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GUIDELINES

UK NEQAS and BSHI guideline: Laboratory testing and clinical interpretation of HLA genotyping results supporting the diagnosis of coeliac disease

Deborah Pritchard[1] | **Arthi Anand**[2] | **Amy De'Ath**[1] | **Helena Lee**[3] | **Margaret Tracey Rees**[1]

[1]UK NEQAS for H&I, Velindre University NHS Trust, Cardiff, UK

[2]H&I Laboratory, North West London Pathology, Imperial College Healthcare NHS Trust, London, UK

[3]Transplantation Laboratory, Manchester Royal Infirmary, Manchester University NHS Foundation Trust, Manchester, UK

Correspondence
Deborah Pritchard, UK NEQAS for H&I, Velindre University NHS Trust, Cardiff, UK.
Email: Deborah.Pritchard3@wales.nhs.uk

Abstract

Coeliac disease is a common immune-mediated inflammatory disorder caused by dietary gluten in genetically susceptible individuals. While the diagnosis of coeliac disease is based on serological and histological criteria, HLA-DQ genotyping can be useful, especially in excluding the diagnosis in patients who do not carry the relevant DQ heterodimers: *DQA1*05 DQB1*02*, *DQB1*03:02* or *DQA1*02 DQB1*02* (commonly referred to as DQ2.5, DQ8 and DQ2.2, respectively). External quality assessment results for HLA genotyping in coeliac disease have revealed concerning errors in HLA genotyping, reporting and clinical interpretation. In response, these guidelines have been developed as an evidence-based approach to guide laboratories undertaking HLA genotyping for coeliac disease and provide recommendations for reports to standardise and improve the communication of results.

KEYWORDS
coeliac disease, histocompatibility, HLA, immunogenetics , immunology, inflammation

1 | INTRODUCTION

Coeliac disease (CD) is an immune-mediated systemic inflammatory disorder that results in intestinal damage. Gastrointestinal mucosal injury occurs after the ingestion of gluten, proteins found in wheat, barley and rye (Lebwohl et al., 2018). Clinical manifestations vary according to age group: infants and young children present with diarrhoea, abdominal distention and failure to thrive, whereas adults that develop CD present not only with diarrhoea but also with silent manifestations such as anaemia, osteoporosis or neurological symptoms (Koning et al., 2012). CD affects approximately 1% of the population in European countries (Mustalahti et al., 2010; Singh et al., 2018) and estimated 0.25%–2% of the worldwide population (Ludvigsson *et al*., 2014; Matzaraki *et al*., 2017).

CD has a strong genetic susceptibility, evident by familial occurrence and high concordance between monozygotic twins (Kuja-Halkola et al., 2016; Singh et al., 2015). Over 99% of patients with CD possess specific HLA-*DQA1* and *DQB1* genes that encode the CD-associated heterodimer proteins DQ2 and/or DQ8 (Megiorni et al., 2012; van Heel et al., 2007; Wolters et al., 2008). Approximately 90% of individuals with CD, across all populations, express either HLA-DQ2 and/or -DQ8 molecules with less than 2% of CD patients lacking these HLA types completely (Brown et al., 2019; De Silvestri et al., 2018; Tolone et al., 2021).

The positive predictive value of HLA testing for DQ2/DQ8 is limited as these types are common in the general population, most of whom will never develop CD. Therefore, HLA testing is generally not considered an initial test for CD diagnosis, instead, diagnosis relies on serological testing to demonstrate the presence of antibodies (e.g., to tissue transglutaminase and endomysium), and histological evaluation of duodenal biopsies (Husby et al., 2020). Nevertheless, HLA testing can provide informative results in certain clinical situations; the absence of DQ2/DQ8 can be used to virtually exclude a diagnosis of CD, which can make it a useful test to overcome

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some of the limitations for serological and histological methods (Brown et al., 2019).

The HLA genetics of CD are complex, especially compared to other HLA-associated diseases (e.g., HLA-B27 and ankylosing spondylitis). CD susceptibility involves several HLA loci and multiple HLA alleles, which are all associated with different levels of risk. It is, therefore, critical that laboratory personnel performing HLA typing for CD have knowledge of HLA genes, nomenclature and risk stratification to be able to provide accurate and unambiguous reports for clinicians.

External quality assessment (EQA) plays an important role in monitoring the accuracy of diagnostic laboratory test results. The UK National External Quality Assessment Service (UK NEQAS) for Histocompatibility and Immunogenetics has identified wide variation in practice, and a concerning number of discrepant results in the Coeliac Disease EQA Scheme (Table 1). These incorrect or misleading reports involved both HLA genotyping results and interpretative comments (De'Ath & Rees et al., 2019), a finding also reported by other EQA providers (Horan et al., 2018). An increase in laboratories that have not traditionally performed HLA testing has also been observed. Reasons for the discrepant results are varied; however, the majority appear to be due to post-analytical errors. Several contributory factors have been identified, which include: (i) lack of understanding of HLA genetics/nomenclature by personnel reporting results, (ii) overly complicated or ambiguous reporting of HLA results and (iii) incorrect interpretation of HLA test results to CD risk.

Numerous clinical guidelines have been produced for the diagnosis and management of CD in adults and paediatrics, including the British Society for Gastroenterology (Ludvigsson et al., 2014), British Society of Paediatric Gastroenterology, Hepatology and Nutrition (Murch et al., 2013); European Society of Pediatric Gastroenterology, Hepatology and Nutrition (Husby et al., 2020); European Society for the Study of Coeliac Disease (Al-Toma et al., 2019). However, specific guidelines covering HLA laboratory testing and reporting for CD are limited (Núñez et al., 2018; Tye-Din et al., 2015). In an attempt to address the issues observed from EQA testing, we have developed these guidelines for reporting HLA results and interpretative comments for CD. We hope that these guidelines, along with the explanation of HLA genetics/nomenclature will serve as a useful resource for laboratories performing HLA CD testing and help provide consistency in reporting between laboratories to allow optimum patient care.

2 | DEVELOPMENT OF RECOMMENDATIONS

This guideline was produced by the following actions:

1. A writing committee (authors of this manuscript) from the UK NEQAS for Histocompatibility and Immunogenetics Steering Committee and British Society of Histocompatibility and Immunogenetics members, comprising scientists providing an H&I clinical service for CD testing, was established.
2. A search of peer-reviewed literature to 30 June 2021 was undertaken.
3. Recommendations were produced from evidence obtained from the literature search and consensus of expert opinion.

(i) The evidence collected was evaluated using a modification of the GRADE nomenclature [https://www.gradeworkinggroup.org/]. For each recommendation, the strength of recommendation has been indicated as one of:

Level 1 (we recommend)
Level 2 (we suggest)
Not graded (where there is not enough evidence to allow formal grading)
Within each level, the quality of evidence has been graded as:
A (high)
B (low)

Executive Summary of HLA Genotyping Service Recommendations

Level 1 (We recommend)	
Evidence GRADE –A	**Guideline Section**
HLA testing for coeliac disease should be performed by an ISO 15189 accredited laboratory. [1A]	**6**
Laboratories must have procedures in place for the HLA testing, interpretation and reporting for coeliac disease. [1A]	**6**
A laboratory performing HLA testing for coeliac disease must participate in a relevant external quality assessment scheme. [1A]	**6**
HLA testing should cover *HLA-DQA1* and *DQB1* loci, and as a minimum, be able to detect the HLA alleles that make up the heterodimers DQ2.5, DQ8 and DQ2.2. [1A]	**6**
Reports should include details of the methodology used for testing, including any limitations of the assay impacting on interpretation of results. [1A]	**6**
HLA typing must be performed at an appropriate level of resolution to separate coeliac disease-associated HLA alleles from non-coeliac disease-associated alleles. [1A]	**6**
Evidence GRADE –B	
Staff undertaking HLA genotyping result interpretation and reporting should have specialist knowledge/experience of HLA genetics and nomenclature. [1B]	**11**
HLA results must be reported using correct HLA nomenclature. [1B]	**5**
Interpretative comments may utilise shortened CD nomenclature (e.g., DQ2.5, DQ2.2) in addition to official HLA nomenclature to aid clinician interpretation of results. [1B]	**5**
Level 2 (We suggest)	
Evidence GRADE A	
A simple summary statement for HLA results should be included in reports to aid clinical interpretation (e.g., the individual is positive for DQ2). [2A]	**5**

(Continues)

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Level 1 (We recommend)	
Evidence GRADE –A	**Guideline Section**
Interpretative comments should be included in reports to clearly inform clinicians if HLA genotype results are associated with coeliac disease. [2A]	**11**
The report should include a comment regarding utility of HLA typing results for CD diagnosis (e.g., high negative predictive value/limited positive predictive value). [2A]	**10**
Evidence GRADE B	
An HLA typing methodology, over single nucleotide polymorphism (SNP) kits (presence/absence result) is recommended. [2B]	**6**
The zygosity status of HLA-DQ should be determined [2B]	**9**
Laboratories should engage with service users to ensure report content and interpretative comments meet the needs of the service users. [2B]	**11**
Interpretative comments may reference the risk of a particular HLA genotype, to highlight the differences in CD risk conferred by different genotypes. [2B]	**11**

3 | DISCLAIMER

These recommendations represent consensus opinion from experts in the field of H&I within the United Kingdom. They represent a snapshot of the evidence available at the time of writing. This evidence may become superseded with time. It is recognised that recommendations have been made even when the evidence is weak. The British Society for Histocompatibility and Immunogenetics (BSHI) and the UK National External Quality Assessment Service for Histocompatibility and Immunogenetics (UKNEQAS for H&I) cannot attest to the accuracy, completeness or currency of the opinions and information contained herein and does not accept any responsibility or liability for any loss or damage caused to any practitioner or any third party as a result of any reliance being placed on this guideline or as a result of any inaccurate or misleading opinion contained in the guideline.

4 | HLA COELIAC DISEASE GENETICS

The HLA complex is located on the short arm of chromosome 6, an area that consists of multiple immune-relevant genes split into three regions: class I, class II and class III (Dendrou et al., 2018). HLA genes exhibit extreme polymorphism, meaning that each HLA locus has many known alleles, with new alleles continuously being detected. The Immuno Polymorphism Database-ImMunoGeneTics (IPD-IMGT/HLA database) is the official repository of HLA allele sequences (Robinson et al., 2015). Polymorphism is required for the crucial role that HLA class I and II molecules have in presenting a vast array of antigenic peptides to T cells, enabling the immune system to discriminate between self and non-self. HLA genes also exhibit strong linkage disequilibrium, with a tendency for certain HLA genes to be inherited en-bloc as an extended haplotype. The frequency of HLA allele and haplotypes varies between populations (Gragert et al., 2013).

The major susceptibility genes for CD are found in the HLA class II region, at the DQ locus: *HLA-DQA1* and *HLA-DQB1* alleles encode α- and β-chains that form a DQ $\alpha\beta$-heterodimer protein on the surface on antigen-presenting cells (Figure 1). One set of HLA alleles (haplotype) is inherited from each parent and both sets of alleles are expressed (co-dominant expression), resulting in potentially two different *DQA1* and *DQB1* alleles available to form up to 4 different $\alpha\beta$-heterodimers in an individual. A heterodimer encoded by *DQA1* and *DQB1* alleles on the same chromosome (inherited from 1 parent) is encoded in *cis*, whereas a heterodimer encoded from alleles on different chromosomes (1 from each parent) is encoded in *trans*. Importantly, if compatible heterodimers are encoded in *cis* or *trans*, functional DQ molecules will be expressed (Figure 1).

