**Prevalence of celiac disease and celiac autoimmunity in the Toba native Amerindian community of Argentina**

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BACKGROUND: Celiac disease (CD) is mostly recognized among subjects with a Caucasian ethnic ancestry. No studies have explored conditions predisposing Amerindians to CD.

OBJECTIVE: To prospectively assess environmental, genetic and serological conditions associated with CD among members of the Toba native population attending a multidisciplinary sanitary mission.

METHODS: An expert nutritionist determined daily gluten intake using an established questionnaire. Genetic typing showed that 73 of 144 (50.7%) subjects had alleles associated with CD; 69 (49.4%) of these subjects had alleles for HLA DQ alleles and four had DQ2 (5.5%). Four and six subjects had antibody concentrations above the cut-off established by the laboratory for IgA endomysial antibodies.

RESULTS: A total of 144 subjects (55% female) were screened. The estimated mean gluten consumption was 43 g/day (range 3 g/day to 185 g/day). Genetic typing showed that 73 of 144 (50.7%) subjects had alleles associated with CD; 69 (49.4%) of these subjects had alleles for HLA DQ double haplotype. Serum antibodies were immunoglobulin (Ig) A tissue transglutaminase (tTG) and the composite deamidated gliadin peptides/tTG Screen test. Positive cases were tested for IgA endomysial antibodies.

CONCLUSION: The present study was the first to detect CD in Amerindians. The native Toba ethnic population has very high daily gluten consumption and a predisposing genetic background. We detected subjects with persistent CD autoimmunity and, at least, three of them fulfilled serological criteria for CD diagnosis.

Key Words: Amerindians; Celiac autoimmunity; Celiac disease; Genetic predisposition; Gluten consumption; Tissue transglutaminase (tTG)

Celiac disease (CD) is a common autoimmune enteropathy, induced by dietary gluten in genetically predisposed individuals (1). The disease has been traditionally recognized among Caucasians; the estimated prevalence in the Western world is approximately 1% (2). CD is increasingly recognized in the Asian-Pacific region (3). In Latin America, there is a variable proportion of the population with European ancestry, with native communities having a diverse degree of mix with European colonizers (4).

The native Toba ethnic population comprises >60,000 individuals living with a cluster distribution in a forest named ‘The Impenetrable’ in Northeastern Argentina. This community experiences highly precarious social, economic, sanitary and educational conditions (5). In recent years, they have undergone a drastic change in dietary habits, with wheat replacing their ancestral food sources (6). This has mainly occurred as a consequence of governmental food aid programs aimed at improving nutritional conditions in the community. CD can only occur in individuals with certain class II human leukocyte antigen (HLA) molecules – namely, HLA DQ2 and/or DQ8. In this context, scarce information exists about the prevalence of HLA DQ2 and DQ8, and of CD in native South Americans (7).
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**TABLE 1**

Demographics, body mass index (BMI) and gluten consumption of the overall population and according to age (children versus adults)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall population</th>
<th>Children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>144</td>
<td>40</td>
<td>104</td>
</tr>
<tr>
<td>Age, years, median (range)</td>
<td>30 (3–79)</td>
<td>8 (3–17)</td>
<td>36 (19–79)</td>
</tr>
<tr>
<td>Sex, female/male, n/n</td>
<td>80/64</td>
<td>20/20</td>
<td>60/44</td>
</tr>
<tr>
<td>Body weight, kg, mean ± SEM</td>
<td>63.5±1.9</td>
<td>38.1±3.1</td>
<td>73.3±1.5</td>
</tr>
<tr>
<td>BMI, kg/m², mean ± SEM</td>
<td>26.1±0.5</td>
<td>28.5±0.5</td>
<td>19±0.8</td>
</tr>
<tr>
<td>Underweight, n (%)</td>
<td>9 (6)</td>
<td>5 (13)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Normal weight, n (%)</td>
<td>44 (31)</td>
<td>16 (37)</td>
<td>28 (27)</td>
</tr>
<tr>
<td>Overweight, n (%)</td>
<td>91 (63)</td>
<td>19 (50)</td>
<td>72 (69)</td>
</tr>
<tr>
<td>Gluten consumption, g/day, mean ± SEM</td>
<td>43.1 (2.7)</td>
<td>27.5 (4.9)</td>
<td>48.9 (3.1)</td>
</tr>
</tbody>
</table>

*Overweight for children was considered to be weight above the 97% percentile.*

Accordingly, we explored CD risk factors in members of the Toba community attending a multidisciplinary sanitary mission. Our specific aims were to estimate the consumption of gluten, determine the associated celiac genetic risk (i.e., HLA DQ2/DQ8), and screen the population for CD.

**METHODS**

Definitions and overall study design

The present prospective population-based study has two major end points: diagnosis of CD autoimmune and serological diagnosis of CD. CD autoimmunity was defined as two positive serological tests (≥3 times the upper limit of normal) in samples taken four months apart. Serological CD diagnosis was established in patients with positive ELISA serology and positive immunoglobulin (IgA) endomysial antibodies (EmA). The study was approved by the Ethics and Scientific Boards of the C. Bonorino Udaondo Gastroenterology Hospital of Buenos Aires, Argentina. Written or expressed consent was obtained from enrolled subjects in the presence of the community leaders and/or their parents.

