CELIAC DISEASE

Jolanta Jedrzkiewicz, MD, 10.15.12

University of Utah CME Statement

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- Speakers are also expected to openly disclose intent to discuss any off-label, experimental, or investigational use of drugs, devices, or equipment in their presentations.
- This speaker has nothing to disclose.

9 month old boy with failure to thrive

- On questioning:
  - 3 months ago, solid foods were introduced to his diet including **cereal**
- Physical exam:
  - Dehydrated, irritable, has a distended abdomen and has not gain weight since the last visit (a month ago)
- **Family history:**
  - Both parents have celiac disease

Diagnosis: CELIAC DISEASE
Treatment: Gluten free diet

Celiac disease (CD)

- **Definition:**
  - Inflammatory disease of the gut that occurs in predisposed individuals after gluten (wheat) ingestion.

CME objectives

- List the symptoms of celiac disease
- Identify the agent that causes celiac disease
- List criteria for diagnosis
- Compare and contrast different tests that are used
- Justify utilization of serology (antibody test)
- List the antibodies that we test for
- Justify utilization of HLA testing
- List the HLA tests that are used in assessing the risk
- Describe current guidelines and recommendations

Topics

- Definitions and historical background
- Clinical symptoms
- Histologic changes in celiac disease
- Pathophysiology
- Serologic tests
- HLA testing
- Screening
- Treatment
- Monitoring adherence to treatment
- Cases

Why celiac disease?

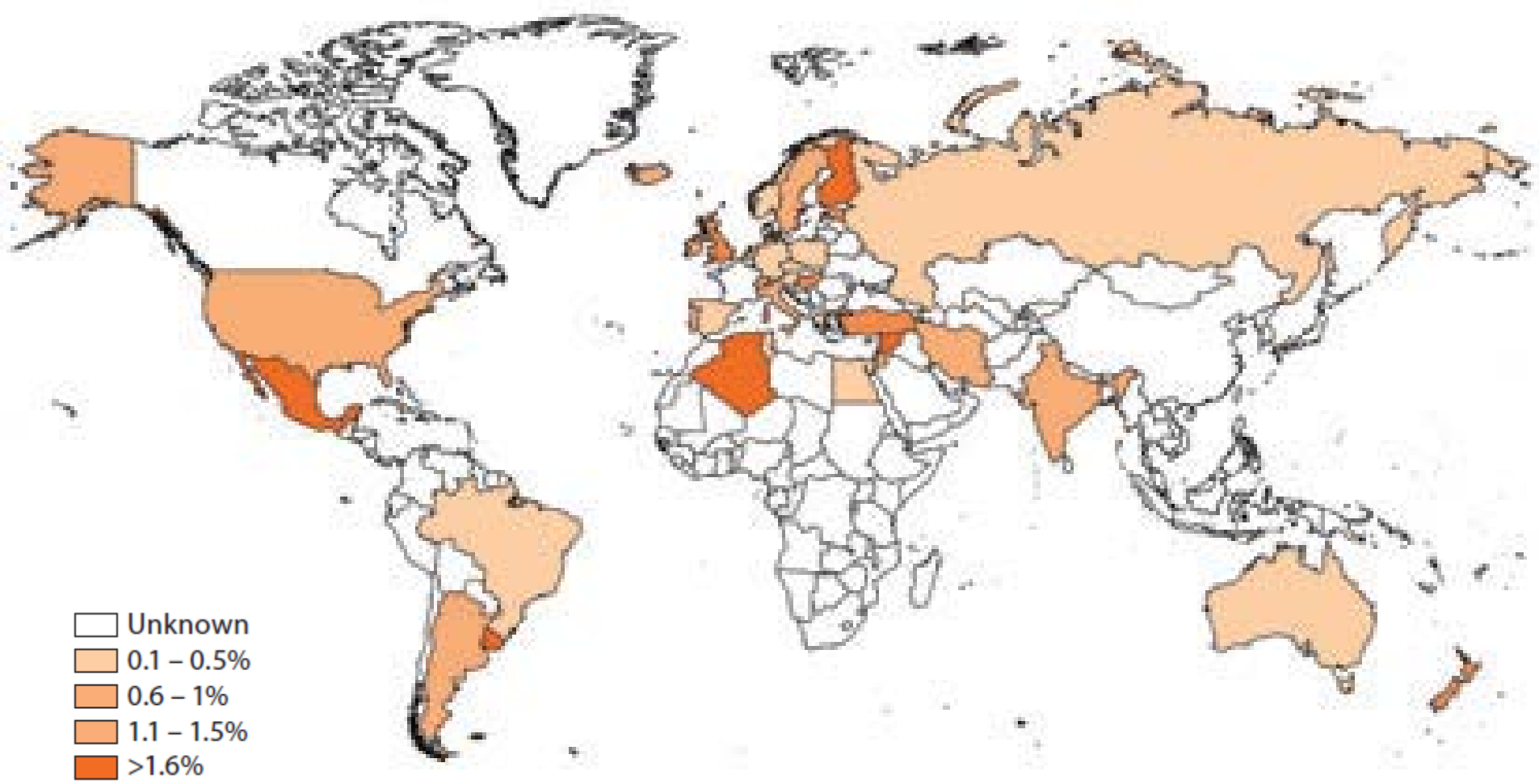
- 1% in the U.S.
  - 5% prevalence in type 1 DM patients
  - 10% higher prevalence in first degree relatives
    - 70% concordance in identical twins
  - Population affected:
    - Age: infancy, 20-30s, 50-60s
    - Historically, Caucasians of North European ancestry
- Consequences for untreated patients
  - Malabsorption
  - Cancer

Historical background

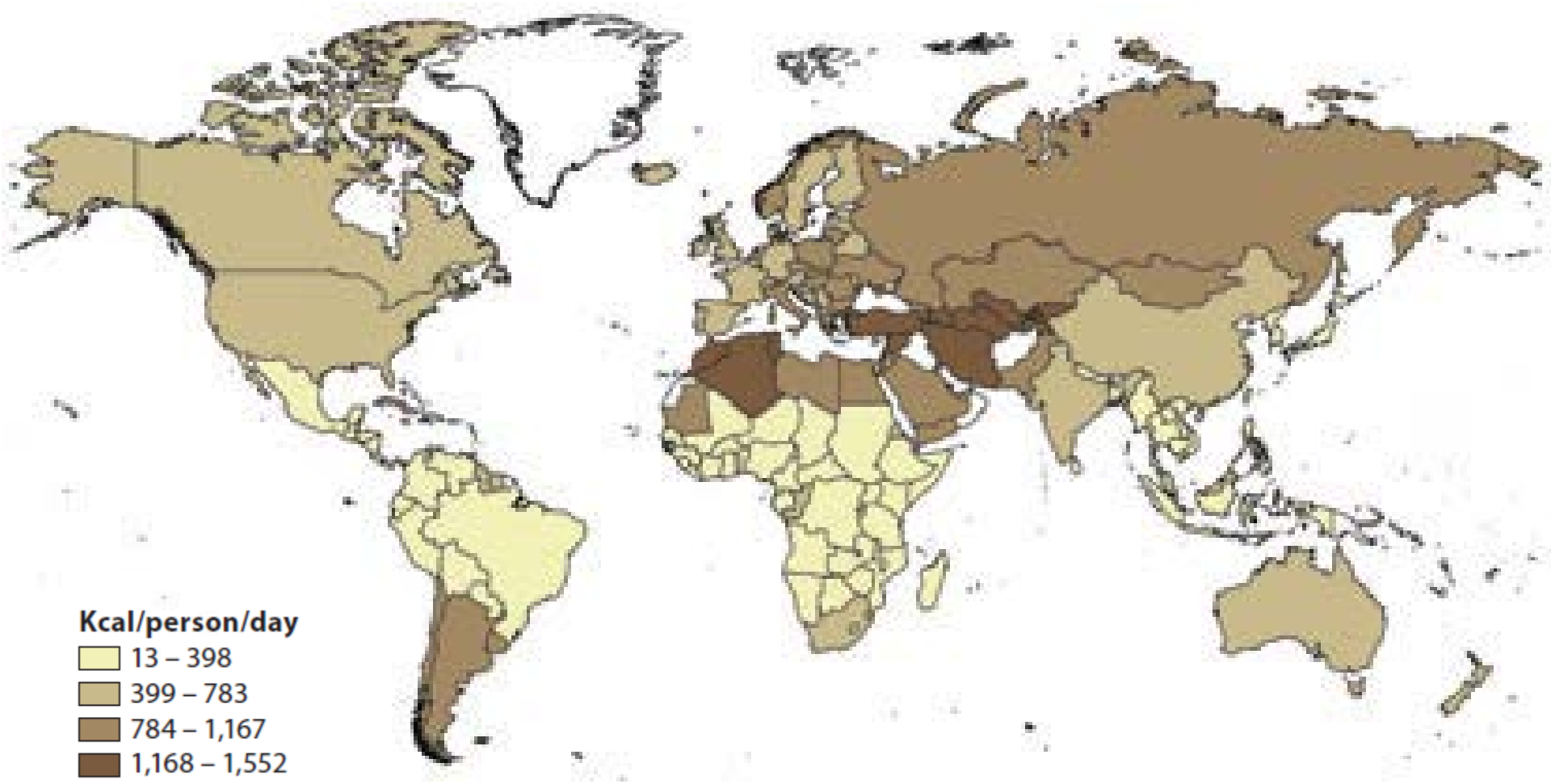
- 100-200 AD: Aretaeus (Greek physician)
  - Description of disease
- 1888: Gee
  - Dietary causation
- 1950s: Dicke and colleagues
  - Wheat and rye brought on celiac disease
- 1954: Paulley
  - Clinical manifestation are linked to small intestine damage
- 1990s: genetic association with HLA
- 1992: Marsh
  - Histologic classification

Abadie, 2011

Prevalence of celiac disease

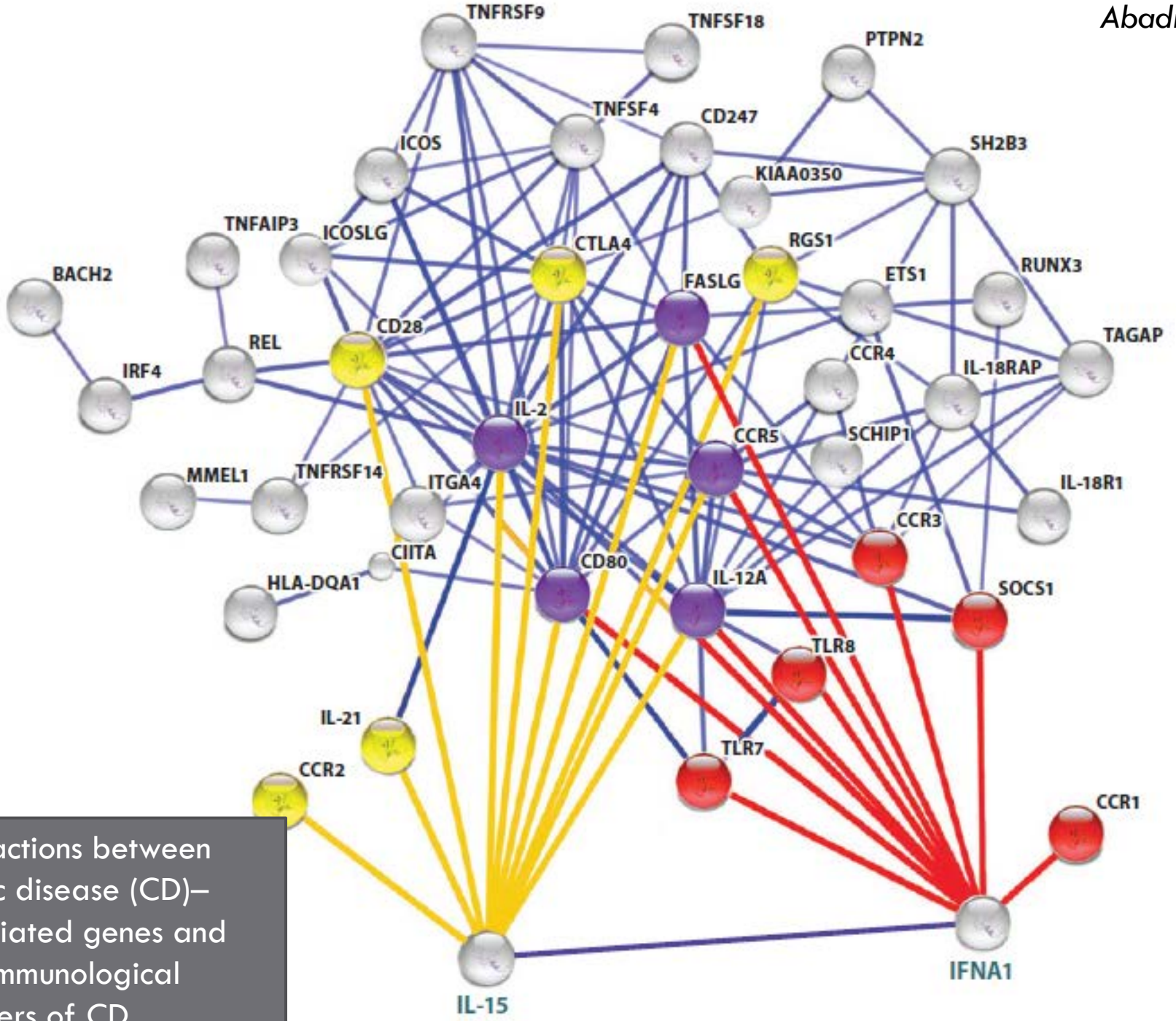


Wheat consumption

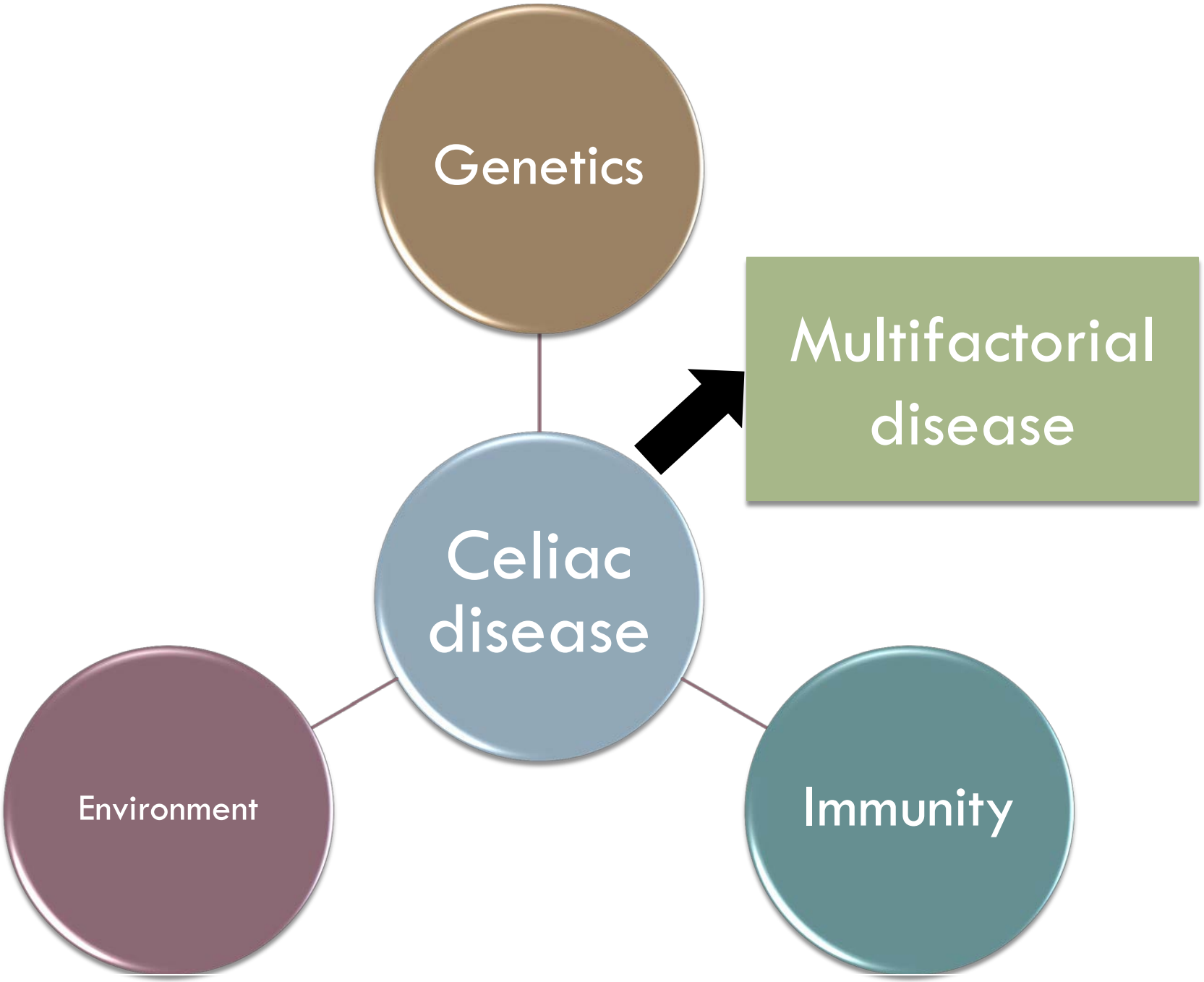


Abadie, 2011

Abadie, 2011



Interactions between celiac disease (CD)–associated genes and key immunological markers of CD.


Genetics
Multifactorial disease
Celiac disease
Environment
Immunity

Clinical manifestation

- Intermittent diarrhea
- Abdominal pain
- Bloating

- Irritability or depression
- Stomach upset
- Joint pain
- Muscle cramps

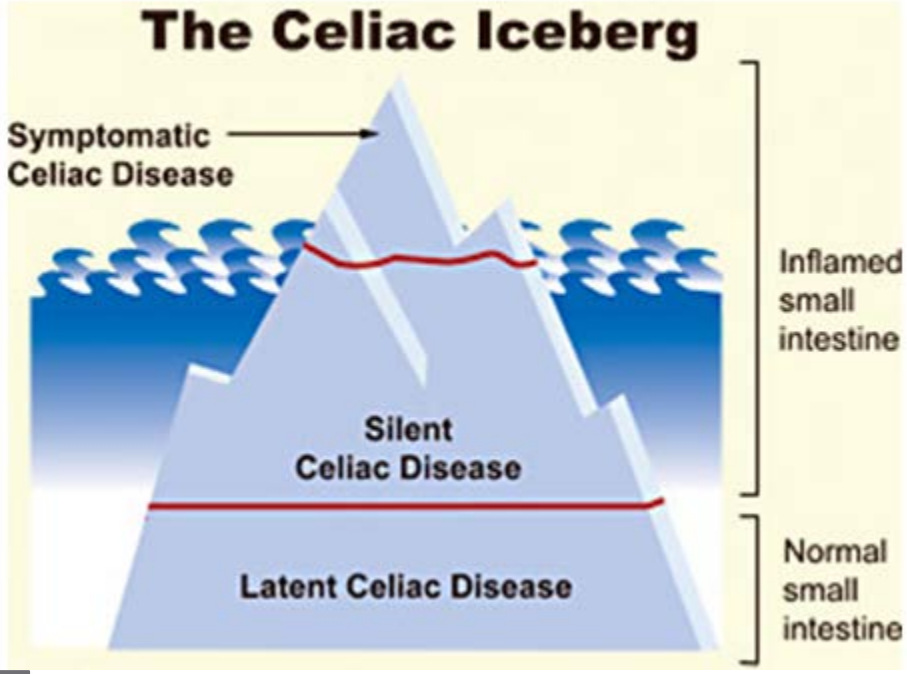
Nonspecific

- Anemia
- Osteoporosis
- Neuropathy

Malabsorption, *common*

- Skin rash
- Mouth sores

Dermatitis herpetiformis

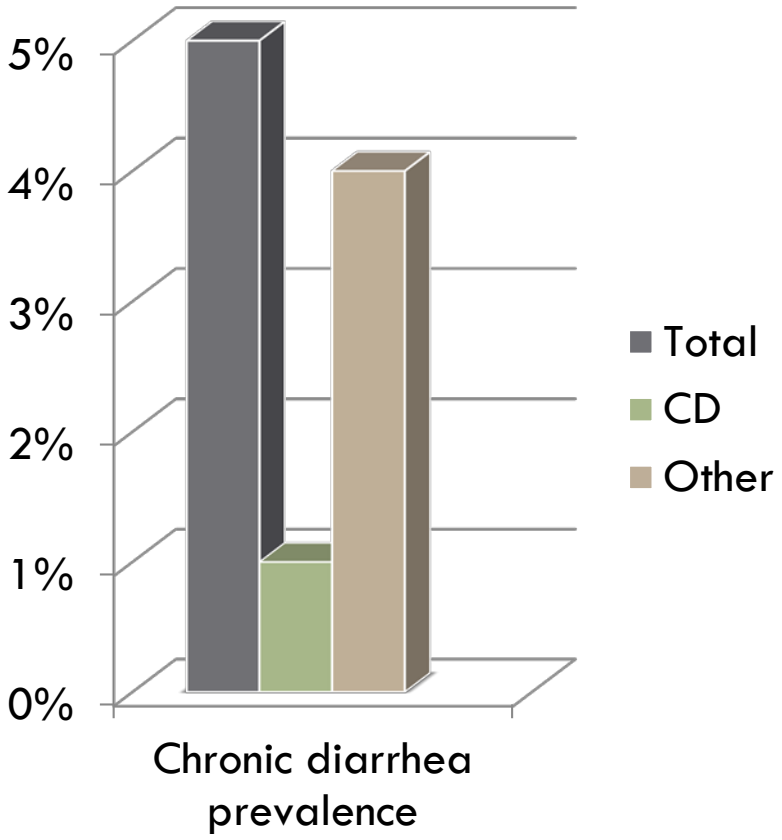


p://fe.blog.images.s3.amazonaws.com/2010/04/celiac_iceberg.jpg

Up-to-date. "Approach to the adult with chronic diarrhea in developed countries" *Last updated on Jun 7,2011*

