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RAPID COMMUNICATION

A functional variant in the CD209 promoter is associated with DQ2-negative celiac disease in the Spanish population

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Abstract

AIM: To address the role of *CD209* in celiac disease (CD) patients. Non-human leukocyte antigen (HLA) genetic factors in CD predisposition are poorly understood, and environmental factors like infectious pathogens may play a role. CD209 is a dendritic and macrophage surface molecule involved in pathogen recognition and immune activation. Recently, a functional variant in the promoter of the *CD209* gene (-336A/G) has been shown to affect the transcriptional *CD209* activity *in vitro* and it has been associated with a higher susceptibility to/or severity of infection.

METHODS: The study population was composed of two case-control cohorts of 103 and 386 CD patients and 312 y 419 healthy controls as well as a panel of 257 celiac families. Genotyping for the -336A/G *CD209* promoter polymorphism was performed using a TaqMan 5´ allelic discrimination assay. HLA-DQ was determined by hybridization with allele specific probes.

RESULTS: Initially, the case-control and familial studies did not find any association of the -336 A/G *CD209* genetic variant with CD susceptibility. However, the stratification by HLA-DQ2 did reveal a significant association of CD209 promoter polymorphism in the HLA-DQ2 (-) group (carrier A *vs* GG in DQ2 (-) *vs* DQ2 (+) patients (P = 0.026, OR = 3.71).

CONCLUSION: The -336G *CD209* allele seems to be involved in CD susceptibility in HLA-DQ2 (-) patients. Our results might suggest a possible role of pathogens in the onset of a minor group of CD patients.

Key words: CD209; HLA-DQ2; Celiac disease; Single nucleotide polymorphism; Susceptibility

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INTRODUCTION

Celiac disease (CD) is a chronic inflammatory disease with a multi-factorial origin. Genetically susceptible individuals show a pathological inflammatory response after exposure to gluten, a protein present in wheat, barley, rye and oats[1]. Genetic studies in CD have revealed a strong influence of human leukocyte antigen (HLA) class II genes, especifically the alleles DQA1*0501 and DQB1*02 (HLA-DQ2) and to a lesser extent DQA1*0301 and DQB1*0302 (HLA-DQ8). However, other genes outside the HLA region must be also involved in CD susceptibility since the genetic susceptibility to CD indicated by the λs (sibling risk/population frequency) is around 30%-60%, but no more than 40% to that sibling familial risk has been attributed to HLA[2,3].

CD can be considered a model of autoimmune diseases, being for the moment the only one in which the environmental factor that triggers the inflammatory response (gluten) has been clearly identified[4]. Nevertheless, it is suggested that other environmental factors may affect the disease onset. It has been speculated that certain viruses or pathogens may somehow alter the tolerance in the intestinal mucosa, or induce an inflammatory state in the intestinal mucosa, prone to react against gluten-derived

peptides[5]. This additional environmental input might be of higher importance in those patients with low genetic susceptibility (e.g., patients without HLA-DQ2). The interaction between the pathogen and gut immune cells might be an essential mechanism in this pathway. In this regard, an important mediator of pathogen recognition by dendritic cells is CD209 or DC-SIGN (dendritic cell specific intercellular adhesion molecule 3 grabbing non-integrin), encoded by the *CD209* gene. CD209 is a type Ⅱ transmembrane protein present in dendritic cells and macrophages and a member of the C-type lectin receptor family. Natural ligands of CD209 include self-molecules, ICAM-2 and ICAM-3, but it also binds to pathogens and pathogen-derived molecules. This interaction may be used by some pathogens as an immunoinvasive strategy through binding and internalization, although always as one among other alternative ways[6]. Furthermore, the *CD209* gene is located within the 19p13.1 chromosomal region, showing a strong linkage with CD susceptibility in a genome-wide study[7].

In the *CD209* promoter region, a putative functional variant (-336A/G; rs4804803) affecting a Sp1-like binding site has been described[8]. This single nucleotide polymorphism (SNP) can affect the transcriptional *CD209* activity *in vitro* and is associated with a higher susceptibility to HIV-1 infection and a higher dengue fever severity.

