beans, lettuce, chives, parsnips, pumpkins, silver beet, spring onions (green part only), tomatoes **> Onion/garlic substitutes** garlic-infused oil	**> Milk** lactose-free, rice milk **> Cheeses** 'hard' cheeses, and brie and camembert **> Yoghurt** lactose-free **> Ice-cream substitutes** gelati, sorbet **> Butter substitutes** milk-free spread	**> Cereals** gluten-free bread/ cereal products **> Bread** 100% spelt bread **> Rice** **> Corn** **> Oats** **> Polenta**	**> Sweeteners** sugar (sucrose), glucose, artificial sweeteners not ending in '-ol' **> Honey substitutes** maple syrup, golden syrup

Food Sources of FODMAPs (where FODMAPs are Problematic Based on Standard Serving Size) and Suitable Alternatives

FODMAP	Excess fructose	Lactose	Oligosaccharides (fructans and/or galactans)	Polyols
Problem high FODMAP food source	*Fruits:* apples, pears, nashi pears, clingstone peaches, mango, sugar snap peas, watermelon, tinned fruit in natural juice *Honey* *Sweeteners:* fructose, high fructose corn syrup *Large total fructose dose:* concentrated fruit sources; large serves of fruit, dried fruit, fruit juice	*Milk:* cow, goat and sheep (regular & low-fat), Ice cream *Yoghurt* (regular & low-fat) *Cheeses:* soft & fresh (e.g. ricotta, cottage)	*Vegetables:* artichokes, asparagus, beetroot, Brussels sprout, broccoli, cabbage, fennel, garlic, leeks, okra, onions, peas, shallots. *Cereals:* wheat & rye when eaten in large amounts (e.g. bread, pasta, couscous, crackers, biscuits) *Legumes*: chickpeas, lentils, red kidney beans, baked beans *Fruits:* watermelon, custard apple, white peaches, rambutan, persimmon	*Fruits:* apples, apricots, cherries, longon, lychee, nashi pears, nectarine, pears, peaches, plums, prunes, watermelon *Vegetables:* avocado, cauliflower, mushrooms, snow peas *Sweeteners:* sorbitol(420), mannitol(421), xylitol(967), maltitol (965), isomalt (953) & others ending in '-ol'
Suitable alternative low-FODMAP food source	*Fruit:* banana, blueberry, carambola, durian, grapefruit, grape, honeydew melon, kiwifruit, lemon, lime, mandarin, orange, passionfruit, paw paw, raspberry, rock melon, strawberry, tangelo. *Honey substitutes:* maple syrup, golden syrup *Sweeteners:* any except polyols	*Milk:* lactose-free, rice milk *Cheese:* 'hard' cheeses including brie, camembert *Yoghurt:* lactose-free *Ice cream substitutes:* gelati, sorbet *Butter*	*Vegetables:* bamboo shoots, bok choy, carrot, celery, capsicum, choko, choy sum, corn, eggplant, green beans, lettuce, chives, parsnip, pumpkin, silver beet, spring onion (green only), tomato *Onion/garlic substitutes:* garlic-infused oil *Cereals:* gluten-free & spelt bread/cereal products	*Fruits:* banana, blueberry, carambola, durian, grapefruit, grape, honeydew melon, kiwifruit, lemon, lime, mandarin, orange, passionfruit, paw paw, raspberry, rock melon *Sweeteners:* sugar (sucrose), glucose, other artificial sweeteners not ending in 'ol'