Abdominal symptoms

- **Villous atrophy/ damage**
  - Chronic diarrhea
    - ≥3 watery or loose bowel movements in a 24h
    - ≥ 4 weeks
    - Osmotic type
      - Water to be pulled into the gut
      - Steatorrhea (diarrhea with high fat content)
- Abdominal bloating
- Abdominal pain



Malabsorption

Malabsorption

- Total malabsorption
  - Vitamins
  - Minerals
  - Proteins
  - Fats

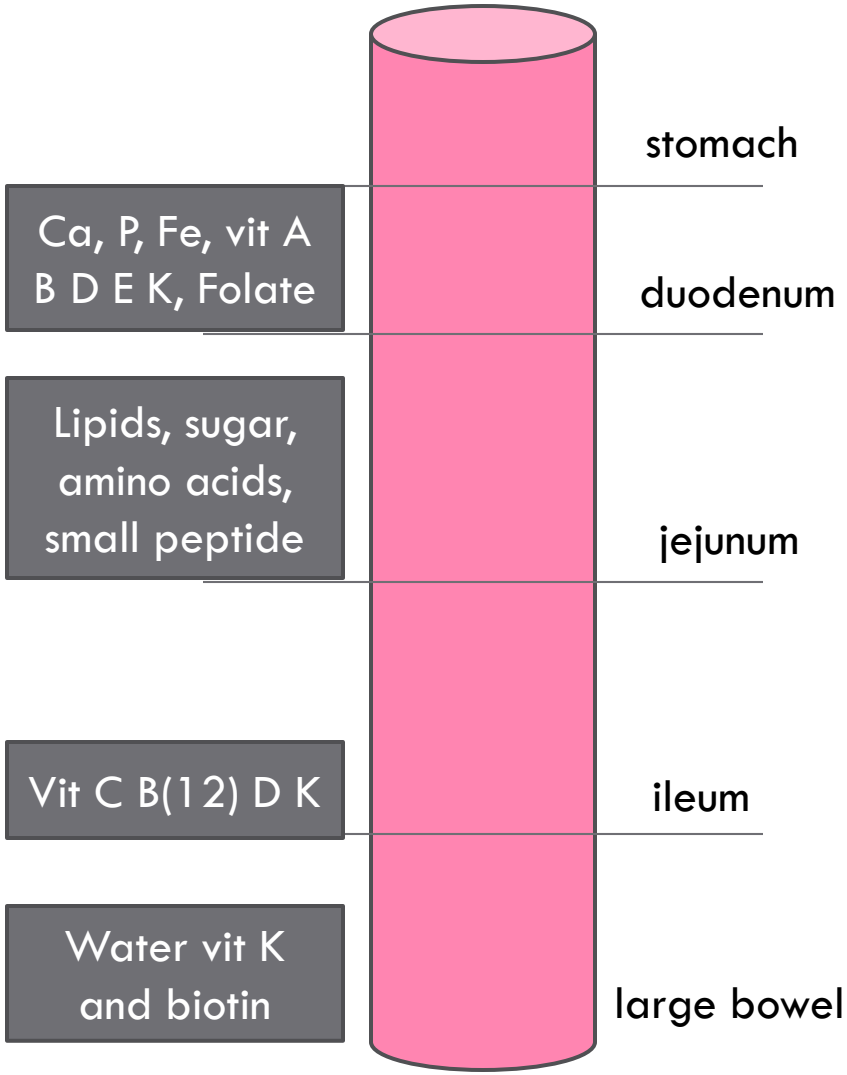
Anemia

Osteoporosis

Neuropathy

Weight loss & weakness

Steatorrhea



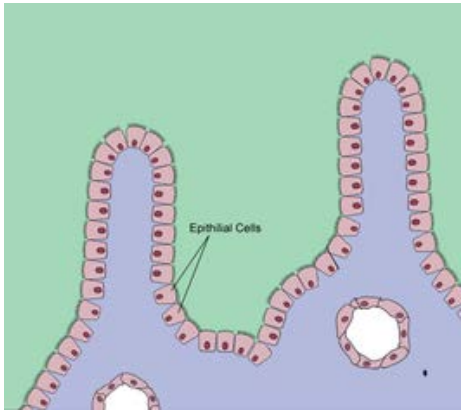
Spada et al, 2008

Imaging

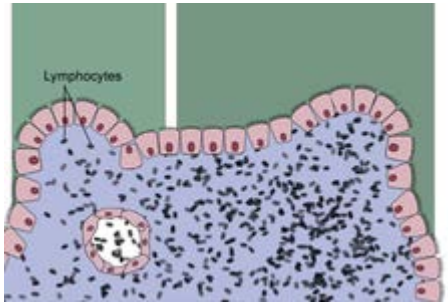
Normal villi

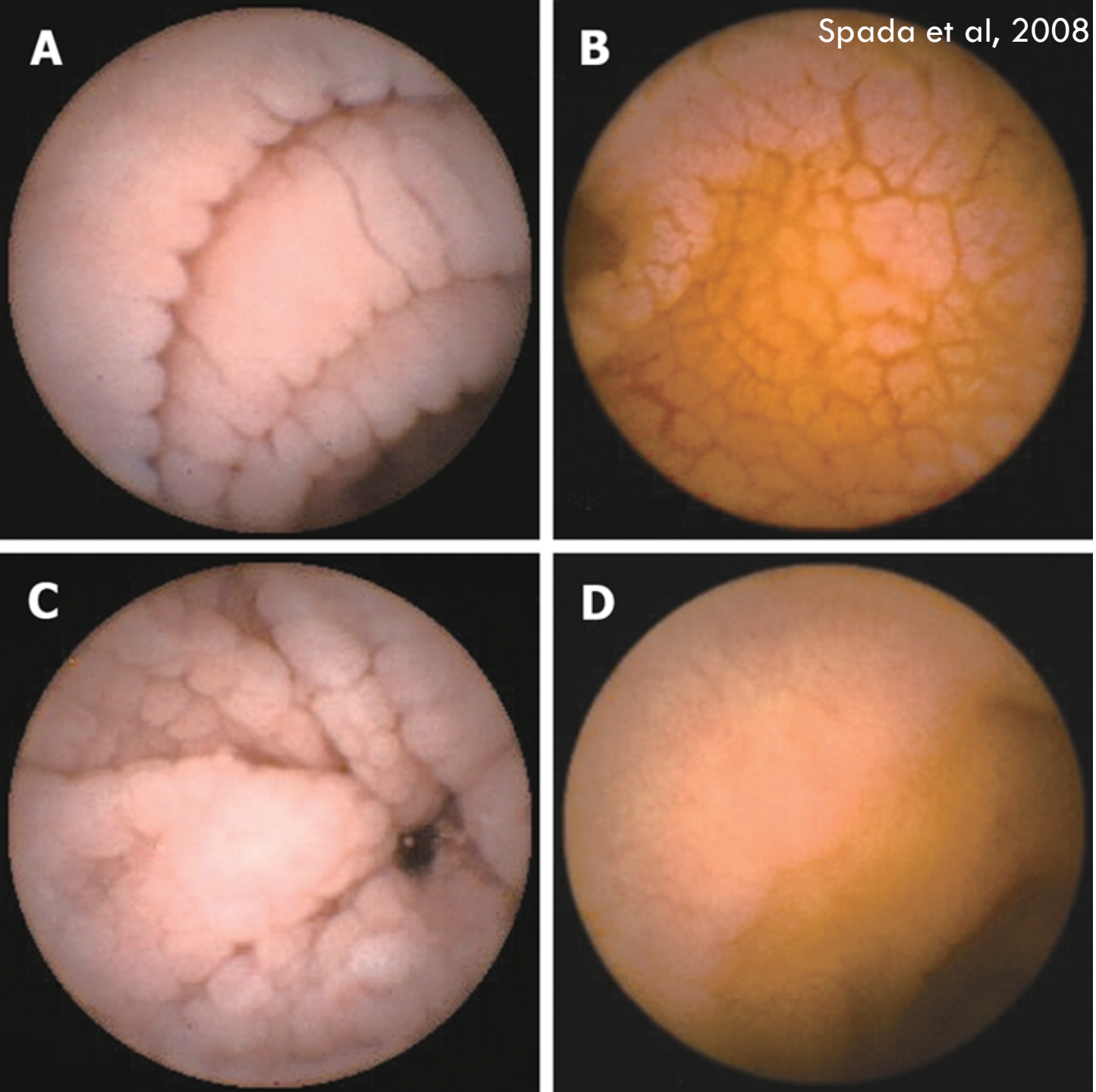
Atrophic villi,
celiac disease

Normal villi



**Atrophic villi,
celiac disease**





Spada et al, 2008

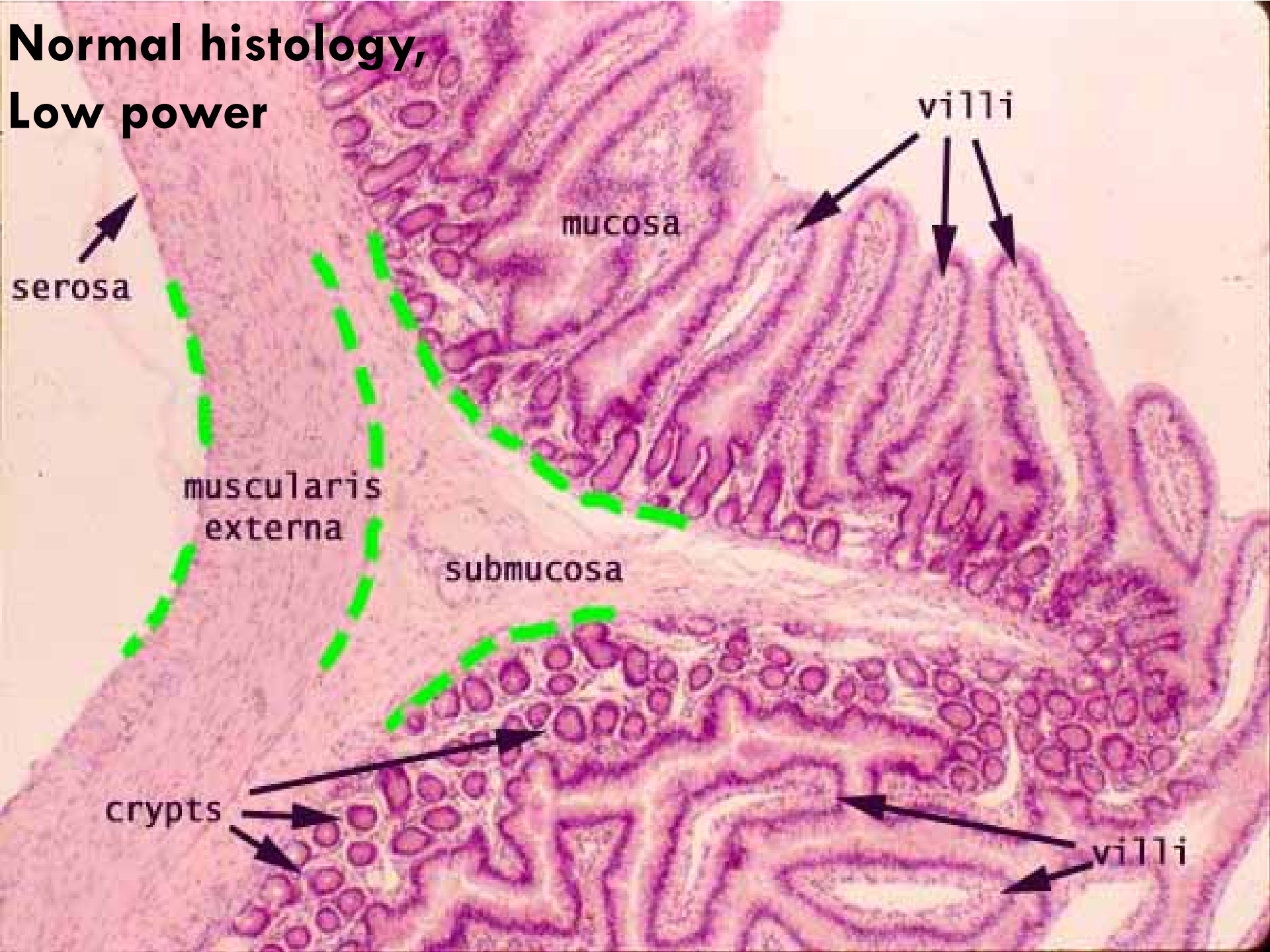
A. Scalloping

B. Mosaic pattern

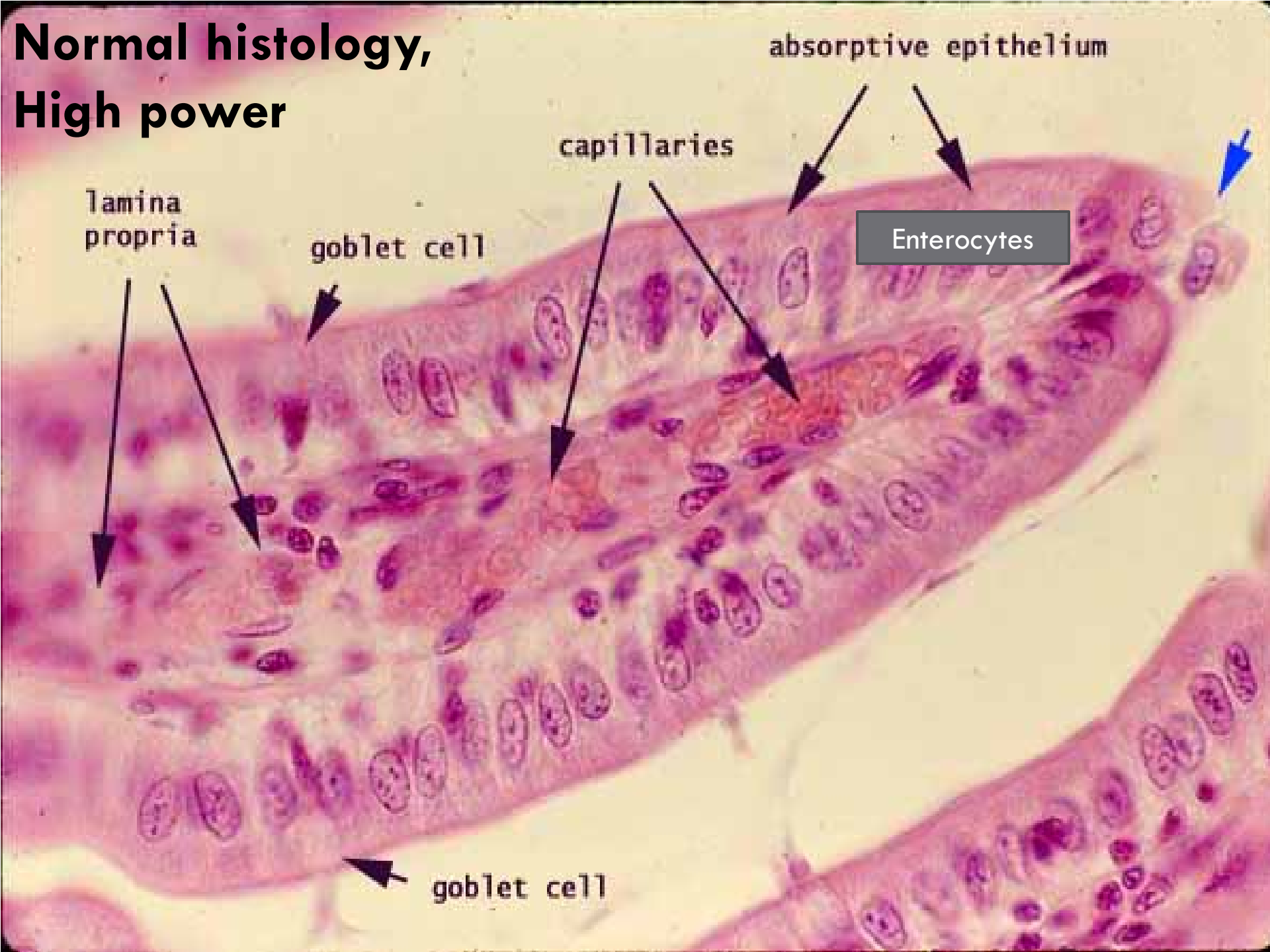
C. Micro-nodularity

D. Reduction of folds

Normal histology, Low power

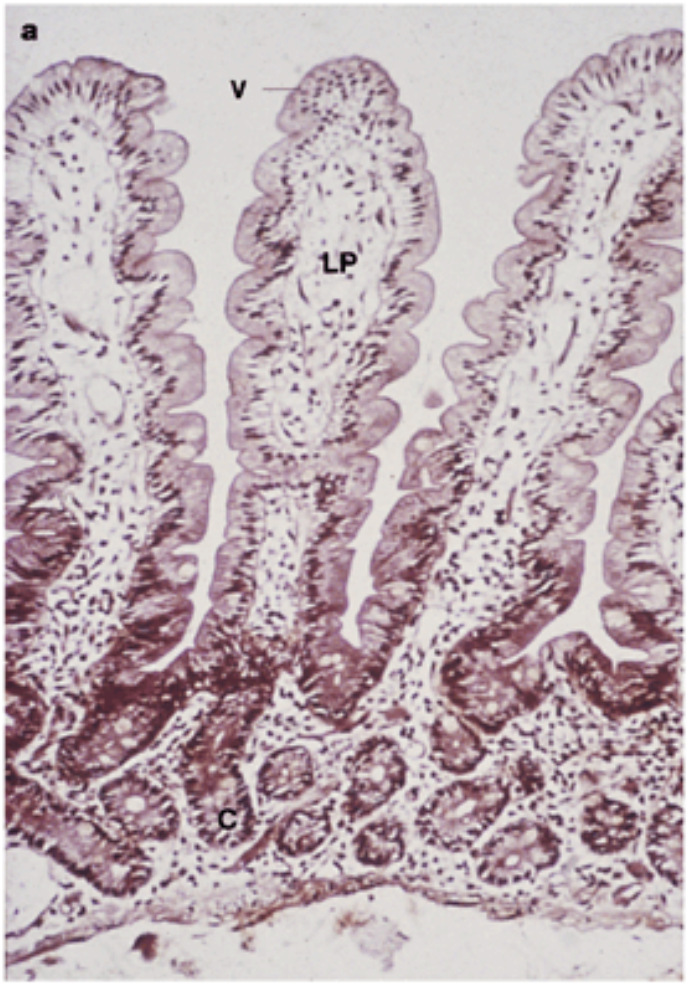


Normal histology, High power

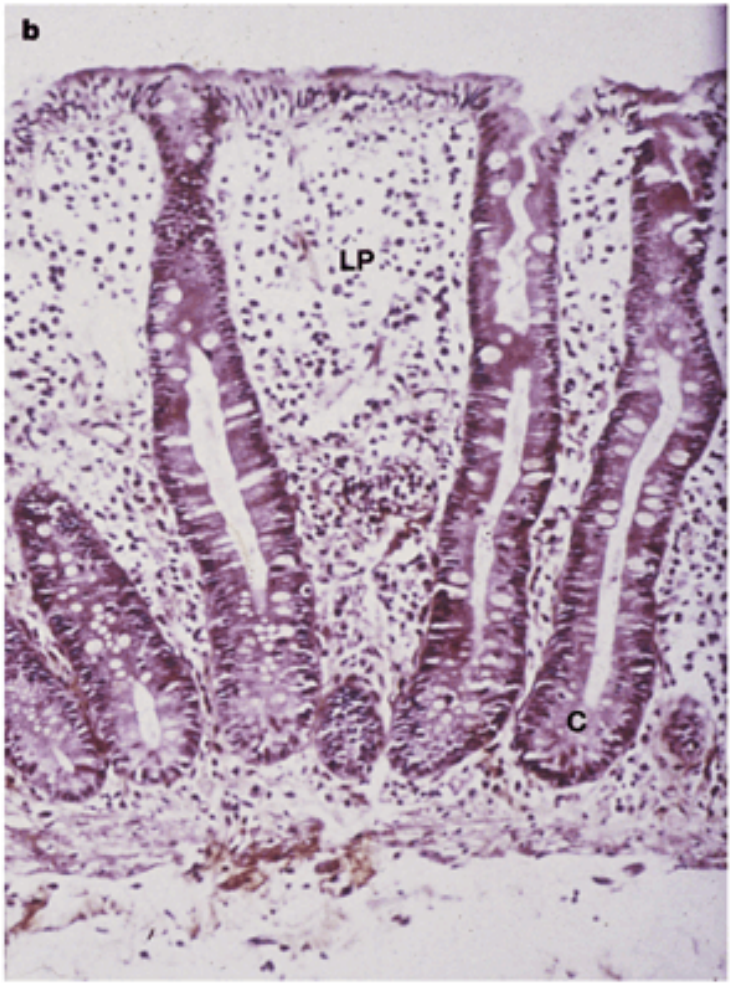


Comparing normal and abnormal

Normal histology, long villi



Abnormal histology, short and bland (atrophic) villi, celiac disease



Microscopic findings in CD

- Intraepithelial lymphocytes
- Increase in plasma cells in lamina propria
- Atrophy or total loss of villi
- Fat globules in surface epithelium
- Enterocytes have stratified nuclei
- Crypt hyperplasia

