Human Immunology 71 (2010) 861–864

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

MSH5 is not a genetic predisposing factor for immunoglobulin A deficiency but marks the HLA-DRB1*0102 subgroup carrying susceptibility

Nadia del Pozo [a], Luz María Medrano [a], M. Carmen Cénit [a], Miguel Fernández-Arquero [a], Antonio Ferreira [b], Mari Cruz García-Rodríguez [b], Emilio G. de la Concha [a], Elena Urcelay [a], Concepción Núñez [a,*]

[a] Clinical Immunology Department, Hospital Clínico, San Carlos, Madrid, Spain
[b] Immunology Department, Hospital Universitario La Paz, Madrid, Spain

ARTICLE INFO

Article history:
Received 27 February 2010
Accepted 4 June 2010
Available online 11 June 2010

Keywords:
Disease susceptibility
Haplotype
IgA deficiency
MSH5

ABSTRACT

The etiology of selective IgA deficiency (IgAD) is clearly influenced by human leukocyte antigen (HLA) genetic composition, although the susceptibility observed has not been ascribed to any specific gene/s. A possible role of the *MSH5* gene, mapping on this chromosomal region, has been proposed based on its function and on the association of some *MSH5* polymorphisms (L85F/P786S and rs3131378) with the disease. However, the extensive linkage disequilibrium in the HLA region makes mandatory additional analyses. We aimed at evaluating the role of those *MSH5* polymorphisms on IgAD susceptibility considering their linkage with other classically associated HLA markers, specifically *DRB1*0102* and *B*08-DRB1*03*. We studied 146 trios composed by IgAD patient and parents to unambiguously establish the gametic phase. Association of those *MSH5* variants with IgAD is observed but stratified analyses considering other HLA alleles rule out the role of *MSH5 per se* as a predisposing factor. However, the minor allele of one of the studied polymorphisms, 85F, defines the subgroup of *DRB1*0102* haplotypes carrying susceptibility. The causal factor present on this haplotype (*MSH5* 85F–*DRB1*0102*) seems to be at the telomeric end of HLA class II or in Class I or III, as the allele composition in more centromeric markers is shared by all the haplotypes containing *DRB1*0102*.

1. Introduction

Selective IgA deficiency (IgAD) is the most common immunodeficiency in the Caucasian population, and it is characterized by very low or undetectable levels of IgA in sera (<0.07 g/l) [1]. Most patients are asymptomatic. Nevertheless, in patients with symptoms, recurrent infections are the dominant complications, frequently as respiratory and gastrointestinal affections in accordance with the presence of IgA in the mucosa. Allergy and autoimmune diseases are also common. A complex etiology characterizes this disease, with an involvement of environmental and genetic factors. An influence of human leukocyte antigen (HLA) has been clearly established, although the specific gene(s) involved is still controversial. Three haplotypes are considered as containing susceptibility factor(s), which are marked by *DRB1*0102*, *B*08-DRB1*03* and *DRB1*07* [2,3]; and *DRB1*1501* is associated with protection against this disease [4]. If those haplotypes mark the same or distinct susceptibility factors remains uncertain [3]. Outside the HLA complex, several genes have been studied as possible susceptibility factors contributing to the disease [5–13], but very few of them rendered significant results [12,13].

The *MSH5* (mutS homolog 5 [*Escherichia coli*]) gene, also located in the HLA region, has been associated with IgAD and common variable immunodeficiency (CVID) risk as reported by Sekine et al. [14]. The authors studied the relevant variation in the *MSH5* gene and found three single nucleotide polymorphisms (SNPs), namely, L85F (rs28381349), P786S (rs28399984) and rs3131378, which increased susceptibility. The role of MSH5 in the regulation of immunoglobulin class switch recombination, together with the functional relevance of L85F and P786S (both SNPs genetically equivalent and resulting in a protein with altered function), led Sekine et al. to suggest that this gene could be predisposing to IgAD and CVID in a subgroup of patients. However, the three *MSH5* variants described are present in known IgAD HLA susceptibility haplotypes and therefore, it could be postulated that either *MSH5* is the etiologic factor on those haplotypes or it is just marking the same etiologic factor/s.