Study participants

From March 2013 through March 2014, four multidisciplinary missions by the same team were performed in the Toba population living in ‘The Impenetrable’, in the province of Chaco, Argentina. All subjects attending the health mission and fulfilling inclusion criteria were invited to participate in the study by a physician in the presence of the community leader. Individuals <3 years of age or having a previous diagnosis of CD were excluded, as well as those refusing blood sampling for cultural reasons. A full clinical history, physical examination and blood samples were obtained.

Nutritional analysis and gluten consumption

A nutritionist-expert in CD interviewed subjects at enrollment. Gluten consumption was estimated using a food frequency intake questionnaire (11) and compared with data obtained from a 48 h food recall recorded by the same nutritionist. The food frequency intake estimates the frequency of consumed foods during a specific period of time and identifies all foods containing wheat, rye and barley; the gluten content was calculated following previously described methods, taking into account only known gluten-containing products (11-14).

CD-specific serology

Serum samples obtained were kept frozen (−20°C) until analysis at Hospital de Gastroenterología C. Bonorino Udaondo. Serology tests included IgA antibodies to tissue transglutaminase (tTG) using ELISA (Quanta Lite h-tTG IgA, Inova Diagnostic Inc, USA), and the dual detection of IgA and IgG isotypes of antibodies to deamidated gliadin peptide (DGP) and tTG in a single assay (DGP/tTG Screen; Quanta Lite h-tTG/DGP Screen, Inova Diagnostic Inc). The cut-off value specified by the manufacturer for the ELISA assays were 20 U/mL but the cut-off used in the present study was based on that estimated by the authors in an earlier study (≥3 times the upper limit of normal [60 U/mL for both tests]) (13). Positive ELISA tests were analyzed for IgA endomysial antibodies (IgA EmA) using immunofluorescence on primate esophagus substrate (dilution 1:5). Positive samples were re-tested after four months during a new visit to the mission to rule out transient positivity.

CD-related HLA genotyping

CD specific gene typing for the detection of HLA class II alleles was performed on DNA from a drop of whole blood obtained from the fingertip and collected on a paper filter card (Whatman 903 FTA, Whatman, USA). Samples were stored at room temperature and analyzed at the BioDiagene laboratory (Italy). A DQ-CD Typing Plus kit (BioDiagene, Italy) was used for the identification of HLA class II alleles: DQA1*0201,*03,*05, DQB1*02,*0301/04 and *0302.

Statistical analysis

Population data are reported as percentage, median and range or mean ± SEM, as appropriate. The Mann-Whitney test for independent samples was used for comparison of patients with and without CD autoimmunity.

**RESULTS**

Study population

A total of 144 subjects (55% female) were enrolled. Patients’ characteristics are summarized in Table 1. Briefly, mean body mass index was above the range of normal weight and most subjects had normal weight or were overweight. Mean biochemical parameters were within normal range (data not shown). No IgA-deficient cases were detected. Genetic typing showed that 73 of 144 (50.7%) subjects had alleles associated with CD; 69 of these subjects (94.5%) had alleles for HLA DQ8 and 4 had DQ2 (5.5%).

Gluten consumption

The estimated mean gluten consumption for all enrolled subjects was almost double of that assessed for populations living in urban areas of Argentina (unpublished data) and in other parts of the world (16) (Table 1).

Identification of subjects with CD and persistent celiac autoimmunity

Two adults (both female, 23 and 38 years of age) and one child (male, 12 years of age) fulfilled serological criteria for CD (Table 2). One additional child (female) was excluded from enrollment due to previous CD diagnosis. From the newly CD diagnosed patients, one adult female had normal body mass index, the other was overweight and the child was obese. The patients were asymptomatic and biochemical parameters were normal (Table 3). High gluten consumption was detected in two of the three (114 g/day and 29 g/day for the adults, and 91 g/day for the child). The child was HLA DQ2+, and both adults were positive for HLA DQ8 (Table 2).

Three additional subjects (two HLA-DQ8+) had serum concentrations of CD antibodies above normal. In three, DGP/tTG was positive while both DGP/tTG and IgA tTG were positive in two. Repeat serology four months apart was positive in all three and, thus, they were considered to have persistent CD autoimmunity. Daily gluten intake was normal in two of these subjects, and high in one (Table 2).