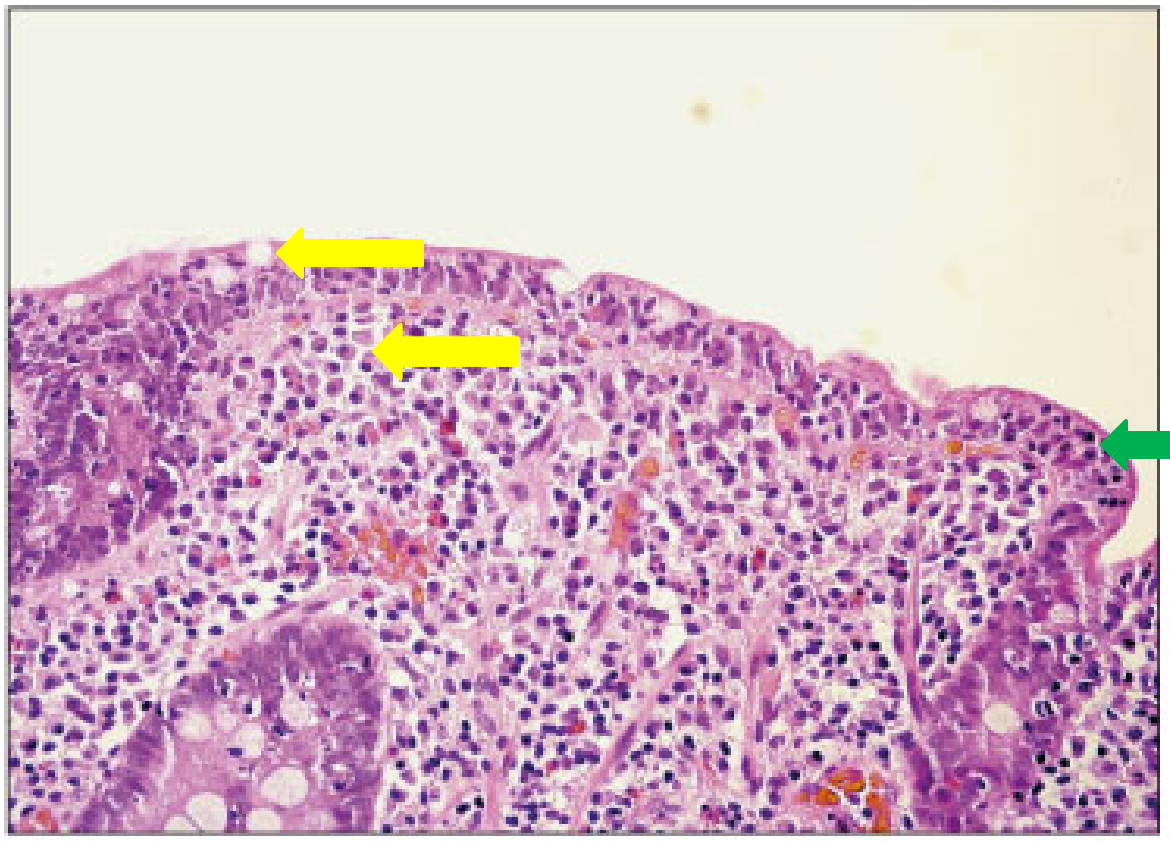
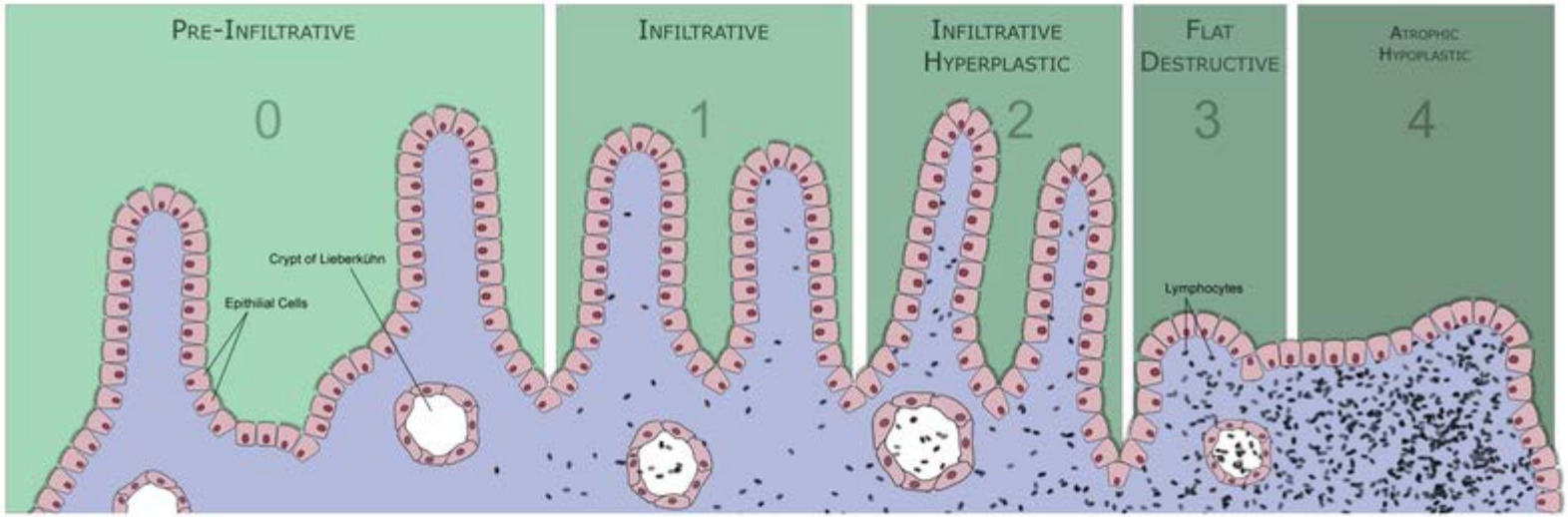


Fig. 2. *Duodenal biopsy.* Complete villous atrophy. The surface epithelium exhibits short cells lacking basal polarity. Intraepithelial lymphocytes amongst them. Lamina propria densely infiltrated with lymphocytes and plasma cells.

Modified Marsh classification used in CD histologic grading

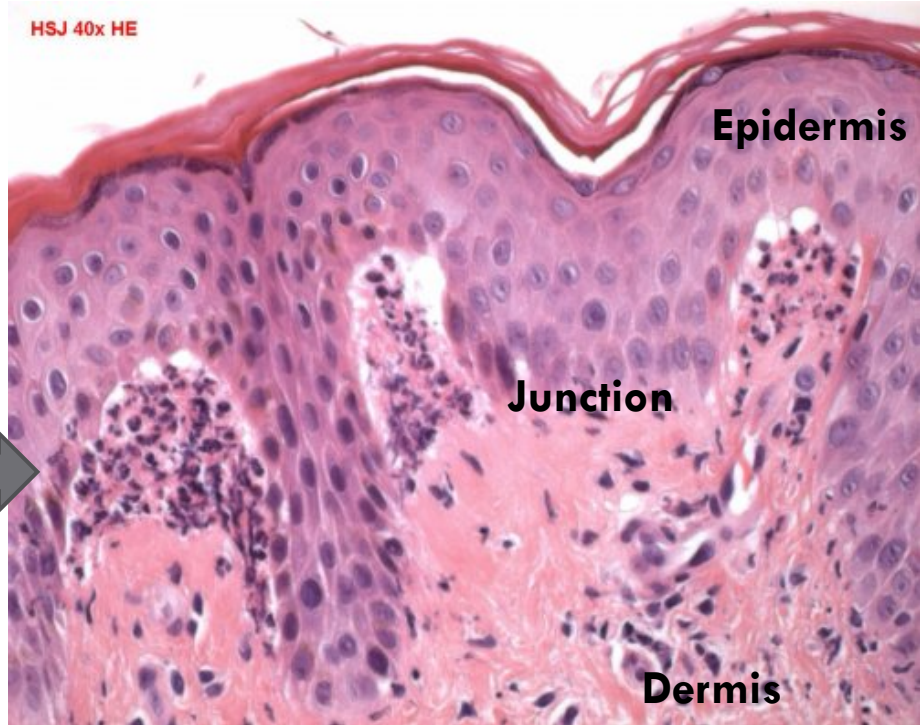
- Duodenal and jejunal biopsy – gold standard for CD diagnosis
  - Marsh 0-1
    - Consider early phase disease
    - Follow-up testing on normal diet
    - Consider false-positive results
  - Marsh ≥2
    - Celiac disease confirmed



Skin manifestation

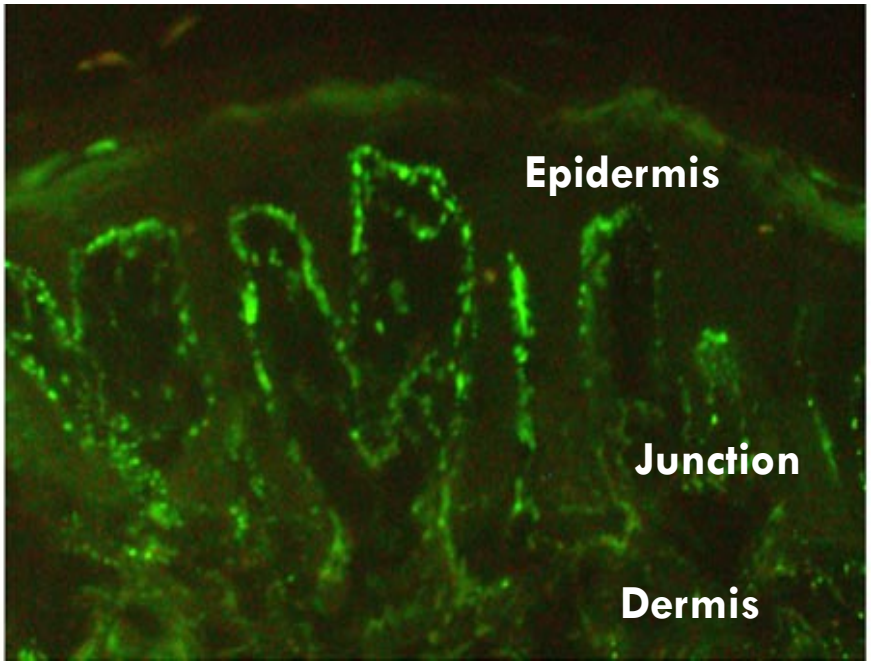
Dermatitis herpetiformis

- Pruritic vesicles
- Symmetric
  - Extensor surfaces
  - Rare mucous membrane involvement
- Microscopy
  - Subepidermal blisters
  - Neutrophilic microabcesses in papillary dermal tips
  - Lymphocytic infiltrate around superficial vascular plexus



Dermatitis herpetiformis

- Incidence – 10-39/100,000
- Age – all ages; peak onset is 20s-40s
  - Most common autoimmune bullous disease of childhood
  - Strong association with HLA-DQ2
- Diagnostic: granular IgA and C3 in papillary dermal tips
- Treatment: gluten free diet, dapsone or sulfapyridine



What is Gluten?

Pathophysiology

Shewry et al, 2002

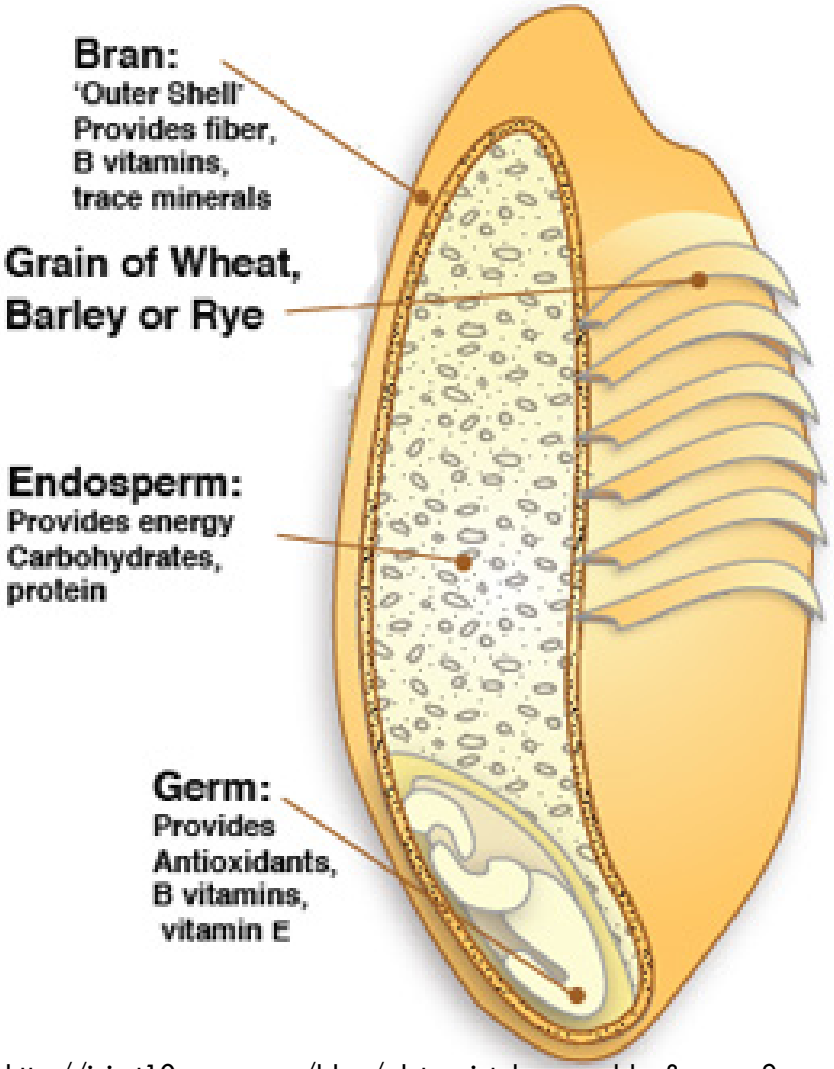
WHAT IS GLUTEN?

PROTEIN

- PROLAMINS
  - 15% proline and 35% glutamine

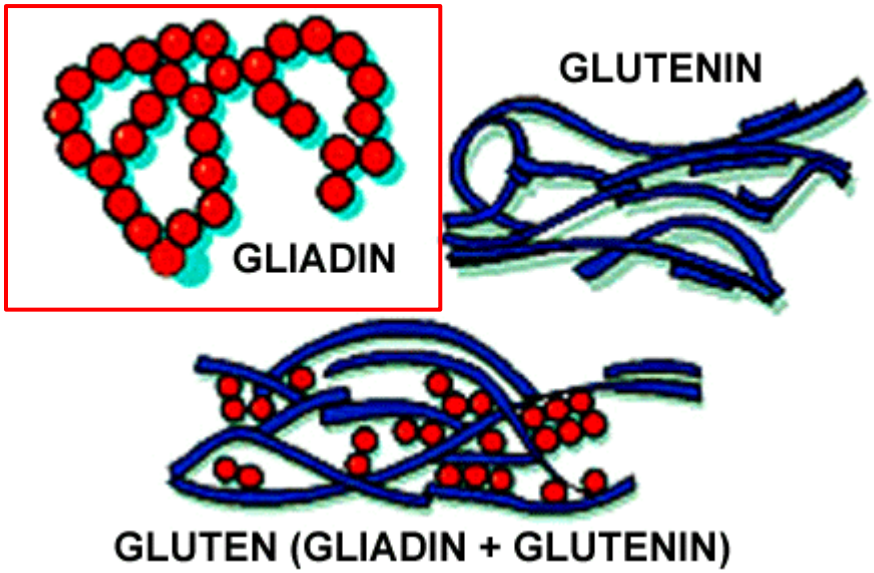
Gluten - 75% and remainder being starch and lipids

