EDITORIAL

The Unfolding Story of Celiac Disease Risk Factors

What causes celiac disease (CD)? On the surface, the answer is straightforward: dietary gluten. In susceptible individuals, ingested gluten is deamidated by tissue transglutaminase in the lamina propria of the small intestine and then bound by antigen-presenting cells to the HLA DQ2 or DQ8. This sets off an immune cascade that results in infiltration of the epithelium with lymphocytes and tissue destruction, leading to villous atrophy that is characteristic of CD.[1]

The identification of dietary gluten as the environmental culprit was a major advance in the history of gastroenterology. Although diet was long considered to trigger or exacerbate CD, it was the Dutch pediatrician Willem Dicke who determined it was the protein fraction of wheat that made his patients ill. These observations were made before World War II, and Dicke's hypothesis was borne out during a blockade-induced famine in 1944, when patients with CD improved markedly during the period of severe wheat shortage, only to relapse on the reintroduction of wheat at the end of the famine.[2] The subsequent discovery that the HLA DQ2 or DQ8 haplotype was necessary for the development of CD[3] led to the present understanding that CD arises when gluten is introduced to the genetically susceptible individual.

Yet this simple explanation is belied by the fact that this gene–environment combination is far more common than the prevalence of CD; gluten is a ubiquitous dietary staple, and the at-risk HLA haplotypes are present in 30%-40% of Western populations.[4] Genome-wide association studies have identified dozens of additional genetic risk loci relating to the immune response, illustrating that CD is a complex, polygenic, immune-based disorder.[5,6] But just as the genetic story of CD is more complicated than HLA inheritance, the environmental trigger of CD is about more than gluten. Ultimately, growing knowledge of the genetic determinants of CD will by itself not be adequate to understand why CD develops. Epidemics are triggered by environmental exposures, because genetic changes are too slow to drive these phenomena. We have now witnessed 2 epidemics of CD: one that was dramatic and limited, and another that, although less visible, is greater in scale and ongoing.

The Swedish epidemic of CD of 1985–1994 has been extensively documented, and resulted in the development of hypotheses regarding environmental risk factors for this disorder.[7] This epidemic was restricted to children younger than 2 years; in that age group, the incidence of diagnosed CD rose from 65 cases per 100,000 person-years to 198 cases per 100,000 person-years. In contrast, incidence data for older children were relatively flat during this period. The epidemic abruptly ended in 1995, although children born during the period of the epidemic have an ongoing increased risk of developing CD. Subsequent investigation led to the hypothesis that infant feeding practices affect the risk of CD in young children. The epidemic occurred during a period of relatively low rates of breastfeeding at the age of 6 months and during the same period of time, the quantity of gluten in infant formula greatly increased. Although it is difficult to separate the relative importance of each feeding practice, it seemed that high quantity of initial gluten intake without overlapping with breastfeeding was responsible for this epidemic. Although a systematic review of the issue has concluded that breastfeeding has not been definitively proved to be associated with risk of CD,[8] subsequent research has indicated that the timing of gluten introduction is important in determining risk.[9] PreventCD, a prospective randomized trial of infants with a family history of CD, is testing specifically whether the introduction of small quantities of gluten beginning at age 4 months of age will induce tolerance to gluten in this high-risk group.[10]

The second epidemic is more diffusely spread over time and space. Studies from the United States and elsewhere have shown that the seroprevalence of CD (as defined by positive tissue transglutaminase and endomysial antibodies) has increased markedly in recent decades. An analysis of stored serum from military recruits at the Warren Air Force Base in the years spanning 1948–1954 found a CD seroprevalence of 0.2%, whereas 2 recent cohorts from Olmsted County (spanning the years 2006–2008) matched by year of birth and age at sampling found a seroprevalence of 0.9% and 0.8%, respectively.[11] An analysis of another cohort in this country found a doubling in seroprevalence during adulthood from 1974 (0.21%) to 1989 (0.45%).[12] The mode of presentation of CD has changed in the past generation, with rising numbers of patients presenting without diarrhea.[13] Patients presenting with anemia may have more severe disease expression (as measured by the degree of villous atrophy and the presence of metabolic bone disease) than patients presenting with diarrhea.[14] Because most individuals in the United States with CD are undiagnosed,[15] this is largely a hidden epidemic, but there is no reason to believe that the prevalence has peaked. In Finland, which had a higher prevalence of CD than the United States to begin with, the seroprevalence of CD doubled between the years 1978 (1.05%) and 2000 (1.99%).[16] It is not known whether this epidemic will subside or if the prevalence of CD will continue to rise to a new set-point. But given the morbidity associated with CD[17] and the cost and difficulty of the gluten-free diet[18,19] these data have sparked interest in identifying the cause of this less visible epidemic.

