Genetic testing for celiac disease

By Edwin Liu, MD

Celiac disease (CD) is a remarkably common immune-mediated disorder determined by both genetic risk [certain human leukocyte antigen (HLA) alleles] and one of the best characterized environmental triggers (gliadin) for any common autoimmune disease. The discovery of transglutaminase autoantibodies in celiac disease has allowed the identification of a large number of both symptomatic and asymptomatic individuals as having intestinal biopsy-confirmed celiac disease. As we gain further understanding of the long-term outcome of asymptomatic celiac disease and the specific interaction between genetic susceptibility and environmental factors, screening for celiac disease — both by autoantibody testing and by genetic testing — may become an integral public-health contribution. Unlike autoantibody testing for celiac disease, however, which has excellent utility and high predictive value, genetic testing should be reserved for very specific indications to be discussed in this overview.

CE CONTINUING EDUCATION

To earn CEUs, see test on page 14.

LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Describe the clinical features associated with celiac disease and identify the environmental trigger.
2. Discuss the serologic testing methods used to identify individuals at risk for developing celiac disease and cite which test(s) are the best assays available.
3. Identify the relevant genes associated with celiac disease and explain how DQ2 can be re-created in individuals with certain other genes.
4. Review which populations carry an increased risk for developing celiac disease.
5. Discuss the advantages and disadvantages of using genetic testing as a screening method for celiac disease in the general population.

Case study

A physician's office calls a laboratory about an 8-year-old boy who was recently diagnosed with type 1 diabetes. Knowing the increased risk of celiac disease in patients with type 1 diabetes and their relatives, the physician wants to know the best test to screen for celiac disease in this child. In addition, he wants to know if there is any type of genetic testing available to evaluate family members if the boy is diagnosed with celiac disease.

This disease is unique in that an environmental factor has been clearly identified and is required for disease expression.

Celiac disease, also known as gluten-sensitive enteropathy, results in an immune-mediated intolerance to ingested wheat gluten or related proteins from rye and barley. Gluten is the water-insoluble fraction of wheat flour, largely composed of two groups of proteins: glutenins (ethanol insoluble) and gliadins (ethanol soluble). Individuals with celiac disease are intolerant to the gliadin fraction of gluten, as well as equivalent prolamins found in rye and barley. This disease is unique in that an environmental factor has been clearly identified and is required for disease expression.

Individuals with celiac disease who continue to ingest gluten develop small intestinal pathology, characterized by villous atrophy, crypt hyperplasia, and intraepithelial lymphocytes that is reversed with the removal of gluten from the diet. While it was previously considered to be only an intestinal disease (hence the classical term "gluten-sensitive enteropathy"), it is really a systemic autoimmune disorder with numerous extra-intestinal manifestations affecting the skin, liver, bones, joints, heart, brain, and other organs. Likewise, traditional clinical features included abdominal pain, distension and bloating, growth failure, vomiting, and diarrhea classically seen in infants and young children. As more individuals are being identified through laboratory screening based on genetic-risk factors, symptoms of celiac disease have drifted away from the traditional clinical features and have become increasingly subclinical, often with patients being asymptomatic (silent celiac disease). Long-term complications of untreated celiac disease include osteoporosis and increased risk of fractures, infertility, and increased risk of small-bowel malignancy, although the natural history of those with silent celiac disease is unknown. The only treatment for an individual with celiac disease is to adhere to a gluten-free diet, which can be extremely difficult since gluten is found in many products ranging from bread and pastas to salad dressings and beer.

Prospective epidemiologic studies ... are evaluating whether timing of the introduction of cereal will influence the eventual development of celiac disease.

Epidemiology of celiac disease

With increased awareness and detection — coupled with more widespread availability of gluten throughout the world — more cases of celiac disease are being identified. Current estimates based on seropositivity rates indicate a frequency of CD in the general population of North America and Europe to be 0.4% to 1%, or 1 in 100 to 250 people.[1] These numbers suggest that for each diagnosed case of CD there may be three to seven undiagnosed cases.

How is celiac disease diagnosed?

In clinical practice, serologic testing specific for celiac disease is usually done and, if positive, intestinal biopsy is needed to confirm the presence of disease. In the research setting, serologic testing is generally used as a screening method to determine

Continues on page 12

Figure 1.

Left – Formation of DQ2 molecule from the a and b chain genes on the same chormosome classified as DR3.
Right – Formation of DQ2 molecule from the a and b chain genes on separate parental chromosomes, one from DR5 and one from DR7.