On this basis, we aimed to investigate the possible involvement of *CD209* -336 genetic variant in CD susceptibility.

MATERIALS AND METHODS

Subjects

A total of 103 celiac disease patients and 312 ethnically matched healthy controls recruited from the Hospital Virgen de las Nieves (Granada, Spain) were initially studied to assess the influence of the -336A/G *CD209* polymorphism in celiac disease. Subsequently, a second larger cohort recruited from the Hospital Clínico San Carlos and Hospital La Paz (Madrid, Spain) and consisting of 386 celiac patients and 419 healthy controls was analyzed to obtain more conclusive results. The age at study of all patients was 7.1 ± 3.9 years, and the mean age for diagnosis was 2.7 ± 2.72 years. A total of 60% were women. We extended our study to a panel of 257 pairs of progenitors of the CD patients studied, specifically 103 families from Granada and 154 from Madrid were obtained, all of them composed of an affected child and their parents.

The participants from both the familial and case-control analyses were of Spanish white origin. All the samples were collected after informed consent was obtained. Celiac disease patients were diagnosed following the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) criteria[9]. This study was approved by the ethics committee of the respective hospitals.

Genotyping

Taqman 5′ allelic discrimination assay was performed to genotype the *CD209* -336 genetic variant. Primers and probes were provided by Custom-Taqman-SNP-Genotyping-Assay Service (Applied Biosystems, Foster City, CA, USA). The primer sequences are 5' - GGACAGTGCTTCCAGGAACT -3' (sense) and 5' - TGTGTTACACCCCCTCCACTAG -3' (antisense). The sequences of Taqman MGB probes are 5'-TACCTGCCTACCCTTG-3' and 5'- CTGCCCACCCTTG -3'. The probes were labeled with the fluorescent dyes VIC and FAM, respectively. Polymerase chain reaction (PCR) was carried out in a total volume of 12.5 l using the following amplification protocol: denaturation at 95℃ for 10 min, followed by 40 cycles of denaturation at 92℃ for 15 s, and annealing and extension at 60℃ for 1 min. Then genotype of each sample was attributed by measuring the allele-specific fluorescence in the ABI Prism 7000 or 7900 Sequence Detection System, using SDS 1.1 software for allele discrimination (Applied Biosystems).

HLA-DQ typing was performed in the respective laboratories as previously described[10,11].

Table 1 Genotypic and allelic CD209 frequencies in celiac patients (n = 103) and controls (n = 312) from the Granada area

Genotype	Celiac patients n (%)	Controls n (%)	P
AA	61 (59)	191 (61)	
AG	36 (35)	109 (35)	
GG	6 (6)	12 (4)	0.7
Allele			
A	158 (77)	491 (78)	
G	48 (23)	133 (21)	0.6

Statistical analysis

Allelic and genotypic frequencies were compared by means of χ^2 tests or Fisher's exact test when necessary (expected values below 5). Statistical analyses were performed with the statistical package EpiInfo v5.00 (CDC, Atlanta, USA). Transmission disequilibrium test (TDT), which assesses the preferential transmission of one allele over the other from heterozygous parents to affected sibs, was used to analyze the familial data.

RESULTS

Allelic and genotypic frequencies for *CD209* in celiac disease patients and controls are shown in Table 1 for the first cohort studied (Hospital Virgen de las Nieves, Granada). No significant differences were observed in any case. A similar result was obtained when family data were analyzed (27 G alleles transmitted *vs* 33 not transmitted, $P = 0.26$). However, due to the overwhelming and potentially masking influence of HLA-DQ2 (DQA1*0501 and DQB1*02) on celiac disease, we divided the patients into two groups according to the presence (+) or absence (-) of those alleles. No allele of *CD209* was significantly increased in any group of patients ($P = 0.11$) (Table 2). However, the different genotype distribution was significant in both groups of patients. The small sample

Table 2 Genotypic frequencies in HLA-DQ2 (+) and HLA-DQ (-) patients from the Granada area

Genotype	DQ2 (-) patients (*n* = 7)	DQ2 (+) patients (*n* = 96)
AA	2	59
AG	5	31
GG	0	6

