

## Recommendations to report and interpret HLA genetic findings in coeliac disease

Concepción Núñez<sup>1</sup> and José Antonio Garrote<sup>2,3</sup>; in the name of the Working Group of Immunology and Genetics of the Sociedad Española de Enfermedad Celíaca (SEEC)

<sup>1</sup>Laboratory of Research in Complex Disease Genetics. Health Research Institute of the Hospital Clínico San Carlos (IdISSC). Madrid, Spain. <sup>2</sup>Genetics Laboratory. Clinical Analysis Service. Hospital Universitario Río Hortega. Valladolid, Spain. <sup>3</sup>Mucosal Immunology Group. IBGM. University of Valladolid-CSIC. Valladolid, Spain

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**Correspondence:** Concepción Núñez. Laboratory of Research in Complex Disease Genetics. Health Research Institute of the Hospital Clínico San Carlos (IdISSC). C/ Profesor Martín Lagos, s/n. 28040 Madrid. Spain

**e-mail:** conchita.npardo@gmail.com

### INTRODUCTION

Coeliac disease (CD) is a chronic autoimmune enteropathy triggered by gluten and related prolamines in genetically predisposed individuals. Although CD is a polygenic disease, there is a strong association with genes of the *human leukocyte antigen* (HLA) region. Most patients present the HLA-DQ2 heterodimer, specifically the DQ2.5 isoform, which is present in around 90-96% of patients of European ancestry. The remaining patients mainly present the HLA-DQ8 heterodimer. This fact and the functional role of these receptors in CD pathogenesis lead us to believe that the presence of these heterodimers is necessary but not enough to develop CD. This confers a very high negative predictive value to the genetic study, almost 100%, although with a low specificity (1,2).

*HLA-DQA1* and *HLA-DQB1* genes encode the  $\alpha$  and  $\beta$  subunits, respectively, of the heterodimeric protein HLA-DQ or DQ. Genotyping of these *loci* or the identification of the specific alleles encoding DQ2.5 and DQ8 (*HLA-DQA1\*05*, *HLA-DQB1\*02*, *HLA-DQA1\*03* and *HLA-DQB1\*03:02*) define the basis of the genetic test used for CD diagnosis.

With the increase in the understanding of CD, HLA studies have gained relevance as a diagnostic tool. This was included in the new CD diagnostic criteria for children and adolescents proposed by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in 2012 (2). The genetic study in adult guidelines has a variable inclusion and relevance, but overall, it is used to support a diagnosis in certain cases. Due to the increased use of genetic studies in CD diagnosis, there is some relevant information that must be considered in order to report and interpret HLA genetic findings. This need has raised some concerns among the members of the Sociedad Española de Enfermedad Celíaca (SEEC), who wanted to address this issue from the working group of Genetics and Immunology. With the aim to establish the recommendations that are referred to in this document, it is important to highlight

that the HLA genetic study can be used for a lifelong exclusion of CD, with the consequential social and quality of life implications (3).

Recommendations have been grouped as follows: necessary, advisable, to be avoided and optional information, in a HLA genetic study. In addition, some information aimed to clarify some notions and an example of a genetic report have been included.

### NECESSARY INFORMATION

As we have mentioned, the vast majority of CD subjects have the HLA-DQ2 heterodimer, meaning the DQ2.5 isoform and/or the HLA-DQ8 heterodimer. However, there are some patients that carry only one of the alleles encoding DQ2.5. This observation was first described in some European populations (4) and was later ratified in Spain (5), as well as in populations from other geographical regions (6-9). Therefore, we consider it mandatory that a genetic report includes the information with regard to the following three points:

1. Reporting whether the subject has the HLA-DQ2 heterodimer, meaning DQ2.5 (presence of *HLA-DQA1\*05* and *HLA-DQB1\*02* alleles) and/or HLA-DQ8 (presence of *HLA-DQA1\*03* and *HLA-DQB1\*03:02* alleles).
2. In the absence of HLA-DQ2 (DQ2.5) and HLA-DQ8, information about the presence of one of the alleles encoding DQ2.5 (*HLA-DQA1\*05* or *HLA-DQB1\*02*) must be included.

