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Short Communication

"Geographical distribution of risk genotypes in pediatric patients with celiac disease in Spain"

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Abbreviations: HLA, Human Lukocytes Antigens; CD, Celiac Disease; REPAC-2, Spanish Celiac Pediatric Registry.

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ABSTRACT

Celiac disease is strongly associated with HLA DQ, specifically with haplotypes.

*DRB1*03-DQA1*05:01/DQB1**02:01 (**DQ2.5),** *DRB1*07-DQA1*02:01/DQB1*02:02* (**DQ2.2**), *DRB1*11-DQA1*05:05/DQB1*03:01* ***(DQ7.5)***, and *DRB1*04-DQA1*03:01/DQB1*03:02* (**DQ8).** The distribution of these risk haplotypes in patients with celiac disease is different in the geographical areas investigated. A high frequency of *DRB1*07- DQA1*02:01/DQB1*02:02* (**DQ2.2)** and *DRB1*11-DQA1*05:05/DQB1*03:01* ***(DQ7.5)***, has been described in Southern Europe.

We analyzed 2102 confirmed CD cases with information on both *DQB1** alelles and their distribution by geographical area in Spain. According to the presence of this haplotype in one or two chromosomes, the genotype is classified in: DQ2 homozygous, DQ2 heterozygous (*cis* or *trans)*, DQ8 homozygous, DQ8/DQ2.5, DQ 2.2 homozygous and genotype known as "half DQ2".

Two different patterns of risks related to CD were identified. In the Basque Country and Navarre, the Mediterranean Area (Aragon, Catalonia, Valencia, Balearic Islands, and Murcia), the South of Spain (Andalucía and Extremadura), and the Canary Islands, higher frequency of DQ2.5 *trans,* and more than 80% of DQ2.5/DQ2.2 homozygosis were described. The Cantabrian Coast (Cantabria, Asturias, and Galicia) and Central Areas (Castilla-León and Castilla-La Mancha) showed a higher percentage of DQ2.5/DQ2.5 homozygosis and a lower DQ2.5 in *trans* frequency, as in Northern Europe. Madrid has an intermediate model between the two described above. 17 cases (0.8%) did not carry any CD risk haplotypes.

1. Introduction

Genetic susceptibility for celiac disease (CD) is located on chromosome 6 in region 6p21. In 1989, evidence of a primary association between Celiac disease and the particular HLA-DQ α/β heterodimer was described [1].

More than 90% of CD patients carry a variant of the HLA-DQ2 heterodimer encoded by *HLA-DQA1*05* and *HLA-DQB1*02* alleles*,* either in *cis* configuration (both alleles on the same chromosome) appearing in the same haplotype as *HLA-DRB1*03* (DQ2.5 extended haplotype), or in *trans* configuration (each allele on a different homologous chromosome) appearing mainly in heterozygous individuals carrying the haplotypes *HLA-DRB1*05-HLA-DQA1*05-HLA-DQB1*03* (DQ7.5 haplotype) and *HLA-DRB1*07-HLA-DQA1*02-HLA-DQB1*02* (DQ2.2 haplotype) [1,2].The remaining patients in whom DQ2 was not expressed were mostly carriers of the HLA DQ8 heterodimer, encoded by *DQA1*03:01* and DQB1**03:02* in *cis* configuration, which appears in the same haplotype as *HLA- DRB1*04.* Finally, a minority of patients carry only one of the two alleles encoding HLA-DQ2, either *DQB1*02* allele (HLA-DQ2.2 molecule) or *DQA1*05* (HLA-DQ7.5 molecule). Those alleles that have not been described as associated with CD are represented as DQX [3,4,5].

The risk intensity for developing CD is associated with the presence or not of risk alleles in individuals [2].This risk has been categorized based on HLA expression. A higher risk has been described in DQ2.5 homozygosis (DQ2.5/DQ2.5 or DQ2.5/DQ2.2), especially in females [6,7,8]. Some authors have reported that DQ2.5, in *trans* configuration, also confers a high risk [9].