- Storage
  - Elasticity and extensibility



http://injust10pages.com/blog/gluten_intolerance_blog?page=2

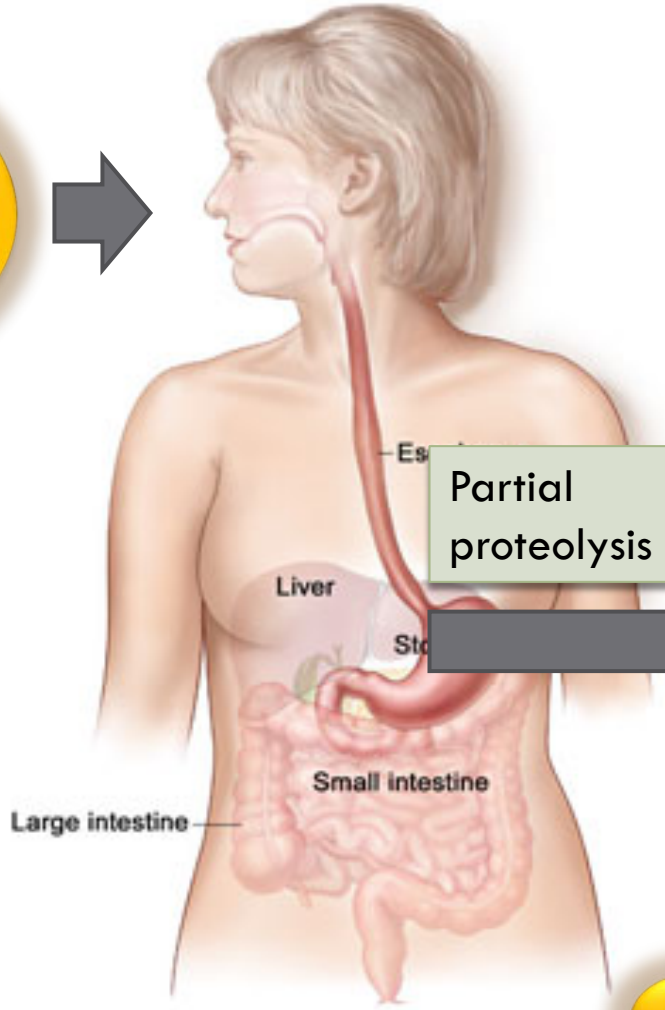
GLUTEN composition



Peptides similar to gliadin are in Rye and Barley

Digestion

Gluten



Partial proteolysis

Gliadin

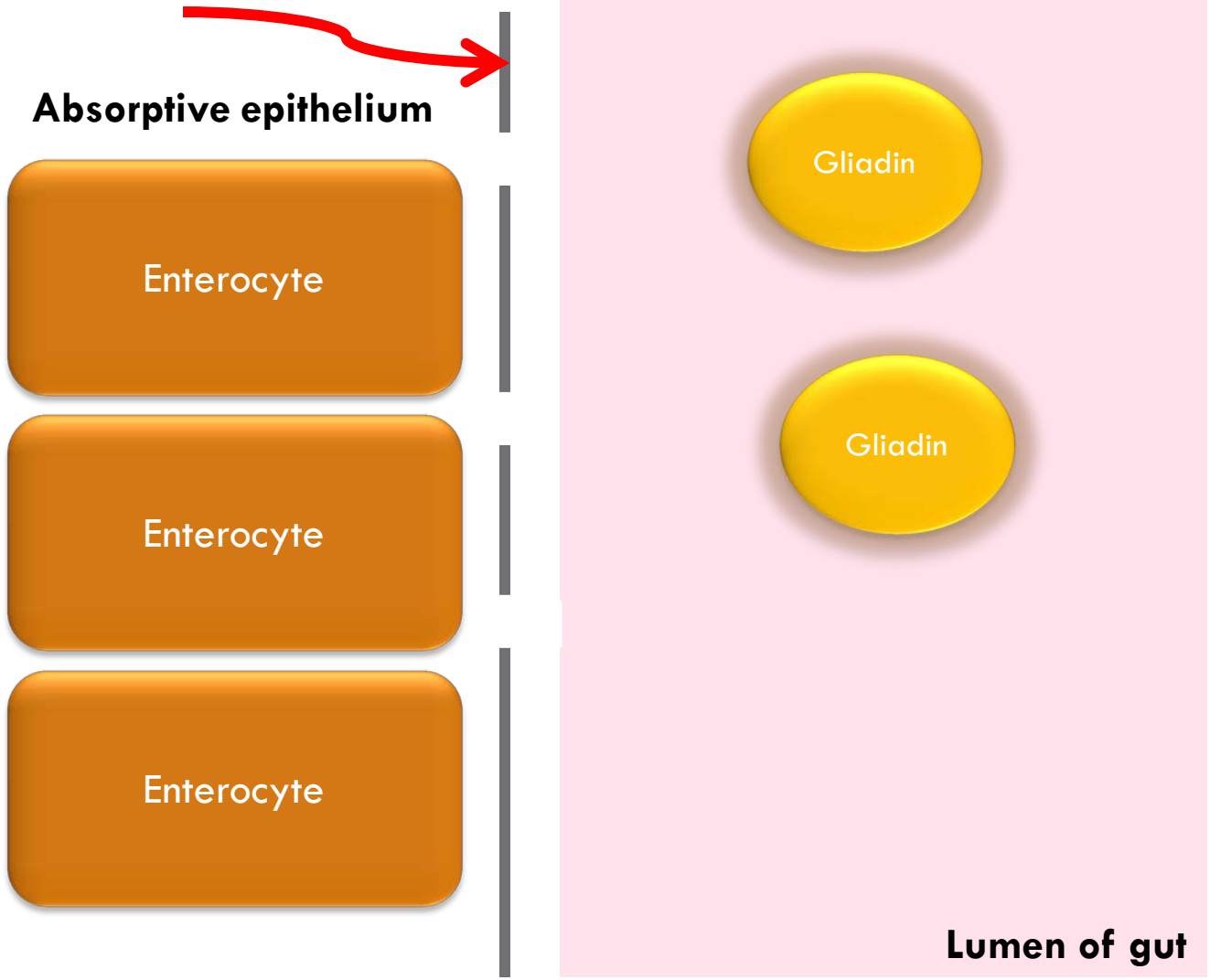
Gliadin

AA

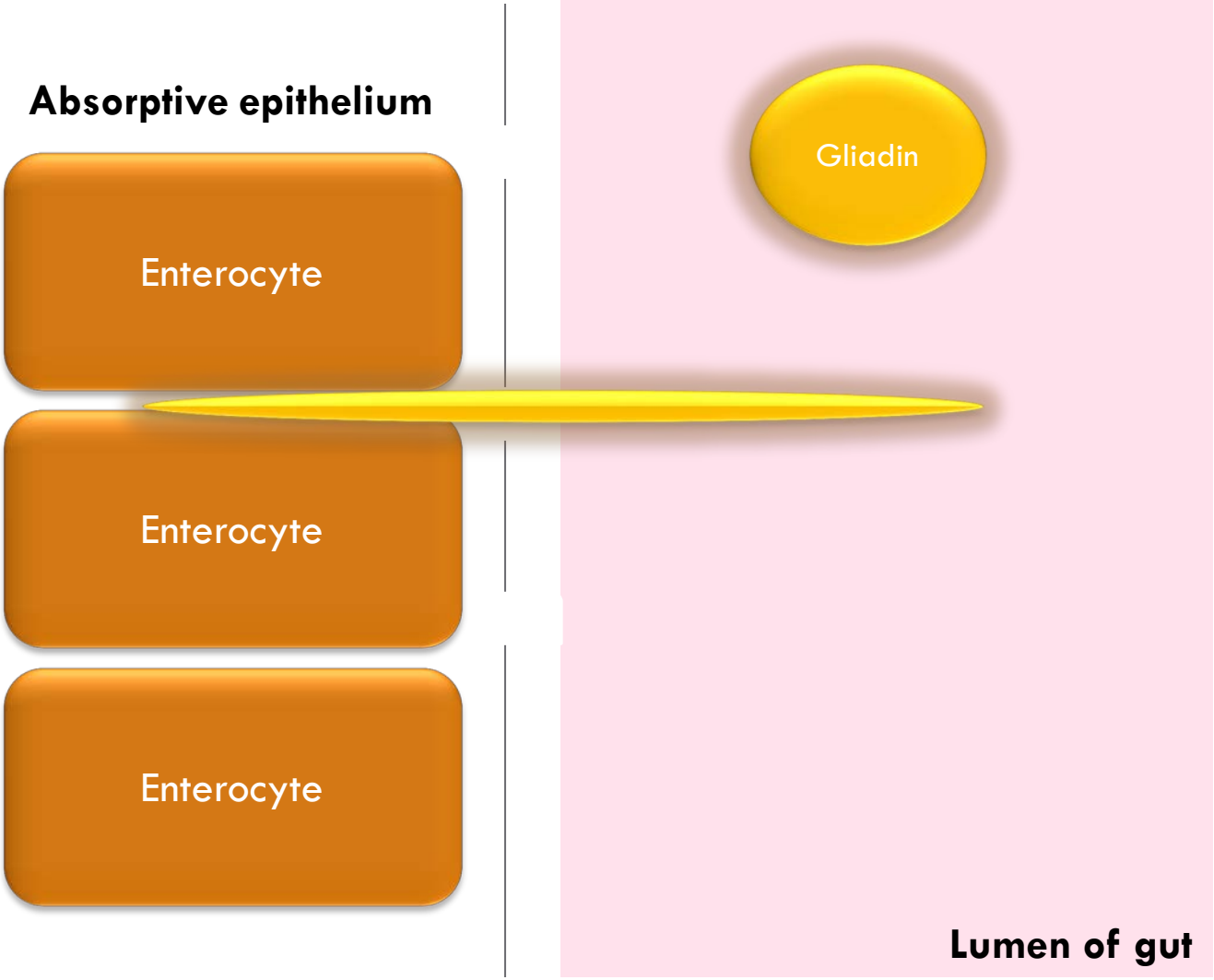
AA

AA

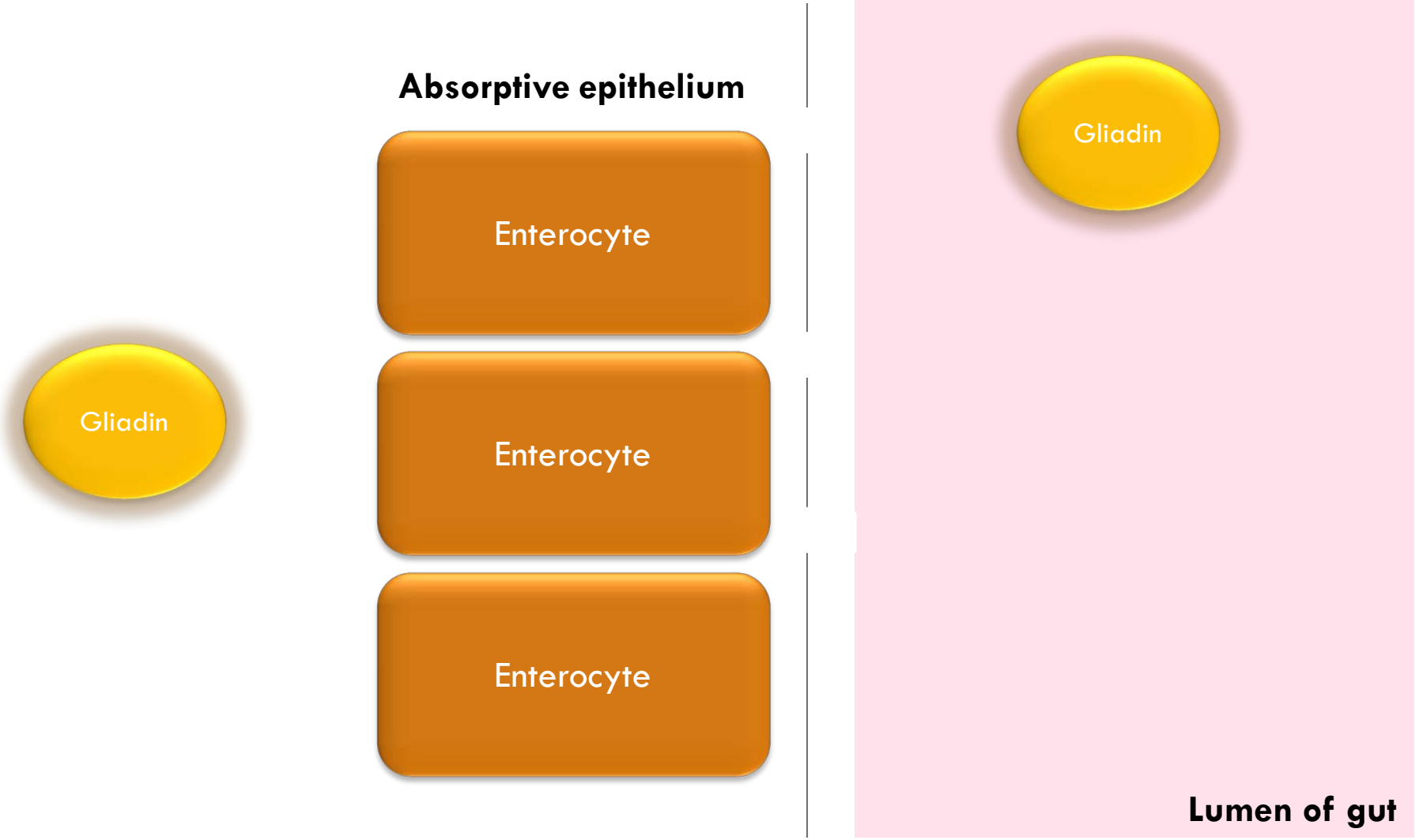
Pathophysiology



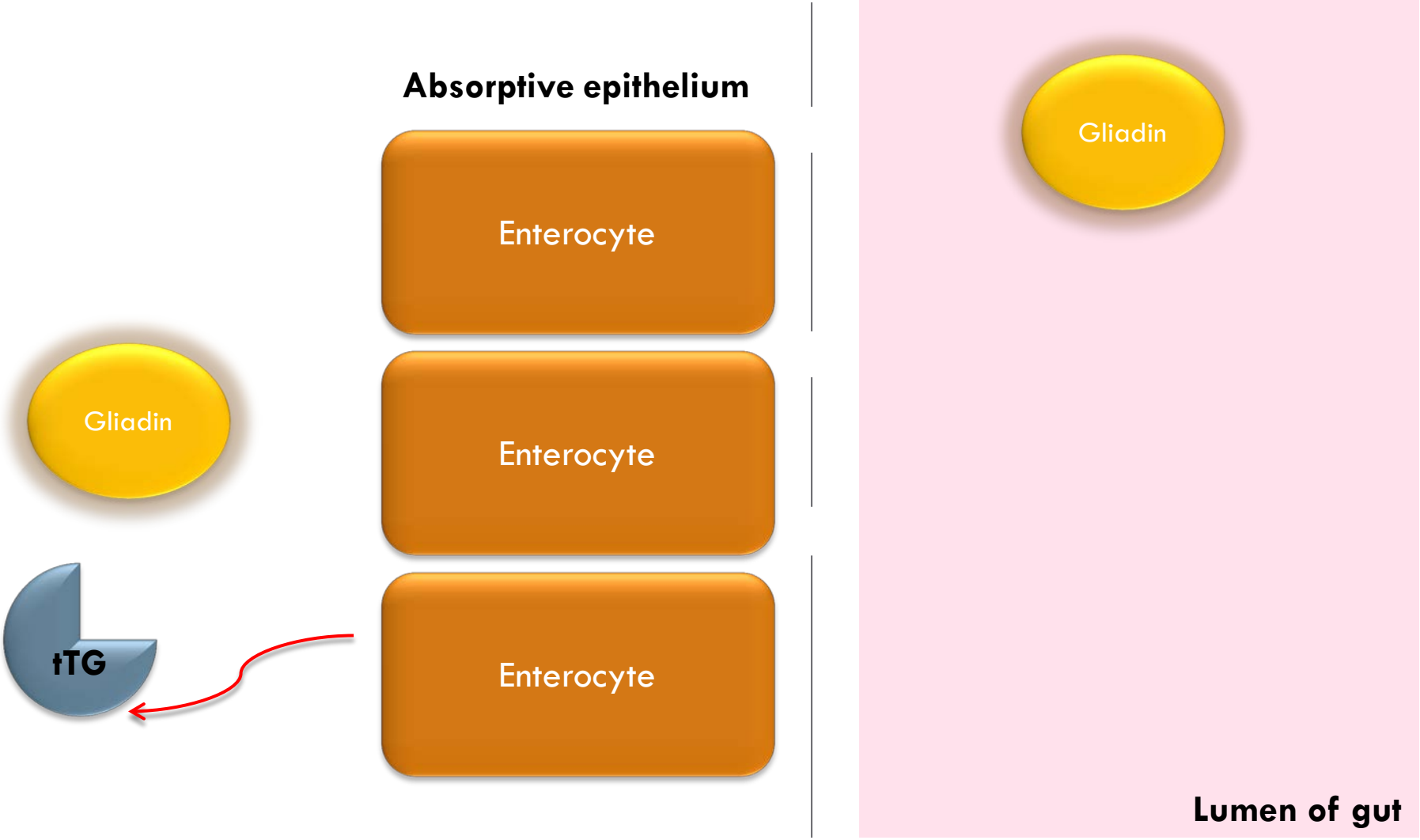
Pathophysiology



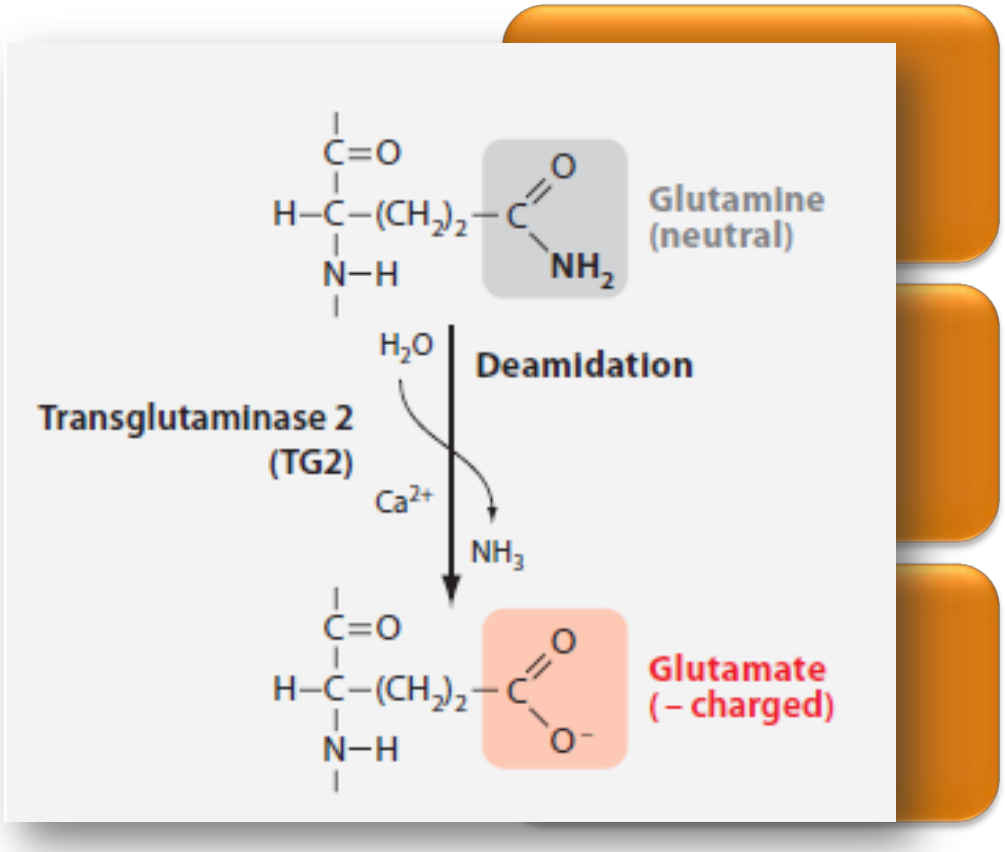
Pathophysiology



Pathophysiology

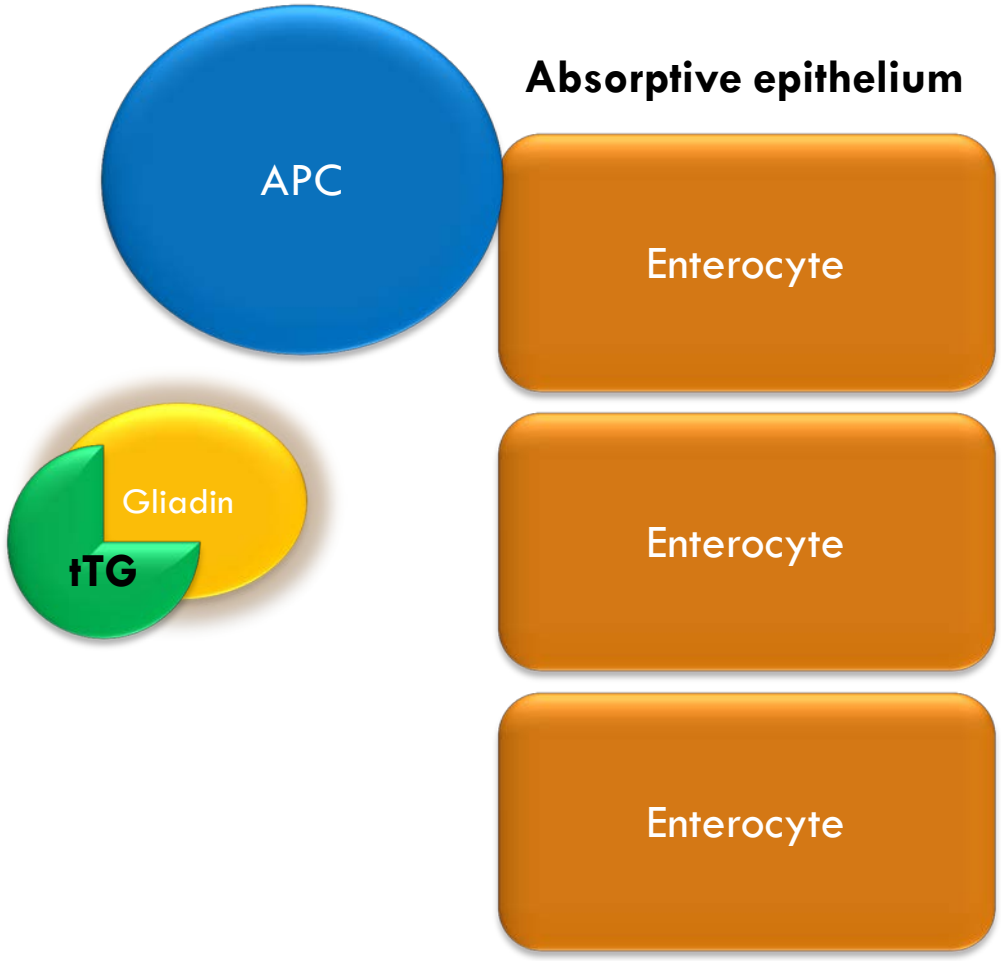


Pathophysiology

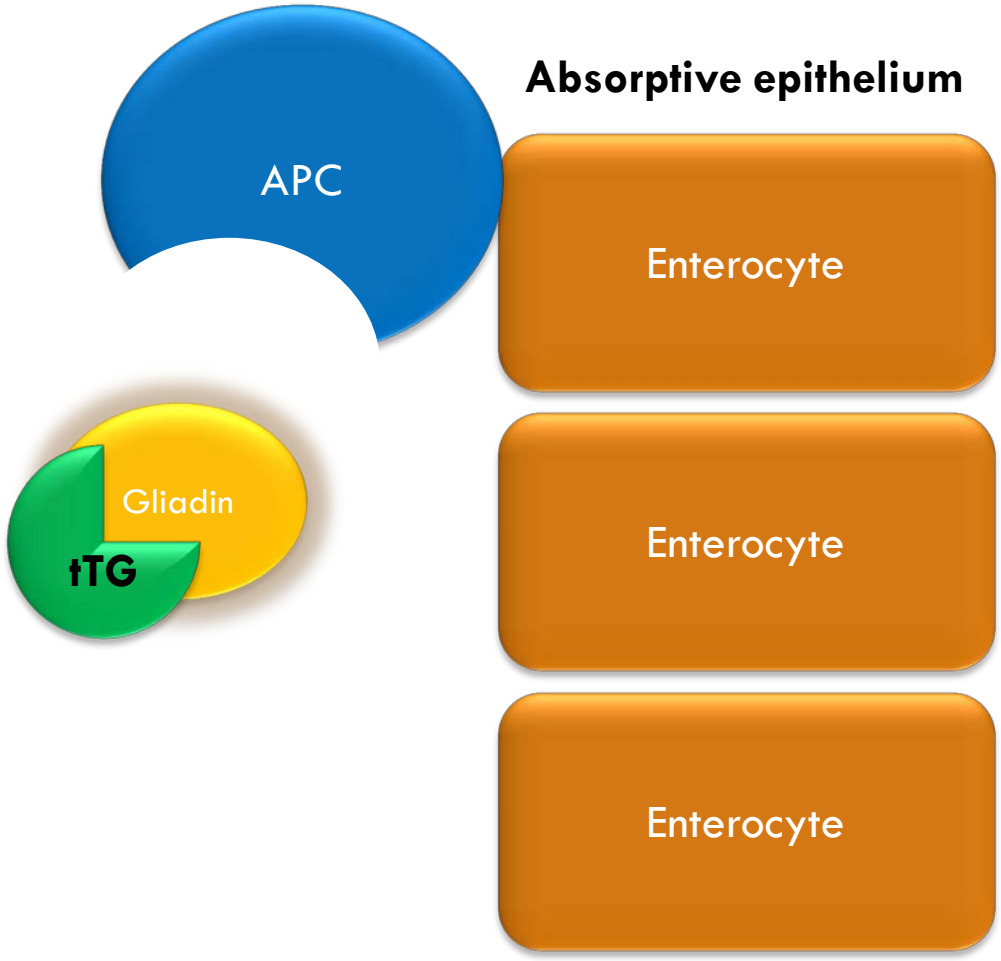


Lumen of gut

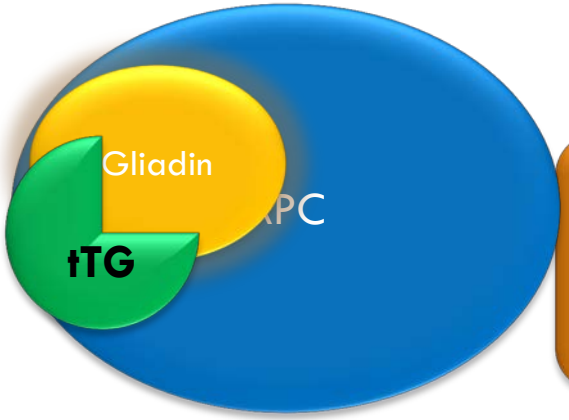
Pathophysiology



Pathophysiology



Sensitization in duodenum



Absorptive epithelium

Enterocyte

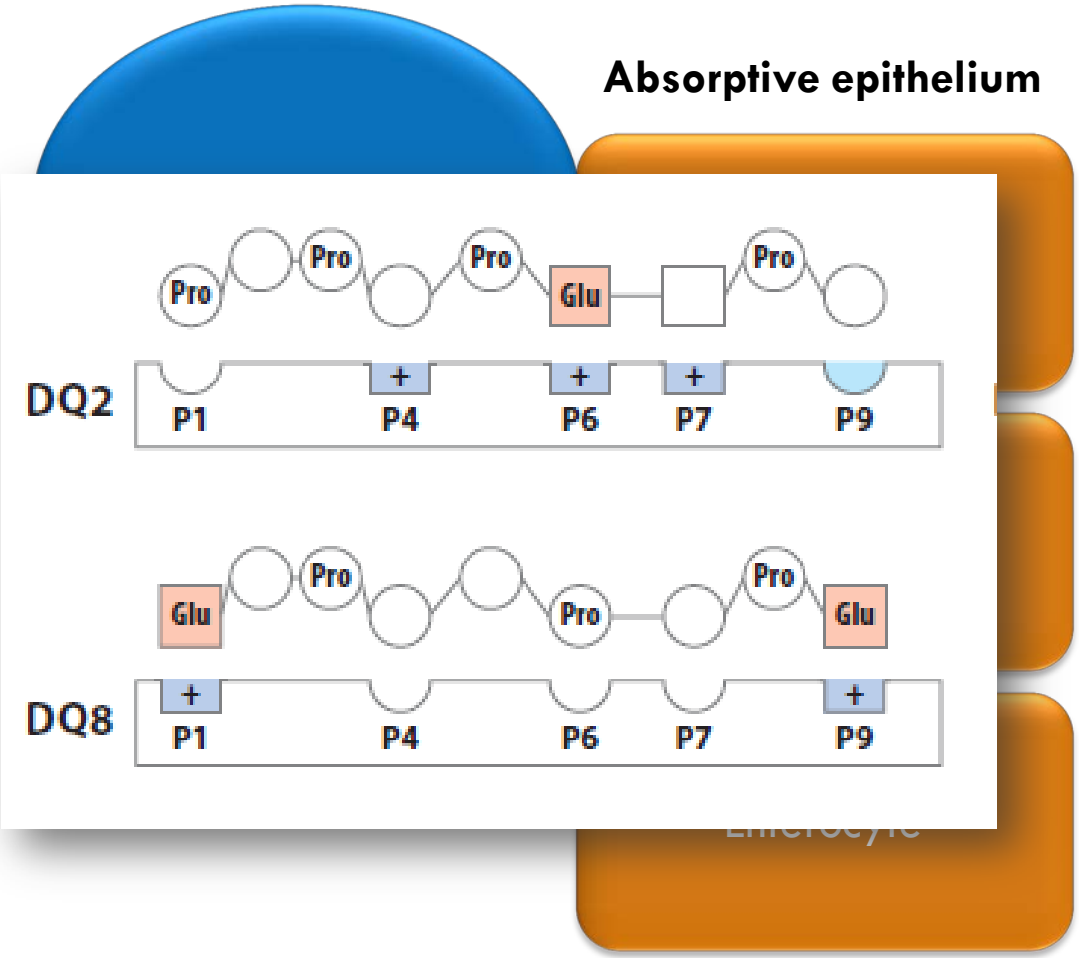
Enterocyte

Enterocyte

