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Clinical utility of celiac disease associated HLA testing

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Abstract

Background—Negative predictive value (NPV) of Celiac Disease (CD) related human leukocyte antigens (HLA) DQ2 and DQ8 approaches 100% in individual patients. However, studies evaluating its exclusionary utility in patient groups are lacking.

Aim—We aim to assess the performance of HLA testing when applied to patient groups with varying characteristics and propose evidence-based recommendations for its clinical use.

Methods—Demographic and clinical information was recorded in patients undergoing HLA testing. Using predetermined criteria, patients were classified as CD, Non CD or indeterminate. Diagnostic yield of HLA testing was defined as the percentage of patients in whom CD could be excluded based on negative HLA test.

Results—256 patients underwent testing for CD related HLA DQ2 and DQ8. 102 (100 Non CD, 2 CD) patients tested HLA negative for a 98% NPV and 39% diagnostic yield. Diagnostic yield was highest (60%) in patients with intraepithelial lymphocytosis plus normal IgA tissue transglutaminase antibody (IgA-tTG) and lowest in patients with positive IgA-tTG plus villous atrophy (0%). CD was diagnosed in 2 HLA negative patients, who carried half of DQ2.5 *trans* genotype.

Conclusions—Diagnostic yield of CD related HLA testing varies widely depending on clinical indication. HLA testing is a practical and valuable test for most patients in whom initial evaluation for CD is inconclusive. A negative HLA result usually obviates the need for further celiac testing including endoscopy and gluten challenge. Rarely, in patients reported as HLA negative, half of HLA DQ2.5 (*cis* or *trans*) is sufficient for development of CD.

Keywords

Celiac Disease; Genetic testing; Human Leukocyte Antigen; HLA DQ2; HLA DQ8

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CONFLICT OF INTEREST

1. Daniel Leffler has served research support and/or consulted for Prometheus Laboratories, Shire Pharmaceuticals, Alvine Pharmaceuticals, Alba Therapeutics, Ironwood pharmaceuticals, Inova Diagnostics.
2. Ciaran P. Kelly has acted as scientific advisor for Alvine Pharmaceuticals, Alba Therapeutics and ImmunosantT.

INTRODUCTION

Celiac disease (CD) affects approximately 1% of the population in the Western world as well as in North Africa, India and South America [1–5]. The incidence is higher in individuals with insulin dependent diabetes mellitus [6, 7], autoimmune thyroid disease [8–10] and a family history of CD [5, 11–13]. Diagnostic rates for CD are on the rise due to increasing prevalence [14, 15], growing awareness, and the high sensitivity and specificity of tests for IgA antibodies to tissue transglutaminase (IgA-tTG) [16–19]. The diagnosis of CD currently requires documentation of characteristic histological findings on duodenal biopsy [20].

Both serological and histological methods currently recommended for diagnosis of CD have limitations. Approximately 5% of CD patients can present with normal serum levels of IgA-tTG (seronegative CD) [17, 19, 21–23]. Small intestinal changes can be limited to intraepithelial lymphocytosis without the presence of duodenal villous atrophy (Marsh 1), which is a sensitive yet nonspecific finding for CD [24–26]. Villous atrophy (VA) can also be patchy [27], leading to a false negative histological evaluation. Conversely, VA, can occur in conditions other than CD [28]. Additionally, institution of a gluten free diet (GFD) leads to serological and histological remission, requiring reintroduction of dietary gluten for a period of 6–8 weeks before meaningful evaluation can be conducted [29].

Nearly all patients with CD express at least one of the two Major Histocompatibility Complex (MHC) class 2 molecules (human leukocyte antigen (HLA) DQ2 and HLA DQ8) critical for pathogenesis of CD. Accordingly, the absence of the corresponding genes virtually excludes a diagnosis of CD with a very high negative predictive value, independent of dietary gluten content [30–38]. The positive predictive value of HLA DQ2/DQ8 is however limited as they can be present in approximately 40% of the general population, most of whom will never develop CD [39, 40].

Available literature on the clinical role of HLA testing in CD is limited to evaluations of risk gradients [41–43], disease severity [43, 44] and sex distribution [45]. HLA testing has also been studied as a screening tool in high-risk populations and as a diagnostic tool for CD in combination with serology [34, 46–49].