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3. Information about how to interpret genetic data must be added, indicating whether the observed genetics is compatible or non-compatible with CD development. With regard to compatible genetics, the presence of HLA-DQ2 (DQ2.5) and/or HLA-DQ8 must be considered and also the presence of only the *HLA-DQA1\*05* or the *HLA-DQB1\*02* allele. The fact that the high negative predictive value of the genetic study is present in the absence of the DQ8 haplotype and of both alleles encoding DQ2 should be highlighted (10).

## ADVISABLE INFORMATION

The presence of DQ2.5 and DQ8 confers susceptibility to develop CD, but there is also a gene dosage effect. Thus, DQ2.5 individuals have a higher risk of CD when they carry two *HLA-DQB1\*02* alleles. DQ8 subjects have a higher risk when they are DQ8 homozygous (4). On the other hand, it is advisable to add the information about genotype when full HLA-DQ genotyping is performed. This allows for the detection of possible errors when reporting HLA heterodimers. However, it must also be noted that there are still unknown CD risk alleles. Full genotyping could provide relevant information in the future for physicians or researchers and also for patients. This can be summarized in the following two bullet points, which are *highly recommended* for inclusion in the genetic report:

1. Indicating the genetic dose (one or two copies) of the *HLA-DQB1\*02* allele and the DQ8 haplotype.
2. Indicating the full genotype of *HLA-DQA1* and *HLA-DQB1* loci, i.e., the two specific alleles present in each gene.

Interpretation of the genetic information is not always obvious. It is common to consider a positive result as indicative of CD at the present moment or in the near future. Therefore, it is important to highlight that the relevance of the genetic test is its *high negative predictive value* (almost 100%). However, it lacks a high positive predictive value and should not be used as a single tool to diagnose CD, as around 35-40% of the general population have the HLA-DQ2 and/or HLA-DQ8 heterodimers and only around 1% develop CD. We *recommend* clarifying that HLA genetics included in the genetic report is considered as *necessary* but *not enough* for CD development.

## INFORMATION TO BE AVOIDED

Related to the aforementioned, assertions that suggest that the presence of HLA-DQ2 and/or HLA-DQ8 are indicative of CD at that moment or in the near future must be avoided. In the same manner, non-compatible genetics does not discard CD with a 100% probability. There are certain CD patients lacking HLA genetic risk, although at very low frequency (4,5).

## OPTIONAL INFORMATION

The risk to develop CD varies depending on the HLA-DQ genotype present. The risk level of each particular subject

can be indicated according to this. When included, we recommend using the following classification:

- Very high risk: the presence of HLA-DQ2 (i.e., DQ2.5) with two copies of the *HLA-DQB1\*02* allele (DQ2.5/DQ2.5, DQ2.5/DQ2.2).
- High risk: the presence of HLA-DQ2 (i.e., DQ2.5) with one copy of the *HLA-DQB1\*02* allele or homozygous for HLA-DQ8 (DQ2.2/DQ7.5 (= DQ2.5 in *trans* configuration), DQ2.5/DQ8, DQ2.5/DQ7.5, DQ2.5/other, DQ8/DQ8).
- Moderate risk: the presence of the HLA-DQ8 and/or the *HLA-DQB1\*02* allele (DQ8/DQ2.2, DQ2.2/DQ2.2, DQ8/DQ7.5, DQ8/other, DQ2.2/other).
- Low risk: the presence of the *HLA-DQA1\*05*: DQ7.5/DQ7.5, DQ7.5/other allele.
- No risk alleles.

“Other” indicates another DQ that is different from DQ2.5, DQ8, DQ2.2 and DQ7.5.

Although this classification can be modified, we recommend to avoid the use of “low risk” in HLA-DQ8 individuals or in those carrying only the *HLA-DQB1\*02* allele. “This can be misunderstood and to consider that it is very unlikely that those individuals present CD. Some differences in the CD risk can be present depending on the specific genotype in the high and moderate risk groups. It is important to highlight that the presence of only the *HLA-DQA1\*05* allele does not increase CD risk, as it is present at a higher frequency in the general population. However, it cannot be used to discard CD. *HLA-DQA1\*05* must be considered as compatible with a CD diagnosis.

