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Case Report

# Histopathological correlations and HLA-DQ/DR typing in coexisting celiac disease and type 1 diabetes in children – case report and literature review

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**Abstract.** Celiac disease is rarely diagnosed in patients with absent Human Leukocyte Antigen (HLA) DQ and the association with diabetes in patients with HLA DQ2 / DQ8 negative genotype is even rarer. Most patients develop first diabetes and celiac disease afterward. We present the case of two brothers: the younger brother (aged 4 years 2 months) came first at the 2nd Clinic of Pediatrics in 2011 and was diagnosed with celiac disease. Then the older brother (aged 9 years 3 months) was investigated too, even though he was clinically asymptomatic. We found that he accomplished the laboratory criteria for celiac disease. We determined the HLA DQ genotype in both brothers. The younger brother was HLA DQ 7 positive and the older brother tested negative for HLA DQ. One year after diagnosis and initiation of gluten-free diet, the older brother was diagnosed with type I diabetes. We determined also the HLA DR and the result was that the younger brother had a protective genotype and the older brother was the carrier of a genotype that predisposes to autoimmune diseases. These cases were outstanding because celiac disease is rarely diagnosed in the absence of predisposing HLA alleles. Consistent with previous studies, the brother who had the predisposing genotype, presented more than one autoimmune disease and the one with the protective genotype developed celiac disease.

**Key Words:** autoimmunity, celiac disease, diabetes mellitus, HLA.



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## Introduction

HLA (human leukocyte antigen) determination in celiac disease (CD) brings numerous information about patient genotype and it is used as a diagnostic tool too: in association with antibodies levels, it can replace the intestinal biopsy. CD has a multifactorial etiology. It appears in genetically predisposed patients who came in contact with trigger factors. Environmental factors known are: viral infections, intestinal flora, breastfeeding and/or introduction of gluten in infant diet. Genetic factors that confer susceptibility to CD are found in approximately 90-95% of patients diagnosed with the disease and are represented by HLA haplotypes Celiac 1 = DQA1, DQB1, DR (about 40% of patients); Celiac 2 = 5q31-33; Celiac 3 = 2q33; Celiac 4 = 19p13.1. Asymptomatic patients who are not positive for any of these genetic polymorphisms are not considered at risk of developing CD. Determination of the HLA genotype is important in patients at high risk for autoimmunity: first-degree relatives of patients with CD, the cases with discrepancies between serology and intestinal biopsy results, those who have other autoimmune diseases (type I diabetes, autoimmune thyroiditis, autoimmune hepatitis, multiple sclerosis) (Megiorni et al 2012). HLA's determination test has a negative predictive value and is designed to patients with increased genetic susceptibility for celiac disease. It is very unlikely that a patient with absent predisposing HLA to develop CD. Patients who are not part of the predisposing HLA class do not require clinical and serological follow-up (Megiorni et al 2009). As CD, diabetes mellitus type 1 (DM) is also a multifactorial disease. Genetic susceptibility is offered by alleles HLA DR3 and DQ2. This susceptibility is essential but not sufficient to produce the disease. The incriminated environmental factors are similar with those responsible for CD.

CD is rarely diagnosed in patients with absent HLA DQ and the association with diabetes in patients with HLA DQ2 / DQ8 negative genotype is even rarer. Most patients develop first diabetes and celiac disease afterward; our case was one of those rarities, breaking all these "rules" (Cronin 1997).

## Case report

We present the case of a family with two male children admitted at the 2nd Clinic of Pediatrics in 2011. There was no significant family history, nor antenatal or perinatal significant antecedents. First, the younger brother (aged 4 years 2 months) presented in our clinic for a constipation type of bowel disorder (1 stool every 4-5 days). On physical examination, the patient did not present any pathological features. Laboratory examinations carried out have revealed lymphocytosis (12.000/mm3 [4.000-8.000/mm$^3$]), iron deficiency anemia (Hemoglobin 10.2 g/dl [12.1-17.2 g/dl], VEM = 69 fl [83-93fl], CHEM=30.2 g/dl [32.4-36 g/dl], Fe=22 µg/dl [70-180 µg/dl]) and an increased level of blood cholesterol (cholesterol = 180 mg/dl). The assumptions included the celiac disease. We dosed the anti-transglutaminase antibodies (TTG) (48 IU/ml; <4.0 IU/mL (negative), 4.0-10.0 IU/mL (weak positive), >10.0 IU/mL (positive)) and anti-endomysium anitibodies (EMA) (7 IU/ml). Despite these results, it was decided that a gastroscopy with duodenal biopsy is needed. The histopathological examination detected CD stage MARCH 3C (fig.1, fig.2). As a result, CD was diagnosed despite the atypical clinical presentation (constipation). Given the diagnosis of CD, the older brother was investigated even though he was clinically asymptomatic. Laboratory parameters were within normal limits, except TTG (200 IU/ml) and surprisingly, EMA were positive (70 IU/ml). An intestinal biopsy was performed and the histologic examination of the intestinal mucosa showed elements of celiac disease but do not fully corresponded with it (flattened villi less than a third of normal height, deep and hyperplastic crypts, modest inflammatory infiltrate in the corium with lymphocytes and eosinophils, exocytosis at the upper limit of normal) (fig.3, fig.4). The conclusion was a celiac syndrome: possible celiac disease or other food allergy as a differential diagnosis. Based on laboratory results, celiac disease was diagnosed and a gluten free diet was carried out.

