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BRIEF ARTICLE

Human leukocyte antigen *DQ2/8* prevalence in non-celiac patients with gastrointestinal diseases

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Abstract

AIM: To investigate the prevalence of human leukocyte antigen (HLA) *DQ2/8* alleles in Southern Italians with liver and gastrointestinal (GI) diseases outside of celiac disease.

METHODS: HLA *DQ2/8* status was assessed in 443 patients from three ambulatory gastroenterology clinics in Southern Italy (University of Federico Ⅱ, Naples, Loreto Crispi Hospital, Ruggi D'Aragona Hospital, Salerno). Patients were grouped based on disease status [pre-post transplant liver disease, esophageal/gastric organic and functional diseases, irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD)] and *DQ2/8* alleles, which correspond to a celiac disease genetic risk gradient. Subject allele frequencies were compared to healthy Italian controls.

RESULTS: One hundred and ninety-six out of four hundred and forty-three (44.2%) subjects, median age 56 years and 42.6% female, were *DQ2/8* positive. When stratifying by disease we found that 86/188 (45.7%) patients with liver disease were HLA *DQ2/8* positive, 39/73 (53.4%) with functional upper GI diseases and 19/41 (46.3%) with organic upper GI diseases were positive. Furthermore, 38/105 (36.2%) patients with IBS and 14/36 (38.9%) with IBD were HLA *DQ2/8* positive (P = 0.21). Compared to healthy controls those with functional upper GI diseases disorders had a 1.8 times higher odds of *DQ2/8* positivity. Those with liver disease had 1.3 times the odds, albeit not statistically significant, of *DQ2/8* positivity. Both those with IBS and IBD had a lower odds of *DQ2/8* positivity compared to healthy controls.

CONCLUSION: The proportion of individuals HLA *DQ2/8* positive is higher in those with liver/upper functional GI disease and lower in IBS/IBD as compared to general population estimates.

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Key words: Human leukocyte antigen *DQ2/8*; Gastrointestinal and liver disease; Celiac disease

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INTRODUCTION

The human leukocyte antigen (HLA) class Ⅱ genes comprise a highly polymorphic region in the short arm of chromosome 6 and are responsible for the creation of molecules involved in exogenous antigen presentation to T cells[1,2]. A subset of class Ⅱ genes, encoding the *DQ2* and *DQ8* serotypes, have been frequently implicated in autoimmune disease pathogenesis. Prevalent in 30%-40% of healthy individuals, *DQ2* and *DQ8* are associated with diseases such as insulin-dependent diabetes mellitus and Hashimoto's Thyroiditis[3,4]. These haplotypes may be best characterized through the gluten dependent relationship with celiac disease, an autoimmune mediated enteropathy affecting approximately 1% of Europeans and North Americans[5-7]. Consequently, many studies have attempted to estimate or infer the proportion of celiac disease risk due to particular *DQ2/8* isoforms. For this reason, a genetic risk gradient has been recently characterized for *DQ2/8* allele variants[8]. The risk associated with celiac disease compared to those healthy depends, incrementally, on the number/type of HLA alleles possessed by an individual. Those with one or both of the *DQ2/8* alleles have a risk ranging from 1:7-1:35, while those lacking all potential immunogenic loci have a near zero chance of contracting celiac disease[8,9]. Beyond celiac disease risk, disease severity and anti-tTg antibody levels are thought to be further tied to this disease/gene-dose relationship[10].

There are several reasons why it may be prudent to study *DQ2/8* alleles in liver/gastrointestinal (GI) disease outside of celiac disease. First, evidence suggests that celiac disease may modify the risk of developing irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), eosinophilic esophagitis, or certain liver diseases[11-14]. Recent research has also shown the presence of HLA *DQ2/8* alleles by themselves, outside of celiac disease, to be associated with GI disease[15-17]. This suggests that *DQ2/8* haplotypes may act as a common factor in liver/GI disease pathogenesis; possibly through a similar mechanism to that of celiac disease. Furthermore, as *DQ2/8* haplotypes contain myriad genes involved in inflammatory processes, such as tumor necrosis factor-α, causal mechanisms between these genes and GI disease may exist[18]. Comparisons of *DQ2/8* prevalence in non-celiac GI diseases, however have not been directly studied.

DQ2/8 associated disease risk is known to be modified across individuals or populations varying in ethnic background, geography or gender[19-23]. Moreover, *DQ2/8* prevalence in Southern Italians has not been characterized. Thus, in this study we sought to first define the prevalence of *HLA DQ2/8* alleles in a Southern Italy non-celiac GI tertiary ambulatory clinic population. Subsequently, we desired to determine what HLA *DQ2/8* haplotypes, if any, were associated with specific liver/GI diseases.

