

Celiac Disease in a Chilean Population Carrying Amerindian Traits

Magdalena Araya, Alexandra Mondragón, *Francisco Pérez-Bravo, †Jose Luis Roessler, ‡Teresa Alarcón, §Gloria Ríos, and §Carmen Bergenfreid

Human Nutrition/Clinical Nutrition Department and *Molecular Biology Laboratory, Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile; †Hospital Felix Bulnes, Santiago, Chile; ‡Hospital San Juan de Dios, Santiago, Chile; §Hospital E Gonzalez Cortés, Santiago, Chile

ABSTRACT

Background: Although clinical manifestations of celiac disease may change throughout life, clinical, histologic, immunologic, and genetic studies show that there are incomplete forms of this condition, making it difficult to define the disease at a given moment. Because there is no information published in the Latin American-Amerindian population, this study was conducted to assess relations between these parameters in Chileans with celiac disease and their first-degree relatives.

Methods: Sixty-two persons with confirmed celiac disease (mean age, 17.9 ± 5.1 years; 78.3% females) and 126 relatives (mean age, 27.9 ± 17.2 years; 65.1% females) were evaluated. Clinical manifestations, antiendomysial antibodies (EMAs), and human leukocyte antigen (HLA) haplotypes were studied in patients. Additionally, jejunal biopsy specimens were assessed (light microscopy) in EMA-positive (EMA+) relatives.

Results: Of the patients, 24.1% adhered to a strict gluten-free diet; 26% were oligosymptomatic, and none were malnour-

ished; 45% were EMA+; 13.8% who ingested gluten were EMA-negative (EMA-); one patient consuming a strict gluten-free diet was EMA+. The DQA1*0501 allele was present in the highest frequency (48%, $P < 0.0005$), whereas combinations of DQ8 were predominant. Of the relatives, 4.8% were EMA+; they had a significantly higher frequency of diarrhea, weight loss, and anorexia ($P < 0.03$); and all had abnormal histology in biopsy specimens.

Conclusions: After childhood, celiac disease is oligosymptomatic and is often unrecognized by patients. Disease in 13.8% of patients and in 4.8% relatives appeared as incomplete forms of celiac disease. Predominance of DQ8 HLA haplotypes reflects the genetic Spanish-Mapuche heritage of this population. *JPGN* 31:381–386, 2000. **Key Words:** Adolescents—Antiendomysial antibodies—Celiac disease—Histology—HLA haplotypes—Relatives—Screening. © 2000 Lippincott Williams & Wilkins, Inc.

Celiac disease (CD) is associated with genetic characteristics and gluten exposure. In recent studies in which genetic markers were used, small intestinal histologic changes, serum immune reactivity and clinical features show that there are subjects with some but not all of its characteristics, posing a difficult question of how to define the disease (1–4). The terms latent, silent, and potential CD have been used to refer to these various manifestations, but it is not yet clear to what extent these patients have increased risk of malignancy later in life (5). Adolescents and young adult patients are of special

interest—particularly, those in whom the natural history of the disease causes symptoms and signs to become less intense and frequent after childhood.

A literature search disclosed no published information about these aspects for the Latin American population, with genetic Amerindian influence. In 1994, a prospective 2-year survey of live births and new cases of CD diagnosed in the seven pediatric public hospitals of Santiago, Chile, showed an incidence of 1:1846 live births (unpublished data, 1994). Human leukocyte antigen (HLA) haplotypes associated with CD have been described in the European population (6,7); however, main HLA haplotypes and the influence of the Amerindian genetic traits are unknown in Hispanic America. This study was performed in patients with confirmed CD to assess the relationship between clinical manifestations, adherence to a gluten-free diet, presence of circulating antiendomysial antibodies, and major-risk HLA haplotypes associated with CD in Chilean patients. Among their relatives we searched for incomplete forms of the disease by using EMA as a screening procedure.

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Address correspondence and reprint requests to Dr. Magdalena Araya, Casilla 138-11, Santiago, Chile.