5 | HLA NOMENCLATURE

There are 2 nomenclature formats for HLA (Marsh et al., 2010), which denote either the genetic information (molecular nomenclature, e.g., *DQB1*03:02*) or the expressed proteins (serological nomenclature, e.g., DQ8) (Table 2). A third informal approach for referring to HLA types is also often used in CD literature (Brown et al., 2019). This has evolved to overcome the limitations of official HLA nomenclature, namely, serological DQ names only reflecting the DQB protein of the heterodimer, and otherwise lengthy molecular nomenclature. This hybrid nomenclature approach captures both the DQB and DQA proteins in a shorter format, e.g., *DQA1*05 DQB1*02* is shortened to DQ2.5 and *DQA1*02 DQB1*02* becomes DQ2.2. Although this is not official HLA nomenclature, we appreciate that it is widely used in publications and understood by those involved in CD diagnosis. Therefore, while we advocate using official HLA nomenclature for reporting HLA genotyping results, laboratories may wish to use the shortened CD nomenclature format, in addition to the genotyping results, or in any interpretative comments present on clinical reports. Indeed, a simple summary statement may be included alongside HLA genotyping results, to aid clinical interpretation.

In practice, due to the polymorphism in HLA genes, molecular testing for CD often detects groups of alleles with identical DNA sequences over the exons/single nucleotide polymorphisms (SNPs) covered by the kit, rather than a single HLA allele. These 'ambiguities' are reported in HLA molecular nomenclature either by reporting only the first field results (e.g., *DQB1*02* includes all *DQB1*02* alleles in the 02 family) or by using '-' or '/' to indicate groups of alleles (e.g., *DQB1*02:01-02:03* or *DQB1*02:01/02:02/02:03* would indicate it is either a DQB1**02:01* or *02:02* or *02:03* allele).

6 | HLA TYPING

Molecular testing allows HLA alleles to be determined to different levels of resolution, which in essence, relates to the number of exons

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TABLE 1 Example EQA errors in HLA reporting and interpretation for coeliac disease.

	Reference HLA Type	Serotype	Reported result /interpretation	Explanation of error/potential clinical impact
1	DQB1*02:02, DQB1*03:01 DQA1*02:01, DQA1*03:03	DQ2.2, DQ7	Negative for **DQ2** and DQ8	False DQ2 negative. The alleles DQB1*02 and DQA1*02, which encode the DQ2.2 heterodimer, are present. Although less frequent than DQ2.5 and DQ8, DQ2.2 is associated with CD; therefore, CD could be incorrectly excluded on the basis of this result.
2	DQB1*03:01, - DQA1*03:03, DQA1*05:01	DQ7 homozygous	***DQB1*02:01***, DQB1*03:01 ***DQA1*03:02***, DQA1*05:01	False DQ2 positive. DQB1*02 (DQ2) is not present in this individual. The DQA1*05 allele is present, which is part of the DQ2.5 heterodimer, but in this case, the DQA1*05 allele is in association with DQB1*03:01 (DQ7). The risk of CD with DQA1*05 only is highly unlikely, and by incorrectly reporting DQ2 as present, could result in the patient undergoing unnecessary monitoring and biopsy. The DQA1*03 allele is also incorrectly reported as DQA1*03:02 instead of DQA1*03:03, although this would not alter the clinical interpretation of the results.
3	DQB1*02:02, DQB1*03:03 DQA1*02:01, DQA1*03:02	DQ2.2, DQ9	***DQB1*07***, DQB1*03:03	False DQ2 negative/incorrect HLA nomenclature: DQB1*07 does not exist (DQ7 in molecular nomenclature is DQB1*03:01). DQB1*03:03 if reported as the serotype would be DQ9. The alleles DQB1*02 and DQA1*02 (DQ2.2) are present, but not reported. CD could be incorrectly excluded on the basis of this result.
4	DQB1*03:01, - DQA1*03:03, *05:05	DQ7 homozygous	Alpha-subunit HLA-DQ2.5 positive **HLA-DQ8 positive**	False DQ8 positive. DQ8 incorrectly reported (DQB1*03:01 is DQ7). Although DQA1*05 is positive, there is no complete heterodimer associated with CD present; this incorrect report could result in the patient undergoing unnecessary monitoring and biopsy.
5	DQB1*02:01, DQB1*03:02 DQA1*03:01, DQA1*05:01	DQ2.5, DQ8	DQB1*03:02: positive DQA1*05: positive DQB1*02: positive DQA1*02: negative **Reported phenotype: DQ2.5**	Incomplete interpretation of HLA type. DQ8 not reported in interpreted phenotype, despite correct report of DQB1*03:02 presence. DQ2.5 has been reported correctly; however, the risk hierarchy is increased when both DQ2.5 and DQ8 are present, compared to DQ2.5 only, which could alter patient management.
6	DQB1*02:01, 03:01 DQA1*03:03, 05:01	DQ2.5, DQ7	Positive for DQB1*02, DQB1*03/06, ***DQA1***03*, DQA1*05, **DQA1***03:02/03, alpha-subunit HLA-DQ2.5, ***alpha-subunit HLA-DQ8,*** beta-subunit HLA-DQ2.5	Overly complex and confusing report. DQA1*03 reported twice (as DQA1*03 then DQA1*03:02/03). 'alpha-subunit HLA-DQ8' report potentially misleading as the presence of DQA1*03 without DQB1*03:02 (DQ8) has not been linked to CD.
7	DQB1*03:01, - DQA1*05:05, -	DQ7 homozygous	***Half DQ2 positive***	Confusing/uninformative report. The report does not state whether it is the alpha or beta part of the heterodimer that is positive and is likely to be confusing for clinicians to interpret.

Abbreviations: CD, coeliac disease; EQA, External Quality Assessment; HLA, human leucocyte antigen.

Note: Examples of incorrect reports/interpretations in the UK NEQAS H&I HLA genotyping for CD EQA scheme. Laboratories are requested to report EQA results in an identical manner to their clinical reports. A common error involves laboratories reporting a sample as DQ2 positive when only the DQA1*05 allele is present (often in conjunction with DQA1*03:01 (DQ7) [e.g. 2]. A number of laboratories also miss reporting DQB1*02 DQA1*02 (DQ2.2), which is found in approximately 5% of patients with CD (Bodd et al., 2012; Mubarak et al., 2013), e.g. [1], [3]. The EQA results highlight multiple various approaches to reporting and interpreting HLA results for CD, as well as numerous incorrect/inappropriate nomenclature errors and, overly complex reporting, e.g. [5], [6], [7].

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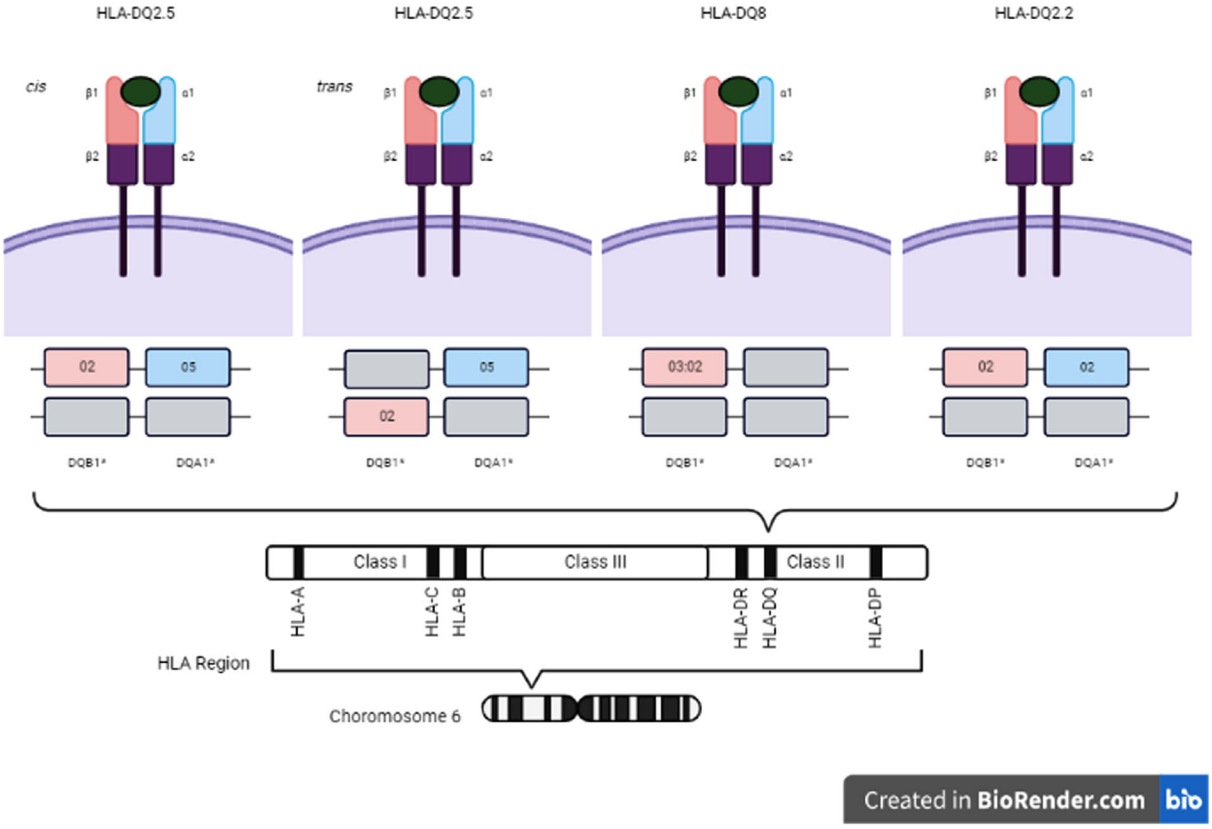

FIGURE 1 Genetic and protein structure for HLA-DQ heterodimers associated with coeliac disease. The genes encoding HLA molecules are found in the MHC complex on chromosome 6. HLA molecules involved in coeliac disease are encoded in the class II region at the DQ loci. HLA-*DQA1* and *DQB1* loci encode for a- and b-chains, respectively, that associate as heterodimers on the cell surface of antigen resenting cells, forming a cleft that binds peptide antigens. Most patients with coeliac disease (90%) express the HLA-DQ2.5 heterodimer, which consists of *HLA-DQA1*05* and *DQB1*02* alleles in either *cis* configuration on the same chromosome or in *trans* configuration, located on different chromosomes. The remaining patients (5%—10%) express HLA-DQ8 encoded by the *HLA-DQB1*03:02* allele, or HLA-DQ2.2, which consists of *HLA-DQA1*02* and *DQB1*02* alleles. HLA, human leukocyte antigen; MHC, major histocompatibility complex. Figure created with BioRender.com.

tested and how well alleles can be differentiated from others. For CD testing, it is important to understand the resolution provided by a chosen technique, to ensure it is able to appropriately determine the required CD HLA alleles. For example, DQ2 is associated with different CD risk depending on if the alpha chain is encoded by *DQA1*05* (DQ2.5) or *DQA1*02* (DQ2.2); therefore, it is important they can be separated. Furthermore, it is not sufficient for a technique to only define *HLA-DQB1*03*, as this can relate to HLA-DQ7 (*DQB1*03:01*), DQ8 or DQ9 (*DQB1:03:03*). It is necessary, therefore, to distinguish DQ8 (*DQB1*03:02*) from other DQB1*03 alleles.