In this work, the role of *MSH5* polymorphisms has been studied by analyzing their concurrence with other HLA markers classically associated with IgAD. A high number of trios composed by the child affected with both parents was used to unequivocally determine the gametic phase.

* Corresponding author.
E-mail address: conchita.npardo@gmail.com (C. Núñez).
N. del Pozo and L.M. Medrano contributed equally to this work.

0198-8859/10/$32.00 - see front matter © 2010 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2010.06.007

2. Subjects and methods

We studied 146 trios composed by unrelated individuals with selective IgA immunodeficiency (IgAD) as defined by the World Health Organization Group on Primary Immunodeficiencies (IgA<0.07 g/l), and their unaffected parents. All the samples were collected from a single reference center, Hospital La Paz (Madrid, Spain) and belong to individuals with Spanish ancestry. Written informed consent was obtained from all the participants. This study was approved by the Ethics Committee of the Hospital Clínico San Carlos (Madrid).

All samples were genotyped for two polymorphisms located in the *MSH5* gene: L85F (rs28381349), in exon 3, and rs3131378, in intron 12. Genotyping was performed by TaqMan technology (Applied Biosystems, Norwalk, CT). The *MSH5* P786S (rs28399984) polymorphism was not included in this study because of its high correlation with L85F [14]. Genotyping for *DRB1* and specifically for *DRB1*1501* and *HLA-B*08* was also performed in all individuals. *DRB1* was typed by polymerase chain reaction (PCR), followed by hybridization with allele-specific probes [3] and *DRB1*1501* and *HLA-B*08* by TaqMan technology using the highly correlated SNPs rs3135388 (*DRB1*1501*), rs6457374, and rs2844535 (*HLA-B*08*). Moreover, information from additional HLA markers or loci, obtained in our laboratory as previously described [3,15], was used for the available samples (83 trios). These additional markers include 16 microsatellites located in (or close to) different genes located in major histocompatibiliy complex (MHC) class III and class II: MICA, TNFa, TNFb, BAT2, D6S273, 9N2, MN6S1424, D6S2670, D3A, NOTCH4, 8105224, D6S2665, D6S2664, DQCARII, DQCAR, G51152, and the polymorphic genes *HLA-B* (class I) and *DQA1* and *DQB1* (class II).

The transmission disequilibrium test (TDT), which measures the overtransmission of one allele (or haplotype) from heterozygous parents to the affected offspring, was initially used to analyze the individual effect of each SNP or haplotype studied. The risk allele of the two *MSH5* polymorphisms analyzed is present on each HLA IgAD susceptibility haplotype, 85F in *HLA-DRB1*0102* and rs3131378-C in *B*08-DRB1*03*. Therefore, haplotypes, including *MSH5* and those classic HLA loci were obtained using the family data and stratified analysis were performed. Comparisons between classes were evaluated using the χ^2 test or a Fisher exact test (two-tailed) when expected values were less than 5.

3. Results

A highly significant overtransmission of the minor allele of L85F was shown by the TDT: 35 85F (rs28381349-T) alleles transmitted vs. 13 untransmitted ($p = 0.001$). A significant result was also observed when studying rs3131378: 36 minor alleles (rs3131378-C) transmitted vs. 15 untransmitted ($p = 0.002$).

Table 1
MSH5 L85F data stratified by the presence of *DRB1*0102*

	*DRB1*0102*	*MSH5* 85F	TDT		T:U
			T:U	*P*	
1	+	+	28:5	3.3×10^{-5}	29:6
2	+	−	7:7	0.60	8:8
3	−	+	7:6	0.50	8:7
4	−	−	127:196	7.3×10^{-5}	130:199

T, transmitted; U, untransmitted.
T:U under the TDT heading includes information from heterozygous parents only. T:U in the last column includes information from homozygous parents. In column 2, (+) denotes carriage of the susceptibility allele *DRB1**0102 and (−) denotes noncarriage of any of the known IgAD susceptibility/protective HLA factors (*DRB1*0102* or *DRB1*03-B*08* or *DRB1*07* or *DRB1*1501*). In column 3, (+) and (−) indicate carriage and noncarriage of the *MSH5* 85F allele, respectively.
Group comparisons: $p = 0.021$; 1 vs. 3: $p = 0.040$; 2 vs. 3: $p = 0.85$.