MATERIALS AND METHODS

Patients

The 95 patients with CD who were more than 12 years of age and registered at hospitals Exequiel Gonzalez, Felix Bulnes, and San Juan de Dios, Santiago, Chile, were requested by phone and/or mail to attend an interview at the hospital. The objective of the study was explained, and the patients and their relatives were invited to participate. In all patients, diagnosis of CD had been made years before, fulfilling the criteria of three positive biopsies (and gluten challenge) originally established by the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (2). Sixty-two of the 95 patients attended the interview, and all agreed to participate. The remaining 33 were not found at the address registered at the hospital. Their clinical records and socioeconomic and demographic characteristics were similar to those who participated. One hundred thirty-six of 219 relatives identified attended the interviews, agreed to participate, and provided informed written consent. Clinical history and socioeconomic and demographic characteristics in the relatives who did not participate were similar to those who agreed to participate. We report the data obtained in the 126 first-degree relatives who were identified in the group.

Clinical Data

Because both patients and relatives considered themselves healthy in response to "open" questions, a questionnaire asking for specific symptoms was used. In both groups this included anorexia, asthenia, weight loss, diarrhea, dyspepsia, abdominal pain, meteorism, anemia, edema, sterility-hypomenorrhea, abortion-premature delivery, depression or psychiatric conditions, diabetes (non-insulin-dependent or insulin-dependent diabetes mellitus), and cancer. Patients' clinical information at the time of diagnosis was obtained from their hospital charts. In relatives, focused questions about symptoms and signs were answered as "I once had it for a period in my life," "I have it now," or "I have had it and have it now." Because there were no data about the incidence of these symptoms in the Chilean population, results were expressed as a comparison between EMA-positive (EMA+) and EMA-negative (EMA-) individuals. Weight and height were measured in patients and relatives, and weight-for-height ratio, or body mass index (BMI), was calculated according to World Health Organization (WHO) criteria for subjects grouped by age as less than or more than 15 years of age. Adherence to diet was evaluated by questionnaire in five categories: "I (never, seldom, sometimes, often, always) eat gluten-containing foods."

Endomysial Antibodies

In 58 patients, a blood sample was obtained, and EMAs were measured in 2 mL of blood without anticoagulant. Immunoglobulin (Ig)A antiendomysial antibodies were detected by indirect immunofluorescence, with commercially available cryostat sections of monkey esophagus serving as the antigen (ImmunoGlo; IMCO Diagnostics, Buffalo, NY, U.S.A.), according to the technique described by Chorzelski et al. (8)

Intestinal Biopsies and Histology

All patients knew that they had confirmed CD, and none agreed to have another intestinal biopsy. In all of them, however, characteristic histologic changes were observed at some stage of their clinical course. In EMA+ relatives, a jejunal specimen was obtained with a double-port Crosby capsule, fixed in Bouin solution, and embedded in paraffin. After staining with hematoxylin-eosin, sections were assessed by light microscopy using the classic 1 to 4 scale (1 = normal, 4 = flat mucosa) (9).

Haplotypes

These studies were performed in 62 patients and 123 relatives who agreed to provide a blood sample. Genomic DNA was extracted from peripheral blood lymphocytes using standard techniques (10,11) and was subjected to polymerase chain reaction with specific primers according to standard protocols. For oligonucleotide dot blot analysis, a combination of DQA1 and DQB1 sequence-specific oligonucleotide (SSO) probes for the determination of known alleles in both homozygous and heterozygous individuals was used. For each locus, alleles studied were 0101, 0201, 0301, 0401, and 0501 in DQA1* and 0201, 0301, 0302, 0303, 0402, 0502, 0601, and 0602 in DQB1*. Evaluation of these alleles was decided on the basis of data reported from Europe, which has led to the concept of "susceptibility" (DQA1*0301, DQA1*0501, DQB1*0201, and DQB1*0302) and "protective" alleles (DQA1*0101, DQA1*0201, and DQB1*0301) (12). The current WHO nomenclature (Committee for Factors of the HLA System) was used for each allele (13).

Alleles and genotype frequencies were computed as sample proportions. The comparison of such frequencies in patients with CD and relatives was statistically assessed by the χ^2 test corrected by multiple comparison (14). Odds ratio (OR) and exact 95% confidence intervals (CIs) were computed from data previously published (15). The reference groups for OR calculations were *0101/*0101 in the DQA1 gene and *0501/*0501 in the DQB1 gene (14,15). In the patient group, comparison of alleles and HLA haplotypes was performed against a previously studied Chilean group (16) and in relatives, against the respective patient and data reported in the literature (16,17). A detailed description and discussion of HLA haplotypes in the studied groups appears elsewhere (18).