HLA-DRB1 has historically been included in CD HLA associations as part of an extended haplotype (e.g., *DRB1*03-DQA1*05-DQB1*02*) due to the strong linkage disequilibrium between DR and DQ loci. However, contemporary HLA typing does not need to include HLA-*DRB1* and should instead focus on *DQA1* and *DQB1* loci testing.

HLA testing can be challenging due to the large number of very similar DQ alleles, which must be differentiated from each other. This is in contrast to most molecular testing which evaluates differences to an accepted 'wild-type'. HLA testing for CD generally falls into two categories: (i) HLA typing to define the *DQA1* and *DQB1* alleles present in an individual and (ii) selected SNP testing to determine presence/absence for specific CD susceptibility alleles (Koskinen et al., 2009) (Table 3; Monsuur et al., 2008).

Full *DQA1 DQB1* typing is often performed by specialist histocompatibility and immunogenetics laboratories who routinely perform HLA typing for other clinical indications, e.g., solid organ or haematopoietic stem cell transplantation. Laboratories using this approach are able to determine the HLA alleles in an individual, regardless of association with CD. The second approach for HLA testing commonly focuses only on the detection of the CD susceptibility alleles using SNP-based tests. These SNP kits are unable to provide a complete *DQA1*, *DQB1* HLA type but indicate the presence/absence of common CD alleles. Laboratories should report the methodology used for CD HLA testing to service users, including any limitations of the assay used.

Full HLA typing of *HLA-DQB1* and *DQA1* alleles is preferential to kit-based detection of specific alleles (Husby et al., 2020; Tye-Din et al., 2015). HLA typing methods clearly define the alleles present in an individual, which allows complete risk stratification, whereas presence/absence kits are likely to have resolution limitations and ambiguities that could hinder full interpretation. An HLA typing approach also allows the determination of homozygosity or

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TABLE 2 Overview of HLA nomenclature.

HLA molecular[1] (HLA Alleles)		HLA serologic[2] (HLA protein)	Commonly used in coeliac disease[3]
DQA1*05:XX[a]	DQB1*02:XX[b]	DQ2	DQ2.5
DQA1*03:XX[c]	DQB1*03:02	DQ8	DQ8
DQA1*02:01[d]	DQB1*02:XX[b]	DQ2	DQ2.2

There are three nomenclature formats in use for coeliac disease: the official molecular nomenclature, the official serological nomenclature and an unofficial system commonly used to refer to the HLA-DQ heterodimers involved in susceptibility to coeliac disease.

[1]For molecular nomenclature, the HLA locus is followed by an asterisk to denote molecular nomenclature followed by sets of numbers arranged in colon-separated fields. The first field numbers refer to the allele family, and second field numbers the specific allele number. *DQA1* encodes the alpha-chain protein, and *DQB1* encodes the beta-chain protein of the DQ molecule.

[2]For serological nomenclature, the HLA protein is followed by a number which denotes the specific HLA antigen. For HLA-DQ, the serological name only represents the DQB protein, and does not reflect the DQA protein of the heterodimer. The DQ serological name usually has the same number as the DQB1 allele family (e.g., DQB1*02 = DQ2), although due to the way HLA nomenclature has evolved, exceptions exist (e.g., DQB1*03:02 = DQ8).

[3]For the coeliac disease specific nomenclature, the serological nomenclature is followed by a ':' and another number to represent the DQA protein of the heterodimer, when it is relevant. This nomenclature captures the DQB and DQA HLA types in a shortened format.

[a]Refers to any allele in the *DQA1*05* family; *DQA1*05:01*, *DQA1*05:03* and *DQA1*05:05* are the most common alleles in European populations (Gonzalez-Galarza et al., 2020).

[b]Refers to any allele in the DQB1*02 family; DQB1*02:01 and DQB1*02:02 are the most common alleles in European populations (Gonzalez-Galarza et al., 2020).

[c]Refers to any allele in the *DQA1*03* family; *DQA1*03:01*, *DQA1*03:02* and *DQA1*03:03* are the most common alleles in European populations (Gonzalez-Galarza et al., 2020).

[d]Refers to any allele in the *DQA1*02* family; *DQA1*02:01* is the only common allele in European populations (Gonzalez-Galarza et al., 2020).

heterozygosity status, which is important when considering CD risk. Regardless of the method used, testing should be undertaken by a laboratory accredited to ISO 15189, and the test must be appropriately validated and/or verified in the laboratory where used. Laboratories must have written procedures in place for HLA testing, interpretation and reporting for CD, and participate in an EQA scheme.

7 | HLA GENES IN COELIAC DISEASE DEVELOPMENT

The development of CD is dependent on the presence of specific HLA-DQ alleles that orchestrate the immunological response to dietary gluten (Abadie et al., 2011). Detailed reviews of CD pathogenesis have been provided by others (Abadie et al., 2011; Caio et al., 2019; Kabri & Sollid 2006), but a brief outline of the involvement of HLA-DQ is provided here.

TABLE 3 Different approaches to CD HLA testing.

	HLA typing	Presence/absence testing
HLA type	DQA1*02:01, 03:02/03; DQB1*02:02, 03:03	DQA1*05 absent DQA1*02 present DQB1*02 present DQB1*03:02 absent
HLA type interpretation	Patient is positive for HLA-DQ2 (DQA1*02, DQB1*02)	
Clinical interpretation	The patient has a genotype which is associated with coeliac disease	

Note: Example of HLA results that may be reported using an HLA typing method and an SNP-based presence/absence detection method.

HLA-DQ heterodimers reside on the surface of antigen-presenting cells and present peptides derived from exogenous proteins to CD4 T cells. Each DQ molecule is capable of presenting a different repertoire of peptides, which is determined by the amino acid sequence present at the peptide-binding region of the heterodimer. In CD, ingested gluten-derived peptides are modified by the enzyme tissue transglutaminase 2 in a process called deamidation, which converts glutamine residues into negatively charged glutamic acid. Unlike other DQ molecules, DQ2 and DQ8 have a high affinity for these negatively charged gluten peptides, which are presented to elicit a gluten-specific T-cell response (Bajor et al., 2019; Jabri & Sollid et al., 2006). Importantly, this pathogenic process is not active in most individuals encoding DQ2 or DQ8 (which are common HLA types in the general population), indicating that other factors besides these genes are involved in disease progression.

Indeed, a variety of genes and environmental influences are critical to CD development. The presence of specific HLA types is necessary but is not a sufficient factor for the development of CD. HLA genotypes confer approximately 35%–40% of the genetic risk (Abadie et al., 2011; Al-Toma et al., 2019), with genome-wide association studies identifying numerous additional non-HLA genomic regions as being associated with CD (Garner et al., 2014; Trynka et al., 2011).

8 | HLA-DQ

There are three specific DQ heterodimers which have been found to account for over 99% of CD cases in Europeans (Karell et al., 2003). However, these genotypes are not all associated with the same CD risk (Pietezac et al., 2009). This is due to differences in their ability to present gluten-derived peptides to T cells (Tjon et al., 2010; Vadar et al., 2003).

8.1 | HLA-DQB1*02, DQA1*05 (DQ2.5)

The HLA-DQ2.5 heterodimer is encoded by *DQB1*02* and *DQA1*05* alleles (usually *DQB1*02:01* and *DQA1*05:01/03/05*), which can be encoded in either *cis* or *trans*. This is the most permissive heterodimer for CD, encoded by approximately 90%–95% of patients with CD

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TABLE 4 The complexity of HLA-DQB1*03.

	Serological specificity	Second field alleles
DQB1*03	DQ7	DQB1***03:01**, 03:04
	DQ8	DQB1***03:02**, *03:05*, *03:10*
	DQ9	DQB1***03:03**
	DQ3	03:06, 03:07, 03:08, 03:09 +

Note: The HLA-DQB1*03 family contains many different alleles, differentiated by the 2nd field HLA nomenclature. Several of these alleles are separated into three different serological groups: DQ7, DQ8 and DQ9. The most common alleles in these groups are DQB1*03:01, 03:02 and 03:03. Other DQB1*03 alleles have not been classified serologically and are categorised as simply DQ3. It is important that the HLA typing methodology used in CD testing is able to distinguish DQB1*03 alleles from other common alleles that encode for DQ7 and DQ9 as these are not implicated in CD. While DQB1*03:02 is the allele which is most commonly associated with DQ8 and has been shown to be associated with CD, other alleles are also classed as DQ8. While DQB1*03:02 is always DQ8, DQ8 may not always be DQB1*03:02, so the use of this nomenclature in reports should be carefully considered alongside the detection capabilities of the methodology used. The other DQ8 alleles are relatively uncommon, and the association with CD is unknown.

(Abadie et al., 2011; Almedia et al., 2016; Liu et al., 2014). The remaining CD patients (5%–10%) have been shown to carry either DQ8 or another DQ2 heterodimer (DQ2.2).

8.2 | HLA-DQB1*03:02 (DQ8)

The HLA-DQ8 heterodimer is usually encoded by *DQB1*03:02* often with *DQA1*03*. Importantly, *DQA1*03* can be found associated with many other *DQB1* genotypes and alone is not associated with CD. CD literature has generally used DQ8/DQB1*03:02 interchangeably; however, it is important to note that DQ8 includes more HLA alleles than just DQB1*03:02, e.g., DQB1*03:05, 03:10 (Table 4). Therefore, while DQB1*03:02 is always DQ8, DQ8 may not always be DQB1*03:02. This issue was highlighted in a sample distributed in 2022 by UK NEQAS for H&I, where 33% of participants reported incorrect results for a sample that was DQB1*03:05. The majority of errors were due to participants reporting the sample as DQ8 negative, whereas if they had reported it as DQB1*03:02 negative, they would have been correct. This highlights the importance for laboratories to understand the detection capabilities of the HLA typing methodology in use and to also be clear with reports.

These other DQ8 alleles are generally rare compared to DQB1*03:02, and no specific published data could be identified on their relevance to CD. Given the structural similarities between DQ8 alleles (e.g. only 1 amino acid difference in the peptide-binding domain between DQB1*03:02 and 03:05), and therefore potential ability to present gluten-derived peptides, the authors feel that in the absence of any data, it is safer to not exclude a diagnosis of CD if DQ8 alleles other than DQB1*03:02 are identified. These results should, however, be carefully communicated to service users.

It is also important to distinguish *DQB1*03:02*, from other *DQB1*03* alleles, as the *03 allele family includes different serologic HLA antigens which are not associated with CD; DQ7 and DQ9, most commonly encoded by *DQB1*03:01* and *DQB1*03:03*, respectively (Table 4).

8.3 | HLA-DQB1*02, DQA1*02 (DQ2.2)

The HLA-DQ2.2 heterodimer is encoded by *DQB1*02* and *DQA1*02* alleles (usually *DQB1*02:02* and *DQA1*02:01*), which can be encoded in either *cis* or *trans*. However, compared to the DQ2.5 heterodimer, DQ2.2 is significantly less capable of presenting gluten-derived peptides (Bodd et al., 2012; Fallang et al., 2009). Whereas the DQ2.5 heterodimer can present a large repertoire of gluten peptides, DQ2.2 can only present a subset of them (Koning et al., 2012; Ting et al., 2020). Bergseng et al. (2015) showed that DQ2.5 and DQ2.2 differ in their peptide-binding preferences which influenced the selection of distinctive gluten epitopes and the development of CD. It has also been reported that DQ2.5 has greater stability when peptides are bound producing sustained presentation and immune response to gluten peptides (Fallang et al., 2009; Lázár-Molnár & Snyder et al., 2018). This likely accounts for the lower prevalence of DQ2.2 in CD patients, compared to DQ2.5. Nevertheless, this HLA type is capable of supporting CD and is found in approximately 2%–5% of patients who lack DQ2.5 or DQ8 (Bodd et al., 2012; Mubarak et al., 2013).

