

# Evaluation of HLA-DQ2/DQ8 genotype in patients with celiac disease hospitalised in 2012 at the Department of Paediatrics

Dorota A. Szałowska-Woźniak<sup>1</sup>, Leokadia Bąk-Romaniszyn<sup>2,3</sup>, Agnieszka Cywińska-Bernas<sup>1</sup>, Krzysztof Zeman<sup>1</sup>

<sup>1</sup>Department of Pediatrics, Preventive Cardiology and Clinical Immunology, Medical University of Lodz, Poland

<sup>2</sup>Department of Nutrition in Gastrointestinal Disease, Medical University of Lodz, Poland

<sup>3</sup>Department of Pediatrics, Immunology and Nephrology, Polish Mothers Memorial Hospital Research Institute, Lodz, Poland

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**Address for correspondence:** Dorota A. Szałowska-Woźniak MD, PhD, Medical University of Lodz, 4 Kościuszki St, 90-419 Lodz, Poland, phone:+48 606 28 28 06, e-mail: d.szalowska@interia.pl

## Abstract

**Introduction:** Celiac disease (CD) is a permanent intolerance to gluten that occurs in genetically predisposed individuals and leads to small intestinal mucosa damage. According to ESPGHAN guidelines from 2012, CD can be diagnosed in a patient with characteristic clinical symptoms, in whom, anti-tissue transglutaminase antibodies (> 10 times the upper limit) are found, endomysial antibodies (EMA) is confirmed and a positive genetic test is obtained. In these conditions no small-bowel biopsies are required.

**Aim:** Evaluation of the presence of HLA-DQ2 and HLA-DQ8 haplotypes in children with previously diagnosed CD, hospitalised in 2012 at the Department of Paediatrics and Immunology and/or the Gastroenterological Outpatient Clinic, and their relatives.

**Material and methods:** Blood samples of 22 subjects, including 9 children with CD diagnosed on the basis of clinical symptoms, serological investigations and small-intestine biopsy, 7 diagnosed on the basis of clinical symptoms and serological investigations, 2 with the suspicion of CD on the basis of clinical symptoms and 4 relatives of a child with CD. Methods: HLA-DQ2/DQ8 test, automatic evaluation by EUROArrayScan.

**Results:** The presence of HLA-DQ2 and/or HLA-DQ8 genotype was confirmed in 16 children with CD diagnosed on the basis of clinical symptoms and serological tests with/without intestinal biopsy, in 2 with the suspicion of CD and in 1 relative of a celiac child.

**Conclusions:** The evaluation of HLA-DQ2/DQ8 haplotype confirms the genetic predisposition to CD in subjects with the disease diagnosed previously on the basis of clinical symptoms, serological tests or intestinal biopsy. Genetic testing is particularly indicated for the diagnosis of CD in infants consuming gluten for a short time and in small amounts.

## Introduction

Celiac disease (CD) is a genetically conditioned abnormal response to gluten, which in the course of autoimmune processes leads to small intestinal mucosa damage. Based on screening for CD performed in Europe and in the USA, its prevalence varies from 1 : 100 to 1 : 300 of the population, and for its symptomatic form – 1 : 3345 individuals [1]. However, despite the availability of serological and genetic testing and intestinal biopsy, the ratio of individuals with diagnosed CD and non-diagnosed cases is 1 : 7, which is associated, among others, with different levels of physicians' knowledge about this disease [1]. The prevalence of CD in children

and adolescents aged 2.5 to 15 years is 3–13/1000 of tested children, in Europe from 1 : 80 to 1 : 300 [2–4]. The results of the epidemiological investigations carried out in Poland in the years 2007–2009 indicated similar to the above for the incidence of celiac disease. It has been estimated that in Poland the number of children and adolescents up to 19 years of age suffering from CD was from 25,000 to as many as 75,000 individuals, and probably a significant amount remain undiagnosed [5]. Gluten is a fraction of proteins present in grains of wheat (gliadin), rye (secalin) and barley (hordein). Exposure to gluten, in genetically predisposed individuals, leads to the development of immune reactions asso-