Statistical Analysis

Analysis of results included calculation of means, standard deviation (SD), medians, and comparisons by χ^2 by computer (Excel 6.0; Microsoft, Santa Rosa, CA, U.S.A.).

ETHICAL CONSIDERATIONS

This investigation was approved by the Committees on Ethics in Human Research of each participating hospital and of the Institute of Nutrition and Food Technology (INTA), University of Chile.

RESULTS

Patients

Mean age ± SD was 17.9 ± 5.1 years, and 78.3% of patients were females. Of the 58 patients who provided all requested information, only 14 (24.1%) were consuming a strict gluten-free diet. All considered themselves healthy; however, a focused questionnaire revealed that 26% had at least some symptoms of mild to moderate intensity and low frequency but consistent over time. No relation was observed between presence of symptoms and adherence to diet. Frequency distributions of symptoms and signs at the time of diagnosis and on present evaluation are shown in Figure 1. No patient was malnourished, but short stature was detected in 21%, whereas 20% were overweight or obese.

Endomysial antibodies were detected in 27 (45%) of 58 patients, with no difference shown by gender. Although significantly more patients who did not adhere to a gluten-free diet were EMA+ (*P* = 0.0086), 8 (13.8%) of 58 patients who reported that they had maintained a complete gluten-containing diet for several years were EMA-, and 1 (3.7%) of the 14 who said they had maintained a strict diet was EMA+.

Among HLA-DQA1* alleles 0501 exhibited the highest frequency in patients with CD, whereas 0302 was the most frequent allele among the DQB1 locus (Table 1). Combinations HLA-DQA1-DQA1, HLA-DQB1-DQB1, and the homozygotic HLA DQB1-DQA1 haplotypes appear in Table 2. Heterozygotic HLA haplotypes were widespread and are shown elsewhere (18). Seven of the eight patients who ingested gluten and were EMA- were typed for HLA. In four, the haplotype consisted of one allele described as a “susceptibility” combination plus one allele considered “protective” (three patients had DQA1-0501-DQA1-0501/DQB1-0302-DQB1-0303, and one had DQA1-0101-DQA1-0501/DQB1-0302-DQB1-0602). In the remaining three patients, the HLA haplo-

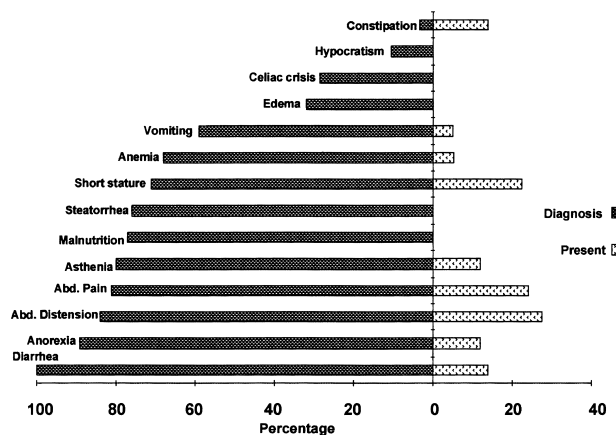


FIG. 1. Symptoms and signs in confirmed celiac patients, at the time of diagnosis and on present evaluation.

TABLE 1. Distribution of main DQA1 y DQB1 alleles in celiac patients and their relatives

Allele	Celiac patients (n = 62)		Relatives (n = 123)	
	n	Frequency	n	Frequency
DQA1				
0101 ^a	20	0.160	64	0.260
0301 ^b	41	0.330	92	0.374
0501 ^c	59	0.480	71	0.289
DQB1				
0201 ^d	31	0.250	64	0.260
0301 ^e	13	0.100	29	0.118
0302 ^f	53	0.430	79	0.321
0501 ^g	14	0.110	42	0.172

Comparison between celiac patients and pediatric Chilean population (17):

- ^a Corrected *P* = 0.01.
- ^b Corrected *P* = NS.
- ^c Corrected *P* = 0.0005
- ^d Corrected *P* = 0.037.
- ^e Corrected *P* = 0.007.
- ^f Corrected *P* = 0.002.
- ^g Corrected *P* = NS.

types coincided with those described as “susceptibility” genotypes. In the patient who apparently maintained a strict gluten-free diet but was EMA+, the genetic study showed a “risk” combination (DQA1-0501-DQA1-0501/DQB1-0302-DQB1-0302).

