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Prevalence estimation of celiac disease in the general adult population of Latvia using serology and HLA genotyping

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Abstract

Background: Prevalence estimates for celiac disease (CD) depend on the method used. The role of deamidated gliadin peptide (DGP) and genetic testing in epidemiological studies and diagnostic settings of celiac disease (CD) has still to be established.

Objectives: The objective of this article is to assess the prevalence of CD in Latvia by combining serological tests with DQ2.5/DQ8 testing.

Methods: A total of 1444 adults from a randomly selected cross-sectional general population sample were tested by ELISA for tTG IgA, DGP IgA and IgG antibodies (QUANTA Lite®, Inova Diagnostics Inc). Samples with tTG IgA ≥20U were tested for EMA IgA by indirect immunofluorescence assay, and all specimens with tTG IgA ≥15U were tested by QUANTA-Flash® chemiluminescent assays (CIA) (Inova Diagnostics Inc) for tTG IgA, DGP IgA and IgG. DQ2.5/8 was detected in individuals with any positive ELISA test and a subgroup of controls.

Results: Forty-three individuals (2.98%; 95% CI: 2.10–3.86%) tested positive by at least one ELISA test; 41.86% of the serology-positive individuals (any test above the cutoff) were DQ positive. Six individuals (0.42%; 95% CI: 0.09–0.75%) were triple ELISA positive, and DQ2.5 or DQ8 was positive in all; 0.35% (95% CI: 0.05–0.65%) were tTG IgA and EMA positive. Two tTG IgA-negative cases were both DGP IgG and IgA positive, both being DQ positive; including them in the "serology-positive" group would increase the prevalence to 0.49% (95% CI: 0.13–0.85%).

CIA tests revealed 2 tTG IgA-positive and EMA-negative cases with a positive genotype. DQ2.5 or DQ8 genotype was positive in 28.6% of the serology-negative population.

Conclusions: Estimates of the prevalence of CD in Latvia based on the serogenetic testing approach range from 0.35% to 0.49% depending on the criteria used. There is a rationale for combining serological tests and DQ2.5/8 genotyping.

Keywords

Celiac disease, prevalence, Eastern Europe, tissue transglutaminase, deamidated gliadin peptide, chemiluminescent assays, DQ2.5/8 genotype

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Introduction

The prevalence of celiac disease in Europe and North America is generally considered approximately 0.7%–1.5%, although five- to eight-fold inter-country variations have been observed among Northern, Western and Southern European countries.[1–3] Countries with the highest prevalence include Finland,[2,4–6] Sweden[7,8]

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(both countries located near Latvia), and Great Britain.[2,9]

The prevalence of celiac disease has grown in parallel with autoimmune and allergic disease; e.g. simultaneous growth of celiac disease and diabetes mellitus type 1 has been well demonstrated.[4] The epidemiology of non-infectious diseases varies considerably among different areas in Europe. Our previous data obtained from the International Study for Asthma and Allergies in Childhood (ISAAC) demonstrated significantly lower prevalence of asthma, allergic rhinoconjunctivitis and atopic eczema symptoms in Latvia (and other countries in Eastern Europe) compared to Nordic countries.[10] It is possible similar differences may also be seen in the prevalence of celiac disease. Kondrashova et al. compared two populations sharing a similar genotypic background but living under different socioeconomic and hygienic conditions in the bordering areas of Finland and Russian Karelia.[11] They concluded celiac disease was significantly more prevalent in areas with better socioeconomic conditions and hygiene.

Currently the standard initial evaluation of patients with suspected celiac disease includes testing for immunoglobulin (Ig)A antibodies to tissue transglutaminase (tTG IgA).[12–14] Recently the use of deamidated gliadin peptide (DGP) tests has emerged.[15,16] DGP (in particular, the DGP IgG test) may identify celiac disease patients by negative tTG IgA and anti-endomysial IgA (EMA) tests,[17] especially IgA-deficient individuals.