ciated with the activation of TCD4, CD8 lymphocytes, production of pro-inflammatory cytokines, excessive proliferation of intraepithelial lymphocytes, migration of macrophages to the intestinal wall and finally to intestinal mucosa damage (flattening of villi to total villous atrophy, crypt hypertrophy and the increase of intraepithelial lymphocytes) [6]. The severity of changes developing in small-bowel mucosa is classified by Marsh scale (1992) and modified by Oberhuber *et al.* [7–13]. In the course of celiac disease, besides the activation of T lymphocytes, there is induction of  $\beta$  lymphocytes, which produces specific antibodies – the markers for the disease. They include: tissue transglutaminase 2 (tTG2), endomysial antibodies (EMA) and the deamidated form of gliadin peptides (DPG). The determination of their concentration is routinely performed in IgA class, but in patients with serum immunoglobulin A deficiency (IgA < 0.2 g/l) in IgG class [1, 14, 15]. HLA haplotype testing is of great value in the diagnosis of CD, and it also allows determination of the risk for this disease occurrence. In the ESPGHAN (European Society for Paediatric Gastroenterology) guidelines from 2012, genetic testing has been included in the diagnostic management of celiac disease. It has been acknowledged that in the case of clinical symptoms of CD occurring in a patient together with the presence of anti-tissue transglutaminase antibodies (> 10 times the upper limit of normal), the presence of anti-endomysial antibodies and the presence of class II HLA-DQ2 and/or DQ8 haplotype, CD can be diagnosed without the necessity of small-bowel biopsy [16]. HLA-DQ2 is present in 90–95% of CD patients, whereas HLA-DQ8 is present in about 5–15% [17]. HLA-DQ2 and/or DQ8 allele is not found in only 1% of celiac patients. The presence of HLA-DQ2 and/or DQ8 is indispensable but insufficient for the development of the disease; HLA-DQ2 genotype is also observed in 25–30% and HLA-DQ8 in 5–10% of the general population [1]. Additionally, other genes not associated with MCH class II family (non-HLA genes), e.g. CTLA4 gene polymorphism (gene located on chromosome 2q33), PTP N22 (protein tyrosine-phosphatase nonreceptor 22), are responsible for susceptibility to CD [18–20]. HLA-DQ2 and HLA-DQ8 genes are located on chromosome 6p21. HLA-DQ2 consists of HLA-DQA1\*05 (subunit  $\alpha$ ) allele and HLA-DQB1\*02 (subunit  $\beta$ ) allele, and HLA-DQ8 genotype consists of HLA-DQA1\*0301 (subunit  $\alpha$ ) allele and HLA-DQB1\*0302 (subunit  $\beta$ ) allele [21].

## Aim

The aim of the study was to evaluate the presence of HLA-DQ2 and HLA-DQ8 haplotypes, predisposing to CD development in children with previously diagnosed celiac disease, hospitalised in 2012 at the Department

of Paediatrics and Immunology and/or the patients of the Gastroenterological Outpatient Clinic and their relatives.

## Material and methods

The study included 22 subjects (13 women, 9 men): 20 children (aged from 11 months to 17.3 years) and 2 adults (father – aged 46.2 years and mother – aged 44 years, of a boy with CD), the mean age was in the whole group was 8.3 years. The investigated group comprised 9 children with CD diagnosed on the basis of characteristic clinical symptoms, serological investigations and positive result of small-bowel biopsy, 7 children with the disease diagnosed on the basis of clinical symptoms, serological investigations, without small-bowel biopsy, 2 children (11 and 14 months old) with suspected CD on the basis of clinical symptoms, and 4 first-degree relatives of a celiac child (mother, father, brother and sister) (Table I). In the group of 18 children 12 (67%) had coexisting diseases: 6 were treated due to allergy, 2 due to Dühring's disease, 2 with bronchial asthma, 1 with diagnosed Crohn's disease and hypothyroidism, 1 congenital optic nerve anomaly and reactive arthritis, 1 nephrotic syndrome and 1 girl was diagnosed with autoimmune hepatitis.

Whole venous blood (2–3 ml) was used. The “EURO-ArrayScan” test was used for HLA-DQ2/DQ8 determination. The tests were performed in a laboratory experienced in performing such determinations. All patients and their parents were informed about the purpose of the determination and gave their written consent to perform their and their child's genetic testing.

## Results

In 19 (86.3%) of 22 individuals HLA-DQ2 or HLA-DQ8 was detected (12 in females and 7 in males). Genetic predisposition to CD was confirmed in all 16 patients with CD diagnosed on the basis of clinical symptoms and positive results of serological investigations with or without small-bowel biopsy, in 2 patients with suspected disease on the basis of clinical symptoms (11- and 14-month-old children receiving gluten for a short time) and in 1 adult (mother of celiac boy), i.e. the presence of HLA-DQ2 and/or HLA-DQ8 genotype (Figures 1, 2). Among individuals with positive haplotype associated with celiac disease, 13 (68%) had HLA-DQ2, 4 (21%) had HLA-DQ8, and 2 (11%) had HLA-DQ2 and HLA-DQ8 (Figure 3). In 3 individuals, first-degree relatives (father, aged 46 years, brother aged 12 years and 7 months, sister aged 19 months) of a boy with CD, no HLA-DQ2/DQ8 alleles were found. The boy's brother and sister remained on a gluten-free diet due to suspicion of CD despite negative results of serological in-