Based on the discrepancies between the serological data and histological findings we decided to determine the genetic predisposition by HLA typing for both patients. In the younger brother (MARCH 3C, serology +) HLA DQ2 and DQ8 were negative but HLA DQ 7 was positive. The older brother (celiac syndrome, serology +++) presented negative HLA DQ2, DQ7 and DQ8 (table 1). Two years after CD diagnosis and following a gluten free diet, the elder brother returned in the clinic presenting a polyuro-polydipsic syndrome. Plasma glucose level was increased (230 mg/dl), the A1c hemoglobin value was 10.8%, glycosuria and ketonuria were also present. A type I diabetes mellitus diagnostic was established. We introduced a low carbohydrates diet (250 grams/day) and a basal-bolus insulin therapy with Humulin R and Humulin N (2011: CD diagnostic, 2013: DM diagnostic). The TTG and EMA levels were determined and the result was negative.

Due to the second autoimmune disease appearance, the younger brother has been tested in terms of pancreatic autoimmunity to reveal a potentially clinically asymptomatic diabetes. We dosed islet cell antibodies, anti-glutamate decarboxylase (GAD II), tyrosine phosphatase antibodies (IA2) and anti-insulin antibodies, all showing negative results. To find long-term prognosis in terms of autoimmunity we decided to type HLA DR in both patients. The younger brother had HLA DR B1 alleles type 07 and 11 and in the older brother HLA DR B1 03 and 16 alleles were detected (table 2). In the case of the patient with CD and DM we verified the existence of other autoimmune diseases with subclinical manifestation: thyroid peroxidase antibodies were negative, with TSH (1.04 microIU/ml) and Free T4 (1.00

Table 1. HLA-DQ genotype presented in the two brothers

| Younger Brother (MARCH 3C) | Older brother (Celiac Syndrome) |
|---|---|
| HLA-DQ 2: negative | HLA-DQ 2: negative |
| HLA-DQ 8: negative | HLA-DQ 8: negative |
| HLA-DQ 7: positive | HLA-DQ 7: negative |

Table 2: HLA-DR alleles in the two brothers

| Younger brother (CD) | Older brother (CD+DM) |
|---|---|
| HLA DRB1 07 | HLA DRB1 03 |
| HLA DRB1 11 | HLA DRB1 16 |

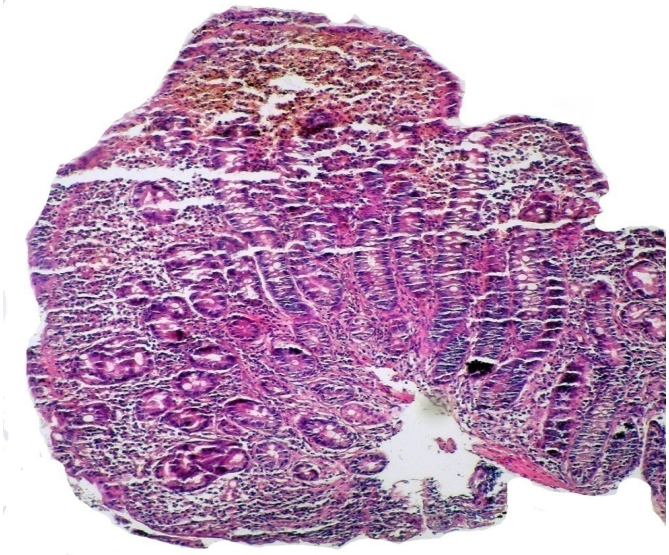


Figure 1. Celiac disease, MARCH 3C stage: totally flattened intestinal villi, deep crypts