Almost all individuals with celiac disease are positive for human leukocyte antigen (HLA)-DQ2.5 or DQ8 genes. While absence of these genes makes the presence of celiac disease extremely unlikely in most populations and thus a strong negative predictor of disease, these genes are present in a considerable proportion of the general population and are only of value to indicate the unlikely presence of celiac disease in those without this genetic background. In Western Europe, the allele frequency of DQ2 ranges from 5% to 20%, while that of DQ8 ranges from 5% to 10%.[1]

Algorithms guiding the routine clinical use of celiac disease-related serological and genetic assays are still evolving. Recent guidelines of the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN)[18] allow consideration of omitting duodenal biopsies for diagnosing celiac disease in children based on transglutaminase, EMA and DQ2/DQ8 test results on particular occasions. Duodenal biopsy is generally required for diagnosing the disease in adults although several studies have suggested adequate accuracy for serology alone along the lines of the ESPGAN guidelines, taking into account cutoff levels for positivity may also be useful for adult patients.[19–22] It should be noted that the prevalence estimates of celiac disease are substantially influenced by the method used in the assessment, i.e. whether seroprevalence only is considered, or biopsy confirmation of the disease is required.[23]

Currently, the clinical value of adding the HLA DQ2/DQ8 test to serology in diagnosing celiac disease in adults is not well defined.[24] The combination of serology with HLA DQ testing has been suggested by a few authors to help guide selection of patients for endoscopy and small-bowel biopsy.[25,26] However, Hadithi et al.[24] have found that the combination of serological and HLA DQ testing is as accurate as either of these strategies alone, and have concluded that for diagnostic purposes these non-invasive strategies cannot substitute for small-bowel biopsy. Recently, a serogenetic approach was proposed by Anderson et al.[27] for determining the community prevalence of celiac disease.

Limited information is available on the prevalence of celiac disease in Eastern Europe. The objective of our study was to assess the prevalence of celiac disease in Latvia based on serology testing alone or in combination with genetic testing. We did not pre-define the criteria for seropositivity since several serological as well HLA DQ2/DQ8 testing methods were used. We employed conventional tTG IgA and DGP enzyme-linked immunosorbent assays (ELISAs) as well as new chemiluminescent-based versions of these immunoassays (CIA). Finally we tested all specimens with positive results by any assay (tTG IgA, DGP IgG, or DGP IgA) for the presence of *HLA DQ2.5/DQ8* genotypes to further clarify the serology results obtained on the study population. Using this methodology, we aimed to investigate the differences in the prevalence results of celiac disease under circumstances when different non-invasive diagnostic approaches are employed.

Methods

Study group selection

The study was performed as a sub-analysis of a larger randomly selected cross-sectional sample of an adult general population aged 24–74, the methodology of which has been described elsewhere.[28] With the primary objective of exploring cardiovascular risk factors, a total of 6000 invitees equally spilt between age groups and genders were randomly selected from the National Latvian population registry covering the entire country in 2008–2009; of these, 3807 accepted the invitation and participated in the study. In exceptional cases, adult members of the family or partners were also included, but not actively invited; a minor proportion of them were outside the invitation group age range. A subgroup of these individuals for whom serum samples

were available was included in our study. Serum samples received from the clinical laboratory following routine clinical testing were stored at −70°C until additional testing was conducted. DNA samples were provided by the Latvia Genome Data Base group.

All specimens with positive results for any assay were referred for genetic testing. In addition, at least six matched control cases to every serology-positive individual from the serology-negative group were genotyped to evaluate the DQ positivity in the serology-negative group.

Serology

All samples were blinded and tested by ELISA for tTG IgA (QUANTA Lite® h-tTG IgA ELISA), DGP IgA (QUANTA Lite® Gliadin IgA II ELISA) and DGP IgG (QUANTA Lite® Gliadin IgG II ELISA). All kits were manufactured by Inova Diagnostics Inc, USA, and performed according to the manufacturer's instructions.

ELISA test results were classified as negative (<20 units), weak positive (20–30 units), and moderate/strong positive (>30 units) according the manufacturer's recommendations.