Certain infections (eg, rotavirus among infants[20] and *Campylobacter* among adults[21]) have recently been shown to be associated with a increased risk of CD, but rates of these infections have not increased markedly, and so are not likely to be driving this epidemic. In contrast, a lack of exposure to certain microbes is a hallmark of modern times, and the increase in CD disease is congruent with the hygiene hypothesis, which states that decreased exposure to microbes may be driving the rise in autoimmune and atopic conditions. This hypothesis is particularly compelling in light of a recent study that found a dramatically different seroprevalence of CD in Finland (1.4%) and the Russian Karelia (0.6%), geographically proximate areas with a similar prevalence of HLA DQ2 and DQ8 but with major differences in economic development.[22]

Further evidence implicating the modern relationship with microbes is now accumulating. Children who were born by elective cesarean section are at increased risk of developing CD, whereas those born by emergent cesarean section (and may have had contact with the birth canal) are not.[23] In addition, there seems to be an inverse relationship between *Helicobacter pylori* colonization and CD.[24] Drugs are another modern innovation that may be affecting the CD epidemic. Population-based studies from Sweden have shown that prescription of antibiotics[25] and proton pump inhibitors[26] are each associated with an increased risk of the subsequent development of CD.

The associations identified in these studies are not necessarily causal. Studies of drug exposure in particular may be prone to protopathic bias, wherein early symptoms of the outcome of interest (CD) may lead to the prescription of the exposure (eg, antibiotics or proton pump inhibitors).[27] Confounding variables, such as another (unmeasured) microbe that correlates positively with *H pylori* and is protective against CD, would create a correlation of this exposure and outcome without direct causation. Although methodologic measures can be taken to address bias and confounding, ultimately these studies should be viewed as hypothesis-generating, mandating investigation to test the potential mechanisms by which exposures inherent to modern life may be contributing to this immune-based disorder.

What about other medications, such as supplements? In this month's issue of *Clinical Gastroenterology and Hepatology*, Størdal and colleagues report on an association between maternal iron supplementation among pregnant women and the risk of the subsequent diagnosis of CD in their offspring.[28] The authors found that the use of iron, whether as sole supplements or in combination in a multivitamin/mineral product, is associated with an increased risk of CD (odds ratio, 1.48), which remained significant after adjusting for maternal CD, gender of the child, age of the child, and the timing of gluten introduction.

This is a methodologically rigorous study and the findings are intriguing. Strengths include its prospective design, in which exposure was measured before outcome; the large cohort size; long follow-up period; and the use of validated food frequency questionnaires to measure dietary iron. A significant limitation is the reliance on a combination of parental reporting and nonvalidated method of using the Norwegian Patient Register for case identification. To compensate for this, the investigators performed a sensitivity analysis in which 10% and 20% of cases were reclassified as control subjects, and found that the association between iron supplementation and CD persisted. This is reassuring, provided that misclassification caused by reliance on claims codes was random, and not differential. Although health-conscious behavior may be associated with iron supplement use and a CD diagnosis, the association between exposure and outcome was seen only in iron supplements and not for other dietary supplements. The finding of a dose-response relationship between estimated cumulative iron supplementation and the subsequent risk of CD also argues for causality.

The mechanism by which iron supplementation could increase the risk of CD is uncertain, but there are a few promising leads. Proteins related to iron metabolism including hepcidin and lactoferrin influence innate immunity,[29] and the host's iron status may influence the makeup of the microbiome.[30,31] The notion that excess iron may lead to a loss of immune tolerance has precedent; a population-based case-control study found a greater than 2-fold risk of CD among individuals with hereditary hemochromatosis.[32] A clear mechanism remains elusive at this time, especially because it is not established that exposure to increased iron in utero has downstream consequences on the development of the immune system after birth.

However, it cannot be ruled out that the association between iron supplementation and CD is correlative but not causal. One important unmeasured confounding variable is undiagnosed maternal CD, which may be associated with both the exposure (iron supplementation) and is certainly associated with the outcome (increased risk of CD in the offspring). Anemia is strongly associated with undiagnosed CD.[33] In a population where approximately 10% of women had anemia, assuming that 3% of such individuals with anemia have undiagnosed CD[33] (compared with a general population rate of 1%), and that 10% of these mothers' children would develop CD, an association between iron supplementation and CD would be observed, with an odds ratio of 1.27, which is similar in magnitude to the effect shown in this study. One way to examine the potential role of undiagnosed CD in women with anemia is to analyze maternal iron supplementation only in women without a diagnosis of anemia to see if the association with offspring CD remains.

Given the uncertainty regarding causality, it is premature to argue that iron supplementation during pregnancy should be avoided in individuals with CD whose offspring are at risk for developing this condition. Overall, although

statistically significant and intriguing, the observed effect (an absolute risk increase of 0.15%) is modest in magnitude. This exposure is not driving the slow, ongoing epidemic of CD. But the signal uncovered by this study may uncover the mechanisms by which many more have been losing immune tolerance of gluten in recent years.

BENJAMIN LEBWOHL, MD, MS
Celiac Disease Center
Department of Medicine
Columbia University College
of Physicians and Surgeons
New York, New York

Department of Epidemiology
Mailman School of Public Health
Columbia University
New York, New York

JONAS F. LUDVIGSSON, MD, PhD
Medical Epidemiology and Biostatistics Unit
Karolinska Institutet
Stockholm, Sweden

Department of Pediatrics
Örebro University Hospital
Örebro, Sweden

PETER H. R. GREEN, MD
Celiac Disease Center
Department of Medicine
Columbia University College
of Physicians and Surgeons
New York, New York

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