Gliadin

Lumen of gut

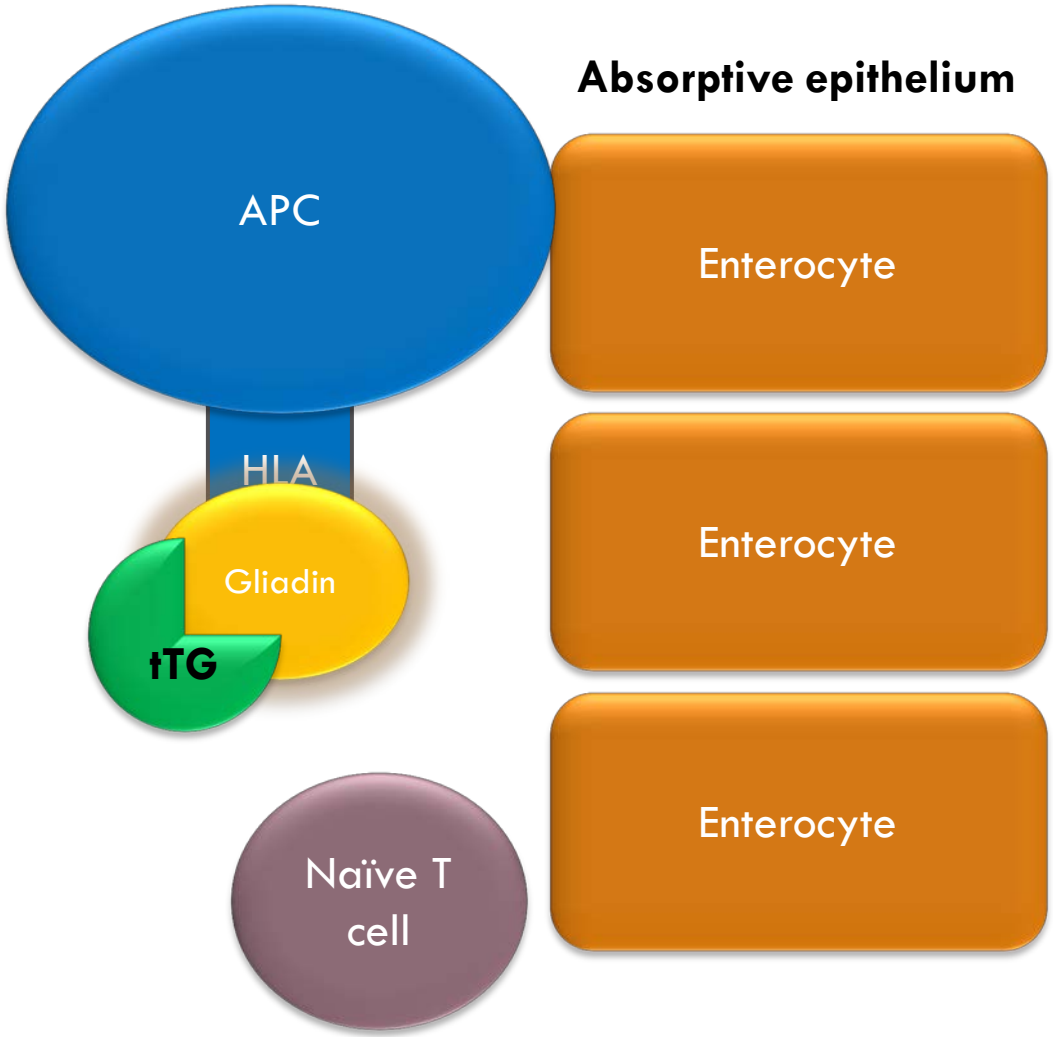
Pathophysiology



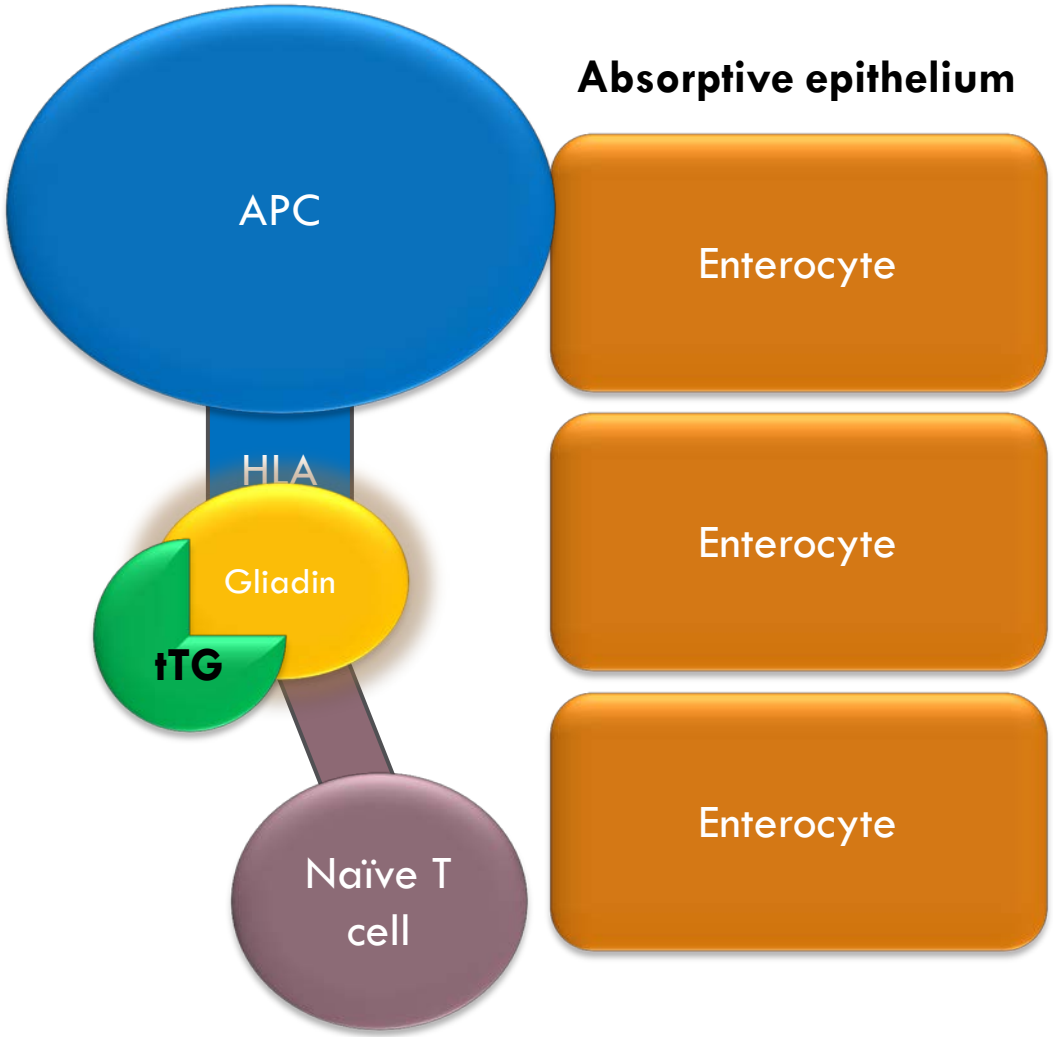
Gliadin

Lumen of gut

Pathophysiology



Pathophysiology



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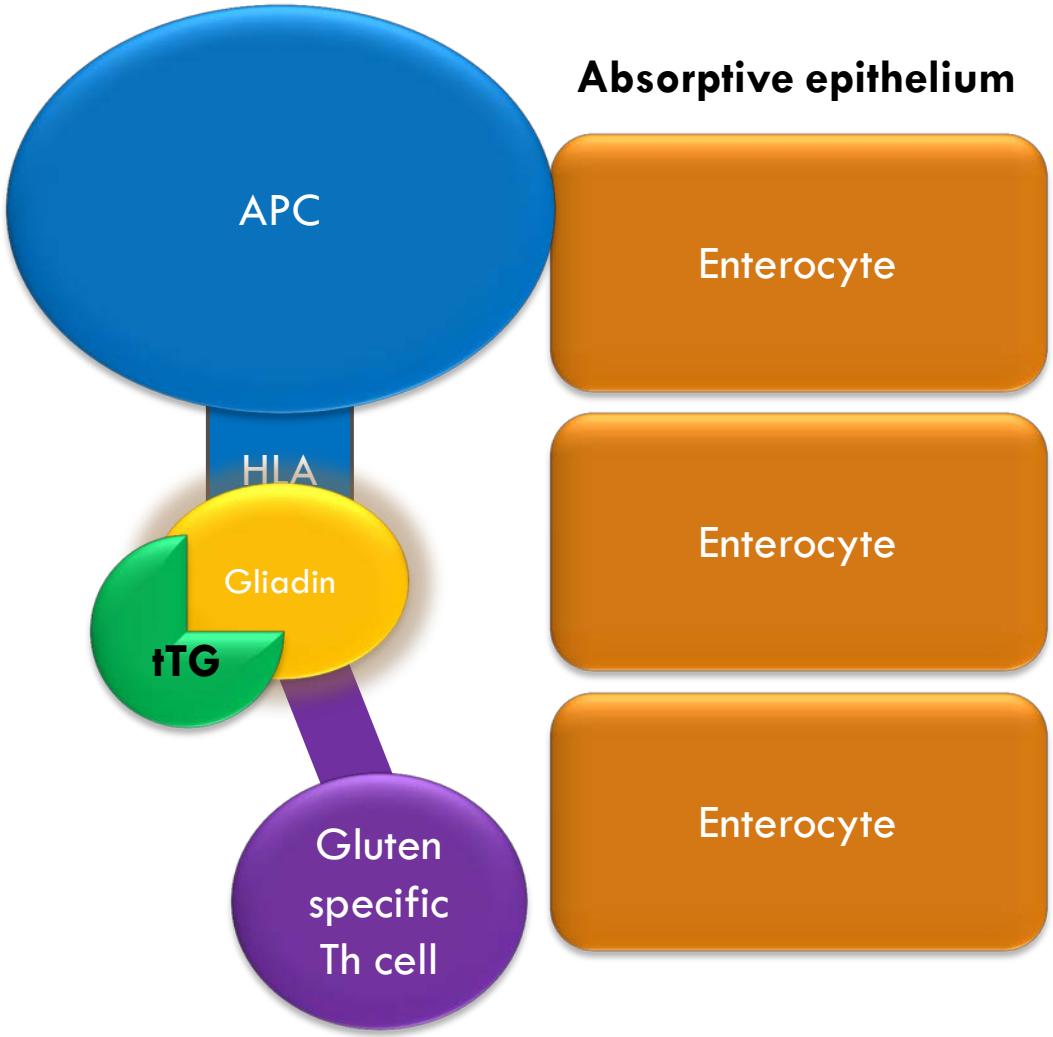

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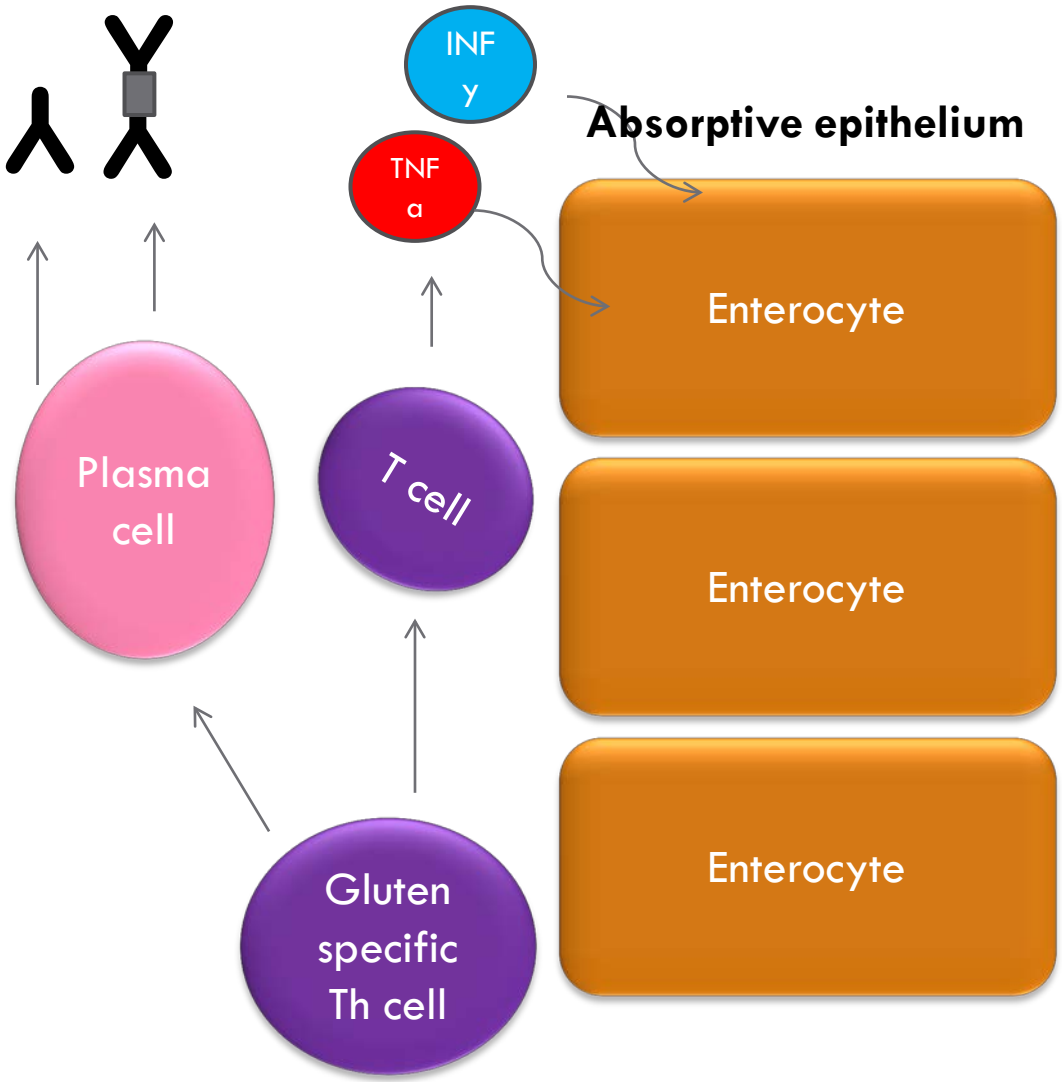


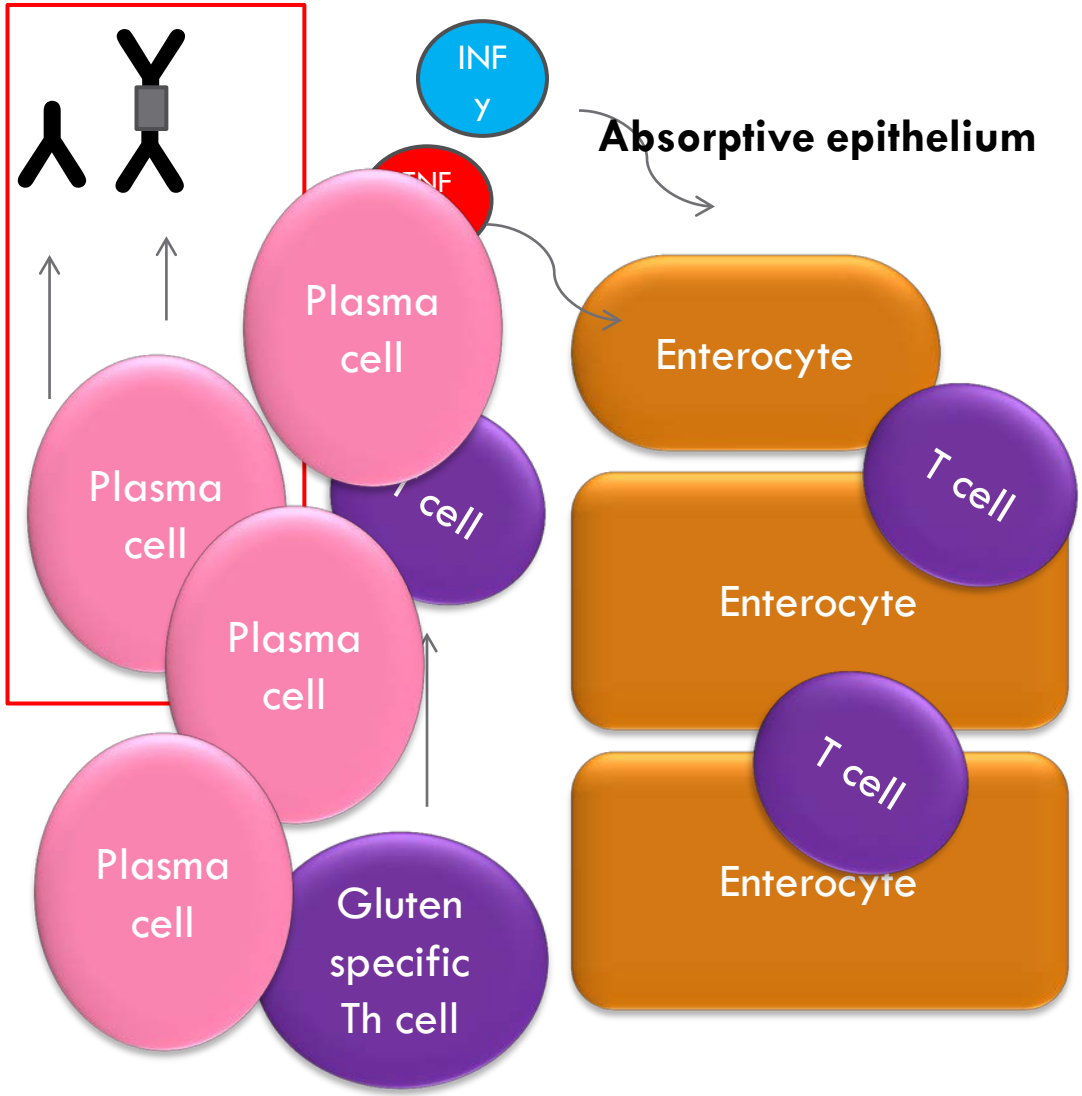
Gliadin

Th cell interacts with cytotoxic cells to facilitated cellular damage via release of INFy and TNFa.

Th cell interacts with B cell to stimulate plasma cells that produce anti-gluten antibodies.

Lumen of gut

Pathophysiology



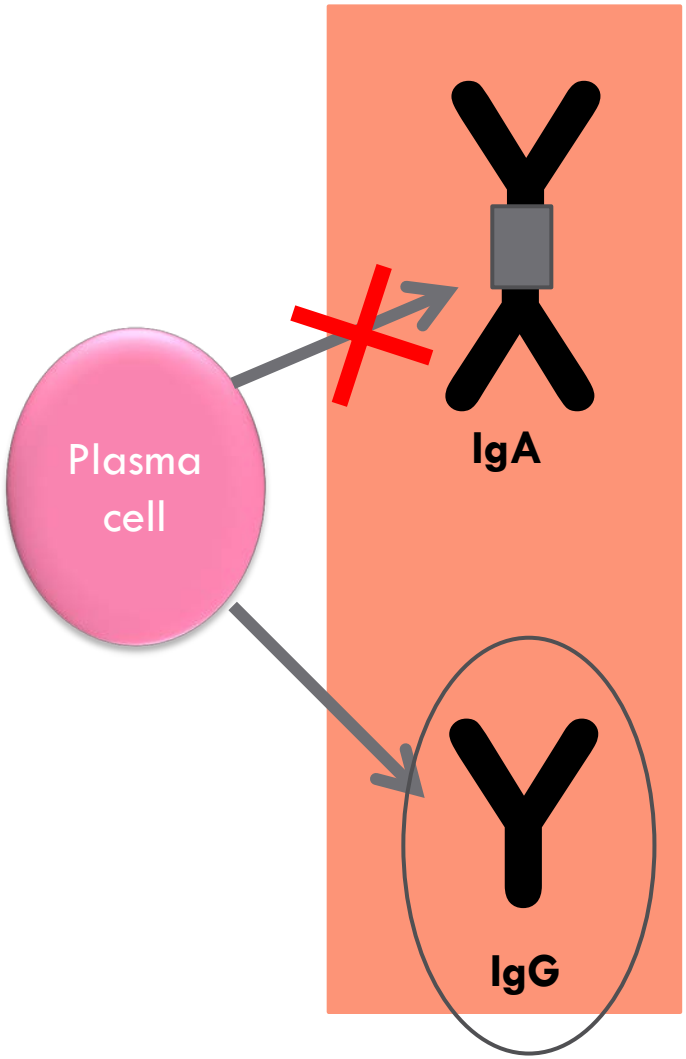
Th cell interacts with cytotoxic cells to facilitated cellular damage via release of INFy and TNFa.

The number of intraepithelial lymphocytes increase.

The number of plasma cells in lamina propria increase.