All specimens positive by the tTG IgA ELISA assay (≥20 units) were tested by indirect immunofluorescence (IFA) for the presence of anti-endomysial IgA antibodies (EMA) when sufficient serum was available. Primate distal esophagus tissue substrate (Nova Lite® Endomysial test system, Inova Diagnostics) was used in the IFA method; detection of EMA at a dilution of 1:5 was interpreted as positive for EMA.

To further assess the performance of the ELISA assays, additional testing was performed on all specimens for which the tTG IgA ELISA test result was above 15 units (five units below the assay's cutoff) using the QUANTA-Flash® tTG IgA, DGP IgA, and DGP IgG CIA assays (all assays United States Food and Drug Administration (FDA) cleared) using the BIO-FLASH chemiluminescent instrument platform (Biokit s.a., Barcelona, Spain).

Genotyping

Direct sequencing of the second exon of the *HLA-DQB1* gene and the second and third exons of the *DQA1* gene was performed. DNA samples were dissolved in water and aliquoted into 96-well polymerase chain reaction (PCR) plates or PCR tubes by Tecan Freedom Evo robotic pipette. The final DNA amount was 28 ng/well. The second exon of *HLA-DQB1* and second and third exons of *DQA1* genes were amplified and sequenced using the BigDye chemistry and ABI Prism 3100 (AME Bioscience, Toroed, Norway) capillary electrophoresis sequencer. All chromatograms were manually inspected and analyzed using the CLC Bio Main Workbench package (CLC Bio Inc). Presence of polymorphisms was confirmed by opposite strand analysis. Allele calling was performed using the IMGT/HLA database[29,30] sequence alignment tool and confirmed by manual alignment of allele sequences from the HLA allele sequence database[31] and sequences obtained by sequencing. DQ 2.5- (*HLA-DQA1**05-DQB1*02 in *cis* or *trans*) and/or DQ8- (*HLA-DQA1**03-DQB1*03:02) positive cases were considered the high-risk genotypes for celiac disease.

Statistics

Descriptive statistics were used to characterize the study group and the proportion of the positive test results according to the predefined criteria. Mean values, range, standard deviation (SD) and 95% confidence intervals (CI) of means were used to describe the distribution of the measurement results.

Ethical considerations

The project was conducted in accordance with the Helsinki Declaration; the study protocol was approved by the Ethics Committee of the Institute of Cardiology, University of Latvia; and genetic testing has been approved by the Central Medical Committee of Ethics in Latvia.

Results

A total of 1444 serum samples were available for the study. The cohort consisted of 982 (68%) women and 462 men; median age was 57. Approximately 39% (558) of the samples were collected in Riga, the capital of Latvia, with the remaining coming from other regions of the country. The age distribution of study participants was as follows: age <40 (217 cases, 15.0%); age 40–54 (438 individuals, 30.3%); age 55–69 (542 individuals, 37.6%); >70 years (70 individuals, 17.1%). Altogether 64 individuals were outside the primary invitation group; seven were younger than 25 and 57 older than 74.

ELISA serology results

Conventional assay testing results are summarized in Table 1, while those obtained with CIA assays are shown in Table 2. Results were combined into several possible profiles and the estimated prevalence calculated based on either serological test results only or by combining serological test interpretation with the presence of DQ2.5 or DQ8 genotype. For the ELISA

Table 1. The prevalence of celiac test positivity obtained with different ELISA assays with and without considering the DQ typing results