8.4 | Other HLA alleles

Less than 1% of patients with a diagnosis of CD have lacked DQ2 or DQ8 (Di Silvestri et al., 2018; Husby et al., 2012; Karell et al., 2003; Pietezac et al., 2009). In rare cases, CD patients have been identified with DQA1*05 only, leading to the assumption that the alpha chain only may be sufficient for CD (Abadie et al., 2011; Karell et al., 2003; Megiorni & Pizzuti, 2012; Pallav et al., 2014). DQA1*05 is often found in conjunction with DQB1*03:01 (DQ7), but as DQ7 heterodimers can be formed with other DQ alpha chains, reports relating to DQ7 should be avoided, and DQA1*05 referred to instead. However, there is no clear evidence that DAQ1*05 alone presents a risk of CD (Brown et al., 2019), and more recent studies suggest that the presence of DQA1*05 alone would not be sufficient for effective antigen presentation of relevant peptides (Bajor et al., 2019). Furthermore, Italian and Brazilian studies identified DQA1*05 at higher frequencies in controls compared with patients (Almeida et al., 2016; Megiomi et al., 2009). Therefore, HLA typing should exclude individuals from a diagnosis of CD if they only carry DQA1*05 or any other HLA-DQ alleles other than those that encode the heterodimers DQ2.5, DQ2.2 and DQ8 (Erlichster et al., 2020).

9 | HLA RISK STRATIFICATION

As discussed above, each of the HLA-DQ heterodimers associated with CD is attributed with a different level of risk. Furthermore, studies have

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also demonstrated a gene dose effect, where patients who possess two of the associated HLA-DQ heterodimers are at a greater risk of developing CD, than individuals with only one heterodimer. This leads to a hierarchy of genetic risk for CD, classified according to HLA type and the number of susceptibility alleles present (Karell et al., 2003; Ploski et al., 1993).

The highest risk is consistently associated with DQ2.5 homozygous individuals (Al-Toma et al., 2019; Bajor et al., 2019a; De Silvestri et al., 2018; Lázár-Molnár & Snyder et al., 2018; Liu et al., 2014). In these cases, all DQ molecules on the cell surface are able to present gluten-derived peptides to T cells, whereas for DQ2.5 heterozygous individuals, 4 heterodimers can form, reducing the total dimers able to present gluten peptides. DQ2.5 homozygosity has been associated with more severe disease, earlier disease onset, an increase in serology antibody positivity, plus a higher rate of refractory CD (Airaksinen et al., 2020; Al-Toma et al., 2006; Karinen et al., 2006; Lionetti et al., 2014; Pietzak et al., 2009; Vader et al., 2003). Gene expression levels may also play a role: Pisapia et al. (2016) demonstrated that DQ2.5 had higher expression than non-CD–associated genes which increased cell surface protein levels which then affected the anti-gluten $CD4^+$ T-cell response.

Heterozygosity for DQ2.5/DQ2.2 also confers a high risk of CD (Choung et al., 2020). Bajor et al. (2019) and Sciurti et al. (2018) performed a meta-analysis and demonstrated that a double dose of HLA-DQB1*02 gene (DQ2.5 homozygous, DQ2.5/DQ2.2 heterozygotes) predisposed patients to developing classical CD, although it did not impact disease severity. Individuals with DQ2.5 and DQ8 are also at greater risk than individuals who only encode one heterodimer (Almeida et al., 2016; Megiorni et al., 2009; Poddighe et al., 2020).

This has led many authors to create risk hierarchies for HLA genotyping results and the risk of CD development. As demonstrated in Table 5, the results of risk stratification based on HLA gene dose vary. Of importance when reviewing published studies is to fully consider the level of HLA typing and resolution performed, to ensure complete definition of the CD heterodimers.

10 | VALUE OF HLA TESTING

Multiple studies have shown that over 99% of CD patients have one or a combination of the DQ heterodimers that confer susceptibility (Clouzeau-Girard et al., 2011; Donat et al., 2016; Klapp et al., 2013; Kurppa et al., 2012; Sandstrom et al., 2013; Tucci et al., 2014; Werkstetter et al., 2017; Wolf et al., 2017), making HLA testing exceptionally useful as a test to exclude CD when the susceptibility genotypes are absent (Tye-din et al., 2018). In other words, HLA testing achieves a high negative predictive value for CD disease. However, as the genes for HLA-DQ2/DQ8 are common in the general population (approximately 40% in Europeans), and only 1% of the population develops CD, the presence of these HLA genotypes gives a poor positive predictive value (Kang et al., 2013).

Although HLA testing alone cannot yield a CD diagnosis, it has some crucial benefits over CD serology testing and histology, including the need to only test patients once, non-reliance on gluten consumption for accuracy and the ability to perform tests on blood or buccal swabs which are less invasive. There are therefore several scenarios where knowledge of a patient's HLA-DQ genotype is useful and in particular might benefit (1) infants never exposed to gluten, (2) young children who might not make antibodies, (3) patients with indeterminate serologies or biopsies, (4) relatives of biopsy-diagnosed individuals, (5) patients with IgA deficiency and (6) patients on a self-imposed gluten-free diet unwilling to undergo gluten challenge (Pietzak et al., 2009). In these scenarios, the strong negative predicate value of HLA testing can be used to confidently exclude a diagnosis for CD and remove the need for ongoing clinical monitoring.

Appropriate and accurate HLA testing for CD is therefore critical, with its major clinical utility to exclude CD. Information on the zygosity and particular HLA alleles detected may help with risk stratification for CD. For example, an at-risk family member's monitoring frequency may be determined by the particular HLA genotype.

11 | RECOMMENDED REPORTING AND INTERPRETIVE COMMENTS

Due to the complexity of the HLA system and CD genetics, an HLA genotyping service should be performed by laboratories with the relevant knowledge/experience to enable the correct HLA genotype and interpretation is obtained. HLA genotyping results need to be reported in a clear and concise manner but contain adequate information to allow full consideration by clinicians undertaking patient diagnosis. Reports should clearly state the genotype identified and include relevant interpretative comments.

Laboratories may choose to adopt one of two approaches for reporting CD results. It is recommended that laboratories engage with their service users to identify which is most appropriate:

1. A simple binary 'risk present' or 'risk absent' approach. Given the strong negative predicative value of HLA genotyping to exclude the diagnosis of CD, this approach is recommended and is likely to be sufficient for the majority of laboratories.
2. A risk stratification for CD based on the HLA genotypes detected. This approach can communicate the differences in risk for CD conferred by the different genotypes but is more complex, and the additional information may not be utilised clinically for the majority of patients.

For either approach, reports must be clear that the presence of an at-risk genotype does not confer a diagnosis and has a low positive predictive value for CD, regardless of the HLA genotype identified.

Table 6 provides suggested reporting templates for the HLA genotyping results depending on (i) the test strategy used (definitive HLA genotyping or SNP kit-based detection of specific alleles) and (ii) the reporting approach (binary presence/absence or risk stratification). Each result scenario contains a simple summary comment for the HLA genotype and a choice of suggested interpretative comments

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TABLE 5 HLA risk groups in coeliac disease in the literature.

Publication	Sample size and population	HLA typing information	HLA risk group				
			Very high	High	Intermediate	Low	Very low
Ramakrishna et al., 2021	Cases 259, controls 300, India.	NGS high-resolution DQA1 and DQB1 typing (NGSgo, Genome Diagnostics).	DQ2.5			DQ8	DQ2.2
Paavola et al., 2021	624 index cases and 2943 relatives. White North European, Tampere University Hospital, Finland	Genotyping performed using SSPTM DQB1 low-resolution kit (Olerup), DELFIA Coeliac Disease Hybridisation Assay Kit (PerkinElmer Life and Analytical Sciences) or tagging SNP approach.		DQ2.5/DQ2.5 DQ2.5/DQ2.2	DQ2.5/DQX DQ2.5/DQ8 DQ2.2/DQ2.2 DQ2.2 DQ2.2/DQ8 DQ8/DQ8 DQ8/DQX	DQX	
Choung et al., 2020	24,339 adult cases Mayo Clinic, USA	PCR-SSO (LABType, One Lambda)	DQ2.5/DQ2.5, DQ2.5/DQ2.2 DQ2.2/DQ2.2	DQ2.5/DQX DQ2.5/DQ8		DQ8 homozygous DQ8/DQX	DQ2.2/DQX
Erlichster et al., 2020	Five European case-control datasets. Total cases 4,487, controls 10,455	HLA DQA1 and DQB1 alleles imputed from SNP results.		DQ2.5/DQ2.5 DQ2.5/DQ2.2	DQ2.5/DQX, DQ2.2/DQ7.5, DQ2.5/DQ8, DQ2.5/DQ7.5, DQ2.2/DQ2.2, DQ2.2/DQ8, DQ8/DQ8, DQ8/DQX, DQ8/DQ7.5 and DQ2.2/DQX	DQ7.5/DQX, DQ7.5/DQ7.5 and DQX/DQX	
Bajor et al., 2019	105 cases, Hungry	PCR-SSP (Inno-Train HLA Ready Gene kit) PCR-SSO (Olerup SSO and One Lambda Luminex kit)		DQ2.5/DQ2.5 DQ2.5/DQ2.2	DQ2.5/DQX	DQ8/DQX DQ2.2/DQX	
Poddighe et al., 2019	184 cases, Pavia, Italy	HLA-DQ typing performed by PCR-SSO (DNA-SSO Inno-LiPA Innogenetics)	DQ2/DQ2 DQ2/DQX	DQ2/DQ8	DQ8	DQ2.2	
Martínez-Ojinag et al., 2019	462 paediatric cases, Spain	Genotyping performed using PCR-SSOP for HLA-DRB1, DQA1 and DQB1	DQ2.5/DQ2.5 DQ2.5/DQ2.2				

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TABLE 5 (Continued)