**DISCUSSION**

We prospectively explored the consumption of gluten by the apparently healthy native Toba community, the frequency of HLA CD-predisposing genotypes, and the prevalence of CD autoimmunity and serological evidence of the disorder. The present study revealed a very high consumption of gluten, almost twofold higher than that of individuals of European ancestry living in urban areas of Argentina.
TABLE 2
Individual estimation of gluten consumption, genetic human leukocyte antigen (HLA) typing and serological status in patients considered to have gluten autoimmunity* or celiac disease†

<table>
<thead>
<tr>
<th>Subject</th>
<th>age consumption, g/day</th>
<th>HLA typing</th>
<th>IgA ITG, U/ml</th>
<th>DGP/ITG Screen test, U/ml</th>
<th>IgA EmA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 33, male</td>
<td>136</td>
<td>DR4-DQ8/ DQ7</td>
<td>12/9</td>
<td>80/101</td>
<td>–ve/+ve</td>
</tr>
<tr>
<td>2: 72, male</td>
<td>26</td>
<td>DQ7</td>
<td>37/32</td>
<td>80/101</td>
<td>–ve/+ve</td>
</tr>
<tr>
<td>3: 38, male</td>
<td>22</td>
<td>DQ8</td>
<td>120/63</td>
<td>109/77</td>
<td>–ve/+ve</td>
</tr>
<tr>
<td>4: 12, male</td>
<td>91</td>
<td>DQ7-DQ8/ DQ7</td>
<td>&gt;200/200</td>
<td>&gt;200/200</td>
<td>+ve/ND</td>
</tr>
<tr>
<td>5: 38, female</td>
<td>114</td>
<td>DQ4-DQ8/ DQ7</td>
<td>131/ND</td>
<td>100/ND</td>
<td>+ve/ND</td>
</tr>
<tr>
<td>6: 23, female</td>
<td>29</td>
<td>DQ8</td>
<td>&gt;200/200</td>
<td>60/ND</td>
<td>+ve/ND</td>
</tr>
</tbody>
</table>

*Patients 1, 2 and 3; †Patients 4, 5 and 6. Serum concentration of ELISA tests (immunoglobulin A [IgA], tissue transglutaminase [ITG] and deamidated gliadin peptides [DGP]/ITG Screen test were re-tested in patients four months after the first positive result. Data above are presented as test 1/test 2 results. EmA: Endomysial antibody; ND: Not determined

and in other parts of the world (16). This alimentary change was the product of a political decision implemented almost 30 years ago to improve alarming nutritional deficits in this population. The initial aim of the alimentary support appears to have achieved relative success because anthropometric evaluations revealed few underweight subjects and normal biochemical parameters. An unintentional consequence of such important alimentary intervention was that 50% and 70% of children and adults, respectively, were overweight or obese at the time of the study. The quality of food support, consisting mainly of wheat, may have played a role in this outcome.

Similar to native populations in Brazil and Chile (17), our study showed that only 3% of subjects had the HLA DQ2alleles (prevalent in European populations), while 48% had the DQ8. This contrasts with the increased HLA-DQ2 allele frequency detected in Asia with high CD prevalence, as demonstrated in several recent studies (17-20).

A significant observation in the present study was the detection of CD autoimmunity in six subjects. Three of these subjects had comonitant positivity in all tests and, therefore, qualified for CD diagnosis. The patients were asymptomatic, did not exhibit weight loss and gluten consumption was very high in two of the cases. The results raise concerns about CD risk in the Toba population and its association with the quality of food aid programs targeted at them.

The present small study was based on serological prevalence due, in part, to some cultural resistance to the implementation of the screening program and to the difficulty of implementing endoscopy assessment in this setting. However, according to our experience and those of others, a positive IgA EmA test is almost absolutely predictive of CD enteropathy.

CONCLUSION
The Toba population has environmental and genetic risk factors for the development of CD. The present study detected a very high consumption of gluten as the result of government food aid programs largely based on wheat products. The genetic background was dominated by alleles coding for DQ8 antigen. Three patients in the present study fulfilled criteria for CD and three others for persistent CD. One additional CD case had previously been identified in this community. These results raise important questions regarding the food quality in aid programs that should include a variety of non-gluten-containing grains and should prompt CD screening in these native populations.

TABLE 3
Demographic and some clinical characteristics of the three patients with serological diagnosis of celiac disease

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>12</td>
<td>38</td>
<td>23</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Clinical categorization*</td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.7</td>
<td>26.6</td>
<td>23.8</td>
</tr>
<tr>
<td>Gluten consumption, g/day</td>
<td>91</td>
<td>114</td>
<td>29</td>
</tr>
</tbody>
</table>

*Clinical categorization was performed according to the Oslo nomenclature


ACKNOWLEDGEMENTS: The authors thank Dr Sonia Kupfer (University of Chicago, Chicago, Illinois) for scientific discussions and critical review regarding the genetic determinations and interpretations of the current study. They also thank the Fundación Pequeños Gestos Grandes Logros, all members of the multidisciplinary sanitary missions, Inova Diagnostic Inc (USA) for generously providing kits for serology, and Biodiagnic Laboratory (Italy) for generously performing CD genetic typing analyses free of charge.

FUNDING SUPPORT: The present study was partially funded by the Consejo de Investigación, MSAL, Buenos Aires City Government and the Asociación para el Estudio de Enfermedades del Intestino (AEDEI). Source for salary support of researchers is provided by the Buenos Aires City Government as part of their research duties. EFV holds a Canada Research Chair and is funded by CIHR MOP 123282.

REFERENCES