Table 2
MSH5 rs3131378 data stratified by the presence of *B*08-DR*03*

	*DRB1*03-B*08*	*MSH5* rs3131378-C	TDT		T:U
			T:U	*p*	
1	+	+	33:10	3.0×10^{-4}	35:12
2	+	−	0:1	0.50	0:1
3	−	+	1:6	0.06	1:6
4	−	−	128:191	2.5×10^{-4}	137:200

T, transmitted; U, untransmitted.
T:U under the TDT heading only includes information from heterozygous parents; T:U in the last column includes information from homozygous parents. In column 2, (+) denotes carriage of the susceptibility allele DRB1*03-B*08 and (−) denotes noncarriage of any of the known IgAD susceptibility/protective HLA factors (*DRB1*0102* or *DRB1*03-B*08* or *DRB1*07* or *DRB1*1501*). In column 3, (+) and (−) indicate carriage and noncarriage of the *MSH5* 85F allele, respectively.
Group comparisons: $p = 0.27$; 1 vs. 3: $p = 0.004$; 2 vs. 3: $p = 1.00$.

Linkage disequilibrium values between L85F and *DRB1*0102* and rs3131378 and *B*08-DRB1*03* obtained from our sample were $D' = 0.66$ and $r^2 = 0.42$ and $D' = 0.98$ and $r^2 = 0.76$, respectively. Therefore, the significant association of *MSH5* with IgAD could be a consequence of the correlation between *MSH5* and HLA susceptibility alleles. To clarify this point, stratified analyses were performed.

In Table 1, the number of transmitted and untransmitted haplotypes constructed considering the isolated or combined presence of *DRB1*0102* and *MSH5* 85F is presented. Only haplotypes containing simultaneously *DRB1*0102* and *MSH5* 85F (class 1 in Table 1) are significantly overtransmitted ($p = 3.3\times10^{-5}$), but not *DRB1*0102* (class 2) or *MSH5* 85F (class 3) separately ($p = 0.60$ and $p = 0.50$ for classes 2 and 3, respectively). Moreover, the number of transmitted vs. untransmitted haplotypes (including information from homozygous parents) containing both *MSH5* 85F and *DRB1*0102* or containing only one of those alleles (class 1 vs 2 and class 1 vs 3, last column of Table 1) was compared with confirm the genuine different susceptibility between them. Class 1 significantly differs from all other classes ($p = 0.021$ vs class 2 and $p = 0.040$ vs class 3), but no differences are observed between classes 2 and 3 ($p = 0.85$).

In Table 2, haplotypic data stratified attending to *B*08-DRB1*03* and *MSH5* rs3131378-C are shown. Haplotypes carrying *B*08-DRB1*03* are almost always bearing the minor allele of *MSH5*-rs3131378 and therefore the individual effect of *B*08-DRB1*03* cannot be evaluated. When the minor allele of rs3131378 appears in non–*B*08-DRB1*03* haplotypes, overtransmission is not observed (1T:6U, $p = 0.06$), and a significant difference exists between this group and that of haplotypes bearing *B*08-DRB1*03* and *MHS5* rs3131378-C ($p = 0.004$).

Note that the undertransmission observed in class 4 of Tables 1 and 2 is a consequence of eliminating all the susceptibility known in the HLA.

Subsequently, we wanted to investigate the allele composition of other HLA markers or loci in the haplotype carrying *DRB1*0102* and *MSH5* 85F. We used, for the 83 available trios, data previously obtained in our laboratory for three loci (*HLA-B*, *DQA1,* and *DQB1*) and 16 microsatellite markers from upstream of *HLA-B* to downstream of *HLA-DQA2* (Fig. 1). We observed almost the same allelic composition for all the markers studied in haplotypes carrying both *HLA-DRB1*0102* and *MSH5* 85F. However, only alleles in markers between G51152 and 8105224 were shared by all haplotypes bearing *DRB1*0102* (independently of the allele present in *MSH5* L85F). The allele composition of the haplotype bearing *DRB1*0102* and *MSH5* 85F for all the markers studied is shown in Figure 1.

4. Discussion

IgAD susceptibility is increased by the presence of several HLA haplotypes, although the specific causal gene(s) has not yet been