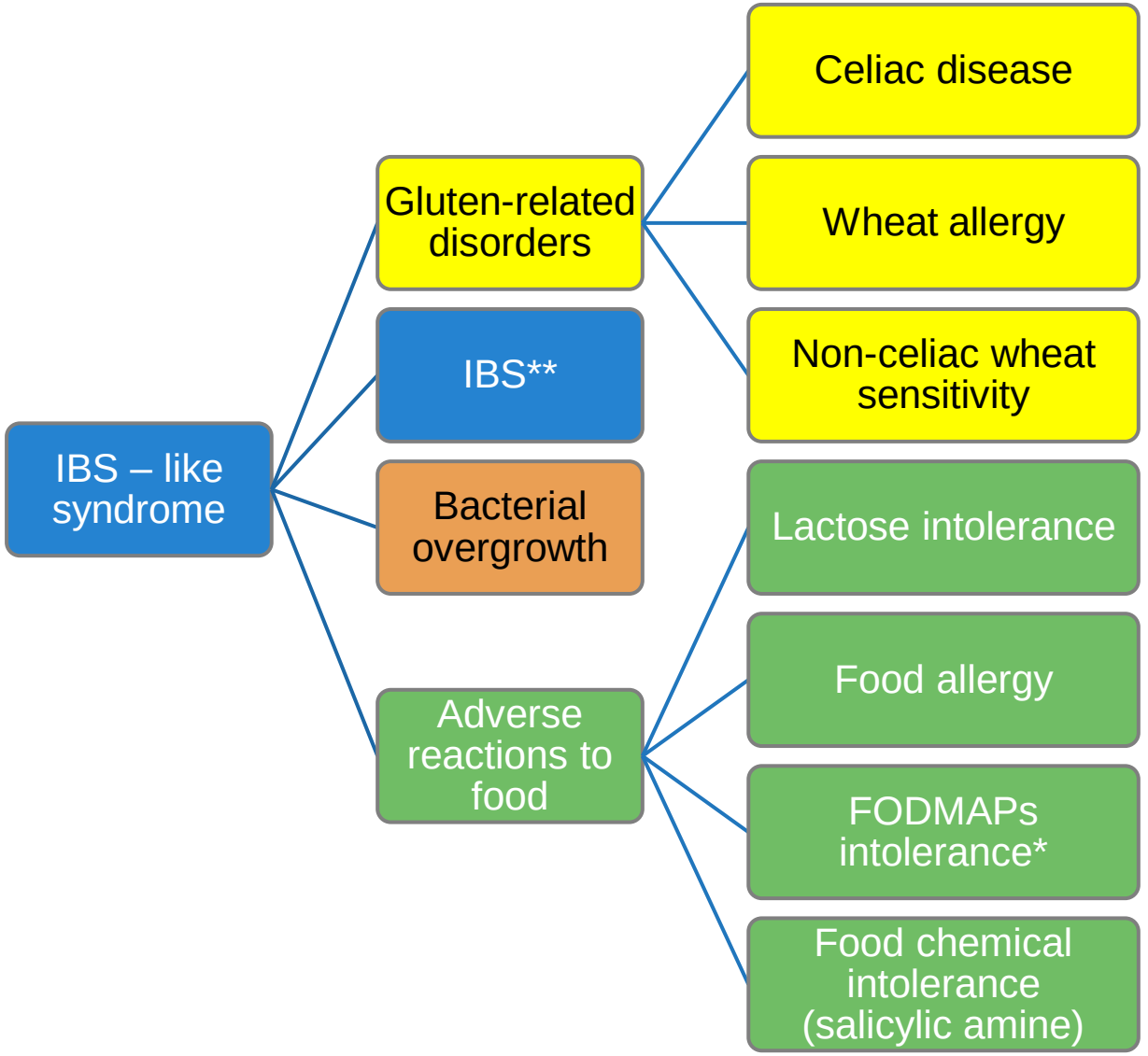
Gibson PR, Sheperd SJ. *J Gastroenterol Hepatol.* 2010;25:252-258.