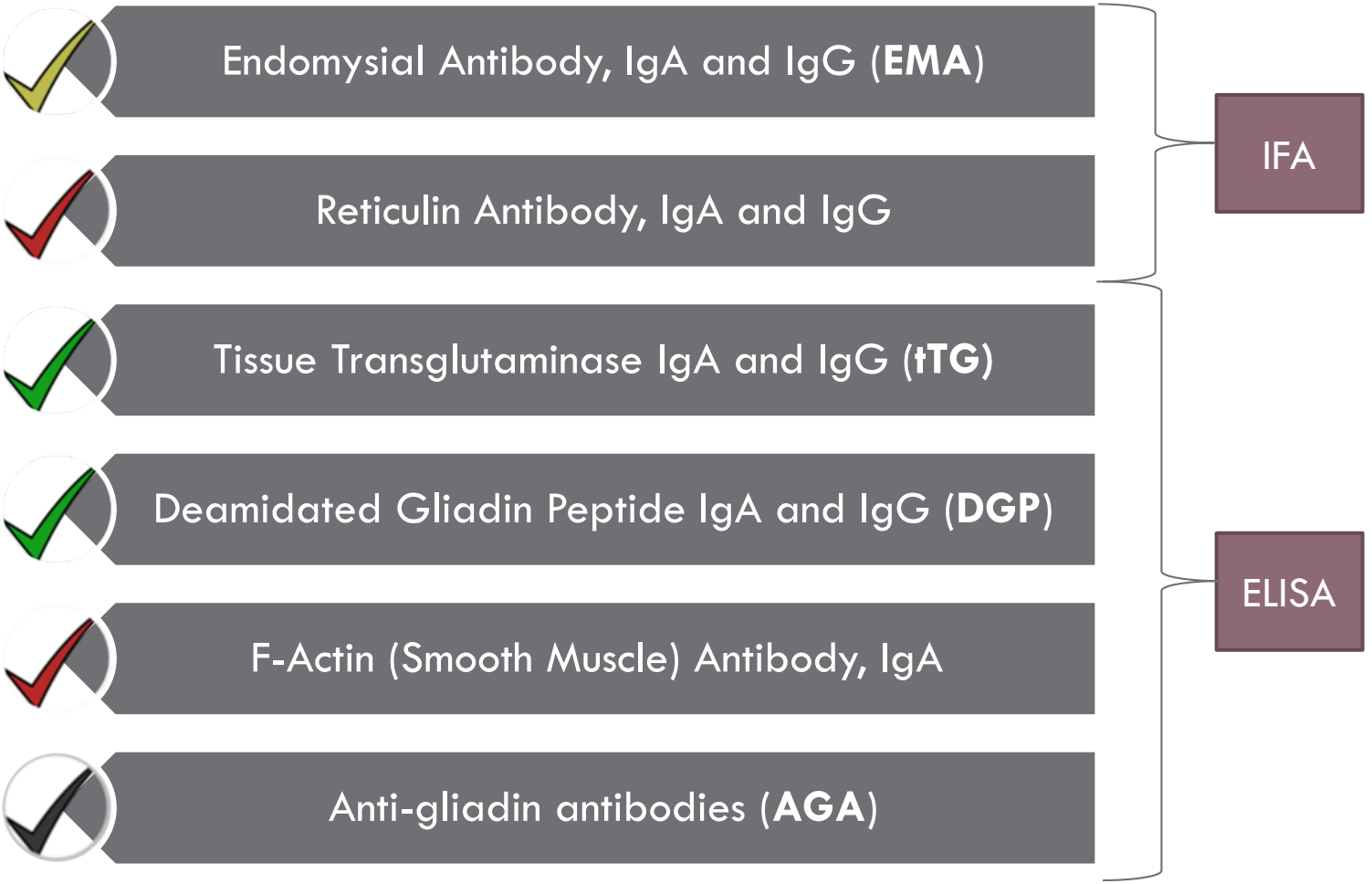
Lumen of gut

Selective IgA immunodeficiency



- Selective IgA immunodeficiency
  - The most common primary immunodeficiency
    - 1 in 223-1000
  - Serum IgA level of less than 5-10 mg/dL
  - Recurrent infections and bronchiectasis
  - Allergy
  - Increased risk of developing atopic or autoimmune disease
    - Including celiac disease

Serologic tests for antibodies



Dieterich et al. 1997

Endomysial Antibody (EMA)

- Identified antigen for EMA Endomysial Antibody
  - Endomysial component of smooth muscle layers or connective tissue stroma covering individual muscle fibers
  - Intracellular tissue transglutaminase (tTG)

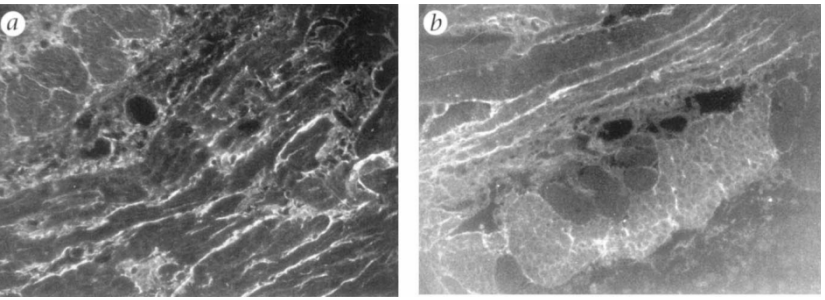


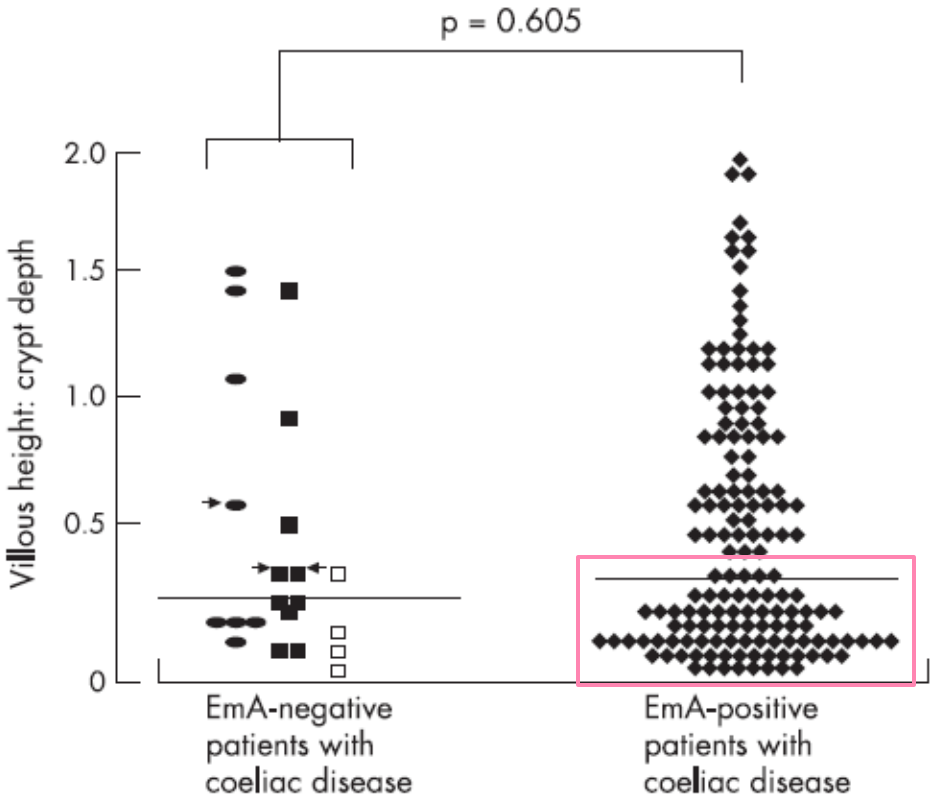
Table 1 Enzyme-linked immunosorbent assay (ELISA) based on tTG for the celiac disease (CD) autoantigen

Serum sample	EmA titer	Serum IgA anti-tTG dilution (1:400)
CD A	(1:640)	>3.500
CD B	(1:320)	>3.500
CD C	(1:320)	3.114 ± 0.024
CD D	(1:320)	1.257 ± 0.024
CD E	(1:320)	1.977 ± 0.018
CD F	(1:320)	1.525 ± 0.085
CD G	(1:160)	1.208 ± 0.019
CD H	(1:160)	0.747 ± 0.048
CD I	(1:160)	2.174 ± 0.291
CD K	(1:80)	0.667 ± 0.037
CD L	(1:80)	1.372 ± 0.093
CD M	(1:40)	0.286 ± 0.009
Ulcerative colitis	(ND)	0.119 ± 0.014
Indeterminate colitis	(ND)	0.092 ± 0.016
Crohn's disease	(ND)	0.090 ± 0.001
Sjögren's syndrome	(ND)	0.137 ± 0.004
Sjögren's syndrome	(ND)	0.082 ± 0.003
Alcoholic liver fibrosis	(ND)	0.059 ± 0.001
Healthy control	(ND)	0.070 ± 0.001

Salmi et al, 2006

Endomysial Antibody (EMA)

- Labor intensive
  - Reading errors
- Expensive
- May be used for confirmation if anti-tTG is equivocal
  - Very specific*
  - Acceptable screen
  - High positive predictive value for active CD



Stevens et al, 1975

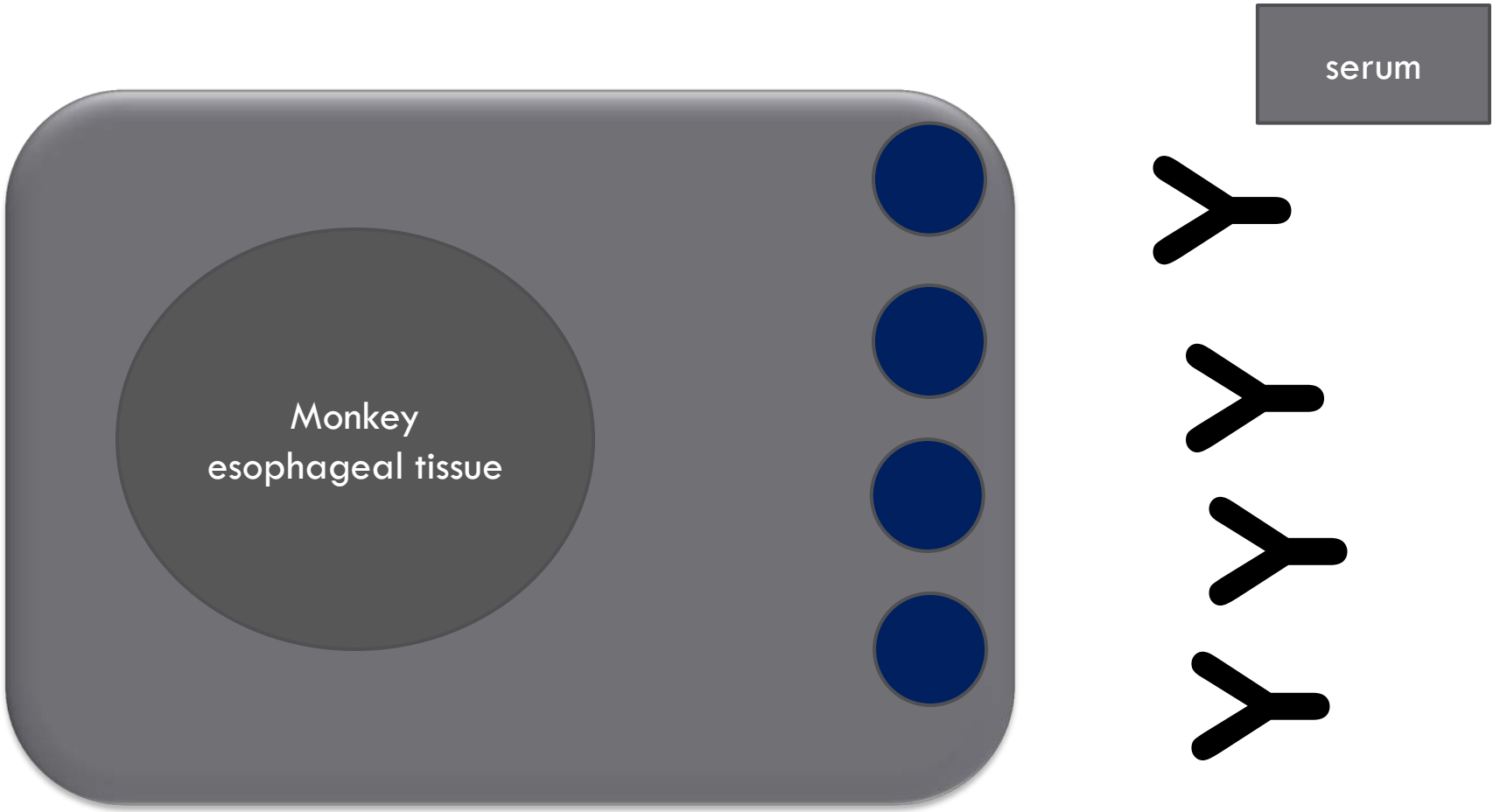
Reticulin Antibody, IgA and IgG

- IgG class reticulin antibodies bullous dermatoses and other diseases, and sometimes in normal patients
  - Reticulin is a type of fiber composed of type III collagen
- Not recommended for CD testing
- IgA 25% dermatitis herpetiformis
- IgA 60% celiac disease

Methodology

Indirect Fluorescent Antibody (IFA)

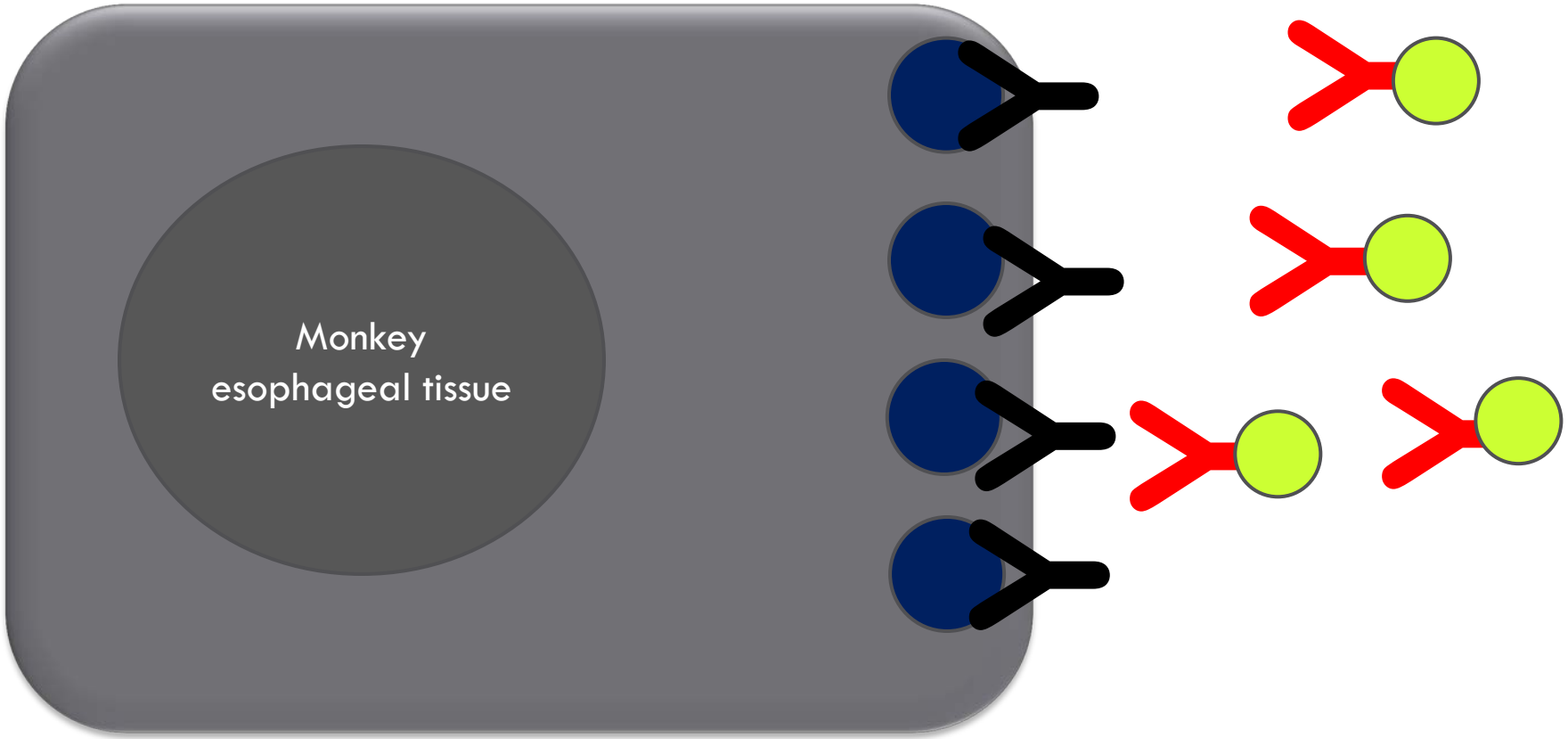
Indirect Fluorescent Antibody (IFA)



Indirect Fluorescent Antibody (IFA)

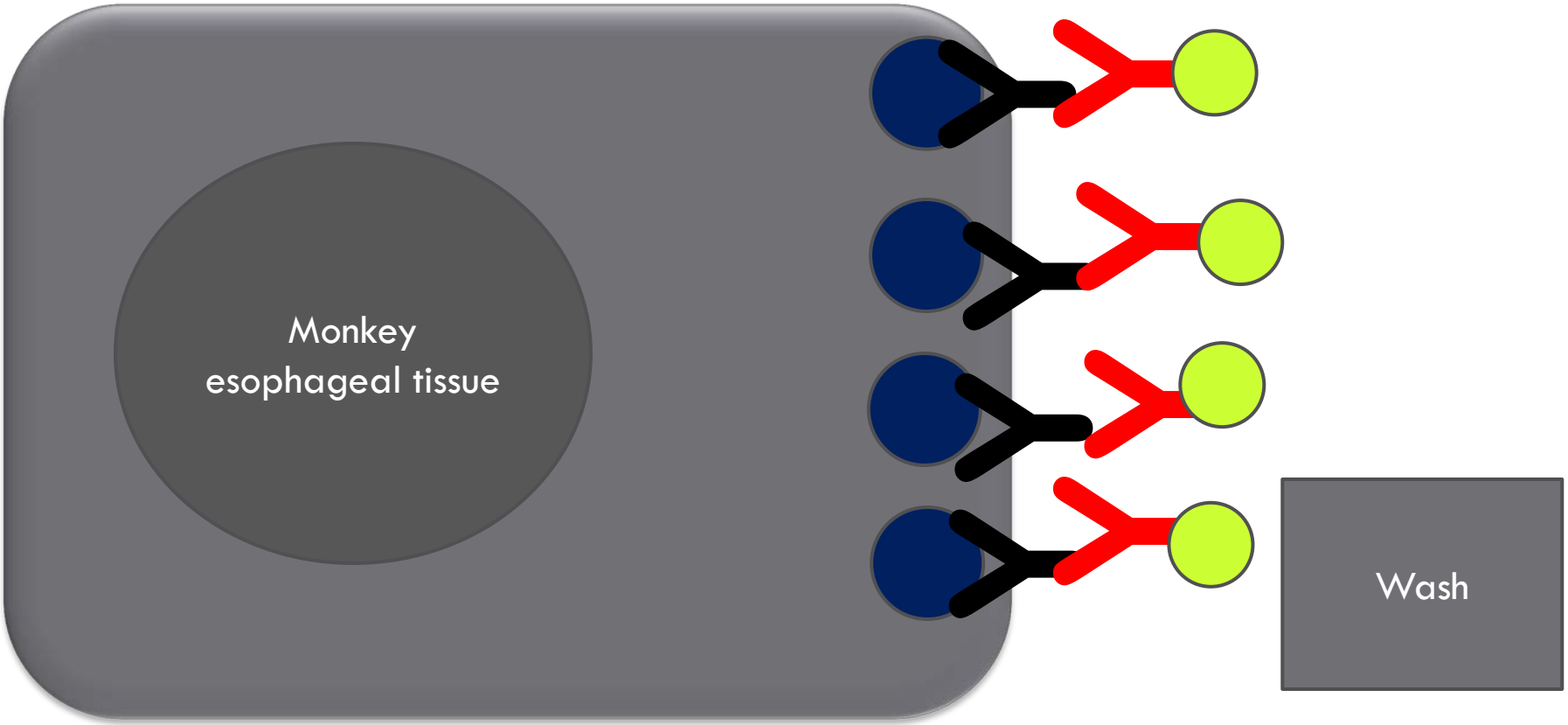
Wash

Indirect Fluorescent Antibody (IFA)



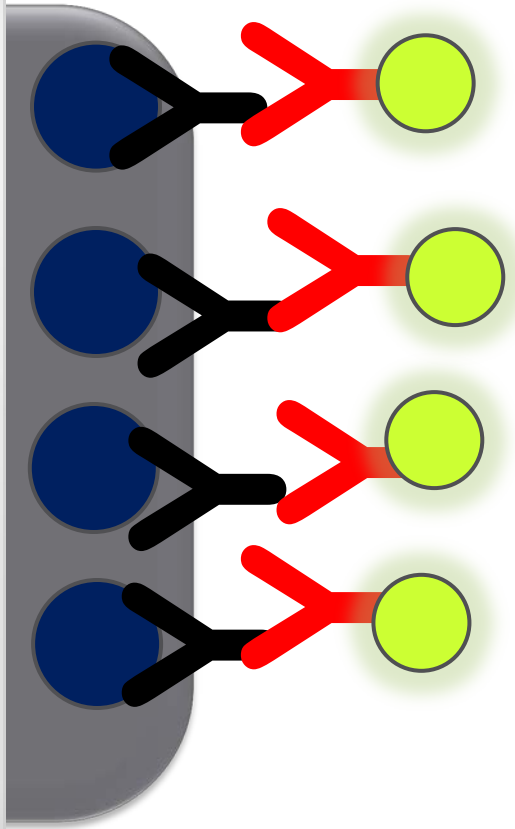
Fluorescein-labeled Antibodies (FITC)