CD-associated HLA frequencies have been reported in several European regions. In 2004, a study including Scandinavia (Norway and Sweden), France, and Italy [10] determined that the distribution of DQ2.5/DQ2.5 was more common in Northern Europe. But the most prominent data finding of Southern Europe and Mediterranean countries is the more frequent distribution of DQ2.2, which allows either the combination with DQ2.5 (in homozygosis DQ2.5/DQ2.2) or with DQ7.5 to form a DQ.2.5 heterodimer in *trans* (DQ2.2/DQ7.5) [9, 10, 11). Globally, CD prevalence figures of 1.4% (based on Antibodies Anti-transglutaminase (IgA-TTG) and/or anti-endomysium (IgA-EMA)) called seroprevalence, have been described, and 0.7% based on biopsy. The prevalence values for CD were 0.4% in South America, 0.5% in Africa and North America, 0.6% in Asia, and 0.8% in Europe

and Oceania [12]. Prevalence figures in Europe are significantly higher in the north (1.60%) than in the east (0.98%) south (0.69%) and west (0.60%). Large increases in the incidence of diagnosed CD across Europe have reached 50 per 100 000 person-years in Scandinavia, Finland, and Spain [13].

In Spain, the highest figures of prevalence by study with serological screening :(IgA-TTG) and/or (IgA-EMA) in children have been described in Granada [14] and Vizcaya [15], with intermediate figures in Madrid [16] and the lowest in Asturias, including the adult and pediatric population [17]. Recently a global incidence rate of 7.9 cases of CD per 1000 live births and 54 cases per 100,000 person-years has been described for the period after the year 2000 [18,19].

The data used here belong to the REPAC-2: Spanish Celiac Pediatric Registry study, whose general characteristics have been recently published, observing that the median age at diagnosis was 4 years. Gastrointestinal symptoms were detected in 71.4% of the patients, and diarrhea was the most frequent of these symptoms (45.9%). The most common clinical presentation was the classical form (65.1%), though 9.8% of the patients were asymptomatic. The clinical presentation of pediatric CD in Spain is evolving in the same direction as in the rest of Europe, with diagnosis at a later age and with a decreased predominance of the gastrointestinal and classical forms, together with an increase in extraintestinal or asymptomatic forms. Nevertheless, there continue to be relevant differences between Spain and other European countries [19].

Considering the importance of these genetic markers and their possible relationship with the epidemiology of CD in the general population, we collected data from a large prospective study of pediatric Spanish populations. The principal aim was to determine the frequencies of the at-risk HLA-DQ genotypes in our group of patients with CD.

2. Patients and methods

2.1. Subjects

Clinical data from CD diagnosed patients under 15 years of age were obtained from the database of a prospective, observational, multicenter, nationwide registry of new cases of CD in Spanish children between January 2011 and December 2013 (REPAC-2: Spanish Celiac Pediatric Registry). This study was conducted in the framework of this project. CD was diagnosed according to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition criteria [20]. We analyzed 2102 patients for whom information for both DQB1 alleles was available. The same investigator reviewed all records to avoid inconsistent data. This study was approved by the Ethics Committee of Clinical Research of the Puerta de Hierro Majadahonda University Hospital, Madrid (No.263.2011). Informed consent was obtained from all patients.

The geographical distribution was determined according to the following areas: the Cantabrian coast (Cantabria, Asturias, and Galicia), the Basque Country and Navarre, the Mediterranean Area (Aragon, Catalonia, Valencia, Balearic Islands, and Murcia), the central area (Castilla-Leon and Castilla-La Mancha), Madrid, South (Andalusia and Extremadura), and the Canary Islands. Geographic distribution was based on aggregation, data coherence, geographical, historical and statistical factors [21].

Children were tested for *DQA1*/DQB1** haplotypes using polymerase chain reaction (PCR) on DNA extracted from peripheral blood. According to the different combinations of HLA risk haplotypes, different HLA categories were considered: DQ2.5, DQ8, DQ2.2, and DQ7.5, as well as non-risk haplotype DQX.