Publication	Sample size and population	HLA typing information	HLA risk group				
			Very high	High	Intermediate	Low	Very low
De Silvestri 2018	Meta-analysis of 10 case control (740 cases and 943 controls) and 3 cohort studies in paediatrics	Various	DQ2/DQ2	DQ8/DQ2 DQ2/DQX	DQ8/DQX	DQA1*05	
Cabrera et al., 2018	196 cases, 206 controls. Malaga, Spain	PCR-SSO LABType (One Lambda) Full DRB1, DQA1 and DQB1 HLA Typing	DQ2.5/DQ2.2	DQ2.5/DQ2.5	DQ2.5/DQX		DQ2.2/DQ2.2 DQ2.2/DQX DQ2.2/DQ8 DQ8/DQ8 DQ8/DQX
Almeida et al., 2016.,	237 patients, 237 controls. Brasilia University Hospital, Brazil	Commercial DQ-CD Typing Plus Kit (BioDiagene) DQA1*05 DQA1*02:01, DQB1*02, DQA1*03, DQB1*03:02 detects homozygosis of DQB1*02 allele	DQ2.5/DQ2.5	DQ2.5/DQ2.2	DQ2.5/DQ8, DQ2.5/DQX DQ2.2/DQ8	DQ2.2/DQ2.2, DQ8/DQX DQ2.2/DQX	DQX.5
Abraham et al., 2015	European (UK 6,785, Finnish 2,476, Dutch 1,649 and Italian 1,040) and American (1,259 cases and 5,437 control) data sets	Illumina genome-wide SNP arrays. HLA DQA1 and DQB1 alleles imputed from SNP results.		DQ2.5/DQ2.5 DQ2.5/DQ2.2	DQ2.2 DQ8 DQ2/DQX	DQX	
Delgado et al., 2014	249 paediatric cases. Sabadell, Spain	PCR-SSP Protrans HLA Celiac Disease Domino System (Pro-trans)DQA1*02:01/03:01/05:01/05:05 HLA-DQB1*02:01/02:02/03:01/03:02 and HLA-DRB1*03/04/07/11	DQ2.5 homozy-gotes	DQ2.5/DQ2.2	DQ2.2/DQ7	DQ2 heterozygotes	DQ2.2/DQ2.2
Rostami-Nejad et al., 2014	59 cases and 151 controls, Iran	PCR assay—six HLA-tagging SNPs to detect DQ2.2, DQ2.5, DQ7 and DQ8		DQ2.5/2.5 DQ2.5/2.2	DQ2 heterozygotes, DQ8 DQ2.2	DQX	

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TABLE 5 (Continued)

Publication	Sample size and population	HLA typing information	HLA risk group				
			Very high	High	Intermediate	Low	Very low
Liu et al., 2014	Six research centres; 3 in the US (Colorado, Georgia, Washington) and 3 in Europe (Finland, Germany and Sweden). 6403 children with DQ2 or DQ8 haplotype.	High-resolution HLA genotyping of DRB1 DQA1 and DQB1		DQ2/DQ2	DQ2/DQX	DQ8/DQ8 DQ8/DQX	
Mubarak et al., 2013	Retrospective cohort 70 cases. Prospective cohort 85 cases. Netherlands	PCR-SSCP heteroduplex assay PCR-SSO Luminex LABType SSO (One Lambda)		DQ2.5/2.5 DQ2.5/2.2	DQ2.5/DQ8 DQ8/DQ2.2	DQX	
Medrano et al., 2012	274 family (trios) and case-control sample (369 cases and 461 controls). Madrid, Spain	PCR-SSOP DRB1, DQA1 and DQB1		DQ2.5/2.5 DQ2.5/2.2	DQ2 heterozygotes DQ2.2/DQ7.5		
Vermeulen et al., 2009	185 paediatric cases. Leiden University Medical Centre, Netherlands	Line probe method for HLA class II low-resolution typing	DQ2.5/2.5 DQ2.5/2.2	DQ2.5/DQ7 DQ2.2/DQ7	DQ2 heterozygotes	DQX	
Thomas et al., 2009	360 cases, 354 controls. Oxford, UK	PCR-SSP		DQ2/DQ2		DQ2.2./DQX DQ2/DQ8 DQ2/DQX	
Romanos et al., 2009	Dutch 508 cases and 888 controls; UK 1,486 cases and 2983 controls; Irish 416 cases and 957 controls; and Italian 508 cases and 593 controls	Tagging SNP approach to predict if individual had 0 1 or 2 HLA DQ2.5 and or DQ2.2 haplotypes		DQ2.5/2.5 DQ2.5/2.2	DQ2.2/2.2, DQ2.2/DQX, DQ2.5/DQX	DQX	

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TABLE 5 (Continued)

Publication	Sample size and population	HLA typing information	HLA risk group				
			Very high	High	Intermediate	Low	Very low
Gudjónsdottír et al., 2009	107 families with at least 2 affected children. Sweden and Southern Norway	PCR-SSOP typing implies DRB1*03-DQA1*05-DQB1*02, DRB1*07-DQA1*0201-DQB1*02, DRB1*11/12-DQA1*05-DQB1*0301 and DRB1*04-DQA1*03-DQB1*0302		DQ2.5/DQ2.5 DQ2.5/DQ2.2	DQ2 heterozygotes DQ2.2/DQ7	DQX	
Nenna et al., 2008	124 cases. Sapienza University, Rome	Commercial PCR-SSP kits (Dynal). DRB1-DQA1 and DQB1 typing		DQ2 homozygotes	DQ2 heterozygotes	DQ negative	
van Belzen et al., 2004	120 unrelated DQ2 positive cases with both parents. Netherlands	PCR-SSCP DQA1 and DQB1 genes		DQ2.5/DQ2.5 DQ2.5/DQ2.2		DQ2.5/DQX	
Margaritte-Jeannin et al., 2004	470 European trio families; Italy (128), France (117), Norway and Sweden (225).	France: DRB1 and DQB1 typing by reverse hybridisation using Murex and Immunogenetics INNO LiPA kits. Italy: DRB1 and DQB1 typing with Dynal AllSet+ SSP low-resolution kits. Sweden and Norway: DQA1 and DQB1 genotyping by PCR-SSO. In French and Italian populations DQA1 alleles were inferred from DQB1 typing using known linkage disequilibrium.		DQ2.5/DQ2.5 DQ2.5/DQ2.2	DQ2.5/DQX	DQX	

Abbreviations: HLA, human leucocyte antigen; NGS, next-generation sequencing.; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; SSCP, single strand conformation polymorphism analysis; SSO, sequence specific oligonucleotide; SSP, sequence specific primers.
Note: DQX, any alleles but not DQ2.5, DQ2.2 or DQ8.

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TABLE 6 Templates for reporting of coeliac HLA genotyping results. It is suggested that laboratories adopt ONE of the formats for reporting HLA typing results (HLA genotype result or HLA coeliac disease heterodimer result) depending on their HLA typing strategy and ONE of the interpretative comments depending on the reporting approach (< 1 > binary presence/absence or < 2 > risk stratification).

i. DQ2.5 homozygous

HLA genotype result:
HLA-DQA1*05, DQB1*02
HLA-DQA1*05, DQB1*02

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) positive
HLA-DQB1*02, DQA1*02 (DQ2.2) negative
HLA-DQB1*03:02 (DQ8) negative

Genotype comment: Positive for DQ2.5 (homozygous)

Interpretative comment < 1 > : The patient has a genotype which is associated with coeliac disease

Interpretative comment < 2 > : This individual has two copies of DQA1*05, DQB1*02 (HLA-DQ2.5) which have a very strong association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

ii. DQ2.5 and DQ2.2 positive result

HLA genotype result:
HLA-DQA1*02, DQB1*02
HLA-DQA1*05, DQB1*02

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) positive
HLA-DQB1*02, DQA1*02 (DQ2.2) positive
DQB1*03:02 (DQ8) negative

Genotype comment: Positive for HLA-DQ2.5 and DQ2.2

Interpretative comment < 1 > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < 2 > : This individual has two copies of DQ2 (DQA1*05, DQB1*02 (HLA-DQ2.5) and DQA1*02, DQB1*02 DQ2.2), which have a very strong association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

iii. DQ2.5 and DQ8 positive result

HLA genotype result:
HLA-DQA1*05, DQB1*02
HLA-DQA1*X, DQB1*03:02

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) positive
HLA-DQB1*02, DQA1*02 (DQ2.2) negative
HLA-DQB1*03:02 (DQ8) positive

Genotype comment: Positive for DQ2.5 and DQ8

Interpretative comment < 1 > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < 2 > : The presence of both DQ2.5 and DQ8 has a strong association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

iv. DQ2.5 heterozygous (cis or trans)

HLA genotype result:
HLA-DQA1*05, DQB1*02
HLA-DQA1*X, DQB1*X
OR
HLA-DQA1*05, DQB1*X
HLA-DQA1*X, DQB1*02

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) positive
HLA-DQB1*02, DQA1*02 (DQ2.2) negative
HLA-DQB1*03:02 (DQ8) negative

Genotype comment: Positive for DQ2.5 (heterozygous)

Interpretative comment < 1 > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < 2 > : This presence of DQA1*05, DQB1*02 (HLA-DQ2.5) has a strong association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

v. DQ8 positive result (homozygous)

HLA genotype result:
HLA-DQA1*X, DQB1*03:02
HLA-DQA1*X, DQB1*03:02

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) negative
HLA-DQB1*02, DQA1*02 (DQ2.2) negative
DQB1*03:02 (DQ8) positive

Genotype comment: Positive for DQ8 (homozygous)

Interpretative comment < 1 > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < 2 > : This individual has two copies of DQ8 (DQB1*03:02) which has a weak association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

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TABLE 6 (Continued)

vi. DQ8 and DQ2.2 positive result

HLA genotype result:
HLA-DQA1*02, DQB1*02
HLA-DQA1*X, DQB1*03:02

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) negative
HLA-DQB1*02, DQA1*02 (DQ2.2) positive
DQB1*03:02 (DQ8) positive

Genotype Comment: Positive for HLA-DQ8 and DQ2.2

Interpretative comment < **1** > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < **2** > : The presence of DQ2.2 and DQ8 has a weak association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

vii. DQ8 positive result

HLA genotype result:
HLA-DQA1*X, DQB1*03:02
HLA-DQA1*X, DQB1*X

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) negative
HLA-DQB1*02, DQA1*02 (DQ2.2) negative
DQB1*03:02 (DQ8) positive

Genotype comment: Positive for DQ8

Interpretative comment < **1** > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < **2** > : This individual carries DQB1*03:02 (DQ8) that has a weak association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

viii. DQ2.2 homozygous positive result

HLA genotype result:
HLA-DQA1*02, DQB1*02
HLA-DQA1*02, DQB1*02

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) negative
HLA-DQB1*02, DQA1*02 (DQ2.2) positive
DQB1*03:02 (DQ8) negative

Genotype comment: Positive for HLA-DQ2.2 (homozygous)

Interpretative comment < **1** > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < **2** > : This individual has two copies of DQA1*02, DQB1*02 (HLA-DQ2.2) which has a weak association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

ix. DQ2.2 positive result

HLA genotype result:
HLA-DQA1*02, DQB1*02
HLA-DQA1*X, DQB1*X

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) negative
HLA-DQB1*02, DQA1*02 (DQ2.2) positive
DQB1*03:02 (DQ8) negative

Genotype comment: Positive for DQ2.2

Interpretative comment < **1** > : This individual has a genotype which is associated with coeliac disease

Interpretative comment < **2** > : This individual carries HLA-DQA1*02, DQB1*02:02 (DQ2.2) that has a weak association with coeliac disease in patients where laboratory tests or symptoms or endoscopic features suggest coeliac disease.

x. DQA1*05 positive result

HLA genotype result:
HLA-DQA1*05, DQB1*X
HLA-DQA1*X, DQB1*X

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) negative
HLA-DQB1*02, DQA1*02 (DQ2.2) negative
DQB1*03:02 (DQ8) negative

Genotype comment: Negative for HLA-DQ2 and DQ8

Interpretative comment < **1** > : This individual does not have a genotype associated with coeliac disease

Interpretative comment < **2** > : This individual does not have a genotype associated with coeliac disease.

xi. DQB1*02 only positive result

HLA Genotype result:
HLA-DQA1*X, DQB1*02
HLA-DQA1*X, DQB1*X

HLA CD heterodimer result:
HLA-DQB1*02, DQA1*05 (DQ2.5) negative
HLA-DQB1*02, DQA1*02 (DQ2.2) negative
DQB1*03:02 (DQ8) negative

Genotype Comment: Negative for DQ2.5 and 2.2

Interpretative Comment < **1** > : This individual does not have a genotype associated with coeliac disease

Interpretative Comment < **2** > : Although this individual has the DQB1*02 variant, it is not present with DQA1*02 or DQA1*05 (to make the DQ2.2 or DQ2.5 heterodimer); therefore, this individual does not carry the HLA-DQ types associated with coeliac disease.