Definition of Food Reactions

(Consensus NIAID 2011)

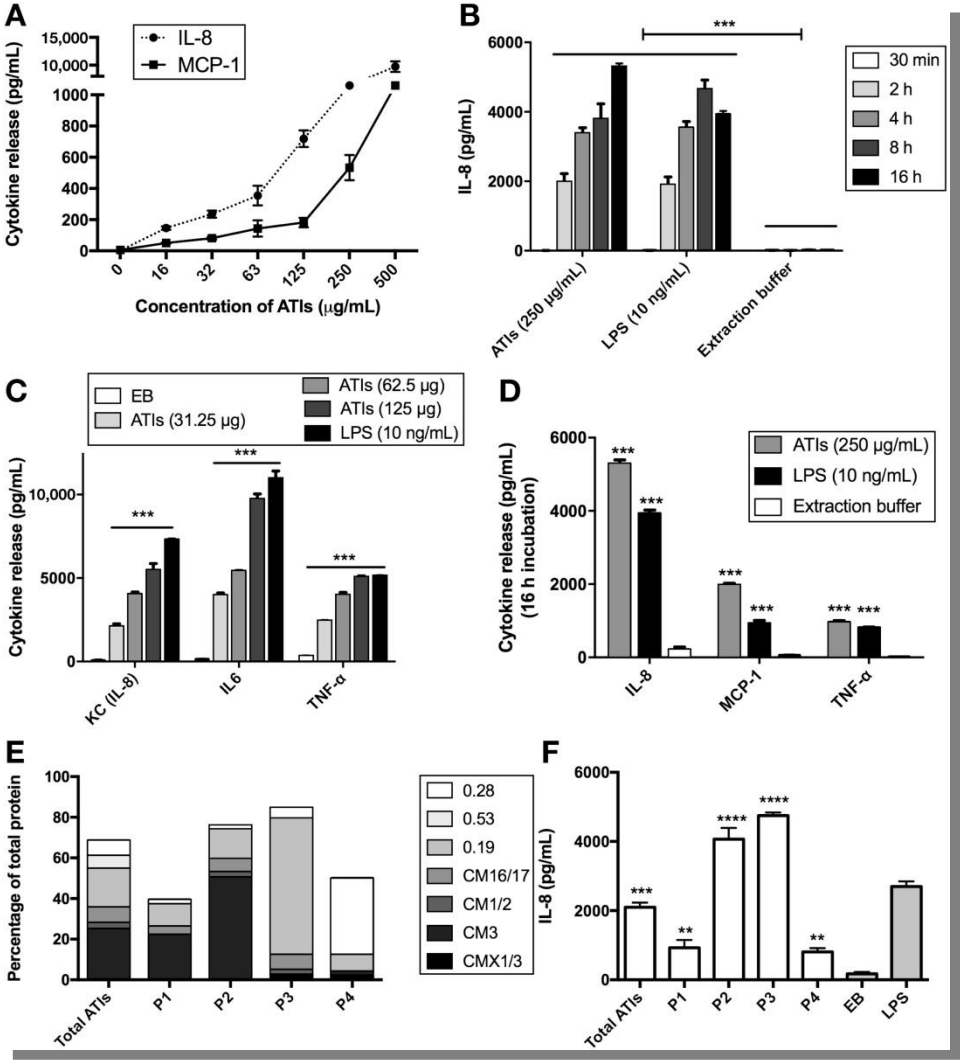
- **Food intolerance** occurs when the body lacks a particular enzyme to digest nutrients, nutrients are too abundant to be completely digested, or a particular nutrient cannot be properly digested, Common examples are lactose intolerance, FODMAP intolerance, or lactulose intolerance (side effect of laxatives).
- **Food sensitivity**, an understudied area, are immune-mediated reaction to some nutrients and these reactions do not always occur in the same way when eating that particular nutrient.
- **Food allergy** is a very specific immune system response involving either the immunoglobulin E (IgE) antibody or T-cells. Both are immune system cells that react to a particular food protein, such as milk protein.