**Table I.** Characteristics of the investigated group

Children							
Patient's no:	Gender	Age	Clinical symptoms of celiac disease	Biopsy	Biopsy result	Coexisting diseases	Serology results
1	M	10 years 8 months	Yes	No	–	Congenital optic nerve anomaly, reactive arthritis	tTGA positive, AGA negative
2	F	2 years	Yes	No	–		tTGA positive, EMA positive, AGA positive
3	F	8 years 1 month	Yes	Yes	III c	Inhaled allergy	EMA positive
4	M	4 years 4 months	Yes	No	–	Food and inhaled allergy, bronchial asthma, atopic dermatitis	tTGA positive, EMA positive
5	M	16 years 3 months	Yes	Yes	IIIa	Crohn disease, hypothyroidism	tTGA negative, AGA positive
6	M	11 years 3 months	Yes	Yes	IIIb	Bronchial asthma, polyvalent allergy	tTGA positive, EMA positive
7	M	12 years 8 months	Yes	Yes	IIIc	Nephrotic syndrome, polyvalent allergy	tTGA positive, EMA positive
8	F	12 years 1 month	Yes	Yes	IIIc	IgA nephropathy	tTGA positive, EMA positive
9	M	3 years 11 months	Yes	No	–		tTGA positive, AGA positive
10	M	1 year 2 months	Yes	No	–		tTGA negative, AGA negative
11	F	15 years 4 months	Yes	Yes	IIIc		tTGA positive, AGA positive
12	F	12 years 3 months	Yes	Yes	IIIb	Duhring's disease	No before-diet results
13	F	5 years 1 month	Yes	Yes	IIIc		tTGA positive, EMA positive
14	F	11 months	Yes	No	–		tTGA negative
15	F	1 year 2 months	Yes	No	–	Duhring's disease	AGA positive
16	F	12 years	Yes	Yes	IIIc		tTGA positive
17	F	17 years 3 months	Yes	No	–	Autoimmune hepatitis	tTGA positive, EMA positive
18	F	6 years 5 months	Yes	No	–		tTGA positive
<b>Celiac child first-degree relatives</b>							
<b>Siblings</b>							
19	F	1 year 9 months	No	No	–	Food allergy	tTGA negative, EMA negative
20	M	12 years 7 months	Yes	No	–	Food allergy	tTGA negative, AGA negative
<b>Parents</b>							
21	M	46 years 2 months	Yes	No	–		On gluten-free diet: tTGA negative, AGA negative
22	F	44 years	No	No	–		tTGA negative

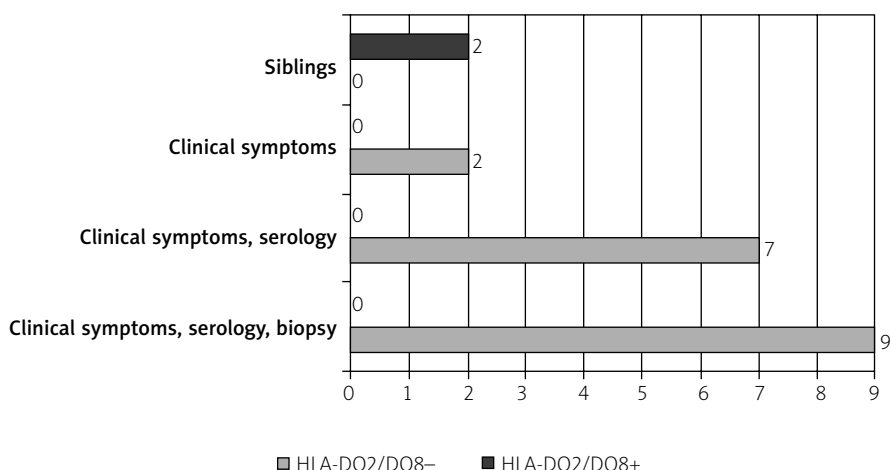


Figure 1. Evaluation of HLA-DQ2/DQ8 genotype in the examined children (n = 20)

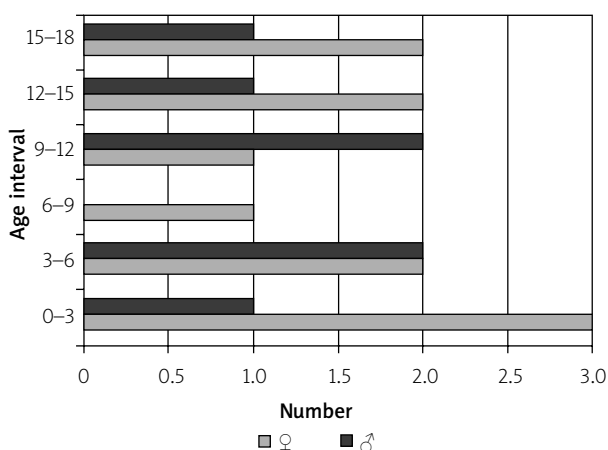


Figure 2. Gender distribution in the population of subjects up to 18 years old of the life with positive results of testing (n = 18)