No.	Tests considered to set a positive result	Cutoff	No of positive cases	Proportion of total study population in % (95% CI)	No of *DQ2.5*- or *DQ8*-positive cases	Proportion of *DQ2.5*- or *DQ8*-positive cases out of serology positives by this criteria in %	Proportion of serology positive by this criteria and *DQ2.5*- or *DQ8*-positive cases of total study population in % (95% CI)
1	Any diagnostic test positive (tTG IgA, DGP IgA, DGP IgG); EMA not considered	>20 U	43	2.98 (2.10–3.86)	18	41.86	1.25 (0.68–1.82)
2	Any diagnostic test positive (tTG IgA, DGP IgA, DGP IgG); EMA not considered	>30 U	20	1.39 (0.79–1.99)	10	50.00	0.69 (0.26–1.12)
3	Any diagnostic test positive (tTG IgA, DGP IgA, DGP IgG) and EMA positive	>20 U	5	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)
4	Any diagnostic test positive (tTG IgA, DGP IgA, DGP IgG) and EMA positive	>30 U	5	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)
5	Two diagnostic tests (tTG IgA, DGP IgA, DGP IgG) positive; EMA not considered	>20 U	8	0.55 (0.17–0.93)	7	87.50	0.49 (0.13–0.85)
6	Two diagnostic tests (tTG IgA, DGP IgA, DGP IgG) positive; EMA not considered	>30 U	6	0.42 (0.09–0.75)	6	100.00	0.42 (0.09–0.75)
7	All three diagnostic tests (tTG IgA, DGP IgA, DGP IgG) positive; EMA not considered	>20 U	6	0.42 (0.09–0.75)	6	100.00	0.42 (0.09–0.75)
8	All three diagnostic tests (tTG IgA, DGP IgA, DGP IgG) positive; EMA not considered	>30 U	4	0.28 (0.01–0.55)	4	100.00	0.28 (0.01–0.55)
9	DGP IgA and DGP IgG positive (irrespective of other tests)	>20 U	7	0.49 (0.13–0.85)	7	100.00	0.49 (0.13–0.85)
10	DGP IgA and DGP IgG positive (irrespective of other tests)	>30 U	5	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)
11	DGP IgA and DGP IgG positive; tTG IgA negative (irrespective of other tests)	>20 U	1	0.07 (0–0.21)	1	100.00	0.07 (0–0.21)

(continued)

Table 1. Continued

No.	Tests considered to set a positive result	Cutoff	No of positive cases	Proportion of total study population in % (95% CI)	No of *DQ2.5*- or *DQ8*-positive cases	Proportion of *DQ2.5*- or *DQ8*-positive cases out of serology positives by this criteria in %	Proportion of serology positive by this criteria and *DQ2.5*- or *DQ8*-positive cases of total study population in % (95% CI)
12	DGP IgA and DGP IgG positive; tTG IgA negative (irrespective of other tests)	>30 U	1	0.07 (0–0.21)	1	100.00	0.07 (0–0.21)
13	tTG IgA positive (irrespective of other tests)	>20 U	24	1.66 (1.00–2.32)	10	41.67	0.69 (0.26–1.12)
14	tTG IgA positive (irrespective of other tests)	>30 U	11	0.76 (0.31–1.21)	7	63.64	0.49 (0.13–0.85)
15	EMA positive (irrespective of other tests)	≥1:5	5	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)
16	tTG IgA and EMA positive (irrespective of other tests)	>20 U/≥1:5	5	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)

ELISA: enzyme-linked immunosorbent assay; tTG IgA: human tissue transglutaminase immunoglobulin A; DGP IgA: deamidated gliadin peptide immunoglobulin A; DGP IgG: deamidated gliadin peptide immunoglobulin G; EMA: anti-endomysial immunoglobulin A antibodies; CI: confidence interval.

assays, profiles were evaluated using both the manufacturer's specified cutoff of ≥20 units, as well as a modified cutoff of >30 units. For the CIA versions of the assays, the manufacturer's cutoff of ≥20 units was used.

The tTG IgA was ≥20 units in 24 cases (1.66%), ≥30 units in 11 cases (0.76%), ≥40 units in seven cases (0.48%), and ≥50 units in five cases (0.35%). The DGP IgA result was ≥20 units in 17 cases (1.18%), ≥30 units in eight cases (0.55%), 40 units in five cases (0.35%), and 50 units in four cases (0.28%). The DGP IgG result was ≥20 units in 16 cases (1.11%); it exceeded 30 units in 11 cases (0.76%), 40 units in seven cases (0.48%), and 50 units in four cases (0.28%).