Pathogenesis Of IBS-Like Syndromes



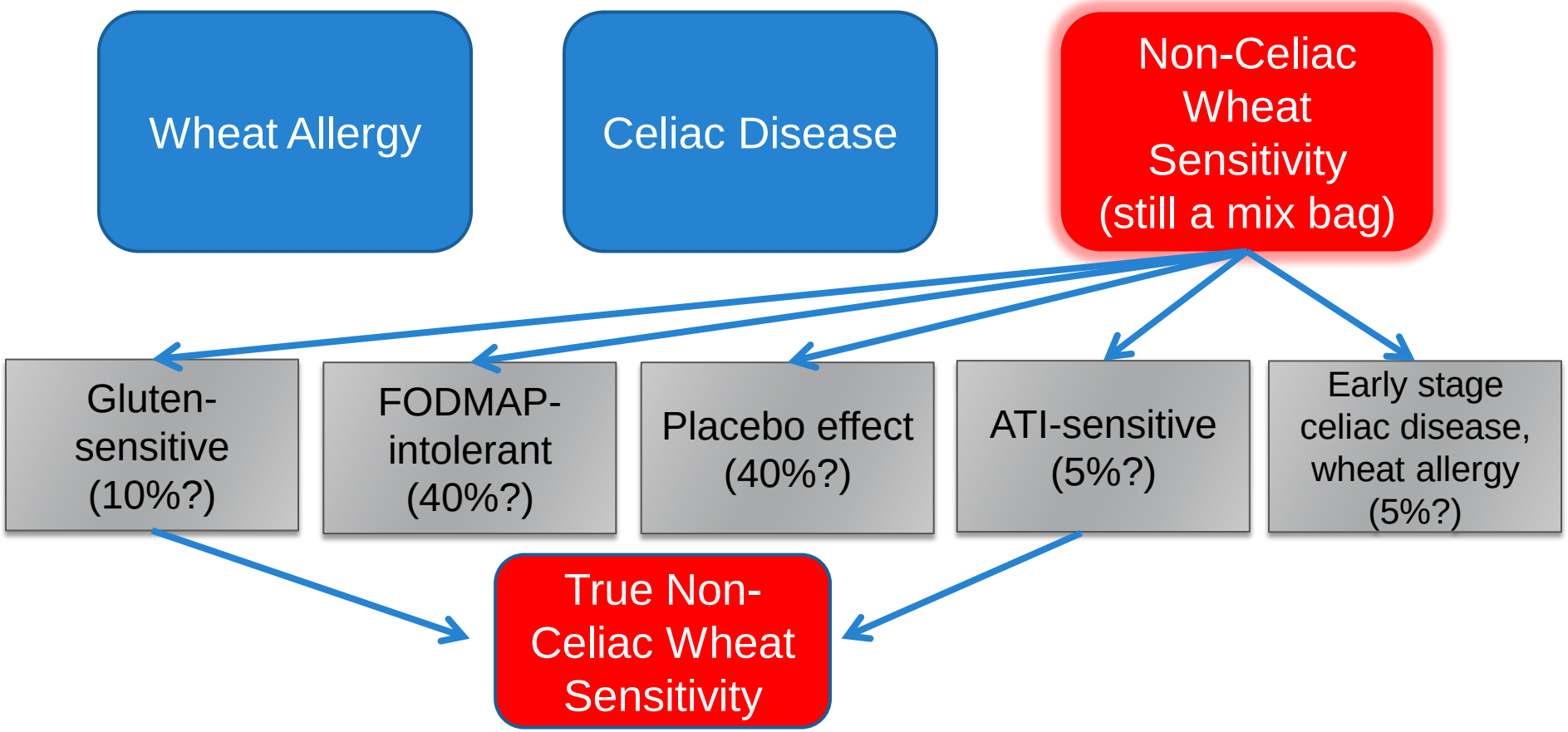
Czaja-Bulsa G et al, *Clin Nutr* 2014

Wheat Amylase-Trypsin Inhibitors (ATI)



Zevallos VF et al., *Gastroenterology* 2017

Adverse Effects of Wheat Ingestion in Humans – Non-Celiac Wheat Sensitivity (cont'd)