MATERIALS AND METHODS

Subject population

Patients (n = 463) from the gastroenterology ambulatory clinics of three hospitals were recruited over a period of three months. Three hundred and twenty-two subjects were recruited from University of Federico Ⅱ, Naples, Italy, 85 from Loreto Crispi Hospital, Naples, Italy and 56 from Ruggi D'Aragona Hospital, Salerno, Italy. During consultation patient's demographics and disease history were recorded. Disease status was classified according to the nature of presenting problem. Separate categories were attributed generally to pre- or post-liver transplant treatment for chronic viral hepatitis, upper functional and organic GI (gastritis, esophagitis) diseases, lower functional (IBS) and lower inflammatory GI (IBD) diseases. Those with IBS were diagnosed *via* Rome Ⅲ criteria. Overall population characteristics are described in Table 1. Participants were excluded from this study if they had missing data on disease status, multiple upper GI diseases or a prior diagnosis of celiac disease (n = 20).

Informed consent was obtained from each patient or patient guardian prior to study enrollment. The study was approved by ethics review board of the University of Naples "Federico Ⅱ" and complied with the Helsinki Ⅱ declaration.

Sample collection and analysis

Peripheral blood was collected in ethylene-diamino-tetra-acetic tubes and stored at 4 ℃. Genomic DNA was isolated and polymerase chain reaction with sequence-specific primer was then performed to test solely for the presence/absence of *DQ2/8* genes (Celiac Gene Screen, BioDiagene, Palermo, Italy). If patients were "susceptible to celiac disease", further analysis was performed to discern specific alleles known to be associated with celiac disease risk (Celiac Gene Alleles, BioDiagene, Palermo, Italy). Fluorescence detection of *DQ2/8* was performed using BioRun Reader (Celiac Gene Alleles, BioDiagene, Palermo, Italy). Patients "susceptible to celiac disease" are generally understood to have at least one of the HLA *DQ2/8* alpha or beta alleles.

Using fluoro-immuno-assay, with human recombinant tTg as an antigen, patients positive for *HLA DQ2/DQ8* alleles were tested for anti-tTg antibodies (a-tTg) and adequate immunoglobulin A (IgA) levels (CeliKey IgA, EliA, Phadia Freiburg, Germany). Anti-tissue transglutaminase levels greater than 10 (EliA U/mL) were considered positive. Those values between 7 and 10 (EliA U/mL) were considered equivocal and those less than 7 (EliA U/mL) negative. In later analysis both positive and equivocal groups were combined to increase power. None of the patients tested for total IgA were found to be deficient. Due to laboratory error several (n = 46) patients' a-tTg levels were unattainable.

Healthy controls

In order to compare the distribution of *DQ2/8* alleles

Table 1 General demographic attributes of study population

	Overall	Liver	Upper functional	Upper organic	IBS	IBD
Total number	443	188	73	41	105	36
Gender						
Male	44.9%	62.8%	27.4%	34.2%	27.6%	50%
Female	55.1%	37.2%	72.6%	65.8%	72.4%	50%
Age (yr)						
Median	56	61	50	57	43	32
Range	72	66	71	66	62	60
Quartiles						
14-41	-	4.81%	30.1%	24.4%	45.6%	55.6%
42-56	-	23.5%	28.8%	24.4%	28.2%	19.4%
57-65	-	38.5%	21.9%	12.2%	10.7%	8.3%
66-86	-	33.2%	19.2%	39%	15.5%	16.7%

IBS: Irritable bowel syndrome; IBD: Inflammatory bowel disease.

in study participants to the general Italian population we incorporated data from a prior published study by Megiorni *et al*[21]. Healthy participants consisted of 292 healthy and 259 family based controls from Rome, Italy. The prevalence of *DQ2.5/8* in healthy controls was 29%. This increased to 39% after incorporating the less common *DQ2* isoforms.

HLA classification

DQ2 and *DQ8* serotypes, if indicated, were tested for the following alleles: *DQA1*0201*, *DQA1*03*, *DQA1*05*, *DQB1*02*, *DQB1*0301/0304* and *DQB1*0302/0305*. The following *DR* alleles were typed in order to determine the presence of *DQ/DR* haplotypes: *DRB1*03*, *DRB1*04*, *DRB1*07*, *DRB1*11*, *DRB1*12*.