(Continues)

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TABLE 6 (Continued)

xii. No CD heterodimers detected	
HLA genotype result: HLA-DQA1*XX, DQB1*XX HLA-DQA1*XX, DQB1*XX	**HLA CD heterodimer result**: HLA-DQB1*02, DQA1*05 (DQ2.5) negative HLA-DQB1*02, DQA1*02 (DQ2.2) negative HLA-DQB1*03:02 (DQ8) negative
Genotype comment: Negative for HLA-DQ2 and DQ8	
Interpretative comment < **1** > : This individual does not have a genotype associated with coeliac disease	
Interpretative comment < **2** > : This individual does not carry the HLA-DQ types associated with coeliac disease.	
Additional report comment	
<A > The presence of an associated HLA genotype does not confer a diagnosis of coeliac disease and has a low positive predictive value for coeliac disease.	
< B > The absence of an associated genotype excludes the diagnosis of coeliac disease with a high probability (likelihood of coeliac disease < 1%).	

Note: DQX, any allele, but not DQB1*02, DQA1*05, DQA1*02 DQB1*03:02.

depending on the reporting approach. The comments are provided as a suggestion for a possible way of reporting, but other reports may be valid, following the principles that the reports have the correct interpretation of the HLA genotype, are easy to understand, unambiguous and not overly complex.

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DATA AVAILABILITY STATEMENT

No.

REFERENCES

Abadie, V., Sollid, L. M., Barreiro, L. B., & Jabri, B. (2011). Integration of genetic and immunological insights into a model of celiac disease pathogenesis. *Annual Review of Immunology*, *29*, 493–525.

Abraham, G., Rohmer, A., Tye-Din, J. A., & Inouye, M. (2015). Genomic prediction of celiac disease targeting HLA-positive individuals. *Genome Medicine*, *7*(1), 72. https://doi.org/10.1186/s13073-015-0196-5

Airaksinen, L., Laurikka, P., Huhtala, H., Kurppa, K., Salmi, T., Saavalainen, P., Kaukinen, K., & Lindfors, K. (2020). Influence of HLA-DQ2.5 dose on clinical picture of unrelated celiac disease patients. *Nutrients*, *12*(12), 3775. https://doi.org/10.3390/nu12123775

Almeida, L. M., Gandolfi, L., Pratesi, R., Uenishi, R. H., Coutinho de Almeida, F., Selleski, N., & Nobrega, Y. K. M. (2016). Presence of DQ2.2 associated with DQ2.5 increases the risk for celiac disease. *Autoimmune Diseases*, *2016*, 5409653.

Al-Toma, A., Goerres, M. S., Meijer, J. W. R., Peña, A. S., Crusius, J. B. A., & Mulder, C. J. J. (2006). Human leukocyte antigen-DQ2 homozygosity and the development of refractory celiac disease and enteropathy-associated T-cell lymphoma. *Clinical Gastroenterology and Hepatology*, *4*, 315–319.

Al-Toma, A., Volta, U., Auricchio, R., Castillejo, G., Sanders, D. S., Cellier, C., Mulder, C. J., & Lundin, K. E. A. (2019). European Society for the Study of Coeliac Disease (ESsCD) guideline for coeliac disease and other gluten-related disorders. *United European Gastroenterology Journal*, *7*(5), 583–613. https://doi.org/10.1177/2050640619844125

Bajor, J., Szakács, Z., Farkas, N., Hegyi, P., Illés, A., Solymár, M., Pétervári, E., Balaskó, M., Pár, G., Sarlós, P., Szűcs, Á., Czimmer, J., Szemes, K., Huszár, O., Varjú, P., & Vincze, Á. (2019). Classical celiac disease is more frequent with a double dose of HLA-DQB1*02 : A systematic review with meta-analysis. *PLoS ONE*, *14*, 1–19.

Bajor, J., Szakács, Z., & Vincze, Á. (2019a). Response to Letter to the Editor: Relevance of HLA-DQB1*02 allele in predisposing to coeliac disease. *International Journal of Immunogenetics*, *46*(4), 276–277. https://doi.org/10.1111/iji.12428

Bergseng, E., Dørum, S., Arntzen, M. Ø., Nielsen, M., Nygård, S., Buus, S., De Souza, G. A., & Sollid, L. M. (2015). Different binding motifs of the celiac disease-associated HLA molecules DQ2.5, DQ2.2, and DQ7.5 revealed by relative quantitative proteomics of endogenous peptide repertoires. *Immunogenetics*, *67*(2), 73–84. Epub 2014 Dec 12. https://doi.org/10.1007/s00251-014-0819-9

Bodd, M., Kim, C.-Y., Lundin, K. E. A., & Sollid, L. M. (2012). T-cell response to gluten in patients with HLA-DQ2.2 reveals requirement of peptide-MHC stability in celiac disease. *Gastroenterol*, *142*, 552–561.

Brown, N. K., Guandalini, S., Semrad, C., & Kupfer, S. S. (2019). A clinician's guide to celiac disease HLA genetics. *American Journal of Gastroenterology*, *114*, 1587–1592. https://doi.org/10.14309/ajg.0000000000000310

Cabrera, C. M., Méndez-López, I. M., & Caballero, A. (2018). Risk variation in celiac disease in a population from Southern Spain: Evaluating the influence of the DQB1*02:02 allele frequency. *Scandinavian Journal of Gastroenterology*, *53*(3), 266–272. Epub 2018 Jan 23. https://doi.org/10.1080/00365521.2018.1430253

Caio, G., Volta, U., Sapone, A., Leffler, D. A., De Giorgio, R., Catassi, C., & Fasano, A. (2019). Celiac disease: A comprehensive current review. *BMC Medicine [Electronic Resource]*, *17*(1), 142. https://doi.org/10.1186/s12916-019-1380-z

Choung, R. S., Mills, J. R., Snyder, M. R., Murray, J. A., & Gandhi, M. J. (2020). Celiac disease risk stratification based on HLA-DQ heterodimer (HLA-DQA1 ~ DQB1) typing in a large cohort of adults with suspected celiac disease. *Human Immunology*, *81*(2–3), 59–64. Epub 2020 Jan 28. https://doi.org/10.1016/j.humimm.2020.01.006

Clouzeau-Girard, H., Rebouissoux, L., Taupin, J.-L., Le Bail, B., Kalach, N., Michaud, L., Dabadie, A., Olives, J.-P., Blanco, P., Morali, A., Moreau, J.-F., & Lamireau, T. (2011). HLA-DQ genotyping combined with serological markers for the diagnosis of celiac disease: Is intestinal biopsy still mandatory? *Journal of Pediatric Gastroenterology and Nutrition*, *52*, 729–733.

De'Ath, A., & Rees, T. (2019). Results from the UK NEQAS for H&I scheme 8: HLA genotyping for coeliac and other HLA associated diseases. *International Journal of Immunogenetic*, *46*, 213.

De Silvestri, A., Capittini, C., Poddighe, D., Valsecchi, C., Marseglia, G., Tagliacarne, S. C., Scotti, V., Rebuffi, C., Pasi, A., Martinetti, M., & Tinelli, C. (2018). HLA-DQ genetics in children with celiac disease: A meta-analysis suggesting a two-step genetic screening procedure starting with HLA-DQ β chains. *Pediatric Research*, *83*, 564–572. https://doi.org/10.1038/pr.2017.307

1744313x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/iji.12649 by Carla Rosser - Test , Wiley Online Library on [03/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Delgado, J. F., Amengual, M. J., Veraguas, A., Rodríguez, E., De Los Santos, M. M., & Guallarte, M. P. (2014). Paediatric celiac patients carrying the HLA-DR7-DQ2 and HLA-DR3-DQ2 haplotypes display small clinical differences. *Acta Paediatrica*, *103*(6), e238–e242. Epub 2014 Mar 17. https://doi.org/10.1111/apa.12605

Dendrou, C. A., Petersen, J., Rossjohn, J., & Fugger, L. (2018). HLA variation and disease. *Nature Reviews Immunology*, *18*, 325–339. https://doi.org/10.1038/nri.2017.143

Donat, E., Ramos, J. M., Sánchez-Valverde, F., Moreno, A., Martinez, M.-J., Leis, R., Peña-Quintana, L., Castillejo, G., Fernández, S., Garcia, Z., Ortigosa, L., Balmaseda, E., Marugán, J.-M., Eizaguirre, F.-J., Lorenzo, H., Barrio, J., & Ribes-Koninckx, C. (2016). ESPGHAN 2012 guidelines for coeliac disease diagnosis: Validation through a retrospective Spanish multicentric study. *Journal of Pediatric Gastroenterology and Nutrition*, *62*(2), 284–291.

Erlichster, M., Bedo, J., Skafidas, E., Kwan, P., Kowalczyk, A., & Goudey, B. (2020). Improved HLA-based prediction of coeliac disease identifies two novel genetic interactions. *European Journal of Human Genetics*, *28*(12), 1743–1752. Epub 2020 Jul 30. https://doi.org/10.1038/s41431-020-0700-2

Fallang, L.-E., Bergseng, E., Hotta, K., Berg-Larsen, A., Kim, C.-Y., & Sollid, L. M. (2009). Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. *Nature Immunology*, *10*, 1096–1101.

Garner, C., Ahn, R., Ding, Y. C., Steele, L., Stoven, S., Green, P. H., Fasano, A., Murray, J. A., & Neuhausen, S. L. (2014). Genome-wide association study of celiac disease in North America confirms FRMD4B as new celiac locus. *PLoS ONE*, *9*, e101428.

Gonzalez-Galarza, F. F., McCabe, A., Santos, E. J., Jones, J., Takeshita, L. Y., Ortega-Rivera, N. D., Del Cid-Pavon, G. M., Ramsbottom, K., Ghattaoraya, G. S., Alfirevic, A., Middleton, D., & Jones, A. R. (2020). Allele frequency net database (AFND) 2020 update: Gold-standard data classification, open access genotype data and new query tools. *Nucleic Acid Research*, *48*, D783–D788.

Gragert, L., Madbouly, A., Freeman, J., & Maiers, M. (2013). Six-locus high resolution HLA haplotype frequencies derived from mixed-resolution DNA typing for the entire US donor registry. *Human Immunology*, *74*, 1313–1320.