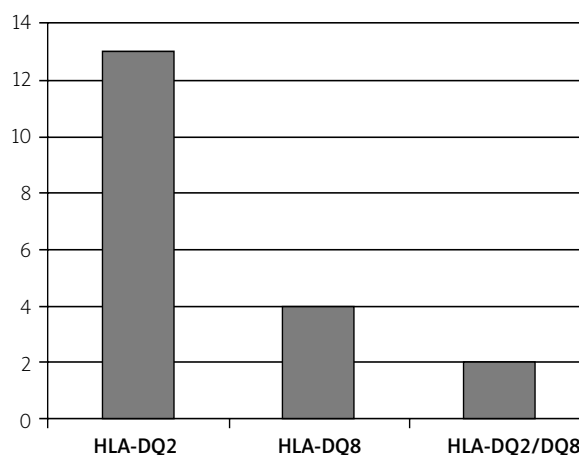


Figure 3. Distribution of HLA-DQ2/DQ8 haplotypes of subjects with positive result of genetic testing (n = 19)

vestigations but with traits of food allergy and irregular bowel movements. The father of the family eliminated gluten from his diet due to recurrent abdominal pain. In the mother, treated as healthy subject, after a positive result of genetic testing for celiac disease, a silent form of the disease was diagnosed.

### Discussion

The genetic susceptibility to CD was confirmed in the tests of all children with intestinal mucosa atrophy (Marsh III), in serologically positive children, and even in two infants only with clinical symptoms of the disease. In the Polish project of genetic testing of celiac disease, performed in the years 2007–2009, including 922 subjects: 594 with diagnosed celiac disease, 131 with suspicion for CD and 141 from celiac families, 79% had a positive result of SNP genotyping (genetic varia-

tions of single nucleotide polymorphism) for DQ2 and/or DQ8. Positive results of genotyping were demonstrated in 97% of patients with diagnosed CD and in 47% of individuals suspected for CD [22]. In the study of Kipatany *et al.* in 2006 from the University in Debreczyn, similarly as in our study, all 40 investigated subjects, in whom the presence of anti-tissue transglutaminase antibodies and EMA was confirmed, had HLA-DQ2 or DQ8 allele [10]. The evaluation of the genotype for the presence of HLA-DQ2/DQ8, as we became convinced in our study, is a test allowing early recognition of CD in children who receive gluten for a short time and/or in a small amount. In the group investigated by us, a positive result of HLA-DQ2/DQ8 was obtained in a 14-month-old boy and positive HLA-DQ8 in an 18-month-old girl with insufficient weight gain and growth despite a negative result of serum tissue trans-

glutaminase. In ESPGHAN guidelines low diagnostic reliability has been emphasized for serological investigations for CD in children < 2 years of age remaining on a gluten-free diet or consuming gluten for a short time and in a small amount, with accompanying diseases, in the evaluation of familial predisposition to the disease and in subjects treated with immunosuppressive agents [16]. It has been emphasized that a negative result of HLA-DQ2/DQ8 test excludes CD in 99% and a positive result points to the disease or to a predisposition to it [1, 16]. The performance of genetic testing is indicated to recognise or exclude CD in patients with ambiguous or contradictory results of serological and histopathological investigations and also in individuals remaining on a gluten-free diet (when the results of histopathological and serological tests can be false negative). The evaluation of HLA-DQ2/DQ8 is also indicated in the so-called risk groups, i.e. first-degree relatives of celiac patients, patients with type I diabetes mellitus and pathologies coexisting with CD (Down's syndrome, Turner's syndrome, Duhring's disease and others). Confirmation of a predisposition to celiac disease by genetic testing is, for the patients, an additional confirmation of the validity of the application of a restrictive gluten-free diet. In our studies the negative result of genetic testing in 3 subjects allowed the exclusion of CD and enabled the decision to be made for gradual introduction of gluten into the diet, which allows the development of sensitivity in an allergy. In the group investigated by us, in 67% of children with celiac disease, chronic diseases were present including those of immunological background. This is in compliance with the observations of other authors; CD often coexists with other diseases, most frequently with type I diabetes, autoimmune thyroiditis, IgA nephropathy, Down's syndrome, Turner's syndrome, Williams syndrome, isolated IgA deficiency and nonspecific intestinal diseases [23].

## Conclusions

The evaluation of HLA-DQ2/DQ8 haplotype confirms the genetic predisposition to CD in individuals with previously recognised disease on the basis of clinical symptoms, serological investigations or intestinal biopsy. A positive result of the genetic test confirming a predisposition to CD in a small child receiving gluten for a short time (or with clinical symptoms of the disease) is an indication for further medical supervision and periodic serological testing.

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