DQ2/8 haplotypes were classified by Megiorni *et al*[8]. *DQ2* positivity was defined as *DQA1*05* in cohort with *DQB2*02* (*DQ2.5*), or *DQA1*0201* (*DQ2.2*)/*DQA1*03* (*DQ2.3*) with *B1*02*. *DQ8* positivity was defined as *DQA1*03* with *DQB1*0302*.

Statistical analysis

The Pearson χ^2 test was performed on categorical data regarding demographics and the overall relationship of prevalence data. Fisher's exact test was used for analysis of data with cell counts $n < 5$. Basic tabular analysis was also performed to obtain odds ratios. A cut-off of $P = 0.05$ was considered significant; all intervals were reported at 95% confidence. The analysis was performed using SAS 9.2 and SPSS 19.

RESULTS

We performed a cross-sectional analysis of HLA *DQ2/8* allele prevalence in a Southern Italian population of patients afflicted with either liver or other digestive diseases outside of celiac disease. *DQ2/8* haplotypes were stratified using a prior defined risk gradient relevant to celiac disease and prevalence in our disease population was compared to estimates in healthy controls.

Table 2 Prevalence of human leukocyte antigen *DQ2/8* by age, gender and gastrointestinal disease

	Proportion of positive subjects	Prevalence	*P* value
Overall	196/443	44.2%	0.02
Gender[1]			
Male	92/199	46.2%	
Female	104/244	42.6%	0.45
Age (yr)			
14-41	48/108	44.4%	
42-56	42/111	37.8%	
57-65	53/107	49.5%	
66-85	52/114	45.6%	0.37
Disease groups			
Liver	86/188	45.7%	
Upper functional	39/73	53.4%	
Upper organic	19/41	46.3%	
IBS	38/105	36.2%	
IBD	14/36	38.9%	0.21

[1]$P > 0.05$ for differences between genders in each disease group except for those with upper functional disorders. In these patients significantly more males were positive than females ($P = 0.05$). IBS: Irritable bowel syndrome; IBD: Inflammatory bowel disease.

Prevalence of HLA alleles in study population

From the patients included in our analysis, 196/443 (44.2%; 95%CI: 39.6%-48.9%) were considered to be HLA *DQ2/8* positive, regardless of disease status. Within those who were positive 144/197 (73.1%) had *DQ2.5* and/or *DQ8*. Table 2 details the prevalence of HLA *DQ2/8* by age, gender and GI disease in the study's participants. The overall difference in *DQ2/8* prevalence between these disease groups was not statistically significant ($P = 0.21$).

Comparison of DQ2/8 prevalence in study population to healthy controls

Subjects *DQ2/8* alleles were organized highest to lowest genetic risk of celiac disease, as described in Megiorni *et al*[8] and compared to healthy controls. No statistically significant difference in HLA *DQ2/8* prevalence between our subject population and healthy controls was found ($P = 0.16$). As with healthy controls, study subjects clustered towards lower celiac disease risk *DQ2/8* alleles with *DQ2.5* heterozygotes lying in the majority. Odds ratio calculations revealed that those with functional gastric/esophageal disorders had a 1.8 fold higher odds of being HLA *DQ2/8* positive as compared to healthy controls. Patients with organic gastric/esophageal disorders had 1.3 higher odds of *DQ2/8* positivity as compared to healthy controls. The odds were also increased in the liver disease group and decreased in IBS/IBD groups although these values were not significant (Table 3).

α-tTg

Out of the patients with a-tTg data available no a-tTg positive patients were found in the liver disease/transplant and inflammatory bowel group. One out of sixty-

Table 3 Magnitude of associations between human leukocyte antigen positivity and specific gastrointestinal disease

Disease group	Odds ratio	95%CI
Overall	1.2	0.96-1.6
Liver	1.3	0.94-1.8
Upper functional	1.8	1.1-2.9
Upper organic	1.3	0.71-2.7
IBS	0.89	0.57-1.4
IBD	0.99	0.49-1.9

IBS: Irritable bowel syndrome; IBD: Inflammatory bowel disease.

Table 4 Positive anti-tTg subject stratified by disease group

Disease group	*n*	Prevalence
Liver	0/173	0%
Upper functional	1/64	1.54%
Upper organic	0/35	0%
IBS	4/90	4.26%
IBD	0/36	0%

$P = 0.04$, for any difference in prevalence of anti-tTg positive between disease groupings. IBS: Irritable bowel syndrome; IBD: Inflammatory bowel disease.

four (1.54%) subjects with functional gastric/esophageal issues and 4/90 (4.26%) with lower functional syndrome were found to be positive ($P = 0.04$; Table 4).