While a negative HLA test rules out CD in a given patient with almost 100% certainty, there are limited data evaluating its ability to rule out CD (diagnostic yield) in groups of patients undergoing evaluation for celiac disease. The objectives of this study are to determine the diagnostic yield of celiac HLA testing and to develop evidence-based recommendations regarding its utility in the evaluation of subsets of patients suspected to have CD.

METHODS

This retrospective study was carried out at The Celiac Center at Beth Israel Deaconess Medical Center (BIDMC), which serves as a tertiary care referral center for patients with known, suspected or complicated CD. HLA testing was performed on blood samples by high-resolution PCR using commercially available sequence specific primers (DQB1 and DQA1: Invitrogen, Carlsbad, California; DQB1: Biotest Diagnostics Denville, NJ). Eight

major alleles that encode HLA DQ2.5-*cis* (DQA⁰⁵⁰¹/DQB⁰²⁰¹), HLA DQ2.5-*trans* (DQA⁰⁵⁰⁵/DQB⁰³⁰¹ + DQA⁰²⁰¹/DQB⁰²⁰²), HLA DQ2.2 (DQA⁰²⁰¹/DQB⁰²⁰²) and DQ8 (DQA⁰³⁰¹/DQB⁰³⁰²) were sought. Patients were considered to be positive for CD enabling HLA genes if they carried the alleles that encode DQ2.5 and/or DQ2.2 and/or DQ8 genotype or alpha chain of DQ2.5-*cis* genotype (DQA⁰⁵⁰¹) [50–52]. Quantitative IgA-tTG testing was performed through INOVA diagnostics using ELISA technique. Absolute values of serum IgA-tTG were reported according to the manufacturer's cutoff as IU/ml and classified as i) Normal (0–19 IU/ml) ii) Borderline positive (20–39 IU/ml) and iii) Positive (>39 IU/ml).

Detailed information regarding indications and results of HLA typing, demographics, symptoms at presentation, investigations, duration and type of treatment including GFD and response to therapy were recorded for all patients undergoing testing for CD related HLA genes. Based on predetermined criteria (Table 1), patients were divided into those with CD, those in whom CD was confidently excluded (Non CD), and those in whom the diagnosis of CD remained indeterminate.

Fisher's exact test and student's t-test (for normally distributed data) and Mann–Whitney U test (for non-normally distributed data) were used to assess observed differences between variables. A p-value <0.05 was considered to represent significance. The institutional review board at BIDMC approved this study.

RESULTS

Demographics and final diagnosis

256 patients underwent CD related HLA testing between December 2007 and August 2011. The mean age at testing was 47.7 years (range 18 to 93 years). The mean age at symptom onset was 40.1 years (range 3 to 77 years) and 200 (78.1%) patients were female. A comorbid autoimmune condition was present in 21.5% patients and none of the above mentioned attributes were significantly different between HLA positive and negative subjects (Table 2). Family history of CD was reported in 17.6% patients and was more likely to be present in HLA positive than negative individuals (23% vs 10% p=0.01) (Table 2). Thorough evaluation of clinical and laboratory data led to a diagnosis of CD in 44 and exclusion of CD in 173 patients. A final diagnosis was not possible in 39 patients of which 31 initiated GFD prior to definitive diagnosis and refused gluten challenge.

Frequency of celiac-related HLA genotypes

Of the 256 patients included in this study, CD related HLA DQ2 or DQ8 were absent in 40% (100 Non CD + 2 CD) patients with an overall diagnostic yield of 39% and negative predictive value of 98%. 154 (60%) patients were found to carry CD permissive HLA DQ2 and/or DQ8, of which 107 (69.5%) were DQ2 positive, 39 (25.3%) were DQ8 positive and 8 (5.2%) patients tested positive for both HLA DQ2 and DQ8.

Forty-two (27%) HLA positive and 2 (2%) HLA negative patients were diagnosed with CD. Hence 42 of 44 (95.5%) CD patients tested positive for one or more permissive HLA genes. 29 (66%) CD patients were DQ2 positive, 9 (20.5%) CD patients were DQ8 positive, 4 (9%) CD patients were positive for DQ2 and DQ8 alleles. 2 (4.5%) CD patients tested negative

for all conventional genotypes but tested positive for half of the DQ2.5-*trans* genotype (DQA⁰⁵⁰⁵/DQB⁰³⁰¹).