## ADDITIONAL INFORMATION

Table 1 shows the alleles in the *HLA-DQA1* and *HLA-DQB1* genes and the haplotypes that they form, which are responsible for the different CD-associated DQ heterodimers or isoforms. DQ heterodimers and haplotypes can have a similar nomenclature. However, it should be noted that each subject has two haplotypes, each one inherited from one progenitor. Consequently, the DQ2.5 heterodimer can be present in the absence of the DQ2.5 haplotype. The DQ2.5 heterodimer is encoded by the *HLA-DQA1\*05* and *HLA-DQB1\*02* alleles and therefore can appear in the presence of the DQ2.5 haplotype (*cis* configuration, inherited by

**Table 1.** HLA-DQ alleles and haplotypes conferring a risk for celiac disease, including their encoded proteins

HLA genetics			DQ protein
<i>HLA-DQA1</i> alleles	<i>HLA-DQB1</i> alleles	HLA-DQ haplotype	
<b>05*</b>	<b>02<sup>†</sup></b>	DQ2.5	DQ2.5
<b>03<sup>‡</sup></b>	<b>03:02</b>	DQ8	DQ8
02	<b>02<sup>†</sup></b>	DQ2.2	DQ2.2
<b>05*</b>	<b>03:01</b>	DQ7.5	DQ7.5

The alleles conferring a risk for celiac disease are in bold. The alleles in plain text are included as they form haplotypes with accompanying risk allele. \*It includes *HLA-DQA1\*05:01* and *HLA-DQA1\*05:05* alleles. †It includes *HLA-DQB1\*02:01* and *HLA-DQB1\*02:02* alleles. ‡It includes *HLA-DQA1\*03:01* and *HLA-DQA1\*03:02* alleles.

only one progenitor); as well as in presence of the DQ2.2 and DQ7.5 haplotypes (*trans* configuration, each haplotype inherited from one progenitor). It must be considered that the presence of only the *HLA-DQA1\*03* allele (i.e., in absence of *HLA-DQB1\*03:02*) does not confer a risk to develop CD.

There are several techniques to identify HLA risk alleles. Some offer the full HLA-DQ genotyping and others only identify the risk alleles. It can be useful to indicate the exact methodology performed in order to know if the information provided in the genetic report is complete.

## GENETIC REPORT

Figure 1 shows an example of how to report HLA genetic findings for a CD diagnosis.

### CONCLUSIONS

CD is a chronic disease with a lifelong treatment: a gluten free diet. This highlights the relevance of a proper diagnosis. The genetic test provides strong support for a diagnosis in certain cases. However, when interpreting or reporting HLA genetic findings, it is necessary to clearly understand several ideas to avoid misdiagnosis.

Genetic markers of celiac disease (CELIAC1, OMIM#212750)

*DQA1*\*\_\_/\*\_\_:

*DQB1*\*\_\_/\*\_\_:

Technique: specify the genotyping method

Protein isoforms/heterodimers: DQX/DQX, indicate if present in *cis* or in *trans* for DQ2.5 individuals)

Risk alleles:

<i>HLA-DQA1*05</i>	(Positive/Negative)
<i>HLA-DQB1*02</i>	(Positive/Negative)
<i>HLA-DQA1*03</i>	(Positive/Negative)
<i>HLA-DQB1*03:02</i>	(Positive/Negative)

Interpretation: Presence of (DQ2.5 heterodimer/DQ8 heterodimer/one of the two alleles encoding the DQ2.5 heterodimer/no risk alleles)

The patient shows a (compatible/non compatible) with the development of celiac disease genotype

Risk: (very high/high/moderate/low/no risk) risk of celiac disease development

The peptides encoded by the *HLA-DQA1\*05* and *HLA-DQB1\*02* alleles form the DQ2.5 heterodimer (commonly referred to as DQ2). The DQ8 heterodimer is formed by the peptides encoded by the *HLA-DQA1\*03* and *HLA-DQB1\*03:02* alleles. The heterodimers DQ2.5/DQ8 are present in around 95% of patients with celiac disease, but also in 30-40% of the general population. A small percentage of patients with celiac disease carry only one of the two alleles encoding the DQ2.5 heterodimer.

Haplotypes commonly associated with celiac disease: DQ2.5/OTHER, DQ2.2/DQ7.5, DQ8/OTHER. OTHER indicates whatever DQ, including the one specified.