As shown in Table1, for **HLA-DQ2** carriers **DQ2 homozygosis**, DQ2.5/DQ2.5, and DQ2.5/DQ2.2 were included. Cases within the cis configuration (DQ2.5/DQX) were considered **DQ2 heterozygosis.** Additionally, **DQ2/DQ8** (DQ2.5/DQ3.3) and **DQ2** *trans* configuration (DQ2.2/DQ7.5) were distinguished. Between **HLA-DQ8** carriers were included homozygous DQ8/DQ8, DQ8/DQ2.2, DQ8/DQ7.5 and DQ8/DQX. Lastly those considered to carry **Half DQ2** include DQ2.2/DQ2.2, DQ2.2/DQX, DQ7.5/DQ7.5 and DQ7.5/DQX. The last two columns of Table 1 show a comparative study between the percentages of DQ2.5/DQ2.5 or DQ2.5/DQ2.

3. The statistic analysis

The frequency distribution of the CD risk haplotypes in the pediatric population for the total sample and by region was described using frequencies and percentages. Two types of comparisons of the risk haplotype frequencies across regions were conducted. First, we compared the proportion of each CD risk haplotype (versus any other CD risk haplotype) by region, and second, within the homozygous DQ2 risk haplotype, we compared the frequency of DQB1*02:01/02:02 homozygosis (versus DQB1*02:01/02:01 homozygosis) by region. Comparisons of relative frequencies across regions were conducted using the chi-square test with continuity correction or F-Fisher test, depending on the expected number of cases of the cells. P-values were adjusted for multiple comparisons (false discovery rate) using the 'fdr' method implemented in the library statistics of the statistical package R, 3.6.3. Finally, two complementary analyses were conducted to assess if age at onset and presentation type differed by HLA type (both aggregated as DQ2 yes or no and by subtype). To do so, analysis of variance with Tukey post-hoc comparisons was used to analyze age at onset, and Fisher test was used to analyze presentation type. Analyses were performed using the SPSS Windows 21 statistical package.

4. Results

Seventeen cases carry non-risk HLA. The results shown refer to the 2085 patients with CD Risk Haplotypes.

The results of the geographical distribution of alleles at risk (*DQB1**) in the CD pediatric Spanish population are shown in Table 1 **and** Fig. 1. In Table 1 we can observe that 92.6% of patients carry DQ2 haplotype as follows: 32.2% **DQ2 Homozygosis** (DQ2.5/DQ2.5 or DQ2.5/DQ2.2); 38.3% **DQ2 heterozygosis** (DQ2.5/DQX); 10.6% **DQ2/DQ8** (DQ2.5/DQ3.3); 11.5% **DQ2 in *Trans*** (DQ7.5/ DQ2.2). The remaining 5.4% are **DQ8** (DQ3.3) and 2.0% carry **half DQ2: DQ2.2** (DQ2.2/DQ2.2 or DQ2.2/DQX) or **DQ7.5** (DQ7.5/DQ7.5 or DQ7.5/DQ7X).

Regarding the distribution of different haplotypes according to the geographical areas, once the p-values had been adjusted for multiple comparisons, we observed a remarkable prevalence of **DQ2 Homozygosis** (DQ2.5/DQ2.5 or DQ2.5/DQ2.2) in the Basque Country and Navarre (39.7%, $p = 0.001$) and Madrid (38.5%, $p = 0.007$). This was significantly lower in central areas (17,6%, $p < 0.001$), while in other areas, the results were between 27.1% and 32.5%. The presence of **DQ2 Heterozygosis (**DQ2.5/ DQX) was significantly more frequent in central areas (48.5%, $p = 0.012$) and lower in the Basque Country and Navarre (31,3% $p = 0.002$). In the other areas, the percentages were between 37.3% and 45%. **DQ2/DQ8** haplotype (DQ2.5/ DQ3.3) was similarly represented in all regions between 9.2% and 11.6%, except for the central area at 21.2% ($p < 0.001$). **DQ2 trans** (DQ2.2/DQ7.5) was similarly distributed in all regions (between 11.9% and 12.9%) apart from the Cantabrian coast and central areas, where its frequency was lower, 7.5% ($p < 0.230$) and 6.1% ($p < 0.045$), respectively. On the Cantabrian coast, the percentage of trans DQ2 is 7.5% vs. 11.5% in the general population, but when adjusting for multiple combinations, the significance is lost, possibly because of the smaller sample size in this area. The frequency of **DQ8** (DQ3.3) in geographical areas of Spain ranged between 2.9% and 8.4%, with no significant differences. Finally, the percentage of "**Half DQ2**" (DQ2.2 or DQ7.5) patients ranges between 0% and

Table 1
Distribution of CD risk haplotypes in pediatric population in Spain.