Gudjónsdóttir, A. H., Nilsson, S., Naluai, Å. T., Ek, J., Amundsen, S. S., Wahlström, J., & Ascher, H. (2009). Association between genotypes and phenotypes in coeliac disease. *Journal of Pediatric Gastroenterology and Nutrition*, *49*(2), 165–169. https://doi.org/10.1097/MPG.0b013e318196c362

Horan, M. P., Chai, S. Y., Munusamy, N., Tay, K. H., Wienholt, L., Tye-Din, J. A., Daveson, J., Varney, M., & Badrick, T. (2018). High rates of variation in HLA-DQ2/DQ8 testing for coeliac disease: Results from an RCPAQAP pilot program. *Journal of Clinical Pathology*, *71*(10), 900–905. Epub 2018 May 15. https://doi.org/10.1136/jclinpath-2018-205209

Husby, S., Koletzko, S., Korponay-Szabó, I. R., Mearin, M. L., Phillips, A., Shamir, R., Troncone, R., Giersiepen, K., Branski, D., Catassi, C., Lelgeman, M., Mäki, M., Ribes-Koninckx, C., Ventura, A., & Zimmer, K. P. (2012). European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. *Journal of Pediatric Gastroenterology and Nutrition*, *54*, 136–160. https://doi.org/10.1097/MPG.0b013e31821a23d0

Husby, S., Koletzko, S., Korponay-Szabó, I., Kurppa, K., Mearin, M. L., Ribes-Koninckx, C., Shamir, R., Troncone, R., Auricchio, R., Castillejo, G., Christensen, R., Dolinsek, J., Gillett, P., Hróbjartsson, A., Koltai, T., Maki, M., Nielsen, S. M., Popp, A., Størdal, K., … Wessels, M. (2020). European Society Paediatric Gastroenterology, Hepatology and Nutrition guidelines for diagnosing coeliac disease. *Journal of Pediatric Gastroenterology and Nutrition*, *70*, 141–156. https://doi.org/10.1097/MPG.0000000000002497

Jabri, B., & Sollid, L. M. (2006). Mechanisms of disease: Immunopathogenesis of celiac disease. *Nature Clinical Practice Gastroenterology & Hepatology*, *3*, 516–525.

Kang, J. Y., Kang, A. H. Y., Green, A., Gwee, K. A., & Ho, K. Y. (2013). Systematic review: Worldwide variation in the frequency of coeliac disease and changes over time. *Alimentary Pharmacology & Therapeutics*, *38*(3), 226–245. Epub 2013 Jun 18. https://doi.org/10.1111/apt.12373

Karell, K., Louka, A. S., Moodie, S. J., Ascher, H., Clot, F., Greco, L., Ciclitira, P. J., Sollid, L. M., & Partanen, J. (2003). HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: Results from the European genetics cluster on celiac disease. *Human Immunology*, *64*(4), 469–477. https://doi.org/10.1016/S0198-8859(03)00027-2

Karinen, H., Kärkkäinen, P., Pihlajamäki, J., Janatuinen, E., Heikkinen, M., Julkunen, R., Kosma, V.-M., Naukkarinen, A., & Laakso, M. (2006). Gene dose effect of the DQB1*0201 allele contributes to severity of coeliac disease. *Scandinavian Journal of Gastroenterology*, *41*, 191–199.

Klapp, G., Masip, E., Bolonio, M., Donat, E., Polo, B., Ramos, D., & Ribes-Koninckx, C. (2013). Celiac disease: The new proposed ESPGHAN diagnostic criteria do work well in a selected population. *Journal of Pediatric Gastroenterology and Nutrition*, *56*(3), 251–256.

Koning, F. (2012). Celiac disease: Quantity matters. *Seminars in Immunopathology*, *34*, 541–549. https://doi.org/10.1007/s00281-012-0321-0

Koskinen, L., Romanos, J., Kaukinen, K., Mustalahti, K., Korponay-Szabo, I., Barisani, D., Bardella, M. T., Ziberna, F., Vatta, S., Széles, G., Pocsai, Z., Karell, K., Haimila, K., Ádány, R., Not, T., Ventura, A., Mäki, M., Partanen, J., Wijmenga, C., & Saavalainen, P. (2009). Cost-effective HLA typing with tagging SNPs predicts celiac disease risk haplotypes in the Finnish, Hungarian, and Italian populations. *Immunogenetics*, *61*, 247–256. https://doi.org/10.1007/s00251-009-0361-3

Kuja-Halkola, R., Lebwohl, B., Halfvarson, J., Wijmenga, C., Magnusson, P. K. E., & Ludvigsson, J. F. (2016). Heritability of non-HLA genetics in coeliac disease: A population-based study in 107 000 twins. *Gut*, *65*, 1793–1798.

Kurppa, K., Salminiemi, J., Ukkola, A., Saavalainen, P., Löytynoja, K., Laurila, K., Collin, P., Mäki, M., & Kaukinen, K. (2012). Utility of the new ESPGHAN criteria for the diagnosis of celiac disease in at-risk groups. *Journal of Pediatric Gastroenterology and Nutrition*, *54*, 387–391.

Lázár-Molnár, E., & Snyder, M. (2018). The role of human leukocyte antigen in celiac disease diagnostics. *Clinics in Laboratory Medicine*, *38*(4), 655–668. Epub 2018 Oct 5. https://doi.org/10.1016/j.cll.2018.07.007

Lebwohl, B., Sanders, D. S., & Green, P. H. (2018). Coeliac disease. *The Lancet*, *391*, 70–81.

Lionetti, E., Castellaneta, S., Francavilla, R., Pulvirenti, A., Tonutti, E., Amarri, S., Barbato, M., Barbera, C., Barera, G., Bellantoni, A., Castellano, E., Guariso, G., Limongelli, M. G., Pellegrino, S., Polloni, C., Ughi, C., Zuin, G., Fasano, A., & Catassi, C. (2014). SIGENP (Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition) working group on weaning and CD risk. Introduction of gluten, HLA status, and the risk of celiac disease in children. *New England Journal of Medicine*, *371*(14), 1295–1303. https://doi.org/10.1056/NEJMoa1400697

Liu, E., Lee, H.-S., Aronsson, C. A., Hagopian, W. A., Koletzko, S., Rewers, M. J., Eisenbarth, G. S., Bingley, P. J., Bonifacio, E., Simell, V., & Agardh, D. (2014). Risk of pediatric celiac disease according to HLA haplotype and country. *New England Journal of Medicine*, *371*, 42–49.

Ludvigsson, J. F., Bai, J. C., Biagi, F., Card, T. R., Ciacci, C., Ciclitira, P. J., Green, P. H. R., Hadjivassiliou, M., Holdoway, A., Van Heel, D. A., Kaukinen, K., Leffler, D. A., Leonard, J. N., Lundin, K. E. A., Mcgough, N., Davidson, M., Murray, J. A., Swift, G. L., Walker, M. M., … Sanders, D. S. (2014). Diagnosis and management of adult coeliac disease: Guidelines from the British Society of Gastroenterology. *Gut*, *63*, 1210–1228. https://doi.org/10.1136/gutjnl-2013-306578

Margaritte-Jeannin, P., Babron, M. C., Bourgey, M., Louka, A. S., Clot, F., Percopo, S., Coto, I., Hugot, J. P., Ascher, H., Sollid, L. M., Greco, L., & Clerget-Darpoux, F. (2004). HLA-DQ relative risks for coeliac disease in European populations: A study of the European genetics cluster on coeliac disease. *Tissue Antigens*, *63*(6), 562–567. https://doi.org/10.1111/j.0001-2815.2004.00237.x

Marsh, S. G. E., Albert, E. D., Bodmer, W. F., Bontrop, R. E., Dupont, B., Erlich, H. A., Fernández-Viña, M., Geraghty, D. E., Holdsworth, R., Hurley,

1744313x, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jji.12649 by Carla Rosser - Test , Wiley Online Library on [03/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

C. K., Lau, M., Lee, K. W., Mach, B., Maiers, M., Mayr, W. R., Müller, C. R., Parham, P., Petersdorf, E. W., Sasazuki, T., … Trowsdale, J. (2010). Nomenclature for factors of the HLA system 2010. *Tissue Antigens*, *75*, 291–455. https://doi.org/10.1111/j.1399-0039.2010.01466.x

Martínez-Ojinaga, E., Fernández-Prieto, M., Molina, M., Polanco, I., Urcelay, E., & Núñez, C. (2019). Influence of HLA on clinical and analytical features of pediatric celiac disease. *BMC Gastroenterology [Electronic Resource]*, *19*(1), 91. https://doi.org/10.1186/s12876-019-1014-0

Matzaraki, V., Kumar, V., Wijmenga, C., & Zhernakova, A. (2017). The MHC locus and genetic susceptibility to autoimmune and infectious diseases. *Genome Biology*, *18*, 76. https://doi.org/10.1186/s13059-017-1207-1

Medrano, L. M., Dema, B., López-Larios, A., Maluenda, C., Bodas, A., López-Palacios, N., Figueredo, M. Á., Fernández-Arquero, M., & Núñez, C. (2012). HLA and celiac disease susceptibility: New genetic factors bring open questions about the HLA influence and gene-dosage effects. *PLoS ONE*, *7*(10), e48403. Epub 2012 Oct 31. https://doi.org/10.1371/journal.pone.0048403

Megiorni, F., Mora, B., Bonamico, M., Barbato, M., Nenna, R., Maiella, G., Lulli, P., & Mazzilli, M. C. (2009). HLA-DQ and risk gradient for celiac disease. *Human Immunology*, *70*, 55–59.

Megiorni, F., & Pizzuti, A. (2012). HLA-DQA1 and HLA-DQB1 in celiac disease predisposition: Practical implications of the HLA molecular typing. *Journal of Biomedical Science*, *19*, 88. https://doi.org/10.1186/1423-0127-19-88

Monsuur, A. J., De Bakker, P. I. W., Zhernakova, A., Pinto, D., Verduijn, W., Romanos, J., Auricchio, R., Lopez, A., Van Heel, D. A., Crusius, J. B. A., & Wijmenga, C. (2008). Effective detection of human leukocyte antigen risk alleles in celiac disease using tag single nucleotide polymorphisms. *PLoS ONE*, *3*, e2270. https://doi.org/10.1371/journal.pone.0002270

Mubarak, A., Spierings, E., Wolters, V., Van Hoogstraten, I., Kneepkens, C. M. F., & Houwen, R. (2013). Human leukocyte antigen DQ2.2 and celiac disease. *Journal of Pediatric Gastroenterology and Nutrition*, *56*(4), 428–430. https://doi.org/10.1097/MPG.0b013e31827913f9

Murch, S., Jenkins, H., Auth, M., Bremner, R., Butt, A., France, S., Furman, M., Gillett, P., Kiparissi, F., Lawson, M., Mclain, B., Morris, M.-A., Sleet, S., & Thorpe, M. (2013). BSPGHAN. Joint BSPGHAN and Coeliac UK guidelines for the diagnosis and management of coeliac disease in children. *Archives of Disease in Childhood*, *98*(10), 806–811. Epub 2013 Aug 28. https://doi.org/10.1136/archdischild-2013-303996

Mustalahti, K., Catassi, C., Reunanen, A., Fabiani, E., Heier, M., Mcmillan, S., Murray, L., Metzger, M.-H., Gasparin, M., Bravi, E., & Mäki, M. (2010). The prevalence of celiac disease in Europe: Results of a centralized, international mass screening project. *Annals of Medicine*, *42*(8), 587–595.