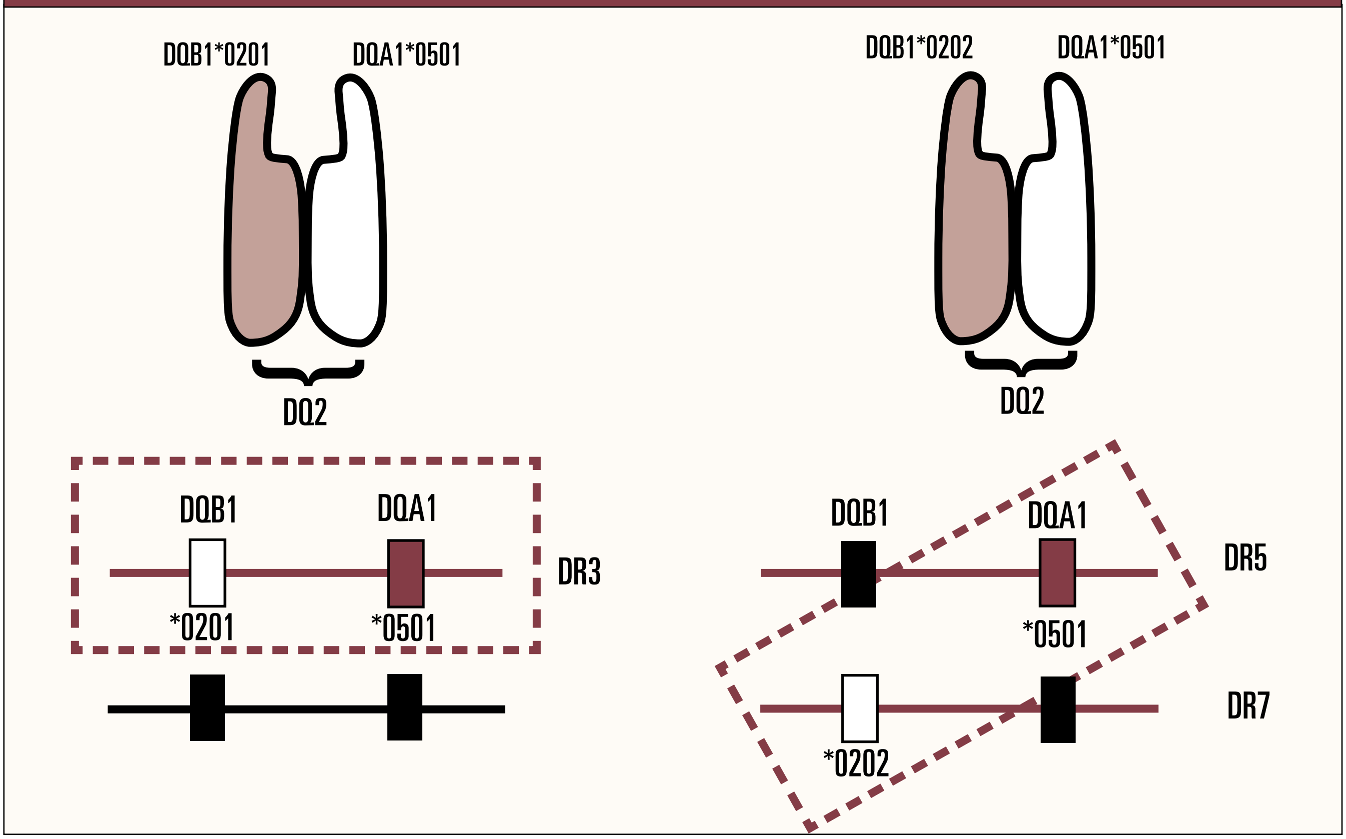

Table 1.
Overall performance of common antibody tests for celiac disease

	Sensitivity	Specificity
Anti-gliadin (IgG and IgA)	52%-100%	47%-100%
EMA IgA	86%-100%	90%-100%
TG IgA	77%-100%	91%-100%

disease prevalence of a population. Tests most commonly offered by commercial laboratories include immunoglobulin IgG- and IgA-based antigliadin antibodies (AGA IgG and AGA IgA); IgA endomysium antibody (EMA IgA); IgA tissue transglutaminase antibody (TG IgA); IgA antireticulin antibody; and anti-actin antibody. The overall sensitivity and specificity of anti-gliadin, EMA, and TG antibodies are summarized in Table 1.[2]