	DQ2 Homocygosis	DQ2 Heterocygosis[b]	DQ2/DQ8	DQ2 *trans*	Total DQ2[c]	DQ8	Half DQ2	Total	Homozygosis DQB1* 0201/0201	Homozygosis[d] DQB1*0201/ 0202
Regions[a]	DQ2.5/DQ2.5or DQ2.5/DQ2.2	DQ2.5/DQX	DQ2.5/ DQ3.3	DQ2.2/ DQ7.5	Total DQ2.5	DQ3.3	DQ2.2 or DQ7.5		DQ2.5/ DQ2.5	DQ2.5/DQ2.2
Cantabrian coast	52(32.5%)	72(45.0%)	16(10.0%)	12(7.5%)	152 (95.0%)	8(5.0%)	0(0%)	160	34(65.4%)	18(34.6%)
p-value	0.999	0.230	0.999	0.230		0.999	0.230			**<0.001**↓
CAPV-Navarra	170(39.7%)	134(31.3%)	40(9.3%)	54(12.6%)	398 (93.0%)	25 (5.8%)	5(1.2%)	428	25(14.7%)	145(85.3%)
p-value	**0.001**↑	**0.002**↓	0.522	0.525		0.755	0.446			**<0.001**↑
Central Areas	29(17.6%)	80(48.5%)	35(21.2%)	10(6.1%)	154 (93.3%)	9(5.5%)	2(1.2%)	165	17(58.6%)	12(41.4%)
p-value	**<0.001**↓	**0.012**↑	**<0.001**↑	**0.045**↓		1.0000	0.774			**0.001**↓
Madrid	163(38.5%)	142(33.6%)	39(9.2%)	52(12.3%)	396 (93.6%)	18 (4.3%)	9(2.1%)	423	68(41.7%)	95(58.3%)
p-value	**0.007**↑	0.066	0.459	0.708		0.459	0.943			**<0.001**↓
Mediterranean	126(28.4%)	186(41.9%)	42(9.5%)	53(11.9%)	407 (91.6%)	27 (6.1%)	10(2.3%)	444	25(19.8%)	101(80.2%)
p-value	0.212	0.213	0.691	0.788		0.691	0.788			0.064
Souther areas	66(29.3%)	84(37.3%)	26(11.6%)	27(12.0%)	203 (90.2%)	19 (8.4%)	3(1.3%)	225	12(18.2%)	54(81.8%)
p-value	0.768	0.875	0.875	0.875		0.141	0.875			0.141
Canary Islands	65(27.1%)	101(42.1%)	24(10.0%)	31(12,9%)	221 (92.1%)	7(2.9%)	12(5.0%)	240	11(16.9%)	54(83.1%)
p-value	0.148	0.320	0.815	0.536		0.115	**0.006**↑			0.060
Total	671(32.2%)	799(38.3%)	222 (10.6%)	239 (11.5%)	1931 (92.6%)	113 (5.4%)	41(2.0%)	2085	192(28.6%)	479(71.4%)

↓ indicates significantly lower frequency of the CD risk haplotype compared with the rest of the regions and ↑ indicates significantly higher frequency. Mediterranean áreas: Aragón, Cataluña, Baleares, Comunidad Valenciana, Murcia; Souther areas: Andalucia and Extremadura.

[a] Regions: Cantabrian Coast: Cantabria, Asturias,Galicia; CAPV and Navarra: Comunidad Autónoma del País Vasco and Navarra; Central áreas: Castilla-León and Castilla la Mancha.

[b] DQ2 heterocygosis any other than DQ2/DQ8 or DQ2 trans.

[c] Includes all those with at least one copy of DQ2.5 in *cis* or in *trans*.

[d] All test in the table are conducted comparing proportion of each CD risk haplotype (versus having any other CD risk haplotype) across regions, except for the last two columns, which compare proportion of homozygosis DQB1*02:01/02:02 within the homozygosis CD risk haplotypes.