Histologic and laboratory data

Of the 256 patients, IgA-tTG levels and duodenal biopsy were checked in 202 and 178 patients respectively while on a gluten containing diet. HLA positive patients were significantly more likely to have positive serology (33.6% vs 9.1% $p < 0.0001$) and villous atrophy (54.4% vs 17.2% $p < 0.0001$) than HLA negative patients. HLA negative patients were significantly more likely to have intraepithelial lymphocytosis (54.7% vs 24.3% $p < 0.0001$) and negative serology (84.4% vs 58.4% $p < 0.0001$) when compared with HLA positive patients. These differences are likely explained by the difference in frequency of CD patients in HLA positive and negative groups (27% vs 2% $p < 0.0001$).

Diagnostic Yield of HLA testing in various Subgroups

For calculation and comparison of indication-specific diagnostic yields of HLA testing, patients were divided into 5 major groups based on results of initial serologic and histologic evaluation. The diagnostic yields of these major groups as well as various subgroups are summarized in Table 3.

Group 1: Patients with abnormal histology and elevated IgA-tTG while consuming gluten

There were 43 patients in this group. Thirty-five patients had villous atrophy and eight patients had Marsh1 histology. Three patients each with VA and Marsh 1 histology were HLA negative. CD was ruled out in all three HLA negative Marsh 1 patients. Amongst the three HLA negative patients with VA, CD was ruled out in just one patient with borderline positive IgA-tTG.

CD was diagnosed in the 2 remaining HLA negative patients with VA. The diagnosis was supported by positive IgA-tTG (Anti-tTG > 2 x ULN) and histologic response to GFD in both these patients. Both patients were reported to be HLA DQ2/DQ8 negative by the testing laboratory but carried half of the DQ 2.5-*trans* genotype (DQA⁰⁵⁰⁵/B⁰³⁰¹). Overall CD was diagnosed in 34 patients, confidently ruled out in 6 patients and remained indeterminate in 2 patients within this group. Diagnostic yields for this group overall as well as subgroups within this group are listed in table 3.

Group 2: Patients with normal histology and normal IgA-tTG while consuming Gluten

There were 34 patients in this group. These patients underwent HLA testing despite negative serology and histology either for ongoing gastrointestinal symptoms ($n = 24$) or for assessment of future risk of CD in those with family history of CD ($n = 10$). CD was confidently excluded in all 34. There were no CD or indeterminate patients in this group. HLA testing was negative in 15 patients for an overall diagnostic yield of 44.4%. HLA negative patients included 2 (20%) of 10 patients with family history of CD and 13 (54.2%) of 24 with ongoing GI symptoms.

Group 3: Patients with abnormal histology and normal IgA-tTG while consuming Gluten

Of the 79 patients in this group, VA and Marsh 1 histology was seen in 29 and 50 patients respectively. CD was confidently excluded in 68 patients overall, including all patients with Marsh 1 histology. Final diagnosis was CD in five and remained indeterminate in 6 patients with VA. Negative HLA testing excluded CD in 8 (27.6%) patients with VA and 30 (60%) patients with Marsh1 histology for an overall diagnostic yield of 48.1%.

Non celiac causes of VA seen in this group included common variable immune deficiency, small intestinal bacterial overgrowth, autoimmune enteropathy, collagenous sprue, peptic duodenitis, and eosinophilic gastroenteritis. The HLA genotypes associated with the above mentioned causes of non celiac villous atrophy are presented in table 4. Alternative causes of intraepithelial lymphocytosis seen in this group included small intestinal bacterial overgrowth, *Helicobacter pylori* infection and peptic duodenitis.

Group 4: Patients with normal histology and elevated IgA-tTG while consuming Gluten

Of the 16 patients in this group, 9 and 7 patients respectively had positive and borderline positive IgA-tTG. HLA testing was negative in two (22.2%) patients with positive and two (28.6%) with borderline positive IgA-tTG, giving an overall diagnostic yield of 25.0%. Although HLA was positive in the remaining 12 patients, CD was confidently excluded in all 16 patients due to absence of typical histology.