Mrs. Smith

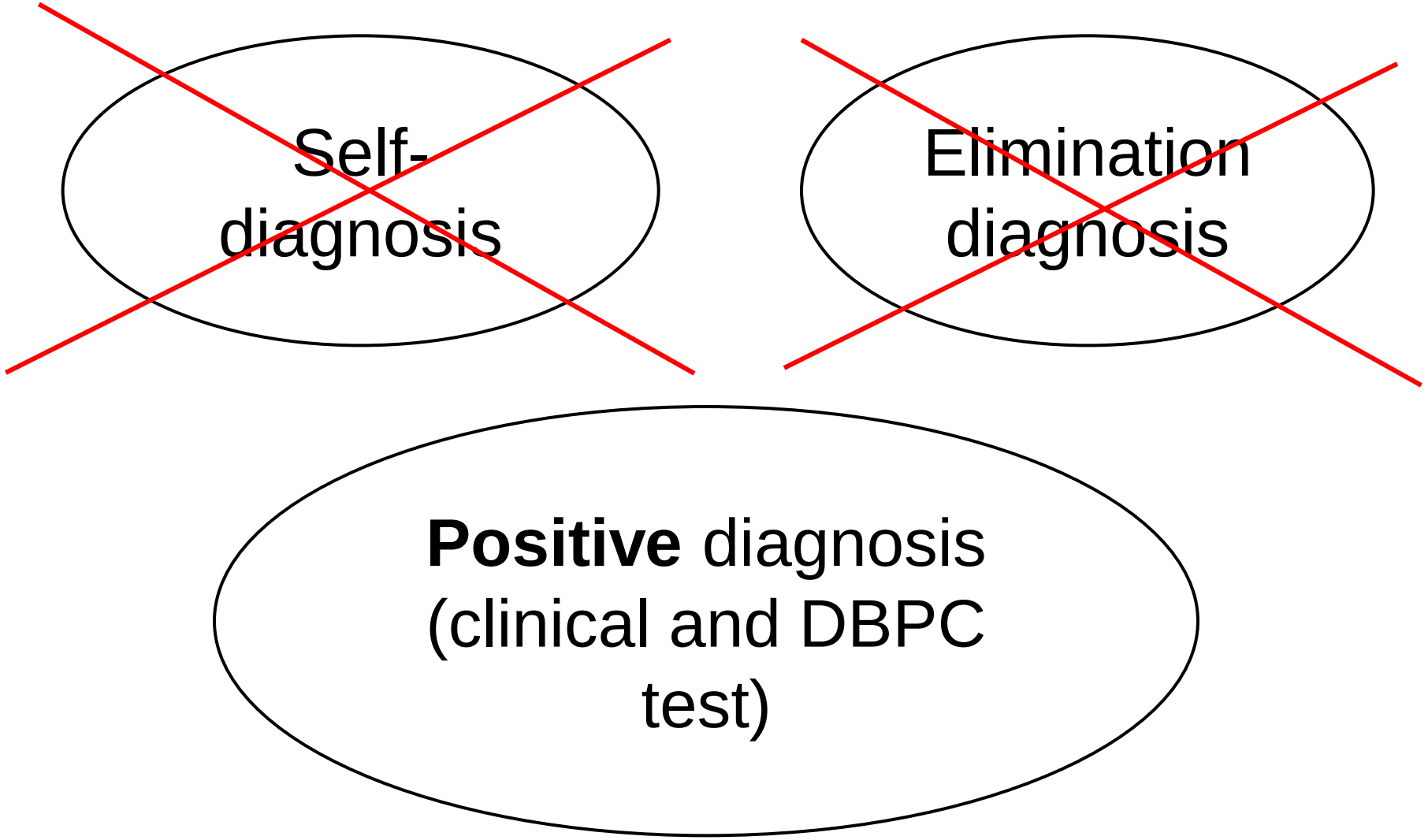
- 42 year old woman, who had headaches, foggy mind, some bloating, occasional abdominal pain. Much better when off wheat.