Indirect Fluorescent Antibody (IFA)



Fluorescein-labeled Antibodies (FITC)

Indirect Fluorescent Antibody (IFA)



Fluorescein-labeled Antibodies (FITC)

Immunofluorescence

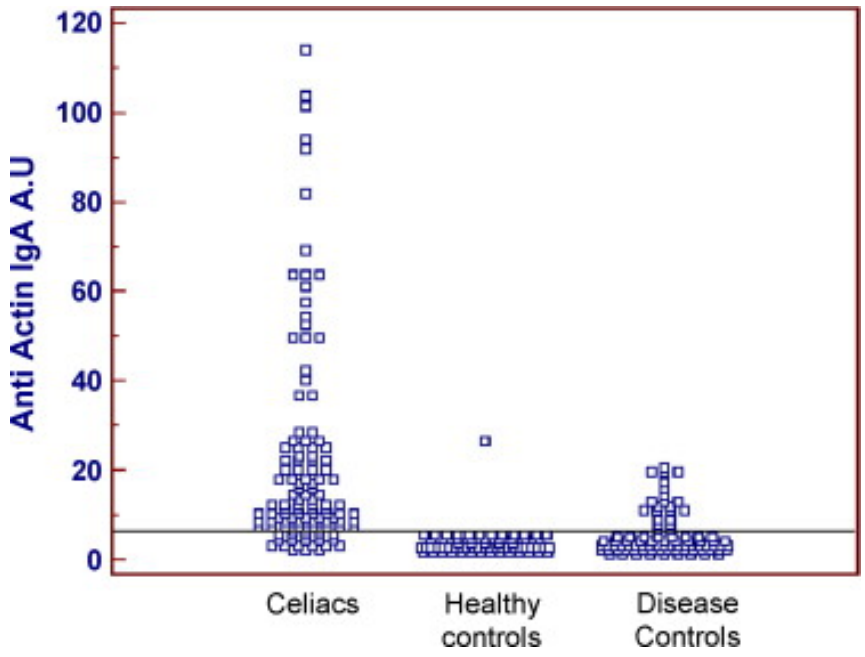
DGP, tTG and AGA antibodies

- **DGP and tTG are used in screening population at risk or with symptoms**
- DGP IgG antibodies more sensitive but less specific markers for disease compared with IgA class antibodies
- Anti-gliadin antibody (AGA) can be found in healthy individuals or in other bowel diseases
  - No longer a recommended test
- tTG antibody may be less reliable in children

Carroccio et al, 2007

F-Actin (Smooth Muscle) Antibody

- Adjunct test
- Monitoring tool for patients with severe disease
  - Correlate with severity
- Test results alone are not diagnostic

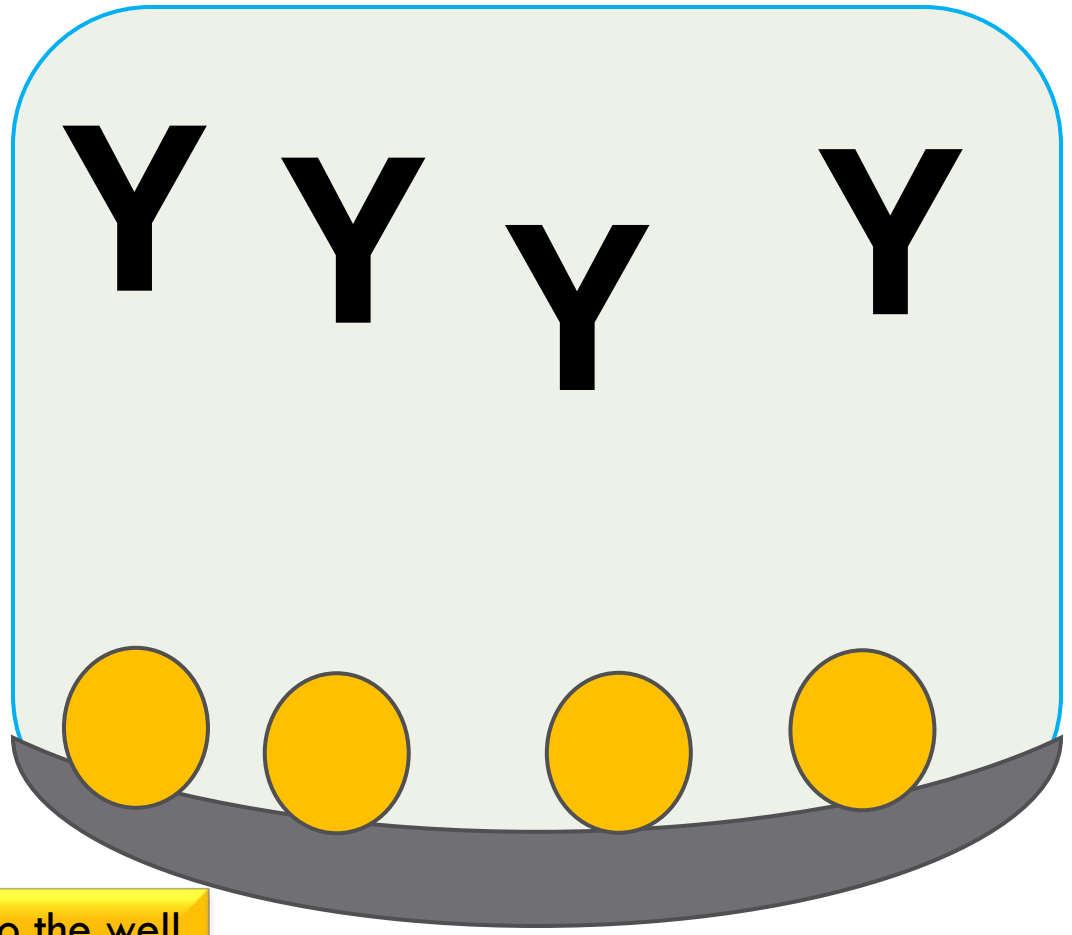


CD with partial villi atrophy	CD with total villi atrophy	Healthy controls	"Disease" controls
41/60 (69%)	123/145 (85.3%)	1/80 (1.3%)	23/81 (28%)

Methodology

Enzyme linked immunosorbent assay (ELISA)

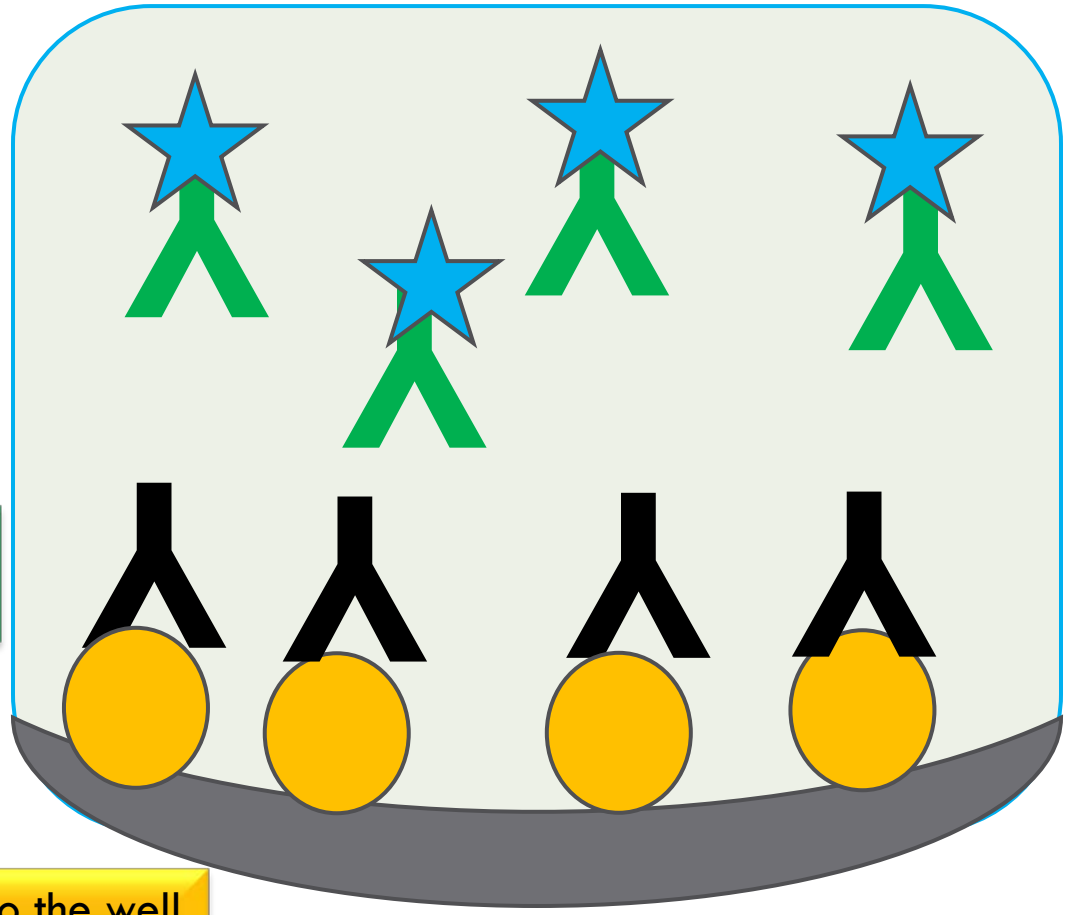
Enzyme linked immunosorbent assay (ELISA)



Patient's serum

Epitope of interest fixed to the well

Enzyme linked immunosorbent assay (ELISA)



Horseradish peroxidase (HRP)-labeled Ab

Incubate and wash

Patient's serum

Epitope of interest fixed to the well

Enzyme linked immunosorbent assay (ELISA)

Tetramethylbenzidine (TMB)

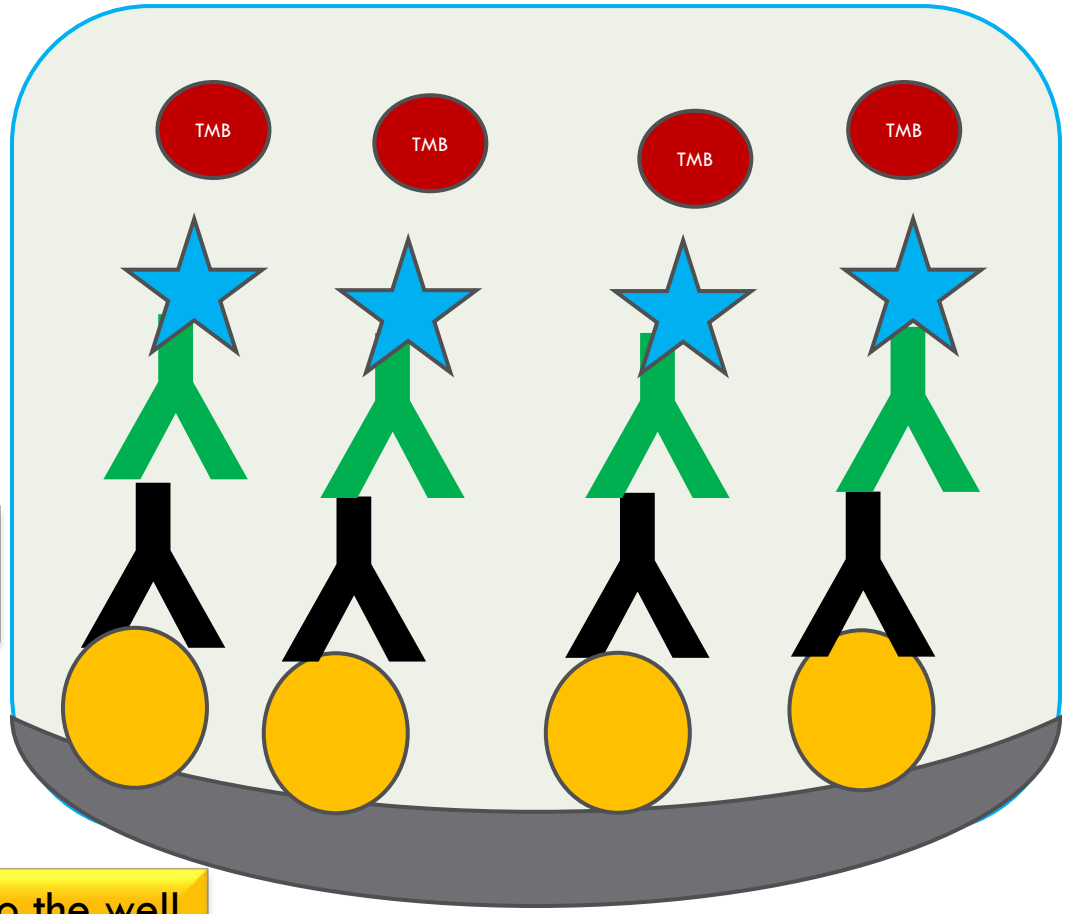
Incubate and wash

Horseradish peroxidase (HRP)-labeled Ab

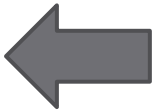
Incubate and wash

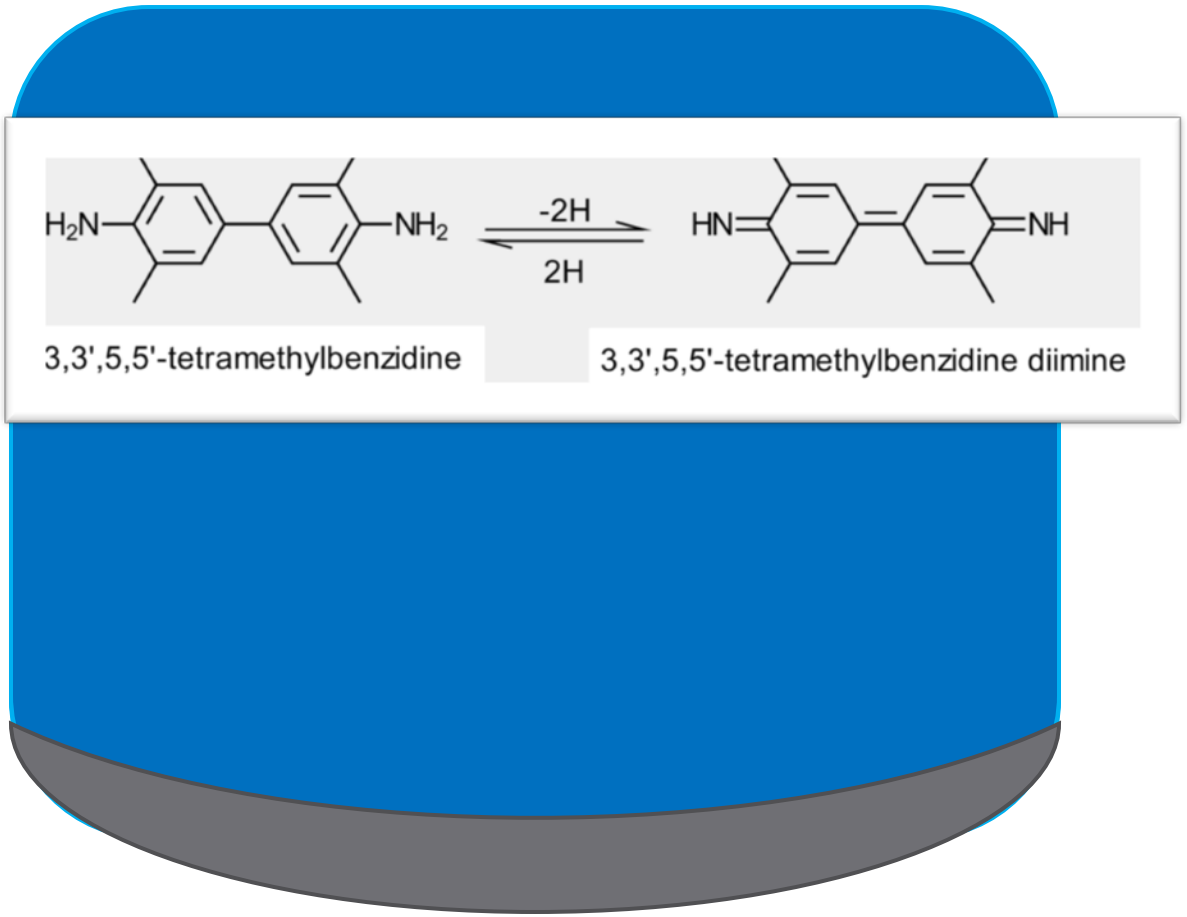
Patient's serum

Epitope of interest fixed to the well



Enzyme linked immunosorbent assay (ELISA)





Summary for serological tests

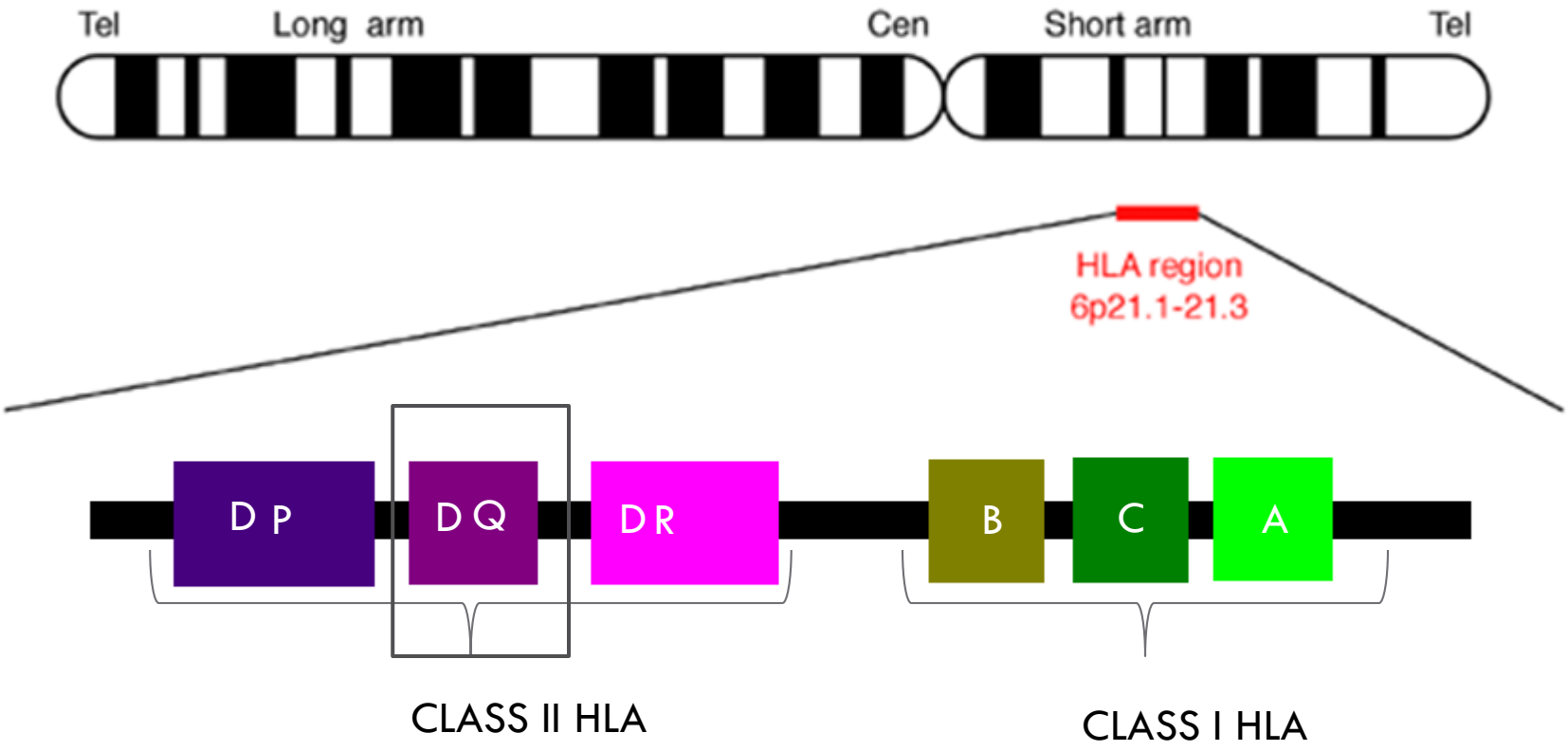
IgA	Method	Sensitivity	Specificity
tTG	**ELISA**	**>90%**	**>99%**
DGP	**ELISA**	**>90%**	**>90%**
EMA	**IFA**	**80-100%**	**>99%**
reticulin Ab*	IFA	24-30%	>90%
gliadin	ELISA	75-95%	>95%

Diagnosis of celiac disease

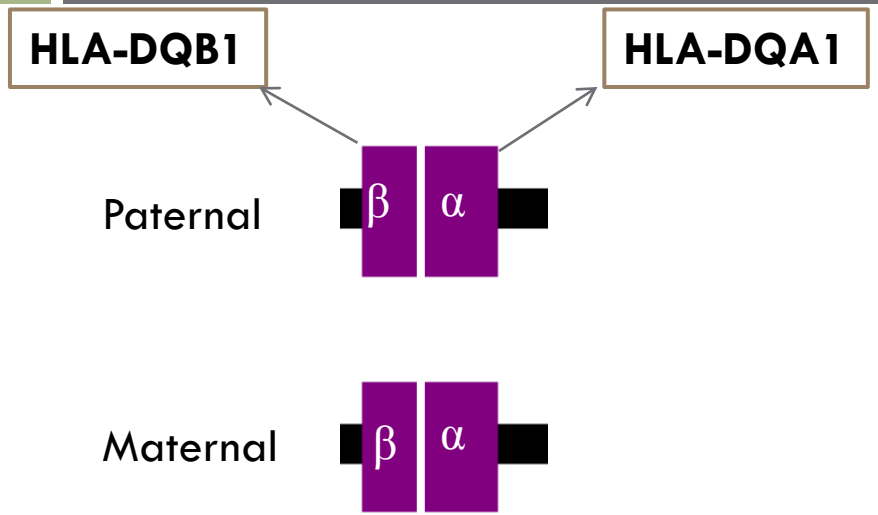
1. Clinical manifestations ✓
2. Antibody/ serology ✓
3. HLA test
4. Biopsy ✓