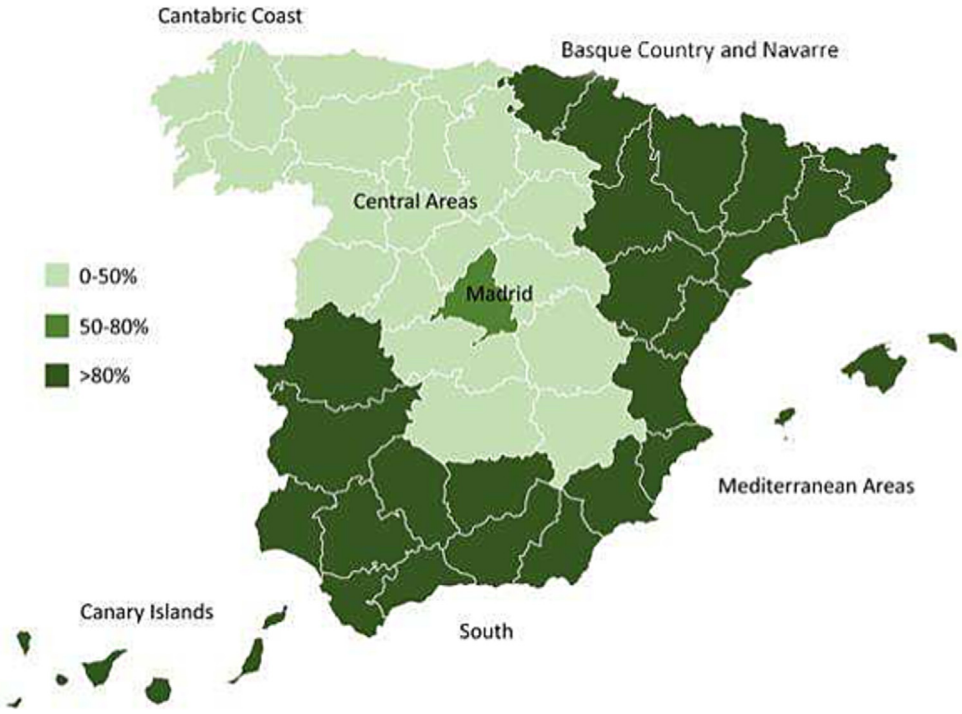

Fig. 1. Spain mapping of homozygous condition DQB1 0201/0202 (DQ2.5/DQ2.2) in Pediatric Celiac Disease.

2.3% in the peninsular area, with 5.0 % (p = 0.006) on the Canary Islands. Table 1.

Specifically related to **DQ2 Homozygosis** our results show that in Spain, 192 (28.6%) out of 671 cases are DQ2.5/DQ2.5 and 479 (71.4%) cases are DQ2.5/DQ2.2. The last two columns of Table 1 show the homozygous distribution DQ2.5/DQ2.5 or DQ2.5/DQ2.2, (%) from each region. We can observe high rates of homozygosis DQ2.5/DQ2.2 in the Basque Country and Navarre (85.3%, p < 0.001), the Mediterranean area (80.1%, p = 0.064), the South (81.8%, p = 0.141), and the Canary Islands (83.1, p = 0.060) have high percentages without significance. However, on the Cantabrian coast (34.6%,p < 0.001), in the central area (41.4%).,p = 0.001), and Madrid (58,3% ,p < 0.001) we can observe lower percentages of homozygosis DQ2.5/DQ2.2. Fig. 1 shows the geographical distribution of DQ2.5/DQ2.2.

Complementary analysis results assessing the role of HLA type on age in months at onset and clinical presentation type: classical or not classical, are given in Supplementary Material. Those patients with genotype DQ2 full, which comprise Dq2 heterocigosis, Dq2 homocigosis, Dq2 trans and Dq2/Dq8, were diagnosed at a much younger age than those with genotype Dq8 or Dq2 half, with mean age = 68.0 (sd = 43.9) when presence and mean age = 82.5 (45.9) when not (p < 0.001) (Table S1 in Supplementary Material). The proportion of patients that had clinical presentation no classical were 11.4%, and this did not vary across HLA types, ranging from 7.9% (in patients with Dq2 trans) to 15.3% (in patients with Dq2/Dq8), with p = 1.000 (Table S2 in Supplementary Material).No significant differences can be observed in the age of CD diagnosis for DQ2.5/DQ2.2 patients = 67,5 months (sd = 45.7), or DQ2.5/DQ2.5 patients 67,9 months (sd = 44.), or in their form of clinical presentation.