There are insufficient studies to determine sensitivity and specificity of anti-reticulin and anti-actin antibodies. Even though most laboratories offer them, the anti-gliadin antibody has fallen out of favor by most gastroenterologists given its poor sensitivity and specificity, leading to poor predictive values for celiac disease. Based on the higher predictive values, EMA and TG autoantibody testing are the best available assays and are highly associated with individuals having celiac disease. While the EMA was previously the most commonly used serologic test for celiac disease, it is performed by indirect immunoflouresence and requires human umbilical cord or primate esophagus as substrate. TG IgA has now largely replaced EMA due to equally high predictive value and ease of use. The use of human recombinant TG has yielded better results over guinea-pig TG and has now been widely accepted as the substrate of choice for the TG IgA assay. TG IgA can also be utilized quantitatively such that the higher the positive test, the more likely the individual will have abnormal histology consistent with celiac disease.[3]

Table 2.
Prevalence of celiac disease in groups considered to be at "high risk"

	Prevalence
General population	0.7%-1%
Non-DQ2 individuals	0.3%
DQ2 positive individuals	3%
First degree relative of someone with type 1 diabetes or celiac disease	2%
Type 1 diabetes	8%-10%
Type 1 diabetes + DQ2/DQ2	33%
Down's, Turner's, or William's syndromes	5%-10%

Relevant genes in celiac disease

Testing for the celiac disease-specific HLA genotypes located on chromosome 6 (DQ2 and DQ8) are now available to the practicing physician. HLA are certain molecules found on the cell surface involved in immune regulation — the same molecules that help identify a person's tissue type for organ transplantation. More than 90% of patients with celiac disease have the HLA DQ2 (DQA1*0501, DQB1*0201) — often referred to by the serological description DR3 having strong linkage with HLA DRB1*0301 allele — and most of the remaining patients (8% to 10%) carry HLA DQ8 (DQA1*0301, DQB1*0302).

The molecules formed by DQ2 or DQ8 present important gluten antigens to the immune system that drive the disease process. Therefore, patients with celiac disease almost always have DQ2, DQ8, or both molecules. Thirty-six percent of the general population, however, carry DQ2 as well, and 40% carry DQ2 or DQ8. HLA molecules are heterodimers, composed of an alpha and beta chain, which — when combined — indicate a specific HLA allele. For each HLA genotype, a person inherits two alleles — one from each parent. Therefore, a person can have two copies of DQ2 if both parents transmitted that particular allele to the offspring, or zero copies if neither parent carried that allele.

There are certain populations at particularly high risk for celiac disease, as it is associated with other autoimmune diseases, such as type 1 diabetes, Addison's disease, and thyroid disease.

In addition, the HLA-DQ2 molecule can be constructed from the alpha and beta chain of either of the parentally inherited genes. Thus, DQA1*0501 and DQB1*0201, encoded either in cis or trans on the sixth chromosome, can form the DQ2 (DQA1*0501, DQB1*0201) molecule. This is an important concept because individuals with HLA-DR5 (DQA1*0501, DQB1*0301) on one chromosome and DR7 (DQA1*0201, DQB1*0202) can form DQ2 (as DQA1*0501, DQB1*0202) — since DQB1*0201 and DQB1*0202 are virtually identical. Thus, DQ2 is re-created in individuals also having DR5/DR7, except that the sequence information is split between two chromosomes (see Figure 1).

Other non-HLA genes have also been studied for association with celiac disease, such as MIC-A and MIC-B found in the HLA region, as well as CTLA-4. Studies, however, have had inconsistent results with much weaker associations than that seen for HLA-DQ2 and DQ8. Therefore, even though celiac disease is a complex genetic disorder, the most useful gene in identifying individuals at risk for celiac disease is DQ2 and DQ8.

Who is at risk for celiac disease?

Children expressing at least one DQ2 allele have a 3% risk of developing evidence of celiac disease autoimmunity (TG IgA positive) by age five years, compared to a 0.3% risk in those without DQ2. There is a 75% concordance of celiac disease in monozygotic twins, while dizygotic twins are not different from siblings, indicating the strong genetic contribution to disease. There are certain populations at particularly high risk for celiac disease, as it is associated with other autoimmune diseases, such as type 1 diabetes, Addison's disease, and thyroid disease, in addition to genetic diseases such as Down's, Turner's, and William's syndrome. Table 2 shows the relative risk of various genetic scenarios for celiac disease.

Screening for celiac disease

In many ways, celiac disease would be the ideal HLA-associated disorder for public-health screening. It is a relatively common disease associated with long-term complications, which is treatable (gluten-free diet) when identified. Antibody testing with TG IgA is inexpensive, sensitive and specific, and — when positive — highly predicts intestinal lesions on biopsy. Under debate, however, is whether or not screening for celiac disease should be done. At the present time, we do not know the clinical/biological significance of an asymptomatic individual found on screening to have celiac disease. Will he have similar long-term complications as someone with celiac disease presenting with anemia, growth failure, and intestinal malabsorption? Furthermore, a single negative TG IgA test does not necessarily exclude the future possibility of disease development at a later time. Therefore, antibody testing for celiac disease might become cumbersome, requiring multiple antibody determinations over time.