Group 5: Patients who initiated GFD before definitive celiac testing

Of the 84 patients in this cohort, 30 underwent IgA-tTG testing without duodenal biopsy and 7 underwent duodenal biopsy without having IgA-tTG tested prior to initiation of GFD. The average GFD duration before HLA testing was 141 weeks (range 4 weeks to 25 years). Negative HLA testing led to exclusion of CD in 39 of the 84 patients for a diagnostic yield of 46.4%. Five of 45 HLA positive patients were diagnosed with CD. Of the remaining 40 HLA positive patients CD could be excluded in nine patients and diagnosis remained indeterminate in 31(68.9%).

Above mentioned groups and subgroups were categorized as having a high (>50%), Intermediate (20% – 50%) or Low (<20%) diagnostic yield for HLA testing. All the indications and respective diagnostic yields for HLA testing are listed in Table 3.

DISCUSSION

While the genetics of CD are considered to be multifactorial [53], HLA DQ2 and DQ8 genes remain the most important genetic factors and are responsible for up to 40% of the genetic heritability of CD as compared to other known associated genes that are collectively responsible for approximately 5% heritability [40, 53]. With a negative predictive value approaching 100%, HLA testing provides critical information in the evaluation of patients with suspected CD where the serological, histological and clinical pictures are discrepant and in those where standard modalities of testing are not applicable due to prior institution of a GFD. However, despite clear utility of celiac HLA testing in individual patients, data on

diagnostic yield for specific clinical indications are limited, contributing to both underuse and misuse of this test.

It is notable that while the cost of HLA testing is high (approximately \$350 at our center) when compared to IgA-tTG, it is much lower than endoscopy with duodenal biopsy and histological evaluation (approximately \$2,000). Endoscopy is also invasive and carries associated risks and patient burden in contrast to HLA testing. Given these estimated costs, in situations where negative HLA testing obviates the need for endoscopy and biopsy, the diagnostic yield of HLA testing need only be greater than 18% to be cost effective.

As expected, the frequency of DQ2 alleles (DQ2.5) was highest in CD patients followed by those with DQ8 genotype and a small fraction were positive for both DQ2 and DQ8 genotype. A small fraction (N = 2) of CD patients were DQ2.2 positive. Also there were no significant differences between DQ2 positive and DQ8 positive patients in terms of disease phenotype or demographics. (Results not presented)

In our evaluation we found that the 256 patients who underwent testing for CD related HLA genes could be subdivided into 5 major categories based on histological and serological abnormality. The diagnostic yield of testing, defined as the percent of patients in which negative HLA testing lead to the exclusion of CD, varied widely within the groups (Table 3).

Seronegative CD occurs in approximately 5% of all patients with CD and can present a diagnostic dilemma. In patients with VA with normal IgA-tTG, negative HLA testing (27.6%), allowed the important differentiation between seronegative CD and non-celiac enteropathy [28, 54].

Our data also highlight the utility of HLA testing in non-celiac gluten sensitivity, which is increasingly encountered in clinical practice [55, 56]. Given the sharp increase in awareness of non-celiac gluten sensitivity [57], a high rate (3.24% –34.9%) of self-reported food sensitivity in the Western population [58] and the improved availability of gluten free options, it is not uncommon for patients to initiate GFD before presenting to a healthcare provider. The finding that a substantial number of patients on a GFD without a diagnosis of CD were negative for HLA DQ2 and DQ8 (46.4%) is in line with previous reports [59]. A diagnostic yield of close to 50% in this group supports HLA testing as an efficient initial step in evaluation prior to considering gluten challenge in patients where institution of GFD precludes serological and histological exclusion of CD.

Approximately 7%–30% CD patients fail to respond to GFD and are considered as having non-responsive CD (NRCD) [60]. In this study, approximately 20% of such patients were found to lack CD related HLA DQ2 and DQ8. This suggests that HLA testing can be considered early in the evaluation of NRCD however further study in this area is needed.

In patients groups with low diagnostic yield for HLA testing but high risk of developing CD such as those with normal histology and positive serology [61, 62], or negative serology with positive family history HLA testing may provide a cost benefit.