DISCUSSION

The clinical importance of HLA genetic testing has been established in several diseases[3,24]. In this study we performed a cross-sectional analysis on a Southern Italian population with the goal of investigating the prevalence of several HLA *DQ2/8* serotypes in those with GI issues outside of celiac disease.

HLA *DQ2/8* prevalence in Italy is thought to be between 30% and 40%, although this estimate may vary by geographic subpopulation[8,24]. Nearly half of the subjects in this study (44%) were considered HLA *DQ2/8* positive. A lesser proportion of those with IBS/IBD were HLA *DQ2/8* positive although these differences were not significant ($P = 0.21$) (Table 2). Within those who were HLA *DQ2/8* positive the majority possessed low risk celiac disease alleles (Table 5). Thus, our results suggest that *DQ2/8* haplotypes may play a role in liver/digestive disease through pathological mechanisms different from those of celiac disease.

Several studies have established significant associations between *DQ2*, primary sclerosing cholangitis and hepatitis C virus recurrence after transplant[25,26]. The large proportion (46%) of *DQ2/8* positive viral hepatitis patients in our study population agrees with the hypothesis that these haplotypes may be involved in certain liver disease pathogenesis.

Differentiating between functional and organic GI disease can be difficult yet is important due to the impacts on clinical decision-making. In this study we stratified our patient population based on upper and lower functional or organic GI disorders. Several studies have directly compared *DQ2/8* haplotype prevalence in upper organic GI disease. Lucendo *et al*[14] previously demonstrated a null association between *DQ2/8* and eosinophilic esophagitis. Interestingly, *DR3* and *DR4* have been significantly linked with atrophic gastritis in a similar Italian population[27]. The positive yet non-significant relationship between our organic gastric/esophageal patients and *DQ2/8* may be a consequence of the various upper GI organic diseases captured in our patient population. Unfortunately, due to sample size restrictions, we were not able to stratify by specific disease. Ultimately these findings suggest that it may be inappropriate to generalize upper organic GI disease as one confluent group because it is unknown whether the lack of a significant association was truly due to causal or confounding disease factors.

Table 5 Prevalence of specific human leukocyte antigen *DQ2/8* alleles between gastrointestinal and control populations *n* (%)

Overall	Gastrointestinal	Controls	Risk
DQ2 and *DQ8*	2 (0.45)	1 (0.2)	1:45
DQ2, *β1*02/*02*	17 (3.8)	13 (2.4)	1:63
DQ8, *β1*02* positive	8 (1.8)	4 (0.7)	1:39
β2, *β1*02/*02*	11 (2.5)	2 (0.4)	1:16
DQ2, *β1*02*/X	85 (19.2)	106 (19.2)	1:100
DQ8, *β1*02* negative	32 (7.2)	36 (6.5)	1:90
β2, *β1*02*/X	41 (9.3)	53 (9.7)	1:104
α5 + other	247 (55.8)	336 (60.9)	1:101
Total	443	551	

Omnibus *DQ2/8* positive *vs* *DQ2/8* negative χ^2; $P = 0.16$. IBS: Irritable bowel syndrome; IBD: Inflammatory bowel disease.

Functional GI disorders represent the majority of GI cases yet many have etiologies, which are poorly understood. Whether it is genetic abnormalities, psychological factors or other environmental variables, functional disorders can represent complex, difficult to solve cases[28]. The strongest evidence of an association with *DQ2/8* in this study was found in patients with functional upper GI disorders. Patients in this study had 1.8 higher odds of *DQ2/8* positivity if they had an upper functional GI disorder as compared to healthy controls. This may signify that the risk of functional upper GI onset or recurrence is modified by the presence of particular *DQ2/8* haplotypes. Currently, the only published data, which could be used to comment on these findings, relates to celiac disease and *DQ2/8*. For example, Ford *et al*[29] conducted a meta-analysis, which found no association between celiac disease and functional dyspepsia. Overall, it is too early to decide whether *DQ2/8* could be used to differentiate functional *vs* organic disease or at least be incorporated into a clinical algorithm that dictates likelihood of disease.