Table 3 Genotypic CD209 frequencies in DQ2 (-) (*n* = 29) and DQ2 (+) (*n* = 357) celiac patients and controls (*n* = 419) from the Madrid area

Genotype	DQ2 (-) patients *n* (%)	DQ2 (+) patients *n* (%)	Controls *n* (%)
AA	18 (62)	207 (58)	255 (61)
AG	6 (21)	131 (37)	142 (34)
GG	5 (17)	19 (5)	22 (5)

3*2 contingency table DQ2- *vs* DQ2+: $P = 0.017$; 3*2 contingency table DQ2- *vs* controls: $P = 0.019$; carriers A *vs* GG DQ2 (-) *vs* DQ2 (+): $P = 0.026$ OR = 3.71 (1.10-11.78); carriers A *vs* GG DQ2 (-) *vs* controls: $P = 0.023$ OR= 3.76 (1.14-11.73).

size of the DQ2 (-) group ($n = 7$) could be responsible for the lack of statistical significance, therefore we decided to repeat the study using a higher number of patients.

A second cohort was then studied (Madrid) (Table 3) and we did obtain significant results. Genotypic frequencies were significantly different when DQ2 (+) celiac disease patients were compared with DQ2 (-) patients ($P = 0.017$) or with controls ($P = 0.019$) and an even higher significance was observed when DQ2 (+) celiac disease patients and controls (grouped because there are no significant differences between them) were considered together ($P = 0.013$). The differences seemed to be mostly due to the increased susceptibility to celiac disease occurrence in DQ2 (-) individuals carrying two copies of the allele *CD209* -336G (AA + AG individuals vs GG individuals, $P = 0.021$, OR = 3.73 (1.18-11.03), DQ2 (-) *vs* DQ2 (+) individuals and controls considered together). A family study with samples from Madrid was also performed to determine the influence of *CD209* on celiac disease after DQ2 stratification. However, the scarce number of families with DQ2 (-) children did not allow us to obtain any conclusion (there were only 4 families and one of the parents was heterozygous for the studied polymorphism and the allele A was transmitted just in one case).

DISCUSSION

The contribution of HLA class Ⅱ genes to CD susceptibility is for the moment in the most reproducible and well established fact regarding CD genetics[12]. Thus, 92%-93% of celiac disease patients carry DQA1*0501-DQB1*02 (DQ2), in sharp contrast with only 28% of the Spanish healthy controls. However, less is known regarding the genetic factors contributing to CD predisposition in patients lacking the DQ2 allele. In this study we analyzed for the first time the contribution of -336 A/G *CD209* genetic variant to CD susceptibility, investigating the contribution of this polymorphism in both DQ2+ and DQ2- CD patients. Interestingly, we have shown that the *CD209*-336 polymorphism seems to be a genetic risk factor for CD in DQ2- patients in our population. We observed that in DQ2- patients the CD209-336 GG genotype was significantly overrepresented, suggesting that additional predisposition factors are more relevant in patients lacking the major susceptibility determinant, namely the antigen presenting HLA class Ⅱ molecule DQA1*0501-DQB1*02. According to the results obtained in this study, *CD209*, more precisely, the promoter allele-336G, is one of those additional secondary genetic factors.

The CD209 -336G allele is also associated with increased predisposition to parenteral HIV infection[13]. The G allele favors the binding to the ubiquitous transcription factor Sp1, but paradoxically the transcription rate from the G allele seems to be lower than that from the A allele[8]. Perhaps the lower amount of DC-SIGN protein on the cell surface might reduce the surveillance activity of sentinel cells, and therefore may promote the persistence of pathogens in the gut. The continued presence of pathogens may underlie in turn the inflammatory down-regulation in celiac disease patients. In fact, it has been suggested that some bacterial infections might play a relevant role in CD development[1,5] and the hypothesis proposed could support the maintenance or facilitation of bacterial infection in CD onset.

In summary, the *CD209*-336 polymorphism for CD susceptibility exists in DQ2- patients in our population. It is necessary to analyze the possible contribution of other C-type lectin receptors (mannose receptor, endo-180, SIGNR1, dectin-1, dectin-2) that are able to bind to exogenous ligands as interesting candidate genes in CD predisposition, especially in DQ2 negative patients.

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