This disease is unique in that an environmental factor has been clearly identified and is required for disease expression.

HLA testing for DQ2/DQ8: the best genetic test

If it is determined that screening should be done for celiac disease, then there could, indeed, be a role for genetic testing of mass populations. HLA-DQ2 and DQ8 would be the best genetic test for celiac disease in these circumstances.[4] A major caveat for HLA screening exists, however; 40% of the general population will have DQ2 or DQ8, and only a subset of those individuals with DQ2 or DQ8 will actually go on to develop biopsy-confirmed celiac disease. Therefore, for mass genetic testing, the utility of HLA typing would only be to exclude 60% of the general population (those not having DQ2 or DQ8) from future TG IgA screening for celiac disease. Of those that are DQ2 or DQ8 positive, there would be a 3% risk of developing celiac autoimmunity by age seven (higher risk if there is an affected family member with celiac disease or type 1 diabetes).

What is the utility of genetic testing in the clinic?

A positive genetic test for DQ2 or DQ8 does not mean that a person has or will develop celiac disease. It can, however, give insight as to the risk of celiac disease. For example, an individual with DQ2 or DQ8 has a 3% overall risk, and a type 1 diabetic homozygous for DQ2 has a 33% risk of having celiac disease. Furthermore, at the individual level, if a person is already considered "high risk" for celiac disease (see Table 2), then genetic testing could also serve to exclude the likelihood of disease. A negative genetic test (not having DQ2 or DQ8) would virtually eliminate the possibility of that individual ever developing celiac disease. Therefore, since genetic testing for DQ2 or DQ8 only provides insight as to risk of celiac disease, most gastroenterologists do not recommend genetic testing in the clinic. Two reasons as to why testing might be done are:

- to help exclude disease as a possibility — occasionally an individual might have inconclusive serology (particularly in IgA deficiency) and inconclusive intestinal biopsies; and
- for peace of mind – occasionally families may be so concerned about the possibility of celiac disease, given a family history, that a negative genetic test might be reassuring. On the other hand, a positive test for DQ2 or DQ8 might serve only to exacerbate patient anxiety.

Finally, prospective epidemiologic studies, particularly studies of diet headed by Jill Norris of the Celiac Disease Autoimmunity Research (CEDAR) study in Denver, are evaluating whether timing of the introduction of cereal will influence the eventual development of celiac disease. If a significant dietary influence on celiac disease is found (such as delaying the introduction of cereals), then there might be a major impetus for newborn screening of HLA.[5]

Conclusion

Celiac disease is an important candidate for public-health newborn genetic screening based on HLA-DQ alleles. Until such screening recommendations are established, however, genetic testing for celiac disease has a specific and limited role. For large population screening, genetic testing of individuals could eliminate 60% of the population (low risk non-DQ2 or DQ8) for consideration of serial autoantibody testing with TG IgA. The role of individual genetic testing in the clinic is only to help assess risk, and diagnosis of celiac disease still rests on the presence of autoantibody positivity and in abnormal intestinal histology. ☐

Edwin Liu, MD, is assistant professor in Pediatrics, Section of Gastroenterology, Hepatology, and Nutrition, at The Children's Hospital, Denver, CO.

References

1. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Arch Intern Med.* 2003;163(3):286-292.
2. Hill ID. What are the sensitivity and specificity of serologic tests for celiac disease? Do sensitivity and specificity vary in different populations? *Gastroenterology.* 2005; 128(4 Suppl 1):S25-S32.
3. Liu E, Li M, Bao F, Miao D, Rewers MJ, Eisenbarth GS, et al. Need for quantitative assessment of transglutaminase autoantibodies for celiac disease in screening-identified children. *J Pediatr.* 2005;146(4):494-499.
4. Liu E, Rewers M, Eisenbarth GS. Genetic testing: who should do the testing and what is the role of genetic testing in the setting of celiac disease? *Gastroenterology.* 2005;128(4 Suppl 1):S33-S37.
5. Norris JM, Barriga K, Klingensmith G, Hoffman M, Eisenbarth GS, Erlich H, et al. Timing of cereal exposure in infancy and risk of islet autoimmunity. The Diabetes Autoimmunity Study in the Young (DAISY). *JAMA.* 2003;290:1713-1720.