Sounds like NCWS

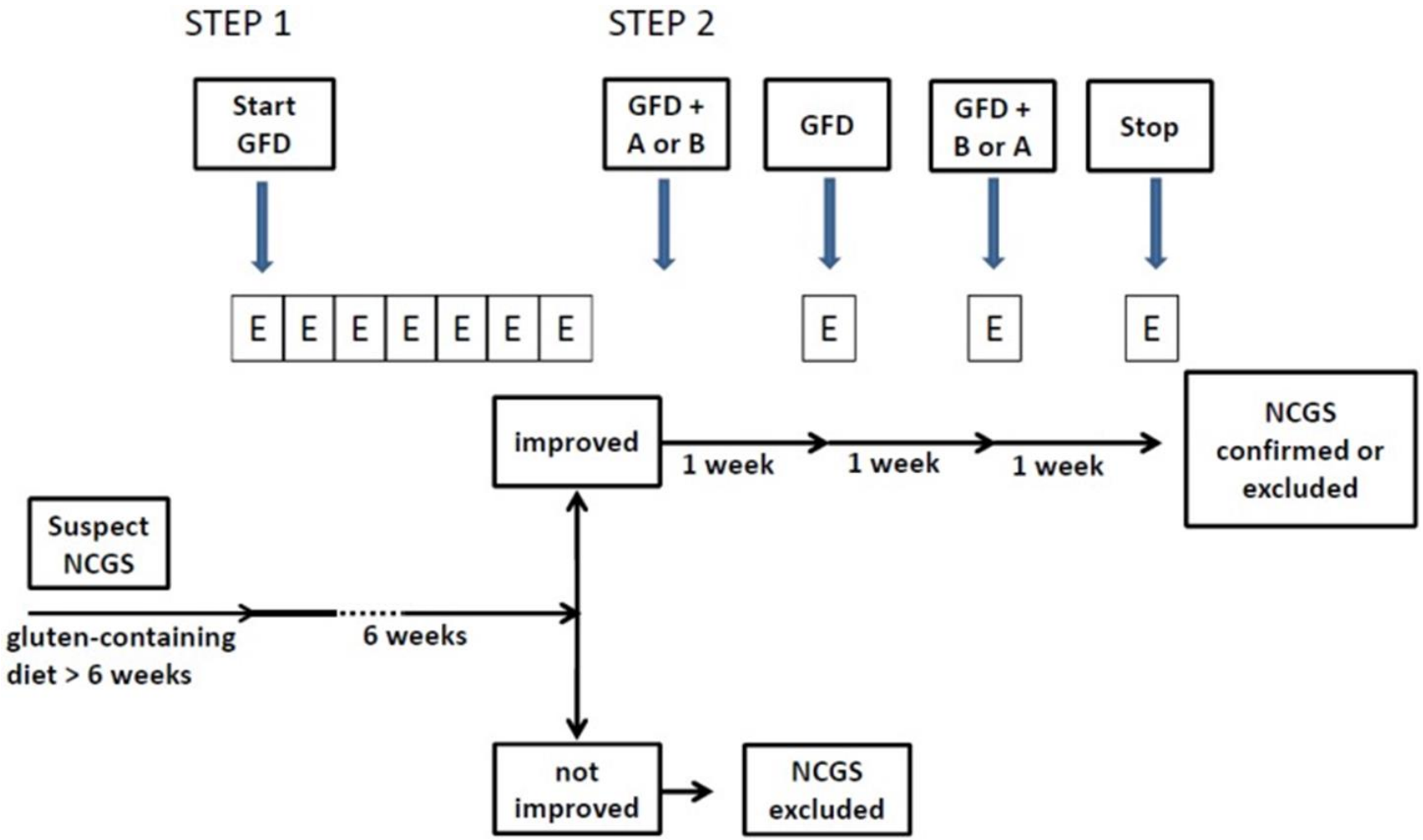
Diagnostic Algorithm for Suspected NCWS

Remember: NO biomarker!