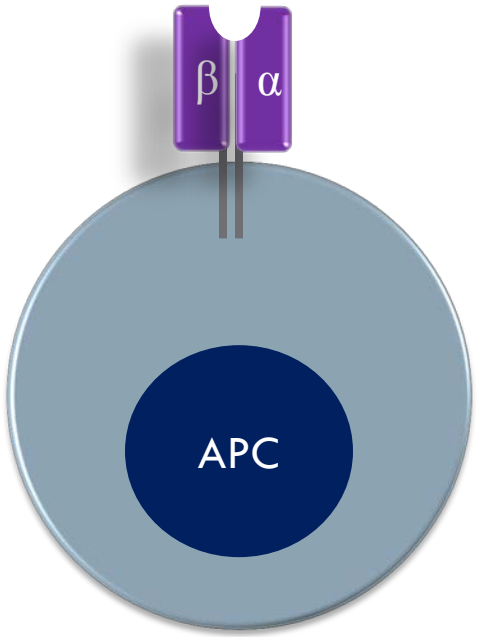
Major histocompatibility complex (Human leukocyte antigen - HLA)

Chromosome 6



HLA-DQ as heterodimeric (αβ) receptor

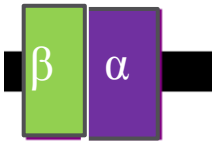




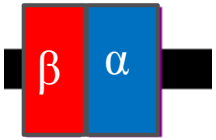
There are many slightly different α and β

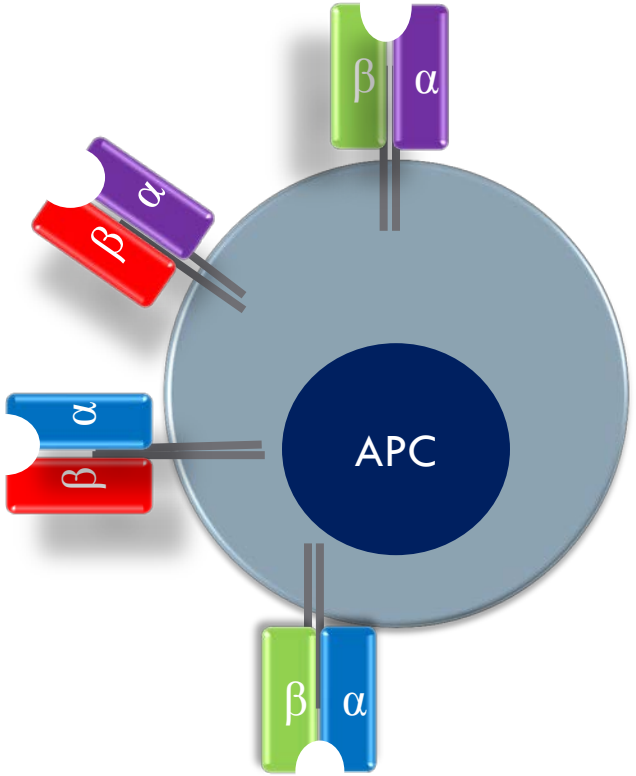
Everybody has 2 HLA-DQ genes

Paternal



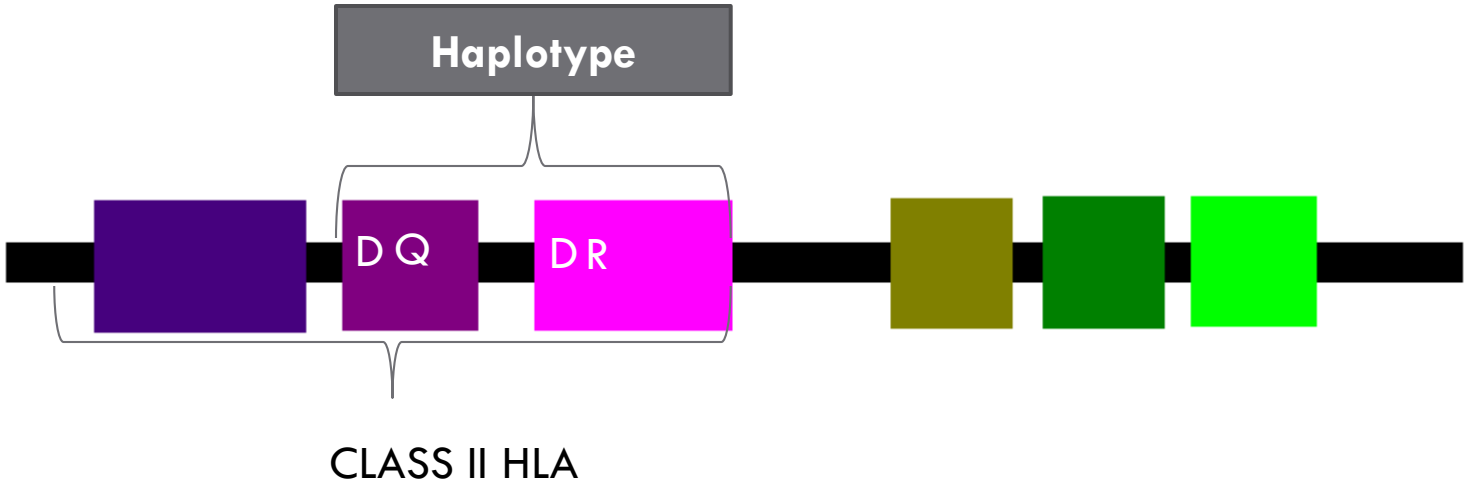
Maternal



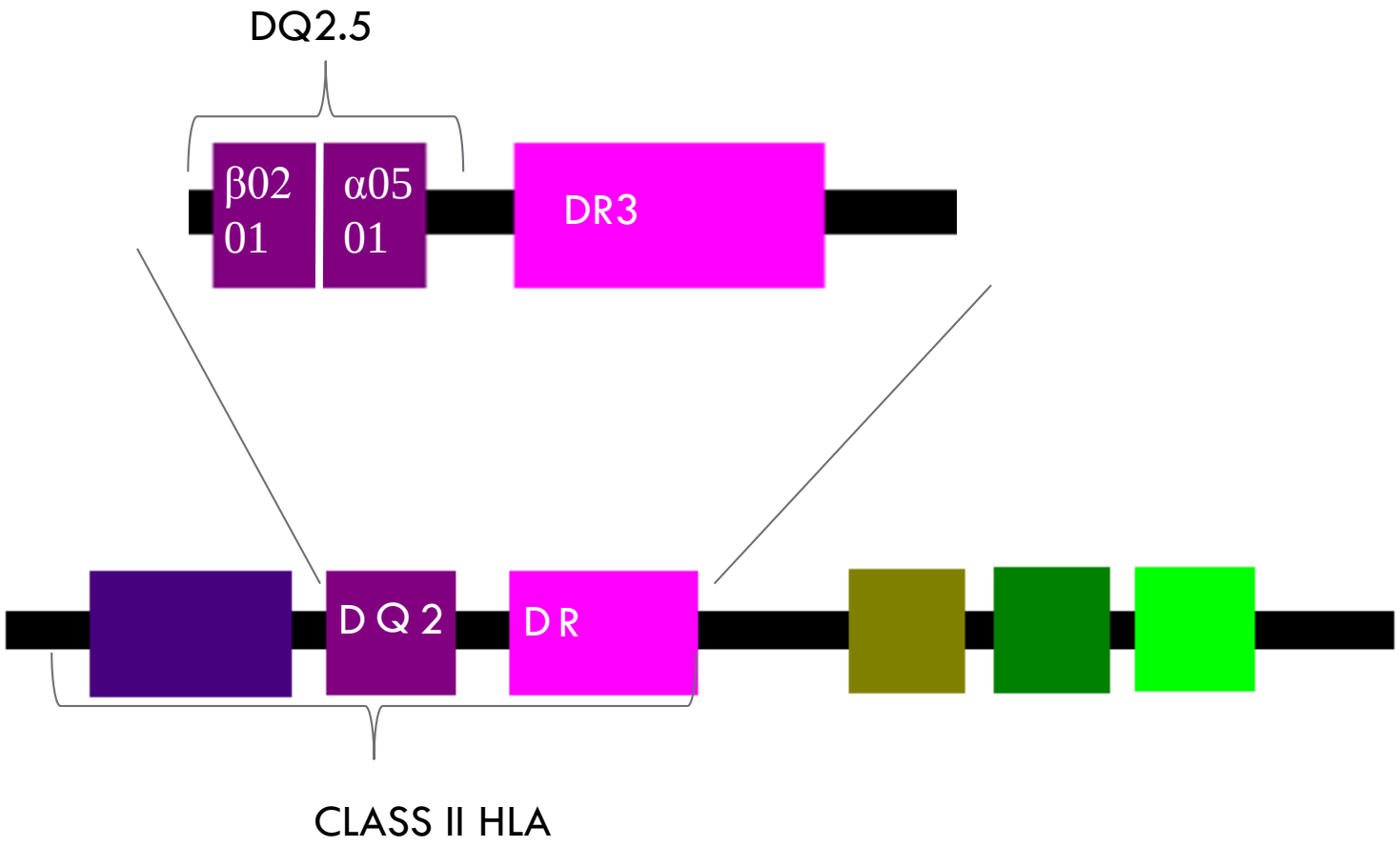


Haplotype

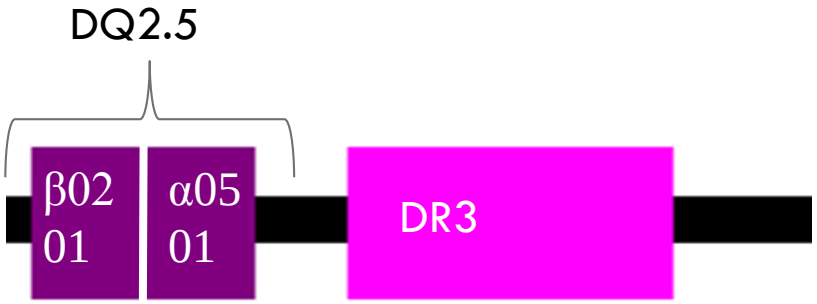
- **Definition:** Combination of alleles of DNA sequences at adjacent locations on the chromosome that are transmitted together



Haplotype



Haplotype

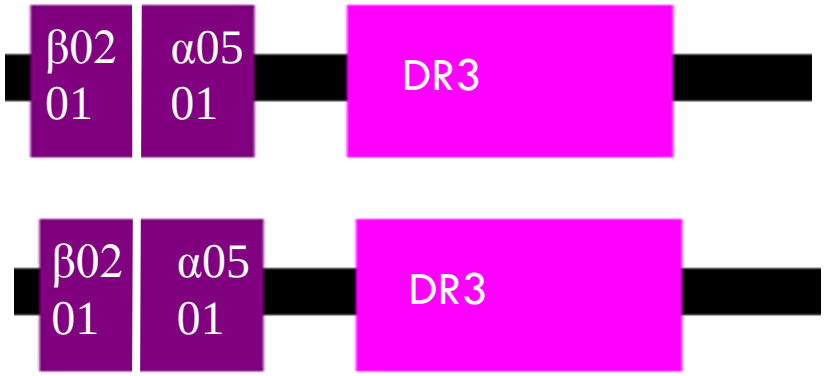


- Haplotype DR3-DQ2
  - The most common haplotype associated with celiac disease
    - European prevalence up to 21.9 %
    - African prevalence of up to 17.3 %
    - Rare in Asian and Native Americans population

Abadie, 2011

Frequency of HLA DR3-DQ2

Trans vs. cis alleles in DQ2



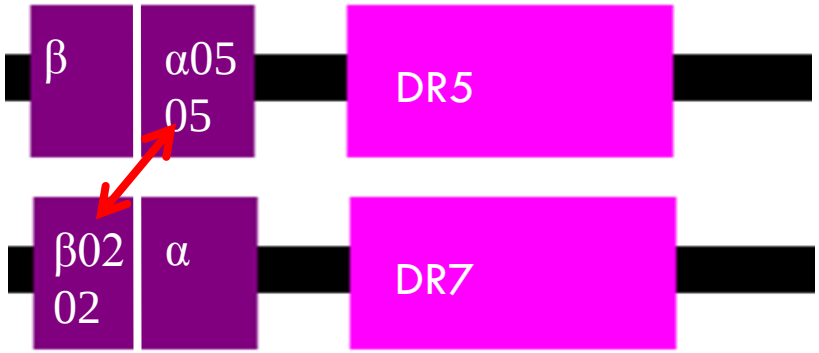
Allele 1, *DQ2.5* **cis**

Allele 2, *DQ2.5* **cis**

2x

Dosage

If patient has two alleles both encoding DQ2 (homozygous), the risk ↑↑↑



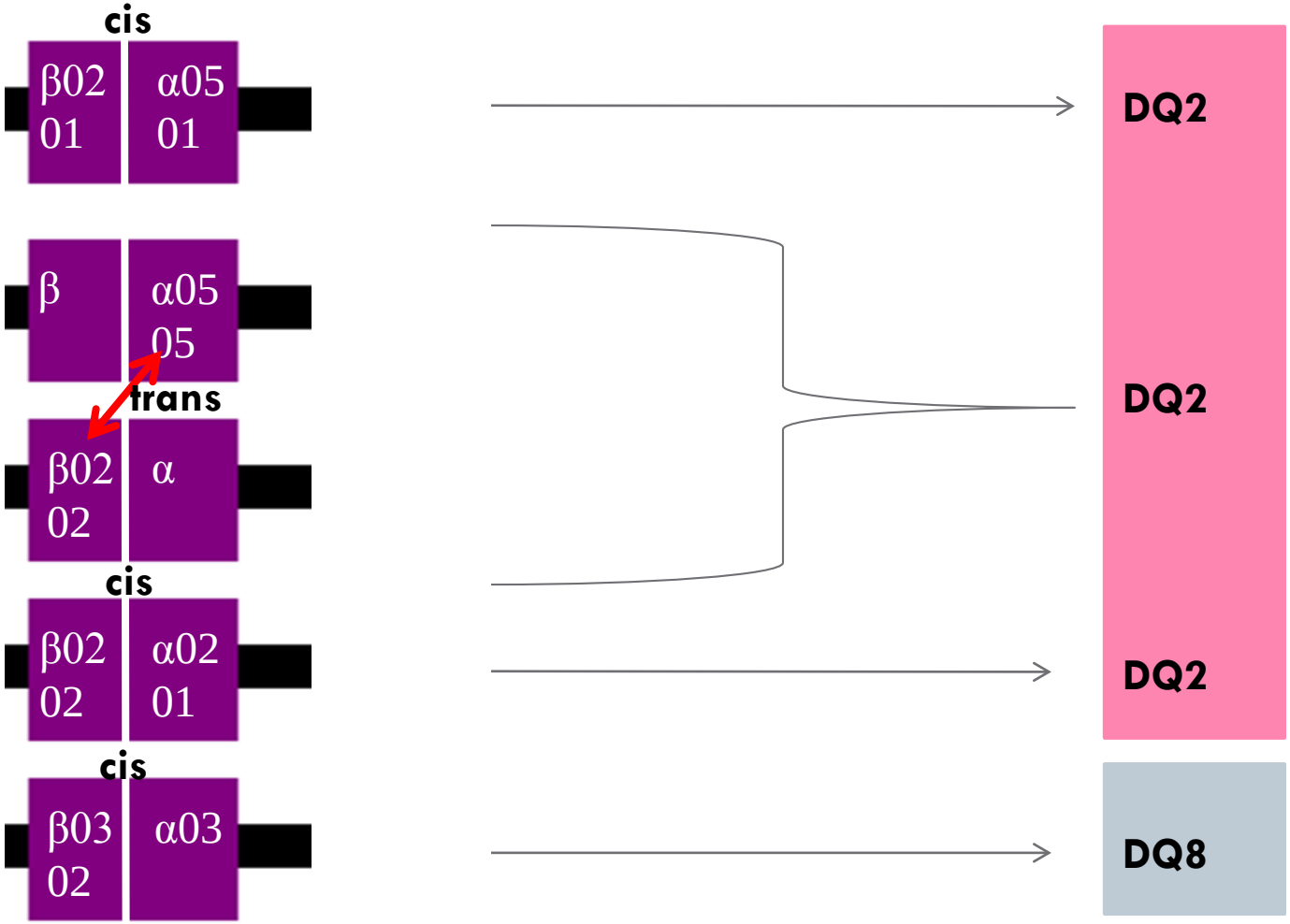
Allele 1

DQ2.2 **trans**

Allele 2

1x

HLA-DQ 2 and 8 predispose for CD

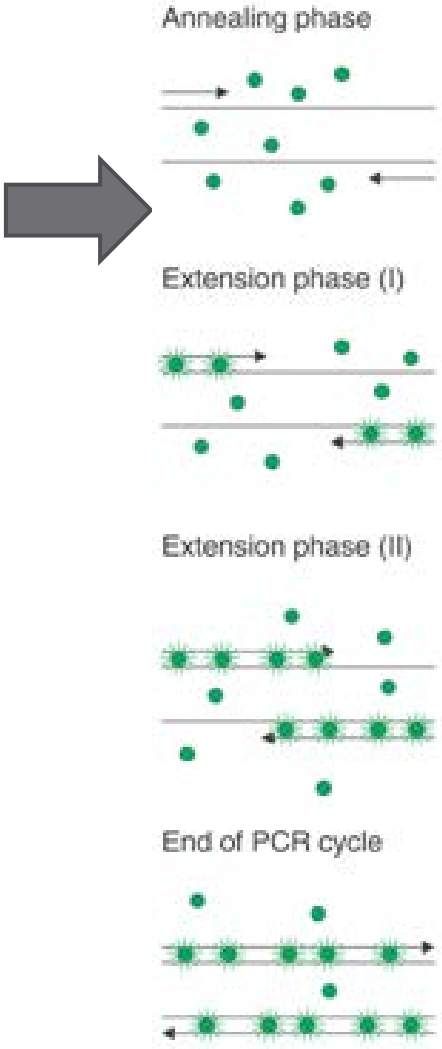


Methodology

PCR with SYBR Green I technique

PCR

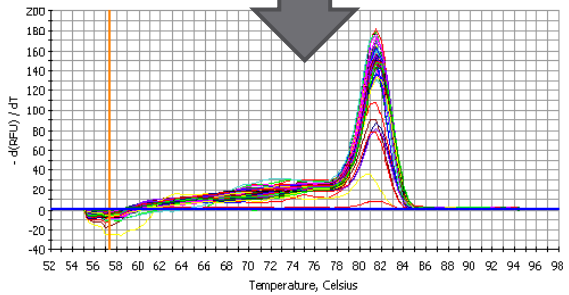
Polymerase
Primers:
DQB1*02
DQA1*05 } **DQ2**
DQB1*03:02 → **DQ8**
Patient's DNA



SYBR
Green I technique/
fluorescent DNA binding
dye

Fluorescence is increases upon binding to double-stranded DNA

Signal ↑ as the DNA concentration ↑



Melting analysis

HLA summary

- *HLA-DQ2* is positive in 90-95% of patients with CD
- Presence does not confirm CD
- Found in 30-40% of general population
- Absence virtually excludes CD
- May be indicated in individuals at risk for CD or individuals who are repeatedly seropositive but biopsy-negative
- Results
  - HLA positive (and other serology/ clinical history +) – CD confirmed
    - Not necessary to confirm by biopsy
    - HLA negative – likely false-positive tTG test

Screening for celiac disease

- Not recommended for the general population
- DGP and tTG IgA and IgG
- Utility of HLA tests:
  - Type 1 DM, Autoimmune thyroid disease, IBS, Dermatitis herpetiformis, Selective IgA deficiency
    - Absence of *DQ2* and *DQ8* render CD highly unlikely
  - Equivocal biopsy

Treatment of celiac disease

- Gluten-free diet (GFD)

Foods containing gluten

Gluten-free foods

Monitoring adherence to treatment

- Monitoring adherence to gluten-free diet (GFD) or disease activity is part of American Gastroenterological Association (AGA) guidelines
  - tTG and/or DGP IgA and IgG assays
    - Typically ordered every 3-6 months
    - If high for >12 months consider rebiopsy
  - F-actin IgA antibody
    - Declining levels of F-actin IgA

Cases and questions

22 yo F with weakness and fatigue when exercising

- Upon further questioning
  - Tingling and numbness in her fingers
  - Itchy rash
  - Diarrhea for past 2 years with associated abdominal pain
  - Serology was done
    - IgA WNL
      - DGP IgA high
      - tTG IgA high
    - HLA typing
      - DQ2 positive

Diagnosis: Celiac disease

What is the treatment?

Gluten free diet

Conclusion

- Gluten free diet is treatment of celiac disease

60 yo M w/ diarrhea

- Upon questioning
  - Diarrhea started yesterday after he ingested a very old potato salad
  - On exam scaly rash was observed (upon questioning he first noticed the rash 10 years)

Should tTG and DGP be done in this case?

No

This patient has probably psoriasis and ingested potato salad with *Staph aureus* toxin.

Conclusion

- Chronic diarrhea is a symptom of celiac disease

31 yo M with allergies, weakness and diarrhea

- Diarrhea for past 3 years
- Patient has been losing weight for past 2 years
- IgA screen was performed
  - IgA - not detected
- What immunoglobulin should be tested in this patient?
  - DGP and tTG IgG
- Results came back negative, what now?
  - HLA testing/biopsy
  - Patient is HLA DQ2 and DQ8 positive
  - Biopsy showed short villi

Celiac disease was diagnosed

Conclusions

- If patient has selective IgA immunodeficiency, use IgG in serologic tests
- HLA DQ2 and DQ8 predispose for CD
- Patient with CD will show short villi on biopsy

Which of the following foods contains gluten?

A. Orange juice
B. Honey
C. Whiskey
D. Seaweed
E. None of the above

Whiskey is a type of distilled alcoholic beverage made from fermented grain mash.

Acknowledgment

- Dr. Tebo
- Dr. Delgado
- Dr. Rockwood
- ARUP
- University of Utah

Thank you

Questions?

References

- ARUP consult
- ARUP directory
- Master control
- Henry's textbook
- Tietz textbook
- Pubmed