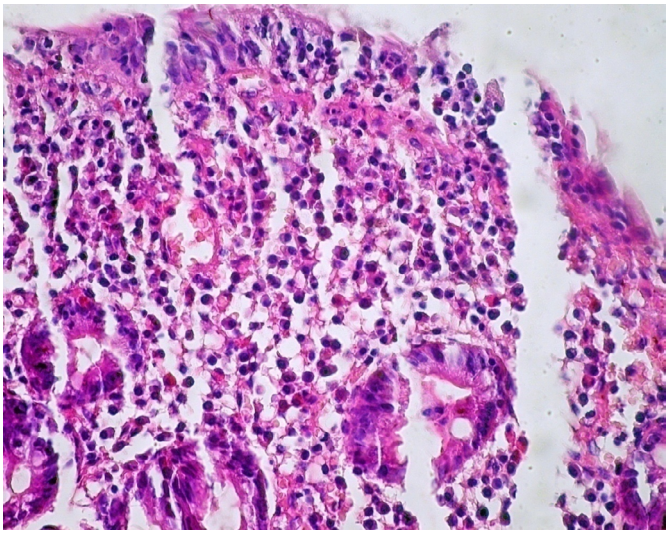


Figure 2. Celiac disease, MARCH 3C stage: intense inflammatory infiltrate in the corium (lymphocytes, representing pathological exocytosis)

Figure 3. "celiac syndrome": flattened villi less than a third of normal height, crypt hyperplasia.

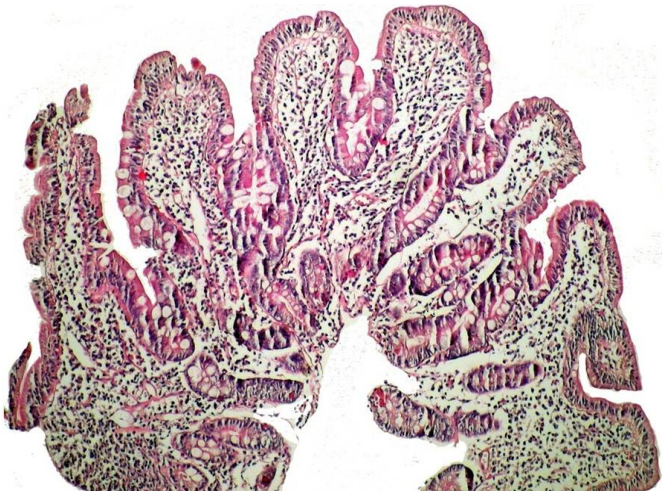
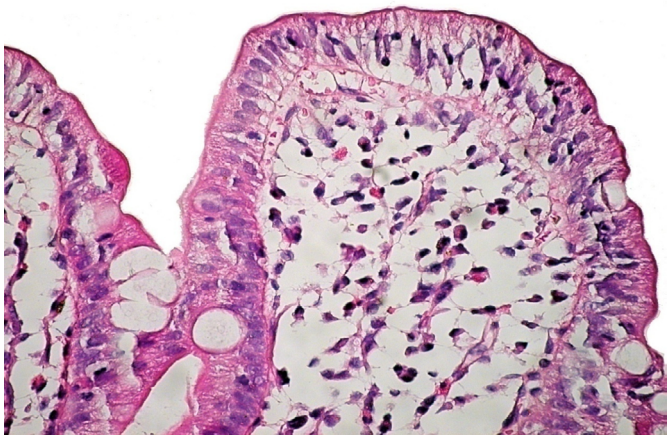


Figure 4. "celiac syndrome": modest inflammatory infiltrate in the corium with lymphocytes and eosinophils, exocytosis at the upper limit of normal



ng/dl) within the limits, so the diagnosis of autoimmune thyroid disease was eliminated. We determined the anti-adrenal antibodies which were also negative.

## Discussion

Class II HLA brings much information regarding autoimmune pathology. The HLA typing is considered by the new ESPGHAN guidelines a diagnostic tool in CD. Given the risks of pediatric intestinal biopsy under general anesthesia, it can be omitted from the CD diagnostic algorithm in patients who have high levels of TTG (10 times the normal value), EMA present in serum and at risk HLA (Hill & Horvath 2012). These alleles are also present in 30-40% of healthy patients so its presence is essential but not decisive. Type II HLA genotypes are responsible for 40-50% of cases of DM (Szaflarska-Popławska 2014). There are predisposing HLA sites in autoimmune diseases such as HLA-DR B1 03 and others that protect against autoimmune diseases (diabetes mellitus, Addison's disease) - HLA-DR B1 07; those were positive in the two presented cases. The risk HLA regarding celiac disease is the HLA DQ2 (in 90% of cases) and HLA DQ8. Patients with negative HLA DQ2/DQ8 are rare, representing about 5% of those diagnosed with CD (Karell et al 2003; Megiorni et al 2008). In the case of these brothers, the big brother had DR B1 03 genotype – giving the predisposition to autoimmunity and explaining why he developed two autoimmune diseases (CD and DM). The younger brother presented DR B1 07 genotype which confers protection against diabetes and Addison's disease which is why he developed CD. What is the role of HLA in CD? In the small intestine, gluten binds on HLA DQ2 and DQ8 on antigen-presenting cells leading to activation of cellular and humoral immunity. Reactive T lymphocytes are stimulated and they synthesize pro-inflammatory cytokines (TNF-α/INF-ɣ) stimulating the production of metalloproteinases. These are arriving at the brush border leading to villous atrophy, then crypt hypertrophy. Stimulated B cells produce autoantibodies that are infiltrating the mucosa (Abadie et al 2011). Studies have shown that intestinal immune system plays an important role in the pathogenesis of type I diabetes. This is sustained by the fact that anti isle T cells express intestinal receptors. In addition, gluten alters the proportion of immune cells and cytokines profile toward an inflammatory pattern (Antvorskov et al 2014).