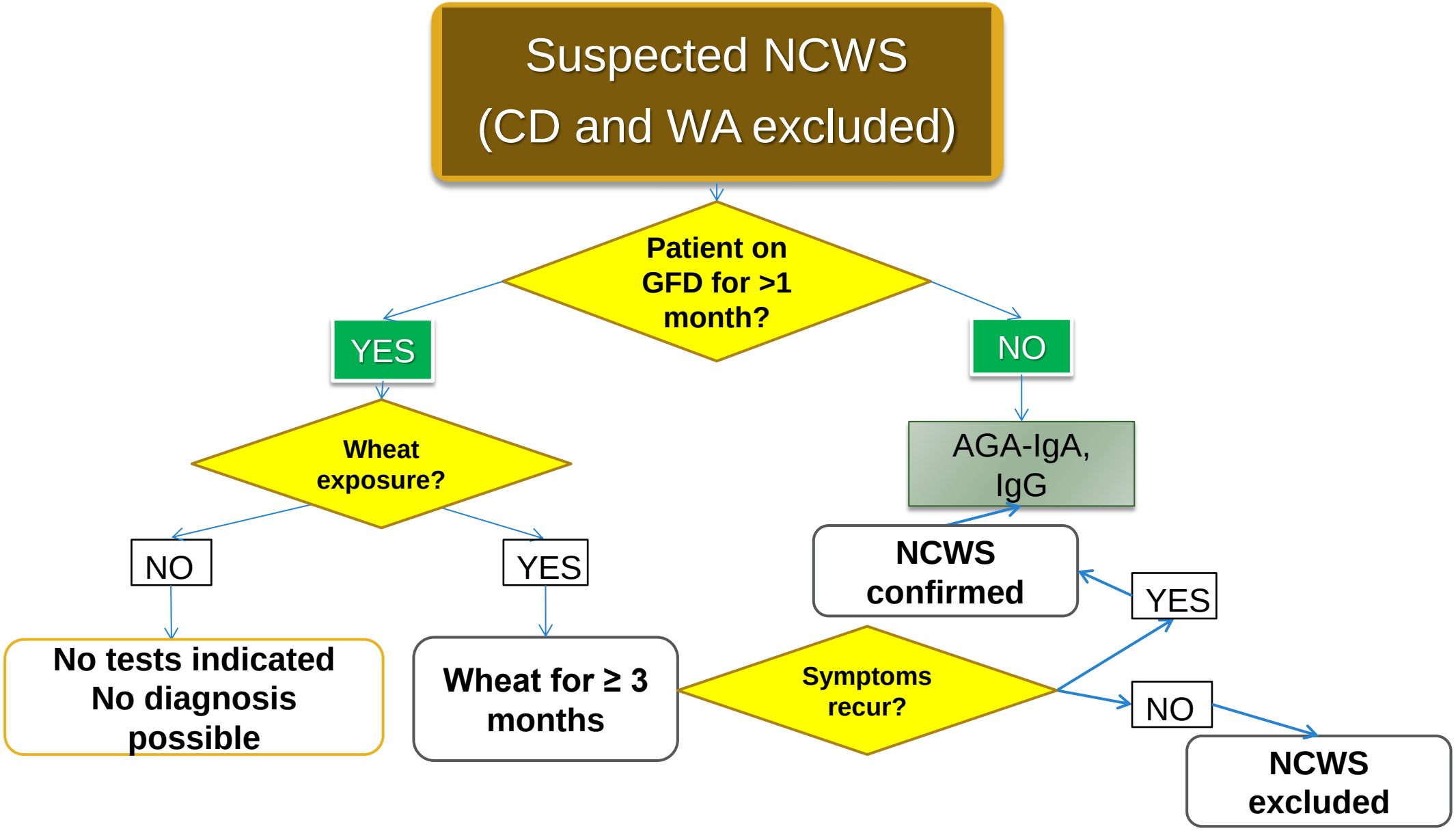
The Diagnosis of NCWS



Proposed Algorithm for NCWS Diagnosis



Catassi C. et al. *Nutrients* 2015; 7:4966-77

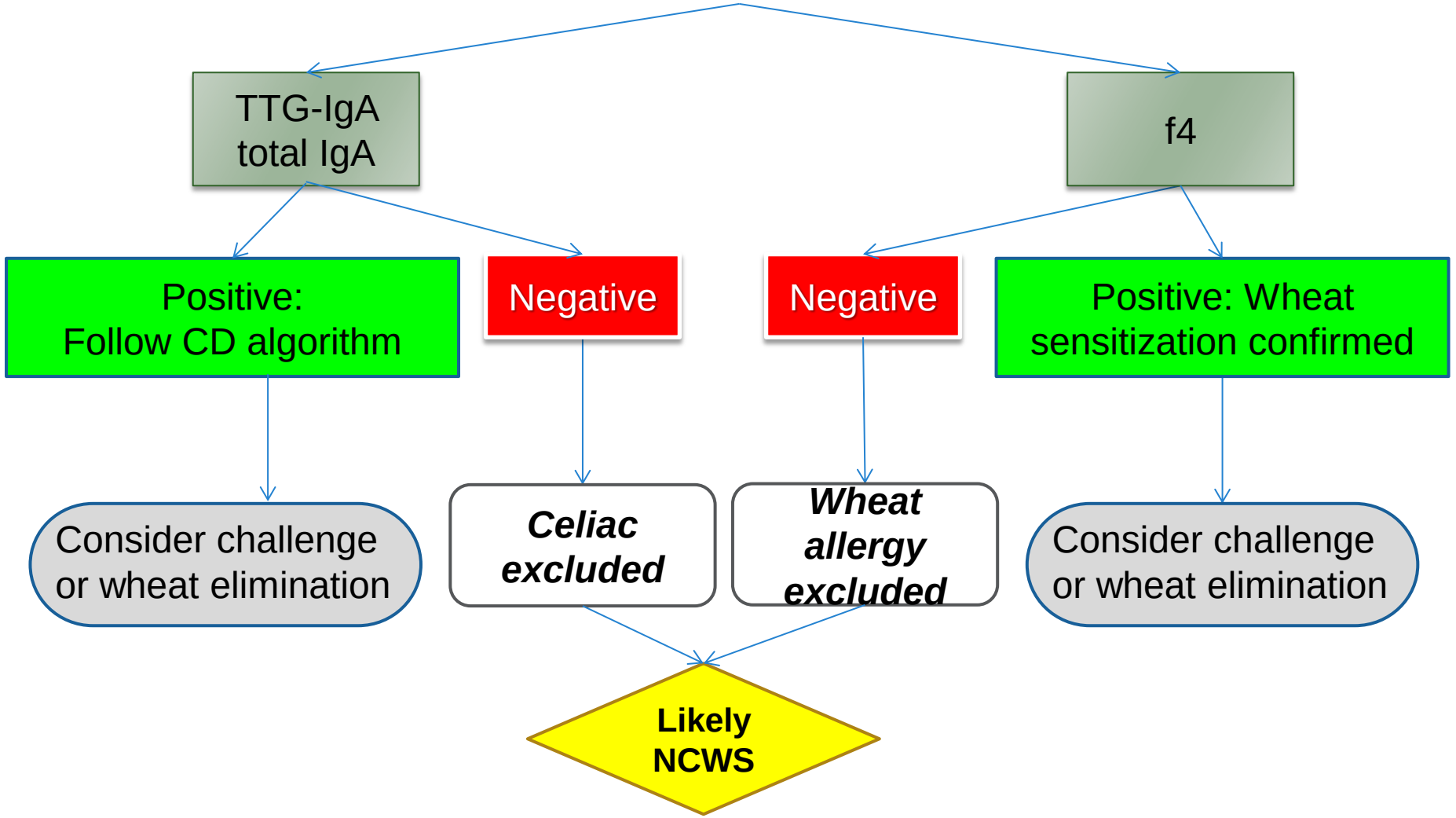

Suspected NCWS
(CD and WA excluded)
Patient on GFD for >1 month?
YES
NO
Wheat exposure?
AGA-IgA, IgG
NO
YES
NCWS confirmed
YES
No tests indicated
No diagnosis possible
Wheat for ≥ 3 months
Symptoms recur?
NO
NCWS excluded

Ms. Jones

- 25 year old lady, with c/o itching rash, headaches, bloating, nausea and occasional diarrhea when ingesting wheat foods

A wheat-related disorder, obviously.
But… which one of the 3?

A Lab Approach to Generic Wheat-Related Disorders



Thank you for your Attention

Questions?