Six individuals (0.42%) were triple positive at ≥20 U for tTG IgA, DGP IgG, and DGP IgA, and four of these (0.28% of the entire group) had all three markers >30 U. Among the triple-positive group, the mean tTG IgA value was 180.7 (range 21.64–269.19; SD 95.67; 95% CI of the mean 80.20–281.10), the mean DGP IgA was 108.77 (range 21.75–205.97; SD 66.33; 95% CI 39.16; 178.38), and the mean DGP IgG was 49.02 (range 40.06–58.01; SD 6.98; 95% CI 41.69; 56.34). All four cases exceeding the 30-unit level for the ELISA tests were EMA positive. Of the remaining two individuals in whom at least one of the test results ranged between 20 and 30 units, one was EMA positive and one was EMA negative.

Eight participants (0.55%) were positive by at least two markers. Forty-three specimens (2.98%) were positive by at least one marker with magnitude over 20 units, while 20 cases (1.39%) were positive by at least one marker with magnitude over 30 units.

CIA serology results

Serology results obtained using CIA were available for 82 cases selected as described in the Methods section. The selection of the cases allowed us to compare the result to the group of positive ELISA cases, but also to the entire study population. Thirteen specimens (0.9% of the total study group) were positive by one or more of the CIA assays. Using more stringent criteria of any two tests positive found seven (0.48%) individuals positive. Five cases (0.35%) were triple positive. The difference between the double and triple positive is a result of two tTG IgA-negative specimens being detected by the DGP IgG and IgA assays. All triple-positive specimens were EMA positive. The two specimens that were only DGP IgG and IgA dual positive were EMA negative as expected, since they were tTG negative.

Table 2. The prevalence of celiac test positivity obtained with different chemiluminescent-based immunoassays (CIA) with and without considering the DQ typing results

No.	Tests considered to set a positive result	Cutoff	No of CIA positive cases	Proportion of positives in the CIA tested group (%)	Proportion of positives from the entire study group in % (95% CI)	No of *DQ2.5* or *DQ8* positives	Proportion of *DQ2.5* or *DQ8* positives from CIA-positive cases (%)	Proportion of CIA positives and DQ2.5 or DQ8 positives from the entire study group in % (95% CI)
1	Any diagnostic test positive (tTG IgA, DGP IgA, DGP IgG); EMA not considered	>20 U	13	15.85	0.90 (0.41–1.39)	9	69.23	0.62 (0.22–1.02)
2	Any diagnostic test positive (tTG IgA, DGP IgA, DGP IgG) and EMA positive	>30 U	5	6.10	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)
3	Two diagnostic tests (tTG IgA, DGP IgA, DGP IgG) positive; EMA not considered	>20 U	7	8.54	0.49 (0.13–0.85)	7	100.00	0.49 (0.13–0.85)
4	All three diagnostic tests (tTG IgA, DGP IgA, DGP IgG) positive; EMA not considered	>20 U	5	6,10	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65
5	DGP IgA and DGP IgG positive (irrespective of other tests)	>20 U	7	8.54	0.49 (0.13–0.85)	7	100.00	0.49 (0.13–0.85)
6	DGP IgA and DGP IgG positive; tTG IgA negative (irrespective of other tests)	>30 U	2	2.44	0.14 (0–0.33)	2	100.00	0.14 (0–0.33)
7	tTG IgA positive (irrespective of other tests)	>20 U	5	6.10	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)
8	EMA positive (irrespective of other tests)	≥1:5	5	6.10	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)
9	tTG IgA and EMA positive (irrespective of other tests)	>20 U/≥1:5	5	6.10	0.35 (0.05–0.65)	5	100.00	0.35 (0.05–0.65)

ELISA: enzyme-linked immunosorbent assay; tTG IgA: human tissue transglutaminase immunoglobulin A; DGP IgA: deamidated gliadin peptide immunoglobulin A; DGP IgG: deamidated gliadin peptide immunoglobulin G; EMA: anti-endomysial immunoglobulin A antibodies; CI: confidence interval.