Known immunological associations between IBD and *DR7*, which is linked to both *DQ2* and *DQ8* hap-

lotypes have been established[17,30]. Prior prevalence data though suggests that IBD, particularly Crohn's disease, is lower in individuals with the *DQ2/8* linked celiac disease[12]. The relative modest prevalence of IBD (39%) in our study supports this notion. IBS has also been linked to HLA *DQ2/8* haplotypes and bowel transit speeds[16,31]. Additionally several studies have demonstrated that those with IBS and *DQ2/8* positivity tend to present with symptoms indicative of gluten sensitivity and are responsive to a gluten-free diet[31,32]. Out of all of the disease groupings those with IBS had the lowest prevalence of *DQ2/8* positivity (36%). As those on a gluten-free diet were excluded from this study this may account for the low prevalence *DQ2/8* positivity in those with IBS.

A small part of this study wished to obtain a baseline level of a-tTg positive patients in a previously diagnosed GI population (Table 4). If these patients were assumed to have celiac disease, these results correspond with known prevalence estimates of the disease[5].

Those who had prior diagnosed celiac disease or were adhering to a gluten-free diet were excluded from the study. It is thought to be common for patients with general abdominal pain, diarrhea and/or nausea to experiment with a gluten-free diet in an attempt to ameliorate symptoms[33]. As such, removing individuals who may have experimented with this type of diet eliminated a potentially large source of bias. An additional strength of this study was the precision with which HLA haplotypes and disease types were measured. Barring laboratory error, the HLA typing assays in this study have been shown to have near perfect sensitivity and specificity[34]. During blood collection patient's disease status was recorded. Thus, it was also unlikely that the classification of disease was subject to recall bias.

This study aimed to generally define HLA *DQ2/8* prevalence in diseases/disorders that may be linked to celiac disease. As such there were several limitations, which could have potentially biased the results. The cross-sectional nature limited the collection of subject's lifestyle and disease history. Therefore, data such as age of disease onset and severity were unavailable. The Southern Italian population is typically generically and environmentally homogenous thus unmeasured confounders would not significantly influence the results. Controls from the study were described as "healthy". We know from Megiorni *et al*[8] that these participants did not have celiac disease but it is possible they were afflicted with a liver or GI disease. This type of bias would have most likely pushed the magnitude of our estimates towards the null, masking potential associations. The small sample size in our study also limited our ability to make statistically significant conclusions and investigate specific *DQ2/8* allele associations.

Due to the limitations of the present study it may be difficult to make truly suggestive conclusions regarding the relationship between HLA *DQ2/8* positive patients and liver/GI conditions. This study though has taken the first step through implying a potential association between specific HLA *DQ2/8* alleles and GI disease pathogenesis. Reproducibility of these results may eventually lead to the creation of clinical markers of elusive disease onset, such as in IBS or other clinically ambiguous disorders[11,16,28,35,36]. Future studies should involve expanding the number of study participants in order to look at specific *DQ2* alleles or investigating the shared influence of non-HLA celiac disease risk alleles.

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COMMENTS

Background

Human leukocyte antigen (HLA) *DQ2/8* alleles and associated haplotypes are important players in the pathogenesis of several autoimmune diseases, particularly celiac disease. The distribution of *DQ2/8* alleles in gastrointestinal (GI) disease outside of celiac disease though has been poorly established.

Research frontiers

HLA *DQ2/8* alleles in patients can be easily tested with currently laboratory capabilities. Knowledge of HLA *DQ2/8* frequencies related to autoimmune disease is being used to identify high risk populations and drive clinical recommendations regarding *DQ2/8* gene testing.

Innovations and breakthroughs

Similar studies have described HLA *DQ2/8* risk alleles in diseases such as type 1 diabetes or celiac disease. For example, in celiac disease those with one or more of the *DQ2.5/8* alleles are at highest risk of disease onset. To date no studies have directly compared *DQ2/8* prevalence in GI disease outside of celiac disease. By comparing the prevalence of HLA *DQ2/8* to that of healthy controls we demonstrated that, like celiac disease, those with liver disease and esophageal/gastric disorders (both organic and functional) are more likely to be *DQ2/8* positive.

Applications

Although this study is preliminary in nature, the results suggest that the development of novel clinical susceptibility markers of GI disease may exist. Particular *DQ2/8* polymorphisms may point to increased risk of certain liver or esophageal/gastric disease.

Peer review

The study investigated the prevalence of HLA *DQ2/8* alleles in Southern Italians with liver and GI diseases outside of celiac disease. The proportion of individuals HLA *DQ2/8* positive resulted higher in those with liver/gastric or esophageal GI disease and lower in irritable bowel syndrome/inflammatory bowel disease as compared to general population estimates. The study is well designed and conduced.

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