Almost all CD patients are positive for HLA DQ2.5, DQ2.2 or DQ8. However, this general rule is not absolute and occasional exceptions have been reported in the literature. Best documented is the occurrence of CD in individuals carrying the HLA DQ2.5-*cis* α allele DQA⁰⁵⁰¹ in the absence of the β allele. In this study we identified 2 subjects with CD who were negative for DQ2.5, DQ2.2 and DQ8. However, the contributing alleles were different to those previously reported in that they represent half of the DQ2.5-*trans* genotype (DQA⁰⁵⁰⁵/DQB⁰³⁰¹). Thus, in patients who test negative for CD associated HLA types the presence or absence of half of DQ2.5, either *cis* (DQA⁰⁵⁰¹) or *trans* (DQA⁰⁵⁰⁵/DQB⁰³⁰¹), should be sought before completely ruling out CD especially in those with positive serology and histology.

While we feel that our results are robust and reflective of standard practices regarding HLA testing in CD, we recognize the limitations that this is a retrospective study, conducted in a single referral center and that the absence of HLA DQ2 and DQ8 does not exclude CD in 100% of cases. While our 5 main groups were large enough to allow data generation and extrapolation, same is not true for some of the subgroups. This is probably secondary to the scarcity of patients with those particular traits.

In conclusion, the yield of HLA testing for exclusion of CD varies significantly depending on the clinical indication. A negative HLA result usually obviates the need for further celiac testing including possible endoscopy and gluten challenge. HLA testing can be a critical and cost effective diagnostic modality. Diagnostic yield of HLA needs to be higher than 18% in a group of patients for the testing to be cost effective. In patients with very high pre-test probability for CD, who test negative for HLA DQ2.5, DQ2.2 and DQ8, certain DQ2.5 alleles, either in *cis* (DQA⁰⁵⁰¹) or *trans* (DQA⁰⁵⁰⁵/DQB⁰³⁰¹) configuration should be sought as these may rarely confer CD susceptibility. The data presented in this study should assist in the effective use of HLA testing in CD evaluation.

Acknowledgments

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Table 1Inclusion and Exclusion criteria for Celiac Disease.¹

	CD Permissive HLA-DQ alleles	Serology	Duodenal histology	Histological response to
Celiac disease diagnosed	Present	Not Required	Villous Atrophy	Yes
	Present	Positive ²	Villous Atrophy	Not required
	Present	Positive ³	Intraepithelial Lymphocytosis	Yes
	Present or absent ⁴	Positive	Villous Atrophy	Yes
Celiac disease excluded	Absent ⁴	Not required	Not required	Not required
	Present or absent	Negative ⁵	Not performed	Not applicable
	Present or absent	Negative	Intraepithelial Lymphocytosis	Not required
	Present or absent	Not required	Normal ⁶	Not required
	Present or absent	Negative	Abnormal but not indicative of CD ⁷	Not required

¹ All cases not meeting these criteria were considered indeterminate² IgA-tTg IgA greater than 39 IU/mL³ Positive serology was required on two occasions at least 1 month apart to exclude transient positive serology during self-limited, infectious enteritis.⁴ Absence of permissive HLA genes rules out CD except in cases where Marsh III histology, positive serology and histological response to GFD were all present.⁵ IgA-tTg IgA 0–19 IU/mL on a gluten-containing diet with normal serum total IgA concentration.⁶ Biopsy performed when subject was consuming a normal, gluten-containing diet.⁷ Histology, laboratory findings and response to therapy confirmatory of an alternative diagnosis.

Table 2

Comparison between HLA positive and HLA negative patients

	Total	HLA Negative	HLA Positive ^a	p value
	N = 256	N = 102	N = 154	
Diagnosed with Celiac Disease	44 (28%)	42 (27%)	2 (2%)	< 0.0001
Mean Age of symptom onset	40.1 yrs	39.4 yrs	40.8 yrs	0.61
Mean age at celiac testing	47.7 yrs	47.2 yrs	48.2 yrs	0.59
Gender (n/% female)	200 (78.1%)	78 (76.5%)	122 (79.2%)	0.64
Family history of CD ^b	43 of 245 (17.6%)	10 of 101 (9.9%)	33 of 144 (22.9%)	0.01
Presence of a comorbid autoimmune disease	55 (21.5%)	17 (16.7%)	38 (24.7%)	0.16
Celiac Disease related Serology ^c	N = 202	N = 77	N = 125	
Negative IgA tTg 0 – 19 IU/ml	138 (68.3%)	65 (84.4%)	73 (58.4%)	< 0.0001
Positive IgA-tTg > 39 IU/ml	49 (24.3%)	7 (9.1%)	42 (33.6%)	< 0.0001
Borderline positive IgA-tTg 20 – 39 IU/ml	15 (7.4%)	5 (6.5%)	10 (8.0%)	0.78
Duodenal Histology ^d	N = 178	N = 64	N = 103	
Marsh 1	60 (33.7%)	35 (54.7%)	25 (24.3%)	<0 .0001
Villous Atrophy	67 (37.6%)	11 (17.2%)	56 (54.4%)	<0 .0001
Normal Histology	51 (28.7%)	18 (28.2%)	33 (32.1%)	0.13