The underlined information will be different depending on the specific alleles present in each individual. The different options are in brackets.

**Fig. 1.** Example of a genetic report for a celiac disease diagnosis.

## COMMENTS

Members of the Sociedad Española de Enfermedad Celíaca (SEEC) collaborating in the present report:

Eduardo Arranz. Mucosal Immunology Group. IBGM. Universidad de Valladolid-CSIC. Valladolid (Spain).

José Ramón Bilbao. Department of Genetics, Physical Anthropology and Animal Physiology, Universidad del País Vasco (UPV-EHU), Instituto de Investigación del Hospital Universitario de Cruces. BioCruces. Leioa, Vizcaya (Spain).

Fernando Fernández-Bañares. Service of Gastroenterology. Hospital Universitari Mutua Terrassa. Terrassa, Barcelona (Spain); CIBERehd of Hepatic and Digestive Diseases. Terrassa, Barcelona (Spain).

Juana Jiménez. Service of Clinical Analysis and Biochemistry. Hospital Universitario Severo Ochoa. Leganés, Madrid (Spain).

Teresa Perucho. Laboratory of Genetics. Genyca Innova. Majadahonda, Madrid (Spain).

Eva Ruiz-Casares. Laboratory of Genetics. Genyca Innova. Majadahonda, Madrid (Spain).

Félix Sánchez-Valverde. Pediatric Gastroenterology and Nutrition Unit. Pediatrics Service. Complejo Hospitalario de Navarra. Pamplona, Navarra (Spain).

Juan I. Serrano-Vela. Research and Training. Asociación de Celíacos y Sensibles al Gluten. Madrid (Spain).

## REFERENCES

- Hadithi M, Von Blomberg BM, Crusius JB, et al. Accuracy of serologic tests and HLA-DQ typing for diagnosing celiac disease. *Ann Intern Med* 2007;147:294-302. DOI: 10.7326/0003-4819-147-5-200709040-00003
- Husby S, Koletzko S, Korponay-Szabo IR, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012;54:136-60. DOI: 10.1097/MPG.0b013e31821a23d0
- Rodríguez Almagro J, Hernández Martínez A, Lucendo AJ, et al. Health-related quality of life and determinant factors in celiac disease. A population-based analysis of adult patients in Spain. *Rev Esp Enferm Dig* 2016;108:181-9. DOI: 10.17235/reed.2016.4094/2015
- Karell K, Louka AS, Moodie SJ, et al. HLA types in celiac disease patients not carrying the *DQA1\*05-DQB1\*02* (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Hum Immunol* 2003;64:469-77. DOI: 10.1016/S0198-8859(03)00027-2
- Fernández-Bañares F, Arau B, Dieli-Crimi R, et al. Systematic review and meta-analysis show 3% of patients with celiac disease in Spain to be negative for HLA-DQ2.5 and HLA-DQ8. *Clin Gastroenterol Hepatol* 2017;15:594-6.
- Mubarak A, Spierings E, Wolters V, et al. Human leukocyte antigen DQ2.2 and celiac disease. *J Pediatr Gastroenterol Nutr* 2013;56:428-30. DOI: 10.1097/MPG.0b013e31827913f9
- Kotze LM, Nisihara R, Utiyama SR, et al. Absence of HLA-DQ2 and HLA-DQ8 does not exclude celiac disease in Brazilian patients. *Rev Esp Enferm Dig* 2014;106:561-2.

8. Pallav K, Kabbani T, Tariq S, et al. Clinical utility of celiac disease-associated HLA testing. *Dig Dis Sci* 2014;59:2199-206. DOI: 10.1007/s10620-014-3143-1
9. Araya M, Oyarzun A, Lucero Y, et al. DQ2, DQ7 and DQ8 distribution and clinical manifestations in celiac cases and their first-degree relatives. *Nutrients* 2015;7:4955-65. DOI: 10.3390/nu7064955
10. Díaz-Redondo A, Miranda-Bautista J, García-Lledo J, et al. The potential usefulness of human leukocyte antigen typing for celiac disease screening: a systematic review and meta-analysis. *Rev Esp Enferm Dig* 2015;107:423-9. DOI: 10.17235/reed.2015.3758/2015