Nenna, R., Mora, B., Megiorni, F., Mazzilli, M. C., Magliocca, F. M., Tiberti, C., & Bonamico, M. (2008). HLA-DQB1*02 dose effect on RIA anti-tissue transglutaminase autoantibody levels and clinicopathological expressivity of celiac disease. *Journal of Pediatric Gastroenterology and Nutrition*, *47*(3), 288–292. https://doi.org/10.1097/MPG.0b013e3181615ca7

Núñez, C., Garrote, J. A., Arranz, E., Bilbao, J. R., Fernández Bañares, F., Jiménez, J., Perucho, T., Ruiz Casares, E., Sánchez-Valverde, F., & Serrano, N. (2018). Recommendations to report and interpret HLA genetic findings in coeliac disease. *Revista Espanola De Enfermedades Digestivas*, *110*(7), 458–461. https://doi.org/10.17235/reed.2018.5269/2017

Pallav, K., Kabbani, T., Tariq, S., Vanga, R., Kelly, C. P., & Leffler, D. A. (2014). Clinical utility of celiac disease-associated HLA testing. *Digestive Diseases and Sciences*, *59*(9), 2199–2206. Epub 2014 Apr 6. https://doi.org/10.1007/s10620-014-3143-1

Pietzak, M. M., Schofield, T. C., Mcginniss, M. J., & Nakamura, R. M. (2009). Stratifying risk for celiac disease in a large at-risk United States population by using HLA alleles. *Clinical Gastroenterol Hepatol*, *7*, 966–971. https://doi.org/10.1016/j.cgh.2009.05.028

Pisapia, L., Camarca, A., Picascia, S., Bassi, V., Barba, P., Del Pozzo, G., & Gianfrani, C. (2016). HLA-DQ2.5 genes associated with celiac disease risk are preferentially expressed with respect to non-predisposing HLA genes: Implication for anti-gluten T cell response. *Journal of Autoimmunity*, *2016*, 63–72. Epub 2016 Apr 12. https://doi.org/10.1016/j.jaut.2016.03.016

Ploski, R., Ek, J., Thorsby, E., & Sollid, L. M. (1993). On the HLA-DQ(alpha 1*0501, beta 1*0201)-associated susceptibility in celiac disease: A possible gene dosage effect of DQB1*0201. *Tissue Antigens*, *41*(4), 173–177. https://doi.org/10.1111/j.1399-0039.1993.tb01998.x

Poddighe, D., Capittini, C., Gaviglio, I., Brambilla, I., & Marseglia, G. L. (2019). HLA-DQB1*02 allele in children with celiac disease: Potential usefulness for screening strategies. *International Journal of Immunogenetics*, *46*(5), 342–345. https://doi.org/10.1111/iji.12441

Poddighe, D., Rebuffi, C., Silvestri, A. D., & Capittini, C. (2020). Epidemiological importance of HLA-DQB1*02 carrier status in CD patients. *World Journal of Gastroenterology*, *26*(12), 1365–1381. https://doi.org/10.3748/wjg.v26.i12.1365

Ramakrishna, B. S., Venugopal, G., Singh, A., Pugazhendhi, S., Dutta, S., Ahuja, V., & Makharia, G. K. (2021). Human leukocyte antigen DQ (HLA-DQ) genotypes and haplotypes and their association with phenotype in patients with celiac disease in India. *JGH Open*, *5*(10), 1190–1196. https://doi.org/10.1002/jgh3.12651

Robinson, J., Halliwell, J. A., Hayhurst, J. D., Flicek, P., Parham, P., & Marsh, S. G. E. (2015). The IPD and IMGT/HLA database: Allele variant databases. *Nucleic Acids Res.*, *43*(Database issue), D423–D431. Epub 2014 Nov 20. https://doi.org/10.1093/nar/gku1161

Romanos, J., Van Diemen, C. C., Nolte, I. M., Trynka, G., Zhernakova, A., Fu, J., Bardella, M. T., Barisani, D., Mcmanus, R., Van Heel, D. A., & Wijmenga, C. (2009). Analysis of HLA and non-HLA alleles can identify individuals at high risk for celiac disease. *Gastroenterology*, *137*(3), 834–840.e3. Epub 2009 May 18. https://doi.org/10.1053/j.gastro.2009.05.040

Rostami-Nejad, M. (2014). Allele and haplotype frequencies for HLA-DQ in Iranian celiac disease patients. *World Journal of Gastroenterology*, *20*(20), 6302–6308. https://doi.org/10.3748/wjg.v20.i20.6302

Sandström, O., Rosén, A., Lagerqvist, C., Carlsson, A., Hernell, O., Högberg, L., & Ivarsson, A. (2013). Transglutaminase IgA antibodies in a celiac disease mass screening and the role of HLA-DQ genotyping and endomysial antibodies in sequential testing. *Journal of Pediatric Gastroenterology and Nutrition*, *57*, 472–476.

Sciurti, M., Fornaroli, F., Gaiani, F., Bonaguri, C., Leandro, G., Di Mario, F., & De' Angelis, G. L. (2018). Genetic susceptibility and celiac disease: What role do HLA haplotypes play? *Acta Biomedica*, *89*(9–S), 17–21. https://doi.org/10.23750/abm.v89i9-S.7953

Singh, P., Arora, S., Lal, S., Strand, T. A., & Makharia, G. K. (2015). Risk of celiac disease in the first- and second-degree relatives of patients with celiac disease: A systematic review and meta-analysis. *American Journal of Gastroenterology*, *110*, 1539–1548.

Singh, P., Arora, A., Strand, T. A., Leffler, D. A., Catassi, C., Green, P. H., Kelly, C. P., Ahuja, V., & Makharia, G. K. (2018). Global prevalence of celiac disease: Systematic review and meta-analysis. *Clinical Gastroenterology and Hepatology*, *16*, 823–836.e2. https://doi.org/10.1016/j.cgh.2017.06.037

Thomas, H. J., Ahmad, T., Rajaguru, C., Barnardo, M., Warren, B. F., & Jewell, D. P. (2009). Contribution of histological, serological, and genetic factors to the clinical heterogeneity of adult-onset coeliac disease. *Scandinavian Journal of Gastroenterology*, *44*(9), 1076–1083. https://doi.org/10.1080/00365520903100473

Ting, Y. T., Dahal-Koirala, S., Kim, H. S. K., Qiao, S.-W., Neumann, R. S., Lundin, K. E. A., Petersen, J., Reid, H. H., Sollid, L. M., & Rossjohn, J. (2020). A molecular basis for the T cell response in HLA-DQ2.2 mediated celiac disease. *PNAS*, *117*(6), 3063–3073. Epub 2020 Jan 23. https://doi.org/10.1073/pnas.1914308117

Tjon, J. M.-L., Van Bergen, J., & Koning, F. (2010). Celiac disease: How complicated can it get? *Immunogenetics*, *62*, 641–651. https://doi.org/10.1007/s00251-010-0465-9

Tolone, C., Piccirillo, M., Dolce, P., Alfiero, S., Arenella, M., Sarnataro, M., Iardino, P., Pucciarelli, A., & Strisciuglio, C. (2021). Celiac disease in pediatric patients according to HLA genetic risk classes: A retrospective

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observational study. *Italian Journal of Pediatrics*, *47*(1), 107. https://doi.org/10.1186/s13052-021-01052-1

Trynka, G., Hunt, K. A., Bockett, N. A., Romanos, J., Mistry, V., Szperl, A., Bakker, S. F., Bardella, M. T., Bhaw-Rosun, L., Castillejo, G., De La Concha, E. G., De Almeida, R. C., Dias, K.-R. M., Van Diemen, C. C., Dubois, P. C. A., Duerr, R. H., Edkins, S., Franke, L., Fransen, K., … Van Heel, D. A. (2011). Dense genotyping identifies and localizes multiple common and rare variant association signals in celiac disease. *Nature Genetics*, *43*, 1193–1201.

Tucci, F., Astarita, L., Abkari, A., Abu-Zekry, M., Attard, T., Ben Hariz, M., Bilbao, J. R., Boudraa, G., Boukthir, S., Costa, S., Djurisic, V., Hugot, J.-P., Irastorza, I., Kansu, A., Kolaček, S., Magazzù, G., Mičetić-Turk, D., Misak, Z., Roma, E., … Greco, L. (2014). Celiac disease in the Mediterranean area. *BMC Gastroenterology [Electronic Resource]*, *14*, 24.

Tye-Din, J. A., Cameron, D. J. S., Daveson, A. J., Day, A. S., Dellsperger, P., Hogan, C., Newnham, E. D., Shepherd, S. J., Steele, R. H., Wienholt, L., & Varney, M. D. (2015). Appropriate clinical use of human leukocyte antigen typing for coeliac disease: An Australasian perspective. *Internal Medicine Journal*, *45*(4), 441–450. https://doi.org/10.1111/imj.12716

Tye-Din, J. A., Galipeau, H. J., & Agardh, D. (2018). Celiac disease : A review of current concepts in pathogenesis, prevention, and novel therapies. *Front Pediatr*, *6*, 1–19. https://doi.org/10.3389/fped.2018.00350

Vader, W., Stepniak, D., Kooy, Y., Mearin, L., Thompson, A., Van Rood, J. J., Spaenij, L., & Koning, F. (2003). The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T cell responses. *Proceedings National Academy of Science USA*, *100*(21), 12390–12395.

Van Belzen, M. J., Koeleman, B. P. C., Crusius, J. B. A., Meijer, J. W. R., Bardoel, A. F. J., Pearson, P. L., Sandkuijl, L. A., Houwen, R. H. J., & Wijmenga, C. (2004). Defining the contribution of the HLA region to cis DQ2-positive coeliac disease patients. *Genes and Immunity*, *5*(3), 215–220. https://doi.org/10.1038/sj.gene.6364061

Van Heel, D. A., Franke, L., Hunt, K. A., Gwilliam, R., Zhernakova, A., Inouye, M., Wapenaar, M. C., Barnardo, M., Bethel, G., Holmes, G. K. T., Feighery, C., Jewell, D., Kelleher, D., Kumar, P., Travis, S., Walters, J. R., Sanders, D. S., Howdle, P., Swift, J., … Wijmenga, C. (2007). A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. *Nature Genetics*, *39*(7), 827–829. https://doi.org/10.1038/ng2058

Vermeulen, B. A. N., Hogen Esch, C. E., Yüksel, Z., Koning, F., Verduijn, W., Doxiadis, I. I. N., Schreuder, G. M. T., & Mearin, M. L. (2009). Phenotypic variance in childhood coeliac disease and the HLA-DQ/DR dose effect. *Scandinavian Journal of Gastroenterology*, *44*(1), 40–45. https://doi.org/10.1080/00365520802116422

Werkstetter, K. J., Korponay-Szabó, I. R., Popp, A., Villanacci, V., Salemme, M., Heilig, G., Lillevang, S. T., Mearin, M. L., Ribes-Koninckx, C., Thomas, A., Troncone, R., Filipiak, B., Mäki, M., Gyimesi, J., Najafi, M., Dolinšek, J., Dydensborg Sander, S., Auricchio, R., Papadopoulou, A., … Eftekhar Sadat, A. T. (2017). Accuracy in diagnosis of celiac disease without biopsies in clinical practice. *Gastroenterology*, *153*, 924–935.

Wolf, J., Petroff, D., Richter, T., Auth, M. K. H., Uhlig, H. H., Laass, M. W., Lauenstein, P., Krahl, A., Händel, N., De Laffolie, J., Hauer, A. C., Kehler, T., Flemming, G., Schmidt, F., Rodrigues, A., Hasenclever, D., & Mothes, T. (2017). Validation of antibody-based strategies for diagnosis of pediatric celiac disease without biopsy. *Gastroenterology*, *153*(2), 410–419.e17.

Wolters, V. M., & Wijmenga, C. (2008). Genetic background of celiac disease and its clinical implications. *American Journal of Gastroenterology*, *103*, 190–195. https://doi.org/10.1111/j.1572-0241.2007.01471.x

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