5. Discussion

The diagnostic criteria for celiac disease published in 2012 include the determination of HLA as a key element in the diagnosis of the disease [20]. The need for an HLA study in clear CD cases was ruled out in the 2020 revision, but it is still necessary in doubtful cases and studies of risk groups [22]. Greater or lower risk of developing CD has been related to the type of HLA, DQ2 or DQ8, the patient carries [6,7,8,9,11]. However, the risk of celiac disease associated with the genotype could depend on the geographical area in which the individual lives [10].

Although at *http://www.allelefrequencies.net/* [23], there are data on healthy controls in different populations in Spain, these do not coincide with the distribution made in the article and therefore, the differences in the CD data cannot be discussed with the differences in the distribution of these alleles in different Spanish populations.

The high frequency of DQ2 haplotypes was confirmed in many North African and Southern European populations: 87% in Moroccans [24], 96.8% in Libyans [25], 91% in Italians [7], and 95.8% in Greeks [26]. In Spain, the DQ2 genotype was more common than the DQ8 genotype. Within Spain, homozygosis DQ2 is predominant in the Basque Country and Navarre, and Madrid. However, there is a low percentage of homozygosis DQ2 in the central area and on the Cantabrian coast, although in the latter it does not reach significance. Heterozygosity (DQ2.5/DQX) was the most common haplotype across all areas, except for the Basque Country, Navarra, and Madrid, where homozygosis DQ2 is more frequent, with 39.7% and 38.5%, respectively. We observe in Cantabrian and Central areas a low occurrence of DQ2.5 *trans*, with the higher percentage of DQ2.5/DQ2.5 homozygosis. In Basque Country and Navarre, Mediterranean area, South, and Canary Islands, more than 80% of all homozygotes were DQ2.5/DQ2.2, with a high frequency of DQ2 *trans*. Finally, Madrid has an intermediate behavior between the two models, which is probably related to the migration and diversity of the city.

There seem to be 2 distinctive patterns in Spain, with an east–west distribution gradient of DQ2.5/DQ2.2 to DQ2.5/DQ2.5 homozygosis, and DQ2 *trans*, and a south-north gradient, albeit to a lesser extent. These data are consistent with previous HLA studies carried out in our country [27] and are highly compatible with those available on the allelic frequency of HLA in the Spanish population [23].

Given the variety of methodologies used to study the prevalence and incidence of CD in children in Spain, the published data is not directly comparable. Nonetheless, it can be observed that the highest prevalence figures are those found in Vizcaya [15], and Granada [14], which coincides with the areas where DQ2.5/DQ2.2 Homozygosis predominates. The figures from the Cantabrian coast present lower percentages of DQ2.5/DQ2.2 and Asturias is lower still [17] while the figures for Madrid are intermediate [16].

According to several hypotheses, the genetic composition of the Iberian population is influenced by a founder effect (original Iberian population, possibly descended from the steppes) and because of the influence of North Africa and throughout history by various Mediterranean cultures, and later still, migrations of Central European origin [27,28,29].

In summary, our data suggest an HLA risk model in our country in pediatric patients with CD, which we can name Mediterranean or Southern European, more present in the Basque Country and Navarre, the Mediterranean area, the South and the Canary Islands, and another predominant in the Cantabrian coast and Central area, like the one described for some Central and Northern European countries. These results show that the distribution of CD risk haplotypes in our country is derived from different populations that have inhabited the Iberian Peninsula throughout history. The DQ2.2 allelic frequency in different geographic areas of Spain seems to play an important role in the presence in the paediatric CD population of DQ2 *trans* and DQ2.5/DQ2.2 homozygosis [23,27,30].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2023.01.010.

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