What is the role of gluten-free diet in diabetes? The answer is important due to an increasing number of patients with CD and DM association, especially in those who have HLA DQ2 and DR3 genotype. Also, in these patients, DM appears first and then the CD (Cronin 1997). The first studies in this direction have started from the premise that in regions that consume grain with a low amount of gliadin (Asia, Polynesia), the incidence of diabetes is much lower (Antvorskov 2014; Scott 1990). In animal models that were fed with gluten-free diet, the incidence of diabetes was considerably reduced. When gluten-free diet was administered to pregnant animals, in their offspring which had no exposure to gluten (nor in utero), diabetes incidence was reduced by 6%. In patients who developed diabetes, however, the onset was delayed. The conclusion was that the withdrawal of gluten from the diet is largely sufficient to prevent diabetes or delay its onset (Hoorfar et al 1993). In the next stage of trials, they tried to demonstrate the hypothesis that an enriched gluten diet increases the risk of diabetes. But the surprise was that the enriched gluten diet prevented as well as the gluten-free diet the appearance of DM. So the conclusion was that dietary factors are not themselves diabetogenic but rather modulate the penetrance in individuals who are genetically predisposed (Funda et al 2008). Following these discrepancies between studies, a new hypothesis was submitted: the gluten enigma in type I DM. Future evaluations led to the conclusion that eliminating gluten from the diet was associated with a reduced incidence of diabetes only in people who have a decreased risk of developing diabetes. In patients with increased risk for diabetes, this assumption is not valid because their immune system is already activated. Persons at increased risk for developing diabetes are those with present autoantibodies, those with first-degree relative with diabetes or older ages. So, any intervention, including the elimination of gluten from the diet, has no protective effect. However, gluten-free diet has some benefit in patients with diabetes such as: decreased nutrient absorption including glucose, leading to lower glucose plasma level (but with increased frequency of hypoglycemic episodes), and islet protection, preserving the insulin secretion. Initially, it was believed that gluten-free diet had no influence on A 1c hemoglobin values (that reflects

the metabolic control), but this theory was denied by the recent studies (Abid N et al 2011; Goh et al 2010). In our case, even with a gluten-free diet, the older brother developed diabetes.

Is there a pathophysiological mechanism that influences the occurrence of DM by gluten? Gluten induces changes of the immune system (cell populations and cytokine/chemokine) towards an inflammatory profile. In addition, gluten causes increased intestinal permeability, thus facilitating antigen contact with the intestinal immune system. In the pathogenesis of diabetes, the intestinal immune system plays an important role; diabetogenic T lymphocytes from the intestine are activated by GALT (gut associated lymphoid tissue) and T lymphocytes that infiltrate the islet express intestinal receptors (Bilabo et al 2003). The gluten-free diet is recommended only for patients who associate DM and CD. Regarding DM, the only advantage of this diet is the delaying onset in some patients (low risk) (Mackinder et al 2014).

One question that arises from this case is why two brothers having a similar genotype and the same environment have such different phenotype? The evolution is dictated by genotype and environmental triggers only determine the organ to be affected (celiac disease, diabetes, Addison's disease, autoimmune thyroiditis, etc.) (Norsa et al 2012). Alleles responsible for this predisposition are situated on the short arm of chromosome 6, in DRB1, DQ A1 and DQA2 region. DR B1 03 haplotype is the one that confers increased risk of developing autoimmune diseases, which is currently present in the big brother genotype. DR B1 07 haplotype provides protection against diabetes and Addison's disease. This haplotype was present in the younger brother who developed only CD, with positive DQ7 (Riddell et al 2009).

Regarding big brother's future, a series of questions are arising. His nutritional status and growth development will be affected by the two autoimmune diseases? Studies developed in this direction have shown no changes in somatic development for children who associate DM and CD (Lukács et al 2015).

The research concerning gluten diet and type 1 diabetes has proven to be very interesting. Even if a strict gluten-free diet is followed, the onset of diabetes is dictated by the inherited genotype. Is remarkable the different genotype between the two brothers, and the different predisposition to autoimmune diseases. Further researches are needed in this direction, to clarify the potential role of the environmental factors in the development of diabetes and celiac diseases and also to pinpoint the follow-up in the autoimmune associations.

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