Relatives

Of the 126 first-degree relatives, 69 were parents, 48 were siblings, and 9 were sons or daughters (65.1% females; mean age ± SD, 27.9 ± 17.2 years; range, 1–75; median, 30.5). All were consuming complete gluten-containing diets. Nutritional anthropometry showed that there were no subjects below the cutoff point. Among

TABLE 2. Main HLA-DQA1*, DQB1* and HLA-DQB1/DQA1 haplotype determinations in celiac patients and their relatives

Genotypes frequencies	Relatives (n = 123)	Celiac patients (n = 62)
DQA1*/DQA1*		
0101-0301	0.179	0.145
0101-0501	0.196	0.065
0301-0301	0.179	0.113
0301-0501	0.138	0.242
0501-0501	0.098	0.323
DQB1*/DQB1*		
0201-0201	0.106	0.113
0201-0302	0.122	0.081
0302-0501	0.179	0.048
^aDQB1*/DQA1*		
0302/0301	0.129	0.258
0201/0501	0.145	0.113
0301/0501	0.174	0.120

^a Heterozygotic combinations not shown.

men and women, respectively, 50.1% and 60.5% were within normal limits, 6.5% and 2.4% between the lower cutoff point and the mean, and 43.4% and 36.2% above the normal range (overweight or obese). Characteristics of the 6 EMA+ relatives (4.8%) are shown in Table 3.

Although all relatives considered themselves asymptomatic, the focused questionnaire revealed the presence of some mild and nonspecific symptoms in some. All who reported symptoms were interviewed by one of the gastroenterologists in this study, who evaluated the subjects' clinical features. In no relative was the clinical history obtained suggestive of CD. No cancer, diabetes, or autoimmune diseases were detected. Analysis of responses to the questionnaire showed that only history of weight loss ($P = 0.03$), anorexia ($P = 0.027$), and diarrhea ($P = 0.029$) were significantly more frequent among EMA+ relatives.

Only relatives who had a positive EMA test result agreed to a small intestinal biopsy. The results of their histologic evaluations and HLA haplotypes are shown also in Table 3. Alleles and genotypes are shown in Tables 1 and 2, respectively.

DISCUSSION

Patients selected for this study had previously confirmed CD (1,2). That they had few symptoms despite frequent and gross dietary transgressions may have been due to their ages (1,19,20). In contrast with other studies, anemia was infrequent in the women in our patient group (20–22). Although at the time of assessment, age in our group was similar to that reported by Ciacci et al. (20), in our patients, diagnosis was made during childhood, whereas in most other published studies patients had disease first diagnosed during adulthood. Whether this explains the differences deserves further study. The rather large proportion of patients with short stature suggests that dietary transgressions had taken place since child-

hood. This indeed has been our experience. Stunted growth is probably also related to high wheat consumption in the Chilean society, which may represent an adverse situation for those with potential CD.

The finding that in 19% of patients the presence of EMAs was not concurrent with the advocated diet is similar to data reported in European patients with CD (23), but in our study it must be taken into account that gluten ingestion was massive and chronic. Patients who ingested gluten and were EMA– may have latent disease (5,24). It is interesting that among these patients, in three of seven who were typed, the presence of “susceptibility” haplotypes suggests that they had a genetic risk.

As for the patient who reported consuming a gluten-free diet but was EMA+, our clinical experience indicates that in most cases, patients who are EMA+ ingest gluten. However, we observed this patient for many years, and to the best of our knowledge, his statement was reliable. If this is the case, the finding is difficult to explain; he would represent the small group of very sensitive patients who react immunologically to marginal ingestion of gluten or whose serologic response lasts for long periods (25). Unfortunately, because he would not agree to another biopsy it was not possible to relate these findings to mucosal appearance. Findings in this patient could also represent a failure of EMA specificity.