HLA DQ testing results

All 43 individuals positive for any of the serological tests along with 280 individuals negative for all serological tests were available for the analysis. In the serology-negative group the prevalence of a positive DQ2.5 genotype was 18.93%, the prevalence of a DQ8 genotype was 10.36%, but the combination of a positive DQ2.5 or DQ8 genotype was 28.57%. DQ2.2 was positive in 10.71%, of whom all were DQ2.5 and DQ8 negative.

In the group of 43 patients in whom at least one serological test was positive, 37.21% were DQ2.5 positive, 6.98% were DQ8 positive; 41.86% were positive for either DQ2.5 or DQ8. A total of 46.5% of the DQ2.5/DQ8-negative cases in this subgroup were DQ2.2 positive.

The proportion of the risk genotype (DQ2.5 or DQ8 genotype) in the subgroups of positive serological tests is presented in Tables 1 and 2.

Discussion

Although celiac disease is considered common, the general estimates of 1%–1.5% are based on studies conducted mainly in Northern and Western Europe and could be an overestimate for other regions of Europe.[23] Even in Western Europe, estimates of the prevalence of the disease vary, e.g. in Germany the prevalence in the adult population is estimated to be approximately 0.3%.[2,32]

The present study is the first on celiac disease epidemiology in Latvia, one of the three Baltic countries in Northeastern Europe where considerable political and socioeconomic changes have taken place over the last century. A previous study conducted in schoolchildren from Estonia, another Baltic country, revealed a 0.34% prevalence of celiac disease.[33,34] In Polish children the prevalence of confirmed celiac disease is 0.25%, but the prevalence of positive serology is 0.8%.[35]

Prevalence figures in epidemiological studies are substantially influenced by the criteria set for diagnosing celiac disease.[2] This was clearly demonstrated in our study. If the standard cutoff value ($\geq$20 U) was used, a positive result was obtained in 2.98% of the population, suggesting a high seroprevalence of celiac disease. However, when more stringent criteria were applied, the results suggested a lower disease prevalence. Using a triple-positive criterion (tTG IgA, DGP IgA, and DGP IgG), positivity ranged between 0.28% and 0.42% depending on the cutoff value used. Adding a positive EMA test as a requirement for positivity decreased the prevalence to 0.35%. It should be mentioned that our prevalence estimates have been based on non-invasive testing only; therefore, the prevalence of biopsy-confirmed celiac disease in this population could be even lower. Furthermore, since the proportion of women in our study group was higher than men and considering the fact that celiac disease is more prevalent in women,[36] this could lead to an additional overestimation of the prevalence in the general population of the country.

Our study confirmed the usefulness of adding genetic testing to the conventional serology for establishing the prevalence figures of the disease as recently suggested by Anderson et al.[27] When the interpretative criteria for celiac disease positivity was a positive result of any ELISA test (cutoff $\geq$20 U), the DQ2.5/DQ8 positivity was only 41.9%, suggesting that a proportion of the serology positives were unlikely to be true celiacs. By design, screening algorithms should have high sensitivity to detect all individuals with the specific disease, in this case, celiac disease. Presumed false-positive results (usually low positives) are expected in screening, and follow-up testing is used to increase specificity of the testing. As criteria are made more stringent, the proportion of positive DQ test results increased; e.g. when test positivity by two ELISAs is required with a cutoff of 20 U, the DQ2.5/8 test was positive in 87.5% cases, but when the cutoff limit was set to >30 U, the DQ2.5/8 test was positive in 100% of the cases. Similarly when all three serology tests were positive, DQ2.5/8 was positive in 100%. Correspondingly, for the CIA assays, when any of the tTG IgA, DGP IgG, or DGP IgA tests were positive, 69.2% were DQ2.5 or DQ8 positive. When any two tests or three tests were positive, 100% were DQ positive.