^a 115 DQ2 positive, 39 DQ8 positive, 8 positive for both DQ2 and DQ8.^b Family history available in 245 patients at the time chart review.^c Serology tested while consuming gluten containing diet and normal serum IgA level.^d Duodenal histology available when subject was consuming a gluten containing diet.

Table 3

Indications for HLA testing with high, intermediate and Low Yield

Indication for HLA Testing	Diagnostic
Indications associated with a high yield	More than 50%
Normal IgA-tTG with Intraepithelial lymphocytosis (50)	60%
Intraepithelial lymphocytosis overall (60)	58.3%
Indications associated with an Intermediate yield	20% – 50%
Borderline positive IgA-tTG and villous Atrophy (2)	50%
Normal IgA-tTG in the absence of reliable histology ¹ (25)	48%
Normal IgA-tTG overall (138)	47.1%
Patients who initiated GFD before definite diagnosis ² (84)	46.4%
Normal IgA-tTG with Normal Histology ³ (34)	44.1%
Borderline positive IgA-tTG and Intraepithelial lymphocytosis (5)	40%
Borderline positive IgA-tTG overall (15)	33.3%
Positive IgA-tTG and Intraepithelial Lymphocytosis (3)	33.3%
Normal histology overall (43)	32.6%
Borderline positive IgA-tTG and normal Histology (7)	28.6%
Normal IgA-tTG with Villous atrophy ⁴ (29)	27.6%
Positive IgA-tTG and Normal Histology ⁵ (9)	22.2%
Family history of CD (10)	20%
Indications associated with a Low yield	Less than 20%
Villous Atrophy overall (67)	16.4%
Positive IgA-tTG overall (49)	14.3%
Positive IgA-tTG with Villous Atrophy ⁶ (33)	0

¹ Patients with negative Anti-tTG who initiated GFD without undergoing endoscopy² Discussed as Group 5 in results section³ Discussed as Group 2 in results section⁴ Discussed as Group 3 in results section⁵ Discussed as Group 4 in results section⁶ Discussed as Group 1 in results section

Table 4

HLA typing results for patients with Non Celiac Enteropathy

Diagnosis	HLA Typing results	DQ Type
Autoimmune Enteropathy	DQB1*0301,DQB1*0402 DQA1*0303,DQA1*0401	DQ2 & DQ8 negative
Common variable Immune deficiency	DQB1*0301,DQB1*0601 DQA1*0103,DQA1*0509	DQ2 & DQ8 negative
Common variable immune deficiency	DQB1*0301,DQB1*0503 DQA1*0104,DQA1*0303	DQ2 & DQ8 negative
Collagenous sprue	DQB1*0202,DQB1*0301 DQA1*0201,DQA1*0505	DQ2.5 <i>trans</i>
Eosinophilic enteritis	DQB1*0301,DQB1*0502 DQA1*0102,DQA1*0505	Half DQ2.5 <i>trans</i>
Peptic duodenitis	DQB1*0202,DQB1*0602 DQA1*0102,DQA1*0201	DQ2.2
Peptic duodenitis	DQB1*0201,DQB1*0604 DQA1*0102,DQA1*0501	DQ2.5 <i>cis</i>
Small intestinal bacterial overgrowth	DQB1*0303,DQB1*0602 DQA1*0102,DQA1*0201	DQ2 & DQ8 negative
Small intestinal bacterial overgrowth	DQB1*0201,DQB1*0201 DQA1*0501,DQA1*0505	DQ2.5 <i>cis</i>