In northern Europe up to 95% of patients with CD carry the DQ2 heterodimer (DQA1*0501-DQB1*0201) (26,27) whereas in southern Europe, although DQ2 is also prevalent, up to 20% of patients carry DQ8 (28). In this study, although the DQA1*0501 allele was present in the highest frequency, combinations of DQ8 HLA haplotypes were more frequently observed. This is in association with the strong Spanish genetic background (29–31). The high frequency of DQB1*0302 may be an accurate finding, because the specific probes used in this study make it unlikely to be a mistyping or deduction-associated error. Genes encoding DQ8 (DQA1*0301-DQB1*0302) are found on DR4 HLA haplotypes. It is

TABLE 3. Characteristics of 7 EMA (+) relatives

Code	Relation to patient	Age (years)	Nutritional status (W/H or BMI) ^b	Symptoms ^a	Histology	Haplotype
1	Mother	39	Overweight	Yes	4	DQA1*0301/0501 DQB1*0302/0302
2	Mother	49	Overweight	No	2–3	DQA1*0501/0101 DQB1*0302/0501
3	Mother	45	Obesity	No	2–3	DQA1*0501/0501 DQB1*0301/0302
4	Mother	30	Low normal	No	3–4	DQA1*0301/0501 DQB1*0201/0302
5	Father	49	Obesity	No	4	DQA1*0301/0501 DQB1*0201/0302
6	Brother	7	Overweight	Yes	4	DQA1*0201/0301 DQB1*0201/0201

^a For symptoms investigated see text.

^b Weight/Height was used in subjects <15 years of age. Body mass index was used in those ≥ 15 years.

worth noting that a high frequency of DR4 is described in previous studies on type I diabetes in the Chilean population (13–15, 32). This may be because the second most important genetic influence in this society is the Mapuche Indian group; they constitute 38% of the genetic pool (33) and in them DR4-DQ7 is found two times more frequently than in white Chilean subjects (34). Thus, the rather low frequency of DQ2 observed in this study is concordant with the ethnic composition of the studied population.

The familial trend of CD is well-documented (35). Among EMA+ relatives, the absence of malnutrition and the 71.4% (5/7 cases) rate of overweight or obesity were unexpected, but they are well in accordance with current national nutritional figures for the general Chilean population (36). Other investigators have also reported a low prevalence of malnutrition in adults with CD (37). Although symptoms detected were nonspecific digestive symptoms, that these were more frequent among EMA+ subjects ($P < 0.003$) suggests that adults may have some symptoms but may be unaware of them.

As in studies performed in school-aged and adult groups in Europe (3,38,39), our search for the presence of CD by means of EMA testing among apparently asymptomatic subjects also suggests that the “celiac iceberg” is greater than expected (45% of patients who declared themselves “cured” and 5.1% of relatives). Taking into consideration that the overt disease is infrequent in the Amerindian population (unpublished results) further surveys should be undertaken in Latin America.

As expected, DQA1* and DQB1* alleles distribution in the relatives was concordant with those of the patients, except for DQA1*0101 which had a higher frequency among relatives. This allele is not associated with the disease, and it is in linkage disequilibrium with DQB1*0501. DQA1-0101/DQB1-0501 has been described as a frequent haplotype in a European population (40) without CD. The higher frequency of alleles DQA1*0101 and DQB1*0301 and DQB1*0501 in our group of relatives compared with patients suggests a protective rather a neutral role for these alleles (Table 1). In relation to the EMA+ relatives, it is worth noting that they all had some degree of histologic damage and all except one carried at least one 0501 in the DQA1 chain and one 0302 in the DQB1 chain (Table 3). Identification of adults with subclinical manifestations and risk haplotypes may be most important when we consider that they may have increased risk of enteropathy-associated T-cell lymphoma (41).

In summary, these results shed light on the characteristics of CD in a Latin American population. They stress that CD in adolescents and adults is oligosymptomatic and is often unrecognized by affected patients. The EMA studies helped to identify individuals who should have long-term follow up, both among patients and relatives. The genetic studies revealed the predominance of DQ8

combinations, which is in agreement with the Spanish-Mapuche genetic stock of the Chilean population.

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