Based on the assumption that virtually all celiacs must have *HLA DQ2.5* or *DQ8* genes, the combined results of ELISA, CIA, EMA, and DQ2.5/8 testing suggest at a minimum the prevalence of celiac disease in Latvia is approximately 0.35% (95% CI: 0.05–0.65%). This estimate is based on specimens being triple positive (tTG IgA, DGP IgA, DGP IgG), EMA positive and possessing *HLA DQ2.5* or *DQ8*. Inclusion of the two individuals who were DGP IgG and IgA dual positive and *DQ2.5* or *DQ8* positive would increase the prevalence to 0.49% (95% CI: 0.13–0.85%). We would speculate that similar prevalence could also be present in neighboring countries, e.g. other Baltic States or East European countries with similar socioeconomic backgrounds.

The accepted standard for the serological screening for celiac disease is detection of IgA antibodies to tTG. There are many tTG assays available and overall most show greater than 93%–95% sensitivity and specificity.[37] At least 0.21% of the Caucasian population has selective IgA deficiency and more than 2% of the population presents with subnormal IgA levels.[38] Individuals with IgA deficiency have a higher likelihood of celiac disease than the general population,[1] but the presence of this condition could lead to misdiagnosis if only IgA-based tests are applied.[7] Guidelines commonly recommend simultaneous detection of total serum IgA for ruling out IgA deficiency;[14] however, the use of IgG-based tests is an alternative.[39]

EMA, performed and interpreted by experienced individuals, remains an extremely specific test for celiac disease, but its sensitivity is less than tTG IgA[37] and clinically proven patients with the disease can be missed if this is the only test employed. Moreover, many laboratories in Europe do not have the EMA test available; e.g. EMA detection is currently not provided by any laboratory in Latvia. Therefore, improved serological or serogenetic approaches are of special importance to select individuals requiring further diagnostic workup, including duodenal biopsies, and also to

avoid unnecessary investigations. A combination of two or more assays, especially the addition of DGP IgA and DGP IgG to tTG tests, was recently suggested by Schyum and Rumessen.[37] Our study confirms the rationale for such an approach and suggests the addition of the HLA risk-genotype can assist in clarifying results.

In the present study we used tTG and DGP assays in the conventional ELISA format as well as a new chemiluminescent format. Chemiluminescence allows an assay with a high signal-to-noise ratio and wide dynamic range.

The strongest evidence for the presence of celiac disease is the case where all tests are positive. Using ELISA, six individuals (0.42%, six of 1444) were found to be positive for tTG IgA, DGP IgG, and DGP IgA. All six were also DQ2.5 or DQ8 positive; five of these were EMA positive. CIA testing found five of the six also positive for all three assays, but found one specimen tTG IgA negative (this specimen was very low positive on the ELISA tTG IgA). The CIA assay picked up two additional specimens that were tTG IgA negative, but DGP IgG and IgA positive (and DQ2.5/DQ8 positive). The ELISA assay also picked up one of these two additional specimens.

The CIA and ELISA tests, in combination with the *HLA DQ 2.5/DQ8* results, detected all five EMA-positive cases. For specimens with double or triple positivity by either CIA or ELISA and the presence of *HLA DQ2.5/DQ8* genotype, the EMA test did not add additional diagnostic value.

Our study has several limitations. By following the protocol of the study, EMA tests and CIA tests were not run on the entire study population partly because of limited serum volume. However, since all specimens without EMA testing (except for one) were negative for all celiac serology, it is exceedingly unlikely EMA testing would have found additional positive specimens. A major limitation of the current study is the unavailability of duodenal biopsy results, therefore making definitive diagnosis of celiac disease impossible. Follow-up of laboratory-positive individuals with endoscopic evaluation is being pursued, but is practically difficult to accomplish.

In conclusion, our study suggests the prevalence of celiac disease in Latvian adults is lower than in many neighboring Nordic countries. Furthermore, our results suggest the rationale for combining several serological tests, in particular the new CIA versions of the tTG and DGP assays, may offer higher performance than conventional ELISAs and that the addition of *HLA DQ2.5/DQ8* genotyping can help clarify serology results and avoid unnecessary endoscopies.

Conflict of interest

Gary L. Norman, Zakera Shums, and Jay Milo are employees of Inova Diagnostics. All other authors have nothing to declare.

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